STUDY PROTOCOL

Protocol Title: A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First-, Second-, or Third-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer

Protocol Number: ATI001-203

Phase: Phase Ib/II

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Sponsor: ZIOPHARM Oncology, Inc.
[redacted]

Medical Monitor: [redacted]

Safety Reporting: [redacted]

PROTOCOL SYNOPSIS

Clinical Protocol ATI001-203

Title

A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First-, Second-, or Third-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer

Protocol Number

ATI001-203

Clinical Phase

Phase Ib/II

Name of Investigational Product(s)

[redacted]-human interleukin-12 (Ad-RTS-hIL-12 [INXN-2001]) + Veledimex (INXN-1001) RTS activator ligand

Ad-RTS-hIL-12 + veledimex may also be referred to in the synopsis and protocol as Ad-RTS-hIL-12 immunotherapy.

Research Hypothesis

A single cycle of Ad-RTS-hIL-12 immunotherapy will be safe and well tolerated and may allow subjects with human epidermal growth factor receptor 2-negative (HER2-) advanced metastatic breast cancer to maintain or improve their pre-study response (partial response [PR] or stable disease [SD]) during a 12-week treatment break from standard chemotherapy. Ad-RTS-hIL-12 immunotherapy also will be safe and well tolerated in patients with HER2-positive (HER2+) metastatic breast cancer who are receiving an established anti-HER2 antibody therapy.

Primary Objective

- To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-, second-, or third-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first-, second-, or third-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by Section 12.4.2.

Secondary Objectives

- To estimate the progression rate at 12 weeks after the start of one cycle of Ad-RTS-hIL-12 immunotherapy
- To evaluate the overall response rate (ORR), defined as the rate of complete response (CR) plus the rate of PR at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy
- To evaluate the disease control rate (DCR), defined as the proportion of subjects who have a CR, PR, or SD at 12 weeks following the start of one cycle of Ad-RTS-hIL-12 immunotherapy
- To evaluate the number of subjects whose baseline tumor status (SD or PR) improves to PR or better at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy
- To compare radiographic responses by irRC with conventional reporting by RECIST
- To explore the impact of treatment on tumor and blood immune biomarkers

Study Design

This is a single-arm, single-center phase Ib/II study to examine the safety, tolerability and preliminary efficacy of one cycle of Ad-RTS-hIL-12 immunotherapy in women with advanced breast cancer and pre-study SD or PR after completion of a minimum 12 week course of standard first-, second-, or third-line chemotherapy. The patient population will include patients with locally advanced or metastatic breast cancer of all subtypes. The safety of this therapy and the preliminary evidence of efficacy will guide further studies. Subjects who have progressive disease (PD) or a (CR) after the standard chemotherapy are not eligible for the study. Following

entry into the trial, patients will go on a treatment holiday from chemotherapy and enter an immunotherapy phase of treatment. Continuation of HER2-targeted antibody therapy is permitted during this immunotherapy phase for women with HER2+ disease. Scans will be conducted at 6 and 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy to determine tumor response. Radiographic PD at week 6 must be confirmed at least 4 weeks later, either at week 12 or earlier if clinically necessitated, except in cases of unequivocal progression.

The assessment of response will be based on Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 using tumor measurements from contrast-enhanced computed tomography (CT). Tumor response will also be assessed by Immune-Related Response Criteria (irRC) (Wolchok et al, 2009). Eligible subjects will also be asked to consent to allow ZIOPHARM to obtain scans and tumor measurements taken both a) prior to initiating the pre-study standard chemotherapy and b) after a minimum of 12 weeks of pre-study standard chemotherapy, and not more than 4 weeks prior to study enrollment, in order to confirm their SD, PR status.

For subjects with HER2- disease, the Ad-RTS-hIL-12 immunotherapy is intended to be given as a chemotherapy break, for example, to avoid intolerable or undesirable toxicity of the standard chemotherapy. Ad-RTS-hIL-12 immunotherapy must be started within 4 weeks of stopping the pre-study standard chemotherapy.

Subjects with HER2+ disease will receive the Ad-RTS-hIL-12 immunotherapy in conjunction with first- or second-line anti-HER2 antibody therapy, i.e., subjects may continue anti-HER2 antibody directed therapy during the study period. Anti-HER2 therapy will be administered according to the manufacturer's recommendations. Subjects with HER2+ breast cancer who continue the anti-HER 2 antibody therapy need to recover from any anti-HER2 related adverse event (AE) or serious adverse events (SAE), i.e., the event must have resolved to Grade 1 or baseline, before starting Ad-RTs-hIL-12 immunotherapy.

The Ad-RTS-hIL-12 immunotherapy cycle is a 21-day cycle comprised of investigational product administration on days 1 through 7 followed by rest on days 8 through 21. On day 1, enrolled subjects will receive the first dose of veledimex followed 3 hours ± 3 hours later by an intratumoral injection of Ad-RTS-hIL-12. The veledimex will continue to be given once daily on days 2 through 7 (see Synopsis subsection "Investigational Product Description, Dose, and Administration," below, for details).

A safety review committee (SRC) will convene after every 5th subject with HER2- disease has completed the Ad-RTS-hIL-12 immunotherapy cycle (i.e., after all 5 subjects have completed 21 days on study). The SRC will also convene after the first 5 subjects with HER2+ disease have completed the Ad-RTS-hIL-12 immunotherapy cycle (i.e., after all 5 subjects have completed 21 days on study). The SRC will consist of the investigator, medical monitor, and other appropriate Sponsor representatives. The SRC will review all available safety information (AEs/SAEs, laboratory parameter data, electrocardiogram [ECG], etc.). Tolerability and efficacy will be evaluated separately for subjects with HER2- and HER2+ disease.

The safety evaluation will be based on the occurrence of related, treatment refractory Grade 3 and 4 AEs within 21 days following the start of Ad-RTS-hIL-12 immunotherapy.

The primary endpoint is safety/tolerability, defined as the proportion of patients who are able to complete 12 weeks on study without Grade 3 or higher toxicity as defined by Section 12.4.2.

The secondary endpoints include the progression rate calculated 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy. For the overall study, a rate of progression at 12 weeks of 50% is deemed acceptable, while an 80% rate is deemed unacceptable. From the progression rate, which is defined below under the efficacy analysis of the Statistical Methods section, the proportion of subjects who are progression-free can also be derived.

Additional secondary endpoints are the ORR, the DCR and the proportion of subjects who experience an improvement from a baseline tumor status from SD to PR/CR or from PR to CR. Disease responses by RECIST versus irRC will be explored.

The effect of treatment on blood biomarkers [including IL-12, IL-10 and interferon-gamma (IFN-γ)] and tumoral immune cell subtypes (including $CD8^+$ effector cells and T-regulatory cells) will also be explored.

Upon completion of week 12, patients who are considered to be SD or better will enter into long-term follow-up for up to an additional 36 weeks. In the long-term follow-up period, scans will be performed at 18, 24, 36, and 48 weeks until a radiographic response of PD is confirmed.

Number of Centers

Single center: Memorial Sloan Kettering Cancer Center, New York, New York, United States

Number of Subjects

40 subjects may be enrolled, including up to 20% (8 subjects) with HER2+ breast cancer.

Study Population

Inclusion Criteria

1. Female, age ≥ 18 years
2. Histologically-confirmed, locally advanced or metastatic adenocarcinoma of the breast, irrespective of hormone receptor or human epidermal growth factor receptor 2 (HER2) status (triple negative receptor status is permitted)
    a. Endocrine resistant hormone receptor-positive breast cancer subject who in the opinion of the physician requires chemotherapy is eligible
    b. Inflammatory breast cancer permitted with 2 injectable lesions
    c. Subjects who have received prior (neo) adjuvant chemotherapy, hormonal therapy or trastuzumab are eligible
3. Achievement of SD or PR after a minimum of 12 weeks of pre-study first-, second-, or third-line standard chemotherapy

    NOTE: A prior therapy that is not completed due to toxicity will not be considered a line of therapy for eligibility determination.
4. Presence of at least 2 measurable lesions as assessed by RECIST v1.1 criteria: one lesion is to be injected with Ad-RTS-IL-12 and defined as a non-target lesion
5. Standard treatment interrupted, except if anti-HER2 therapy
6. All treatment-related or radiation-related toxicities resolved to Grade 1 or lower
7. Submission of copies of tumor measurements and scans taken a) prior to starting the pre-study standard chemotherapy and b) at the end of a minimum of 12 weeks of the pre-study therapy
8. Life expectancy > 12 weeks at study entry
9. Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (see Appendix 16.1)
10. Adequate bone marrow function as assessed by the following:
    a. ANC ≥ 1500/µL (without use of growth factors within 7 days of screening)
    b. ALC > 700/µL
    c. PLT ≥ 100,000/µL
    d. Hb ≥ 9 g/dL
11. Adequate liver function as assessed by the following:
    a. Serum bilirubin ≤ 1.5 mg/dL, except as follows
        i. Patients with Gilbert's disease: serum bilirubin < 5 mg/dL
    b. AST, ALT, and ALP ≤ 2.5 × ULN, except as follows
        i. Patients with hepatic metastases: ALT and AST ≤ 5 × ULN
        ii. Patients with hepatic and/or bone metastases: alkaline phosphatase ≤ 5 × ULN
12. Adequate renal function as assessed by the following:
    a. Serum creatinine ≤ 1.5 mg/dL OR
    b. Creatinine clearance of ≥ 60 mL/min based on a 24-hour urine collection
13. Female subjects and their male partners must agree must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the screening visit through 28 days after the last dose of investigational product. Women of childbearing potential (perimenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential) must have a negative pregnancy test at screening
14. Able to swallow oral medication
15. Willing to comply with study procedures for the duration of the study and able to provide informed consent

Exclusion Criteria

1. Metastatic breast cancer patients currently on hormonal therapy as first- or second-line are not permitted
2. Prior radiation therapy encompassing > 25% of bone marrow
3. Any congenital or acquired condition leading to compromised ability to generate an immune response, including concomitant immunosuppressive therapy
4. Immunosuppressive therapy
    a. Use of systemic immunosuppressive drugs including corticosteroids (prednisone equivalent ≥ 10 mg/day) within 6 weeks
    b. Requirement for continual immune suppression with immunosuppressive drugs (eg, subjects with organ allografts)
5. Major surgery within 4 weeks of study treatment, or major surgery planned for duration of study participation
6. An active, second potentially life-threatening cancer, adequately treated basal cell or squamous cell skin cancer, or carcinoma in situ of the cervix, low grade thyroid is permitted
7. Presence of brain or subdural metastases, unless local therapy has been completed and corticosteroids have been discontinued for this indication for ≥ 4 weeks before starting study treatment:
    a. Any signs (e.g., radiologic) and/or symptoms of brain metastases must be stable for ≥ 4 weeks before starting study treatment
    b. Radiographic stability should be determined by comparing contrast-enhanced CT or magnetic resonance imaging (MRI) scans at screening to scans obtained by the same method at least 4 weeks earlier

NOTE: Screening for brain lesions is not required for all potential subjects; however, if there are any neurological signs or symptoms consistent with brain metastases, then a brain CT or MRI should be performed as clinically indicated.

8. Presence or documented history of any of the following autoimmune conditions:
    a. Inflammatory bowel disease
    b. Rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus, autoimmune vasculitis (e.g., Wegener's granulomatosis)
    c. Motor neuropathy considered of autoimmune origin (e.g., Guillain-Barre Syndrome)
9. Presence of meningeal carcinomatosis
10. Use of any medications that induce, inhibit, or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of investigational product without consultation with the medical monitor
11. History or evidence of cardiac disease as indicated by any of the following:
    a. Congestive heart failure greater than New York Heart Association (NYHA) Class II (see protocol Appendix 16.4)
    b. Unstable angina (anginal symptoms at rest), or new-onset angina (begun within the last 3 months), or myocardial infarction within the 6 months prior to enrollment
    c. Cardiac ventricular arrhythmias requiring anti-arrhythmic therapy
    d. Congenital long QT syndrome or taking drugs known to prolong the QT interval
12. Current use of any drugs with a known risk of causing torsades de pointes
13. Evidence or history of thromboembolic, venous, or arterial events such as a cerebrovascular accident including transient ischemic attacks within the past 3 months
14. Evidence or history of bleeding diathesis or coagulopathy at the time of screening
15. International normalized ratio (INR) and activated partial thromboplastin time (aPTT) > 1.5 x ULN, in subject who is not therapeutically anticoagulated. Subjects who are being therapeutically anticoagulated with an agent such as Coumadin (warfarin sodium) or subcutaneous heparin may be included provided there is not prior evidence of underlying abnormality in coagulation parameters, the screening test results are in an appropriate therapeutic range, and the anticoagulation regimen is stable and closely monitored
16. History of malabsorption syndrome or other condition that would interfere with enteral absorption

17. Presence of active clinically serious infection (Grade 3 or higher by National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE v.4.03, see Appendix 16.5)
18. Diagnosis of infection with human immunodeficiency virus (HIV) or chronic infection with hepatitis B or C virus
19. Any other unstable or clinically significant concurrent medical condition that would in the opinion of the investigator or medical monitor jeopardize the safety of a subject and/or their compliance with the protocol
20. Pregnant or breast-feeding
21. Use of any investigational, non-United States Food and Drug Administration (US FDA) approved drug within 28 days or 5 half-lives, whichever is longer, preceding screening
22. Current participation in any other clinical trial without consultation with the medical monitor
23. Presence of any condition which, in the investigator or medical monitor's opinion, makes the patient unsuitable for the study participation

Investigational Product Description, Dose, and Administration

Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter activated in the presence of the activator ligand, veledimex. Veledimex is a small molecule RTS specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the optimal veledimex dose and schedule.

Veledimex oral activator ligand

Veledimex will be supplied by the Sponsor as gelatin capsules for oral administration. Subjects will receive 80 mg of veledimex on days 1 through 7. On day 1, subjects will receive veledimex 3 hours ± 3 hours before intratumoral injection of Ad-RTS-hIL-12. On days 2 through 7, subjects will be directed to take the veledimex in a fed state (preferably within 30 minutes following a normal meal), once daily, and at the same time (± 1 hour) each day. All daily capsules must be taken together on the same day and within a few minutes. Subjects should NOT make up any missed doses. If any dose is missed, a subject should take the next dose as scheduled on the next day.

Ad-RTS-hIL-12

Ad-RTS-hIL-12 will be supplied by the Sponsor as a sterile suspension for injection. Subjects will receive an intratumoral injection of [redacted] a volume of 0.5 mL on day 1, 3 hours ± 3 hours after administration of veledimex.

The 0.5 mL dose of Ad-RTS-hIL-12 should be injected into the same lesion and should be delivered by multiple injections with a fine needle (no finer than 27-gauge) directly into each quadrant of the lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. Should the tumor selected for injection not support the entire Ad-RTS-hIL-12 injection volume (approximately 0.5 cc), another tumor should be injected with the remaining volume to ensure that all subjects receive [redacted]. If another tumor is not available, then the remaining volume should be injected into a draining, pathologic lymph node of the injected tumor. Lesions displaying signs of localized infection should not be injected. The lesion chosen for injection will be designated as a non-target lesion.

Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions for at least 2 hours following the Ad-RTS-hIL-12 injection.

All subjects receiving Ad-RTS-hIL-12 immunotherapy must be adequately hydrated with approximately 2000 ml of oral liquid preferably containing electrolytes every 24 hours for the first 72 hours. Subjects must be instructed to maintain good oral hydration on and between dosing days. Additional details regarding the number and selection of lesions and injection of Ad-RTS-hIL-12 is provided in Section 6.1 of the protocol.

Only one cycle of Ad-RTS-hIL-12 immunotherapy will be given.

Study Assessments and Criteria for Evaluation

Safety Assessments

Safety parameters will include AEs, SAEs, physical examinations (PEs), ECGs, vital signs, clinical laboratory evaluations, medical history, and prior/concomitant medications. All AEs/SAEs will be collected from the signing of informed consent until the end of Week 12. Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt/discontinue further dosing at the discretion of the investigator. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on treatment will be made by the investigator in conjunction with the Sponsor medical monitor.

Efficacy Assessments

Tumor response will use RECIST v 1.1 (see Section 9 of the main protocol). Injected and non-injected lesions will be assessed and recorded separately. Up to 5 measureable lesions may be selected and designated as target lesions. One measurable lesion will be injected and will be consequently designated a non-target lesion. Target lesions will be used to evaluate the systemic effect of Ad-RTS-hIL-12, as defined by RECIST v1.1. Additional non-injected, non-target lesions also may be followed, according RECIST v1.1. Target and non-target lesions will be assessed by CT at 6 weeks and 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy. Tumor response will also be assessed by Immune-Related Response Criteria (irRC) (Wolchok et al, 2009). The imaging method used for the week 6 and week 12 assessments of response must be the same as that used for the pre-study assessments. The tumor assessment at 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy will be compared with the pre-study tumor assessment when SD or PR was achieved.

Biologic/immunologic assessments will be performed using blood samples obtained at screening, days 1, 3, 8, and weeks 6 and 12. Tumor biopsies will be obtained by interventional radiology prior to the intratumoral vector injection and again at 6 weeks.

Duration of Each Subject's Participation

Enrolled subjects will participate for up to 52 weeks, including up to 4 weeks for screening activities.

Statistical Methods

Because there is little data on this treatment approach, there will be two stopping rules based on repeated significance testing to ensure safety (Jennison and Turnbull, 2000; Ivanova et al, 2005). The first rule is based on related, treatment refractory Grade 3 or 4 AEs. A rate of 50% is deemed unacceptable, a rate of 25% is deemed acceptable. Tolerability and efficacy will be evaluated separately for subjects with HER2- and HER2+ disease. The safety evaluation will be based on the occurrence of related, treatment refractory Grade 3 and 4 AEs within 21 days following the start of Ad-RTS-hIL-12 immunotherapy. The boundaries to stop the study are given as follows:

If 4 of the first 5 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 6 of the first 10 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 8 of the first 15 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 9 of the first 20 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 11 of the first 25 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 12 of the first 30 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 14 of the first 35 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

If 15 of the first 40 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

The second stopping rule is based on the progression rate during the 12 weeks of the study. A progression rate of 50% is deemed acceptable, while an 80% rate is deemed unacceptable. The boundaries to stop the study using a repeated significance testing approach are given as follows:

If 5 of the first 5 patients progress, stop the study.

If 9 of the first 10 patients progress, stop the study.

If 12 of the first 15 patients progress, stop the study.

If 15 of the first 20 patients progress, stop the study.

If 18 of the first 25 patients progress, stop the study.

If 20 of the first 30 patients progress, stop the study.

If 23 of the first 35 patients progress, stop the study.

If 26 of the first 40 patients progress, stop the study.

Primary endpoints:

- To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-, second-, or third-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first-, second-, or third-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by Section 12.4.2.

Secondary endpoints

- To estimate the progression rate at 12 weeks after the start of one cycle of Ad-RTS-hIL-12 immunotherapy
- To evaluate the overall response rate (ORR), defined as the rate of complete response (CR) plus the rate of PR at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy
- To evaluate the disease control rate (DCR), defined as the proportion of subjects who have a CR, PR, or SD at 12 weeks following the start of one cycle of Ad-RTS-hIL-12 immunotherapy
- To evaluate the number of subjects whose baseline tumor status (SD or PR) improves to PR or better at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy
- To compare radiographic responses by irRC with conventional reporting by RECIST
- To explore the impact of treatment on tumor and serum immune biomarkers

Analysis Populations

- The safety population will comprise all subjects who have received either the injection of Ad-RTS-hIL-12 or any doses of veledimex
- The veledimex-treated population will comprise subjects who have received the injection of Ad-RTS-hIL-12 and at least one dose of veledimex
- The veledimex-evaluable population will comprise subjects who have received the injection of Ad-RTS-hIL-12 and all 7 doses of veledimex

Safety Analysis

Exposure to investigational product and reasons for discontinuation of study treatment will be tabulated. All treatment-emergent AEs will be coded according to the system organ class and preferred term using the Medical Dictionary for Regulatory Affairs (MedDRA®), and will be tabulated by number and percent of subjects, and according to relationship to the investigational product, severity, and seriousness. Laboratory parameters will be summarized by visit. Vital signs and physical examination data will be presented by visit.

Toxicity stopping rules, when applicable, will be determined based on a clinical assessment made by the SRC after a formal evaluation of safety data after every 5 subjects with HER2- disease have completed one cycle of Ad-RTS-hIL-12 immunotherapy. The SRC will review data separately for subjects with HER2+ disease to assess toxicity after the first 5 subjects with HER2+ disease have completed one cycle of Ad-RTS-hIL-12 immunotherapy.

Efficacy Analysis

Tumor response

Tumor response (CR, PR, SD, and PD) will be determined according to RECIST v1.1 (see Section 9 of the main protocol). Tumor response will also be assessed by Immune-Related Response Criteria (irRC) (Wolchok et al, 2009).

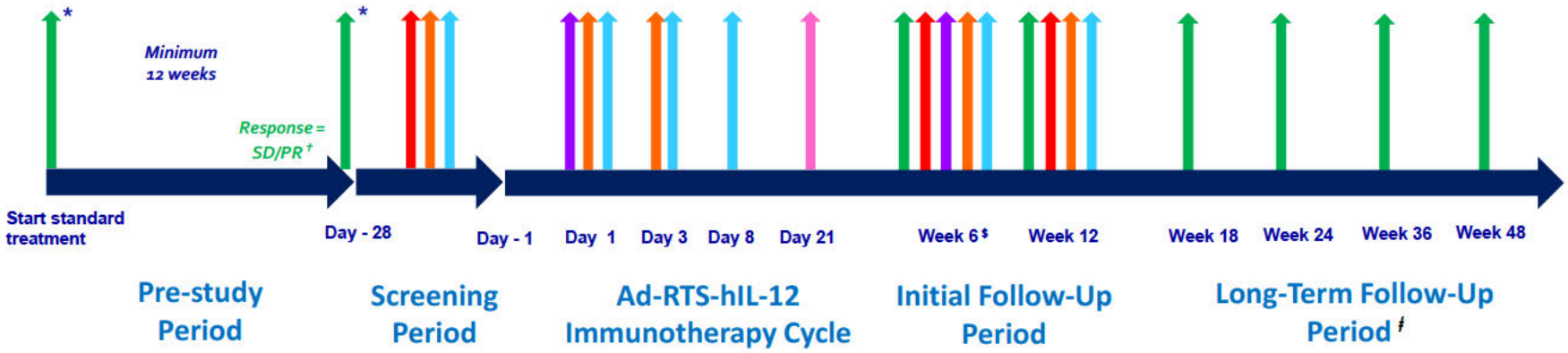
Progression-free rate

Proportion of subjects who are progression-free 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy.

ORR

The ORR is defined as the percentage of subjects who have CR or PR at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy. *DCR* The disease control rate is the proportion of subjects who have a CR, PR, or SD at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy.
Sample Size Determination Approximately 40 patients will be treated. This patient population will be heterogeneous and as such, it is difficult to define a threshold for a clear null and alternative hypothesis. This sample size will allow us to estimate an overall progression rate with a maximum 95% exact confidence interval half-width of 0.16.

STUDY SCHEMA



Tumor response imaging
Tumor markers
Tumor biopsy
Research blood
Serum chemistry and/or hematology
Safety assessment

* *Copies of scans required for study entry (imaging)*
† *Study entry requires SD or PR after minimum of 12 weeks of standard treatment*
$ *If PD – confirm at least 4 weeks later*
ǂ *Tumor response imaging will continue until PD is confirmed*

SCHEDULE OF STUDY PROCEDURES

ACTIVITY	SCREENING	Ad-RTS-hIL-12 IMMUNOTHERAPY CYCLE				INITIAL FOLLOW-UP		LONG-TERM FOLLOW-UP			
	D-28 to D-1	D1	D3	D8	D21[p]	W6[u]	W12[u]	W18[u]	W24[u]	W36[u]	W48[u]
Informed Consent	X										
Medical/Cancer History	X										
Physical Exam[a]	X	X	X	X		X	X				
ECOG performance status	X	X	X	X		X	X				
Height	X										
Weight	X	X				X	X				
Vital Signs[b]	X	X	X	X		X	X				
Adverse Events	X →	→	→	→	→	X[t]					
Concomitant Medications[c]	X →	→	→	→	→	→	X				
Pregnancy[d]	X										
Hematology [e]	X	X	X	X		X	X				
Coagulation[f]	X			X							
Serum Chemistry[g]	X			X		X	X				
Urinalysis[h]	X	X									
Tumor markers (CEA, Ca 15-3)	X					X	X				
TSH, Hepatitis B/C, HIV	X										
ECG[i]	X										
Oral veledimex[j,k]		X	X								
Intratumoral Ad-RTS-hIL-12		X[l]									
Dispensing of veledimex for home administration and diary[m]		X									
Diary collection[m]		X	X	X							
Imaging Studies[n]	X					X[q]	X[r]	X[r]	X[r]	X[r]	X[r]
Tumor biopsy		X[s]				X					
Research bloods[o]	X	X	X	X		X	X				

Abbreviations: D, day; W, week; ECOG, Eastern Cooperative Oncology Group

a. A physical exam is required during screening and at Weeks 6 and 12. Otherwise, a symptom-directed physical exam should be performed where indicated.

b. Vital signs include blood pressure, pulse, temperature, and respirations. On Days 1 and 3 vital signs will be recorded prior to administration of veledimex. On Day 1, vital signs will also be recorded prior to administration of Ad-RTS-hIL-12. At all indicated time points, blood pressure should be monitored closely, [redacted] Subjects must be instructed to maintain adequate oral hydration while taking veledimex; sites must closely monitor subjects' hydration status. Oral hydration (approximately 2000 mL oral electrolyte solution over 24-hours x 3 days) and administration of prophylactic antipyretics are highly recommended after injection of Ad-RTS-hIL-12.

c. Concomitant medications should be collected from screening through Week 12. In addition, any new anti-cancer medication started prior to confirmation of radiographic response of PD should be collected.

d. Females of childbearing potential will have a serum pregnancy test during screening (within 14 days of dosing) and a urine pregnancy test at Day 1. A serum pregnancy test should be completed on Day 1 if the urine pregnancy test is positive.

e. The hematology panel includes complete blood count (CBC) and white blood cell (WBC) count, differential white blood cell count, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices (MCV, MCH, MCHC), and platelet count.

f. The coagulation panel includes activated partial thromboplastin time (aPTT) and the international normalized ratio (INR).

g. The serum chemistry panel includes aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, sodium, potassium, chloride, calcium, phosphorus, and bicarbonate.

h. The urinalysis panel (dipstick) includes appearance, pH, specific gravity, glucose, protein/albumin, blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated.

i. Standard, 12-lead ECG.

j. Oral veledimex to be taken daily on Days 1-7. The first dose of veledimex will be given at the clinical site in a fasting state. The remaining 6 doses can be self-administered at the same time as on Day 1 (±1 hour) by subjects, in the fed state, on Days 2-7. Study sites must verify adherence with veledimex dosing. Subjects should return bottles of veledimex to the clinical site to determine extent of subject adherence to self-administration preferably on Day 8.

k. Days 2, 4, 5, 6, and 7 are not shown, but veledimex is also to be taken on those days.

l. The intratumoral Ad-RTS-hIL-12 ([redacted]) should be administered 3 hours ± 3 hours after oral veledimex. Lesions displaying signs of localized infection should not be injected. Subjects must be instructed to maintain good oral hydration while taking veledimex. Oral hydration (approximately 2000 mL in the first 24h) and administration of prophylactic antipyretics is highly recommended after injection of Ad-RTS-hIL-12 [redacted]. Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions for at least 2 hours following the Ad-RTS-hIL-12 injection. A detailed description of physical location(s) of the injected tumor(s) and surrounding tissue, and any visible local reactions will be documented. The subject should be instructed to call the clinical site if any such reactions develop or don't resolve within 24 to 48 hours.

m. Study sites must determine compliance of veledimex dosing. Veledimex will be dispensed to the subject on Day 1 for the next 6 days along with the veledimex subject diary. Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate last meal, the number of capsules taken, whether subject missed any veledimex doses and the study day and reason for any missed doses. Investigational product container(s) with any remaining capsules should be returned to the study staff on Day 8 (or after last dose), so that staff can properly assess dose compliance.

n. Appropriate cancer staging procedures should be performed during screening. Copies of tumor measurements and scans taken a) before the start of the pre-study standard chemotherapy and b) following a minimum of 12 weeks of the pre-study chemotherapy are required for study entry. In the event that IV contrast cannot be used, the medical monitor should be consulted. All imaging should be of diagnostic quality and include intravenous (IV) contrast. Please refer to Appendix 16.3 for RECIST v 1.1. guidelines.

o. Research bloods consist of blood for cytokines and immune cell activation by flow cytometry as noted in Section 11.

p. The Day 21 visit will collect adverse events and concomitant medication. This visit may be completed via phone call.

q. If radiographic PD is recorded at 6 weeks, PD must be confirmed 4 weeks later. In cases of slow tumor growth, the investigator may wait until confirmation of the PD at 12 weeks to resume chemotherapy. In cases of unequivocal progression, the investigator may resume chemotherapy prior to 12 weeks.

r. Subjects who have SD or better at Week 12 will continue into the long-term follow-up period where imaging should continue per the schedule until a radiographic response of PD is confirmed.

s. Biopsy on Day 1 is to be taken 3 hours ± 3 hours after administration of veledimex and prior to injection of Ad-RTS-hIL-12.

t. After Week 6, only SAEs suspected of being related to investigational product will be collected.

u. The follow-up visits can be performed within ± 1 week.

