A Phase 1-2 multicenter, open-label trial of H3B-6545, a covalent antagonist of estrogen receptor alpha, in women with locally advanced or metastatic estrogen receptor-positive, HER2 negative breast cancer

SPONSOR STUDY PROTOCOL NUMBER:	H3B-6545-A001-101
IND NUMBER: **EUDRACT NUMBER:**	**133,282** **2018-000570-29**
STUDY DRUG:	H3B-6545
SPONSOR:	Eisai Inc. 200 Metro Boulevard Nutley, NJ 07110 USA
STUDY CHAIR:	PPD Sarah Cannon Research Institute 1100 Charlotte Avenue, Suite 800 Nashville, TN 37203 USA 1-877-MY-1-SCRI

DATE FINAL:	19 May 2017
AMENDMENT NUMBER 1	CCI [redacted]
AMENDMENT NUMBER 2	
AMENDMENT NUMBER 3	
AMENDMENT NUMBER 4	
AMENDMENT NUMBER 5	
AMENDMENT NUMBER 6	
AMENDMENT NUMBER 7	
AMENDMENT NUMBER 8	
AMENDMENT NUMBER 9	

CLINICAL STUDY SIGNATURE APPROVAL FORM

A Phase I-II multicenter, open-label trial of H3B-6545, a covalent antagonist of estrogen receptor alpha, in women with locally advanced or metastatic estrogen receptor-positive, HER2 negative breast cancer

SPONSOR STUDY PROTOCOL NUMBER:	H3B-6545-A001-101
IND NUMBER:	**133,282**
EUDRACT NUMBER:	**2018-000570-29**
DATE FINAL:	19 May 2017
AMENDMENT 9:	29 Aug 2022

By signing this page, I confirm I have read and approved the contents of this amendment to this clinical study protocol.

DocuSigned by:
PPD
Signer Name: PPD
Signing Reason: I approve this document
Signing Time: 07-Sep-2022 | 10:36:36 EDT
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07 Sep 2022

SIGNATURE
Sponsor Medical Director
PPD
Eisai Inc.

Date

DocuSigned by:
PPD
Signer Name: PPD
Signing Reason: I approve this document
Signing Time: 07-Sep-2022 | 11:54:12 EDT
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07 Sep 2022

SIGNATURE
Sponsor Biostatistician Lead
PPD
Eisai, Inc.

Date

CLINICAL STUDY PRINCIPAL INVESTIGATOR SIGNATURE FORM

A Phase I-II multicenter, open-label trial of H3B-6545, a covalent antagonist of estrogen receptor alpha, in women with locally advanced or metastatic estrogen receptor-positive, HER2 negative breast cancer

SPONSOR STUDY PROTOCOL NUMBER:	H3B-6545-A001-101
IND NUMBER: **EUDRACT NUMBER:**	**133,282** **2018-000570-29**
DATE FINAL:	19 May 2017
AMENDMENT NUMBER 9:	29 Aug 2022

By signing this protocol acceptance page, I confirm I have read, understand, and agree to conduct the study in accordance with the current protocol.

Principal Investigator Name <<Insert Site Name and ID info as applicable>> <<Insert Site Location>>	**Principal Investigator Signature**	**Date**

H3B-6545-A001-101 PROTOCOL SUMMARY OF CHANGES

Date	Highlights of Major Changes Section/Change
19 May 2017	Original Protocol
CCI [redacted]	CCI [redacted]
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Date	**Highlights of Major Changes** **Section/Change**
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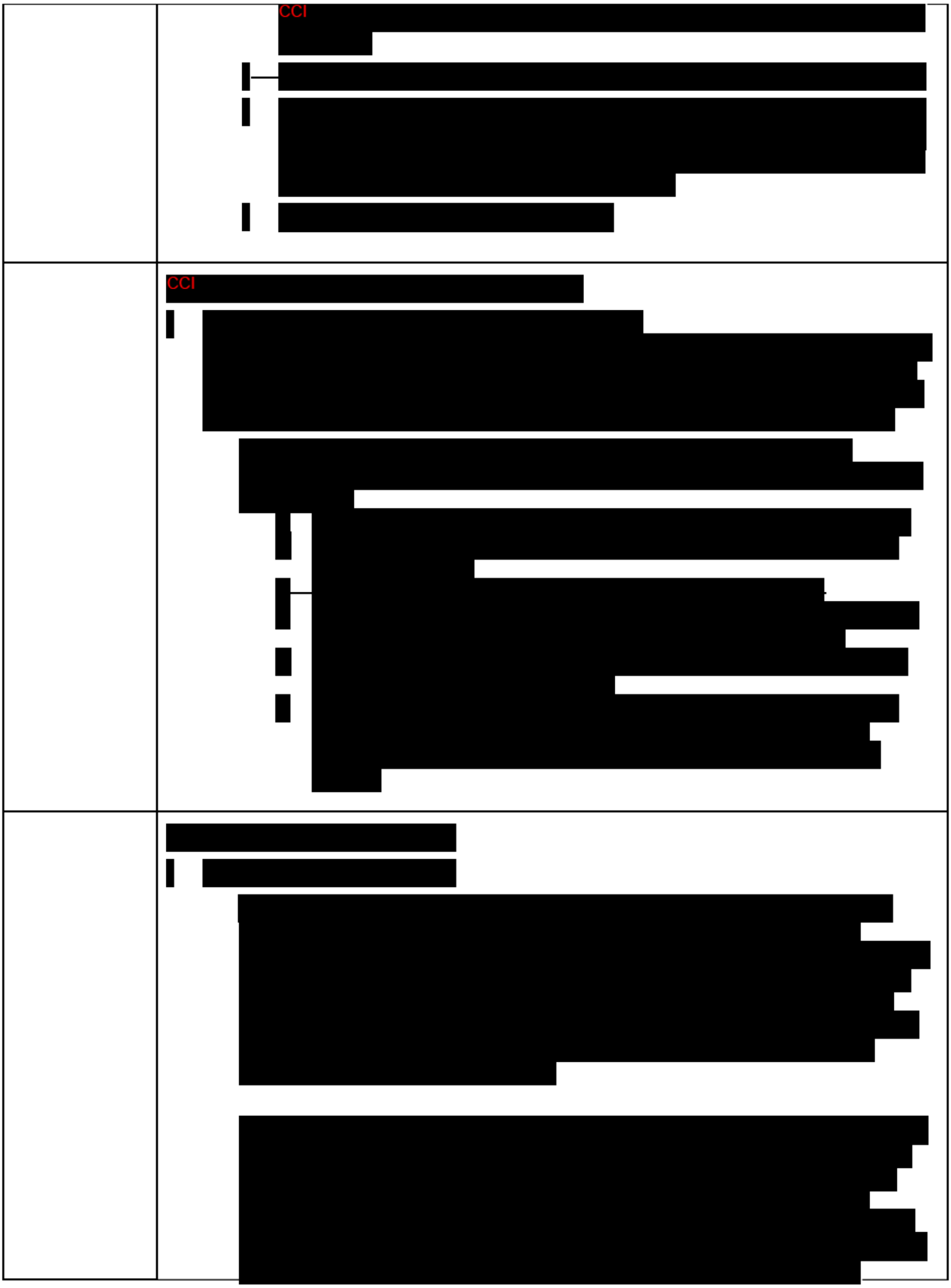
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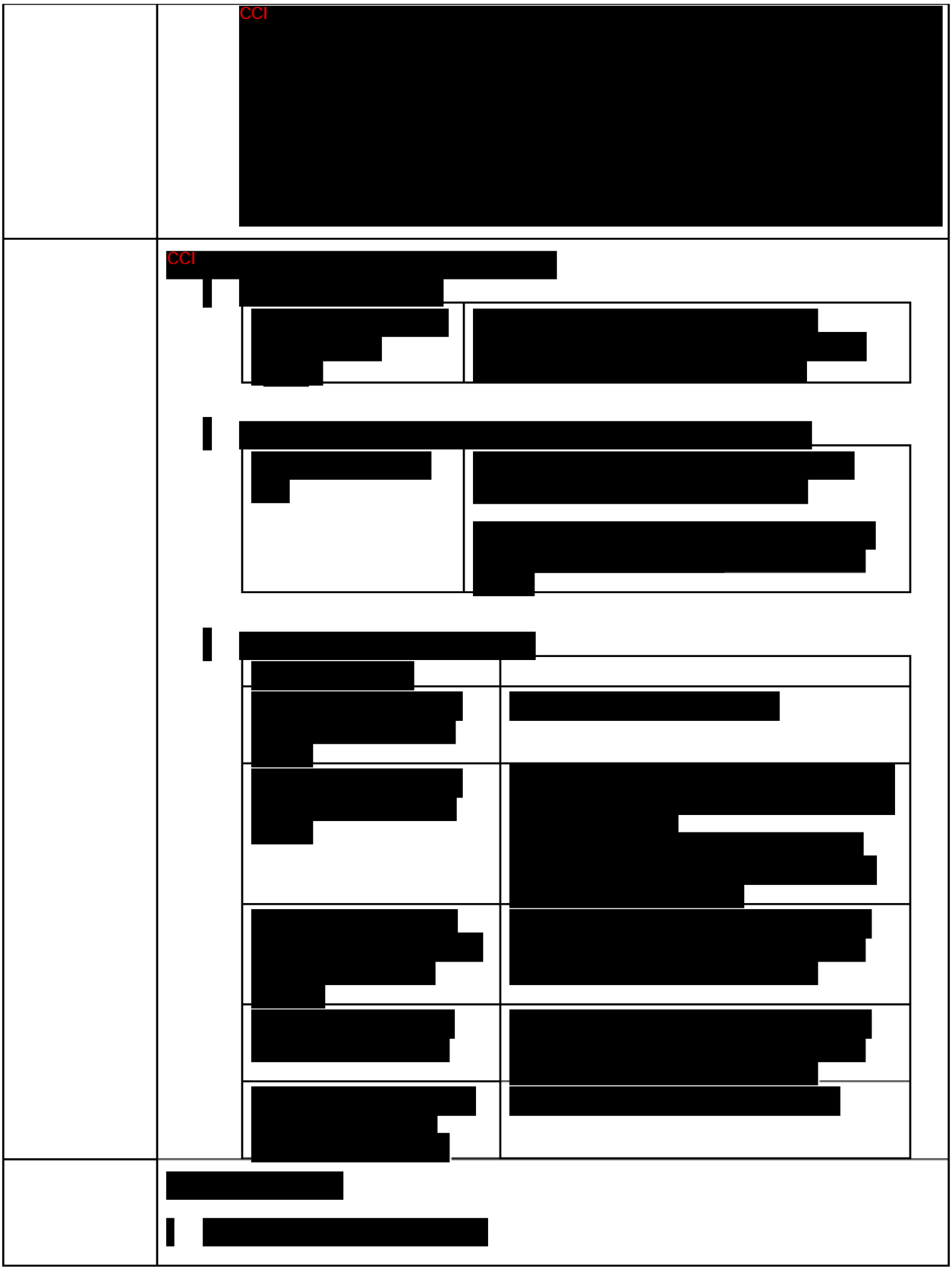
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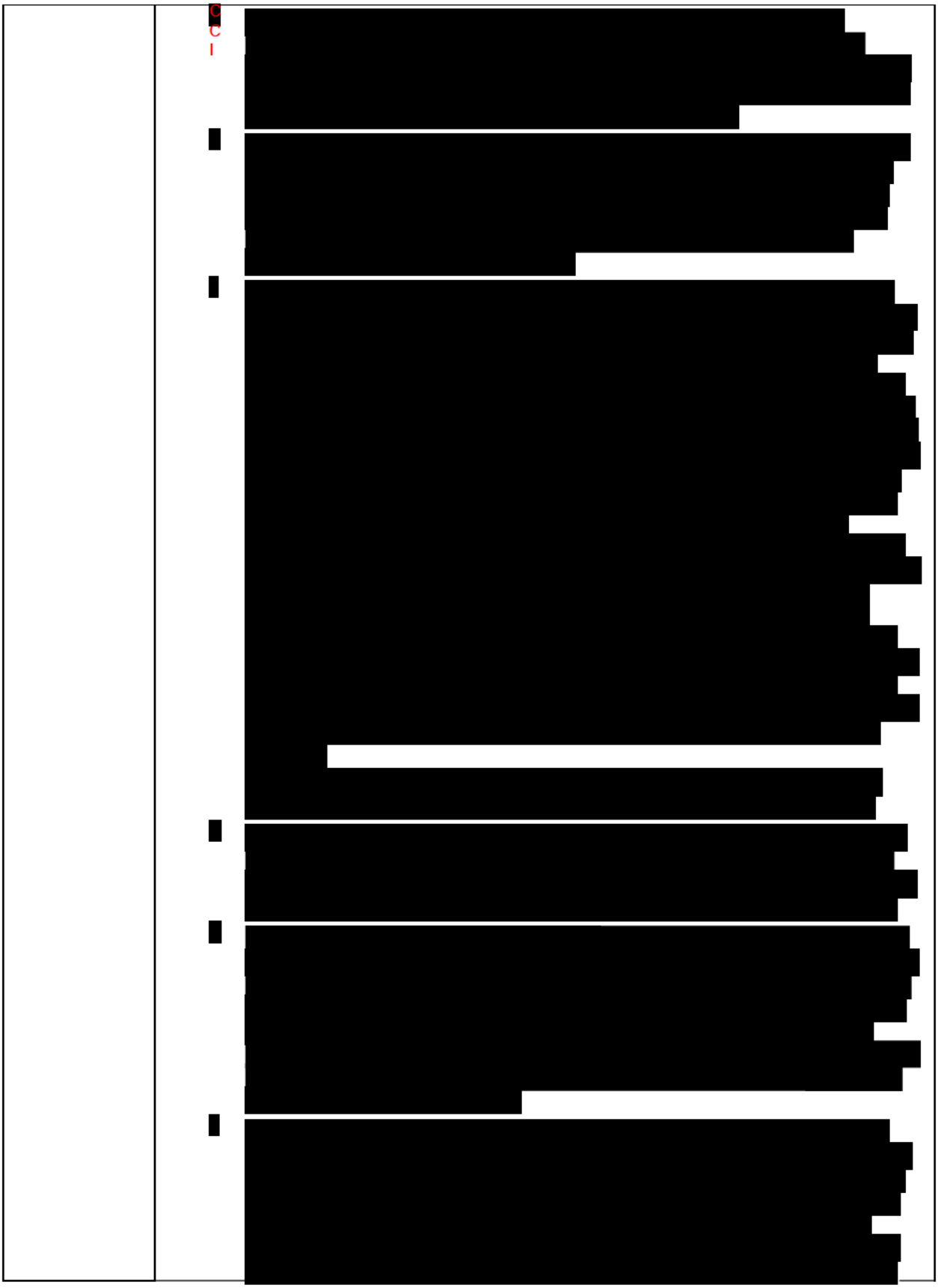


