Randomized, Double-Masked, Pilot Study Comparing the Safety and Efficacy of Two Dosing Regimens of TP-03 for the Treatment of Meibomian Gland Dysfunction in Patients with *Demodex* Lid Infestation (Ersa)

Protocol Number: TRS-008

[redacted]

IND Sponsor: Tarsus Pharmaceuticals, Inc.

NCT05454956

PROTOCOL SUMMARY

TITLE	Randomized, Double-Masked, Pilot Study Comparing the Safety and Efficacy of Two Dosing Regimens of TP-03 for the Treatment of Meibomian Gland Dysfunction in Patients with *Demodex* Lid Infestation (Ersa)
TEST ARTICLE	TP-03, lotilaner ophthalmic solution, 0.25%
OTHER TEST ARTICLE	Vehicle of TP-03 to maintain masking of BID dosing
OBJECTIVE	To evaluate the safety and efficacy of TP-03, 0.25%, BID versus TID dosing for the treatment of meibomian gland dysfunction in patients with *Demodex* lid infestation. Efficacy will be evaluated by the assessment of lower lid meibomian gland secretion, erythema of the lid margins, [redacted]. Safety will be determined by assessing adverse effects related to treatment [redacted]
CLINICAL HYPOTHESIS	Treatment with TP-03, 0.25%, TID will result in a greater improvement from baseline than BID treatment for 85 days in the signs [redacted] of MGD with no significant increase in ocular irritation.
STUDY DESIGN	Prospective, randomized, two-arm, double-masked, parallel treatment study
NUMBER OF PARTICIPANTS	Up to 40
PARTICIPANT POPULATION	Participants with evidence of meibomian gland dysfunction in the presence of *Demodex* infestation
DESCRIPTION OF SITES	Up to six sites in the United States
STUDY DURATION	Approximately 9 months
RANDOMIZATION	1:1
STUDY VISITS	Study visits will take place at Day 1, Day 15, Day 29, Day 43, Day 57, Day 85 and Day 115
SAFETY MEASURES	• Adverse events • [redacted] • Slit lamp biomicroscopy findings
EFFICACY MEASURES	• Lower lid meibomian gland secretion assessment o Total meibomian gland score

	[redacted] • Lid margin erythema [redacted]
STATISTICAL CONSIDERATIONS	This is a pilot study to compare the safety and efficacy of two different dosing regimens, BID and TID, of TP-03 for the treatment of MGD. There are no prior data available to perform a sample size estimate. The sample size of 40 participants was selected based on clinical and practical considerations.
STATISTICAL ANALYSIS PLAN	Safety measures will be summarized for each treatment regimen and both treatment regimens combined using descriptive statistics. Efficacy measures will be compared between dosing regimens as the change from baseline using either the t-test or Wilcoxon rank-sum test as appropriate. Comparisons will be two-sided using an α of 0.05. No adjustment will be made for multiple comparisons. [redacted]

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[redacted]

[redacted]

1. ABBREVIATION TABLE

ADE	Adverse drug effect
AE	Adverse event
APEC	Asociación para Evitar la Ceguera
BID	Twice a day
[redacted]	[redacted]
CA	California
CDVA	Corrected distance visual acuity
CRF	Case report form
[redacted]	[redacted]
eCRF	Electronic case report form
EMEA	European Medicines Evaluation Agency
ETDRS	Early Treatment Diabetic Retinopathy Study
EU	European Union
FDA	Food and Drug Administration
GABACls	GABA-gated chloride channels
GCP	Good clinical practice
GLP	Good laboratory practice
ICH	International Conference on Harmonisation
ID	Identification
IND	Investigational New Drug
IOP	Intraocular pressure
IPL	Intense pulsed light
IRB	Institutional Review Board
IUPAC	International Union of Pure and Applied Chemistry
LOCS	Lens opacities classification system
MedDRA	Medical Dictionary for Regulatory Activities
MD	Doctor of medicine
MGD	Meibomian gland dysfunction
NADA	New Animal Drug Application
NaFl	Sodium fluorescein
NC	North Carolina
NEI	National Eye Institute
NIBUT	Non-invasive breakup time
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OD	Doctor of optometry
[redacted]	[redacted]
SAE	Serious adverse event
SPEED	Standard Patient Evaluation of Eye Dryness questionnaire
SUSAR	Serious and unexpected suspected adverse reaction

[redacted]	[redacted]
TID	Three times a day
TP-03, 0.25%	Lotilaner ophthalmic solution, 0.25%
TRS	Tarsus
US	United States
VA	Visual acuity
VAS	Visual analog scale
WOCBP	Women of childbearing potential

2. INTRODUCTION AND RATIONALE

2.1. MEIBOMIAN GLANDS

Meibomian glands are sebaceous glands within the eyelids that secrete meibum, a compound primarily made of lipids that form the superficial layer of the tear film. Meibum coats the aqueous layer and provides tear film stability and protection against microbial agents.[1] In healthy individuals, there are approximately 25 to 40 glands in the upper eyelid and approximately 20 to 30 in the lower eyelid.[2] Proper functioning of the glands serves many purposes including minimizing tear film evaporation, enhancing tear film stability, providing a smooth optical surface for the cornea, and sealing the opposing lid margins during sleep.[3]

2.2. MEIBOMIAN GLAND DYSFUNCTION

Meibomian gland dysfunction (MGD) is a common eyelid disorder[4] that may well be the leading cause of dry eye disease throughout the world.[5,6] Published literature cites a global incidence ranging widely from 3.5% to almost 70%.[7] The incidence of MGD has been found to increase with age[8–12] and is markedly higher in Asian populations.[13–15] According to the International Workshop of Meibomian Gland Dysfunction, MGD is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation and ocular surface disease.[16] Characteristic signs of MGD include the release of cloudy meibum or more viscous material on gland expression or an absence of expressible secretion.[4] Meibomian gland dysfunction should be regarded as a progressive but treatable disease in which therapy may prevent irreversible changes.[4]

Demodex mites have been implicated in several ocular surface diseases including dry eye syndrome[17] associated with MGD.[18–20] Luo and colleagues demonstrated that the *Demodex*-positive rate in MGD patients is higher than that in the control group and that *Demodex*-positive patients experienced more severe meibomian gland loss.[21] Other studies found that *Demodex*-positive patients had more corneal and conjunctival pathologic features.[22,23] In a retrospective review of clinical results, Gao et al[24] identified MGD in addition to ocular demodicosis in seven of 11 subjects. These subjects manifested abnormal lipid film with slow lipid film spread, intermittent trichiasis (n = 5) and subjective lash loss (n = 4) suggestive of damage to the meibomian glands and lash follicles. The authors concluded that *Demodex* potentially causes

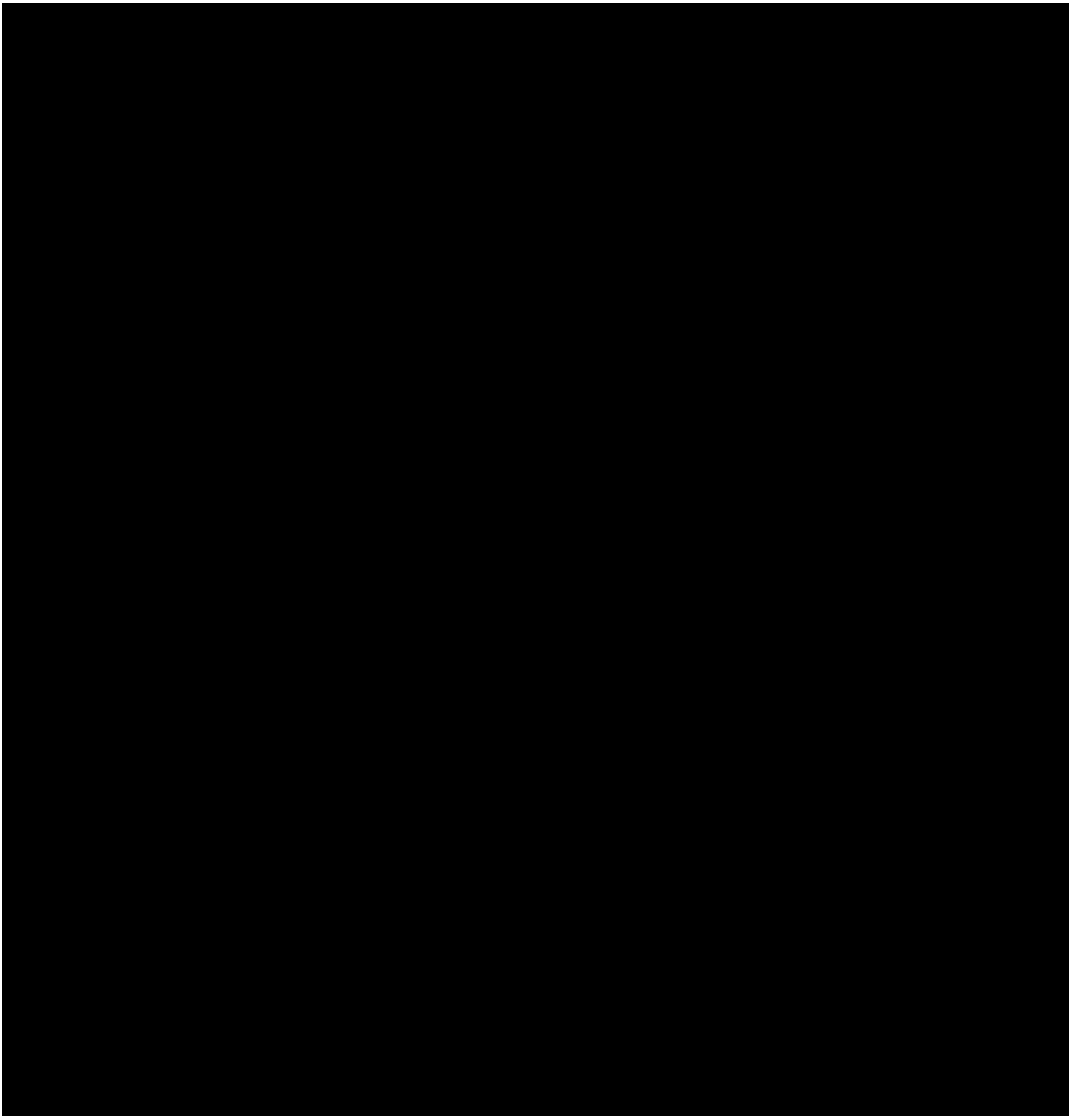
MGD, ocular surface inflammation and lash abnormalities. A study by López-Ponce et al[25] found that the prevalence of infestation by *D. folliculorum* is high in patients with posterior blepharitis. In addition, they found that parasite density was positively correlated with age and with the occurrence of collarettes on the eyelid border.