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Abbreviation	Definition
Ad	Adenovirus
AE	Adverse event
ALC	Absolute lymphocyte count
ALT	Alanine transaminase
ALP	Alkaline Phosphatase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
BUN	Blood Urea Nitrogen
CBC	Complete blood count
GCP	Good Clinical Practice
CBC	Complete blood count
CD	Cluster of differentiation
CR	Complete response
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria For Adverse Events
CYP	Cytochrome p
DCR	Disease control rate
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EcR	Ecdysone receptor
FDA	Food and Drug Administration
FNA	Fine needle aspiration
Hb	Hemoglobin
HER2	Human epidermal growth factor receptor 2
HIPAA	Health Insurance Portability and Accountability Act of 1996
hIL-12	Human interleukin-12
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IFN-γ	Interferon gamma
IL	Interleukin
INXN-1001	Veledimex oral activator ligand
INXN-2001	Ad-RTS-hIL-12 vector for injection
IV	Intravenous
INR	International Normalized Ratio
IRB	Institutional Review Board
irRC	Immune-Related Response Criteria
LDH	Lactate dehydrogenase
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NK	Natural killer
NYHA	New York Heart Association
ORR	Overall Response Rate
PD	Progressive disease
PE	Physical examination
PET	Positron emission tomography
PLT	Platelet
PR	Partial response
PTT	Partial thromboplastin time
QOL	Quality of life
RBC	Red Blood Cell Count
RECIST	Response Evaluation Criteria in Solid Tumor
[redacted]	[redacted]
RXR	Retinoid X receptor
SAE	Serious adverse event
SD	Stable disease
SOP	Standard operating procedure
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
TNF	Tumor necrosis factor
ULN	Upper limit of normal
US FDA	United States Food and Drug Administration
Vp	Viral particles
WBC	White Blood Cell Count

1 INTRODUCTION

1.1 Disease Background

It is estimated that there are nearly 3 million women living in the United States with a history of invasive breast cancer, and an additional 226,870 women were diagnosed in 2012 (Siegel et al, 2012). Approximately 50% of women diagnosed with primary breast cancer will eventually relapse and develop metastatic or advanced disease. In addition, around 10% of patients present with metastatic disease at first diagnosis. The 5-year relative survival rate for women diagnosed with localized breast cancer is 98.6%; survival declines to 83.8% for regional stage and to 23.3% for distant stage. In addition to stage, factors that influence survival include tumor grade, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) status.

Loco regional recurrence in breast cancer is an indicator of an aggressive tumor, and early recurrence carries a poor prognosis. Approximately 11 and 20 percent of patients treated with adjuvant therapies develop loco regional recurrence within 5 and 10 years, respectively (Brewster et al, 2008). If disease is localized, surgical excision can be attempted. Breast cancer recurrences after mastectomy pose a therapeutic challenge with few surgical options. Many patients have systemic disease either at the time of their cutaneous or other recurrence or soon after, some may manifest disease only at the dermal site of disease, and a few are cured with aggressive treatment. These lesions often are widespread throughout the chest wall or involve heavily irradiated tissue.

Recent advancements in targeted therapy and improvements in treatment have heralded a significant improvement in survival, but metastatic breast cancer remains essentially incurable. Advanced metastatic breast cancer may be treated with systemic therapy (chemotherapy, biological therapy, targeted therapy, hormonal therapy), local therapy (surgery, radiation therapy), or a combination of these treatments (Morris et al, 2009). Guidelines from the National Comprehensive Cancer Network for treatment of women with a loco regional breast cancer recurrence and metastatic breast cancer suggest consideration of systemic therapy, but do not specify the type of therapy or the duration. There is no standard of treatment among first- and second-line chemotherapy for locally advanced or metastatic breast cancer disease. The initial choice of chemotherapy is highly influenced by the subject's personal history of previous drug exposure. Most subjects have been exposed to both an anthracycline and a taxane in the adjuvant setting. In case instant response is required, such as in subjects with widespread visceral disease, heavy tumor burden or rapidly progressive, threatening disease or massive symptoms, combination cytostatic therapy is usually administered. Monotherapy is a choice for patients in whom a long-term stabilization of metastatic disease is the objective. The prolonged administration of chemotherapy in the metastatic setting may be challenging because of residual toxicity after taxanes such as lingering neuropathy, problems with edema, especially after docetaxel, cumulative cardiotoxicity after anthracyclines or mucositis and diarrhea on capecitabine.

Almost all patients who have received first-line chemotherapy for metastatic disease will relapse or experience progressive disease (PD) and require subsequent treatment. For these patients requiring second- and subsequent-line therapy, the goals of treatment are to maintain a good

quality of life and to prolong survival (Howlader et al, 2012). Combination therapy is a mainstay of anticancer treatment, with optimal combinations producing synergistic antitumor responses, achieved by combining agents with non-overlapping mechanisms of action and safety profiles.

1.2 Interleukin-12 and Cancer Immunotherapy

Interleukin-12 (IL-12) is a pro-inflammatory cytokine and has been recognized as a master regulator of cell mediated immunity in response to intracellular pathogens and neoplastic transformation. Structurally IL-12 is a heterodimeric protein composed of p35 and p40 subunits covalently linked to form the biologically active IL-12p70 molecule (Carra et al, 2000). It has been shown that IL-12 has the profound impacts upon the tumor environment. The IL-12 could mediate the recruitment of lymphocytes, activation of tumor infiltrating lymphocytes as well as direct effects on tumor cells to decrease angiogenesis.

Initial studies identified that IL-12 was produced by innate immune cells in response to pathogens and that it led to the production of interferon gamma (IFNγ) and tumor necrosis alpha (TNFα) by T and natural killer (NK) cells (Micallef et al, 1996; Trinchieri, 2003). When it was discovered that IL-12 could, indeed, drive naïve T helper cell (Th0) differentiation to the inflammatory Th1 phenotype (Hsieh et al, 1993) a role for IL-12 was established as a bridge between innate immune cells and the adaptive immune response through polarization of naïve CD4+ cells. More recent data demonstrate additional functional roles of IL-12 directly influencing CD8+ T cell differentiation (Kalinski et al, 1999; Curtsinger et al, 2003) and the reactivation and survival of memory CD4+ T cells (Yoo et al, 2002). This is particularly important in the context of the tumor microenvironment where high levels of IL-12 have been shown to repolarize antigen-experienced CD4+ T cells back to the functional antitumor Th1 phenotype (Wesa et al, 2007).

Evidence that IL-12 is able to trigger innate and adaptive immunity and modulate the tumor microenvironment supports the relevance of IL-12 as an important immunotherapeutic agent. Its ability to activate and recruit dendritic cells that facilitate the cross-priming of tumor antigen-specific T cells, along with its influence on NK and $CD8^+$ T cell cytotoxic activities and antigen-specific antitumor responses (Mosmann and Coffman, 1989; Trinchieri, 1995; Tsung et al, 1997; Mailliard et al, 2002) warrant further study in cancer therapy. Additionally, IL-12 has also been shown to stimulate the production of superoxides and nitric oxide and possess potent antiangiogenic activity through INFγ (Voest et al, 1995; Wigginton et al, 1996; Coughlin et al, 1998). The potent anti-tumor activity of IL-12 has been well documented in various cancer mouse models including melanoma, mammary carcinoma, sarcoma, colon and renal carcinoma (Colombo et al, 2002). The potent nature of its biological activity and signaling complexity has also prompted the study of different delivery mechanism with a focus on intratumoral delivery and tumor microenvironment modulation.

Based on such data, human studies of IL-12 as an anticancer agent were initiated. The first of these studies was a Phase 1 dose escalation of IV administered recombinant human IL-12 in patients with either melanoma or renal cell carcinoma. The study reported a transient complete response (CR) in melanoma and a partial response (PR) in renal cell carcinoma with significant toxicities. The Phase 2 trial observed similar toxicities and two IL-12 related deaths prompting the FDA to suspend the trial (Atkins et al, 1997; Leonard et al, 1997). Additional studies

confirmed that systemic administration of recombinant IL-12 resulted in significant toxicity limiting its potential for clinical development (Leonard et al, 1997; Salem et al, 2006). These results prompted the investigation of alternative delivery routes focusing on loco regional administration either by sub cutaneous injection or intratumoral delivery implementing IL-12 as a direct anticancer therapeutic or as an adjuvant to vaccination.

1.3 Local IL-12 Delivery and Development of an Inducible IL-12 Immunotherapy

The potent effects of cytokines, particularly IL-12, as mediators of an anticancer immune response remain compelling. This is especially true since the advent of immunotherapies such as anti CTLA-4 and anti PD1 antibodies provide proof of concept that inhibiting immune checkpoints translates into clinical benefit. IL-12 biology including the level of activation, location of initial expression, immune effector function and biologically active combination with other cytokines remain incompletely understood.

Several human studies that have implemented the local delivery of cytokines or chemotherapeutic agents have already shown that such an approach reduces systemic toxicity and produces signals of clinical benefit (Chiocca et al, 2008).

[redacted]

1.4 Adenoviral Vectors for Gene Therapy

1.4.1 Adenoviral Safety

Adenoviral vectors have been used extensively to deliver a variety of gene products to human subjects, including cancer patients. Although adenoviral vectors are immunogenic, virtually all recipients have pre-existing humoral immunity to adenoviruses and they are generally considered a safe and well-tolerated vehicle for gene delivery. To date, numerous studies utilized adenoviral vectors to achieve intratumoral expression of a variety of genes. In a Phase I/II clinical trial of subjects with prostate cancer, direct intra-prostatic injection of a replication-defective adenoviral vector encoding bacterial nitroreductase (dose levels 5×10^{10}-1×10^{12} vp) was well tolerated, with minimal side effects (Patel et al, 2009). A Phase 1 study of subjects with oral leukoplakia, implemented multiple intraepithelial injections of recombinant adenovirus (rAd)-p53 (1×10^{8} vp/cm^2) and demonstrated good tolerance of the vector, with no evidence of DLTs (Zhang et al, 2009). Another, Phase I/II study of subjects with chemoradiation-resistant advanced esophageal carcinoma, intratumoral injections of adenovirus vector containing p53 (Ad.5CMV-p53) were

well tolerated at doses ranging from 10×10^{11} to 25×10^{11} vp, with no DLTs, and generally mild to moderate adverse events (AEs) (Shimada et al, 2006). The most common AEs were fever (all 10 subjects), pain (30% of subjects), and hyperglycemia, which was attributed to the use of total parental nutrition (30% of subjects). Hypocalcemia was reported in two subjects (20%) and one subject each (10%) experienced activated partial thromboplastin time (aPTT) elongation, abnormally high serum amylase, and abnormally high serum creatinine.

In a Phase 1 study of subjects with advanced pancreatic, colorectal, or primary liver tumors, intratumoral injection of an adenoviral vector encoding hIL-12 (Ad.IL-12) was well tolerated at doses of up to 3×10^{12} vp. Common AEs were similar to symptoms observed with gene delivery by other adenoviral vectors, including transient, mild to moderate fever, malaise, sweating, and lymphopenia (Sangro et al, 2004).

1.4.2 Safety of Intratumoral Injection of IL-12 Gene Vectors

In contrast with the systemic toxicity resulting from administration of recombinant IL-12 protein, local administration of IL-12 via injection of plasmid or adenoviral vectors containing the hIL-12 gene has proven to be well tolerated in patients with various cancers, and therefore appears to provide an effective delivery method for this potent immunomodulatory cytokine. Several studies have investigated the safety of intratumoral expression of IL-12 in patients with metastatic melanoma. A Phase 1 study investigated intratumoral expression of IL-12 together with the co-stimulatory molecule B7.1 via two separate canarypox virus viral vectors in patients with metastatic melanoma and reported mild to moderate injection site reactions, fever, chills, myalgia, and fatigue as AEs (Triozzi et al, 2005). However, all patients also developed antibodies to canarypox virus viral vectors. Notably, serum IL-12 and IFN-gamma levels were not increased after treatment. Another Phase I trial showed that delivery by electroporation of a plasmid containing interleukin-12 to tumors in patients with metastatic melanoma resulted in minimal systemic toxicity, with transient pain after electroporation being the most common AE (Daud et al, 2008). Results from another Phase 1 study showed that intratumoral injection of DNA encoding hIL-12 in patients with metastatic melanoma was well tolerated overall (Heinzerling et al, 2005). Eight of nine patients experienced a transient response at the intratumoral injection site, and some patients who had tumor responses also showed some increases in systemic IL-12, IP-10, and IFN-γ.

Localized production of IL-12 also has been reported as well tolerated in subjects with other malignancies. For example, a Phase 1 study in 17 patients with metastatic pancreatic, colorectal, or primary liver cancer examined intratumoral injection of dendritic cells engineered to secrete interleukin-12 via a recombinant adenovirus vector (Mazzolini et al, 2005). In that study, the most common AEs were lymphopenia, fever, and malaise. Patients also developed antibodies to the adenoviral capsid proteins. Intraperitoneal injection of a plasmid containing the hIL-12 gene in women with chemotherapy-resistant, recurrent, ovarian cancer also was found to be generally safe and well tolerated (Anwer et al, 2010). Low-grade fever and abdominal pain were the most common side effects. Plasmid DNA was not detected in the subjects' serum samples, and treatment-related increases in IFN-γ levels were observed in pleural fluid but not in serum. Similar data were reported in a study of subjects with advanced pancreatic, colorectal, or primary liver malignancies who received intratumoral injections of adenoviral vectors encoding hIL-12 at doses ranging from 2.5×10^{10} to 3×10^{12} vp (Sangro et al, 2004). In that study, treatment was

well tolerated and a maximum tolerated dose (MTD) was not reached. Transient lymphopenia was observed in 86% of subjects, and the severity was increased at higher vector doses. Transient, mild to moderate fever, sometimes accompanied by malaise and sweating, was observed in ≈ 60% of subjects during the first 2 days after the injection. Five of the 21 subjects (24%) experienced nausea and/or vomiting, on the day of the injection. No cumulative toxicity was observed. These events were deemed related to injection of the virus and not to transgene expression.

[redacted]

ctor.

[redacted]

[redacted]

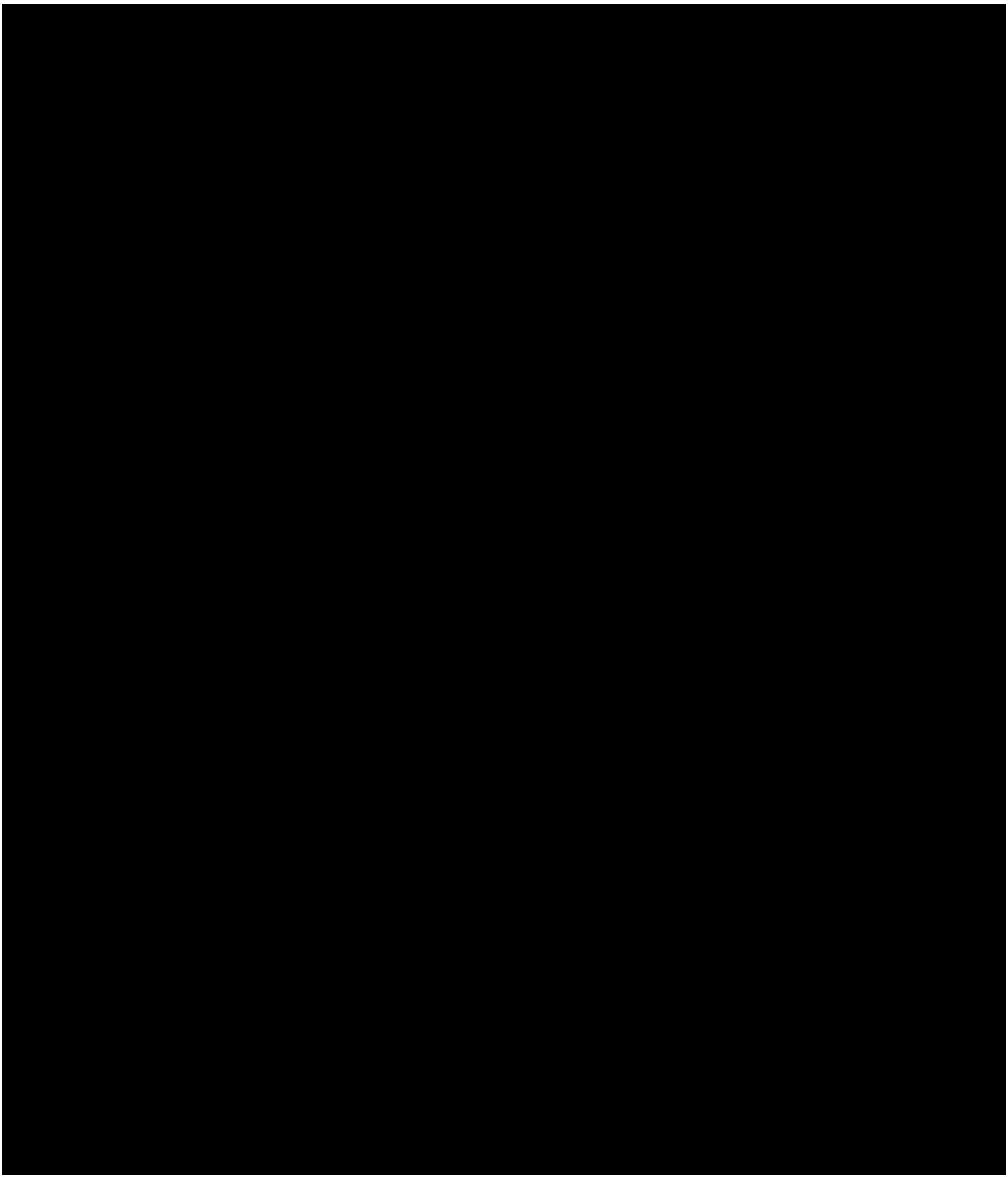
[redacted]

1.5.3 Veledimex

Veledimex is a diacylhydrazine that is fully active at the RTS[redacted] receptor. Veledimex is a liquid formulation with the active dissolved in the excipients. This formulation has been encapsulated in gelatin capsules for oral administration in clinical trials.

Nonclinical studies in vitro and in vivo demonstrate that veledimex interacts with the receptor component EcR of RTS[redacted] to induce the activation of therapeutic gene transcription, leading to the production of transgene mRNA and, ultimately, protein (Anderson et al, 2000; Palli et al, 2003; Karzenowski et al, 2005).

[redacted]



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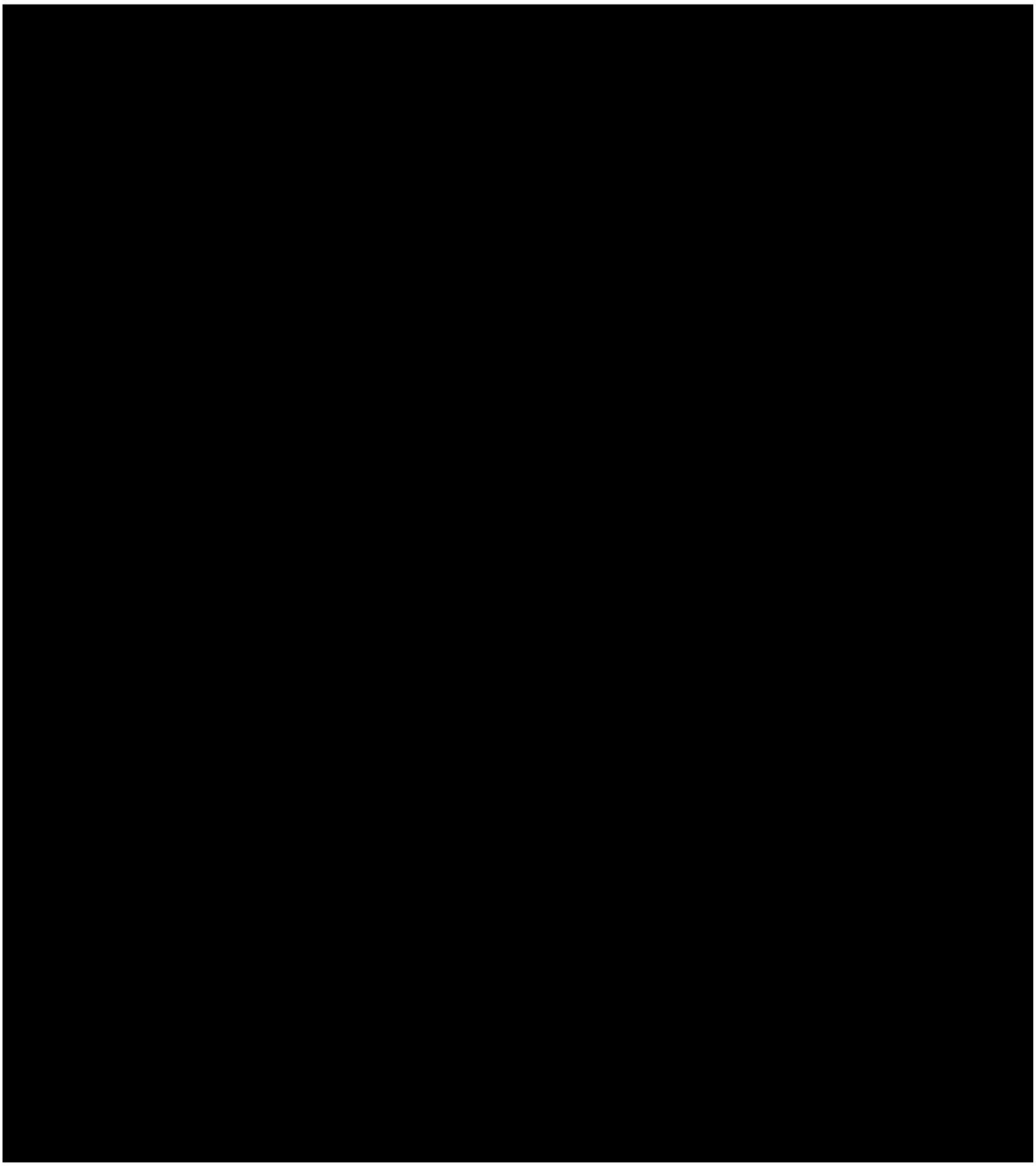
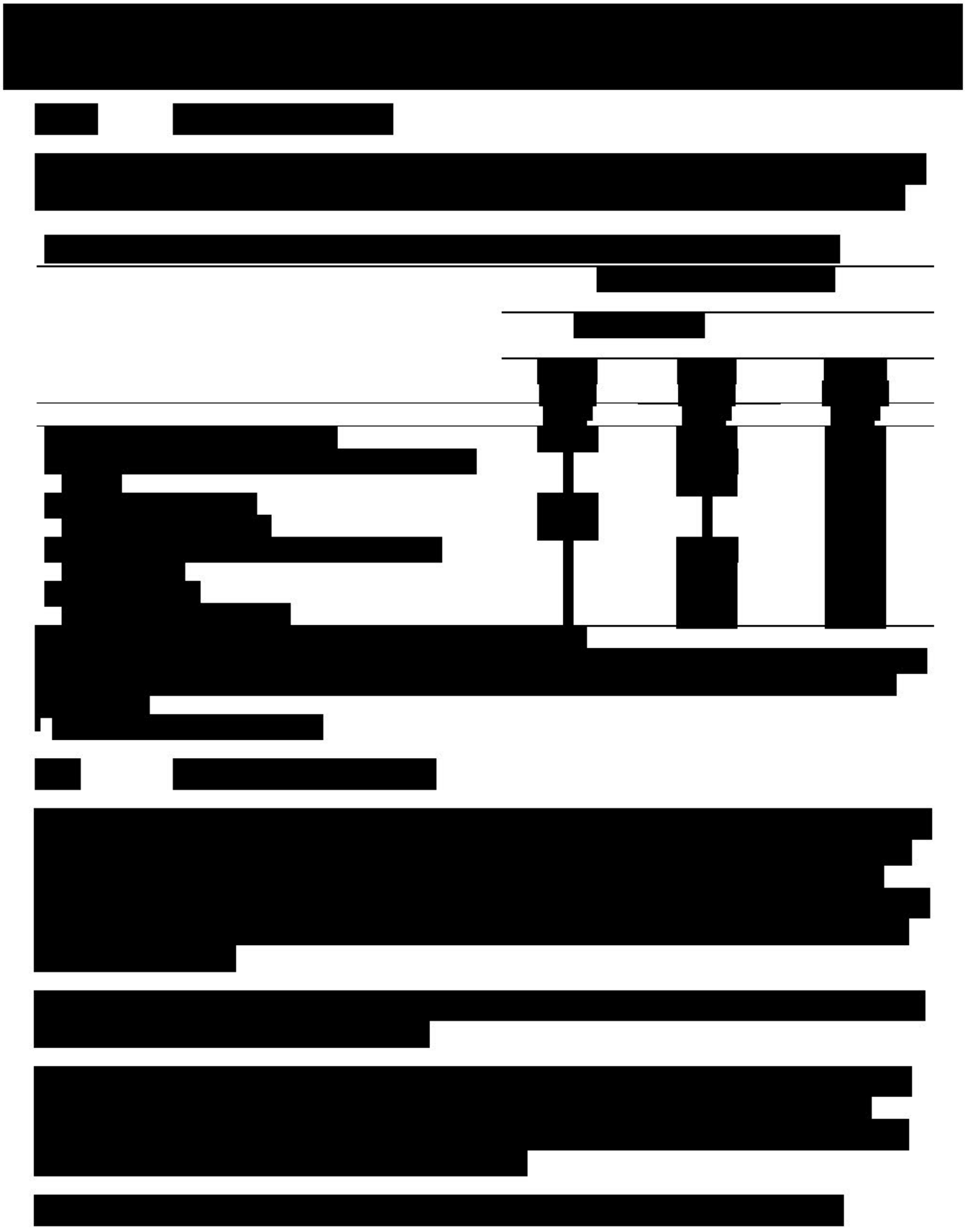




1.7 Rationale for Current Study

Metastatic breast cancer is an incurable disease with palliative therapeutic goals that include prolongation of survival with good quality of life (QOL) and symptom control. Chemotherapy is generally recommended in patients with HR negative tumors, endocrine resistant disease and rapidly proliferative and/or symptomatic disease. Although endocrine therapy, chemotherapy, and, more recently, targeted or biotherapy, may produce an objective response, almost all patients subsequently experience PD. Numerous monotherapy drugs as well as combinations of cytotoxic drugs have been used to treat patients with advanced breast cancer. The optimal duration of therapy in first-line chemotherapy in the treatment of metastatic breast cancer is unknown and controversial. Clinical trials have reported prolonged duration of remission with continuous chemotherapy beyond an initial induction of several weeks; however, its effect on survival and QOL are less consistent (Coates et al, 1987; Ejlertsen et al, 1993; Falkson et al, 1998; Nooij et al, 2003)

The relative benefits of tumor regression and improvement in disease related symptoms must be balanced with treatment induced toxicity and its impact on QOL. The physicians are generally using a continuous chemotherapy despite the insufficient and inconsistent evidence of improved overall survival because continuous chemotherapy has consistently been associated with longer median time to progression than a short, mostly four to six-course induction regimen followed by observation (Muss et al, 1991). In addition, reports have indicated that in patients with objective PR or disease stabilization, no difference in survival is obtained when chemotherapy is continued (Harris et al, 1990). There are however, known risks from cumulative drug-associated toxicities, such as peripheral neuropathy associated with the prolong use of taxanes, as well as mucositis, hand-foot syndrome, and diarrhea associated with the use of capecitabine, and cardiac toxicity associated with doxorubicin.

Although patients who achieve benefit with initial therapy may continue to receive chemotherapy until disease progression or unacceptable toxicity, chemotherapy can also be

stopped after maximal benefit has been obtained and patients can then be followed for the occurrence of disease progression.

Immunotherapy has recently shown evidence as a potentially promising approach to treating patients with invasive and metastatic breast cancers (Hudis, 2003; Zhou and Zhong, 2004; Emens et al, 2005). Continued chemotherapy must be assessed in relationship to QOL and increased frequency of AEs compared to stopping chemotherapy. Patients and their physicians may discuss how long to continue the chemotherapy treatment and if some chemotherapy breaks may be considered either when AEs occur to allow the patient to recover or to allow the patient to carry on other personal plans and re-start chemotherapy later on.

An effective and convenient therapy that could be used to maintain benefits from prior cytotoxic chemotherapy such as prolonged disease stability might offer a useful addition to the pharmacologic management of patients with locally advance, recurrent or metastatic breast cancer.

This Phase Ib/II study was designed to assess the safety and efficacy of a new immunotherapy product, Ad-RTS-hIL-12 (also known as INXN-2001) + veledimex (also known as INXN-1001), in patients with locally advanced, recurrent or metastatic breast cancer who achieved clinical benefit, i.e., stable disease (SD) or a PR with standard therapy. The study will evaluate whether Ad-RTS-hIL-12 + veledimex (also referred to in this protocol as Ad-RTS-hIL-12 immunotherapy) allows maintenance of clinical benefit in patients who stop first-, second-, or third-line standard therapy after having achieved a PR or SD.

2 STUDY OBJECTIVES

2.1 Primary Objective

- To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-, second-, or third-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first-, second-, or third-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by Section 12.4.2.