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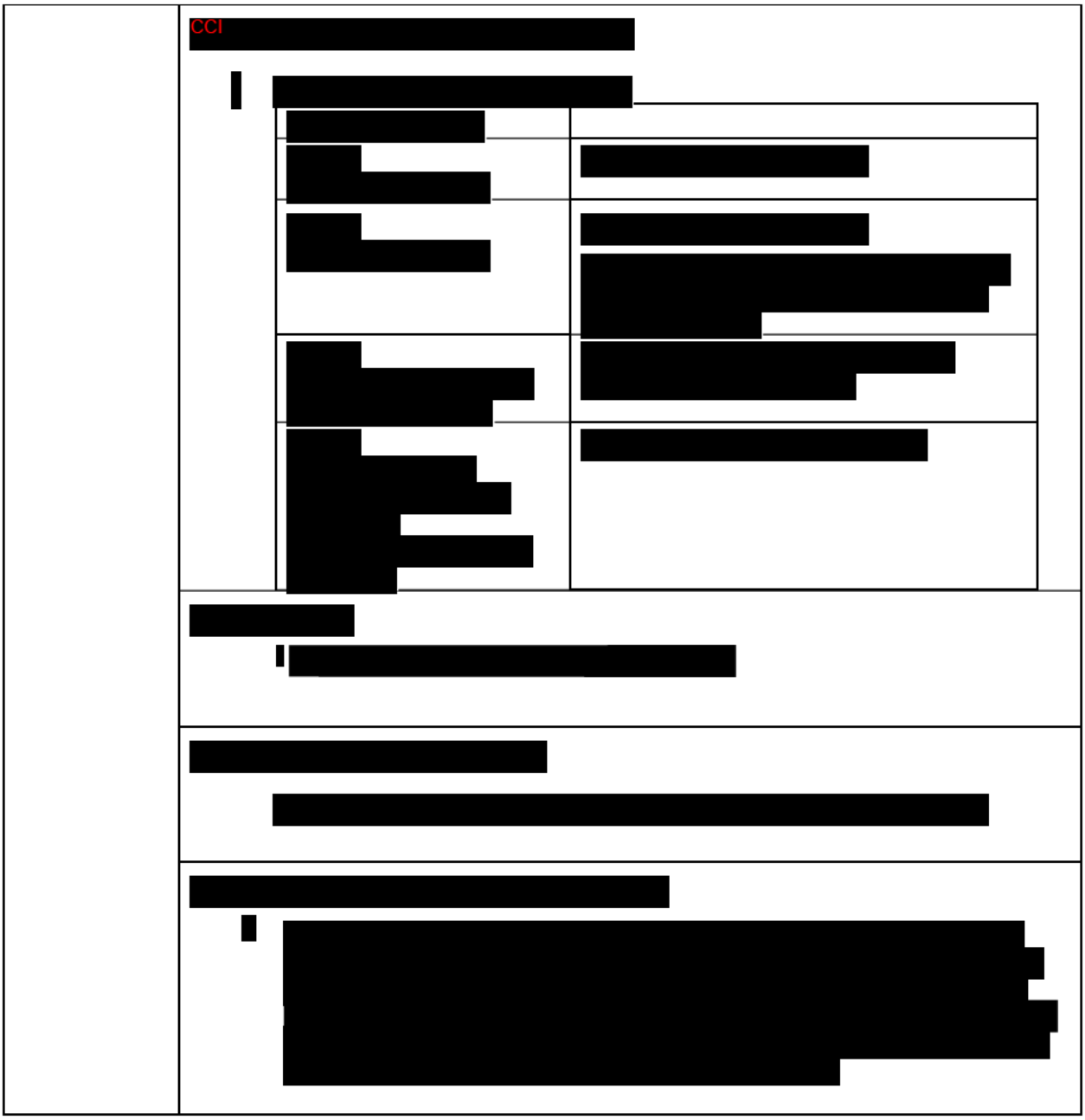
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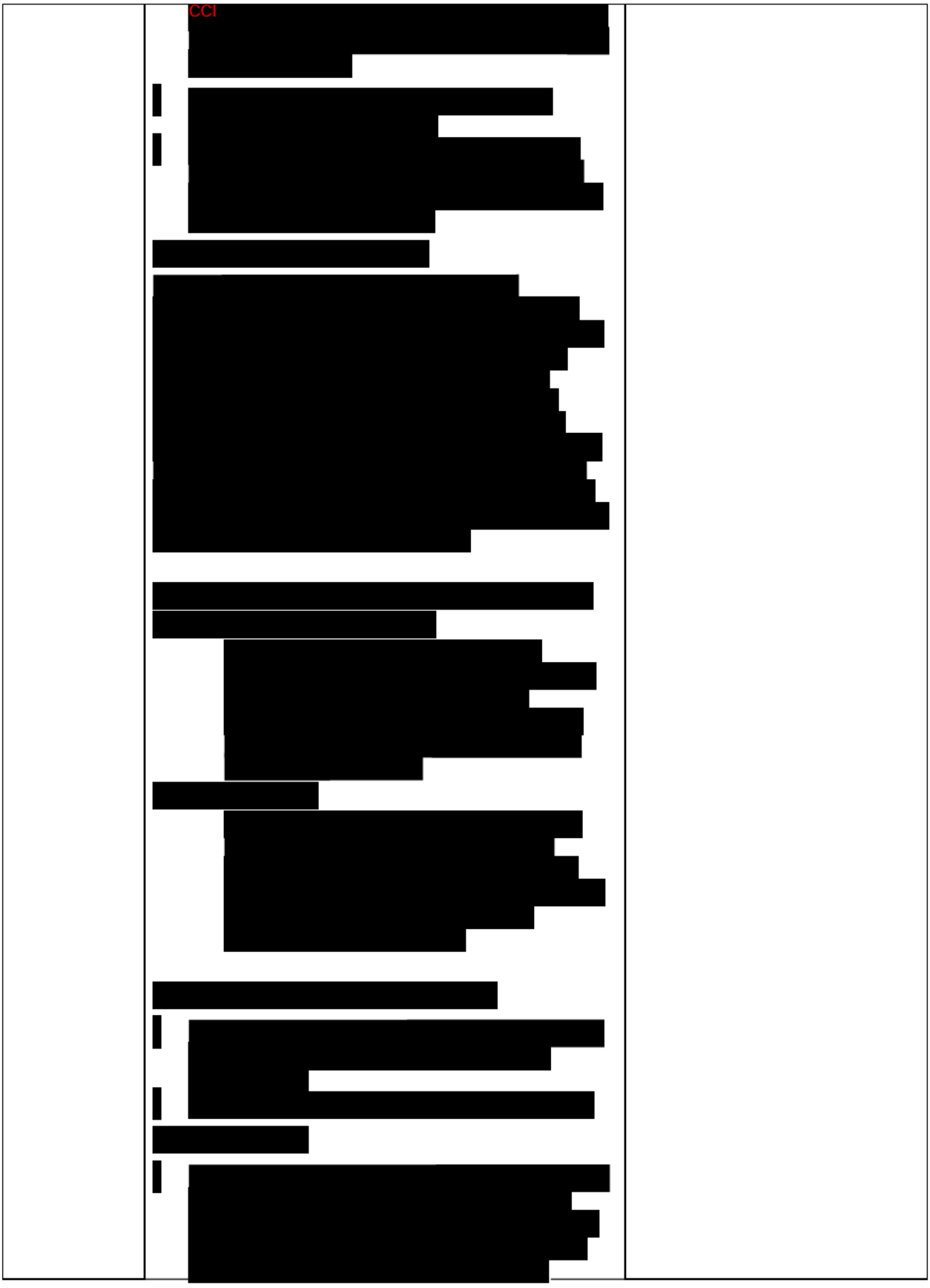
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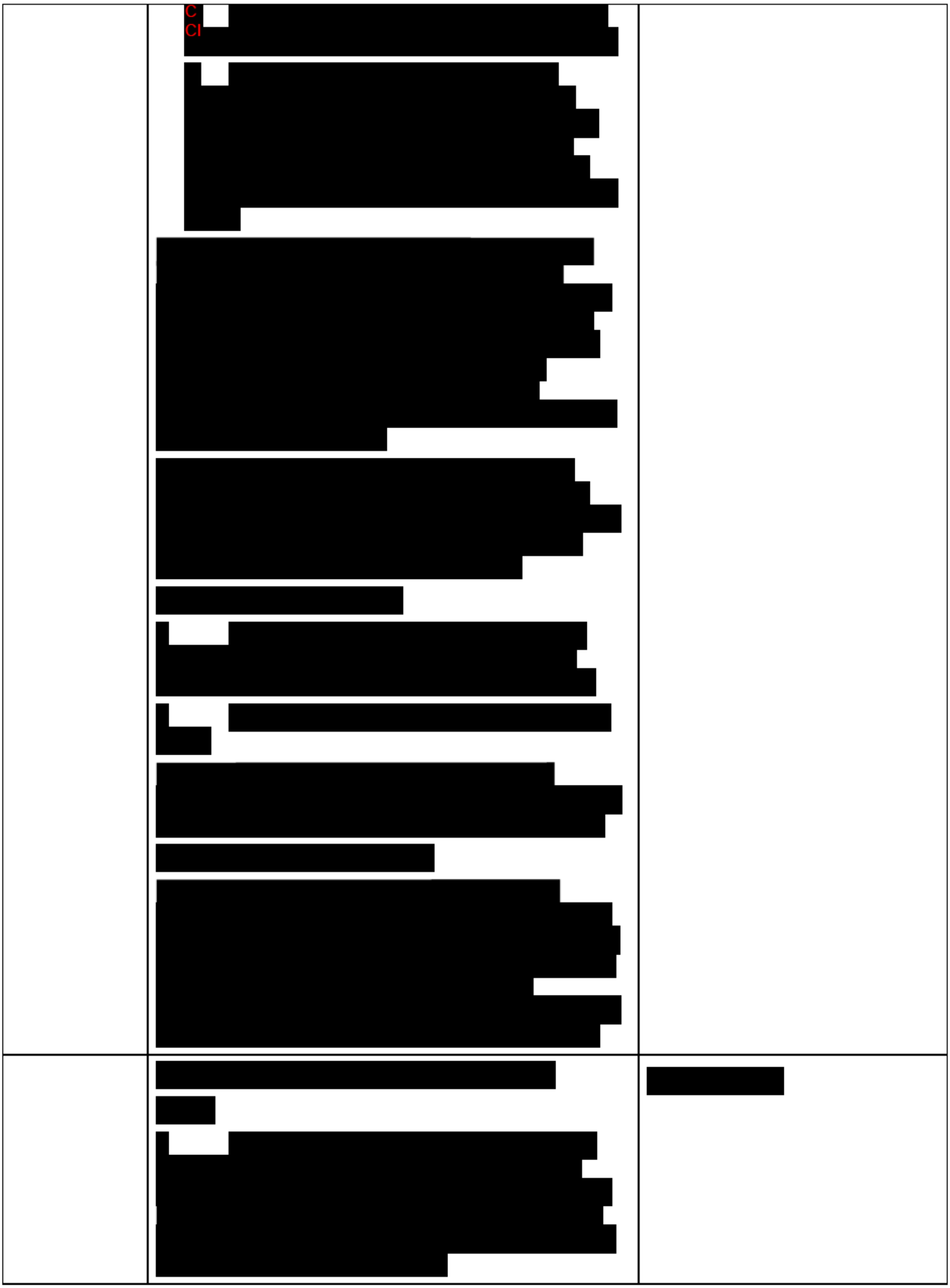