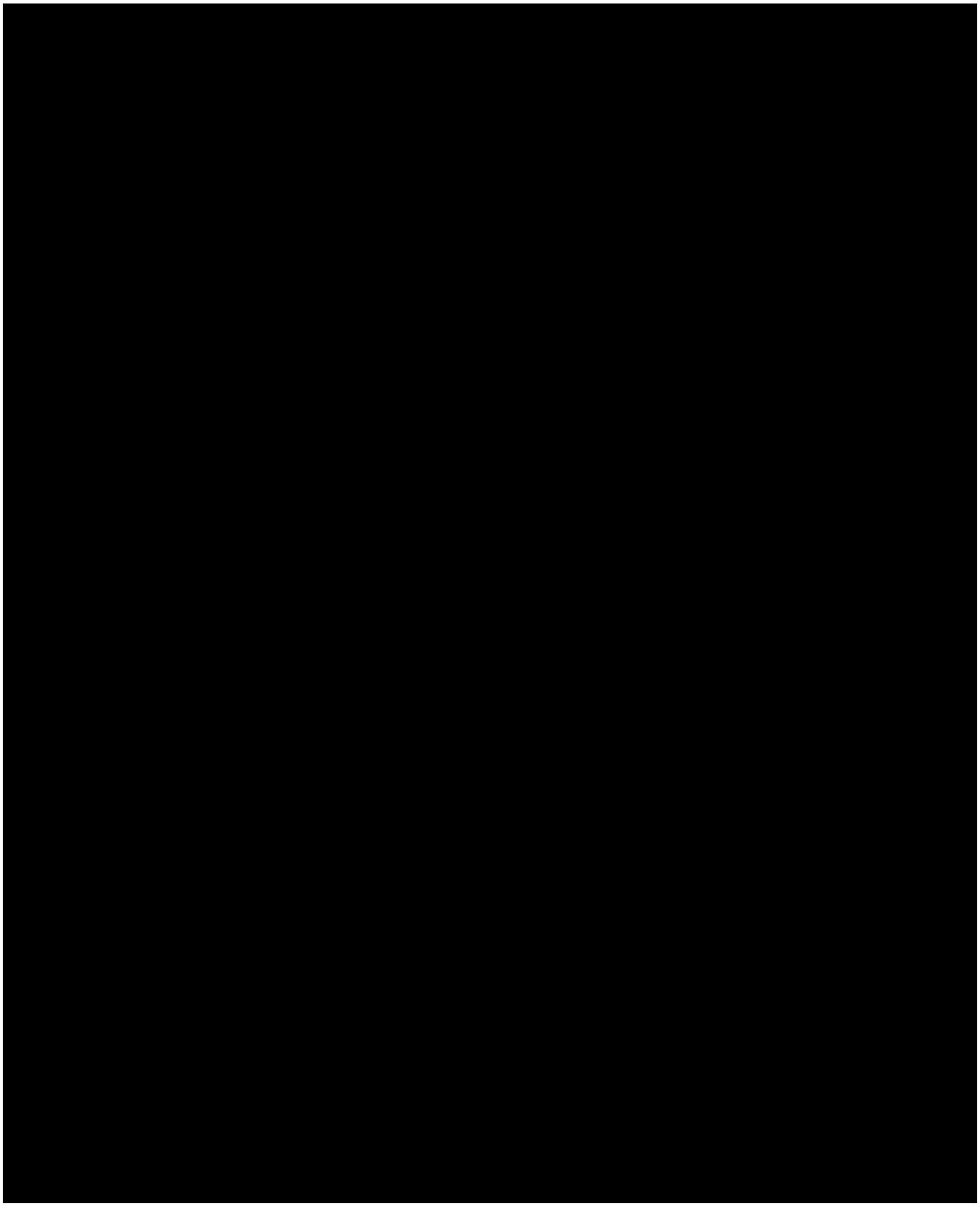
Previous studies have found that *Demodex* mites can cause microstructural changes in meibomian glands, which can result in or aggravate MGD.[26] A recent comparative study sought to understand the impact of *Demodex* infection on the lipid component of meibum in patients.[27] The authors found a significant increase in the levels of (O-acyl)-*w*-hydroxy fatty acids (OAHFAs) in the *Demodex*-positive group suggesting that OAHFAs may be associated with the progression of ocular *Demodex* infections. The authors concluded that *Demodex* exert a significant effect on meibum composition in the human meibomian gland and proposed several possible mechanisms contributing to this change, all involving the movement of bacteria carried or secreted by *Demodex* mites.

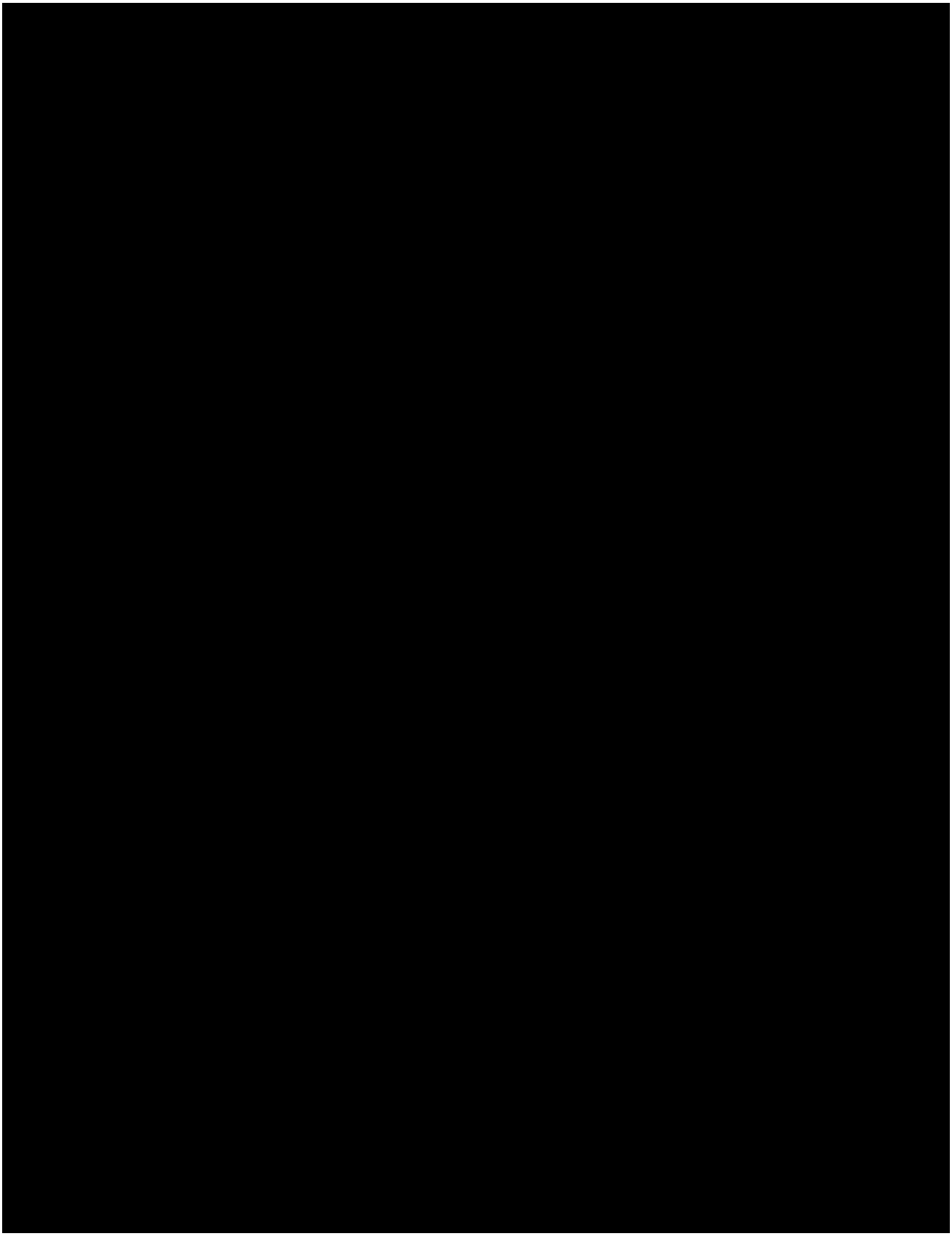
Other studies investigated the relationship between the area of meibomian gland loss and parameters of subjective symptoms and tear film. Pult et al[28] reported that meibomian gland loss was significantly correlated with lipid layer thickness, tear breakup time and subjective symptoms. Ban et al[29] reported that parameters of meibomian gland morphology such as the mean length of meibomian gland ducts and the percentage area of meibomian glands are significantly correlated with tear film parameters including tear breakup time and fluorescein staining. These findings suggest that morphometric analysis using meibography may prove useful for assessing ocular surface conditions.

Currently, there are no FDA-approved pharmaceutical treatments for MGD or *Demodex* blepharitis. For MGD, the LipiFlow thermal pulsation system (Johnson & Johnson Vision), the iLux system (Tear Film Innovations) and the TearCare System (Sight Sciences) are the only devices to receive FDA clearance to date. Other recommendations for the management and treatment of MGD include lid heating, massage, and lid hygiene; topical antibiotics and/or topical steroids; systemic anti-inflammatory antibiotics such as tetracycline; lubricants; meibomian gland probing; and meibomian gland expression. For *Demodex* blepharitis, commercial products are prescribed without substantial evidence of effectiveness.[30] In previous clinical trials, systemic ivermectin, metronidazole, their combination and topical tea tree oil have been used to treat blepharitis due to *Demodex* infestation with varying degrees of success.[31–34] It should be noted, however, that terpinen-4-ol, a tea tree oil component, has been shown to be toxic to human meibomian gland epithelial cells in vitro, even at levels 10-fold to 100-fold lower than demodicidal concentrations.[35] This study is being performed to compare two dosing regimens of a topical ophthalmic preparation of an established acaricide, TP-03, 0.25%, for the treatment of MGD in the presence of *Demodex* infestation.

[redacted]







[redacted]

3. TREATMENTS

3.1. TP-03 AND VEHICLE

Lotilaner is the investigational drug substance. Lotilaner is an isoxazoline and inhibits the γ-aminobutyric acid (GABA)-gated chloride channels (GABACls) of arthropods. The GABA-mediated chloride influx leads to hyperpolarization of the cellular membrane and generates an inhibitory postsynaptic potential, which decreases the probability of an action potential.[36] Ectoparasites exposed to isoxazolines will exhibit spastic paralysis leading to their starvation and death. Isoxazolines as a chemical class have been shown to have no to very low affinity to the human GABA receptor and the GABA pathway is not activated in humans.

[redacted]

Vehicle of TP-03

The vehicle of TP-03 will be used to maintain masking of the BID dosing. Specifically, the concentrations of the excipients match TP-03, lotilaner ophthalmic solution, 0.25%.

[redacted]

4. STUDY OBJECTIVE

This study is intended to compare the safety and efficacy of two dosing regimens of TP-03, 0.25%, for the treatment of MGD in participants with *Demodex* lid infestation. Efficacy objectives will be evaluated by the assessment of the lower lid meibomian gland secretion score; assessment of erythema of the lid margins; [redacted] tear breakup time; [redacted] Safety will be determined by assessing adverse effects related to treatment [redacted].

5. STUDY DESIGN

This is a prospective, randomized, two-arm, double-masked, parallel pilot study of participants who have MGD and have *Demodex* lid infestation. Up to 40 participants will be enrolled. The treatment period will last for approximately 85 days and the follow-up period will last up to 175 days. The diagnosis of ocular demodicosis will be based on the presence of significant collarettes on the eyelashes and by the presence of mites. The diagnosis of MGD will be based on the specified meibomian gland secretion score and the tear breakup time criteria.

6. STUDY POPULATION

On Day 1, participants must be qualified to participate and baseline assessments are performed prior to the first administration of the study drug.

[redacted]

Inclusion criteria:

1. Male or female, aged ≥18 years of age
2. Participants must have all of the following **in at least one eye**:
   - More than 10 lashes with collarettes / cylindrical dandruff present on the upper lid
   - Presence of one or more mites with lash epilation of the upper and lower lids
   - For the 15 glands evaluated on the lower lid, have evidence of meibomian gland dysfunction based on a total meibomian gland secretion score between 12 to 32
   - Have at least Grade 1 erythema of the lower lid
   - Fluorescein tear breakup time < 10 seconds
   - Per meibography, the participant must have intact partial to full meibomian glands in at least 33% of the total meibomian gland area of the lower lid in the opinion of the investigator

[redacted]

4. Have a corrected distance visual acuity better than or equal to logMAR +0.7 as assessed by the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in each eye at Day 1
5. Willing to sign the informed consent, deemed capable of complying with the requirements of the study protocol and stated willingness and availability to comply with all study procedures for the duration of the study
6. For females of childbearing potential: use of acceptable methods of contraception* for at least 1 month prior to Day 1 and agreement to use such a method during study participation and for an additional four weeks after the end of study drug administration

[redacted]

Exclusion criteria:

1. Have used any artificial tear product within 24 hours of Day 1 or anticipated use during the study (with the exception of rescue therapy described in Section 15 of the protocol)
2. Use of systemic antihistamines within 30 days of Day 1

[redacted]

[redacted]

7. Treatment of blepharitis within 14 days of Day 1 (e.g., topical tea tree oil or hypochlorous acid) or unwilling to forego the use of these treatments for the duration of the study
8. The use of lid hygiene products such as lid scrubs, warm compresses or lid massage within 7 days of Day 1 or unwilling to forego the use of these treatments for the duration of the study

[redacted]

10. Have used artificial eyelashes, eyelash extensions or had other cosmetic eyelash or eyelid procedures (e.g., eyeliner tattooing, eyelash tinting, eyelash curling perm, etc.) within 7 days of Day 1 or be unwilling to forego their use during the study
11. Contact lens wear within 7 days of Day 1 or unwilling to forego contact lens wear for the duration of the study

[redacted]

21. Be pregnant or lactating at Day 1

[redacted]

7. STUDY OUTCOME MEASUREMENTS

7.1. Efficacy Parameters

Efficacy measures include the assessment of lower lid meibomian gland secretion; assessment of erythema of the lid margins; [redacted]; tear breakup time; [redacted].