2.2 Secondary Objectives

- To estimate the progression rate at 12 weeks after the start of one cycle of Ad-RTS-hIL-12 immunotherapy.
- To evaluate the overall response rate (ORR), defined as the rate of complete response (CR) plus the rate of PR at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy.
- To evaluate the disease control rate (DCR), defined as the proportion of subjects who have a CR, PR, or SD at 12 weeks following the start of one cycle of Ad-RTS-hIL-12 immunotherapy.
- To evaluate the number of subjects whose baseline tumor status (SD or PR) improves to PR or better at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy.
- To compare radiographic responses by irRC with conventional reporting by RECIST

- To explore the impact of treatment on tumor and serum immune biomarkers

3 STUDY DESIGN

3.1 Overall Study Design

This is a single-arm, single-center phase Ib/II study to examine the safety, tolerability and preliminary efficacy of providing one cycle of Ad-RTS-hIL-12 immunotherapy following achievement of SD or PR on standard first-, second-, or third-line chemotherapy in breast cancer subjects. The patient population will include patients with locally advanced or metastatic breast cancer of all subtypes. The safety of this therapy and the preliminary evidence of efficacy will guide further studies. Patients will be required to be in SD or PR after completion of a minimum of 12 weeks on standard chemotherapy. Subjects who have PD or a CR after the standard chemotherapy are not eligible for the study. Following entry into the trial, patients will go on a treatment holiday from the standard chemotherapy and enter an immunotherapy phase of treatment.

Scans will be conducted 6 and 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy for determination of tumor response. Radiographic PD at week 6 must be confirmed at least 4 weeks later either at week 12 or earlier if clinically necessitated. In cases of slow tumor growth, the investigator may wait until confirmation of PD at 12 weeks to resume chemotherapy. In cases of unequivocal progression, the investigator may resume chemotherapy prior to 12 weeks and the subject will be considered as treatment failure at 12 weeks.

Upon completion of Week 12, subjects who are considered to have SD or better will enter into long-term follow-up for up to an additional 36 weeks. In the long-term follow-up period, scans will be performed at 18, 24, 36, and 48 weeks until a radiographic response of PD is confirmed.

The assessment of response will be based on Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 using tumor measurements from contrast-enhanced computed tomography (CT). Tumor response will also be assessed by Immune-Related Response Criteria (irRC) (Wolchok et al, 2009). Eligible subjects will also be asked to consent to allow ZIOPHARM to obtain scans and tumor measurements taken both a) prior to initiating the pre-study standard chemotherapy and b) after a minimum of 12 weeks of pre-study standard chemotherapy, and not more than 4 weeks prior to study enrollment, in order to confirm their SD, PR status.

For subjects with HER2- disease, the Ad-RTS-hIL-12 immunotherapy is intended to be given as a chemotherapy holiday, for example, to avoid intolerable or undesirable toxicity of the standard therapy. Ad-RTS-hIL-12 immunotherapy must be started within 4 weeks of stopping the pre-study standard chemotherapy.

Subjects with HER2+ disease will receive the Ad-RTS-hIL-12 immunotherapy in conjunction with first-, second-, or third-line anti-HER2 antibody therapy, i.e., subjects may continue anti-HER2-directed antibody therapy during the study period. Anti-HER2 antibody therapy will be administered according to the manufacturer's recommendations. Subjects with HER2+ breast cancer who continue the anti-HER2 antibody therapy need to recover from any anti-HER2 related AE/SAE, i.e., the event must have resolved to Grade 1 or baseline, before starting Ad-RTS-hIL-12 immunotherapy.

The Ad-RTS-hIL-12 immunotherapy cycle is a 21-day cycle comprised of investigational product administration on days 1 through 7 followed by rest on days 8 through 21. On day 1, enrolled subjects will receive the first dose of veledimex followed 3 hours ± 3 hours later by an intratumoral injection of Ad-RTS-hIL-12. The veledimex will continue to be given once daily on days 2 through 7 (see Section 5 below, for details).

A safety review committee (SRC) will convene after every 5th sujbject with HER2- disease have completed the Ad-RTS-hIL-12 immunotherapy cycle (i.e., after each subject has completed 21 days on study). The SRC will also convene after the first 5 subjects with HER2+ disease have completed the Ad-RTS-hIL-12 immunotherapy cycle. The SRC will consist of the investigator, medical monitor, and other appropriate Sponsor representatives. The SRC will review all available safety information (AEs/SAEs, laboratory parameter data, ECG etc.). Tolerability will be evaluated separately for subjects with HER2- and HER2+ disease.

The safety evaluation will be based on the occurrence of related, treatment refractory Grade 3 and 4 AEs within 21 days following the start of Ad-RTS-hIL-12 immunotherapy. A post-treatment safety assessment visit will be conducted on Day 21.

The primary endpoint is safety/tolerability, defined as the proportion of patients who are able to complete 12 weeks on study without a Grade 3 or higher toxicity as defined by Section 12.4.2.

The secondary endpoints include the progression rate calculated at 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy. For the overall study, a rate of progression at 12 weeks of 50% is deemed acceptable, while an 80% rate is deemed unacceptable. From the progression rate, the proportion of subjects who are progression-free can also be derived (see Section 12.4.3.3) Additional secondary endpoints are the ORR, the DCR and the proportion of subjects who experience an improvement from a baseline tumor status from SD to PR or from PR to CR.

Immunologic biomarkers of response in blood and tumor will also be explored.

3.2 Dose Escalation Cohorts

Not applicable.

3.3 Study Oversight for Safety Evaluation

The first level of safety oversight will occur through the site investigator and medical monitor. An SRC, consisting of the study investigators, medical monitor, and other appropriate Sponsor representatives, will provide overall safety oversight.

4 SUBJECT SELECTION

The eligible study population includes adult subjects with locally advanced, recurrent or metastatic breast cancer.

4.1 Inclusion Criteria

1. Female, age ≥ 18 years

2. Histologically-confirmed, locally advanced or metastatic adenocarcinoma of the breast, irrespective of hormone receptor or human epidermal growth factor receptor 2 (HER2) status (triple negative receptor status is permitted)
    a. Endocrine resistant hormone receptor-positive breast cancer subject who in the opinion of the physician requires chemotherapy is eligible
    b. Inflammatory breast cancer permitted with 2 injectable lesions
    c. Subjects who have received prior (neo) adjuvant chemotherapy, hormonal therapy or trastuzumab are eligible
3. Achievement of SD or PR after a minimum of 12 weeks of pre-study first-, second-, or third-line standard chemotherapy

    NOTE: A prior therapy that is not completed due to toxicity will not be considered a line of therapy for eligibility determination.
4. Presence of at least 2 measurable lesions as assessed by RECIST v1.1 criteria: one lesion is to be injected with Ad-RTS-IL-12 and defined as a non-target lesion
5. Standard treatment interrupted, except if anti-HER2 therapy
6. All treatment-related or radiation-related toxicities resolved to Grade 1 or lower
7. Submission of copies of tumor measurements and scans taken a) prior to starting the pre-study standard chemotherapy and b) at the end of a minimum of 12 weeks of the pre-study chemotherapy
8. Life expectancy > 12 weeks at study entry
9. Eastern Cooperative Oncology Groups (ECOG) performance status of 0 to 1 (see Appendix 16.1)
10. Adequate bone marrow function as assessed by the following:
    a. ANC ≥ 1500/µL (without use of growth factors within 7 days of screening)
    b. ALC > 700/µL
    c. PLT ≥ 100,000/µL
    d. Hb ≥ 9 g/dL
11. Adequate liver function as assessed by the following:
    a. Serum bilirubin ≤ 1.5 mg/dL, except as follows
        i. Patients with Gilbert's disease: serum bilirubin < 5 mg/dL
    b. AST, ALT, and ALP ≤ 2.5 × ULN, except as follows
        i. Patients with hepatic metastases: ALT and AST ≤ 5 × ULN
        ii. Patients with hepatic and/or bone metastases: alkaline phosphatase ≤ 5 × ULN
12. Adequate renal function as assessed by the following:
    a. Serum creatinine ≤ 1.5 mg/dL OR

b. creatinine clearance of ≥ 60 mL/min based on a 24-hour urine collection

13. Female subjects and their male partners must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the screening visit through 28 days after the last dose of investigational product. Women of childbearing potential (perimenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential) must have a negative pregnancy test at screening
14. Able to swallow oral medication
15. Willing to comply with study procedures for the duration of the study and able to provide informed consent

4.2 Exclusion Criteria

1. Metastatic breast cancer patients currently on hormone therapy as first- or second-line are not permitted
2. Prior radiation therapy encompassing > 25% of bone marrow
3. Any congenital or acquired condition leading to compromised ability to generate an immune response, including concomitant immunosuppressive therapy
4. Immunosuppressive therapy
    a. Use of systemic immunosuppressive drugs including corticosteroids (prednisone equivalent ≥ 10 mg/day) within 6 weeks
    b. Requirement for continual immune suppression with immunosuppressive drugs (e.g., subjects with organ allografts)
5. Major surgery within 4 weeks of study treatment, or major surgery planned for duration of study participation
6. An active, second potentially life-threatening cancer, adequately treated basal cell or squamous cell skin cancer or carcinoma in situ of the cervix, low grade thyroid is permitted
7. Presence of brain or subdural metastases, unless local therapy has been completed and corticosteroids have been discontinued for this indication for ≥ 4 weeks before starting study treatment:
    a. Any signs (e.g., radiologic) and/or symptoms of brain metastases must be stable for ≥ 4 weeks before starting study treatment
    b. Radiographic stability should be determined by comparing contrast-enhanced CT or magnetic resonance imaging (MRI) scans at screening to scans obtained by the same method at least 4 weeks earlier

    NOTE: Screening for brain lesions is not required for all potential subjects; however, if there are any neurological signs or symptoms consistent with brain metastases, then a brain CT or MRI should be performed as clinically indicated.
8. Presence or documented history of any of the following autoimmune conditions:
    a. Inflammatory bowel disease

   b. Rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus, autoimmune vasculitis (e.g., Wegener's granulomatosis)
   c. Motor neuropathy considered of autoimmune origin (e.g., Guillain-Barre Syndrome)

9. Presence of meningeal carcinomatosis
10. Use of any medications that induce, inhibit, or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of investigational product without consultation of the medical monitor
11. History or evidence of cardiac disease as indicated by any of the following:
   a. Congestive heart failure greater than New York Heart Association (NYHA) Class II (see protocol Appendix 16.4)
   b. Unstable angina (anginal symptoms at rest), or new-onset angina (begun within the last 3 months), or myocardial infarction within the 6 months prior to enrollment
   c. Cardiac ventricular arrhythmias requiring anti-arrhythmic therapy
   d. Congenital long QT syndrome or taking drugs known to prolong the QT interval
12. Current use of any drugs with a known risk of causing torsades de pointes
13. Evidence or history of thromboembolic, venous, or arterial events such as a cerebrovascular accident including transient ischemic attacks within the past 3 months
14. Evidence or history of bleeding diathesis or coagulopathy at the time of screening
15. International normalized ration (INR) and activated partial thromboplastin time (aPTT) >1.5 × ULN, in subject who is not therapeutically anticoagulated. Subjects who are being therapeutically anticoagulated with an agent such as Coumadin (warfarin sodium) or subcutaneous heparin may be included provided there is not prior evidence of underlying abnormality in coagulation parameters, the screening test results are in an appropriate therapeutic range, and the anticoagulation regimen is stable and closely monitored
16. History of malabsorption syndrome or other condition that would interfere with enteral absorption
17. Presence of active clinically serious infection (Grade 3 or higher by National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE v.4.03)
18. Diagnosis of infection with human immunodeficiency virus (HIV) or chronic infection with hepatitis B or C virus
19. Any other unstable or clinically significant concurrent medical condition that would in the opinion of the investigator or medical monitor jeopardize the safety of a subject and/or their compliance with the protocol
20. Pregnant or breast-feeding

21. Use of any investigational, non-United States Food and Drug Administration (US FDA) approved drug within 28 days or 5 half-lives, whichever is longer, preceding screening
22. Current participation in any other clinical trial without consultation with the medical monitor
23. Presence of any condition which, in the investigator or medical monitor's opinion, makes the patient unsuitable for the study participation

4.3 Subject Enrollment

Forty subjects may be enrolled, including up to 8 subjects (20% of overall cohort) with HER2+ breast cancer.

4.4 Withdrawal of Subjects from Study Treatment and/or Study

The Sponsor may terminate this study at any time. The investigator and/or the subject have the right to terminate the subject's participation in the study at any time.

A subject **may** discontinue study treatment prematurely for any of the following reasons:

- The investigator determines further participation is not in subject's best interest (for example, subject experiences rapid clinical deterioration in the absence of confirmed disease progression)
- Subject has confirmed disease progression

A subject **MUST** discontinue study treatment in the event of any of the following:

- Subject request to end study treatment
- Any treatment-related AE(s) that meet withdrawal criteria
- Substantial noncompliance with study requirements
- Confirmed positive pregnancy test
- Any intercurrent illness that would, in the judgement of the investigator or Sponsor's medical monitor, affect assessments of clinical status to a significant degree and require discontinuation of protocol therapy

A subject **MUST** be withdrawn from the study in the event of any of the following:

- Withdrawal of informed consent for the study
- The Ad-RTS-hIL-12 injection was not received

NOTE: Any subject receiving veledimex will be included in the safety analysis group even if the Ad-RTS-hIL-12 injection was not received.

Every effort should be made to follow subjects who withdraw from study treatment for ongoing treatment-related AEs until resolved, no resolution is expected, or the study has ended.

4.5 Replacement of Subjects

Subjects who withdraw from the study during the screening period and prior to starting Ad-RTS-hIL-12 immunotherapy, or those who discontinue treatment at any time during the initial 7 days of veledimex dosing for reasons other than toxicity or disease progression may be replaced. All dosed subjects will be included in the overall safety assessment.

4.6 Premature Termination of Study

The Sponsor has the right to close the study at any time, although this should occur only after mutual consultation between the Sponsor and the investigators. The Institutional Review Board (IRB)/Independent Ethics Committee (IEC) must be informed of such action. Should the study/center be closed prematurely, all study materials (completed, partially completed, and blank case report forms, study medication, etc.) must be stored or disposed of according to the Sponsor's instructions. Events that may trigger premature termination of the study or closure of a center include, but are not limited to the following: new toxicity findings; decision to re-challenge patient who has experienced a Grade 4 event; interim analysis results; noncompliance with the protocol; changes in the development plans for the investigational product; slow recruitment; and poor-quality data.

5 INVESTIGATIONAL PRODUCT

The investigational product has two components: [redacted]-human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand). Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter activated in the presence of the activator ligand, veledimex. Veledimex is a small molecule, RTS-specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the optimal veledimex dose and schedule.

[redacted].

5.1 Preparation of Ad-RTS-hIL-12

Ad-RTS-hIL-12 will be supplied in single-dose vials. [redacted]

5.2 Preparation of Veledimex

Sponsor will provide veledimex capsules to be dispensed by the study site pharmacy to subjects for oral administration. [redacted]

5.3 Handling and Storage

Investigational product must be stored in a restricted access area under the storage conditions indicated in the Investigator's Brochure [redacted] All necessary precautions while handling potentially toxic compounds must be strictly followed.

5.4 Monitoring of Subject Adherence and Managing Missed Veledimex Doses

The first veledimex dose will be given in the clinic 3 hours ± 3 hours before Ad-RTS-hIL-12 injection is expected under careful medical supervision by the clinic staff to ensure that the subject does not have difficulty swallowing the capsules. Thereafter, subjects may be allowed to self-administer the remaining once daily doses as described in Section 6.2. Subjects are to be instructed to take the appropriate number of capsules in the same way for each of the remaining treatment period days. All daily capsules must be taken together on the same day and within a few minutes.

Subjects should NOT make up any missed doses. If any dose is missed, a subject should take the next dose as scheduled on the next day.

Each subject will be carefully monitored for possible local reactions at the injection site and/or hypersensitivity reactions for at least 2 hours following the Ad-RTS-hIL-12 injection. The subject should be instructed to call the clinical site if any such reactions develop or do not resolve within 24 to 48 hours.

Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate last meal prior to administration of veledimex, the number of capsules taken, whether subject missed any veledimex doses and the study day and reason for any missed doses. Investigational product container(s) with any remaining capsules should be returned to the study staff on Day 8, so that staff can properly assess dose compliance.

5.5 Disposition of Unused Drug

All unused investigational product should be destroyed at the study site in accordance with standard institutional practice and in accordance with US Occupational Safety and Health Administration procedures, after full accountability has been documented. Any investigational product destruction at study site must be documented and the records maintained in the investigator's study file.

5.6 Accountability and Dispensation

The investigator must maintain accurate records accounting for the receipt and dispensation of investigational product. The investigational materials are to be prescribed only by the investigator or the sub-investigators named on the United States Food and Drug Administration (US FDA) Form 1572, and may only be dispensed by authorized personnel at the institution(s) listed therein. Under no circumstances will the investigator allow the investigational drug(s) to be used for purposes or in patients other than as directed by the protocol.

6 TREATMENT PLAN

6.1 Ad-RTS-hIL-12 Dosage and Administration Procedures

The 0.5 mL dose of Ad-RTS-hIL-12 [redacted] must be injected into the same lesion and should be delivered by multiple injections with a fine needle (no finer than 27 gauge) directly into each quadrant of the lesion. Attention must be paid to adequately infiltrate the circumference of the tumor margins. Should the tumor selected for injection not support the entire Ad-RTS-hIL-12 injection volume (approximately 0.5 cc), another tumor should be injected with the remaining volume to ensure that all subjects receive [redacted] If another tumor is not available, then the remaining volume should be injected into a draining, pathologic lymph node of the injected tumor if possible. Lesions displaying signs of localized infection should not be injected.

Note: In the event that Ad-RTS-hIL-12 injection is not performed, subject will not continue in the study. The reason why injection was not given will be documented and the subject will be withdrawn from the study.

Administration of prophylactic antipyretics is strongly recommended during the first week after Ad-RTS-hIL-12 injection (see Section 6.9 and Appendix 16.2). See note in Section 6.2 concerning importance of proper hydration during treatment with Ad-RTS-hIL-12 immunotherapy.

6.2 Veledimex Dosage and Administration

Veledimex will be supplied by the Sponsor as gelatin capsules for oral administration. Subjects will receive 80 mg of veledimex on days 1 through 7. On day 1, veledimex will be administered in a fasting state 3 hours ± 3 hours before the tumor biopsy and intratumoral injection of Ad-RTS-hIL-12. On days 2 through 7, subjects will be directed to take the veledimex in a fed state (preferably within 30 minutes following a normal meal), once daily, and at the same time (± 1 hour) each day. All daily capsules must be taken together on the same day and within a few minutes. Subjects should NOT make up any missed doses. If any dose is missed, a subject should take the next dose as scheduled on the next day.

Note: Subjects should be carefully monitored for possible local reactions and/or hypersensitivity reactions, according to standard practice. [redacted]

Administration of prophylactic antipyretics is strongly recommended during the first week after Ad-RTS-hIL-12 injection (see Section 6.9 and Appendix 16.2).

6.3 Dose Escalation

Not applicable.

6.4 Modification of Dose Schedule

Subjects will receive only one cycle of Ad-RTS-hIL-12 immunotherapy. If a dose of veledimex (up to 2 doses) is held for related toxicity, the treatment related AEs must have resolved to Grade 2 or less for dosing to resume. The held doses will not be made up; the patient will complete dosing according to the remaining schedule.

If a patient misses a dose, the patient is allowed to resume dosing at the next scheduled dose, but must complete dosing according to the remaining schedule. The missed dose will not be made up.

6.5 Dose-Limiting Toxicity

Not applicable.

6.6 Maximum Tolerated Dose

Not applicable.

6.7 Dose Modifications and Dose Delays

Please refer to Section 6.4.

Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt/discontinue further dosing at the discretion of the investigator. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor medical monitor.

6.8 Severity Grading and Management of Local Reactions

Injection of agents into tissue carries a potential risk of local reactions that may be characterized as intense immunologic reaction at or near the injection site. Local reactions will be graded according to the NCI CTCAE v.4.03 criteria.

As with all signs and symptoms, events should be recorded and graded as AEs according to NCI CTCAE v.4.03 criteria. Study stopping rules will not apply to a specific event if it is clearly unrelated to the Ad-RTS-hIL-12 injection.

6.9 Prophylactic Antipyretic and/or Analgesic Administration

The use of antipyretics and/or analgesics is allowed as a prophylactic measure. Antipyretics and/or analgesics can be used anytime during study treatment, as indicated and required for patient safety and must be recorded as concomitant medications. Please refer to exclusion criteria for acute clinically significant and/or chronic infections.

NOTE: Since fever and flu-like symptoms (e.g., fever, headache, chills, dehydration, etc.) are commonly experienced following adenoviral vector administration, it is highly recommended that subjects be treated with prophylactic, antipyretic and/or analgesic medication prior to Ad-RTS-hIL-12 injection and during the first week after injection.

Please refer to Appendix 16.2 for the recommended regimen for the prophylactic administration of antipyretics and/or analgesics.

7 CONCOMITANT THERAPY

Information on concomitant medications, including all medications, blood products, vitamins, and other supplements, will be collected from the Screening visit through the end of the Initial Follow-up Period (Week 12). Any new anti-cancer therapy that is started prior to confirmation of a radiographic response of PD, should be collected and entered as a concomitant medication.

7.1 Permitted Medications

- HER-directed antibody therapies in patients with HER2+ disease
- Subjects may receive standard treatments, including palliative and supportive care for any illness or symptom management during study treatment.
- Anti-pyretics, analgesics, NSAIDs; Antidiarrheal therapy is permitted for investigational product-induced diarrhea.
- Anti-emetics, with the exception of CYP450 3A4 inducers, inhibitors and substrates, are permitted for investigational product-induced nausea and vomiting.

7.2 Prohibited Medications

- Any other investigational agent or anticancer therapy (chemotherapy, radiotherapy, including corticosteroids, etc.) while receiving study treatment.

NOTE: Care should be given when prescribing medications that are classified as CYP450 3A4 inducers, inhibitors, or substrates due to potential interactions with the investigational product. In the event that one is prescribed, consultation with the medical monitor is advised. All medications should be recorded in the case report form (CRF) as indicated in the CRF completion guidelines.

8 STUDY PROCEDURES

8.1 Written Informed Consent

The provided written informed consent form (ICF) must be signed before any protocol specific procedures and assessments can be performed. A copy of the signed ICF will be given to the subject and a copy should be filed in the medical record. The original ICF should be kept on file with the study reports. Standard of care evaluations performed as part of the subject's routine treatment prior to signing the ICF can be used if they were conducted within the timeframe of the screening period. Refer to Section 13.8 for further information.

8.2 Subject Registration

Centralized registration of subjects will be completed according to a process defined by the Sponsor. Eligible subjects are to be enrolled and assigned a unique study identification number before the planned intratumoral Ad-RTS-hIL-12 injection. Once assigned, a subject's identification number will not be reused.

8.3 Description of Procedures and Observations

Screening assessments must be performed within 28 days prior to the Ad-RTS-hIL-12 injection. Any screening tests, exams or procedures outside of this range may be repeated at the investigator's discretion. All study visits must be completed as described in the protocol while subjects are taking veledimex capsules.

8.3.1 Study Tests, Exams and Procedures

8.3.1.1 Demographics, Medical and Cancer History, and Concomitant Medications

Each subject's complete medical history will be documented during screening, including demographic information, relevant medical history, current primary cancer diagnosis, and prior cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries). In addition, concomitant medications, including blood products, vitamins, and other supplements received during the screening period (28 days) prior to initiating study treatment will be recorded. Concomitant medications will continue to be collected through the Initial Follow-up Period (Week 12).

8.3.1.2 Physical Examinations

A physical examination (PE) will include examination of the subject's general appearance, and the following organ systems: heart, lung, head and neck, abdomen, skin, and extremities.

8.3.1.3 Vital Signs, Height and Weight

Vital signs will include blood pressure, pulse rate, temperature, and respiration rate. Subject's blood pressure is to be monitored closely, [redacted] for 72 hours after administration of Ad-RTS-hIL-12. Assessment of vital signs is required prior to injection of Ad-RTS-hIL-12, and prior to veledimex dosing on day 1. The assessment of vital signs on day 3 and 7 should be performed prior to administration of veledimex.

Height and weight will be measured and recorded according to Schedule of Study Procedures.

8.3.1.4 ECOG Performance Status

See Appendix 16.1. Subjects will need to have ECOG performance status of 0 or 1 to be eligible for the study.

8.3.1.5 Pregnancy Testing

Females of childbearing potential will have a serum pregnancy test at the screening visit within 14 days of dosing and a urine pregnancy test at Day 1. The pregnancy test must be negative prior to administration of veledimex and injection of Ad-RTS-hIL-12. A serum pregnancy test should be completed on Day 1 if the urine pregnancy test is positive.

8.3.1.6 Monitoring of Adverse Events

Monitoring and recording of AEs and SAEs will be conducted throughout the study. AEs and SAEs from the signing of informed consent until the end of week 6 will be collected and entered into the case report forms (CRFs). After Week 6, only SAEs suspected of being related to Ad-RTS-hIL12 immunotherapy will be collected.

Adverse events will be recorded on the CRFs using NCI CTCAE v.4.03 criteria. MedDRA will be used for evaluating AEs. Definitions, documentation, and reporting procedures for adverse events are described in detail in Section 10.

8.3.1.7 Clinical Laboratory Assessments

The hematology panel comprises a complete blood count (CBC), including white blood cell (WBC) count with differential, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices (MCV, MCH, MCHC), and platelet count.

The serum chemistry panel comprises the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, sodium, potassium, chloride, calcium, phosphorus, and bicarbonate (as CO_2).

The coagulation panel includes aPTT and the INR.

The urinalysis panel (dipstick) includes appearance, pH, specific gravity, glucose, protein/albumin, presence of blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated.

8.3.1.8 Imaging Studies

Subjects must consent to allow ZIOPHARM to collect their scan and tumor measurements that were taken a) prior to starting the pre-study standard chemotherapy, and b) prior to study entry, i.e., at the end of a minimum of 12 weeks of pre-study chemotherapy. Tumor response imaging for the pre-study standard chemotherapy must be completed within 4 weeks of study enrollment and must meet the criteria listed below in order to be used as screening/baseline scans. In the event that the scans are not adequate, they should be repeated.

i. Computed tomography (CT) of the chest, abdomen +/- pelvis
ii. PET or Bone Scintigraphy (BS) at the treating physician's discretion
iii. MRI or CT of the brain, if brain metastasis are known or suspected
iv. MRI of other anatomical regions as clinically indicated

All imaging should be of diagnostic quality and include intravenous (IV) contrast. Please refer to Appendix 16.3 for RECIST v 1.1 guidelines. NOTE: In the event that IV contrast cannot be used, the medical monitor should be consulted.

The same imaging method used for screening/baseline scans should be used for the tumor assessment performed at 6 and 12 weeks for the primary assessment of efficacy. Scans should be available for collection upon Sponsor request.

8.3.1.9 Electrocardiogram

A standard 12-lead ECG for evaluation of the QT/QTc interval will be performed at screening.

8.4 List of Assessments by Study Period

8.4.1 Screening

The screening exams, tests and procedures must be done within 28 days of Ad-RTS-hIL-12 injection.

- Signed Informed Consent Form
- Medical/Cancer History, including history of prior treatments and any associated residual toxicity
- Physical Examination
- ECOG performance status
- Height and weight
- Vital signs
- Adverse events
- Concomitant medications, i.e., medications taken within 28 days of Ad-RTS-hIL-12 injection
- Serum pregnancy test
- Hematology panel: CBC, WBC count with differential, RBC count, hematocrit, hemoglobin, red blood cell indices (MCV, MCG, MCHC), and platelet count
- Coagulation panel: aPTT and INR
- Serum chemistry panel: AST, ALT, LDH, ALP, creatinine, total bilirubin, total protein, albumin, BUN, glucose, sodium, potassium, chloride, calcium, phosphorus, and bicarbonate (as CO2)
- Screening hepatitis B, hepatitis C, and HIV serology in subjects without documented negative results, or in subjects with known risk factors for infection
- Baseline TSH
- Tumor markers: CEA and Ca 15-3
- Research bloods: for cytokines and immune cell activation by flow cytometry
- Urinalysis panel: appearance, pH, specific gravity, glucose, protein/albumin, presence of blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated
- ECG at screening

- Receipt of copies of tumor measurements and scans taken a) before the start of the pre-study standard chemotherapy and b) at after a minimum of 12 weeks of the pre-study chemotherapy. Tumor response imaging must be completed within 4 weeks of study enrollment.