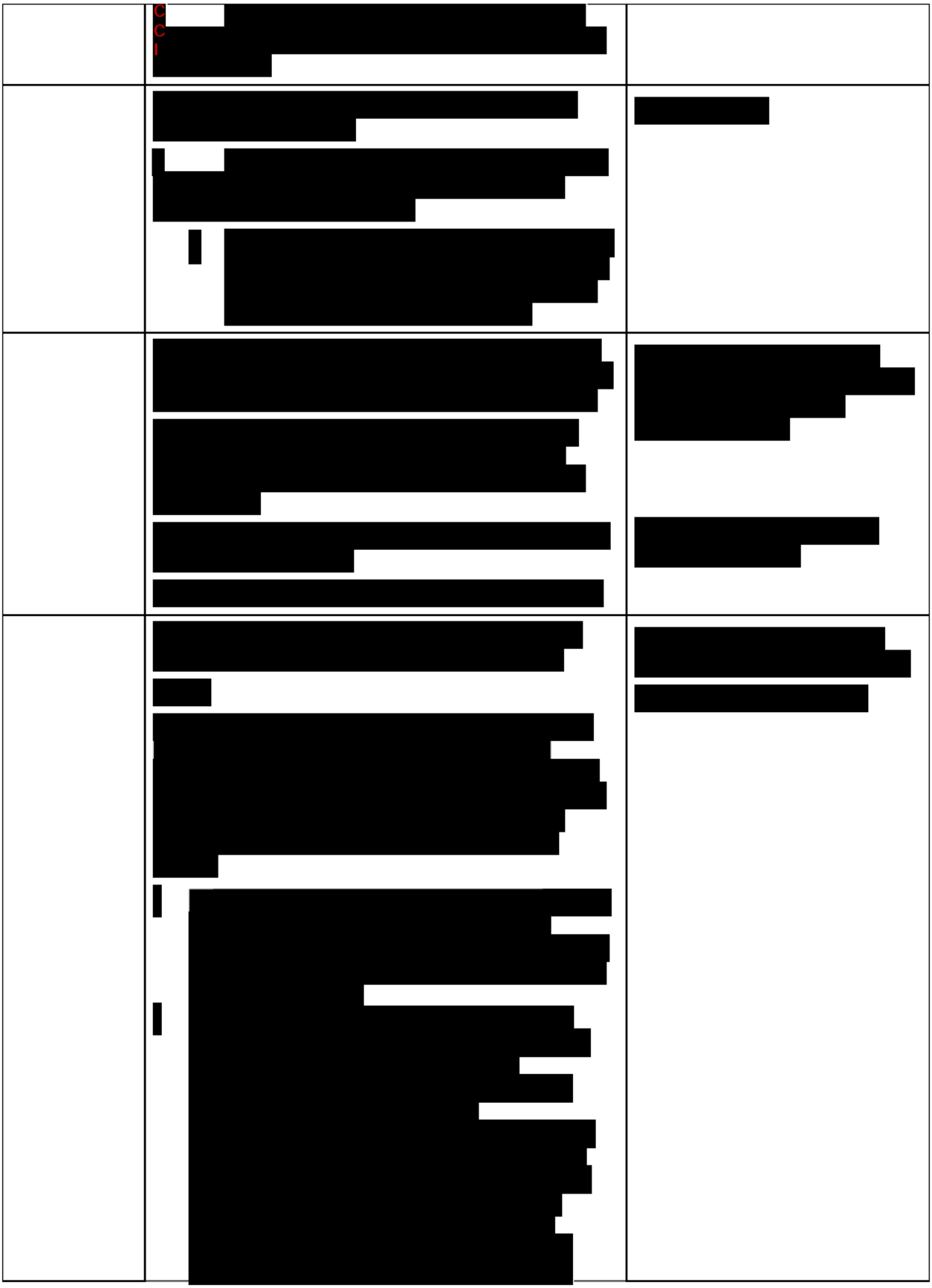

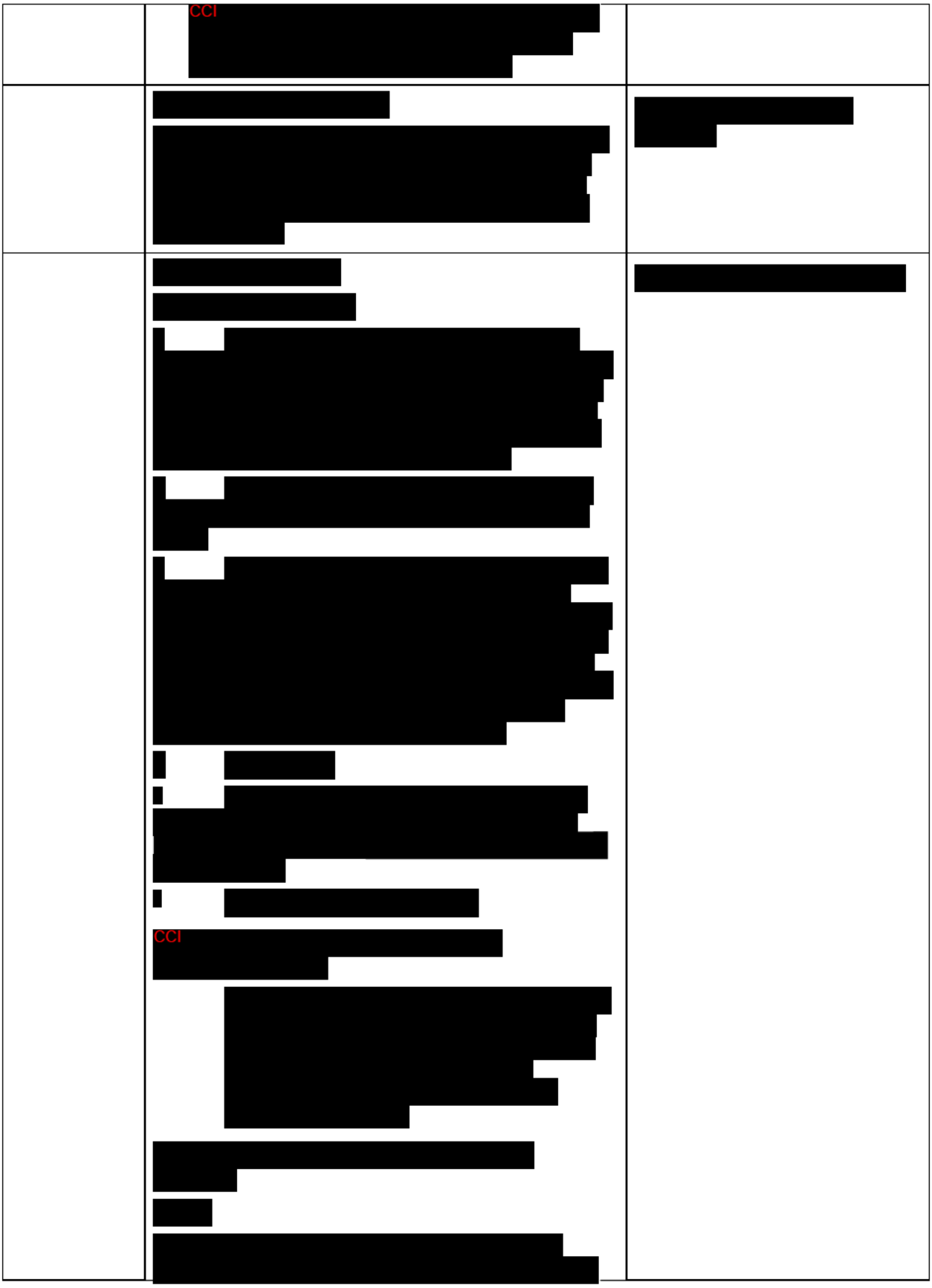
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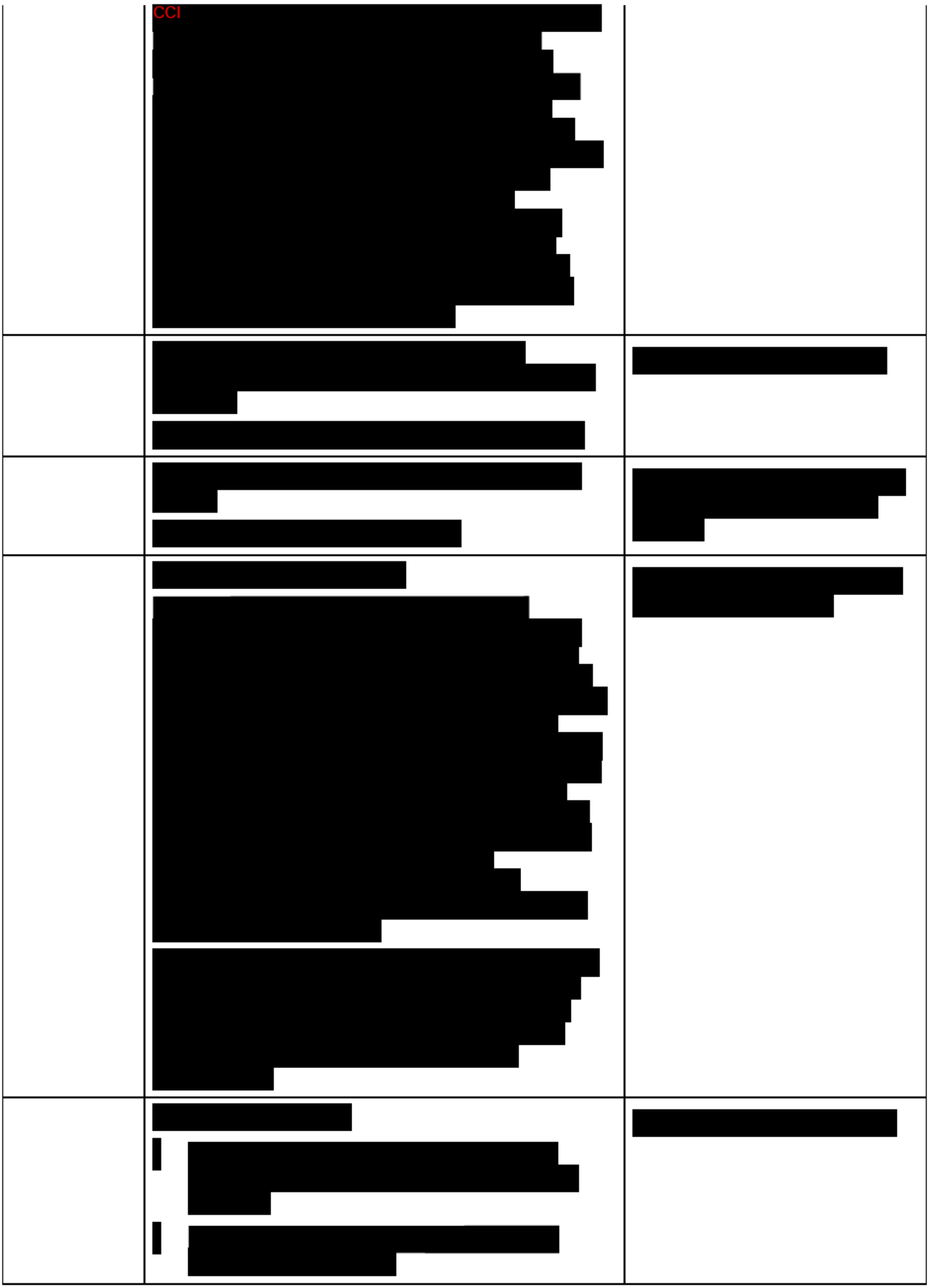
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PROTOCOL SYNOPSIS

Title of Study:	A Phase I-II multicenter, open-label trial of H3B-6545, a covalent antagonist of estrogen receptor alpha, in women with locally advanced or metastatic estrogen receptor-positive, HER2 negative breast cancer
Sponsor Study Numbers:	H3B-6545-A001-101
Sponsor:	Eisai, Inc.
Study Duration:	The total duration of the study is planned to be five (5) years. **Phase of Study:** 1-2
Study Sites:	Approximately 4 sites planned for Phase 1 and approximately CCI sites for Phase 2.
Number of Subjects:	Up to approximately 170 subjects with locally advanced or metastatic breast cancer are planned to be enrolled in this study.
Objectives:	**Phase 1** The primary objective of the Phase 1 portion of this study is to: • Determine the maximum tolerated dose (MTD) and/or the recommended Phase 2 dose (RP2D) of H3B-6545 in women with locally advanced or metastatic estrogen receptor (ER)-positive, human epidermal growth factor receptor-2 (HER2)-negative breast cancer. The secondary objectives of the Phase 1 portion of this study are to: • Evaluate the safety and tolerability of H3B-6545 as a single agent administered orally once daily (QD) over a 28-day cycle in this subject population • Characterize the plasma pharmacokinetics (PK) of H3B-6545. • Estimate the efficacy of H3B-6545 in terms of response rate, duration of response (DoR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS). The exploratory objectives of the Phase 1 portion of this study are to: CCI [redacted] **Phase 2** The primary objective of the Phase 2 portion of this study is to: • Estimate the efficacy of H3B-6545 in terms of best overall response rate, DoR, DCR, clinical benefit rate, PFS, and OS in all subjects with ER-positive HER2-negative breast cancer and in those with and without ERα mutation (ERαMUT) (including a clonal estrogen receptor 1 [ESR1] Y537S mutation) The secondary objectives of the Phase 2 portion of this study are to: • Further characterize the safety of H3B-6545 in this subject population • Further characterize the PK of H3B-6545. At least sparse PK samples will be collected from all subjects on study. • Evaluate the effect of a high-fat meal on the relative bioavailability of H3B-6545. • Assess the effect of H3B-6545 on serum bone turn-over markers, namely bone-specific alkaline phosphatase (BSAP; for osteoclast metabolism), amino-terminal