7.2. Safety Parameters

Safety measures include adverse events related to treatment [redacted].

8. CLINICAL EVALUATIONS

8.1. Day 1

At the Day 1 visit, potential participants will be evaluated for eligibility and, if qualified, enrolled in the study. Prior to performing any study-specific procedures, potential participants must provide informed consent using the current IRB-approved informed consent form.

If potential participants fail to meet eligibility criteria, they can be screened one additional time on a different day. Rescreened participants should be assigned the same participant number that was used for the initial screening.

Procedures to be performed at the Day 1 visit include:

- Obtaining informed consent
- Obtaining the medical and ophthalmic history; concomitant medications

[redacted]

- CDVA

[redacted]

[redacted]

- TBUT
- Slit lamp biomicroscopy, including assessment of lid margin erythema

[redacted]

- Meibomian gland secretion assessment of lower lids

[redacted]

There will be two dosing arms, a BID active dosing and a TID active dosing arm. [redacted]

Participants will be instructed to administer a single drop per eye from the first bottle in the morning and evening; a single drop per eye from the second bottle should be administered in the afternoon. The treatment period will be for approximately 85 days.

At Day 1, site staff will supervise the participant's first dose of the study medication. Participants should wash their hands prior to administration of the dose. Following drop administration, the participant will be asked to close their eyes for approximately 15 to 30 seconds and apply gentle pressure to the upper lid to express the medication across the upper and lower lid margins. The participant should be instructed to let the medication air dry on the lid without dabbing with a tissue.

On the day of in-office study visits, participants will be instructed not to use the study medication prior to the visit; the study medication can be administered after the visit has been completed.

If a dose is missed, participants will be instructed to administer the drop if it is at least an hour prior to the next dose. If it is less than an hour, the participant should simply dose the next drop.

8.2. DAYS 15 (±4 DAYS) AND 29 (±5 DAYS)

Procedures to be performed include:

- Concomitant medications review

[redacted]

- CDVA

[redacted]

[redacted]

- Slit lamp biomicroscopy, including assessment of lid margin erythema

[redacted]

- Meibomian gland secretion assessment of lower lids
- Assessment of adverse events

8.3. Day 43 (-7/+6 days) and Day 57 (-7/+10 days)

Procedures to be performed include:

- Concomitant medications review

[redacted]

- CDVA

[redacted]

- Slit lamp biomicroscopy, including assessment of lid margin erythema

[redacted]

- Meibomian gland secretion assessment of lower lids
- Assessment of adverse events

[redacted]

8.4. Day 85 (-13/+7 days)

Procedures to be performed include:

- Concomitant medications review

[redacted]

- CDVA

[redacted]

- Slit lamp biomicroscopy including assessment of lid margin erythema

[redacted]

- [redacted]
- Meibomian gland secretion assessment of lower lids
- Assessment of adverse events
- Collect study drug

At the Day 85 visit, the remaining study drug will be collected.

8.5. DAY 115 (+60 DAYS)

Participants who were exited from the study after completing dosing of study drug through Day 85 and participants who have not exited the study will be invited for an additional post-treatment follow-up visit on Day 115.

Procedures to be performed include:

- Reconsent the participants who were exited per protocol version 3.0 and participants who have not exited the study
- Concomitant medications review
- [redacted]
- CDVA
- [redacted]
- Slit lamp biomicroscopy including assessment of lid margin erythema
- [redacted]
- Meibomian gland secretion assessment of lower lids
- Assessment of adverse events
- Study exit

At this visit, participants will be exited from the study provided they do not have any ongoing adverse events.

9. STUDY DRUG

Contents and Packaging

Lotilaner has been formulated as a topical ophthalmic preparation, TP-03. The vehicle of TP-03 is the same formulation without the inclusion of lotilaner. Both are supplied as a 10 mL fill in an 11-ml-low-density polyethylene ophthalmic dropper bottle. [redacted]

Storage and Handling

Both preparations should be stored at ambient room temperature, 20° to 25° C (68° to 77° F) per USP. Excursions between 15° and 30° C (59° and 86° F) that are experienced in pharmacies, hospitals, ophthalmic practices, warehouses, and during shipping are allowed. There are no light requirements, but extreme humidity conditions should be avoided.

Participants will be instructed to store the study drug at room temperature in a climate-controlled environment avoiding extreme heat or cold and extreme humidity conditions. In addition, participants will be advised that the study drug should not be kept at temperatures higher than 40° C (or 104° F) for longer than 24 hours.

10. STUDY PROCEDURES AND ASSESSMENTS

10.1. INFORMED CONSENT

Prior to performing any study-specific procedures, potential study participants will be provided with an IRB-approved informed consent form and be given a chance to review and ask questions before written informed consent is obtained.

The potential study participant will be asked to sign and date the informed consent form. The individual explaining the consent will also sign and date the consent form as required. The original will be retained with the potential study participant's medical records and a copy will be provided to the potential study participant.

10.1.1. RECONSENTING

Prior to performing any study-specific procedures specific to Day 115, potential study participants who exited the study per protocol version 3.0 and participants who have not exited the study will be provided with an IRB-approved informed consent form and given a chance to review and ask questions before written informed consent is obtained.

The potential study participant or enrolled study participant will be asked to sign and date the informed consent form. The individual explaining the consent will also sign and date the consent form as required. The original will be retained with the study participant's medical records and a copy will be provided to the study participant.

10.2. PARTICIPANT ID ASSIGNMENT

While undergoing screening, potential study participants who sign an informed consent form will be identified by their initials and unique subject ID. For the initials, a dash (-) will be used in place of the middle initial for potential participants who have no middle name. The unique subject ID will consist of the site number followed by a hyphen and then the screening number. For each site, the screening number is a 4-digit number assigned in sequential order beginning with 0101.

If a potential study participant has provided written informed consent and met all eligibility criteria, the participant will be considered enrolled in the study. Once the participant has been enrolled, the site will supervise the administration of the first dose of the study medication.

Potential participants who have provided written informed consent but fail to meet eligibility criteria and are screened again on a different day will be assigned a new unique subject ID that is

different from their initial unique subject ID (next sequential screening number available at the site).

Participants who enrolled and exited the study but agree to return for the Day 115 visit will retain their originally assigned unique subject ID. Rescreened participants who enrolled and exited the study will retain their second unique subject ID assigned.

10.3. Study Treatment Compliance

Participants will be instructed on proper instillation and storage of the study drug and provided with written instructions. Participants will be instructed to record in a dosing compliance diary on a daily basis. During in-office study visits, dosing compliance will be assured by a review of the dosing diary to assess adherence to the protocol regarding the administration of the study product.

[redacted]

10.4. Visit Schedule and Exams

Participants will remain in the study for up to 175 days and will be seen at the following intervals: Day 1, Day 15, Day 29, Day 43, Day 57, Day 85 and Day 115.

The schedule of exams and procedures performed at these visits can be found in [redacted] Descriptions of exams and procedures performed during these visits can be found in Appendix B.

For participants reporting for an unscheduled visit, assessments may be performed at the discretion of the investigator. At a minimum, corrected distance visual acuity and the occurrence of any adverse events should be assessed.

Participants who are exited from the study due to unanticipated adverse effects will be followed, if possible, until their medical outcome is determined. The investigator will provide written reports to the IRB as required.

10.5. Study Exit

Participants will be exited at the Day 115 visit.

11. EARLY TERMINATION AND PARTICIPANT DISCONTINUATION

For each participant, the study will be considered completed when the Day 115 assessment has been performed in accordance with the study protocol.

Any participant may voluntarily discontinue the study at any time without prejudice. The investigator may elect to discontinue a participant for reasons unrelated to the study drug (e.g., failure to comply with the study protocol, missed visits, etc.) or for reasons related to the study drug (e.g., AE, unsatisfactory therapeutic response, etc.). Reason(s) for discontinuation should be recorded on the CRF.

Possible reasons for study discontinuation include the following:

- Lack of efficacy: the participant does not feel that the study drug has adequately relieved his/her symptoms
- AEs necessitating discontinuation from the study
- The participant is lost to follow-up
- Participant decision unrelated to lack of efficacy or AEs
- Reasons relating to COVID-19
- Investigator decision (specify)
- Other reason (specify)

If a participant chooses to discontinue his/her participation or is discontinued, the Exit Form will be completed regardless of other information available. Should a participant experience any difficulties requiring review between planned visits, or in case of early exit between planned visits, the Unscheduled Visit Form will be completed. Reason(s) for such a visit will be recorded on the CRF. Discontinued participants will not be replaced. Participants to be discontinued for AE(s) will be followed until the event is resolved, if possible, or considered medically stable by the investigator.

The data to be collected at the time of study treatment discontinuation may include the following:

- An AE assessment and performance of safety assessments including visual acuity and slit lamp biomicroscopy
- Any follow-up evaluations required per investigator determination

Temporary discontinuation of the study treatment could include the onset of such conditions as allergic conjunctivitis or signs or symptoms of eyelid allergy (e.g., injection, swelling, itching, etc.). Per investigator discretion, the study treatment could be discontinued until such a time that the adverse event has shown sufficient improvement such that the study treatment could be re-started. Re-challenging with the study treatment could help to determine if the adverse event is related to the study drug. It should be noted that discontinuation from use of the study treatment does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit as soon as possible, counseling the participant on the importance of continuing the administration of the study drop and of maintaining the assigned visit schedule. In addition, the site should ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have discontinued the study with a primary reason of lost to follow-up.

The principal investigator reserves the right to discontinue the study for any safety, ethical or administrative reason at any time.

12. STATISTICAL CONSIDERATIONS AND ANALYSIS

12.1. SAMPLE SIZE

There is no prior data available for TP-03 in the treatment of MGD to use as a performance estimate. The sample size of 40 participants was selected based on clinical and practical considerations. Results from this study will be used to inform the design of subsequent studies to demonstrate a therapeutic effect.

12.2. RANDOMIZATION

This is a randomized, double-masked study. A blocked randomization schedule will be prepared and used to package the afternoon dose of the drug (all participants receive TP-03 for the morning and evening doses). The drug will be dispensed at the site sequentially from the available inventory. The bottle number dispensed will be recorded.

12.3. GENERAL CONSIDERATIONS

The sample size, mean and standard deviation will be presented for continuous analysis variables or, if appropriate, the sample size, median and range. Categorical variables will be presented as the count and percentage for each category. Statistical tests will be two-sided and evaluated at an α of 0.05 unless otherwise specified. No adjustment will be made for multiplicity.

12.4. MISSING DATA

Missing data will not be imputed.

12.5. STUDY OUTCOMES

Efficacy outcomes for this study will include:

- Total meibomian gland secretion score (MGSS) computed as the sum of the grades for all 15 glands evaluated on the lower eyelid with a range from 0 to 45

[redacted]

- Lid margin erythema

[redacted]

The primary safety endpoint of this study is incidence of treatment-related adverse events (AE). Descriptive statistics of adverse events will be provided, as will narratives of any serious, unexpected, drug-related AEs. Any clinically meaningful changes in visual acuity or slit lamp biomicroscopy findings will be tabulated.

12.6. Statistical Hypotheses

For the comparisons between dosing regimens, the null hypothesis is the efficacy outcomes for the dosing regimens is equal. The alternative hypothesis is the efficacy outcomes are not equal, ie a two-sided test. The hypothesis can be expressed as follows:

H_0: $E_1 = E_2$

H_A: $E_1 \neq E_2$

where E_1 and E_2 are the efficacy outcomes for each dosing regimen.

For the analyses of the change from baseline in efficacy outcomes for both treatments combined, the null hypothesis is there is no difference between the baseline and follow-up values. The alternative hypothesis is the follow-up outcome has improved from baseline. The hypothesis can be expressed as follows:

H_0: $D \leq 0$

H_A: $D > 0$

where D is the improvement from baseline (note, for some variables, an improvement may be a decrease from baseline).