8.4.2 Immunotherapy Cycle: Day 1 (Ad-RTS-hIL-12 injection)

- Physical examination (symptom-directed)
- ECOG performance status
- Weight
- Vital signs
- Adverse events
- Concomitant medications
- Hematology panel
- Research bloods
- Urinalysis
- Urine pregnancy test
- Tumor biopsy (prior to injection of Ad-RTS-hIL-12)
- Oral veledimex 3 hours ± 3 hours before injection of Ad-RTS-hIL-12
- Ad-RTS-hIL-12 injection
- Veledimex dose compliance/subject diary
- Veledimex dispensed for the following 6 days (the first dose is to be given in clinic)

8.4.3 Immunotherapy Cycle: Day 2

- Oral veledimex
- Veledimex dose compliance/subject diary

8.4.4 Immunotherapy Cycle: Day 3

- ECOG performance status
- Vital signs
- Physical examination (symptom-directed)
- Adverse events
- Concomitant medications
- Hematology panel
- Research bloods
- Oral veledimex
- Veledimex dose compliance/subject diary

8.4.5 Immunotherapy Cycle: Day 4-7

- Oral veledimex
- Veledimex dose compliance/subject diary

8.4.6 Immunotherapy Cycle: Day 8

- ECOG performance status
- Vital signs
- Physical examination (symptom-directed)
- Adverse events
- Concomitant medications
- Hematology panel
- Coagulation panel
- Serum chemistry panel
- Research bloods
- Collect veledimex dose compliance/subject diary

8.4.7 Immunotherapy Cycle: Day 21

- Adverse events
- Concomitant medications

8.4.8 Initial Follow-Up Period: Week 6

- ECOG performance status
- Vital signs
- Physical examination
- Adverse events
- Weight
- Concomitant medications
- Hematology panel
- Serum chemistry panel
- Research bloods
- Tumor markers
- Tumor Biopsy
- Imaging studies for tumor response. If radiographic PD is detected at 6 weeks, PD must be confirmed with repeat imaging 4 weeks later. In case of slow tumor growth, the investigator may wait until confirmation of the PD at 12 weeks to resume chemotherapy. In case of unequivocal progression, the investigator may resume chemotherapy prior to 12 weeks and the subject will be considered as treatment failure at 12 weeks.

8.4.9 Initial Follow-Up Period: Week 12

- ECOG performance status
- Vital signs
- Physical examination
- Adverse events
- Weight
- Concomitant medications (including any new anti-cancer therapies)

- Hematology panel
- Serum chemistry panel
- Research bloods
- Tumor markers
- Imaging studies for tumor response

8.4.10 Long-Term Follow-Up Period: Week 18

- Imaging studies for tumor response

8.4.11 Long-Term Follow-Up Period: Week 24

- Imaging studies for tumor response

8.4.12 Long-Term Follow-Up Period: Week 36

- Imaging studies for tumor response

8.4.13 Long-Term Follow-Up Period: Week 48

- Imaging studies for tumor response

9 TUMOR RESPONSE ASSESSMENTS

Tumor response is based on the RECIST v.1.1. A copy of the full article is provided as Appendix 16.3. Key elements of the criteria are summarized below. Tumor response will also be assessed by Immune-Related Response Criteria (irRC) (Wolchok et al, 2009).

9.1 Measurability of Tumor at Baseline

RECIST v.1.1 provides the following definitions for designating the tumor lesion/lymph node as measurable or non-measurable at baseline.

9.1.1 Measurable Lesions

9.1.1.1 Tumor Lesions

Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size as follows:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II of Eisenhauer et al, 2009 for imaging guidance)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with caliper should be recorded as non-measurable)
- 20 mm by chest X-ray

9.1.1.2 Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. For additional information on lymph node measurement, see notes in Eisenhauer et al, 2009 under the heading "Baseline documentation of target and non-target lesions," as well as a companion paper on lymph node assessment using RECIST (Schwartz et al, 2009).

9.1.2 Non-measurable Lesions

Non-measurable lesions are all other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Truly non-measurable lesions include leptomeningeal disease, ascites, pleural or pericardial effusions, inflammatory breast disease, lymphangitic involvement of skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measureable by reproducible imaging techniques.

9.1.3 Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment.

9.1.3.1 Bone Lesions

Bone scan, PET scan, or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components,* that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

Blastic bone lesions are non-measurable.

9.1.3.2 Cystic Lesions

Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (i.e., neither measurable nor non-measurable) since they are, by definition, simple cysts.

Cystic lesions thought to represent cystic metastases can be considered as measurable lesions if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same individual, these are preferred for selection as target lesions.

9.1.3.3 Lesions with Prior Local Treatment

Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

9.1.4 Methods of Tumor Measurement

Please refer to Eisenhauer et al, 2009, Section 3.2 for details concerning details of how measurements of lesions/lymph nodes should be done. For the current study, CT is the only acceptable measurement method.

9.2 Tumor Response Evaluation

9.2.1 Designation of Target and Non-Target Lesions

When more than one measurable lesion is present at baseline, all lesions, up to a maximum of 5 lesions (and a maximum of 2 lesions per involved organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. In the current study, the baseline measurement will be based on the scans and measurements taken at the end of the pre-study standard chemotherapy, as submitted by the investigator.

Study entry requires the presence of at least 2 measurable lesions at baseline, one of which will be injected with Ad-RTS-hIL-12 and the other of which will be followed for response. Per RECIST v.1.1 (see Appendix 16.3), the injected lesion must be considered a non-target lesion. Remaining measurable lesions should be designated as target or non-target lesions as described above.

Target lesions should be selected on the basis of their size (lesions with longest diameter) and be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that on occasion, the largest lesion does not lend itself to reproducible measurement; in such cases, the next largest lesion that can be reproducibly measured should be selected.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as the reference to characterize any objective changes in the measurable dimension of disease.

All other lesions or sites of disease (i.e., non-measurable lesions), including pathological lymph nodes, should be identified as non-target lesions. Measurements of non-target lesions, even if measurable, are not required, as they are intended to be assessed qualitatively and should be designated as present or absent at baseline. It is also possible to record multiple non-target lesions in the same organ as a single item, e.g., “multiple enlarged pelvic lymph nodes.”

9.2.2 Definitions of Response

9.2.2.1 Target Lesions

Definitions of response for individual patients at each assessment time point for target lesions are shown in Table 9.2.2.1-1.

Table 9.2.2.1-1 Evaluation of Response in Target Lesions

Response Category	Definition
CR	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
PR	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
PD	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression). *For this study, PD should be confirmed at least 4 weeks later.*
SD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

Abbreviations: CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease

9.2.2.1.1 Lymph Nodes as Target Lesions

Please refer to RECIST v.1.1 in Appendix 16.3 for special notes on the assessment of lymph nodes as target lesions, lesions that become too small to measure, and lesions that split or coalesce after treatment.

9.2.2.2 Non-target Lesions

Definitions of response for individual patients at each assessment time point for non-target lesions are shown in Table 9.2.2.2-1. Non-target lesions, even if measurable, are to be evaluated qualitatively, as noted above, and are to be designated as CR, non-CR/non-PD, or PD, as defined in Table 9.2.2.2-1.

Table 9.2.2.2-1 Evaluation of Response at Each Time Point in Non-target Lesions

Response Category	Definition
CR	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
Non-CR/Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
PD	Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).[a]

Abbreviations: CR, complete response; SD, stable disease; PD, progressive disease.

[a] Although a clear progression of "non-target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail.

9.2.2.2.1 Special Notes on Designation of Progression in Non-Target Disease

The concept of progression of non-target disease in the setting of concurrent measurable disease as in this study requires further explanation. In this setting, to assign unequivocal progression on the basis of the non-target disease (see determination of overall response in Section 9.2.2.5), there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in the target disease, the overall tumor burden has increased sufficiently to warrant discontinuation of therapy (see examples in the RECIST v.1.1 guidelines in Appendix 16.3). A modest increase in the size of one or more non-target lesions is usually not sufficient to qualify for a designation as unequivocal progression. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

9.2.2.3 New Lesions

The appearance of new malignant lesions denotes disease progression. Refer to RECIST v.1.1 in Appendix 16.3 for notes concerning the detection of new lesions.

9.2.2.4 Missing Data

When no imaging/measurement is done at all at a particular time point, the subject is not evaluable (NE) at that time point. If only a subset of lesion measurements is made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesions would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a subject had a baseline sum of 50 mm with three measured lesions and at the assessment only two lesions were assessed, but those gave a sum of 80 mm, the subject will have achieved PD status regardless of the contribution of the missing lesion measurement.

9.2.2.4.1 Additional Notes Relevant to Designations of Disease Progression

Per RECIST v.1.1, patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression should be reported as "symptomatic deterioration," and every effort should be made to document objective progression

even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response of such subjects is to be determined by evaluation of target and non-target disease as shown in Table 9.2.2.1-1 and Table 9.2.2.2-1. In the current study, scans are to be done at week 6 and 12 weeks, and at any time deemed appropriate by the investigator (i.e., when progression is suspected). PD at week 6 must be confirmed at least 4 weeks later. The definition of progression rate for this study, is given in Section 12.4.3.2.

9.2.2.5 Overall Response for Each Subject at Each Assessment Time Point

Overall response at each assessment time point is based on the totality of the responses for target and non-target lesions, as shown in Table 9.2.2.5-1.

Table 9.2.2.5-1 Rubric for Determination of Overall Response at Each Assessment Time Point for Each Patient

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

9.2.2.6 Additional Information Concerning RECIST v1.1

Full details concerning the use of RECIST v.1.1 may be found in the full article by Eisenhauer et, al (2009), which is attached as Appendix 16.3.

9.2.2.7 Immune Related Response Criteria (irRC)

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions ($\geq 5 \times 5$ mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden:

Tumor Burden = $SPD_{index\ lesions} + SPD_{new,\ measurable\ lesions}$

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening).

Overall response using the irRC: The overall response according to the irRCis derived from time-point response assessments (based on tumor burden) as follows:

- irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented
- irPR, decrease in tumor burden ≥50% relative to baseline confirmed by a consecutive assessment at least 4 weeks after first documentation
- irSD, not meeting criteria for irCR or irPR, in absence of irPD
- irPD, increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented

10 SAFETY ASSESSMENTS

10.1 Adverse Events (AEs) and Definitions

Adverse Event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship to the treatment. An AE can therefore be any unfavorable and unintended sign including an abnormal laboratory finding, symptom, or disease temporarily associated with the use of a medicinal product, and any worsening of pre-existing conditions, regardless of causality to the medicinal product.

Suspected Adverse Reaction: Any AE for which there is evidence to suggest a causal relationship (reasonable possibility) between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Adverse Reaction: Any AE caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Unexpected Adverse Reaction: Any AE that is (a) not listed in the Investigator's Brochure, (b) not listed with the specificity and severity that is being observed, (c) not consistent with the risk information described in the general investigational plan or elsewhere in the current application (in the absence of an investigator brochure), and (d) listed as occurring with a class of drugs, but not specifically mentioned as occurring with the particular drug under investigation.

Adverse events may be treatment-emergent (i.e., occurring after initial receipt of investigational product) or nontreatment-emergent. A nontreatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the subject has received investigational product.

10.2 Evaluation of Adverse Events

Adverse events include:

- Suspected adverse drug reactions
- Reactions from investigational product overdose, abuse, withdrawal, sensitivity, or toxicity
- Significant changes or abnormalities when compared to baseline, in signs, symptoms, clinical laboratory results, or physiological testing. This includes any worsening of a pre-existing condition temporally associated with the use of investigational product.
- Other untoward medical events, regardless of their relationship to the investigational product, such as injury, events that require surgery, accidents, extensions of symptoms, or apparently unrelated illnesses

The following items should be considered when identifying an AE:

- Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered AEs.
- In the event that a constellation of symptoms results in a confirmed diagnosis, the diagnosis (not the symptoms) should be recorded as the AE term.
- If a diagnosis cannot be established, the symptoms should be recorded as the AE(s).
- If an ongoing symptom has been included in the medical history, an associated severity grade and frequency should also be documented so that a worsening in severity or frequency of a symptom can be readily identified as an AE.
- Progression of disease is not itself an AE; however, the presenting sign or symptom of the disease progression should be documented as an AE (e.g. pleural effusion). *Exception:* If a subject experiences progression of disease that results in death, 'progression of disease' may be reported as an SAE if, by medical opinion, the term best describes the cause of death.

10.3 Determination of Seriousness

10.3.1 Serious Adverse Event (SAE)

An AE is considered an SAE if any of the following conditions applies:

- Death: An AE that results in death during the active study period or within 30 days following investigational product administration. In addition, a reported death at any time post-study that is thought to be related to investigational product administration;
- Life-threatening adverse event: An AE that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e. this does not include a reaction that, had it occurred in a more severe form, might have caused death);
- Permanent, persistent, or significant disability: A disability is defined as any substantial disruption of a person's ability to conduct normal life functions;
- Inpatient hospitalization or prolongation of existing hospitalization: In general, hospitalization refers to admission of a subject into a hospital for at least a 24-hour stay.

Hospitalizations for routine blood transfusions, hospitalization for an elective or diagnostic procedure, or surgery for a pre-existing condition that has not worsened, are not considered SAEs. (Emergency room visits that do not result with admission are not considered as SAEs);

- A congenital anomaly/birth defect in the offspring of a subject: A fixed, permanent impairment established at or before birth;
- An important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above. Medical judgment should be used to decide if an adverse event should be considered an important medical event. Examples of medically important events are intensive treatment for allergic bronchospasm or convulsions that do not result in hospitalizations
- New cancer: Occurrence or diagnosis of a new cancer during the trial is considered an SAE. (This does not pertain to metastasis of current disease);
- Any AE associated with an overdose of the investigational product: An overdose of investigational product is defined as an occurrence of administered dose exceeding that which is prescribed by the investigator per protocol.

10.3.2 Non-Serious Adverse Event

An AE that does not fulfill the criteria for a SAE is classified as a non-serious AE. See Section 10.1.

10.4 Determination of Severity

Assessment of severity is one of the responsibilities of the investigator in the evaluation of AEs and SAEs. The severity of AEs will be assessed according to the NCI CTCAE, v.4.03, which will be provided to the site as a separate document. If the AE is not defined in the NCI CTCAE, v.4.03, the investigator will determine the severity of an AE based on the following definitions:

- Mild (Grade 1): The AE is noticeable to the subject, but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the investigational product.
- Moderate (Grade 2): The AE interferes with routine activity, but responds to symptomatic therapy or rest. The AE may require reducing the dose, but not discontinuing administration of the investigational product.
- Severe (Grade 3): The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. In addition, the AE leads to discontinuing administration or reducing the dose of the investigational product.
- Life Threatening (Grade 4): The AE requires discontinuing administration of the investigational product. The subject is at immediate risk of death.
- Death (Grade 5): The subject dies as a direct result of the complication or condition

10.5 Determination of Causality

The investigator is required to provide an assessment of relationship of AEs and SAEs to the investigational product. A number of factors should be considered in making this assessment including:

- Alternative possible causes of the AE, including the subject's underlying disease or comorbid conditions, other drugs, other host, and environmental factors
- The temporal sequence between the exposure to investigational product and the AE
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the investigational product or similar drugs
- Whether the AE resolved or improved with decreasing the dose or stopping the investigational product (i.e., dechallenge); or recurred or worsened with re-exposure to the drug (i.e., rechallenge).

Relationship assessments that indicate *"Not Related"* to investigational product:

- None: The event is related to an etiology other than the investigational product (the alternative etiology must be documented in the study subject's medical record and/or SAE form).
- Unlikely or Remote: The event is unlikely to be related to the investigational product and likely to be related to factors other than investigational product.

Relationship assessments that indicate *"Related"* to investigational product:

- Possible: There is an association between the event and the administration of the investigational product and there is a plausible mechanism for the event to be related to investigational product; but there may also be alternative etiology, such as characteristics of the subject's clinical status or underlying disease.
- Probable: There is an association between the event and the administration of investigational product, a plausible mechanism for the event to be related to the investigational product and the event could not be reasonably explained by known characteristics of the subject's clinical status or an alternative etiology is not apparent.
- Definite: There is an association between the event and the administration of investigational product, a plausible mechanism for the event to be related to the investigational product and causes other than the investigational product have been ruled out and/or the event re-appeared on re-exposure to the investigational product.

For AEs that occur prior to the administration of investigational product, an assessment of protocol relatedness must be made. AEs may occur as a result of procedures required during the screening process (e.g., blood collection, washout of an existing medication) prior to the initial administration of investigational product. For AEs that occur before administration of investigational product, only those that are assessed by the investigator as protocol-related should be reported to the Sponsor. The following guidelines should be used by investigators to assess the relationship of an AE to a protocol-required procedure:

- Protocol related: The event occurred due to a procedure/intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.
- Not protocol related: The event is related to an etiology other than the study procedure (the alternative etiology must be documented in the study subject's medical record).

10.6 Documenting Adverse Events

All AEs (including SAEs) are to be accurately recorded on the Adverse Event page of the subject's electronic CRF from the time the subject signs the informed consent through the Follow-up Period. Each event will be assessed for serious criteria, severity, and causality (see Section 10.5). The date of onset, as well as the duration of the event will be recorded. In addition, treatments provided to the subject, actions taken with the investigational product, and the outcome of the AE will also be noted.

10.7 Reporting Serious Adverse Events

Time Frame for Reporting

All SAEs must be reported to the Sponsor or Sponsor designee within 24 hours of awareness, regardless of initiation of new anticancer therapy including the following:

- Any death or SAE experienced by the subject from the signing of informed consent to 30 days after the last dose of investigational product, regardless of relationship to investigational product.
- Any death or SAE that the Investigator becomes aware of, and believes to be investigational product related, that occurs more than 30 days after the subject last received investigational product.

All and SAEs must be reported to the following fax line within 24 hours of awareness:

[redacted]

Additional data concerning the SAE (eg diagnostic test reports, hospital summaries, etc.) must be promptly reported (within 24 hours of receipt) to the Sponsor or Sponsor's designee, until resolution of the SAE. Should the FDA or National Regulatory Authorities require that the Sponsor submit additional data on the event, the investigator will be asked to provide those data to the Sponsor in a timely fashion.

Information to be provided by the Investigator

Within 24 hours of becoming aware of the SAE or subject death, the investigator must notify the Sponsor or designee and transmit information to the Sponsor or designee. Information (initial and follow-up) should be provided on an electronic and/or paper SAE Report form signed and

dated by the investigator. The SAE Report form and copies of source documents (with subject identifiers redacted) will be transmitted by fax. A hospital discharge summary should be provided if the subject was hospitalized. An SAE report will be considered final once all relevant information has been received and reviewed by the Sponsor.

The SAE Report form is provided in the investigator study files. Please refer to the investigator study files for instructions on how to complete this form. The investigator will provide all of the following information related to the event:

- Investigator identification
- Subject identification (e.g., subject number, initials, sex, age or date of birth)
- Information regarding investigational product administration (e.g. start/stop date, dose, and frequency)
- Cycle and day of SAE occurrence documentation on SAE report forms
- Description of event
- Action taken with the investigational product in relation to the SAE
- Outcome of the SAE

In addition to the above information, the investigator must provide, for each event term, an assessment of:

- Severity/intensity
- Relationship to the investigational product (causality assessment)

10.8 Sponsor and Investigator Responsibility for Reporting Adverse Events

All AEs and SAEs will be reported to regulatory authorities, IRBs/IECs, and investigators in accordance with all applicable global laws and regulations. The investigator must submit all Safety Letters received from the Sponsor to his/her IRB/IEC per agreements and local requirements. The investigator must keep copies of all safety reports/letters, including correspondence with ZIOPHARM and the IRB/IEC, in the study file.

10.9 Follow-up Information for Adverse Events

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, per the investigator, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has resolved, stabilized, returned to baseline, or is otherwise explained by the investigator.

Required Follow-up for Adverse Events
All AEs and SAEs will be collected from the signing of the ICF through Week 6. After Week 6, only SAEs suspected of being related to investigational product will be collected. Follow up information for ongoing SAEs will be requested by the Sponsor until one of the following occurs:

- The event resolves
- The event returns to baseline, if a baseline value is available
- The event stabilizes (following consultation and agreement by the medical monitor)
- The event can be attributed to factors other than the investigational product or other than study procedure

10.10 Pregnancy

Subjects who become pregnant during the study should immediately discontinue treatment. The Sponsor must be immediately notified.

An initial Pregnancy Report Form and a Pregnancy Outcome Form are to be completed by the investigator or designee. The Pregnancy Report form and the completion guidelines will be provided in the investigator study files. Please refer to the investigator study files for details on how to complete these forms.

10.11 Overdose

Investigational product overdose of study subject, with or without associated AEs/SAEs, should be reported within 24 hours of awareness to Sponsor [redacted] All AEs and SAEs as a result of overdose should be reported as described previously in Section 10.6 and Section 10.7, respectively.

11 BIOLOGIC AND IMMUNE RESPONSE ASSESSMENTS

11.1 Blood Cytokine and Immune Function Assessments from Blood

Soluble factors, such as cytokines, chemokines, soluble receptors, and antibodies to tumor antigens will be characterized and quantified by immunoassays in blood (serum or plasma). Analyses may include, but are not necessarily limited to blood IL-12, IL-10, and IFN-γ. The proportion of specific lymphocyte subsets and expression levels of T cell co-stimulatory markers in peripheral blood mononuclear cell (PBMC) preparations will be quantified by flow cytometry. Analyses may include, but not necessarily be limited to, the proportion of T, B, and NK cells, proportion of memory and effector T cell subsets, and expression levels of PD-1, PDL1, ICOS, and Ki67.

11.2 Cytokine and Immunologic Markers Assessment from Tumor Tissue

Cytokines and immunologic markers will be examined in core biopsy (punch or FNA biopsy accepted in the absence of the core biopsy) taken on day 1 and at week 6. Biopsies will be by evaluated by immunocytochemistry for evidence of tumor infiltration by effector cells such as T cells and their subsets, as well as immune suppressor elements, such as T-regulatory cells and myeloid-derived suppressor cells, and other immunological markers. Antibodies to be used in the immunocytochemistry analysis may include, but are not limited to those against cluster of differentiation (CD) antigens CD3, CD4, CD8, CD25, FOX-P3, and CD56, and CD45RO.

12 STATISTICAL METHODS

12.1 Populations for Analysis

The following subject populations will be evaluated and used for presentation and analysis of the data:

- The safety population will comprise all subjects who have received either the injection of Ad-RTS-hIL-12 or any doses of veledimex
- The veledimex-treated population will comprise subjects who have received the injection of Ad-RTS-hIL-12 and at least one dose of veledimex
- The veledimex-evaluable population will comprise subjects who have received the injection of Ad-RTS-hIL-12 and all 7 doses of veledimex

12.2 Sample Size and Power Calculations

A maximum of 40 patients will be treated. This patient population will be heterogeneous and as such, it is difficult to define a threshold for a clear null and alternative hypothesis. This sample size will allow us to estimate an overall progression rate at 12 weeks with a maximum 95% exact confidence interval half-width of 0.16. This is based on an exact binomial calculation and is an estimate. However, analysis of progression-free survival will be conducted using Kaplan-Meier methods.

12.3 Endpoints

12.3.1 Primary Endpoint

- To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-, second-, or third-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first-, second-, or third-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by section 12.4.2.

12.3.2 Secondary Endpoints

- To estimate the progression rate at 12 weeks after the start of one cycle of Ad-RTS-hIL-12 immunotherapy.
- To evaluate the overall response rate (ORR), defined as the rate of complete response (CR) plus the rate of PR at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy.
- To evaluate the disease control rate (DCR), defined as the proportion of subjects who have a CR, PR, or SD at 12-weeks following the start of one cycle of Ad-RTS-hIL-12 immunotherapy
- To evaluate the number of subjects whose baseline tumor status (SD or PR) improves to PR or better at 12 weeks following the start of Ad-RTS-hIL-12 immunotherapy
- To compare radiographic responses by irRC with conventional reporting by RECIST

- To explore the impact of treatment on tumor and serum immune biomarkers

12.4 Analyses

12.4.1 Baseline Characteristics

Demographic and baseline disease characteristic data will be summarized. Data to be tabulated will include at least demographic features such as sex, age, and race, as well as disease-specific status and medical history.

Categorical data will be summarized using counts and percentages based on non-missing values. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented. Data will be summarized according to the defined populations for analysis.

12.4.2 Safety Analyses

Exposure to investigational product and reasons for discontinuation of study treatment will be tabulated. All treatment-emergent AEs will be coded according to the system organ class and preferred term using the Medical Dictionary for Regulatory Affairs (MedDRA®), and will be tabulated by number and percent of subjects, and according to relationship to the investigational product, severity, and seriousness. 'Treatment-emergent' is defined as any AE that occurs during or after administration of the first dose of investigational product through the evaluation period for safety defined above, regardless of relationship to investigational product; or any event that is present at baseline that worsens in intensity or is subsequently considered to be drug related by the investigator.

Deaths, SAEs, and AEs resulting in patient discontinuation will be listed. Laboratory parameters, vital signs, and physical examination data will be summarized by visit. Data listings will be presented by visit.

Toxicity stopping rules (Jennison and Turnbull, 2000; Ivanova et al, 2005), when applicable, will be determined based on a clinical assessment made by the SRC after a formal evaluation of safety data after every 5 subjects with HER2- disease have completed one cycle of Ad-RTS-hIL-12 immunotherapy. The SRC will review safety data separately for subjects with HER2+ disease to assess toxicity after the first 5 HER2+ subjects complete one cycle of Ad-RTS-hIL-12 immunotherapy.

The first stopping rule is based on related, treatment refractory Grade 3 or 4 adverse events. A rate of 50% is deemed unacceptable, a rate of 25% is deemed acceptable. The boundaries to stop the study are given as follows:

- If 4 of the first 5 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 6 of the first 10 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 8 of the first 15 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

- If 9 of the first 20 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 11 of the first 25 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 12 of the first 30 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 14 of the first 35 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.
- If 15 of the first 40 patients have related, treatment refractory Grade 3 or 4 AE, stop the study.

12.4.3 Efficacy Analyses

Efficacy will be based on the tumor response at 12 weeks.

12.4.3.1 Tumor response

Tumor response (CR, PR, SD, and PD) will be determined according to RECIST v.1.1 (see Section 9).

12.4.3.2 Progression rate

The proportion failed by 12 weeks is denoted as the progression rate survival and is derived based on the sum of progression events, death events and subjects who discontinue the trial due to an AE.

The second stopping rule is based on the progression rate during the 12 weeks of immunotherapy. A progression rate of 50% is deemed acceptable, while an 80% rate is deemed unacceptable. The boundaries to stop the study using a repeated significance testing approach are given as follows:

- If 5 of the first 5 patients progress, stop the study.
- If 9 of the first 10 patients progress, stop the study.
- If 12 of the first 15 patients progress, stop the study.
- If 15 of the first 20 patients progress, stop the study.
- If 18 of the first 25 patients progress, stop the study.
- If 20 of the first 30 patients progress, stop the study.
- If 23 of the first 35 patients progress, stop the study.
- If 26 of the first 40 patients progress, stop the study.

12.4.3.3 Progression-free Rate

Proportion of subjects who have 12-week progression free survival (proportion failed) after the start of Ad-RTS-hIL-12 immunotherapy.

12.4.3.4 ORR

The ORR is defined as the percentage of subjects who have CR or PR at 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy.

12.4.3.5 DCR

The disease control rate is the proportion of subjects who have a CR, PR, or SD at 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy.

12.4.3.6 Multi-Center Study

Not applicable. This is a single center study at the Memorial Sloan Kettering Cancer Center (MSKCC).

12.4.4 Adjustments for Covariates

No adjustments for covariates will be made.

12.4.5 Procedures for Handling Missing, Unused, and Spurious Data

No imputation of values for missing data will be performed. Standard clinical monitoring and data management practices will be used to ensure the integrity of data.

12.4.6 Interim Analysis

See pre-specified stopping rules in Section 12.4.2 and Section 12.4.3.2.

12.4.7 Procedures for Reporting Deviations to Original Statistical Analysis Plan

A formal statistical plan for the analysis and presentation of data from this study will be prepared prior to database lock. Deviations from the statistical analyses outlined in this protocol will be indicated in this plan; any further modifications will be noted in the final clinical study report.

13 STUDY MANAGEMENT

13.1 Electronic Case Report Forms and Source Documentation

For each subject, eCRFs and corresponding source records will be maintained at the clinical site. The Sponsor or designee will provide the study site with secure access to and sufficient training on the electronic data capture application, to permit site personnel to enter or correct information in the eCRFs for the subjects for whom they are responsible.

The eCRFs should be completed in a timely manner, and every effort should be made to have forms completed and up-to-date in anticipation of a visit by the Sponsor's monitor. Specific instructions will be provided to the site. All requested information must be entered on the eCRF in the spaces provided. If an item is not available or is not applicable, it should be documented as such; do not leave a space blank.

It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's eCRF. Through the electronic data capture application, the investigator must provide formal approval of all subject information in the eCRFs and changes to the eCRFs to endorse the final submitted data for the subjects for whom he/she is responsible. The audit trail entry will show the user's identification information and the date and time of any corrections.

eCRF completion may be delegated to other study personnel; however, such delegation must be documented in writing. If, for any reason, certain data are lacking to complete an individual report form, the investigator will provide a written statement explaining the reasons for the lack of data.

Sponsor or designee will retain the eCRF data and corresponding audit trails. A copy of the final archival eCRF in the form of a compact disk or other electronic media will be placed in the investigator's study file.

13.2 Good Clinical Practice

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, applicable regulatory requirements, and ZIOPHARM policies.

13.3 Sponsor Monitoring

After satisfactory receipt of all necessary regulatory paperwork, the Sponsor's monitor will arrange that all study material be delivered to the study site at a mutually convenient time. An initiation visits by ZIOPHARM and its monitoring personnel will be made. At this meeting, all personnel expected to be involved in the conduct of the study will undergo an orientation to include review of study protocol, instruction for CRF completion and overall responsibilities, including those for drug accountability and study file maintenance.

Throughout the course of the study, the Sponsor's monitor will make frequent contact with the investigator, and this will include telephone and/or on-site visits. During these visits, CRFs will be reviewed for completeness and adherence to protocol. As part of the data audit, it is expected that source documents (e.g. hospital records, office records) will be made available for review by the monitor. The monitor also will perform drug accountability checks, and may periodically request review of the investigator's study file to assure completeness of documentation in all respects of study conduct.