	propeptide of type 1 collagen (PINP; for bone formation), and C-terminal cross-linking telopeptide of type 1 collagen (CTX; for bone resorption) • Assess the effect of H3B-6545 on endometrial thickness and uterine volume. This objective will be assessed in a subgroup of women with intact uteri. The exploratory objectives of the Phase 2 portion of this study are to: CCI [redacted]
Study Design:	This is a first-in-human study of H3B-6545 in women with ER-positive, HER2-negative, locally advanced or metastatic breast cancer. **Phase 1** The Phase 1 dose escalation will follow a standard 3+3 cohort design until the MTD/RP2D is determined in this population. Once a subject has completed Cycle 3 at her assigned dose level of drug, intra-subject dose escalation will be allowed. Only subjects who have not progressed on treatment and did not have a dose reduction because of adverse events, can escalate to the highest dose level that has completed dose-limiting toxicity (DLT) assessment and has been shown to be safe (no DLT in the first 3 subjects or no more than 1 DLT in 6 subjects). Subjects on the intra-subject dose escalation will not be included in the DLT analysis at the higher dose level. Assuming 5 dose levels will be studied and a maximum of 6 subjects will be enrolled per dose level, then approximately 18–30 subjects may be accrued during Phase 1. The primary endpoint will be the determination of the RP2D. In the absence of a MTD, the RP2D will be based on PK and PD information, in addition to all available safety and efficacy data. Additional endpoints will include safety as per the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 criteria, objective response rate (ORR), DoR, DCR, PFS, and OS, in addition to biomarkers (including but not limited to ER, PR, Ki67, HER2, gene expression, and DNA mutations). Safety, tumor response, PK, and PD assessments will be performed for every subject in Phase 1. Phase 1 is closed to enrollment, and the RP2D is CCI [redacted] **Phase 2** The Phase 2 portion of the study will examine the efficacy of the RP2D in an expanded population of subjects with ER-positive HER2-negative locally advanced or metastatic breast cancer. The planned enrollment for the Phase 2 portion of the study is approximately C[redacted] subjects CCI [redacted] The first C[redacted] subjects enrolled in the Phase 2 part of the study will be included in the food-effect cohort and will be randomly assigned to receive the Cycle 1 Day 15 dose of H3B-6545 in a fed or fasted state. Each subject will then receive the Cycle 1 Day 22 dose in the reverse/untried state. In addition, C[redacted] subjects in Phase 2 with an intact uterus will undergo transvaginal ultrasound to examine the effect of H3B-6545 on endometrial thickness and uterine volume. Potential subjects to be enrolled under Amendment 6 and subsequent amendments will sign a prescreening consent to provide a whole blood sample to perform *ESR1* mutation analysis. Eligible subjects must have *ESR1* Y537S mutation at allelic frequency (AF) ≥0.5%, in the absence of D538G mutation at the same AF value. This will be

	determined by central laboratory assessment prior to signing the main study consent. See Section 5.2. Under Amendment 8, approximately [redacted] subjects will be enrolled to allow for approximately [redacted] response evaluable subjects.
Inclusion Criteria:	Subjects must meet the following criteria in order to be included in the research study: 1. Prescreening: Subjects must have clonal ESR1 Y537S mutation at AF ≥0.5%, in the absence of ESR1 D538G at AF ≥0.5%, as determined at prescreening by a Sponsor-designated central laboratory from a Nucleic Acids Whole Blood (plasma cfDNA) sample. 2. Subject has signed informed consent form (ICF) before any trial-related activities and according to local guidelines. Main ICF may only be signed once positive mutation status is confirmed by a sponsor-designated central laboratory, unless prior authorization has been received by the Sponsor, based on local mutation analysis. 3. Only females are eligible. Menopausal status: – Postmenopausal defined by: a) Prior bilateral oophorectomy, b) Age ≥60 years, or c) Age <60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression); or a follicle-stimulating hormone value >40 mIU/mL and an estradiol value <40 pg/mL (140 pmol/L) or in postmenopausal ranges per local reference ranges Or – Premenopausal or perimenopausal concurrently given a luteinizing hormone-releasing hormone (LHRH) agonist starting at least 4 weeks before the start of trial therapy and is planned to continue LHRH during the study. 4. Subject has a histologically and/or cytologically confirmed diagnosis of ER-positive breast cancer by local laboratory. 5. Subject has HER2-negative breast cancer as defined by American Society for Clinical Oncology College of American Pathologists guidelines (Wolff et al. 2013). 6. Subject must have progressed on the most recent therapy. 7. Prior therapy for breast cancer in the advanced/metastatic setting must have included **a minimum of**: a) two prior hormonal therapies, or b) one prior hormonal therapy and one prior chemotherapy regimen, or c) one prior hormonal therapy and a CDK4/6 inhibitor. Note: Subjects may have received treatment for brain metastases, but must be neurologically stable, completed radiotherapy and off corticosteroids for at least one month prior to starting trial therapy. Note: Subjects enrolled under Amendment 6 (or subsequent amendments) must have received prior therapy, including at least one prior hormonal therapy and a CDK4/6 inhibitor. Up to one prior chemotherapy in the metastatic setting is allowed. 8. Subject must have at least one biopsiable lesion in the Phase 1 portion. In the Phase 2 part of the trial, subjects must have either

	a) at least one measurable lesion as per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, or b) at least one predominantly lytic bone lesion. Note: Subjects enrolled under Amendment 6 (or subsequent amendments) must have at least one measurable lesion as per RECIST 1.1. 9. Subject must be willing to undergo tumor biopsies prior to treatment and on Cycle 2 Day 1. In the Phase 2 part of the trial, subjects with bone-only disease, or those for whom a biopsy is contra-indicated, may opt out of providing tumor biopsies following investigator concern and direct discussion and approval from the Sponsor. Note: A subset of subjects in Phase 2 will be required to provide tumor tissue until tumor pairs have been collected from at least 15 subjects with $ER\alpha^{WT}$ and 15 subjects with $ER\alpha^{mut}$ (determined retrospectively by Sponsor-designated central laboratory test). Note: For subjects enrolled under Amendment 6 (or subsequent amendments), a recent archival tumor tissue obtained within 6 months prior to enrollment or a fresh tumor biopsy must be provided. A second biopsy after initiating trial therapy is not required. 10. Subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. 11. Subject has adequate bone marrow and organ function, as defined by the following laboratory values: ● Absolute neutrophil count ≥1.5 × 10^9/L ● Platelets ≥100 × 10^9/L ● Hemoglobin ≥9.0 g/dL (may have been transfused). ● Potassium, sodium, calcium (corrected for serum albumin) and magnesium <CTCAE Grade 2 ● Cockcroft-Gault based creatinine clearance ≥35 mL/min ● Serum albumin ≥3.0 g/dL (≥30 g/L) ● In the absence of liver metastases, alanine aminotransferase (AST) and aspartate aminotransferase (ALT) should be below 3.0 × ULN. If the subject has liver metastases, ALT and AST should be below 5.0 × ULN. ● Total serum bilirubin <ULN. For subjects with documented Gilbert's syndrome, total serum bilirubin <2.5 × ULN 12. Age ≥18 years 13. Willingness and ability to comply with study and follow-up procedures 14. Ability to understand the nature of this study and give written informed consent
Exclusion Criteria:	Subjects who meet any of the following criteria will be excluded from study entry: 1. Subject with bone-only disease (Phase 1 only). 2. Phase 2 subjects may have predominantly lytic bone-only disease. Note: Subjects enrolled under Amendment 6 (or subsequent amendments) must have at least one measurable lesion as per RECIST 1.1. 3. Subject with inflammatory breast cancer. 4. Subject has received more than one prior chemotherapy regimen for metastatic disease (Phase 2 only).

	5. Subject has had prior antineoplastic therapy within 14 days prior to starting study drug. 6. Subject is currently receiving or has received systemic corticosteroids ≤2 weeks prior to starting study drug, or has not fully recovered from side effects of such treatment. Note: The following uses of corticosteroids are permitted: single doses, topical applications (eg, for rash), inhaled sprays (eg, for obstructive airways diseases), eye drops or local injections (eg, intra-articular). 7. Subject has received radiotherapy ≤4 weeks or limited field radiation for palliation ≤2 weeks prior to starting study drug, and who has not recovered to Grade 1 or better from related side effects of such therapy (with the exception of alopecia) and/or for whom ≥30% of the bone marrow was irradiated. 8. Major surgical procedures ≤14 days of beginning study drug, or minor surgical procedures ≤7 days, or has not recovered from major side effects. No waiting required following port-a-cath placement. 9. Subject has active cardiac disease or a history of cardiac dysfunction, including any of the following: ● History of angina pectoris, symptomatic pericarditis, or myocardial infarction within 12 months prior to study entry ● History of documented congestive heart failure (New York Heart Association functional classification III or IV) ● Documented cardiomyopathy ● Subject has a left ventricular ejection fraction <50% as determined by multiple-gated acquisition (MUGA) scan or echocardiogram. ● History of any cardiac arrhythmias, eg, ventricular, supraventricular, nodal arrhythmias, or conduction abnormality, in the previous 12 months ● A prolonged QTcF interval >450 msec as demonstrated by a repeated ECG; a history of risk factors for Torsade de pointes (eg, heart failure, hypokalemia, and family history of long QT syndrome); or the use of concomitant medications that prolonged the QTcF interval ● Subject has a resting pulse rate <60 bpm 10. Subject has impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of H3B-6545 (eg, ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection). 11. Subject has a known hypersensitivity to any of the excipients of H3B-6545. 12. Subject has a known history of human immunodeficiency virus infection or hepatitis C virus, or requires treatment with protease inhibitors (testing not mandatory). 13. Subject has any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, contraindicate subject participation in the clinical study (eg, chronic pancreatitis, active hepatitis, etc). 14. Subject received in the 7 days prior to the administration of study drug or is currently receiving any of the following medications (see Appendix H and Appendix I for details): a. Known strong inducers or inhibitors of cytochrome P450 (CYP) 3A4 or P-glycoprotein b. Medications that have a known risk to prolong the QT interval or induce Torsades de Pointes. c. Medications that have a narrow therapeutic window and are predominantly metabolized through CYP2C8, CYP2C9, CYP2C19, or CYP3A4

	d. Medications that have a narrow therapeutic window and are breast cancer resistance protein substrates e. Herbal preparations/medications; these herbal medications include, but are not limited to, St. John's wort, kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone, yohimbe, saw palmetto, and ginseng 15. Any adverse event related to previous therapies for breast cancer that has not resolved to ≤Grade 1. 16. Females who are breastfeeding or pregnant at Screening or Baseline (as documented by a positive beta-human chorionic gonadotropin [ß-hCG] (or human chorionic gonadotropin [hCG]) test with a minimum sensitivity of 25 IU/L or equivalent units of ß-hCG [or hCG]). A separate baseline assessment is required if a negative screening pregnancy test was obtained more than 72 hours before the first dose of study drug. 17. Females of childbearing potential who: ● Had unprotected sexual intercourse within 30 days before study entry and who do not agree to use a highly effective method of contraception (eg, total abstinence, an intrauterine device, a double-barrier method [such as condom plus diaphragm with spermicide], a contraceptive implant, an oral contraceptive, or have a vasectomized partner with confirmed azoospermia) throughout the entire study period or for 28 days after study drug discontinuation. ● Are currently abstinent, and do not agree to use a double-barrier method (as described above) or refrain from sexual activity during the study period or for 28 days after study drug discontinuation. ● Are using hormonal contraceptives but are not on a stable dose of the same hormonal contraceptive product for at least 4 weeks before dosing and who do not agree to use the same contraceptive during the study or for 28 days after study drug discontinuation All females will be considered to be of childbearing potential unless they are postmenopausal [amenorrheic for at least 12 consecutive months, in the appropriate age group, and without other known or suspected cause] or have been sterilized surgically [ie, bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy, all with surgery at least 1 month before dosing]). 18. Alcohol dependency within 6 months before study entry. 19. Subject has a concurrent malignancy or malignancy within 3 years of enrollment, with the exception of adequately treated basal or squamous cell carcinoma, non-melanomatous skin cancer, or curatively resected cervical cancer. 20. Psychological, familial, sociological, or geographical conditions that do not permit compliance with the protocol. 21. Subjects with advanced, symptomatic visceral spread, that are at risk of life-threatening complications in the short term. 22. Subjects with abnormal coagulation profiles, or any history of coagulopathy within 6 months prior to the first dose of study drug, including history of deep vein thrombosis (DVT) or pulmonary embolism. Note: subjects (except pre/perimenopausal women) with adequately treated catheter-related venous thrombosis occurring more than one month prior to the first dose of study drug will be allowed to participate. 23. Prothrombin time/international normalized ratio (INR) >1.5 times the upper limit of normal (ULN) or outside therapeutic range if receiving anticoagulation that would affect the prothrombin time/INR.