12.7. Statistical Analyses

Efficacy Analyses

Efficacy analyses comparing the outcome between dosing regimens will be performed on the change from baseline, except for the post treatment survey, using either the Student's *t*-test or Wilcoxon rank-sum test as appropriate.

Efficacy analyses examining the change from baseline for both dosing regimens combined will use either a paired *t*-test or a Wilcoxon signed-rank test as appropriate. A one-sided test for an improvement from baseline will be evaluated using an α of 0.025.

[redacted]

Safety Analyses

Clinically meaningful changes from baseline (Day 1) in visual acuity and slit lamp biomicroscopy will be summarized for each follow-up visit. A clinically meaningful change for visual acuity will be a change of more than 2 lines on the ETDRS chart (change greater than 0.2 logMAR). A clinically meaningful change in slit lamp biomicroscopy will be a change of 2 or more grades.

Adverse event data will be summarized by the MedDRA preferred term and listed.

12.8. INTERIM ANALYSIS

This is an initial exploratory study of TP-03 for this indication. If needed for business or project planning purposes including the design of any future studies, Tarsus may perform an interim analysis once 20 or more participants have been enrolled in the study.

12.9. FINAL REPORT

Once all participants have completed the Day 85 assessment or have been exited from the study and all corresponding data has been received and corrected, the study database for visits through Day 85 will be locked. Once the data has been locked, the treatment assignments will be unmasked and a complete analysis of the results will be performed and a final report will be prepared. Once all participants have been exited from the study and all additional data has been received and corrected, the complete database including the additional data collected through Day 115 will be locked. An addendum to the final report will be prepared to include the additional follow-up results through the Day 115 assessment.

13. ETHICAL AND REGULATORY CONSIDERATIONS

Investigator Responsibilities/Performance (Code of Conduct): The investigator will ensure that the clinical study is conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, GCP, ICH guidelines and any regional or national regulations as appropriate. The investigator will also ensure that participants undergo no investigational procedures other than those described in this protocol and approved by the IRB, except those procedures deemed necessary to protect the health and well-being of the participant. Any such procedures will be documented in the participant's records and reported to the IRB as required. If any additional requirements are imposed by the IRB or regulatory authority, these requirements shall be followed.

Informed Consent Process and IRB:

The site will submit the proposed informed consent to the IRB for review and approval. Copies of the informed consent form used in the study should contain the IRB-approved stamp (if applicable) and version date. The following information should be provided in the informed consent:

- The study is research and the purpose of the research

- Purpose of the study including the potential duration of participation by the participant and a description of the procedures
- Possible side effects, risks or discomforts of the investigational drug
- Reasonably expected benefits
- The existence of alternative therapy options and/or alternatives to participating in the study
- Confidentiality of records and possible inspection of records
- Costs, compensation and treatment for injury
- Person to contact regarding participant's rights
- Person to contact for questions on research or research-associated injury
- Participation is voluntary
- The right to discontinue participation in the study at any time without disadvantage to the participant
- Participant will be informed of new information learned during the study, which may affect the participant's decision to continue participation in the study

The investigator or qualified/delegated staff will obtain written informed consent from each participant prior to the initiation of any study-related activities. The potential study participant or the participant's legally authorized representative will be allowed sufficient time to thoroughly read (or have read and explained to them) the informed consent form. The investigator or qualified/delegated staff will answer any questions that the potential study participant or their representative might have. If the potential study participant agrees to participate in the study, all required parties should sign and date the informed consent form. The original signed informed consent form will be retained by the site and a copy will be given to the potential study participant for their records. The obtaining of informed consent and the date of that informed consent should be noted in the participant's medical notes. It should also be noted that informed consent was obtained prior to any study-related activities being conducted.

14. INVESTIGATOR RESPONSIBILITIES

The investigator will ensure that the clinical study is conducted in accordance with Good Clinical Practice (GCP), International Conference on Harmonisation (ICH) and all regulatory and institutional requirements, including those for participant privacy, informed consent, Institutional Review Board approval and record retention.

The investigator is responsible for any expedited reports of SAEs or SUSARs to the regulatory authorities and IRB.

15. CONCOMITANT MEDICATIONS AND THERAPIES

All concomitant medications including over-the-counter medications and nutritional supplements used within 3 months prior to the first visit will be documented. Any changes to concomitant medication use or the initiation of new medications will be reported throughout the study. For the duration of the study, any therapy considered necessary for the maintenance of participant welfare may be given to the participant at the discretion of the investigator. Initiation of new medications, changes to concomitant medications, or new therapies administered to the participant should be recorded on the appropriate case report form. In the event prohibited medications / procedures are indicated for care as a medical necessity, participants will not be discontinued.

If participants experience significant symptoms of dry eye during the study, participants will be allowed to use a sterile saline solution as necessary provided by the investigator, but participants will be instructed not to use the drop for at least one hour before and one hour after administration of the study drug. On the day of in-office study visits, participants should be advised not to use the sterile saline solution prior to the visit; the saline solution can be used after the visit has been completed. The use of nutritional supplements is not restricted.

15.1. PROHIBITED CONCOMITANT MEDICATIONS/PROCEDURES

No other experimental drug or device should be used within three months of the Day 1 visit or during the course of the clinical trial.

Study participants will be asked not to use any topical ophthalmic medications one hour before and one hour after administration of the study drug. In addition, participants should refrain from using any cosmetics (e.g., eyeliner, mascara, foundation or eyeshadow) in the vicinity of the eyelid margin on the day of their visit prior to completion of the visit.

Contact lenses should not be worn for the duration of the study. [redacted]

[redacted]

- Artificial eyelashes, eyelash extensions or other cosmetic eyelash or eyelid procedures

[redacted]

16. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

16.1. DEFINITION OF ADVERSE EVENTS (AE)

Adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related (21 CFR 312.32 (a)).

16.2. DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. Serious adverse events include drug deficiencies that might have led to an SAE if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate.

Events that are considered sight-threatening in the opinion of the investigator will be considered SAEs.

A planned hospitalization for a pre-existing condition without a serious deterioration in health is not considered to be an SAE. Hospitalization for less than 24 hours is not considered an SAE unless the precipitating event was unanticipated and also related to the investigational drug.

16.3. CLASSIFICATION OF AN ADVERSE EVENT

16.3.1. SEVERITY OF EVENT

The following guidelines will be used to describe the severity of AEs.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

Of note, the term "severe" does not necessarily equate to "serious." Severity is a measurement of intensity. Thus, a severe reaction is not necessarily an SAE. For example, a headache may be severe in intensity but would not be serious unless it met one or more of the criteria for a serious adverse event.

16.3.2. RELATIONSHIP TO STUDY TREATMENT

All AEs must have their relationship to study treatment assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – Evidence indicates a reasonable temporal sequence of the event with the study drug administration exists or that the association of the event with study drug administration is unknown and the event is not reasonably supported by other conditions such that:
  - There is a clinically plausible time sequence between onset of the AE and study treatment administration, and/or
  - There is a biologically plausible mechanism for study treatment causing or contributing to the AE, and
  - The AE cannot be reasonably attributed to concurrent/underlying illness, other drugs or procedures.
- **Potentially Related** – Evidence indicates a possible relationship to the study drug such that:
  - The event occurs within a reasonable period of time relative to the treatment that makes a causal relationship possible, but plausible explanations may also be provided by other causes such as other drugs, products, chemicals, underlying disease, environment, etc.
  - The event is possibly related to the study treatment.
- **Not Related** – Evidence indicates no plausible direct relationship to the study drug such that:
  - A clinically plausible temporal sequence is inconsistent with the onset of the AE and drug administration, and/or
  - A causal relationship is considered biologically implausible
  - The AE can be attributed to concurrent/underlying illness, other drugs or procedures.

16.3.3. EXPECTEDNESS

The investigator will determine whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study treatment. Expectedness should be determined if the AE is characterized as related to treatment and is serious (met one or more of the criteria for an SAE).

16.3.4. OUTCOME

The clinical outcome of an AE will be characterized as follows:

- Resolved without sequelae
- Resolved with sequelae (specify)
- Death
- Ongoing (i.e., continuing at the time of study discontinuation)
- Unknown

16.3.5. TREATMENT OR ACTION TAKEN

The clinical treatment or action taken from an AE will be characterized as follows:

- None
- Medical intervention
- Surgical intervention
- Unknown
- Other (specify)

16.3.6. ACTION TAKEN WITH INVESTIGATIONAL PRODUCT

The action taken with the IP will be characterized as follows:

- None
- Drug temporarily withdrawn
- Drug discontinued
- Unknown

16.4. TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care or upon review by a study monitor. At study visits, study personnel should pose general questions to the participant to ascertain if the participant experienced any issues prior to the visit.

All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF/eCRF. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. All AEs occurring while in the study must be documented appropriately regardless of relationship to the study product.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed.

The investigator or designee will record all reportable events with start dates occurring any time after the study drug is first administered until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Whenever possible, recognized medical terms should be used when recording AEs. Colloquialisms and/or abbreviations should not be used. Only one medical concept, preferably a diagnosis instead of individual symptoms, should be recorded as the event (e.g., record congestive heart failure rather than dyspnea, rales and cyanosis). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual sign or symptom should be recorded as a separate AE. A diagnosis that is subsequently established should be reported as follow-up information.

Adverse events occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A "primary" event, if clearly identifiable, should represent the most accurate clinical term to record as the AE event term. For example: Orthostatic hypotension $\Rightarrow$ fainting and fall to floor $\Rightarrow$ head trauma and neck pain. The primary event is orthostatic hypotension and the sequelae are head trauma and neck pain.

16.5. ADVERSE EVENT REPORTING

The investigator must record nonserious adverse events and submit on the CRF/eCRF in a timely manner.

16.6. SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor and Medical Monitor any SAE, whether or not considered study treatment-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the study treatment caused the event [21 CFR 312.64(b)]. Serious adverse events must be reported immediately to the below-listed individuals using the Serious Adverse Event Report (SAER) form. Sites will also be instructed to document the event in the Serious Adverse Event eCRF.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

The study sponsor or designee will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information [21 CFR 312.32I(2)]. In addition, the sponsor must notify the FDA and all participating investigators in an Investigational New Drug (IND) safety report of potentially serious risks from clinical trials or any other source as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting [21 CFR 312.32I(1)]. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information. The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE.

16.7. REPORTING EVENTS TO PARTICIPANTS

It will be the responsibility of the principal investigator to inform participants of any AEs or SAEs deemed related to the study drug and of any study-related results.

16.8. REPORTING OF PREGNANCY

Women of Childbearing Potential (WOCBP) includes any females who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or are not postmenopausal at least 12 months since last menses. WOCBP will be required to use designated methods of birth control during the course of the study. All women who are pregnant, nursing an infant or planning a pregnancy during the duration of this study will be excluded from participation.

If a participant or investigator suspects that the participant may be pregnant prior to study drug administration, the study drug should be withheld until the results of pregnancy testing are available. If pregnancy is confirmed, the participant should not administer the study drug and should not be enrolled in the study.

If a female participant becomes pregnant during the study, use of the study drug should be discontinued immediately. The investigator will notify the sponsor (or designee) immediately after the pregnancy is confirmed. The investigator will (1) obtain a consent from the female participant for pregnancy follow-up and (2) follow the progress of the pregnancy to term. The investigator should document the outcome of the pregnancy and provide a copy of the documentation to the sponsor or designee. The sponsor or sponsor's designee will be responsible for reporting to the IRB and regulatory agencies as appropriate.

17. COMPLIANCE WITH PROTOCOL

Participation in the study will be summarized by presenting the number of participants who received at least one dose of the study treatment, the number of participants who completed the study per protocol, and the number of participants who did not complete the study by reason of early discontinuation.

18. PUBLICATION OF THE RESULTS AND PROTECTION OF COMMERCIAL SECRETS

This study will comply with the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

[redacted]

19. DATA COLLECTION AND REPORTING

Source Documentation: Source documentation for this study will be maintained to document the treatment and study course of each participant and to substantiate the integrity of the trial data submitted for review to the regulatory agencies. Source documentation will include, but may not be limited to: Worksheets, hospital and/or clinic or office records documenting participant visits, including study and other treatments or procedures, medical/ophthalmologic history and physical examination information, laboratory and special assessment results, pharmacy records, drug accountability records and medical consultations.