Upon study completion, the monitor will arrange for a final review of the study files, after which the file should be secured by storage for the appropriate period as specified in Section 13.5. The

investigator or appointed delegate will receive the Sponsor's representative during these on-site visits and will cooperate in providing the documents for inspection and responding to inquiries that may arise as part of this review. The investigator will also permit inspection of the study files by authorized representatives of the FDA.

13.4 Duration of the Study

Enrolled subjects will participate for up to 52 weeks, including up to 4 weeks for screening activities.

The overall duration of the entire study (screening, treatment, and follow-up for all subjects) is expected to be approximately 3.5 years.

13.5 Records Retention

Records of drug disposition, CRFs, and reports of the clinical trial must be maintained by the investigator for a period of at least 2 years following the date on which the test article is approved by FDA for marketing for the purposes that were investigated in the study. If no application is to be filed or if the application is not approved for such indication, the records must be stored for 2 additional years and then returned to ZIOPHARM. No records will be destroyed, but will be indefinitely stored.

13.6 Institutional Review Board

This protocol and the study informed consent form must be reviewed and approved by the Institutional Biosafety Committee (where applicable) and IRB/IEC prior to the start of the study, and a copy of the approval letter supplied to ZIOPHARM. During the course of the study, the investigator shall make timely and accurate reports to the IRB/IEC on study progress at intervals not exceeding one year, as well as satisfying any other local IRB/IEC reporting regulations. Copies of all reports to, and correspondence with, the IRB/IEC must be provided to ZIOPHARM. Further, within three months of the completion or early termination of the study, a final report should be made to the IRB/IEC and ZIOPHARM by the investigator.

All protocol revisions must originate with and be documented by ZIOPHARM. If the requested revision is an amendment, the investigator must sign it. The FDA will be notified of all revisions by ZIOPHARM. The investigator must submit the amendment to his/her IRB/IEC for review and approval prior to implementation. Documentation of approval signed by the chairperson or designee of the IRB/IEC must be sent to ZIOPHARM.

It is the investigator's obligation to maintain an IRB/IEC correspondence file and to make this available for review to ZIOPHARM representatives as part of the routine study monitoring process.

13.7 Confidentiality and HIPAA

The written Informed Consent will explain that study data will be stored in a database, maintaining confidentiality in accordance with national data legislation. All data processed by ZIOPHARM, or its representatives, will be identified by subject number and study code.

The written Informed Consent will also explain that, for data verification purposes, authorized representatives of ZIOPHARM, a regulatory authority (FDA), and/or the IRB/IEC may require direct access to parts of the hospital or clinic records relevant to the study that include the subject's medical history.

The Informed Consent Form will be accompanied by or include a separate document incorporating US Health Insurance Portability and Accountability Act (HIPAA)-compliant wording by which the subjects authorize the use and disclosure of their Protected Health Information.

13.8 Informed Consent

13.8.1 FDA Informed Consent Requirements

The investigator or his/her staff will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential risks involved to the prospective subject prior to enrollment. The Informed Consent should also indicate that, by signature, the subject or, where appropriate, a legal guardian, permits access to relevant medical records by the Sponsor and by representatives of the US FDA. If a prospective subject does not understand English, an appropriate translation into his or her primary language must be made available. The investigator or designee will obtain written, informed, and witnessed consent. The individual will have ample time and opportunity to ask questions. He/she will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Following the discussion regarding the study, the prospective subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the individual voluntarily agrees to sign the informed consent statement and has done so, may he/she enroll into the study. A copy of his/her signed and dated informed consent will be provided to each subject. The signed Informed Consent Form is to remain in the investigator's file.

The Informed Consent Form and any other written information provided to the subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or if there is an amendment to the protocol that necessitates a change to the content of the subject's informed consent. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm continuation of his/her participation in the study by his/her signature on the revised Informed Consent Form (if applicable). Any written Informed Consent Form and written information must receive IRB/IEC approval/favorable opinion in advance of use.

13.8.2 Subject Informed Consent Form

ZIOPHARM will provide a sample subject Informed Consent Form for modification, as appropriate, by the investigator.

14 PROTOCOL APPROVAL PAGE

Title: A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First- or Second-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer

With the exception of a change intended to eliminate an immediate hazard to subjects, the study shall be conducted as described in the approved protocol. All deviations from the protocol will be documented. Any significant deviation or deviation related to dosing or safety evaluation will be reported to ZIOPHARM.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to principles of Good Clinical Practice and local regulations and requirements.

Study Site Center Name: _____

Principal Investigator

Print Name: _____

Signature: _____

Date: _____

[redacted]

[redacted]

[redacted]

16 APPENDICES

16.1 Eastern Oncology Performance Status

ECOG PERFORMANCE STATUS[37]

Grade	Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

[37] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-55, 1982.

16.2 Recommended Regimen for Antipyretic and/or Analgesic Prophylaxis

Recombinant adenoviral vectors have the potential to elicit potent cellular and humoral immune responses. While the mechanism responsible for these effects is poorly understood, transient low-grade fevers are common after systemic recombinant adenovirus vector administration and temperatures up to 104° F with chills and generalized malaise have been observed with treatment. Because low grade fever is very likely to occur, prophylaxis with acetaminophen is strongly recommended.

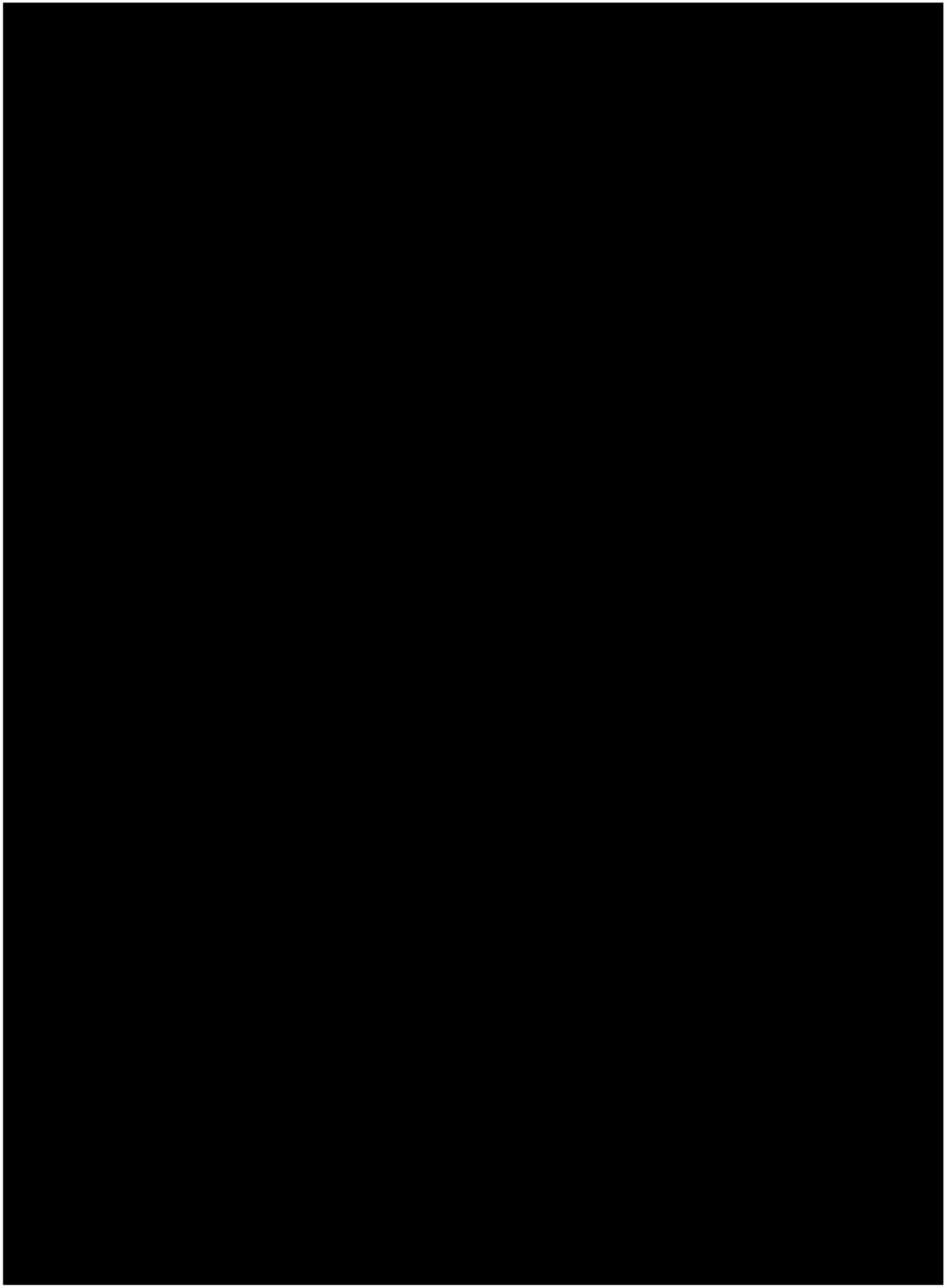
- While meta-analyses suggest that ibuprofen is a better anti-pyretic medication than acetaminophen, acetaminophen also prevents and or reduces a fever. Acetaminophen is available without a prescription in 325 mg or 500 mg tablets. One to two tablets every 4 hours should be used to eliminate fever. The maximum dose of acetaminophen in adults should not exceed 4 grams in a 24-hour period.

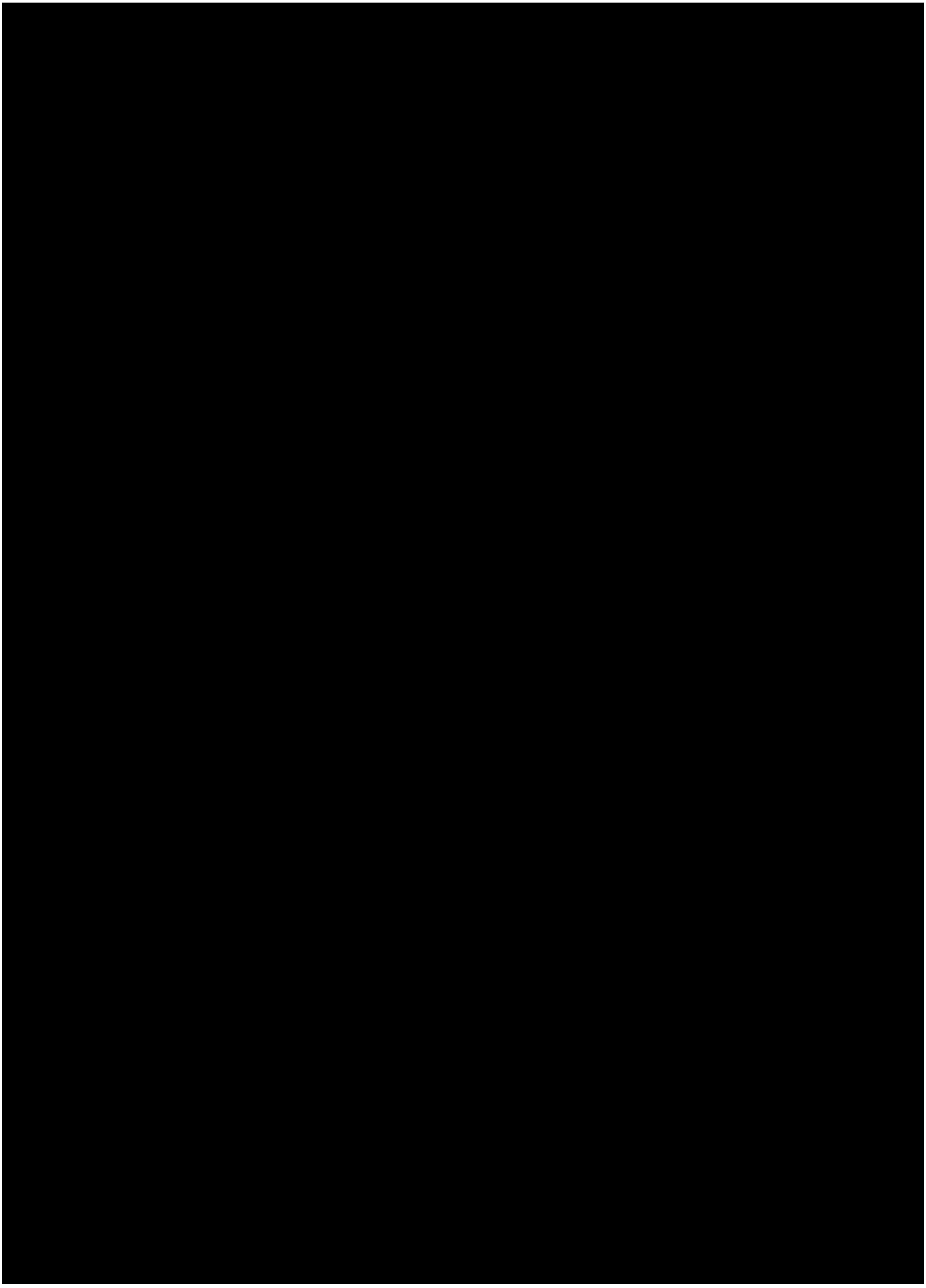
- Side effects are rare, but some people are allergic to the medication. Over doses may cause liver failure. Therefore, people with liver disease and chronic alcohol users should avoid this medication.

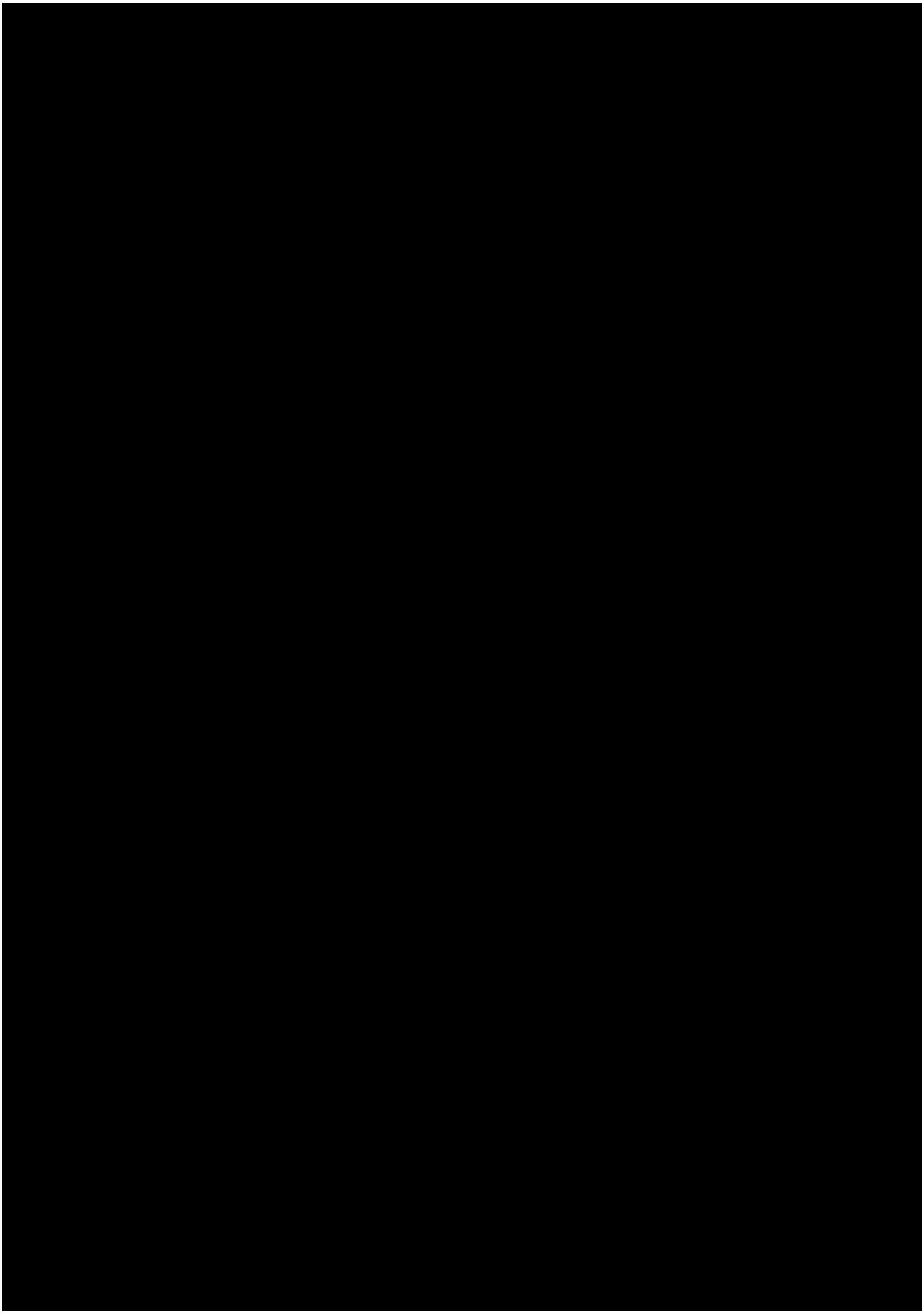
- Common brand names of acetaminophen are Aspirin Free Anacin®, Feverall®, Genapap®, Panadol®, Tempra®, and Tylenol®.

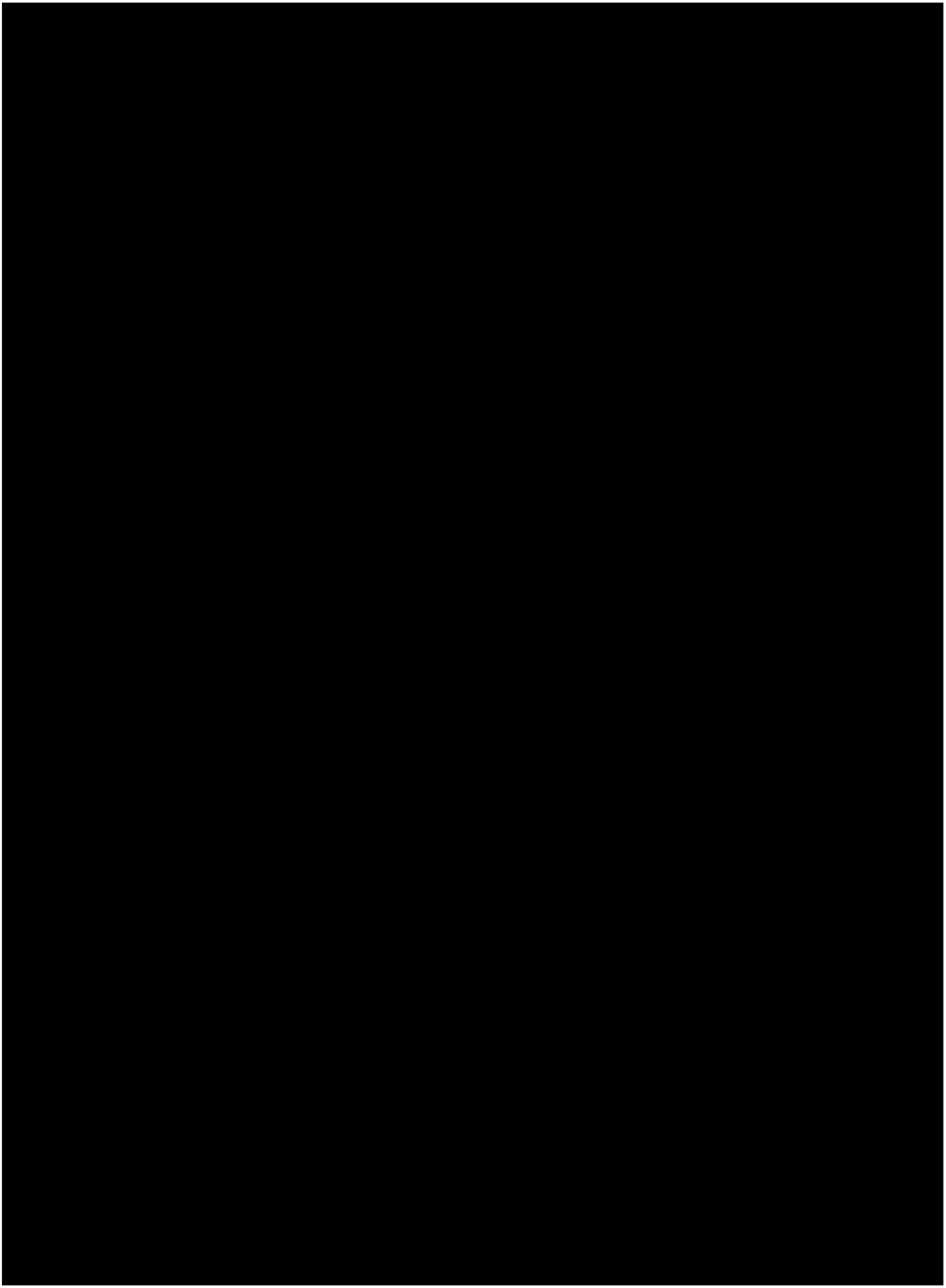
In general, a fever can be treated with acetaminophen. Alternating doses of ibuprofen with acetaminophen will also effectively control fever and prevent accidental overdose. If a fever occurs in spite of prophylactic medication or does not respond to usual doses of acetaminophen, then a combination of both acetaminophen and ibuprofen may be needed to stop the fever.