	Note: subjects (except pre/perimenopausal women) treated with an anticoagulant, that affects prothrombin time/INR, for a stable and allowed medical condition (eg, well controlled atrial fibrillation), will be allowed to participate, provided dose and coagulation parameters (as defined by local standard of care) are stable for at least one month prior to the first dose of study drug (hospital data prior to date of signature of the ICF will be used if necessary).
Correlative Testing:	CCI [redacted]
Statistical Methodology:	**Efficacy Analysis:** CCI [redacted]

CONTACT INFORMATION

Sponsor Contact Information:	PPD Study Director Eisai Inc. 200 Metro Boulevard Nutley, NJ 07110 USA
Study Chair:	PPD

LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
AF	Allelic frequency
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
AUC	Area under the curve
ß-hCG	beta-human chorionic gonadotropin
BCRP	Breast cancer resistance protein
BP	Blood pressure
BSAP	Bone-specific alkaline phosphatase
CBC	Complete blood count
cfDNA	Cell-free DNA
CFR	Code of Federal Regulations
CI	Confidence interval
CL/F	Apparent total body clearance following oral administration
C_{max}	Maximum observed plasma concentration
CMP	Comprehensive metabolic profile
CR	Complete response
CRF	Case report form
CRO	Contract research organization
CT	Computed tomography
CTA	Clinical trial agreement
CTX	C-terminal cross-linking telopeptide of type 1 collagen
CYP	cytochrome P450
DCR	Disease control rate
DDI	Drug-drug interaction
DHEA	Dehydroepiandrosterone
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DoR	Duration of response
EC	Ethics Committee
EC_{50}	Half-maximal exhibitory concentration
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EOT	End-of-Treatment
ER	Estrogen receptor
ERα	Estrogen receptor alpha
$ERα^{WT}$	Estrogen receptor alpha wild-type
$ERα^{mut}$	Estrogen receptor alpha mutant
ESR1	Estrogen receptor 1 gene
FAS	Full Analysis Set

FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP	Good Laboratory Practice
HED	Human equivalent dose
HER2	Human epidermal growth factor receptor-2
HNSTD	Highest non-severely toxic dose
HR	Heart rate
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonisation
INR	International normalized ratio
IRB	Institutional Review Board
ISF	Investigatory study file
LHRH	Luteinizing hormone-releasing hormone
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MRSD	Maximum recommended starting dose
MTD	Maximum tolerated dose
MUGA	Multiple-gated acquisition
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
ORR	Objective response rate
OS	Overall survival
PD	Pharmacodynamic(s)
PFS	Progression-free survival
P-gp	P-glycoprotein
PG	Pharmacogenetic(s)
PHI	Protected health information
PI	Principal Investigator
PINP	Amino-terminal propeptide of type 1 collagen
PK	Pharmacokinetic(s)
PO	Orally/by mouth
PS	Performance status
PT/PTT	Prothrombin time/partial thromboplastin time
QD	Once daily
QRS interval	Q, R, and S heart waves shown on electrocardiogram
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's formula
R_{acc}	Accumulation ratio
RECIST	Response Evaluation Criteria in Solid Tumors
RES	Response-Evaluable Set
RNA	Ribonucleic acid
RP2D	Recommended Phase 2 dose
RR	Respiratory rate
SAE	Serious adverse event

SAR	Suspected adverse reaction
SD	Stable disease
SOC	System Organ Class
SRC	Safety Review Committee
STD_{10}	Severely toxic dose in 10% of animals
$t_{½}$	Terminal elimination half-life
TEAE	Treatment-emergent adverse event
t_{max}	Time of maximum observed plasma concentration
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
V_z/F	Apparent volume of distribution during terminal phase
WBC	White blood count
WHO DD	World Health Organization Drug Dictionary

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1. INTRODUCTION

1.1 Background

In 2015, an estimated 231,840 new cases of invasive breast cancer were diagnosed in women in the United States (US), as well as an estimated 60,290 additional cases of in situ breast cancer. It was estimated that over 3 million women in the US are living with breast cancer (NCI SEER, 2016). The estimated number of deaths from breast cancer in the same year in the US was 40,290 (American Cancer Society, 2015). In the European Union, the predicted number of deaths from breast cancer among women for 2016 was 92,300 (Malvezzi et al., 2016).

The age-standardized incidence rate of breast cancer is 92.9 cases per 100,000 person-years in the US 95.0 cases per 100,000 person-years in the United Kingdom [UK] and 51.5 cases per 100,000 person-years in Japan and the incidence is increasing with time (Ferlay et al., 2013).

1.2 Role of the Estrogen Pathway in Breast Cancer

Approximately 70% of breast cancers express estrogen receptor alpha (ERα), a hormone-regulated transcription factor (Lumachi et al., 2013). Nonclinical and clinical epidemiological studies highlight an important oncogenic role for estrogen and ERα, through genomic- and non-genomic-mediated activation of proliferation and survival pathways, in the genesis and progression of breast cancer (Spicer and Pike, 1993). Mouse models genetically engineered to express ERα induce mammary adenocarcinoma formation (Tilli et al., 2003), whereas small inhibitor ribonucleic acid-mediated knockdown of ERα results in reduced proliferation and enhanced apoptosis in vitro and in vivo (Fu et al., 2006), confirming the critical role for this pathway in breast cancer.

Several estrogen receptor (ER)-directed therapies have been developed to antagonize the oncogenic ERα function in pre- and post-menopausal women with locally advanced, recurrent, or metastatic cancer, including selective ER modulators such as tamoxifen, selective ER downregulators such as fulvestrant, selective nonsteroidal aromatase inhibitors such as anastrozole and letrozole, and steroidal aromatase inhibitors such as exemestane (Beslija et al., 2009). Although these therapies have demonstrated efficacy in the nonclinical and clinical settings, innate and acquired resistance remains a major challenge.

Several mechanisms of resistance to ERα antagonists have been identified including: ERα/human epidermal growth factor receptor-2 (HER2) "crosstalk" (Shou et al., 2004), aberrant expression of ERα coactivators/corepressors (Osborne et al., 2003) and most recently, recurrent mutations in ERα (Li et al., 2013; Robinson et al., 2013; Toy et al., 2013). The recurrent mutations in ERα, which are enriched in nearly 30% of endocrine-therapy resistant metastases, confer ligand-independent activation of the ERα pathway (Li et al., 2013; Robinson et al., 2013; Toy et al., 2013; Merenbakh-Lamin et al., 2013; Yu et al., 2014; Segal and Dowsett, 2014; Jeselsohn et al., 2014; Chandarlapaty et al., 2016), and are associated with more aggressive disease biology with shorter overall survival (OS) relative to WT ERα (Chandarlapaty et al., 2016). Furthermore, ERα mutations ($ER\alpha^{mut}$) confer partial resistance to existing classes of endocrine therapies, likely through promoting constitutive activity.

The fact that current endocrine therapies are only partially effective in the $ER\alpha^{mut}$ setting and that a significant proportion of endocrine-therapy resistant metastases continue to remain

dependent on ERα signaling for growth and survival indicates a continuing need to develop the next generation of ERα antagonists that can overcome aberrant activities of both wild-type ($ERα^{WT}$) and mutant ERα. To this end, we introduce herein an orally bioavailable small molecule inhibitor that potently inhibits both $ERα^{WT}$ and $ERα^{mut}$ function by a novel mode of action.

1.3 Role of H3B-6545 in Breast Cancer

H3B-6545 is an oral, selective, and covalent small-molecule inhibitor of ER. H3B-6545 binds potently to ERα (half-maximal exhibitory concentration [EC_{50}], CCI and ERβ (EC_{50}, CCI and is a potent inhibitor of ERα coactivator recruitment (half-maximal inhibitory concentration [IC_{50}], CCI demonstrating CCI -fold selectivity over other nuclear hormone receptors tested. Addition of H3B-6545 to $ERα^{WT}$ and $ERα^{mut}$ breast cancer cell lines leads to dose-dependent inhibition of ERα-dependent transcription and concomitant reduction in cell proliferation. Oral dosing of H3B-6545 in mice leads to inhibition of ERα-dependent transcription in the $ERα^{mut}$ expressing subject-derived xenograft model. H3B-6545 also leads to dose-dependent tumor growth inhibition and/or stasis in both $ERα^{WT}$ and $ERα^{mut}$ xenograft models at well-tolerated doses.

These nonclinical findings support the clinical investigation of H3B-6545 in subjects with ER-positive ($ERα^{WT}$ and $ERα^{mut}$) HER2-negative breast cancer.

1.3.1 Nonclinical Pharmacokinetics

The pharmacokinetics (PK) of H3B-6545 have been characterized in Sprague Dawley rats and cynomolgus monkeys, species used in the nonclinical safety evaluation of H3B-6545. The PK of H3B-6545 were characterized by a low clearance in rats and a moderate clearance in monkeys after intravenous administration, with terminal elimination half-life ($t_{½}$) of CCI hours in rats and CCI hours in monkeys. Renal clearance was not a significant contributor to the elimination of H3B-6545 in rats.

In both species, H3B-6545 appears to be distributed well into tissues with a volume of distribution at steady state that exceeds total body water. In both species, after oral dosing, t_{max} was typically being achieved at CC hours postdose and H3B-6545 oral bioavailability was moderate. In vitro, H3B-6545 showed similar high plasma protein binding in rats, monkeys, and humans CCI and was predominantly metabolized by human cytochrome P450 (CYP) 3A4, followed by CYP3A5 and CYP2C8. H3B-6545 is a substrate of P-glycoprotein (P-gp), but not the breast cancer resistance protein (BCRP).

1.3.2 Nonclinical Safety

The toxicology program for H3B-6545 was designed to support the oral (PO) administration of H3B-6545 as a single agent given once daily (QD) for at least 28 days to women with metastatic breast cancer. The hydrochloride salt form of H3B-6545 was used in all pivotal safety studies and is intended for clinical use in Phase 1/2 trials.

Rats and cynomolgus monkeys were chosen as appropriate species for toxicology studies based on metabolic profiles similar to that of humans, and only female animals were used in toxicology studies based on the intended subject population.

The nonclinical toxicity findings in the toxicology studies included anticipated pharmacological effects of ER inhibition, lymphocyte depletion in peripheral lymphoid organs, gastrointestinal

(GI) toxicity, and hepatocellular effects. Findings from pivotal repeated-dose toxicology studies in female rats and monkeys were as follows:

- Moribundity in cynomolgus monkeys at [redacted] in the Good Laboratory Practice (GLP) 4-week toxicity study with contributory GI toxicity (histiocytosis of the small intestine and secondarily in the mesenteric lymph nodes and/or spleen) and clinical signs (watery/soft feces, emesis, inappetence, hunched posture, decreased activity, lower body temperature, and distended abdomen) resulted in early euthanasia of the dose group on [redacted]
- Reversible decreases in body weight gain in rats at [redacted] but not at [redacted] and body weight loss in monkeys at [redacted]
- Non-dose-response reproductive tract findings in rat at [redacted] (decreased corpora lutea and increased tertiary and/or cystic follicles in ovary, uterine atrophy and hypertrophic epithelial changes within uterus correlating with decreased uterine weight, and vaginal mucification) and in monkeys at [redacted] (inactive endometrium and minimal adenomyosis of uterus and follicular cysts within ovary). The reproductive findings showed a trend toward reversibility during the 4-week recovery period, whereas the findings in monkeys fully resolved (high-dose animals not available for evaluation of recovery).
- In rats, increases in liver enzyme activities and weights observed at [redacted] correlating dose-responsive, hepatocellular vacuolation at [redacted] and hepatocellular hypertrophy at [redacted] All liver effects reversed at [redacted] as did hepatocellular hypertrophy in the liver at [redacted] hepatocellular vacuolation was lower in incidence at [redacted] and comparable at [redacted] after the 28-day recovery period.
- Concomitant, reversible thyroid follicular cell hypertrophy in rats at [redacted] likely secondary to hepatocellular hypertrophy that reversed after a 28-day recovery period.
- Dose-dependent increases in liver enzymes alanine aminotransferase (ALT) (≥20 mg/kg/day), gamma-glutamyl transferase [redacted] aspartate aminotransferase (AST) (150 mg/kg/day), and total bilirubin [redacted] and correlating increased liver weights were noted at [redacted] with correlating hepatocellular vacuolation noted in monkeys at all doses [redacted] and hepatocellular, single-cell necrosis at 150 mg/kg/day. Findings reversed at [redacted] but ALT and gamma-glutamyl transferase remained elevated at [redacted] after a 28-day recovery period.
- H3B-6545 showed no toxicologically significant (proarrhythmic) cardiovascular effects (ie, no inhibition of human ether-à-go-go related gene [hERG], Nav1.5, and Cav1.2 channels) were observed in vitro or in vivo in the 4-week GLP toxicity study in monkeys; decreased heart rate (HR) was the only observed cardiovascular effect observed at [redacted] respectively, relative to concurrent controls).
- No central nervous system or respiratory effects assessed in 4-week toxicity studies in rats or monkeys, respectively.
- The severely toxic dose level to 10% (STD10) of rats was considered to be the highest dose level of [redacted] and the highest non-severely toxic dose (HNSTD) in monkeys was determined to be [redacted]

The maximum recommended starting dose (MRSD) was determined from the lowest human equivalent dose (HED) of one-tenth the rat STD_{10} (HED of 150 mg QD) compared with one-sixth the monkey HNSTD (HED of [CCI redacted] see Section 1.5).

1.4 Rationale for the Study

While the utilization of endocrine therapy is the basis of therapy for women with hormonal-receptor positive breast cancer, many subjects continue to develop resistance to this therapy. Optimal therapy in the advanced disease setting is directed at preventing the emergence of hormone-resistant disease. Preclinical data suggest H3B-6545 has been shown to target both $ER\alpha^{WT}$ and $ER\alpha^{mut}$, providing potential efficacy against tumors resistant to therapies that target only $ER\alpha^{WT}$. Therefore, the safety and potential efficacy of this drug will be examined in women with ER-positive, HER2-negative breast cancer.

The most common ER 1 (*ESR1*) mutations, Y537S and/or D538G, are detected in about 30% ER+ metastatic breast cancer patients (Gonzalez et al., 2020; Gyanchandani et al., 2016; Fribbens et al., 2016). These two specific mutations have a higher frequency in plasma than other *ESR1* mutations (Razavi et al., 2020). These *ESR1* mutations induce an agonist conformation of ERα, confer an estrogen-independent phenotype, and have been associated with worse prognosis after progression on aromatase inhibitors (AI) therapy, irrespective of subsequent therapies (Gonzalez et al., 2020; Schiavon et al., 2015; Takeshita et al., 2016; Fribbens et al., 2016).

Biophysical and cell-based studies have shown that *ESR1*-Y537S mutation can efficiently stabilize helix 12 of the ERα ligand domain in a conformation resembling that of the wild-type protein bound to estrogen (Nettles et al., 2008; Fanning et al., 2016), leading to estrogen-independent activation to a higher extent binding than other activating *ESR1* mutations (Toy et al., 2013; Puyang et al., 2018). By virtue of the significantly greater constitutive activity and reduced affinity to ER ligands, it is not surprising that several independent studies have confirmed the *ESR1*-Y537S mutation as the most significant driver of resistance to current system organ class (SOC) endocrine therapies, including 4-OHT and fulvestrant (Toy et al., 2013; Robinson et al., 2013; Jeselsohn et al., 2014; Puyang et al., 2018). In light of these data, there is a critical need to develop a more potent, next-generation ERα antagonist for the treatment of patients bearing *ESR1*-Y537S disease.