Medical/ophthalmologic history should be recorded for the past [redacted] of the participant. Concomitant medications taken by the participant 3 months prior to Day 1 should be recorded.

Case Report Forms: All historical data and required study observations obtained during the study will be entered in the participant's source documentation and transcribed to the appropriate case report forms (CRFs/eCRFs). CRFs/eCRFs will be filled out completely for each participant in accordance with instructions from Tarsus Pharmaceuticals or its designee. The investigator should ensure that all data on the CRF/eCRF is accurate and consistent with the source documents. Only the principal investigator or other designated sub-investigator(s) may sign and date the designated CRF/eCRF page(s).

The CRFs/eCRFs will be monitored and corrected on-site and, if necessary, clarified on an ongoing basis. The site will be queried if any data, signatures or forms are missing or incorrect. Case report forms can be used as source documents where appropriate.

Monitoring and Inspection of Records: The investigator must allow regular inspections of all study records including CRFs/eCRFs, source documents and regulatory documents by the study monitor. This measure is to ensure that the study is carried out and documented in accordance with all regulatory requirements and the terms of this protocol.

In cooperation with the on-site staff, a designated study monitor(s) will:

- Review all study documents to ensure compliance with the protocol
- Review data recordings for each visit to verify the accuracy and completeness of the information on the CRFs/eCRFs against appropriate source documents
- Review drug disposition and accountability records
- Review adequacy of enrollment rate
- Review required regulatory documentation

Record Retention: The investigator is responsible for maintaining adequate records to enable the conduct of the study to be fully documented. This includes:

- The Investigator's Brochure, signed protocol and amendments
- Signed and dated informed consents per institutional policy
- Signed, dated, and completed CRFs/eCRFs and documentation of CRF corrections
- Notification of SAEs and related reports
- All study drug dispensing and accountability logs
- Shipping records of investigational drug and trial-related materials
- Dated and documented IRB protocol approvals and all correspondence between the investigator and IRB
- *Curriculum vitae* and current medical licenses for principal investigator and all sub-investigators

Source documents: The investigator is responsible for maintaining adequate case histories in the source documents of each participant. The following data will be noted by the investigator in the source medical records of each participant enrolled in the study:

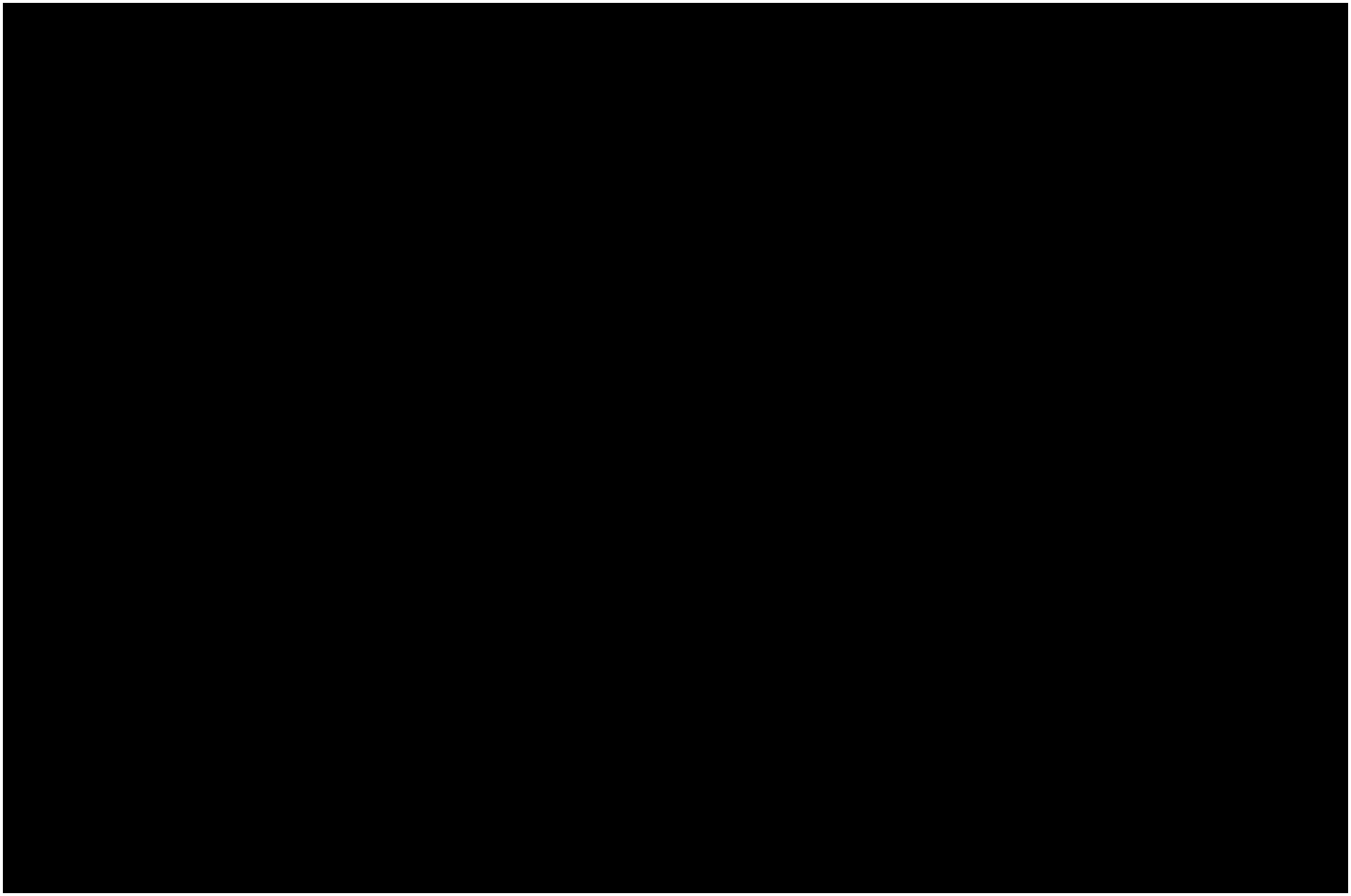
- The participant is participating in this clinical trial
- The date the informed consent form was signed
- Kit numbers
- Date of study completion or withdrawal

The investigator must maintain a copy of all study documents for the period of time that is required by the regulatory authorities. No documents will be destroyed without prior written permission from Tarsus Pharmaceuticals.

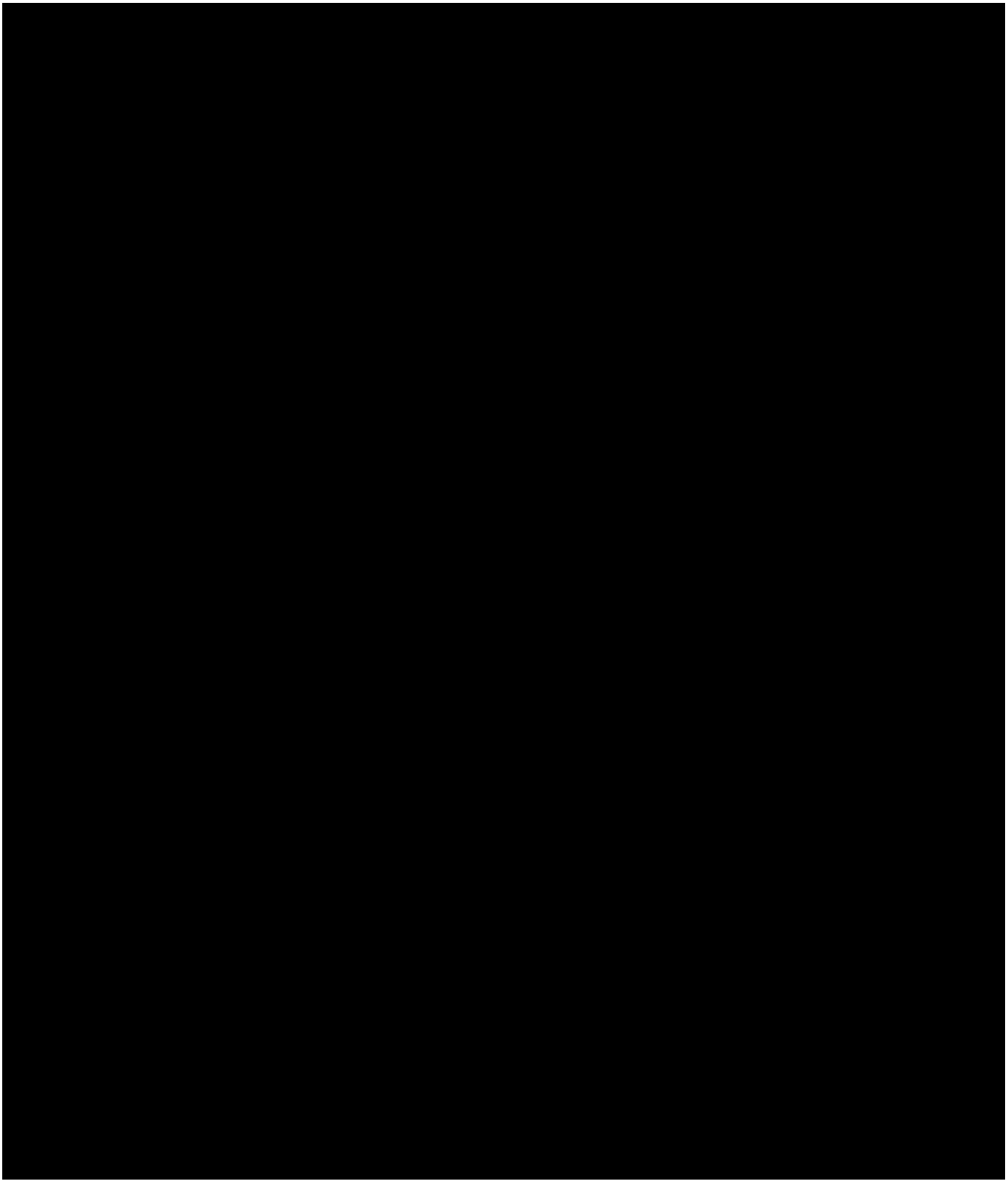
Data Management: The data will be entered into a secure study database. The database will be electronically stored and a copy placed in backup on a regular basis. Case report forms will be stored for reference. Any data inconsistencies will be resolved by a data clarification request and final resolution verified during site monitoring and study closure**.** Study documents should be retained for a minimum [redacted] after the last approval of a marketing application and until there are no pending or contemplated marketing applications or until at [redacted] have elapsed since the formal discontinuation of clinical development of the study treatment. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