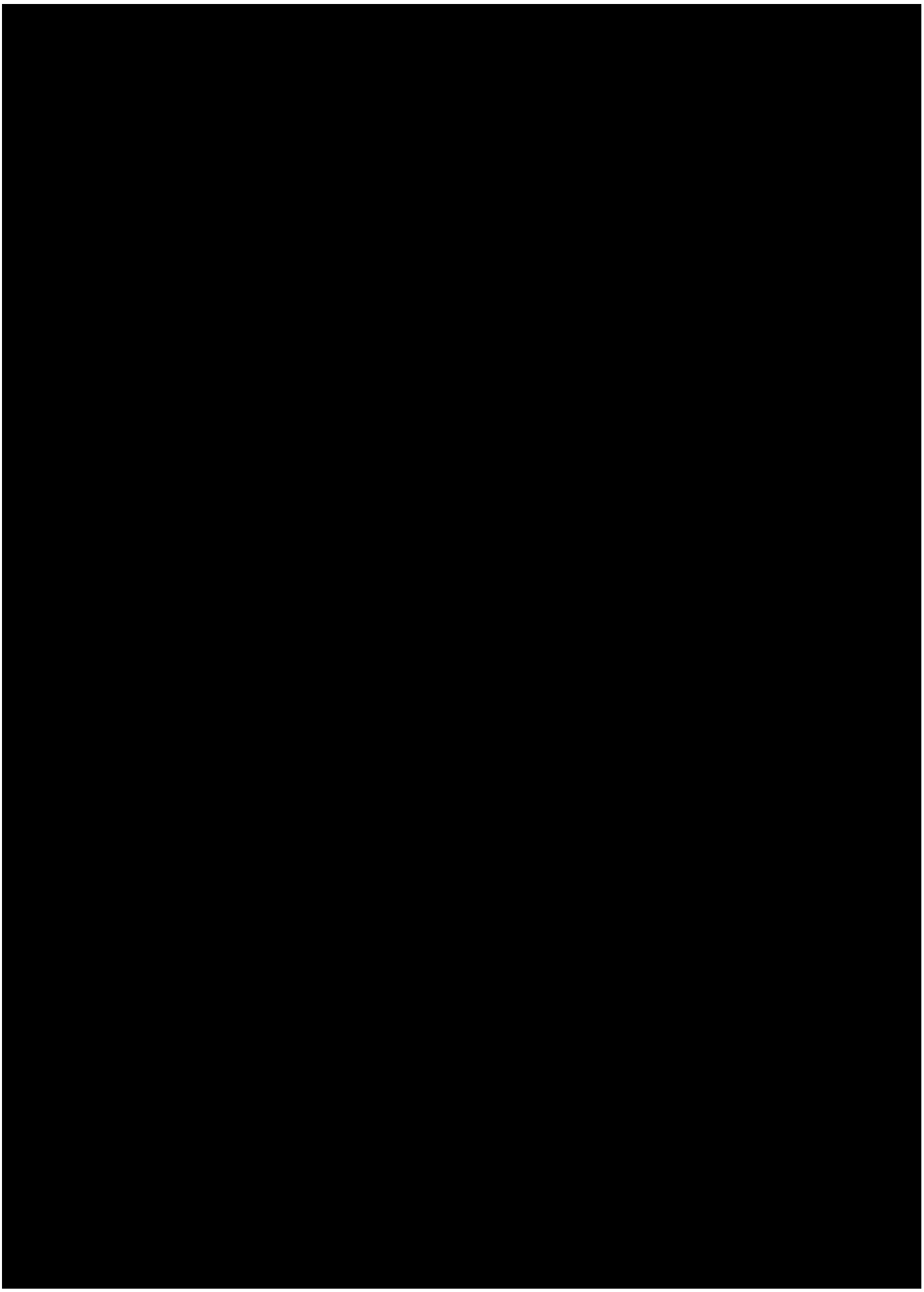
16.3 RECIST v. 1.1

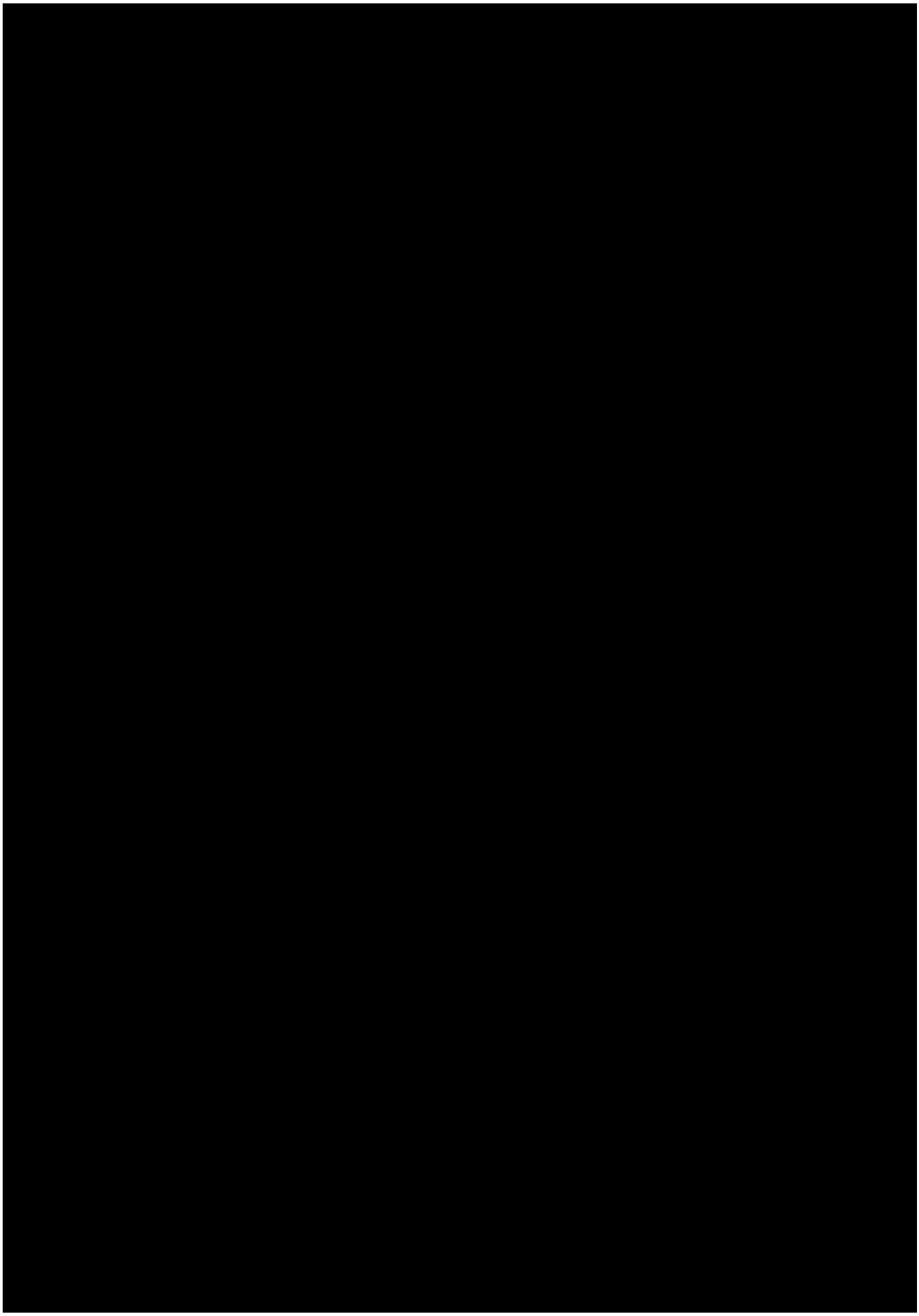


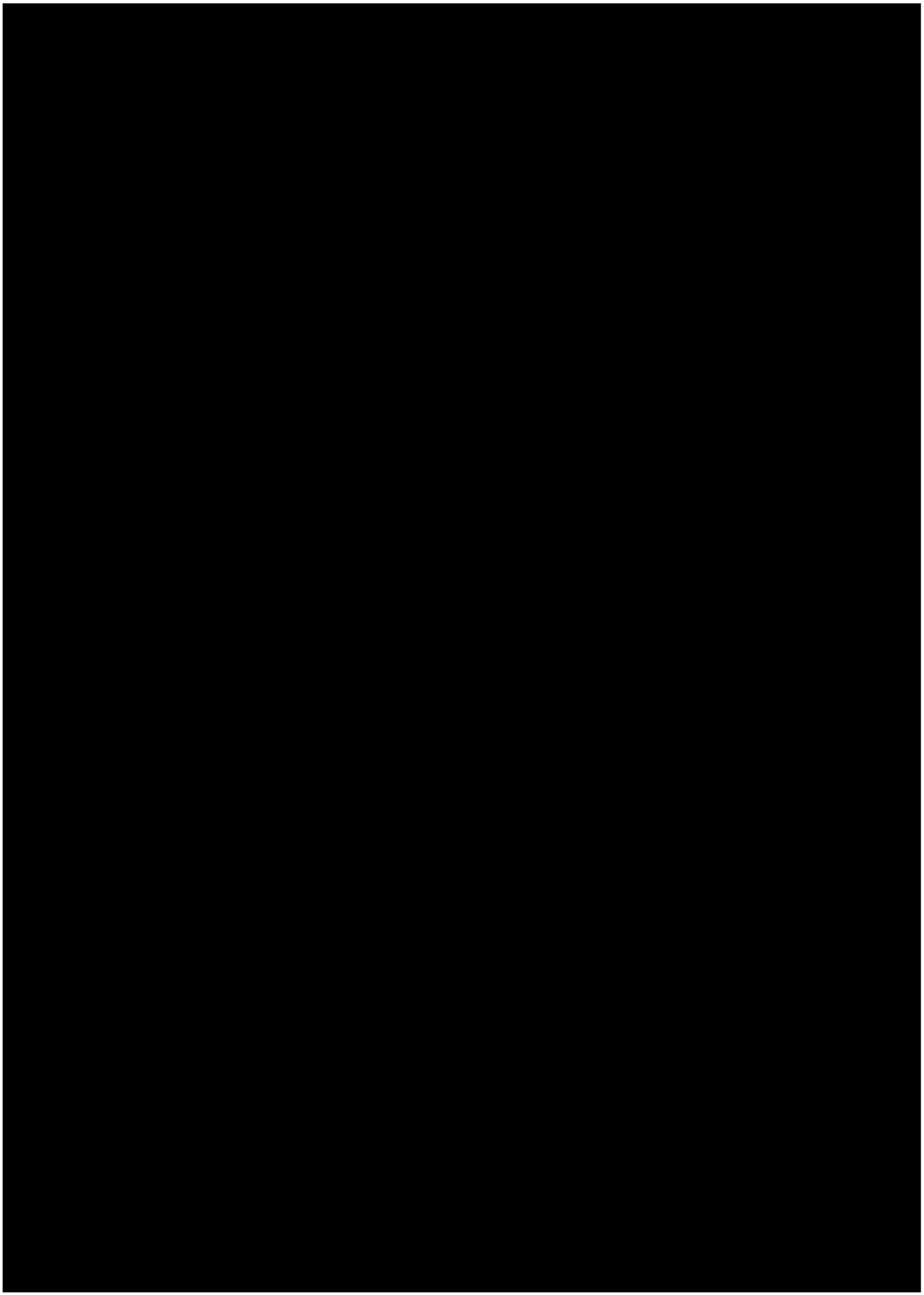


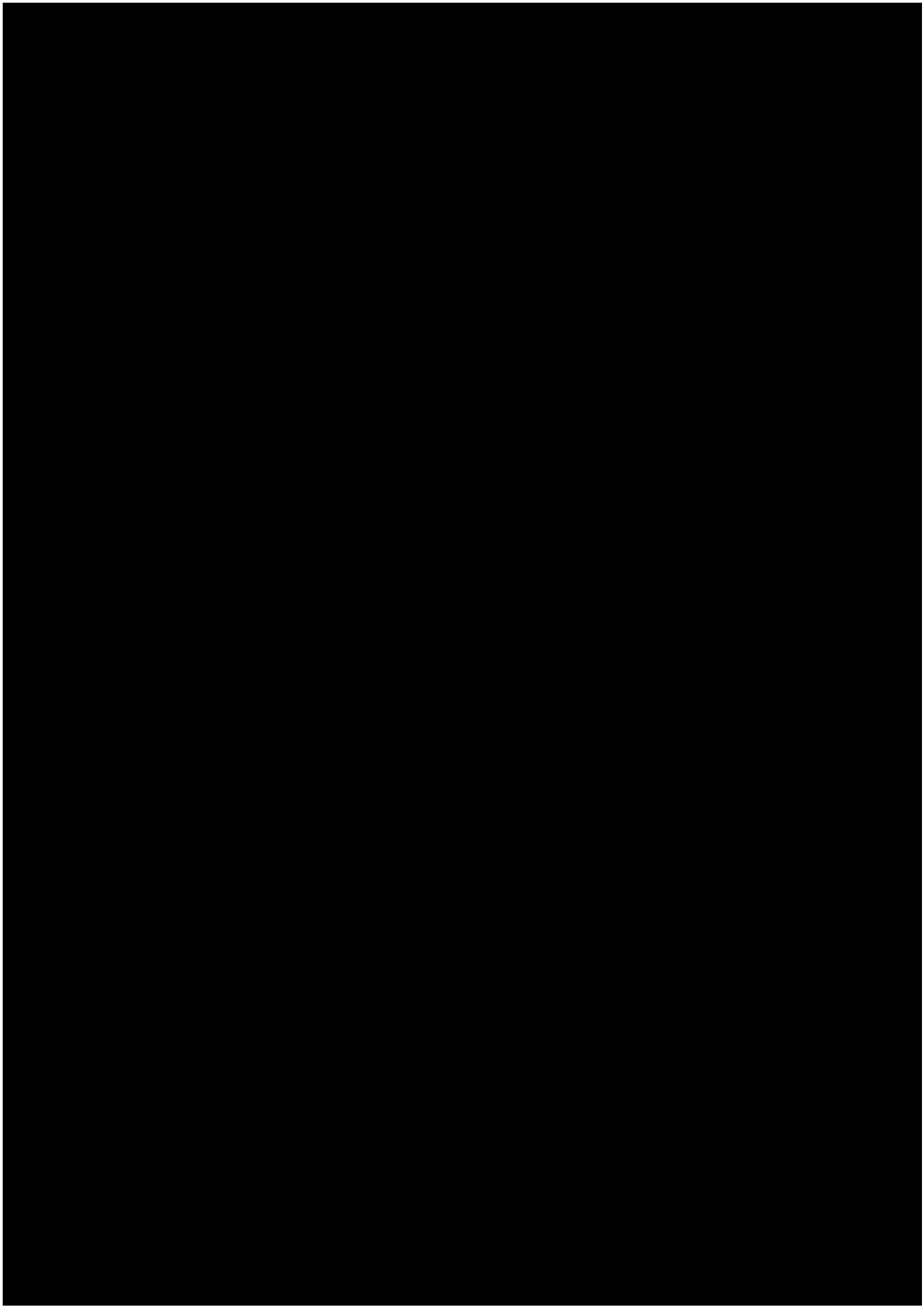


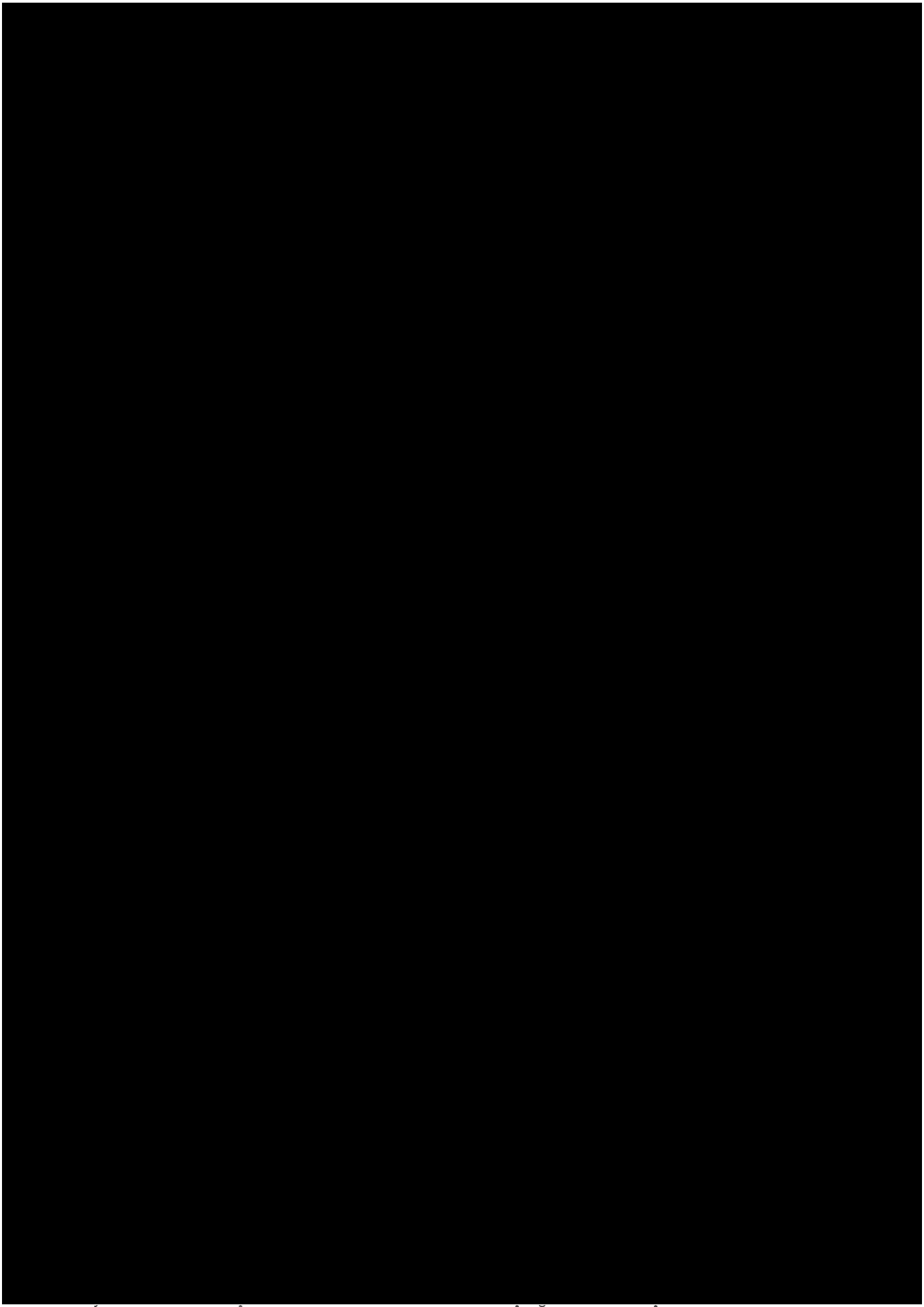


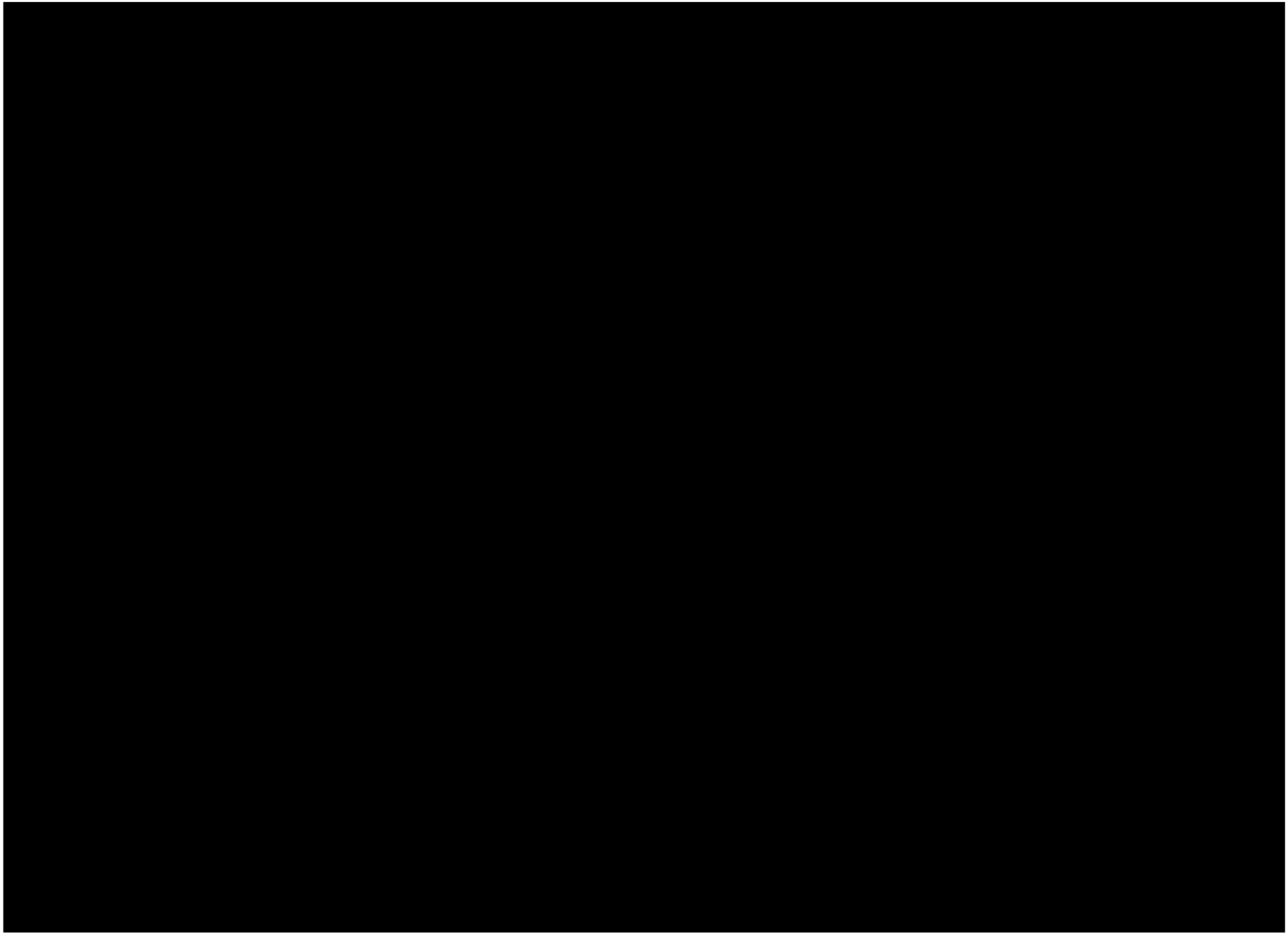


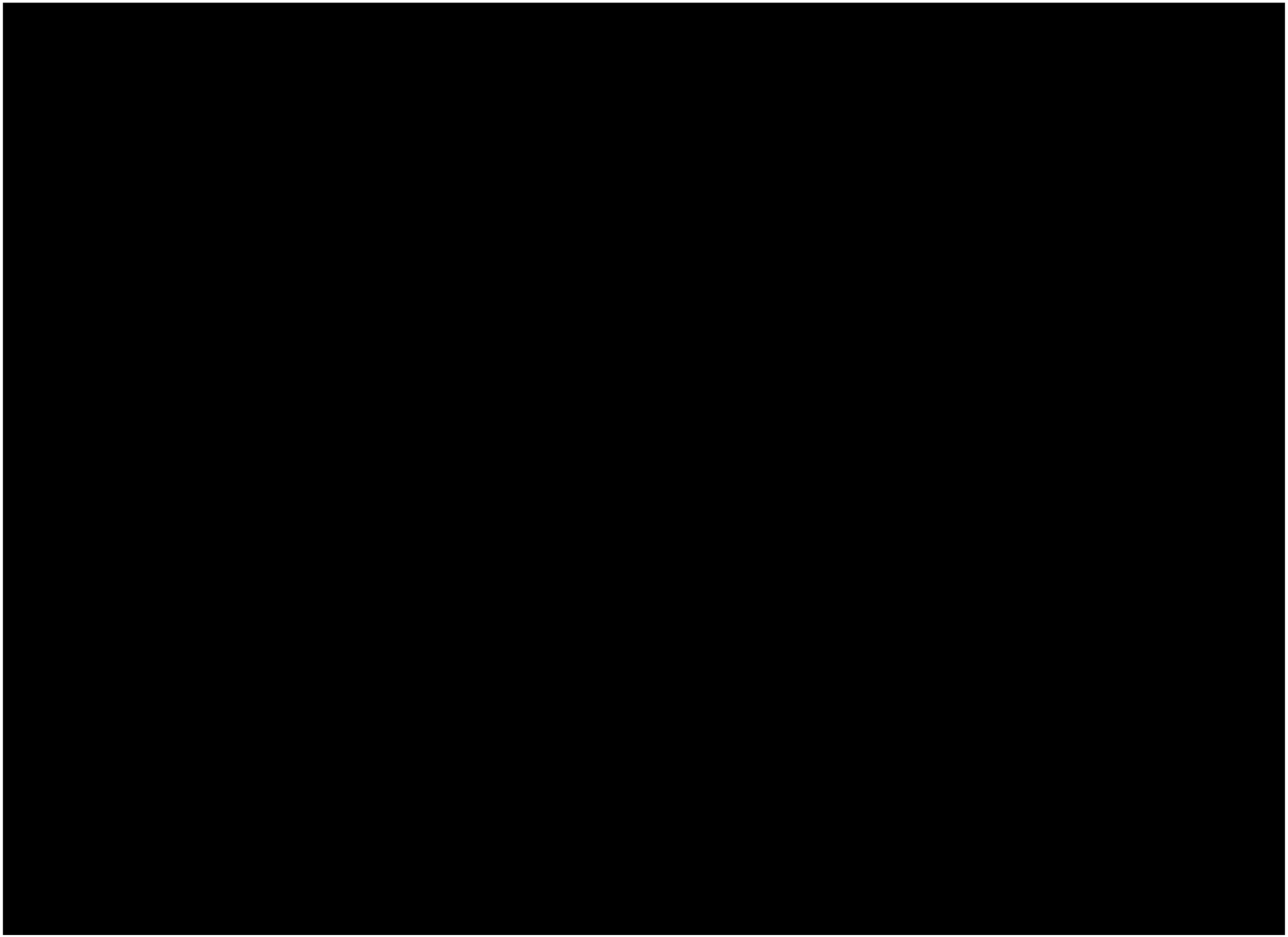


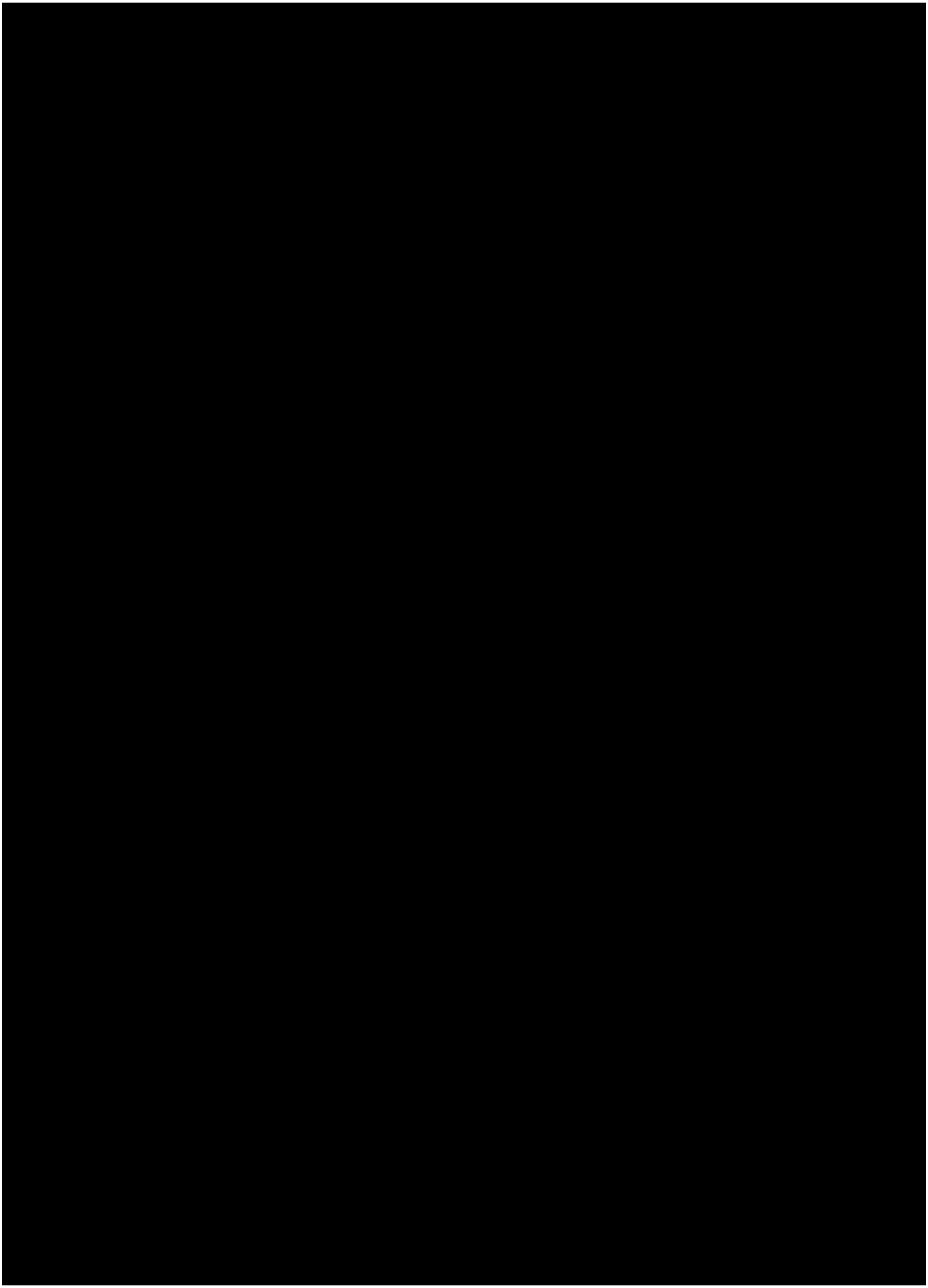


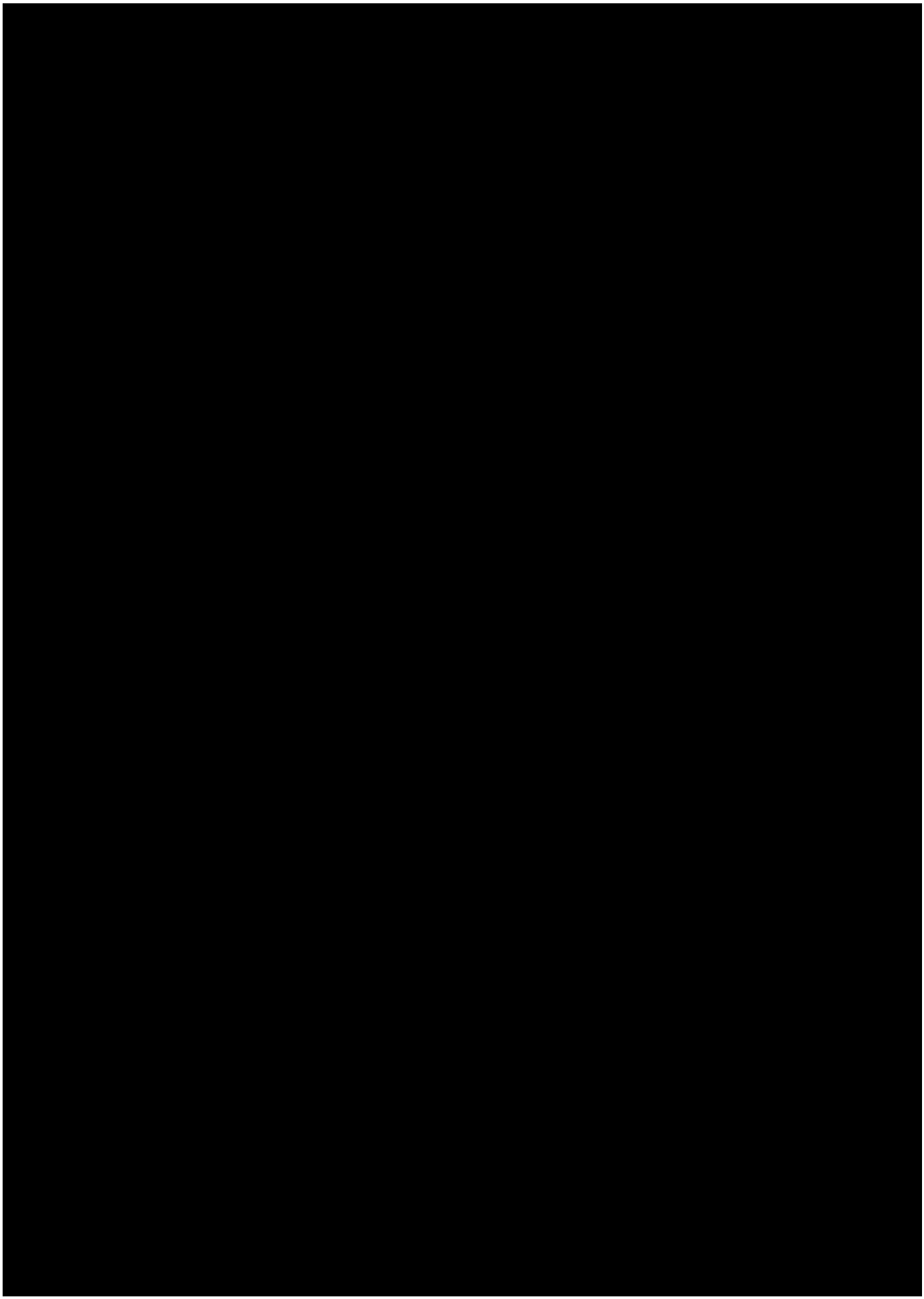


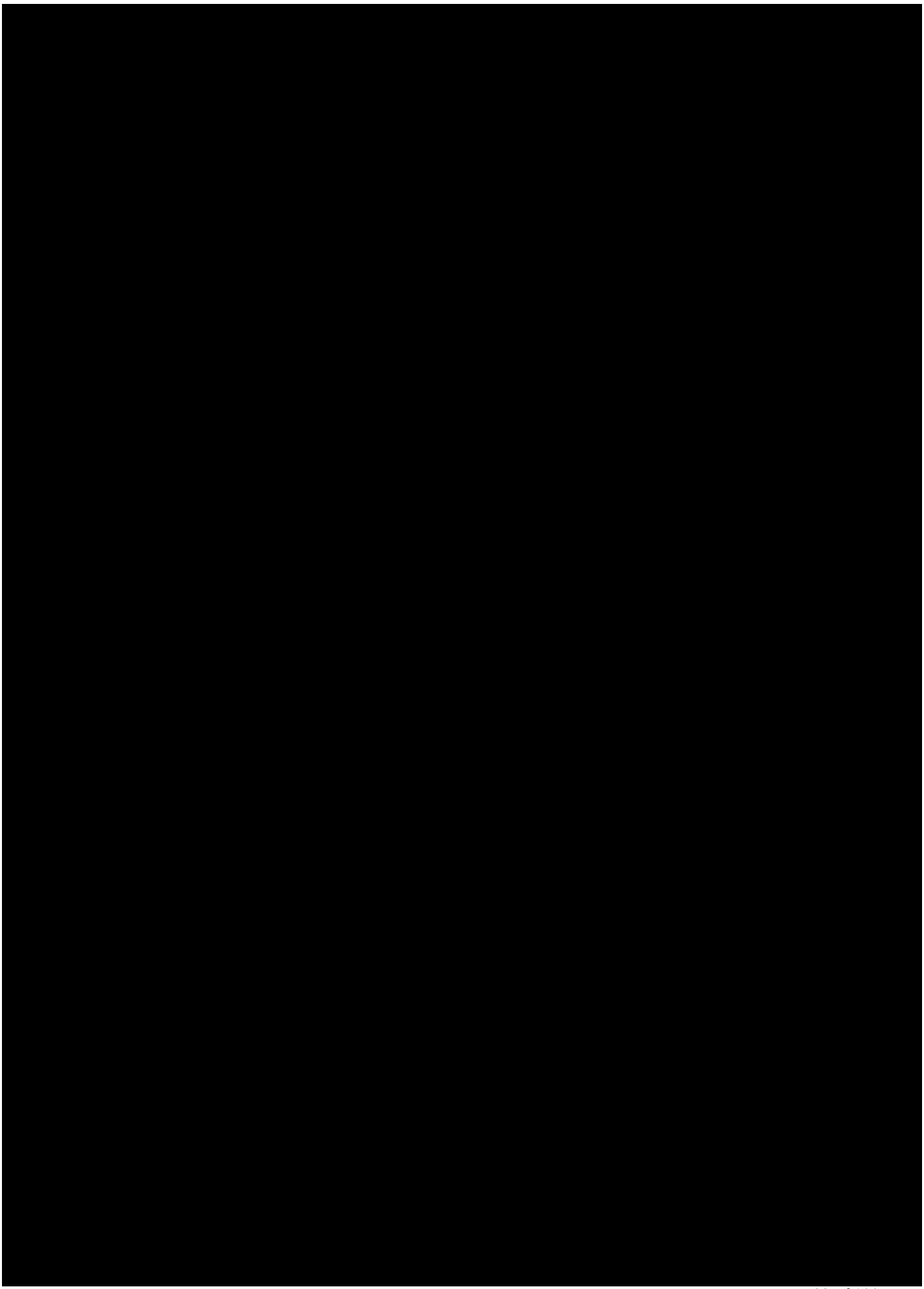


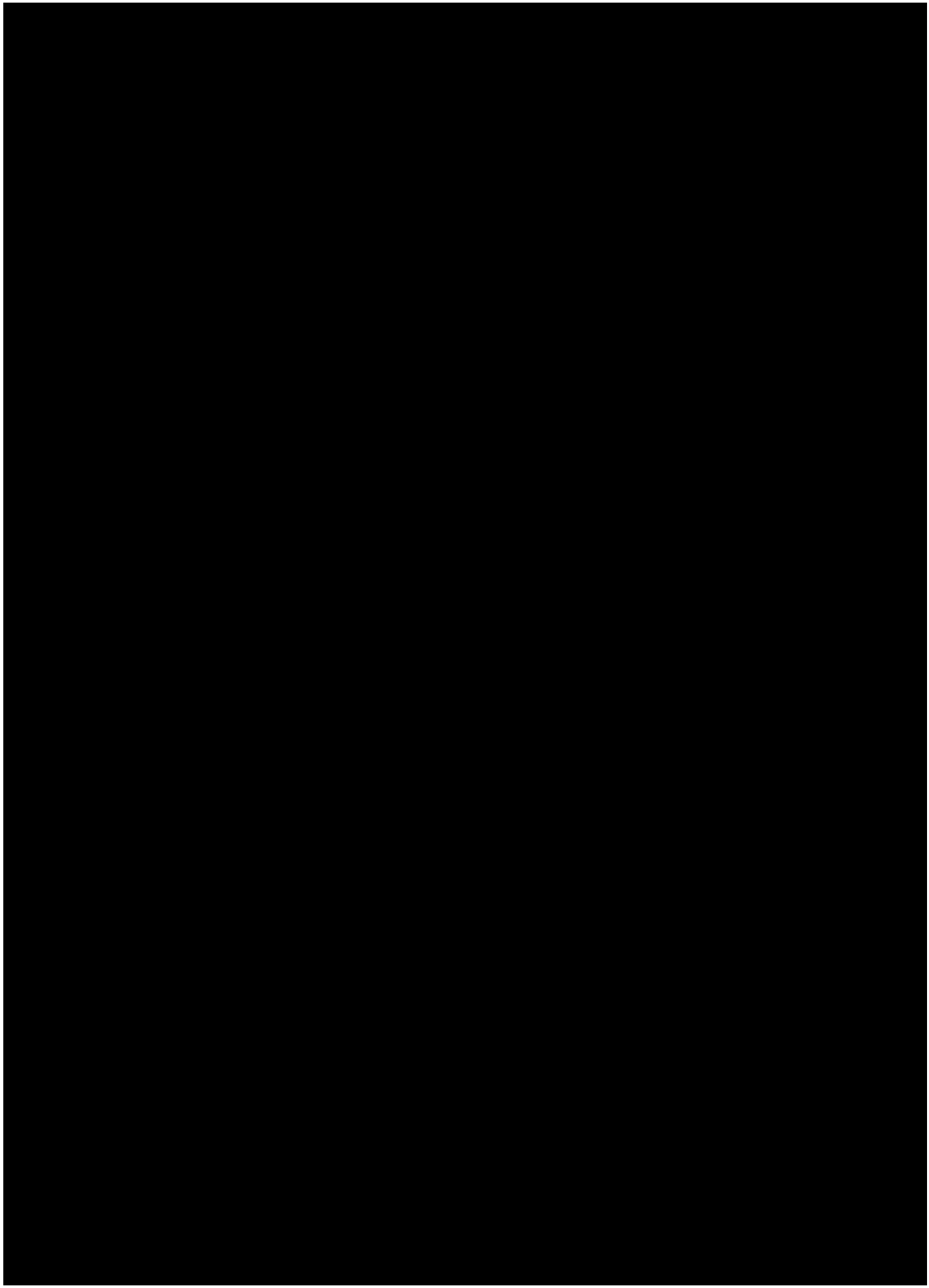


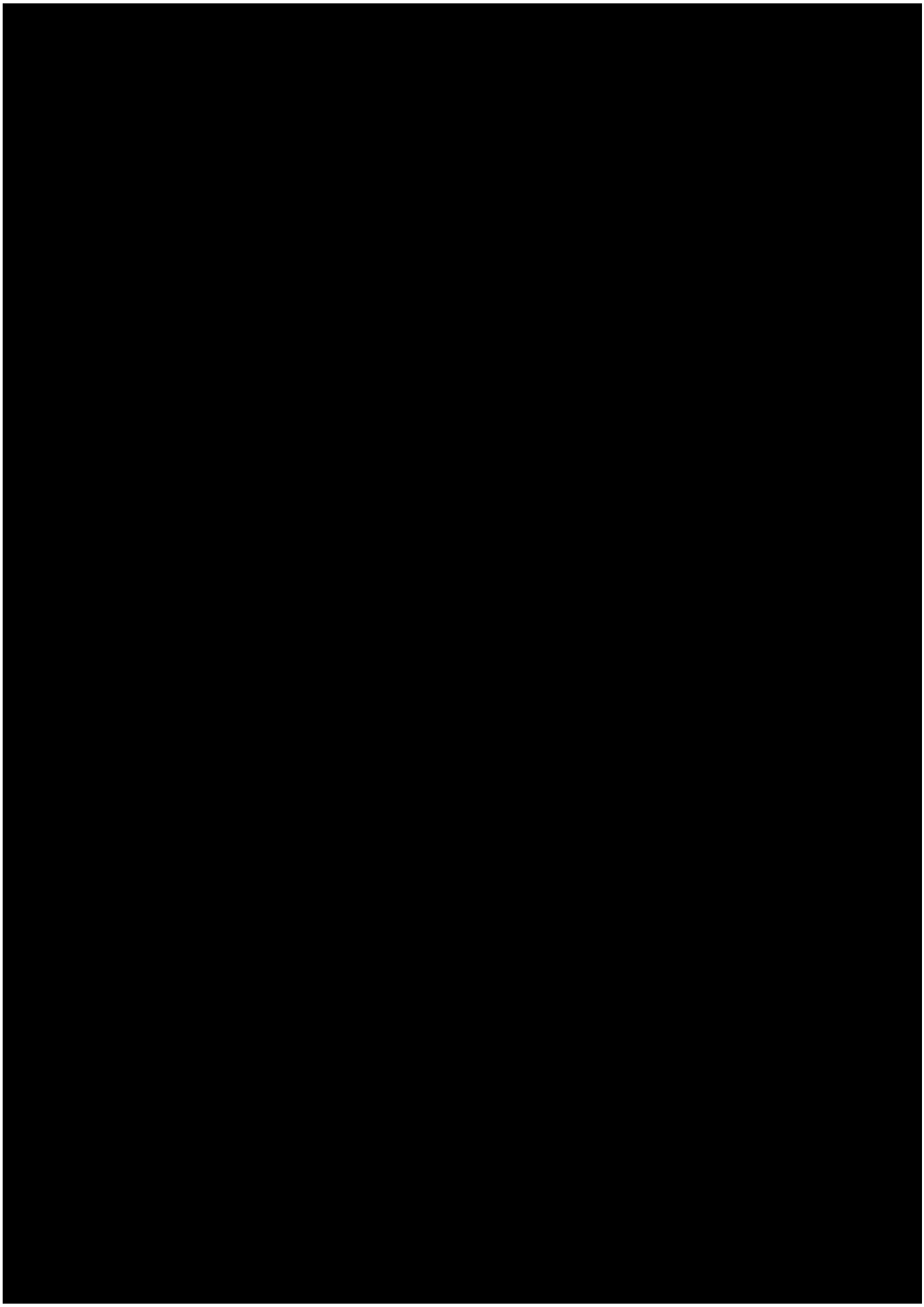


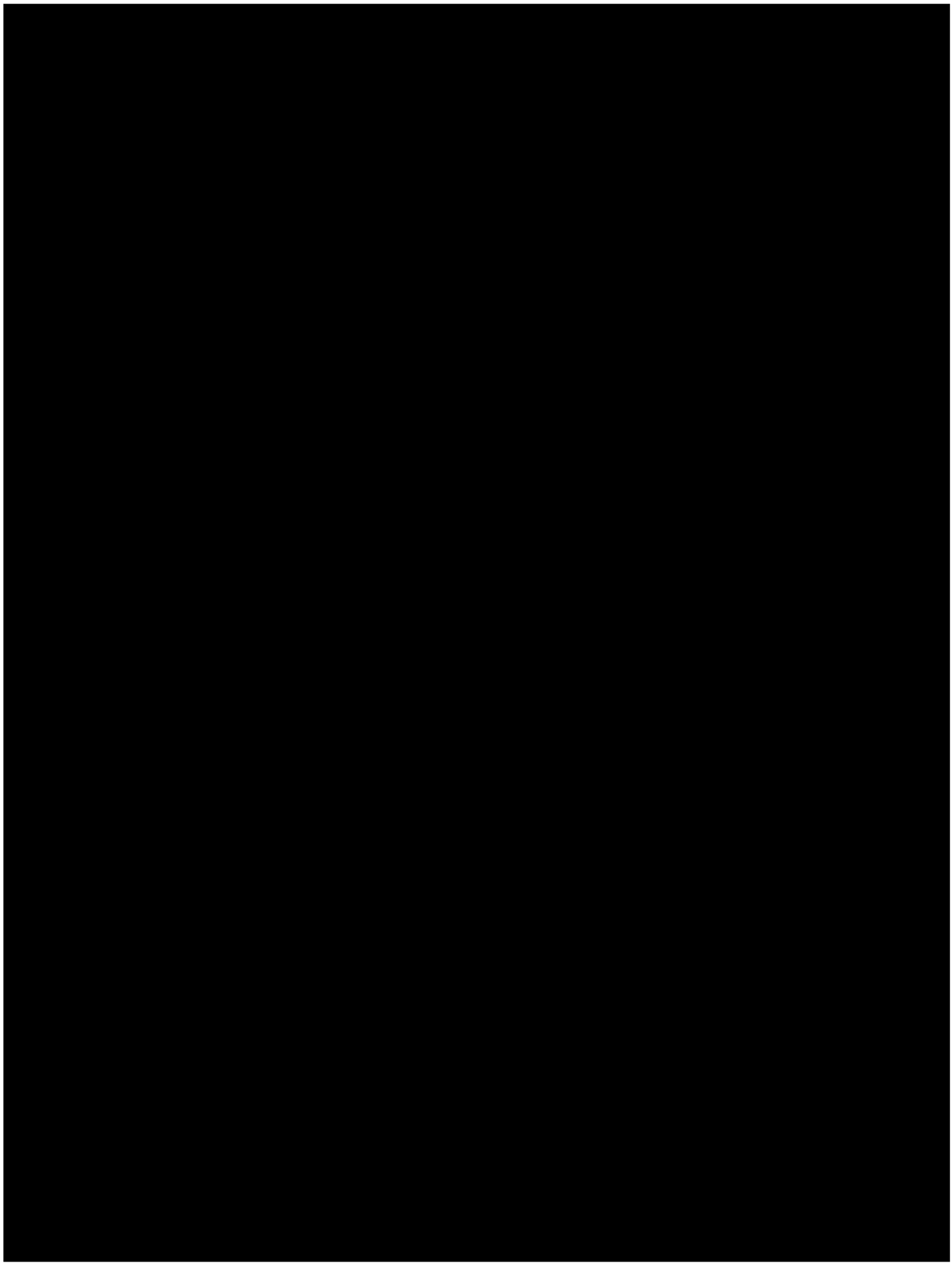












16.4 New York Heart Association Classification

Classes of Heart Failure

Doctors usually classify patients' heart failure according to the severity of their symptoms. The table below describes the most commonly used classification system, the New York Heart Association (NYHA) Functional Classification. It places patients in one of four categories based on how much they are limited during physical activity.

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

Class	Objective Assessment
A	No objective evidence of cardiovascular disease. No symptoms and no limitation in ordinary physical activity.
B	Objective evidence of minimal cardiovascular disease. Mild symptoms and slight limitation during ordinary activity. Comfortable at rest.
C	Objective evidence of moderately severe cardiovascular disease. Marked limitation in activity due to symptoms, even during less-than-ordinary activity. Comfortable only at rest.
D	Objective evidence of severe cardiovascular disease. Severe limitations. Experiences symptoms even while at rest.

For Example:

- A patient with minimal or no symptoms but a large pressure gradient across the aortic valve or severe obstruction of the left main coronary artery is classified:
  - **Function Capacity I, Objective Assessment D**
- A patient with severe anginal syndrome but angiographically normal coronary arteries is classified:
  - **Functional Capacity IV, Objective Assessment A**

[redacted]

16.5 CTCAE v. 4.03

Please refer to the following website for the *Common Terminology Criteria for Adverse Events (CTCAE)*, Version 4.03, published June 14, 2010 by the National Cancer Institute:

[redacted].

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Protocol Title:	A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First- or Second-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer
Protocol Number:	ATI001-203
Study Drugs:	INXN-2001 (Ad-RTS-hIL-12) INXN-1001 (oral activator ligand)
Date of Protocol:	Amendment 1: 05 November 2014 Original protocol: 08 July 2014

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Tabular Summary of Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Title Page, Page Headings and Footers (page 1)	Specified "Original" for Date of Protocol (08 July 2014) and added Amendment 1, 05 November 2014.	To differentiate the original protocol from Amendment 1
Title Page, Page Headings and Footers (page 1)	Updated the primary contact for safety reporting from medical monitor to Safety Hotline Number.	SAEs should be reported by calling the Safety Hotline Number instead of the medical monitor
Protocol Synopsis (page 2)	In Research Hypothesis, specified standard therapy to be "chemotherapy" and changed anti-HER2 regimen to **anti-HER 2 "antibody therapy"**; these changes were also made throughout the document.	Requested by principal investigator
Primary Objective and Primary Endpoints (pages 2, 8, 35, 73)	Primary Objective, changed from "To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-or second-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first- or second-line anti-HER2 treatment in HER2-positive (HER2+) subjects" to "To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-or second-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first- or second-line anti-HER2 **antibody therapy** in HER2-positive (HER2+) subjects **as defined by Section 12.4.2**.	Requested by principal investigator and site staff
Secondary Objective and Primary Endpoints (pages 2, 8, 35, 73)	Secondary Objectives, deleted "To explore the biologic/immunologic effects of treatment on levels of the following cytokines in serum: interleukin-12 (IL-12), interleukin-10 (IL-10), and interferon-gamma (IFN–γ)" and "To explore the effect of treatment on tumor infiltration by effector cells and immune suppressor elements" and replaced them with **"To compare radiographic responses by irRC with conventional reporting by RECIST"** and **"To explore the impact of treatment on tumor and serum immune biomarkers"**	Requested by principal investigator and site staff
Study Design (page 2 - 3)	Study Design language changed from "This is a single-arm pilot, single-center study to examine the safety, tolerability and preliminary efficacy of providing one cycle of Ad-RTS-hIL-12 immunotherapy following achievement of SD or PR on standard treatment as first- or second-line therapy in breast cancer subjects. The patient population will include patients with locally advanced or metastatic breast cancer of	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	all subtypes. The safety of this therapy and the preliminary evidence of efficacy will guide further studies. Patients will be required to have SD or PR after completion of a minimum of 12 weeks on standard therapy. Subjects who have progressive disease (PD) or a CR after the standard therapy are not eligible for the study. Following entry into the trial, patients will go on a treatment holiday from the standard therapy and enter an immunotherapy phase of treatment" to "**This is a single-arm, single-center study to examine the safety, tolerability and preliminary efficacy of one cycle of Ad-RTS-hIL-12 immunotherapy in women with advanced breast cancer and pre-study SD or PR after completion of a minimum 12 week course of standard first- or second-line chemotherapy. The patient population will include patients with locally advanced or metastatic breast cancer of all subtypes. The safety of this therapy and the preliminary evidence of efficacy will guide further studies. Subjects who have progressive disease (PD) or a CR after the standard therapy are not eligible for the study. Following entry into the trial, patients will go on a treatment holiday from chemotherapy and enter an immunotherapy phase of treatment. Continuation of HER2-targeted antibody therapy is permitted during this immunotherapy phase for women with HER2+ disease.**"	
Study Design (page 3)	Study Design language changed from "Radiographic PD at week 6 must be confirmed at least 4 weeks later." To "Radiographic PD at week 6 must be confirmed at least 4 weeks later, **either at week 12 or earlier if clinically necessitated**."	Requested by principal investigator and site staff
Study Design (page 3)	Study Design language specified from standard therapy to **"chemotherapy**" and changed anti-HER2 therapy to anti-HER 2 **"antibody therapy"**	Requested by principal investigator and site staff
Pages 3, 6, 12, 36, 44, 46 and 54	"The first dose of veledimex followed 3 hours ± 30 minutes later by an intratumoral injection of Ad-RTS-hIL-12" to 3 hours ± **3 hours**	Requested by principal investigator and site staff
Study Design (page 3)	Study Design, deleted "A post-treatment safety assessment visit will be conducted 28 days after the last dose of veledimex."	Requested by principal investigator and site staff to lessen burden for patients
Study Design (pages 3 and 37)	Study Design, changed "The primary endpoint is safety/tolerability, defined as the proportion of patients who are able to complete 12 weeks on study without a Grade 3 or higher toxicity" to "The primary endpoint is safety/tolerability, defined as the	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	proportion of patients who are able to complete 12 weeks on study without Grade 3 or higher toxicity **as defined by Section 12.4.2.**	
Study Design (pages 3 and 37)	Study Design, changed "Additional secondary endpoints are the ORR, the DCR and the proportion of subjects who experience an improvement from a baseline tumor status from SD to PR/CR or from PR to CR, biologic/immunologic effects of treatment on levels of the cytokines in serum and the effect of treatment on tumor infiltration by immune cells. Biological markers of response (biomarkers) in serum will include various cytokines; for example, IL-12, IL-10 and interferon-gamma (IFN-γ). Additionally, immunocytochemistry will be performed on core or punch biopsy or fine needle aspiration (FNA) of the tumor and/or associated tumor-involved draining lymph nodes. Cellular infiltration by effector cells and immune suppressor elements will be evaluated using antibodies to various cluster of differentiation (CD) antigens, including CD3, CD4, CD8, CD25, FOX-P3, and CD56. In addition, blood for tumor markers such as CA 15-3 and CEA will be collected on day 1 and at 6 and 12 weeks after the start of immunotherapy" to **"Additional secondary endpoints are the ORR, the DCR and the proportion of subjects who experience an improvement from a baseline tumor status from SD to PR/CR or from PR to CR. Disease responses by RECIST versus irRC will be explored. The effect of treatment on serum biomarkers [including IL-12, IL-10 and interferon-gamma (IFN-γ)] and tumoral immune cell subtypes (including $CD8^+$ effector cells and T-regulatory cells) will also be explored."**	Requested by principal investigator and site staff
Exclusion Criteria (pages 5 and 39)	Exclusion #6 changed from "History of prior malignancy, unless the prior malignancy was diagnosed and definitively treated ≥ 5 years previously and showed no subsequent evidence of recurrence; adequately treated basal cell or squamous cell skin cancer or carcinoma in situ of the cervix, low grade thyroid are permitted to "**An active, second potentially life-threatening cancer, adequately treated basal cell or squamous cell skin cancer or carcinoma in situ of the cervix, low grade thyroid are permitted**."	Requested by principal investigator and site staff
Safety Assessments (page 7)	Safety Assessments changed from "Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt further dosing. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor" to "Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt/**discontinue** further dosing **at the discretion of the investigator**. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor **Medical Monitor**."	
Efficacy Assessments (page 7)	Efficacy Assessments changed from "Biologic/immunologic assessments will be performed using serum samples obtained at days 1, 3, 7, as well as tumor biopsies, preferably core biopsy (punch or FNA biopsy accepted in the absence of the core biopsy), taken on days 1 and 7 following initiation of immunotherapy" to "Biologic/immunologic assessments will be performed using serum samples obtained at screening, days 1, 3, **8**, **and weeks 6 and 12. Tumor biopsies will be obtained by interventional radiology prior to the intra-tumoral vector injection and again at 6 weeks."**	Requested by principal investigator and site staff
Study Schema (page 10)	Updated Study Schema to match the Schedule of Study Procedures	Requested by principal investigator and site staff
Schedule of Study Procedures (page 11)	Updated Schedule of Study Procedures	Requested by principal investigator and site staff