H3B-6545, a first-in-class covalent antagonist that irreversibly engages ERα, has shown clear anti-tumor activity in preclinical models of breast cancer carrying homozygous or heterozygous Y537S mutations (Korpal et al., SABCS Poster, 2017). As of [CCI redacted], among the subjects who started trial therapy at [CCI redacted] subjects had *ESR1*-Y537S mutations in cell-free DNA (cfDNA) with an allele fraction ≥0.5% (and without concurrent D538G). Amendment 6 will be conducted to include approximately an additional [CCI redacted] subjects (expanded to approximately [CCI redacted] subjects in Amendment 8, to further characterize efficacy) carrying the Y537S mutation without concurrent D538G mutations (clonal Y537S) as confirmed by a sponsor-designated central laboratory in order to provide a better estimate of the activity of H3B-6545 in this specific population of breast cancer patients.

1.5 Rationale for the Starting Dose

This is a first-in-human study and H3B-6545 has not been previously administered to humans.

The clinical starting dose of [CCI redacted] was selected. This dosage is less than the MRSD of [CCI redacted], which is the HED of one-tenth the rat severely toxic dose in 10% of rats (STD_{10} of [CCI redacted]

[redacted] based on body surface allometry, determined in the GLP 28-day toxicity study in rats given H3B-6545. In order to gain additional PK and safety data, the starting dose for the Phase 1 trial was selected to be [redacted]

1.5.1 Rationale for Recommended Phase 2 Dose

As of [redacted] subjects were enrolled; [redacted] subjects in the Phase 1 part and [redacted] subjects in the Phase 2 part of the trial. The Phase 1 part evaluated oral, QD doses ranging from [redacted] Consequently, the dose of [redacted] was selected as the recommended Phase 2 dose (RP2D).

1.6 Risk Assessment and Summary

The investigation of H3B-6545 in this subject population appears acceptable, based upon the nonclinical and clinical efficacy and safety profile and the lack of effective alternative treatments available to subjects (See Section 1.5.1 for details of clinical data). Thus, the benefit/risk assessment for this FTIP Phase 1 study supports the administration of H3B-6545 to subjects with locally advanced or metastatic ER-positive, HER2-negative breast cancer.

The risk-benefit continues to be favorable for Phase 2 based on available clinical data (Section 1.5.1)

2. STUDY OBJECTIVES

2.1 Phase 1 Primary Objective

The primary objective of the Phase 1 portion of this study is to:

- Determine the maximum tolerated dose (MTD) and/or the RP2D of H3B-6545 in women with locally advanced or metastatic ER-positive, HER2-negative breast cancer.

2.2 Phase 1 Secondary Objectives

The secondary objectives of the Phase 1 portion of this study are to:

- Evaluate the safety and tolerability of H3B-6545 as a single agent administered orally (PO) QD over a 28-day cycle in this subject population.
- Characterize the plasma PK of H3B-6545.
- Estimate the efficacy of H3B-6545 in terms of response rate, duration of response (DoR), disease control rate (DCR), progression-free survival (PFS), and OS.

2.3 Phase 1 Exploratory Objectives

The exploratory objectives of the Phase 1 portion of this study are to:

[redacted]

2.4 Phase 2 Primary Objective

The primary objective of the Phase 2 portion of this study is to:

- Estimate the efficacy of H3B-6545 in terms of best overall response rate, DoR, DCR, clinical benefit rate (CBR), PFS, and OS in all subjects with (ER)-positive, HER2-negative breast cancer and those with and without ERα mutation (including a clonal ESR1 Y537S mutation).

2.5 Phase 2 Secondary Objectives

The secondary objectives of the Phase 2 portion of this study are to:

- Further characterize the safety of H3B-6545 in this subject population.
- Further characterize the PK of H3B-6545. At least sparse PK samples will be collected from all subjects on study.
- Evaluate the effect of a high-fat meal on the relative bioavailability of H3B-6545.
- Assess the effect of H3B-6545 on serum bone turn-over markers, namely bone-specific alkaline phosphatase (BSAP, for osteoclast metabolism), amino-terminal propeptide of type 1 collagen (PINP, for bone formation), and C-terminal cross-linking telopeptide of type 1 collagen (CTX, for bone resorption).
- Assess the effect of H3B-6545 on endometrial thickness and uterine volume. This objective will be assessed in a subgroup of women with intact uteri.

2.6 Phase 2 Exploratory Objectives

The exploratory objectives of the Phase 2 portion of this study are to:

3. STUDY SUBJECT POPULATION AND DISCONTINUATION

The study will include women with ER-positive, HER2-negative, locally advanced or metastatic breast cancer. In Amendment 6 (and subsequent amendments), potential subjects will sign a prescreening consent to provide a whole blood sample to perform *ESR1* mutation analysis. Eligible subjects must have ESR1 Y537S mutation at allelic frequency (AF) ≥0.5%, in the

absence of D538G mutation at the same AF value. This will be determined by central laboratory assessment prior to signing the main study consent, unless prior authorization has been received by the Sponsor, based on local mutation analysis.

Eligible subjects must have progressed on their most recent therapy. The investigator or designee must ensure that only subjects who meet all the following inclusion criteria and none of the exclusion criteria are enrolled in the study.

3.1 Inclusion Criteria

Subjects must meet the following criteria in order to be included in the research study:

1. Prescreening: Subjects must have clonal ESR1 Y537S mutation at AF ≥0.5%, in the absence of ESR1 D538G at AF ≥0.5%, as determined at prescreening by a Sponsor-designated central laboratory from a Nucleic Acids Whole Blood (plasma cfDNA) sample.
2. Subject has signed informed consent form (ICF) before any trial-related activities and according to local guidelines. Main ICF may only be signed once positive mutation status is confirmed by a sponsor-designated central laboratory, unless prior authorization has been received by the Sponsor, based on local mutation analysis.
3. Only females are eligible. Menopausal status:

   − Postmenopausal defined by:

      a) Prior bilateral oophorectomy,

      b) Age ≥60 years, or

      c) Age <60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression); or a follicle-stimulating hormone value >40 mIU/mL and an estradiol value <40 pg/mL (140 pmol/L) or in postmenopausal ranges per local reference ranges

   Or

   − Premenopausal or perimenopausal concurrently given a luteinizing hormone-releasing hormone (LHRH) agonist starting at least 4 weeks before the start of trial therapy and is planned to continue LHRH during the study.
4. Subject has a histologically and/or cytologically confirmed diagnosis of ER-positive breast cancer by local laboratory.
5. Subject has HER2-negative breast cancer as defined by American Society for Clinical Oncology-College of American Pathologists guidelines (Wolff et al., 2013).
6. Subject must have progressed on the most recent therapy.
7. Prior therapy for breast cancer in the advanced/metastatic setting must have included **a minimum of**:

   a) two prior hormonal therapies, or

   b) one prior hormonal therapy and one prior chemotherapy regimen, or

   c) one prior hormonal therapy and a CDK4/6 inhibitor.

Note: Subjects may have received treatment for brain metastases, but must be neurologically stable, completed radiotherapy and off corticosteroids for at least one month prior to starting trial therapy.

Note: Subjects enrolled under Amendment 6 (and subsequent amendments) must have received prior therapy including at least one prior hormonal therapy and a CDK4/6 inhibitor. Up to one prior chemotherapy in the metastatic setting is allowed.

8. Subject must have at least one biopsiable lesion in the Phase 1 portion. In the Phase 2 part of the trial subjects must also have either:
   a) at least one measurable lesion as per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, or
   b) at least one predominantly lytic bone lesion.

      Note: Subjects enrolled under Amendment 6 (and subsequent amendments) must have at least one measurable lesion as per RECIST 1.1.
9. Subject must be willing to undergo tumor biopsies prior to treatment and on Cycle 2 Day 1. In the Phase 2 part of the trial, subjects with bone-only disease, or subjects for whom a biopsy is contra-indicated, may opt out of providing tumor biopsies following investigator concern and direct discussion and approval from the Sponsor.

   Note: A subset of subjects in Phase 2 will be required to provide tumor tissue until tumor pairs have been collected from at least 15 subjects with $ER\alpha^{WT}$ and 15 subjects with $ER\alpha^{mut}$ (determined retrospectively by Sponsor-designated central laboratory test).

   Note: For subjects enrolled under Amendment 6 (and subsequent amendments), a recent archival tumor tissue obtained within the 6 months prior to enrollment or a fresh tumor biopsy must be provided. A second biopsy after initiating trial therapy is not required.
10. Subject has an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1.
11. Subject has adequate bone marrow and organ function, as defined by the following laboratory values:
    - Absolute neutrophil count $\geq 1.5 \times 10^9$/L
    - Platelets $\geq 100 \times 10^9$/L
    - Hemoglobin ≥9.0 g/dL (may have been transfused).
    - Potassium, sodium, calcium (corrected for serum albumin) and magnesium <National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Grade 2
    - Cockcroft-Gault based creatinine clearance ≥35 mL/min
    - Serum albumin ≥3.0 g/dL (≥30 g/L)
    - In the absence of liver metastases, AST, and ALT should be below 3.0 × upper limit of normal (ULN). If the subject has liver metastases, ALT and AST should be below 5.0 × ULN.
    - Total serum bilirubin <ULN. For subjects with documented Gilbert's syndrome, total serum bilirubin <2.5 × ULN

12. Age ≥18 years
13. Willingness and ability to comply with study and follow-up procedures
14. Ability to understand the nature of this study and give written informed consent

3.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from study entry:

1. Subject with bone-only disease (Phase 1 only).
2. Phase 2 subjects may have predominantly lytic bone only disease.

   Note: Subjects enrolled under Amendment 6 (and subsequent amendments) must have at least one measurable lesion as per RECIST 1.1.
3. Subject with inflammatory breast cancer.
4. Subject has received more than one prior chemotherapy regimen for metastatic disease (Phase 2 only).
5. Subject has had prior antineoplastic therapy within 14 days prior to starting study drug.
6. Subject is currently receiving or has received systemic corticosteroids ≤2 weeks prior to starting study drug, or has not fully recovered from side effects of such treatment.

   Note: The following uses of corticosteroids are permitted: single doses, topical applications (eg, for rash), inhaled sprays (eg, for obstructive airways diseases), eye drops or local injections (eg, intra-articular).
7. Subject has received radiotherapy ≤4 weeks or limited field radiation for palliation ≤2 weeks prior to starting study drug, and who has not recovered to Grade 1 or better from related side effects of such therapy (with the exception of alopecia) and/or for whom ≥30% of the bone marrow was irradiated.
8. Major surgical procedures ≤14 days of beginning study drug, or minor surgical procedures ≤7 days, or has not recovered from major side effects. No waiting required following port-a-cath placement.
9. Subject has active cardiac disease or a history of cardiac dysfunction, including any of the following:
   - History of angina pectoris, symptomatic pericarditis, or myocardial infarction within 12 months prior to study entry
   - History of documented congestive heart failure (New York Heart Association functional classification III or IV)
   - Documented cardiomyopathy
   - Subject has a left ventricular ejection fraction <50% as determined by multiple-gated acquisition (MUGA) scan or echocardiogram
   - History of any cardiac arrhythmias, eg, ventricular, supraventricular, nodal arrhythmias, or conduction abnormality in the previous 12 months
   - A prolonged QT interval corrected for HR using Fridericia's formula (QTcF) >450 msec as demonstrated by a repeated ECG; a history of risk factors for Torsade de pointes (eg,

heart failure, hypokalemia, and family history of long QT syndrome); or the use of concomitant medications that prolonged the QTcF interval

- Subject has a resting pulse rate <60 bpm.

10. Subject has impairment of GI function or GI disease that may significantly alter the absorption of H3B-6545 (eg, ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).
11. Subject has a known hypersensitivity to any of the excipients of H3B-6545.
12. Subject has a known history of human immunodeficiency virus infection or hepatitis C virus, or requires treatment with protease inhibitors (testing not mandatory).
13. Subject has any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, contraindicate subject participation in the clinical study (eg, chronic pancreatitis, active hepatitis, etc).
14. Subject that received in the 7 days prior to the administration of study drug or is currently receiving any of the following medications (see Appendix H and Appendix I for details):
    a. Known strong inducers or inhibitors of CYP3A4 or P-gp
    b. Medications that have a known risk to prolong the QT interval or induce Torsades de Pointes
    c. Medications that have a narrow therapeutic window and are predominantly metabolized through CYP2C8, CYP2C9, CYP2C19, or CYP3A4.
    d. Medications that have a narrow therapeutic window and are BCRP substrates.
    e. Herbal preparations/medications. These herbal medications include, but are not limited to, St. John's wort, kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng.
15. Any adverse event related to previous therapies for breast cancer that has not resolved to ≤Grade 1.
16. Females who are breastfeeding or pregnant at Screening or Baseline (as documented by a positive beta-human chorionic gonadotropin [ß-hCG] (or human chorionic gonadotropin [hCG]) test with a minimum sensitivity of 25 IU/L or equivalent units of ß-hCG [or hCG]). A separate baseline assessment is required if a negative screening pregnancy test was obtained more than 72 hours before the first dose of study drug.
17. Females of childbearing potential who:

- Had unprotected sexual intercourse within 30 days before study entry and who do not agree to use a highly effective method of contraception (eg, total abstinence, an intrauterine device, a double-barrier method [such as condom plus diaphragm with spermicide], a contraceptive implant, an oral contraceptive, or have a vasectomized partner with confirmed azoospermia) throughout the entire study period or for 28 days after study drug discontinuation.
- Are currently abstinent, and do not agree to use a double-barrier method (as described above) or refrain from sexual activity during the study period or for 28 days after study drug discontinuation.

- Are using hormonal contraceptives but are not on a stable dose of the same hormonal contraceptive product for at least 4 weeks before dosing and who do not agree to use the same contraceptive during the study or for 28 days after study drug discontinuation.