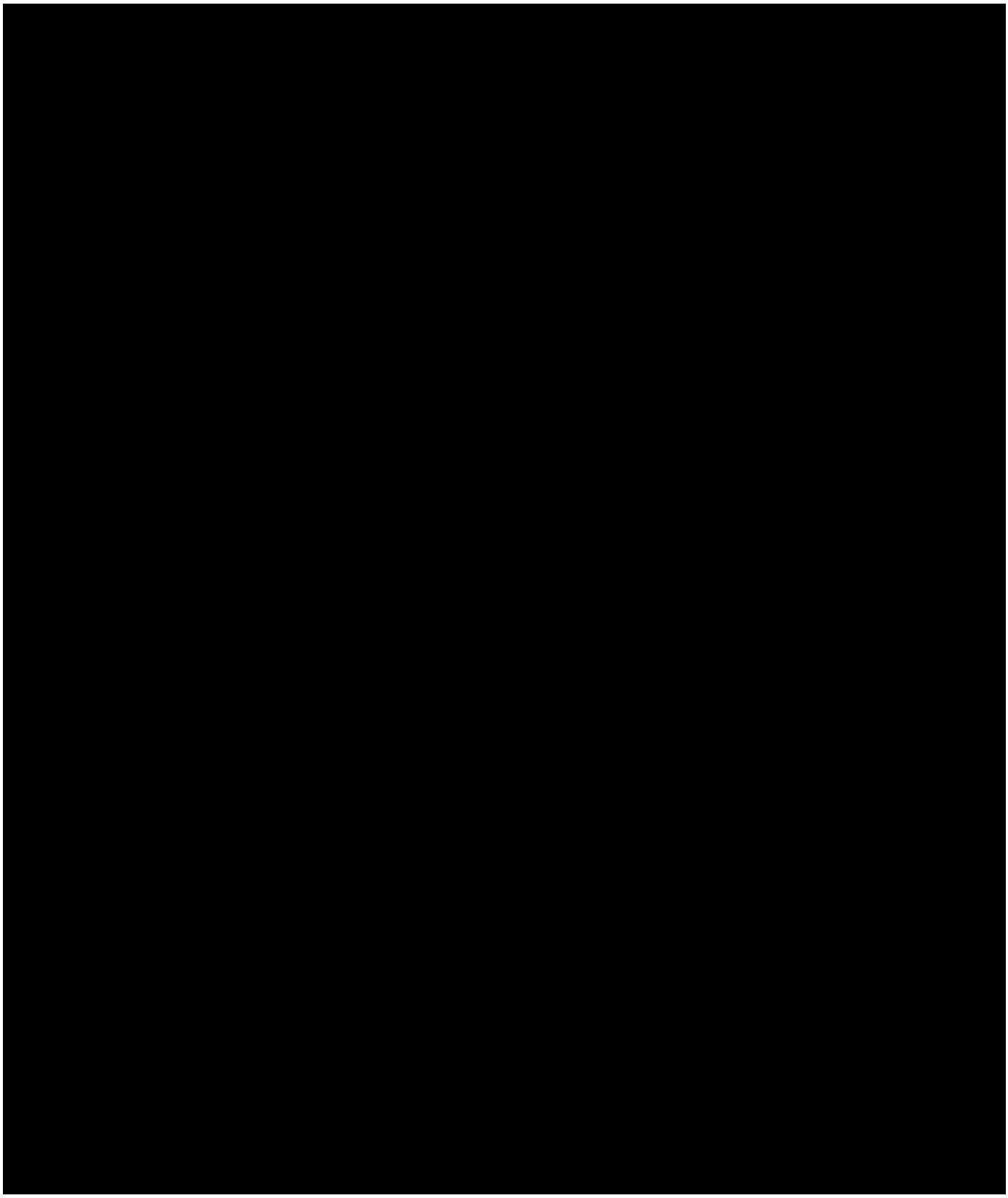
Protocol Amendments: Any amendments to the protocol must be approved by the IRB in advance of implementation.

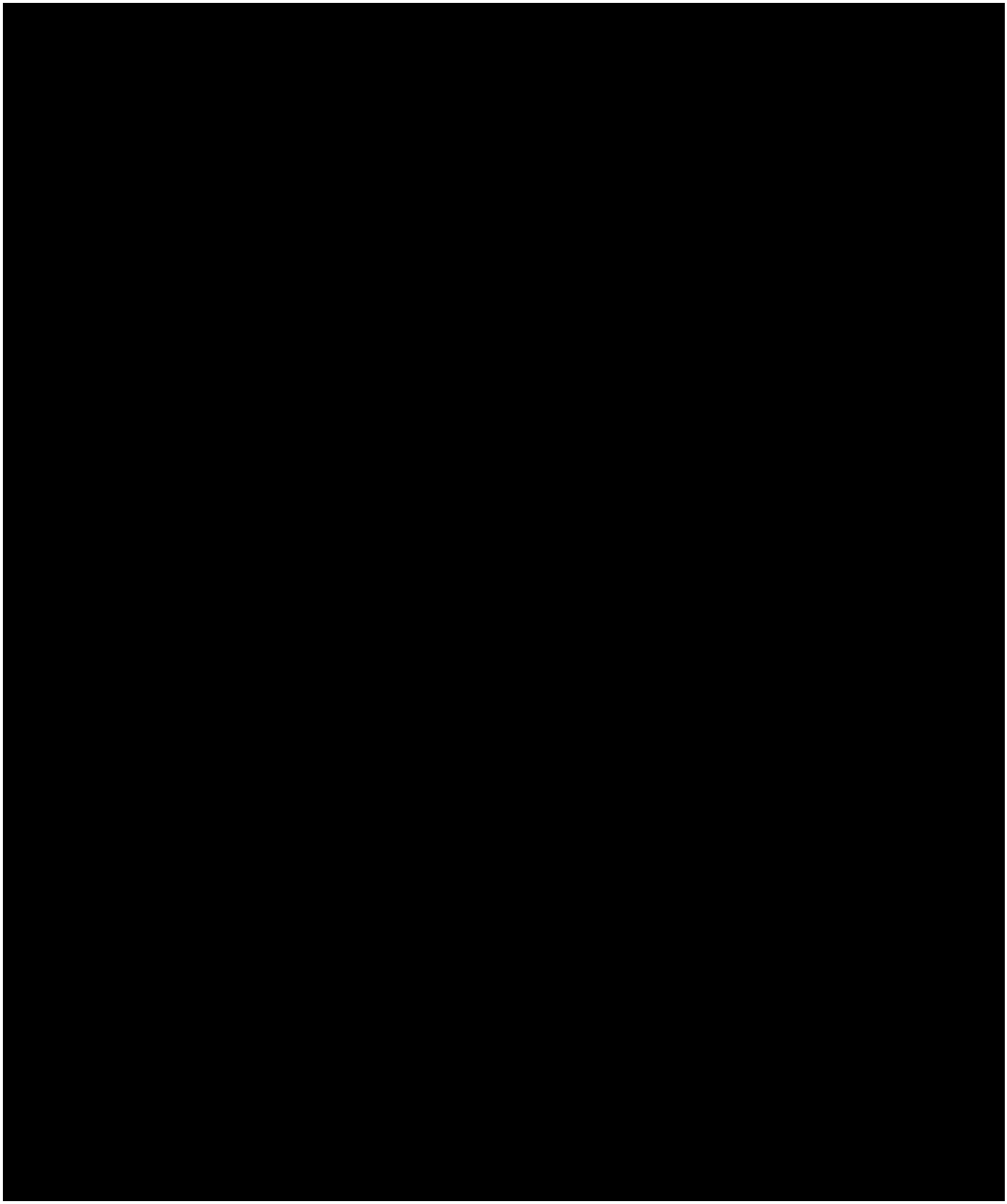
Deviations from Clinical Investigation Plan: All deviations from the protocol should be clearly documented. Deviations conducted to protect a participant from possible hazards should be reported to the IRB as soon as possible.

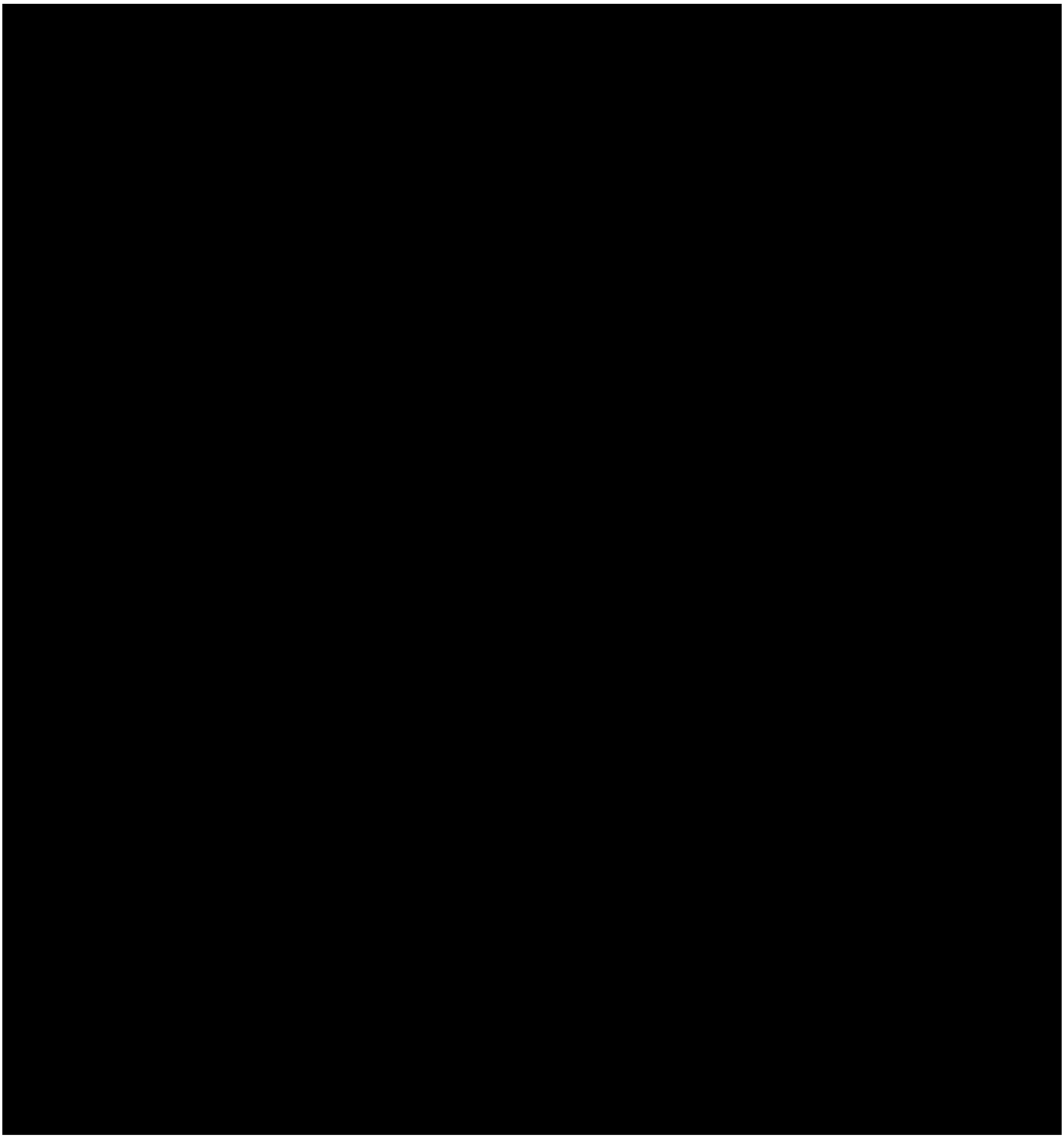


21. PROTOCOL AMENDMENT HISTORY









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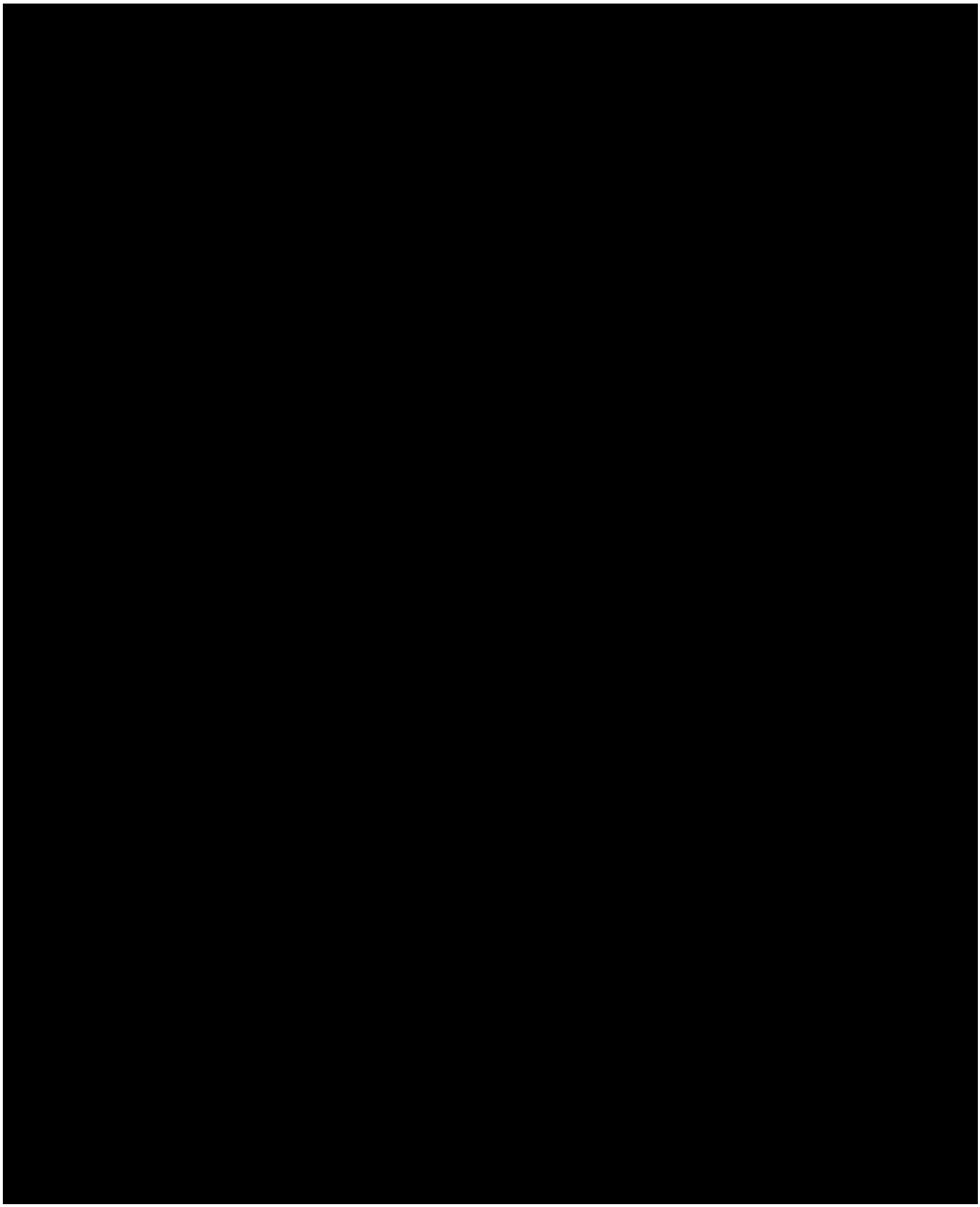