Overall Study Design (page 36)	Overall Study Design, Changed from "This is a pilot single-arm, single-center study to examine the safety, tolerability and preliminary efficacy of providing one cycle of Ad-RTS-hIL-12 immunotherapy following achievement of SD or PR on standard treatment as first- or second-line therapy in breast cancer subjects" to "This is a single-arm, single-center phase **1b/II** study to examine the safety, tolerability and preliminary efficacy of providing one cycle of Ad-RTS-hIL-12 immunotherapy following achievement of SD or PR on standard first- or second-line **chemotherapy** in breast cancer subjects." Also changed, "Subjects who have PD or a CR after the standard therapy are not eligible for the study. Following entry into the trial, patients	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	will go on a treatment holiday from the standard therapy and enter an immunotherapy phase of treatment to "Subjects who have PD or a CR after the standard **chemotherapy** are not eligible for the study. Following entry into the trial, patients will go on a treatment holiday from the standard **chemotherapy** and enter an immunotherapy phase of treatment."	
Overall Study Design (page 36)	Overall Study Design, changed from "For subjects with HER2- disease, the Ad-RTS-hIL-12 immunotherapy is intended to be given as a chemotherapy break, for example, to avoid intolerable or undesirable toxicity of the standard therapy. Ad-RTS-hIL-12 immunotherapy must be started within 4 weeks of stopping the pre-study standard therapy. Subjects with HER2+ disease will receive the Ad-RTS-hIL-12 immunotherapy in conjunction with first- or second-line anti-HER2 therapy, ie, subjects may continue anti-HER2-directed therapy during the study period" to "For subjects with HER2- disease, the Ad-RTS-hIL-12 immunotherapy is intended to be given as a chemotherapy **holiday**, for example, to avoid intolerable or undesirable toxicity of the standard therapy. Ad-RTS-hIL-12 immunotherapy must be started within 4 weeks of stopping the pre-study standard therapy. Subjects with HER2+ disease will receive the Ad-RTS-hIL-12 immunotherapy in conjunction with first- or second-line anti-HER2 **antibody** therapy, ie, subjects may continue anti-HER2-directed **antibody** therapy during the study period."	Requested by principal investigator and site staff
Inclusion Criteria (pages 4 and 38)	Inclusion # 3, and 7, changed therapy to "**chemotherapy**"	Requested by principal investigator and site staff
Exclusion Criteria (page 5 and 41)	Exclusion #15, changed "International normalized ration (INR) and activated partial thromboplastin time (aPTT) <1.5 × ULN" to "**International normalized ration (INR) and activated partial thromboplastin time (aPTT) >1.5 × ULN**"	Requested by principal investigator and site staff
Section 5.4 (page 45)	Section 5.4, changed "Study drug container(s) with any remaining capsules should be returned to the study staff on Day 7" to "Study drug container(s) with any remaining capsules should be returned to the study staff on Day **8**"	Requested by principal investigator and site staff
Section 6.1 (page 46)	Section 6.1, changed "If another tumor is not available, then the remaining volume should be injected into a draining, pathologic lymph node of the injected tumor" to "If another tumor is not available, then the remaining volume should be injected into a draining, pathologic lymph node of the injected tumor **if possible**"	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Section 6.5 (page 47)	Section 6.5, changed "The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor" to "The decision as to whether subjects who experience serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor **Medical Monitor**"	Requested by ZIOPHARM Safety and Medical Monitor for clarity
Section 6.7 (page 47)	Section 6.7, added **"Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt/discontinue further dosing at the discretion of the investigator. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor Medical Monitor."**	Requested by ZIOPHARM Medical Monitor for clarity
Section 7.1 (page 49)	Section 7.1, added **"HER-directed therapies in patients with HER2+ disease"**	Requested by principal investigator and site staff
Section 7.2 (page 49)	Section 7.2 changed "Any other investigational agent or anticancer therapy (chemotherapy, radiotherapy, etc) while receiving study treatment" to "Any other investigational agent or anticancer therapy (chemotherapy, radiotherapy, **including corticosteroids**, etc) while receiving study treatment"	Requested by ZIOPHARM Medical Monitor for clarity
Section 8.3.1.5 (page 51)	Section 8.3.1.5, changed "Females of childbearing potential will have a serum pregnancy test at the screening visit and a urine or serum pregnancy test on Day 1" to "Females of childbearing potential will have a serum pregnancy test at the screening visit **within 14 days of dosing and a urine pregnancy test at Day 1.**"	Requested by principal investigator and site staff
Pages 58 - 65	Pages 58-65, updated assessments and visits to reflect the Schedule of Study Procedures	Requested by principal investigator and site staff
Section 9.2.1 (page 60)	Section 9.2.1, changed "In the current study, the baseline measurement will be based on the scans and measurements taken at the end of the pre-study standard therapy, as submitted by the investigator" to "In the current study, the baseline measurement will be based on the scans and measurements taken at the end of the pre-study standard	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	chemotherapy, as submitted by the investigator"	
Section 10.1 (page 65)	Section 10.1, changed "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship the treatment" to "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship **to** the treatment"	Grammatical Correction
Section 10.2 (page 65)	Section 10.2, changed "Progression of disease is not itself an AE; however, the presenting sign or symptom of the disease progression should be documented as an AE (e.g. increase in pain)" To "Progression of disease is not itself an AE; however, the presenting sign or symptom of the disease progression should be documented as an AE (e.g. **pleural effusion**)."	Requested by ZIOPHARM Safety and Medical Monitor for clarity
Section 10.7 (page 70)	Section 10.7, changed from "The SAE Report form is provided in the investigator study files. A DLT specific form will be used to report DLTs and is also provided in the investigator files. Please refer to the investigator study files for instructions on how to complete these forms" to "**The SAE Report form is provided in the investigator study files. Please refer to the investigator study files for instructions on how to complete this form."** Also deleted all occurrences of "DLT"	Requested by ZIOPHARM Safety for clarity
Section 10.10 (page 71)	Section 10.10, changed "The Sponsor should be immediately notified" to "The Sponsor **must** be immediately notified"	Requested by ZIOPHARM Safety for clarity
Section 11.1 (page 72)	Section 11.1, changed from "Immunologic markers will be examined in core biopsy (punch or FNA biopsy accepted in the absence of the core biopsy) taken on days 1 and 7. Biopsies will be by evaluated by immunocytochemistry for evidence of tumor infiltration by effector cells such as T cells and their subsets, as well as immune suppressor elements, such as T-regulatory cells and myeloid-derived suppressor cells, and other immunological markers. Antibodies to be used in the immunocytochemistry analysis may include, but are not limited to those against cluster of differentiation (CD) antigens CD3, CD4, CD8, CD25, FOX-P3, and CD56, and CD45RO" to **"Soluble factors, such as cytokines, chemokines, soluble receptors, and antibodies to tumor antigens will be characterized and quantified**	Requested by principal investigator and site staff

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	by immunoassays in serum. Analyses may include, but are not necessarily limited to serum IL-12, IL-10, and IFN-γ. The proportion of specific lymphocyte subsets and expression levels of T cell co-stimulatory markers in peripheral blood mononuclear cell (PBMC) preparations will be quantified by flow cytometry. Analyses may include, but not necessarily be limited to, the proportion of T, B, and NK cells, proportion of memory and effector T cell subsets, and expression levels of PD-1, PDL1, ICOS, and Ki67."	
Section 11.2 (page 72)	Section 11.2, changed from "Immunologic markers will be examined in core biopsy (punch or FNA biopsy accepted in the absence of the core biopsy) taken on days 1 and 7" to "Immunologic markers will be examined in core biopsy (punch or FNA biopsy accepted in the absence of the core biopsy) taken on **day 1 and at week 6."**	Requested by principal investigator and site staff
Section 12.1 (page 73)	Section 12.2, changed from **"**This sample size will allow us to estimate an overall progression rate with a maximum 95% exact confidence interval half-width of 0.16" to "This sample size will allow us to estimate an overall progression rate **at 12 weeks** with a maximum 95% exact confidence interval half-width of 0.16. **This is based on an exact binomial calculation and is an estimate. However, analysis of progression-free survival will be conducted using Kaplan-Meier methods."**	Requested by principal investigator and site staff
Section 12.4.2 (page 74)	Section 12.4.2, changed from "Deaths, SAEs, and AEs resulting in study discontinuation will be listed" to "Deaths, SAEs, and AEs resulting in **patient** discontinuation will be listed"	Requested by ZIOPHARM Medical Monitor for clarity
Section 12.4.3.2 (page 75)	Section 12.4.3.2, changed "The proportion failed by 12 weeks is denoted as the progression rate and is derived based on the sum of progression events, death events and subjects who discontinue the trial due to an AE" to "The proportion failed by 12 weeks is denoted as the progression rate **survival** and is derived based on the sum of progression events, death events and subjects who discontinue the trial due to an AE"	Requested by ZIOPHARM Statistician for clarity
Section 12.4.3.3 (page 76)	Section 12.4.3.3, changed "Proportion of subjects who are progression free at Week 12 (proportion failed) by 12 weeks after the start of immunotherapy" to "Proportion of subjects who **have 12-week progression free survival** (proportion failed) after the start of immunotherapy."	Requested by ZIOPHARM Statistician for clarity

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Appendix 16.2	Appendix 16.2, Added **"NOTE: This is not a complete list of CYP450 3A4 substrates, inhibitors and inducers. To use a drug of unclear CYP450 3A4 status, please contact the ZIOPHARM medical monitor."**	Requested by ZIOPHARM Medical Monitor for clarity
Pages 89 and 91	Added "**Fosaprepitant (Emend)"**	Requested by ZIOPHARM Medical Monitor for clarity

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Protocol Title:	A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First-, Second-, or Third-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer
Protocol Number:	ATI001-203
Study Drugs:	[redacted]-human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand)
Date of Protocol:	Amendment 2: 16 May 2016
Sponsor:	ZIOPHARM Oncology, Inc. [redacted]

NOTE TO INVESTIGATORS

Amendment 2 dated 16 May 2016 will be used to conduct the study in place of any preceding version of this protocol. ZIOPHARM Oncology will implement this version as of 16 May 2016. This protocol should be submitted to your IRB promptly.