All females will be considered to be of childbearing potential unless they are postmenopausal [amenorrheic for at least 12 consecutive months, in the appropriate age group, and without other known or suspected cause] or have been sterilized surgically [ie, bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy, all with surgery at least 1 month before dosing]).

18. Alcohol dependency within 6 months before study entry.
19. Subject has a concurrent malignancy or malignancy within 3 years of enrollment, with the exception of adequately treated basal or squamous cell carcinoma, non-melanomatous skin cancer, or curatively resected cervical cancer.
20. Psychological, familial, sociological, or geographical conditions that do not permit compliance with the protocol.
21. Subjects with advanced, symptomatic visceral spread, that are at risk of life-threatening complications in the short term.
22. Subjects with abnormal coagulation profiles, or any history of coagulopathy within 6 months prior to the first dose of study drug, including history of deep vein thrombosis (DVT) or pulmonary embolism.

    Note: subjects (except pre/perimenopausal women) with adequately treated catheter-related venous thrombosis occurring more than one month prior to the first dose of study drug will be allowed to participate.
23. Prothrombin time/international normalized ratio (INR) >1.5 times the upper limit of normal (ULN) or outside therapeutic range if receiving anticoagulation that would affect the prothrombin time/INR.

    Note: subjects (except pre/perimenopausal women) treated with an anticoagulant, that affects prothrombin time/INR, for a stable and allowed medical condition (eg, well controlled atrial fibrillation), will be allowed to participate, provided dose and coagulation parameters (as defined by local standard of care) are stable for at least one month prior to the first dose of study drug (hospital data prior to date of signature of the ICF will be used if necessary).

3.3 Discontinuation from Study Treatment

Subjects will be discontinued from study treatment for any of the following reasons:

- Disease progression
- Irreversible or intolerable toxicity or abnormal laboratory values thought to be related to drug toxicity
- Cycle 1 DLT
- Conditions requiring therapeutic intervention not permitted by the protocol
- Intercurrent illness (at the investigator's discretion

- Inability of the subject to comply with study requirements, or subject is lost to follow-up
- Subject requests to discontinue treatment
- Subject withdraws consent from the study
- Pregnancy

After discontinuation from protocol treatment, subjects must be followed for adverse events (AEs) for 28 days after their last dose of study drug. All new AEs, including serious AEs (SAEs), occurring during this period must be reported and all SAEs must be followed until resolution, unless, in the opinion of the investigator, these events are not likely to improve because of the underlying disease. In this case, the investigator must record his or her reasoning for this decision in the subject's medical records and indicate on the AE pages that the outcome is not resolved on the Electronic Case Report Form (eCRF).

All subjects who have Grade 3 or 4 laboratory abnormalities (per NCI CTCAE v4.03) at the time of discontinuation must be followed until the laboratory values have returned to Grade 1,2 or baseline for the subject, unless it is, in the opinion of the investigator, not likely that these values are to improve. In this case, the investigator must record his or her reasoning for making this decision in the subject's medical records and indicate on the AE pages that the outcome is not resolved on the eCRF.

4. STUDY REGISTRATION

The subject must willingly consent after being informed of the procedures to be followed, the experimental nature of the treatment, and the potential benefits, alternatives, side effects, risks, and discomforts. Human protection committee (Institutional Review Board [IRB]/Ethics Committee [EC]) approval of this protocol and consent form is required. Eligible subjects who wish to participate in the study will be enrolled into the study.

In Phase 1 of the study, the Sponsor or designee will notify sites via e-mail when a new Dose Level/Cohort is opened and enrollment slot(s) becomes available. As soon as a potential subject has been identified following the slot availability announcement, sites will notify the Sponsor or designee via email. Sponsor or designee will reply to verify an available slot, and reserve the slot for the subject. Upon receipt of confirmation, sites will have 5 business days to consent the identified subject. If a subject is not consented within 5 business days, the slot will open to all sites for enrollment.

Sponsor or designee will also communicate to all sites via e-mail when a cohort has been fully reserved and screening is closed. This communication may also include language that describes any opportunity for additional screening should a subject screen-fail and/or meet replacement criteria.

Once a site receives confirmation of successful slot reservation, the subject may be consented and screening procedures may begin. All screening procedures must be completed as outlined in the protocol, and the Principal Investigator (PI) must assess and confirm eligibility of the subject prior to requesting enrollment. Once eligibility is confirmed by the PI and Sponsor or designee, subject registration and dose level assignment will be performed by the Sponsor or designee. The Sponsor or designee will document the subject identification number, dose level, and date of

enrollment on the registration form, and will send the completed form back to the site as soon as possible, no later than 24 hours following the registration request.

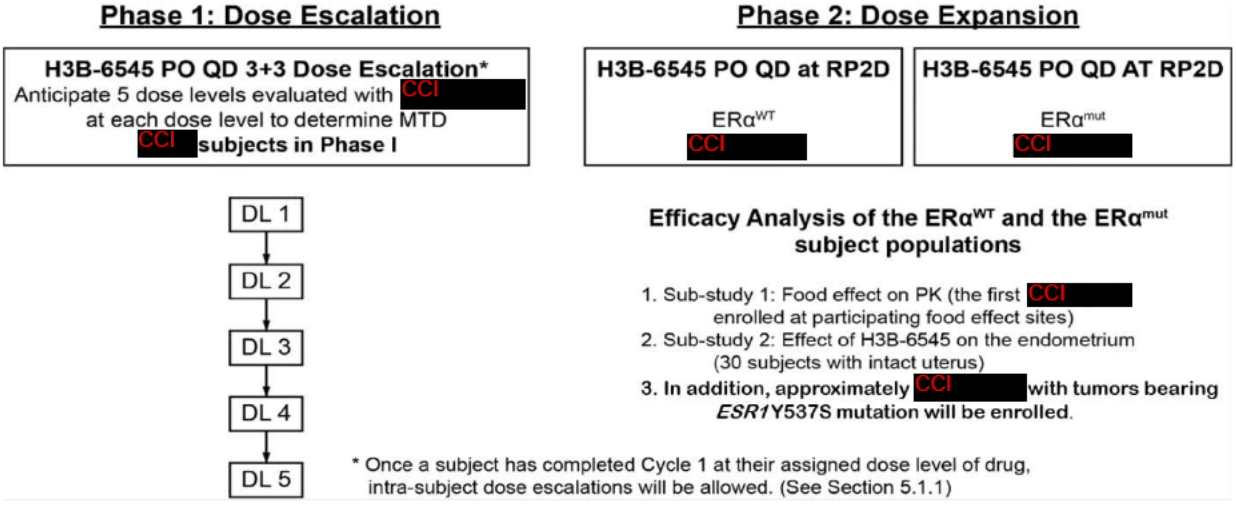
For subjects enrolled under Amendment 6 (and subsequent amendments), the Sponsor or designee will assign a subject number identifier for each subject that is enrolled into the study upon completion of informed consent for prescreening for the clonal *ESR1* Y537S mutation. All subject data collected in the study, including *ESR1* mutational status, will be stored under this number. Only the investigator will be able to link the subject's study data to the subject via an identification list kept at the site. The subject's original medical data that are reviewed at the site during source data verification by the Monitor, audits, and Health Authority inspections will be kept strictly confidential.

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data, including *ESR1* mutational status. Subjects will be informed accordingly and will be requested to give their consent on data handling procedures in accordance with national regulations.

5. STUDY DESIGN

This clinical trial will include a Phase 1 component (dose escalation) and a Phase 2 component, as shown in Figure 1.

Figure 1: Study Design



Abbreviations: DL=dose level; ERα^{mut}=estrogen receptor alpha mutant; ERα^{WT}=estrogen receptor alpha wild-type; *ESR1*=estrogen receptor 1 gene; MTD=maximum tolerated dose; PK=pharmacokinetics; PO=orally (by mouth); QD=once daily; RP2D=recommended Phase 2 dose.

5.1 Phase 1

This Phase 1 dose escalation will follow a standard 3+3 cohort design until the MTD/RP2D is determined in this population. Assuming 5 dose levels will be studied and a maximum of [redacted] subjects will be enrolled per dose level, then approximately [redacted] subjects may be accrued during Phase 1. The primary endpoint will be the determination of the RP2D. In the absence of an MTD, the RP2D will be based on PK and PD information, in addition to all available safety and efficacy data. Additional endpoints will include safety as per the NCI CTCAE v4.03 criteria, objective response rate (ORR), DoR, DCR, PFS, and OS, in addition to biomarkers (including but not limited to ER, PR, Ki67, HER2, gene expression, and DNA mutations).

Safety, tumor response, PK, and PD assessments will be performed for every subject; details are described in Section 7.

[redacted]

5.1.1 Starting Dose and Cohort Management

The objective of the dose escalation phase is to determine the MTD and/or RP2D of H3B-6545 in subjects with ER-positive or HER2-negative locally advanced or metastatic breast cancer.

After all subjects in a cohort have completed Cycle 1 (28 calendar days), all available safety data will be reviewed by the SRC, consisting of the main investigators, and Sponsor personnel, and the decision to proceed to the next dose cohort will be made jointly. Toxicities will be graded using the NCI CTCAE v4.03.

H3B-6545 will be tested in sequential, escalating dose cohorts [redacted] at the dose levels in Table 1. Dose escalation will follow a modified Fibonacci design.

Table 1 Dose Cohorts in H3B-6545 Dose Escalation Phase

Dose Level (Cohort)	No. of Subjects	H3B-6545 Dose CCI	Multiple of Starting Dose
CCI [redacted]			

no. = number; QD = once daily.

CCI [redacted]

CCI

[redacted]

5.1.2 Definition of Dose-Limiting Toxicities

Toxicity will be assessed utilizing the NCI CTCAE v4.03, unless otherwise specified. (NCI CTCAE v 4.03, https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). One cycle is 28 calendar days.

- A DLT is defined as an AE or laboratory abnormality that occurs during Cycle 1 (Phase 1 only) and meets one of the criteria in Table 2 below, according to the NCI CTCAE v 4.03. All AEs of the specified grades in the following table will be counted as DLTs, except those that are clearly and incontrovertibly due to disease progression or extraneous causes.
- A subject who experiences a DLT in Phase 1 Cycle 1 should be discontinued from further study treatment. In rare cases, an exemption to study discontinuation for a non-hepatic, related DLT may be considered on a case-by-case basis after discussion between the treating physician, Sponsor and study medical monitor. The treating physician will need to obtain written Sponsor approval prior to restarting treatment with H3B-6545. Additionally, a dose reduction must be taken upon treatment resumption.
- Any event listed in Table 2 that occurs in Cycle 2 or beyond (including any cycle in Phase 2) will be considered a Dose Modifying Event and should be managed as outlined in Table 3 and Table 4 below.

Table 2 Definition of Dose-Limiting Toxicity (Cycle 1 Phase 1 Only) or Dose-Modifying Event (Cycle 2 or Beyond and Any Cycle in Phase 2)

TOXICITY	CRITERIA*
[redacted]	[redacted]

Subjects whose study treatment is interrupted or permanently discontinued due to an AE or laboratory abnormality must be evaluated at least once a week for 4 weeks (every 2-3 days for hematologic toxicities) and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. If a subject requires a dose delay of ≥4 weeks from the

intended day of the next scheduled dose then a discussion between the treating physician, Sponsor and study medical monitor will need to take place and approval must be granted by the Sponsor before the subject can restart treatment with H3B-6545. If the subject is discontinued from treatment, they will continue to be followed for toxicity as described above. All subjects will be followed for SAEs for 28 days following the last dose of study treatment.

Determination of Dose-Limiting Toxicities

The subject population used for determination of DLTs will consist of subjects who have met the minimum safety evaluation requirements of the study and/or who have experienced a DLT. Minimum safety requirements will be met if, during Cycle 1 of treatment (28 calendar days), the subject receives at least 70% of planned total doses of H3B-6545, completes all required safety evaluations, and is observed for at least 28 days following the first dose of H3B-6545.

Subjects who discontinue treatment early due to disease progression or withdrawal will be asked to have all end-of-treatment (EOT) safety evaluations performed as described in the protocol (see Section 7.5). If a subject withdraws from treatment during Cycle 1 (28 calendar days) due to any reason other than DLT and does not meet the minimum requirements for inclusion in the MTD-determining population described above, that subject will be replaced.

5.1.3 Maximum Tolerated Dose

The MTD is the highest dose at which ≤1 of 6 subjects experience a DLT during the first cycle (28 calendar days) of therapy. If 2 or more subjects in a dosing group of ≤6 subjects experience a DLT, the MTD has been exceeded. If 2 or more subjects in a dose level group of up to 6 subjects experience a DLT and only 3 subjects were evaluated at the previous (ie, next lower) dose, then an additional 3 subjects will be evaluated at this next lower dose and if zero or one have DLTs then this previous dose level is declared the MTD. If two or more have DLTs there is further de-escalation according to the same scheme.

5.1.4 Recommended Phase 2 Dose

The RP2D may not exceed the MTD and will be agreed upon by the SRC based on an integrated evaluation of safety, tolerability, clinical benefit, PK, and PD data for all dose levels tested.

Phase 1 is closed to enrollment, and the RP2D is [redacted]

5.2 Phase 2

The Phase 2 portion of the study will examine the efficacy of the RP2D in an expanded population of ER-positive and HER2-negative subjects. The planned enrollment for the Phase 2 portion of the study (prior to Amendment 6) is approximately [redacted] subjects total [redacted]

In addition, under Amendment 6 and subsequent amendments, approximately [redacted] subjects having a clonal *ESR1* Y537S mutation, in the absence of *ESR1* D538G mutation (determined during the prescreening period by a sponsor-designated central laboratory), will be enrolled. Subjects may enter the screening phases based on an existing sponsor-designated local laboratory result on a case by case basis after consultation with Sponsor. Confirmation of patient eligibility by a sponsor-designated cfDNA test (ie, presence of ESR1 Y537S mutation at AF ≥0.5% and absence of ESR1 D538G at AF ≥0.5%) must be obtained prior to Cycle 1 Day 1.