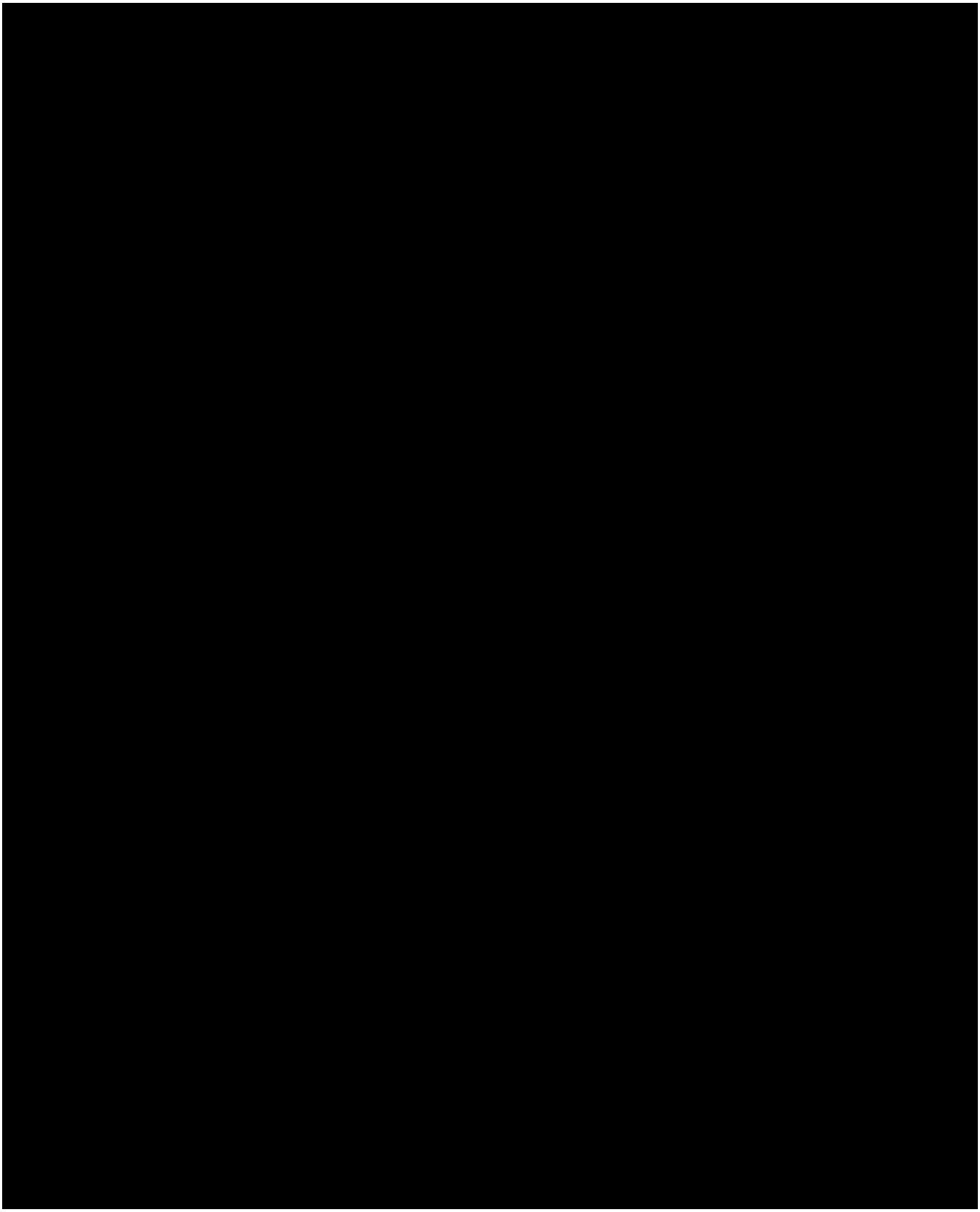

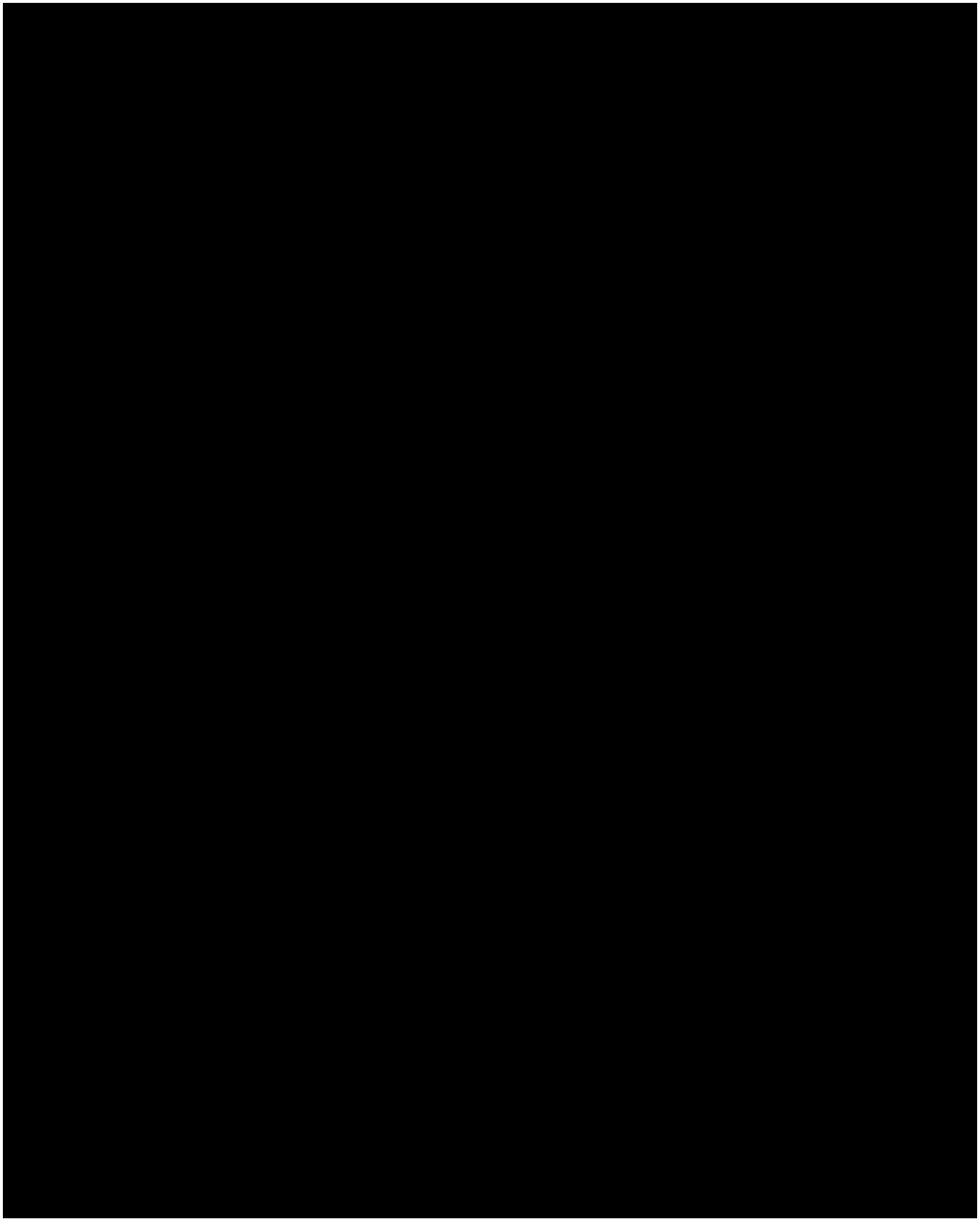

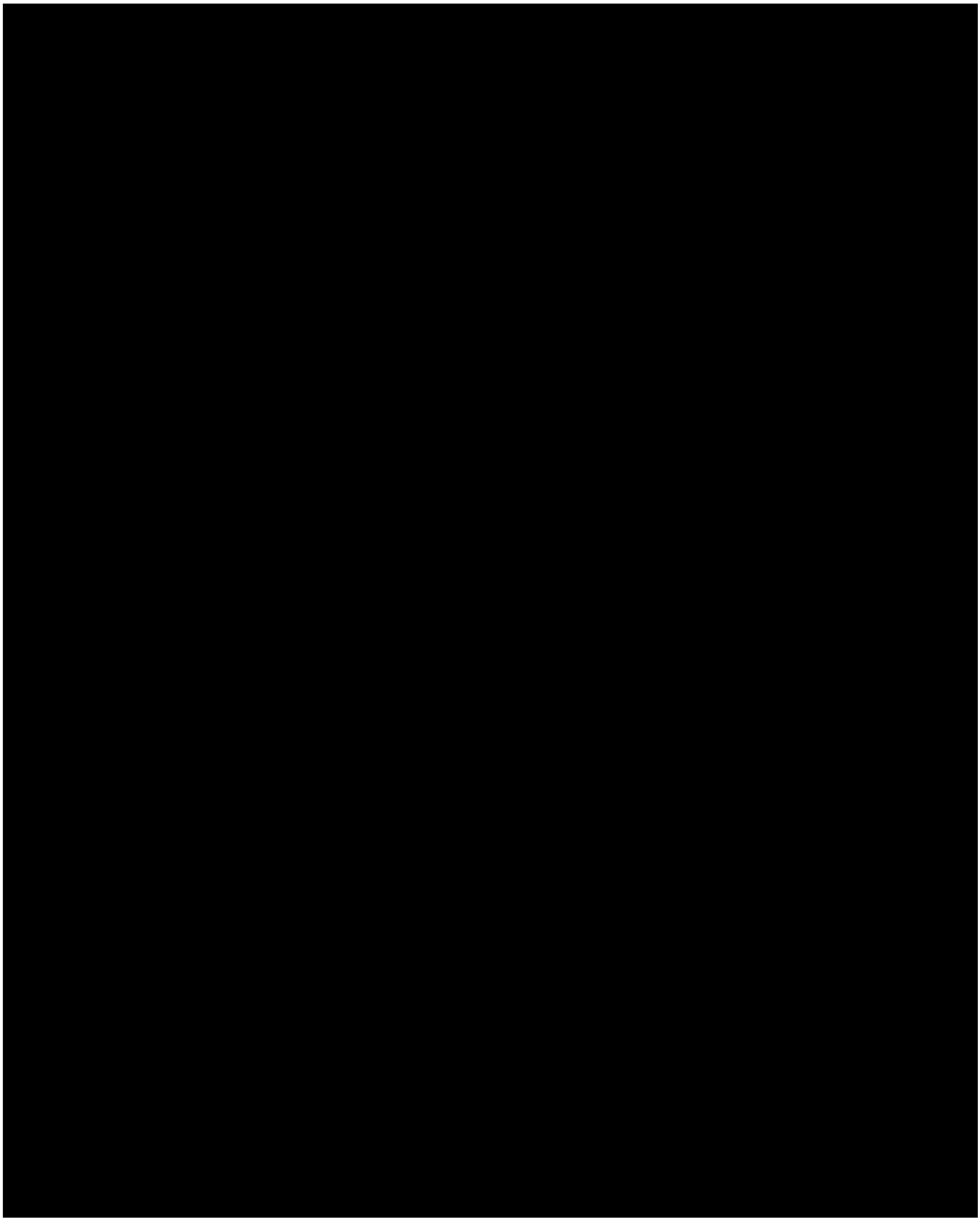

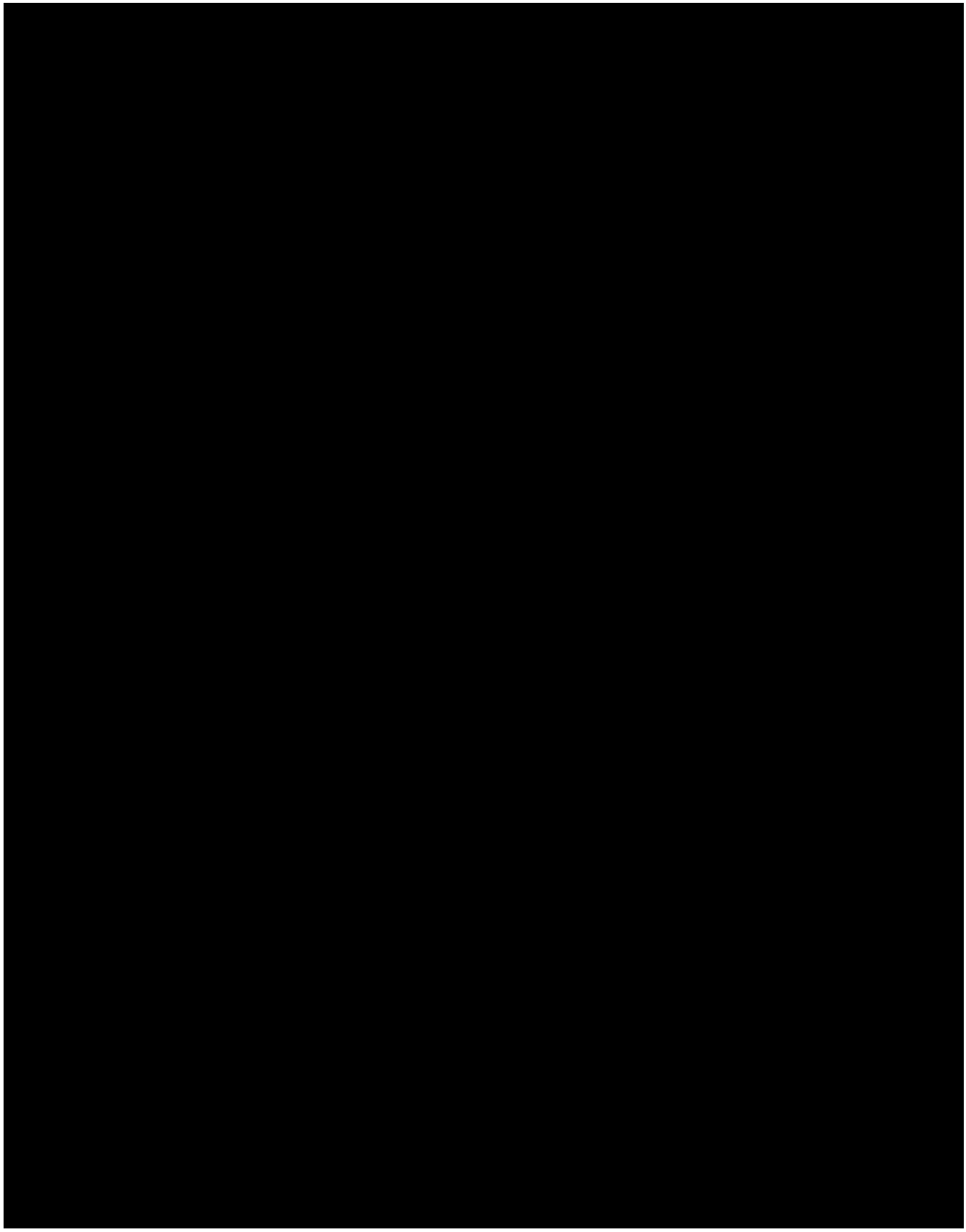