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AMENDMENT 2

ATI001-203

1. Summary and Rationale for Changes

ZIOPHARM is amending the clinical protocol for the ATI001-203 study, "A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First-, Second-, or Third-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer" to allow enrollment of subjects who have received 3rd-line therapy, add long-term follow-up imaging studies, clarify the definition of cycle and the related timing of SRC meetings, and add in safety assessment visit at the end of the cycle (Day 21). Additionally, updates including correction of typos, administrative edits and formatting were made.

The following administrative edits were made throughout the document:

- "serum" to "blood"
- "study drug" to "investigational product"
- Clarification of "immunotherapy" and "study drug treatment" as "Ad-RTS-hIL-12 immunotherapy"

2. Tabular Summary of Key Revisions Implemented in the Amended Protocol

*indicates sections where revision text is not verbatim

Section in Amended Protocol	Revision	Rationale for Change
Title Page; Synopsis Title	**FROM:** A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First- or Second-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer **TO:** A Single-arm, Open-label Study of Ad-RTS-hIL-12 + Veledimex Following First-, Second-, or Third-Line Standard Treatment in Subjects with Locally Advanced or Metastatic Breast Cancer	Title update required due to change in inclusion criteria.
Title Page and Headers	**FROM:** Amendment 1: 05 November 2014 **TO:** Amendment 2: 16 May 2016	Updated to reflect current protocol version and date of amendment.

Section in Amended Protocol	Revision	Rationale for Change
Title Page	[redacted]	Updated to reflect current medical monitor.
Synopsis Primary Objective; Synopsis Statistical Methods; Section 2.1; Section 12.3.1	**FROM:** • To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-or second-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first- or second-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by Section 12.4.2. **TO:** • To evaluate the safety and tolerability of one cycle of Ad-RTS-hIL-12 immunotherapy a) following a first-, second-, or third-line standard treatment in HER2-negative (HER2-) subjects, or b) together with a first-, second-, or third-line anti-HER2 antibody therapy in HER2-positive (HER2+) subjects as defined by Section 12.4.2.	Updated to allow enrollment of subjects who have had 3 lines of chemotherapy and would otherwise qualify for the study.
Synopsis Study Design; Section 1.7; Section 3.1	**FROM:** first- or second-line chemotherapy **TO:** first-, or second-, or third-line chemotherapy	Updated to allow enrollment of subjects who have had 3 lines of chemotherapy and would otherwise qualify for the study.
Synopsis Study Design	**FROM:** Radiographic PD at week 6 must be confirmed at least 4 weeks later, either at week 12 or earlier if clinically necessitated. **TO:** Radiographic PD at week 6 must be confirmed at least 4 weeks later, either at week 12 or earlier if clinically necessitated, except in cases of unequivocal progression.	Updated to clarify confirmation is not required in cases of unequivocal progression as outlined in Section 8.4.

Section in Amended Protocol	Revision	Rationale for Change
Synopsis Study Design; Section 3.1	**FROM:** b) after a minimum of 12 weeks of pre-study standard chemotherapy, ie, just prior to study enrollment, in order to confirm their SD, PR status. **TO:** b) after a minimum of 12 weeks of pre-study standard chemotherapy, and not more than 4 weeks prior to study enrollment, in order to confirm their SD, PR status.	Clarification that scans completed after 12 weeks on pre-study standard chemotherapy can be used as baseline scans only if they are completed within 4 weeks prior to study enrollment.
Synopsis Study Design; Section 3.1	**FROM:** The Ad-RTS-hIL-12 treatment phase comprises one 21-day cycle of Ad-RTS-hIL-12 immunotherapy (treatment administered on days 1 through 7 followed by rest on days 8 through 21). **TO:** The Ad-RTS-hIL-12 immunotherapy cycle is a 21 day cycle comprised of investigational product administration on days 1 through 7 followed by rest on days 8 through 21.	Clarification of a cycle of Ad-RTS-hIL-12 immunotherapy.
Synopsis Study Design; Section 3.1	**FROM:** A safety review committee (SRC) will convene after every 5th subject with HER2- disease has completed the Ad-RTS-hIL-12 immunotherapy as well as after the first 5 subjects with HER2+ disease have completed the Ad-RTS-hIL-12 immunotherapy cycle (ie, after all 5 subjects have completed 21 days on study). **TO:** A safety review committee (SRC) will convene after every 5th subject with HER2- disease has completed the Ad-RTS-hIL-12 immunotherapy cycle (i.e., after all 5 subjects have completed 21 days on study). The SRC will also convene after the first 5 subjects with HER2+ disease have completed the Ad-RTS-hIL-12 immunotherapy cycle (i.e., after all 5 subjects have completed 21 days on study).	Clarification of the scheduling of the SRC meetings.
Synopsis Study Design; Section 3.1	**FROM:** The safety evaluation will be based on the occurrence of refractory Grade 3 and 4 AEs within 21 days following Ad-RTS-hIL-12 immunotherapy. **TO:** The safety evaluation will be based on the occurrence of related, treatment refractory Grade 3 and 4 AEs within 21 days following the start of Ad-RTS-hIL-12 immunotherapy.	Clarification.
Synopsis Study Design; Section 3.1	**ADDED:** Upon completion of week 12, patients who are considered to be SD or better will enter into long-term follow-up for up to an additional 36 weeks. In the long-term follow-up period, scans will be performed at 18, 24, 36, and 48 weeks until a radiographic response of PD is confirmed.	Updated to add long-term follow-up.

Section in Amended Protocol	Revision	Rationale for Change
Synopsis Study Population; Section 4.1	**FROM:** 3. Achievement of SD or PR after a minimum of 12 weeks of pre-study first- or second-line standard chemotherapy **TO:** 3. Achievement of SD or PR after a minimum of 12 weeks of pre-study first-, second-, or third-line standard chemotherapy NOTE: A prior therapy that is not completed due to toxicity will not be considered a line of therapy for eligibility determination.	Updated to allow enrollment of subjects who have had 3 lines of chemotherapy and would otherwise qualify for the study.
Synopsis Study Population; Section 4.1	**FROM:** a. ANC > 1500/μL (without use of growth factors within 7 days of screening) **TO:** a. ANC ≥ 1500/μL (without use of growth factors within 7 days of screening)	Correction of typographical error. This was previously included in a memo.
Synopsis Study Population; Section 4.2	**FROM:** 10. Use of any medications that induce, inhibit, or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of study drug (see Appendix 16.2) **TO:** 10. Use of any medications that induce, inhibit, or are substrates of cytochrome P450 (CYP450) 3A4 within 7 days prior to the first dose of investigational product without consultation with the medical monitor	Updated to allow sites to treat subjects according to their standard practice, with consultation with the medical monitor.
Synopsis Study Population; Section 4.2	**FROM:** 19. Any other unstable or clinically significant concurrent medical condition that would in the opinion of the investigator jeopardize the safety of a subject and/or their compliance with the protocol **TO:** 19. Any other unstable or clinically significant concurrent medical condition that would in the opinion of the investigator or medical monitor jeopardize the safety of a subject and/or their compliance with the protocol	Updated to include medical monitor in decision-making.
Synopsis Study Population; Section 4.2	**FROM:** 22. Participation in any other clinical trial from screening through the final visit 28 days after the completion of the last study treatment **TO:** 22. Current participation in any other clinical trial without consultation with the medical monitor	Updated to allow for long-term follow-up.

Section in Amended Protocol	Revision	Rationale for Change
Synopsis Duration of Each Subject's Participation; Section 13.4	**FROM:** Enrolled subjects will participate for approximately 20 weeks, including up to 4 weeks for screening activities. **TO:** Enrolled subjects will participate for up to 52 weeks, including up to 4 weeks for screening activities.	Updated to add long-term follow-up.
Synopsis Statistical Methods; Section 3.1; Section 12.4.2	**FROM:** treatment refractory Grade 3 or 4 AEs **AND** refractory Grade 3 or 4 AEs **TO:** related, treatment refractory Grade 3 or 4 AEs	Clarification.
Synopsis Statistical Methods; Section 12.4.2	**FROM:** Toxicity stopping rules, when applicable, will be determined based on a clinical assessment made by the SRC after a formal evaluation of safety data for every 5 subjects with HER2- disease as well as after the first 5 subjects with HER2+ disease have completed the Ad-RTS-hIL-12 immunotherapy. **TO:** Toxicity stopping rules, when applicable, will be determined based on a clinical assessment made by the SRC after a formal evaluation of safety data after every 5 subjects with HER2- disease have completed one cycle of Ad-RTS-hIL-12 immunotherapy. The SRC will review data separately for subjects with HER2+ disease to assess toxicity after the first 5 subjects with HER2+ disease have completed one cycle of Ad-RTS-hIL-12 immunotherapy.	Updated to clarify the timing of the SRC meetings.
Study Schema	**The figure has been replaced.**	Updated to add long-term follow-up.
Schedule of Study Procedures	**The following visits have been added as new visits:** End of Cycle, Week 18, Week 24, Week 36, Week 48.	Updated to add long-term follow-up.
Schedule of Study Procedures; Section 6.2*	**FROM:** Subjects must be instructed to maintain adequate oral hydration on and in between veledimex dosing days; sites must closely monitor subjects' hydration status. **TO:** Subjects must be instructed to maintain adequate oral hydration while taking veledimex; sites must closely monitor subjects' hydration status.	Updated to clarify veledimex dosing as there are no days between doses.
Schedule of Study Procedures	**ADDED:** Concomitant medications should be collected from screening through Week 12. In addition, any new anti-cancer medication started prior to confirmation of radiographic response of PD should be collected.	Updated to clarify timing of medication collection.

Section in Amended Protocol	Revision	Rationale for Change
Schedule of Study Procedures;	**FROM:** Females of childbearing potential will have a serum pregnancy test at the screening visit within 14 days of dosing and a urine pregnancy test at Day 1. **TO:** Females of childbearing potential will have a serum pregnancy test during screening (within 14 days of dosing) and a urine pregnancy test at Day 1. A serum pregnancy test should be completed on Day 1 if the urine pregnancy test is positive.	Updated to add requirement of serum pregnancy test at Day 1 to confirm a positive urine pregnancy test.
Schedule of Study Procedures; Section 8.3.1.7*; Section 8.4.1*	**FROM:** The hematology panel includes complete blood count (CBC) and white blood cell (WBC) count, differential white blood cell count, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices, mean corpuscular volume (MCV) and platelet count. **TO:** The hematology panel includes complete blood count (CBC) and white blood cell (WBC) count, differential white blood cell count, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices (MCV, MCH, MCHC), and platelet count.	Updated to correct the list of tests included in the panel.
Schedule of Study Procedures	**FROM:** The first dose of veledimex will be given at the clinical site in a fed state (preferably within 30 minutes after the start of a normal meal). **TO:** The first dose of veledimex will be given at the clinical site in a fasting state.	Updated to note that veledimex will be administered in a fasting state only on day 1 due to the biopsy. This was previously included in a memo.
Schedule of Study Procedures	**FROM:** Veledimex dispensed for the next 6 days (the first dose is to be given in the clinic). The dosing diary will be dispensed at this time point along with the veledimex. **TO:** Veledimex will be dispensed to the subject on Day 1 for the next 6 days along with the veledimex subject diary.	Administrative change.

Section in Amended Protocol	Revision	Rationale for Change
Schedule of Study Procedures*; Section 5.4	**FROM:** Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate breakfast, the number of capsules taken, whether subject missed any veledimex doses and the study day and reason for any missed doses. **TO:** Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate last meal prior to administration of veledimex, the number of capsules taken, whether subject missed any veledimex doses and the study day and reason for any missed doses.	Updated to clarify that the last meal prior to dosing should be captured regardless of which meal that was.
Schedule of Study Procedures	**REMOVED TEXT:** For the purpose of this clinical trial, the following imaging is expected at screening: Computed tomography (CT) of the chest, abdomen +/- pelvis PET or Bone Scintigraphy (BS) at the treating physician's discretion MRI or CT of the brain, if brain metastasis are known or suspected MRI of other anatomical regions as clinically indicated	Captured in section 8.
Schedule of Study Procedures; Section 8.3.1.8	**ADDED TEXT:** NOTE: In the event that IV contrast cannot be used, the medical monitor should be consulted.	Added to request consultation with the medical monitor if contrast cannot be used.
Schedule of Study Procedures	**ADDED TEXT:** The Day 21 visit will collect adverse events and concomitant medication. This visit may be completed via phone call.	Added to clarify that the Day 21 visit is only to collect AEs and concomitant medications and that it may be completed by phone.
Schedule of Study Procedures*; Section 3.1	**ADDED TEXT:** In cases of slow tumor growth, the investigator may wait until confirmation of PD at 12 weeks to resume chemotherapy. In cases of unequivocal progression, the investigator may resume chemotherapy prior to 12 weeks and the subject will be considered as treatment failure at 12 weeks.	This text is already included in Section 8.4 of the protocol and has been added to these sections for clarity.
Schedule of Study Procedures	**FROM:** Imaging should occur every 6-8 weeks until a response is confirmed per irRC (i.e. SD, PD, PR, or CR). **TO:** Subjects who have SD or better at Week 12 will continue into the long-term follow-up period where imaging should continue per the schedule until a radiographic response of PD is confirmed.	Updated to add imaging to the long-term follow-up visits.

Section in Amended Protocol	Revision	Rationale for Change
Schedule of Study Procedures	**FROM:** Biopsy on Day 1 is to be taken prior to veledimex dosing. **TO:** Biopsy on Day 1 is to be taken 3 hours ± 3 hours after administration of veledimex and prior to injection of Ad-RTS-hIL-12.	Updated to correct the order in which the biopsy is completed in relation to administration of veledimex and Ad-RTS-hIL-12. This was previously clarified in a memo.
Schedule of Study Procedures	**ADDED TEXT:** After Week 6, only SAEs suspected of being related to Ad-RTS-hIL12 immunotherapy will be collected.	This is in Section 8.3.1.6 of the protocol and has been added to the Schedule for clarity.
Schedule of Study Procedures	**FROM:** The 6 and 12 week visits can be performed within ± 1 week. **TO:** The follow-up visits can be performed within ± 1 week.	Updated to add long-term follow-up.
Section 3.1	**FROM:** A post-treatment safety assessment visit will be conducted 28 days after the last dose of veledimex. **TO:** A post-treatment safety assessment visit will be conducted on Day 21.	Updated to clarify the timing of the post-treatment safety assessment visit.

Section 4.4	**FROM:** A subject may withdraw (or be withdrawn) from the study treatment prematurely for any of the following reasons: • The investigator determines further participation is not in subject's best interest (for example, subject experiences rapid clinical deterioration in the absence of confirmed disease progression) • Subject has confirmed disease progression A subject MUST be withdrawn in the event of any of the following: • Withdrawal of informed consent • Note: Any subject who wishes to withdraw from the study treatment may do so at any time, but will be asked to be available for the safety if withdrawal occurs before 12 weeks. • Any treatment-related AE(s) that meet withdrawal criteria • Substantial noncompliance with study requirements • Confirmed positive pregnancy test • Any intercurrent illness that would, in the judgement of the investigator or Sponsor's medical monitor, affect assessments of clinical status to a significant degree and require discontinuation of protocol therapy Every effort should be made to follow subjects who withdraw from study treatment for ongoing treatment-related AEs. **TO:** A subject may discontinue study treatment prematurely for any of the following reasons: • The investigator determines further participation is not in subject's best interest (for example, subject experiences rapid clinical deterioration in the absence of confirmed disease progression) • Subject has confirmed disease progression A subject MUST discontinue study treatment in the event of any of the following: • Subject request to end study treatment • Any treatment-related AE(s) that meet withdrawal criteria • Substantial noncompliance with study requirements • Confirmed positive pregnancy test • Any intercurrent illness that would, in the judgement of the investigator or Sponsor's medical monitor, affect assessments of clinical status to a significant degree and require discontinuation of protocol therapy A subject MUST be withdrawn from the study in the event of any of the following: • Withdrawal of informed consent for the study • The Ad-RTS-hIL-12 injection was not received NOTE: Any subject receiving veledimex will be included in the safety analysis group even if the Ad-RTS-hIL-12 injection was not received. Every effort should be made to follow subjects who withdraw from study treatment for ongoing treatment-related AEs until resolved, no resolution is expected, or the study has ended.	Updated to clarify key differences between treatment discontinuation and study withdrawal and expected follow-up for AEs.

Section in Amended Protocol	Revision	Rationale for Change
Section 4.5	**FROM:** Subjects who withdraw from the study during the screening period and prior to starting Ad-RTS-hIL-12 immunotherapy, or those who withdraw at any time during the initial 7 days of veledimex dosing for reasons other than toxicity or disease progression may be replaced. **TO:** Subjects who withdraw from the study during the screening period and prior to starting Ad-RTS-hIL-12 immunotherapy, or those who withdraw or discontinue treatment at any time during the initial 7 days of veledimex dosing for reasons other than toxicity or disease progression may be replaced.	Updated to clarify which subjects may be replaced.
Section 6.2	**FROM:** On day 1, veledimex will be administered 3 hours ± 3 hours before intratumoral injection of Ad-RTS-hIL-12. **TO:** On day 1, veledimex will be administered in a fasting state 3 hours ± 3 hours before the tumor biopsy and intratumoral injection of Ad-RTS-hIL-12.	Clarification that the biopsy should be done between dosing with veledimex and injection of Ad-RTS-hIL-12 and that veledimex will be administered in a fasting state only on day 1.
Section 6.5	**FROM:** Subjects who experience drug-related Grade 3 AEs during the first 7 days of oral dosing with veledimex will interrupt/discontinue further dosing at the discretion of the investigator. Subjects who experience drug-related Grade 4 AEs during the first 7 days of oral dosing with veledimex will discontinue therapy. The decision as to whether subjects who experience severe or serious treatment-related adverse events will remain on study will be made by the investigator in conjunction with the Sponsor Medical Monitor. **TO:** Not applicable.	Dose Limiting Toxicity is not applicable for this study.
Section 7	**FROM:** Information on concomitant medications, including all medications, blood products, vitamins, and other supplements, will be collected from the Screening visit through the End-of-Study visit at 12 weeks after the start of Ad-RTS-hIL-12 immunotherapy. **TO:** Information on concomitant medications, including all medications, blood products, vitamins, and other supplements, will be collected from the Screening visit through the end of the Initial Follow-up Period (Week 12). Any new anti-cancer therapy that is started prior to confirmation of a radiographic response of PD, should be collected and entered as a concomitant medication.	Updated to clarify that the Week 12 visit is no longer the end of study visit and add in requirement for collecting new anti-cancer therapies.

Section in Amended Protocol	Revision	Rationale for Change
Section 7.2	**FROM:** • Medications that are classified as CYP450 3A4 inducers, inhibitors or substrates (see Appendix 16.2) **TO:** NOTE: Care should be given when prescribing medications that are classified as CYP450 3A4 inducers, inhibitors, or substrates due to potential interactions with the investigational product. In the event that one is prescribed, consultation with the medical monitor is advised. All medications should be recorded in the case report form (CRF) as indicated in the CRF completion guidelines.	Updated to allow sites to treat subjects according to their standard practice, with consultation with the medical monitor.
Section 8.3.1.1	**ADDED:** Concomitant medications will continue to be collected through the Initial Follow-up Period (Week 12).	
Section 8.3.1.5	**FROM:** The pregnancy test must be negative prior to Ad-RTS-hIL-12 injection. **TO:** The pregnancy test must be negative prior to administration of veledimex and injection of Ad-RTS-hIL-12. A serum pregnancy test should be completed on Day 1 if the urine pregnancy test is positive.	Clarification that a negative pregnancy test is required prior to administering either investigational product.
Section 8.3.1.6	**FROM:** AEs/SAEs occurring prior to the signing of the informed consent should be entered into the medical history. AEs/SAEs from the signing of informed consent until the end of week 6 will be collected and entered into the safety database. Between weeks 6 and 12, only SAEs suspected of being related to Ad-RTS-hIL12 will be collected. **TO:** AEs and SAEs from the signing of informed consent until the end of week 6 will be collected and entered into the case report forms (CRFs). After Week 6, only SAEs suspected of being related to Ad-RTS-hIL12 immunotherapy will be collected.	Updated to add long-term follow-up.
Section 8.3.1.8	**HEADER FROM:** CT chest/abdomen +/- pelvis **TO:** Imaging Studies	Updated to be consistent with Schedule of Procedures.

Section in Amended Protocol	Revision	Rationale for Change
Section 8.3.1.8	**MOVED TEXT FROM SECTION 8.4.1:** Tumor response imaging for the pre-study standard chemotherapy must be completed within 4 weeks of study enrollment and must meet the criteria listed below in order to be used as screening/baseline scans. In the event that the scans are not adequate, they should be repeated. i. Computed tomography (CT) of the chest, abdomen +/- pelvis ii. PET or Bone Scintigraphy (BS) at the treating physician's discretion iii. MRI or CT of the brain, if brain metastasis are known or suspected iv. MRI of other anatomical regions as clinically indicated All imaging should be of diagnostic quality and include intravenous (IV) contrast. Please refer to Appendix 16.3 for RECIST v 1.1 guidelines.	Moved to include this text in the section that describes the Imaging Studies instead of the section that lists the assessments required at each visit.
Section 8.3.1.8	**FROM:** The same imaging method used in the pre-study phase should be used for the tumor assessment performed at 6 and 12 weeks for the primary assessment of efficacy. PET or bone scintigraphy may also be requested at the treating physician's discretion. Scans should be available for collection upon Sponsor request. **TO:** The same imaging method used for screening/baseline scans should be used for the tumor assessment performed at 6 and 12 weeks for the primary assessment of efficacy. Scans should be available for collection upon Sponsor request.	Updated "pre-study phase" to "screening/baseline" for consistency and removed statement regarding PET as it is already stated in this section.
Section 8.4.2	**ADDED:** Urine pregnancy test	Urine pregnancy test on Day 1 is indicated in the Schedule of Procedures but was not previously listed in this section.
Section 8.4.7	**ADDED TEXT:** Immunotherapy Cycle: Day 21 • Adverse events • Concomitant medications	Updated to add the post-treatment safety assessment visit.
Section 8.4.10-Section 8.4.13	**ADDED TEXT (for Weeks 18, 24, 36, 48):** Long-Term Follow-Up Period: Week [#] • Imaging studies for tumor response	Updated to add long-term follow-up.
Section 8.4	**REMOVED TEXT:** Unscheduled Visits • Physical examination • ECOG performance status • Adverse events • Concomitant medications • Hematology panel • Serum chemistry panel • Research bloods	An unscheduled visit can consist of any assessments requested by the PI and all assessments previously noted should not be required.

Section in Amended Protocol	Revision	Rationale for Change
Section 9.2.2.2	**FROM:** incomplete response/SD **TO:** non-CR/non-PD	Updated to correct RECIST terminology.
Section 9.2.2.2	**REMOVED TEXT:** and the progression status should be confirmed later on by the review panel (or study chair)	Removed as there is no separate review panel for this study.
Section 10	**HEADER FROM:** ADVERSE EVENTS **TO:** SAFETY ASSESSMENTS	Updated to be consistent with ZIOPHARM standard language.
Section 10.1	**FROM:** An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal product, whether or not related to the medicinal product. An AE includes but is not limited to any clinically significant worsening of a subject's pre-existing condition. An abnormal laboratory finding (including electrocardiogram [ECG] finding) that requires an action or intervention by the investigator, or a finding judged by the investigator to represent a change beyond the range of normal physiologic fluctuation, should be reported as an AE. **TO:** An AE can therefore be any unfavorable and unintended sign including an abnormal laboratory finding, symptom, or disease temporarily associated with the use of a medicinal product, and any worsening of pre-existing conditions, regardless of causality to the medicinal product.	Updated to include ZIOPHARM standard language for adverse events and remove statement that appears to limit collection of abnormal laboratory values.
Section 10.1	**ADDED TEXT:** <u>Suspected Adverse Reaction:</u> Any AE for which there is evidence to suggest a causal relationship (reasonable possibility) between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction. <u>Adverse Reaction:</u> Any AE caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. <u>Unexpected Adverse Reaction:</u> Any AE that is (a) not listed in the Investigator's Brochure, (b) not listed with the specificity and severity that is being observed, (c) not consistent with the risk information described in the general investigational plan or elsewhere in the current application (in the absence of an investigator brochure), and (d) listed as occurring with a class of drugs, but not specifically mentioned as occurring with the particular drug under investigation.	Updated to include ZIOPHARM standard language for adverse events.

Section in Amended Protocol	Revision	Rationale for Change
Section 10.2	**ADDED TEXT:** Adverse events include: • Suspected adverse drug reactions • Reactions from investigational product overdose, abuse, withdrawal, sensitivity, or toxicity • Significant changes or abnormalities when compared to baseline, in signs, symptoms, clinical laboratory results, or physiological testing. This includes any worsening of a pre-existing condition temporally associated with the use of investigational product. • Other untoward medical events, regardless of their relationship to the investigational product, such as injury, events that require surgery, accidents, extensions of symptoms, or apparently unrelated illnesses	Updated to include ZIOPHARM standard language for adverse events.
Section 10.3.1	**ADDED TEXT:** • New cancer: Occurrence or diagnosis of a new cancer during the trial is considered an SAE. (This does not pertain to metastasis of current disease); • Any AE associated with an overdose of the investigational product: An overdose of investigational product is defined as an occurrence of administered dose exceeding that which is prescribed by the investigator per protocol.	Updated to include ZIOPHARM standard language for adverse events.
Section 10.5	**FROM:** A number of factors should be considered in making this assessment including: 1) the temporal relationship of the event to the administration of investigational product; 2) whether an alternative etiology has been identified; and 3) biological plausibility. The following guidelines should be used by investigators to assess the relationship of an AE to investigational product administration (study drug administration). **TO:** A number of factors should be considered in making this assessment including: • Alternative possible causes of the AE, including the subject's underlying disease or comorbid conditions, other drugs, other host, and environmental factors • The temporal sequence between the exposure to investigational product and the AE • Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the investigational product or similar drugs • Whether the AE resolved or improved with decreasing the dose or stopping the investigational product (i.e., dechallenge); or recurred or worsened with re exposure to the drug (i.e., rechallenge).	Updated to include ZIOPHARM standard language for adverse events.

Section in Amended Protocol	Revision	Rationale for Change
Section 10.9	**FROM:** All AEs and SAEs will be collected from the signing of the ICF through the end-of-treatment visit at Week 12. Follow up information for ongoing SAEs will be requested by the Sponsor until one of the following occurs: • The event resolves • The event returns to baseline • The event stabilizes **TO:** All AEs and SAEs will be collected from the signing of the ICF through Week 6. After Week 6, only SAEs suspected of being related to investigational product will be collected. Follow up information for ongoing SAEs will be requested by the Sponsor until one of the following occurs: • The event resolves • The event returns to baseline, if a baseline value is available • The event stabilizes (following consultation and agreement by the medical monitor) • The event can be attributed to factors other than the investigational product or other than study procedure	Updated to include ZIOPHARM standard language for adverse events and to clarify requirements for collection of AEs/SAEs.
Section 10.10	**FROM:** Subjects who become pregnant during the study should immediately discontinue participation in the study. **TO:** Subjects who become pregnant during the study should immediately discontinue treatment.	Clarification that subjects must immediately discontinue treatment if they become pregnant.
Section 11.2	**FROM:** Immunologic Markers **TO:** Cytokine and Immunologic Markers	Added to allow for tumor cytokine analyses.
Section 12.4.3	**REMOVED TEXT:** The database will be locked after all subjects who have not discontinued the trial for another reason complete the 12-week tumor assessment.	Removed to allow for long-term follow-up.
Section 13.4	**FROM:** The overall duration of the entire study (screening, treatment, and follow-up for all 40 subjects) is expected to be approximately 2.5 years. **TO:** The overall duration of the entire study (screening, treatment, and follow-up for all subjects) is expected to be approximately 3.5 years.	Updated to add long-term follow-up.

Section in Amended Protocol	Revision	Rationale for Change
Section 14	**FROM:** All deviations from the protocol will be documented in the CRF. Any significant deviation or deviation related to dosing or safety evaluation will be reported to ZIOPHARM and documented in the CRF. **TO:** All deviations from the protocol will be documented. Any significant deviation or deviation related to dosing or safety evaluation will be reported to ZIOPHARM.	Updated as deviations are not captured in the CRF.
Appendix 16.2	**APPENDIX REMOVED:** Appendix 16.2 CYP450 3A4 Substrates, Inhibitors, and Inducers	Updated to allow sites to treat subjects according to their standard practice, with consultation with the medical monitor. A separate guidance document will be issued.