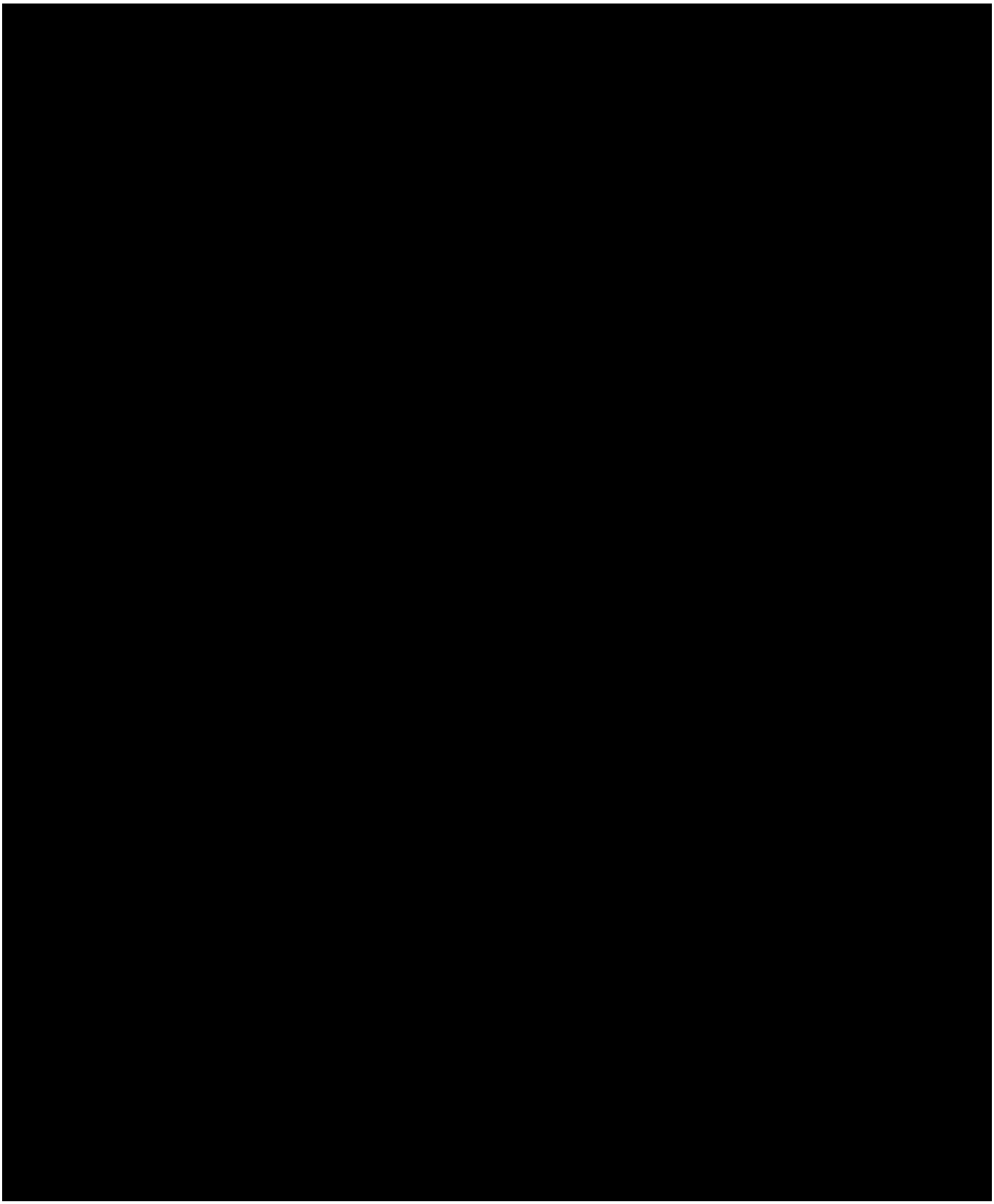

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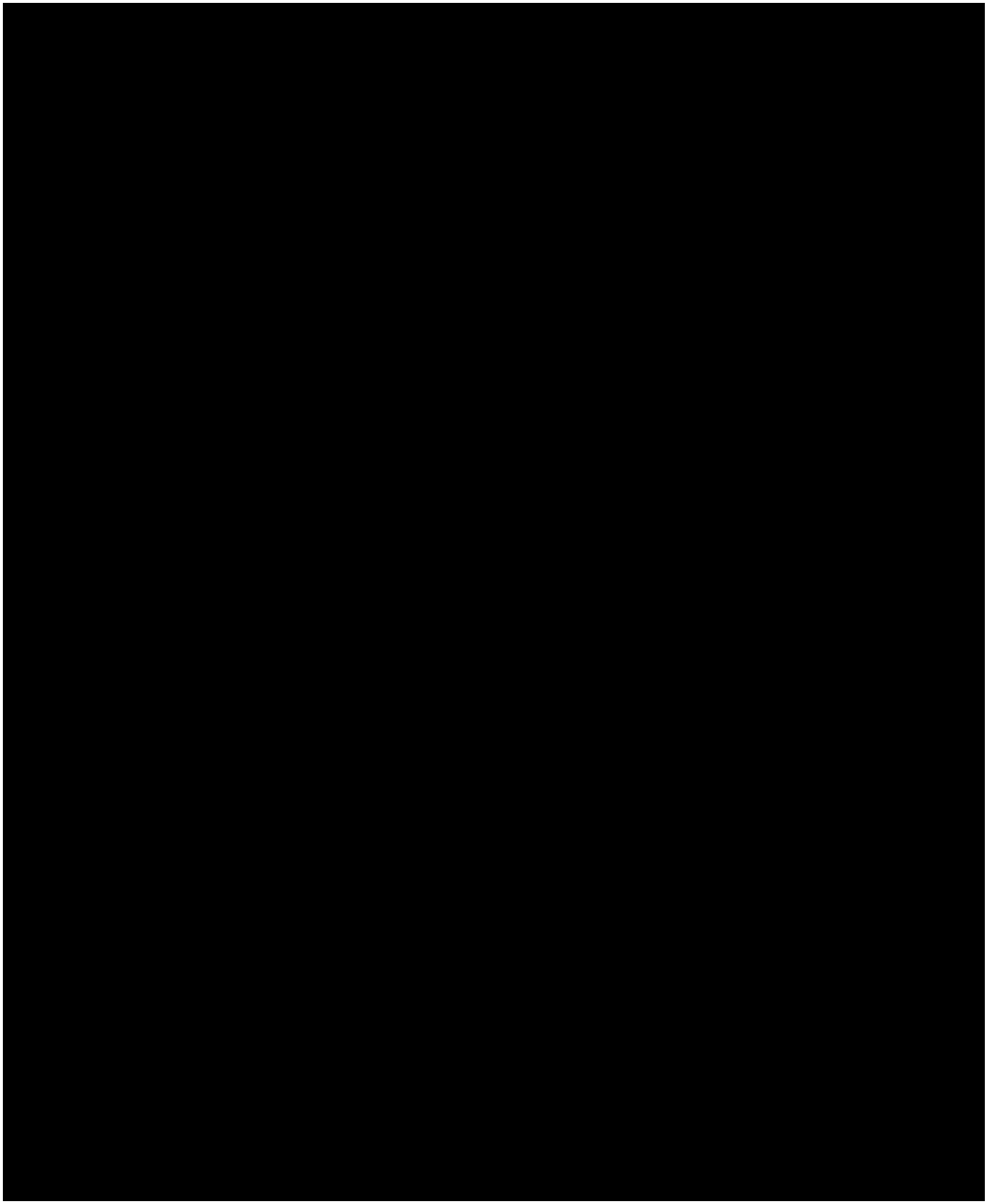

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