The effect of a high-fat meal on the relative bioavailability of H3B-6545 will be determined in the first [redacted] subjects enrolled in the Phase 2 part of the trial at sites able to meet PK requirements.

In addition, subjects with an intact uterus will undergo an assessment of endometrial thickness and uterine volume at baseline and 12 weeks later using transvaginal ultrasound. A total of 30 subjects with paired assessments are needed. For as long as the subject is receiving study drug, endometrial assessment will be performed every 24 weeks thereafter until progression.

A subject may participate in neither, one, or both sub-studies in addition to the primary efficacy analysis.

The eligibility criteria in Phase 2 will be similar to those in the Phase 1 part of the trial, with the following exceptions:

- Only 1 prior chemotherapy regimen in the advanced setting will be allowed for Phase 2 subjects.
- Only subjects with tumor tissue accessible for biopsy will be eligible. Subjects with a contra-indication for tumor biopsy may opt out of providing samples after investigator discussion and approval from the Sponsor.
- Subjects with predominantly lytic bone-only disease will be allowed to enroll in the Phase 2 portion of the study and need not provide tumor biopsies.
- [redacted]
- Phase 2 subjects must either have at least one measurable lesion as per RECIST 1.1, or at least one predominantly lytic bone lesion.
- All Phase 2 subjects will provide blood samples for ESR1 mutational analysis.

For subjects enrolled under Amendment 6 (and subsequent amendments), prior therapy must include a CDK4/6 inhibitor and the subject must have at least one measurable lesion as per RECIST 1.1. In addition, the cfDNA *ESR1* mutation analysis must show clonal Y537S with VAF of ≥0.5% in absence of D538G (absent clone or VAF <0.5%).

5.2.1 Food-Effect Evaluation

A high-fat meal is defined as a meal in which approximately 50% of the total caloric content of the meal is from fat and is between 800–1000 calories. This test meal derives approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively. A typical test meal is two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk. Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity (FDA: Assessing the effects of food on drugs in INDs and NDAs – Clinical pharmacology considerations guidance for industry, 2019). Twelve subjects from the Phase 2 part of the study will be included in the food-effect cohort and

will be randomly assigned to receive the Cycle 1 Day 15 dose of H3B-6545 in a fed or fasted state. Each subject will then receive the Cycle 1 Day 22 dose in the reverse/untried state.

- For the "fasted" treatment, following an overnight fast of at least 10 hours, subjects should be administered a single dose of H3B-6545 with 240 mL (8 fluid ounces) of water. No food is allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration.
- For the "fed" treatment, following an overnight fast of at least 10 hours, subjects will be instructed to eat a high-fat breakfast (800-1000 calories, with approximately 50% of calories from fat) in 30 minutes or less, and take a single dose of H3B-6545 within 30 minutes after the start of the meal. H3B-6545 should be administered with 240 mL of water. No food is allowed for at least 4 hours post-dose. Water is allowed as desired except for one hour before and after drug administration.

If a subject does not consume the entire high-fat breakfast (when appropriate) or complete the Day 15 or Day 22 PK assessments, this subject will be replaced to ensure that there are at least 6 subjects in each of the fed-fasted and fasted-fed sequences.

Enrollment to the food effect cohort is closed.

CCI [redacted]

Subjects enrolled under Amendment 6 (and subsequent amendments) may take their dose of H3B-6545 irrespective of food conditions; under fasting or after a meal.

5.2.2 Endometrium Evaluation in Subjects

This sub-study will assess the endometrial effects of H3B-6545 and include subjects who have an intact uterus at baseline. Transvaginal ultrasonography will be performed at baseline and after 12 weeks post-baseline if the subject is still on the study drug on a total of 30 subjects, and then every 24 weeks after that as long as the subject is on treatment until progression. In order to guarantee enough paired ultrasounds, all subjects with an intact uterus will undergo a transvaginal ultrasound at baseline until the total of 30 subjects with ultrasounds at baseline and 12 weeks later is reached. Whenever possible, the baseline and 12-week ultrasound for a subject should be performed by the same person to minimize variability. Changes from baseline in endometrial thickness and uterine volume will be used as endpoints.

The uterus will be assessed in sagittal and coronal planes with uterine size recorded in three diameters (D1, 2, 3). The volume (cm^3) will be estimated as (D1 × D2 × D3 × 3.14) / 6. Double endometrial thickness will be measured in mm in the sagittal plane, from one endometrial-myometrial interface to another excluding intracavitary fluid. Endometrial abnormalities such as internal cysts and polyps will be recorded. The presence of internal cysts is defined by visualization of more than one anechogenic area greater than 2 mm.

No interventions will be performed on any asymptomatic uterine abnormalities that are detected at baseline. A similar approach was adopted for the assessment of the effect of fulvestrant on the endometrium (Morales et al., 2009).

5.3 Treatment Plan

H3B-6545 Oral (PO) continuous daily dosing

The RP2D was determined to be [redacted] (section 1.5.1 Rationale for Recommended Phase 2 Dose).

All subjects entering this study will receive H3B-6545 PO QD based on data evaluation from the prior cohort(s). With the exception of the fed doses of the food-effect cohort (either Cycle 1 Day 15 or Cycle 1 Day 22), subjects will be instructed to take the dose on an empty stomach (no food 2 hours before or 2 hours after dose) at approximately the same time each morning. Capsules should be swallowed whole. If the subject misses a dose of study drug by less than 8 hours, the subject should take the dose as soon as possible. If a dose is missed by greater than 8 hours, the subject should skip the missed dose and take the next dose as scheduled.

For Amendment 6 and subsequent amendments, subjects may take their dose of H3B-6545 irrespective of food conditions; under fasting or after a meal.

If vomiting occurs after taking the study medication, the subject should be instructed not to retake the dose. Subjects should take the next scheduled dose of H3B-6545. If vomiting persists, the subject should contact the investigator.

No routine prophylactic antiemetics will be given. However, the use of palonosetron, prochlorperazine, promethazine, and cyclizine for management of nausea and vomiting is allowed. The use of ondansetron and granisetron is not permitted because of their potential to prolong QT interval.

Treatment Duration

The Primary Completion Date for the study is defined as the date of the last visit for assessment of the primary endpoint of the last subject on the trial.

Subjects will be evaluated for toxicity at the start of each cycle (28 calendar days). Every 2 cycles, a detailed assessment will occur with imaging, laboratory chemistries, and tumor markers as defined in Appendix E. Subjects will continue on treatment until progression, as defined in Appendix G or intolerance to side effects, or withdrawal of consent.

5.4 Concomitant Medications

Subjects will be instructed not to take any additional medications during the course of the study without prior consultation with the research team. At each visit, the subject will be asked about any new medications she is taking or has taken after the start of the study drug.

5.4.1 Permitted Concomitant Medications

The use of palonosetron, prochlorperazine, promethazine, and cyclizine for management of nausea and vomiting is allowed. The use of ondansetron and granisetron is not permitted because of their potential to prolong QT interval.

Use of antacids is allowed.

Medications may be administered for maintenance of existing conditions prior to study enrollment or for a new condition that develops while on study, including but not limited to the following:

- Growth Factors: Subjects receiving recombinant erythropoietin or darbepoietin-α prior to study start may continue to receive pretreatment doses.
  Following initiation of study treatment, the use of erythropoietic and granulocyte growth factors in accordance with local practice or American Society of Clinical Oncology guidelines may be implemented at the discretion of the treating physician.
- Bisphosphonate use, as recommended according to practice guidelines as long as the subject has been on a stable dose for >30 days prior to study enrollment
- Receptor activator of nuclear factor kappa-B ligand inhibitor use, as recommended according to practice guidelines, as long as the subject has been on a stable dose >30 days prior to study enrollment
- Anticoagulation with warfarin and derivatives will not be permitted. However, a maximum daily dose of 1 mg of warfarin will be permitted for port line patency. Should a thrombotic event occur while the subject is receiving treatment, the subject may continue, but low molecular weight heparin, dabigatran, or edoxaban will be the preferred treatment.
- Subjects who develop hyperglycemia during the study should be treated as determined by the treating clinician, according to the American Diabetes Association guidelines.

Other medications considered necessary for the subject's safety and well-being may be given at the discretion of the investigator with the exception of those listed in Section 5.4.2 or Appendix H and Appendix I.

5.4.2 Prohibited Concomitant Medications

The following treatments are prohibited while on this study:

- No other investigational therapy should be given to subjects. No anticancer agents other than the study medications should be given to subjects. If such agents are required for a subject, then the subject must first be withdrawn from the study.
- In vitro, H3B-6545 is a reversible and time-dependent inhibitor of CYP2C8, 2C9, 2C19, and CYP3A4. Drugs with narrow therapeutic index and known to be metabolized by CYP2Cs or CYP3A4 are not permitted because of the inherent potential risk of either reduced activity or enhanced toxicity of the respective concomitant medication (Appendix H).
- H3B-6545 is a substrate of CYP3A4 and P-gp, thus medications that are strong inducers or inhibitors of CYP3A4 or P-gp (Appendix H) should be avoided. For the most updated information, visit the following Web address: http://medicine.iupui.edu/clinpharm/ddis/.
- Due to a potential drug-drug interaction (DDI), BCRP substrates with narrow therapeutic index are not permitted because H3B-6545 is an inhibitor of BCRP (but not a substrate) (Appendix H).
- If, after a subject has been enrolled, she requires the concomitant use of any of the medications that may cause QTc interval prolongation, then H3B-6545 must be held while

the subject receives the concomitant medication (Appendix I). Resumption of treatment with H3B-6545 may be considered following discontinuation and washout (at least 5 half-lives) of the concomitant medication if the subject has not progressed and is agreed upon with the Sponsor. Excluded medications that may cause QTc interval prolongation are also listed and updated at the following Web address: http://crediblemeds.org.

- Herbal preparations/medications are not allowed throughout the study. These herbal medications include, but are not limited to St. John's wort, kava, ephedra (ma huang), gingko biloba, DHEA, yohimbe, saw palmetto, and ginseng. Subjects should stop using these herbal medications 7 days prior to first dose of study drug.
- Systemic corticosteroids are not allowed during the study. Note: The following uses of corticosteroids are permitted: single doses, topical applications (eg, for rash), inhaled sprays (eg, for obstructive airway diseases), eye drops, or local injections (eg, intra-articular). Short courses of systemic corticosteroids may be considered in consultation with the Medical Monitor.

6. DOSE MODIFICATIONS

If any toxicity occurs, it will be graded utilizing the NCI CTCAE v 4.03, and appropriate supportive care treatment will be administered to decrease the signs and symptoms thereof. Dose adjustments will be based on the organ system exhibiting the greatest degree of toxicity.

6.1 Dose Modifications Due to Toxicity

Dose reductions or holds and initiation of supportive care are allowed as clinically indicated by the treating physician. Subjects who require a treatment interruption due to drug-related toxicity for more than 4 weeks will discontinue study treatment. Treatment with H3B-6545 will be held in any subject experiencing a dose modifying event as described in Section 5.1.2 at any time during the study. Subjects who experience a DLT in Phase 1 Cycle 1 according to Table 2 above will be discontinued from treatment unless otherwise specified in writing from the Sponsor. Dose modifications following a dose modifying event will be according to Table 3 and Table 4 below.

Two dose reductions [redacted] for toxicity will be allowed. In addition, dose reductions will also be allowed based on clinical judgment of the treating physician. If persistent toxicity occurs despite two dose reductions, the subject will discontinue study treatment.

Table 3 Dose Modifications Due to Hematologic Toxicities

Event	H3B-6545 Dose
CCI	

Table 4 Dose Modification for Grade 3, Grade 4, or Intolerable Grade 2 Non-Hematologic Toxicities

Toxicity Grade	H3B-6545 Dose[a]
[redacted]	CCI [redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]

Table 4 Dose Modification for Grade 3, Grade 4, or Intolerable Grade 2 Non-Hematologic Toxicities

Toxicity Grade	H3B-6545 Dose[a]
CCI [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

7. STUDY ASSESSMENTS AND EVALUATIONS

7.1 Overview

All subjects should visit the study site on the days specified within this protocol. The complete Schedule of Assessments for this study is shown in Appendix D, Appendix E, and Appendix F.

With Sponsor approval, in certain situations (eg, due to the COVID pandemic), and as per local regulations, certain assessments and study related activities may be performed remotely.

Prescreening Visit - starting from Amendment 6 (or subsequent amendments):

If all the following criteria are satisfied:

a) An ESR1 mutation status report is already available for the subject from one of the sponsor-designated laboratories based on cfDNA test report
b) The blood sample for this report was drawn within 3 months of the date of main study consent
c) The report clearly shows ESR1 Y537S mutation at an AF ≥0.5% in absence of ESR1 D538G at AF ≥0.5% (that is, clonal Y537S)

Then the subject may proceed to sign the main consent of the study and no prescreening blood sample is needed for re-testing the ESR1 mutation status.

Otherwise, potential subjects will be asked to sign a prescreening consent in order to determine if their tumor carries a clonal *ESR1* Y537S mutation. Only after confirmation of the presence of a ESR1 Y537S mutation at an AF ≥0.5% and absence of ESR1 D538G at AF ≥0.5% from a sponsor-designated laboratory will the subject be invited to sign the main study consent.

-During the Prescreening Visit:

- Pre-screening consent for the collection of a whole blood sample for cfDNA testing -
- Collection of a whole blood sample for cfDNA testing

If a redraw is necessary due to quality concerns at the analysis laboratory (eg, not acceptable for analysis), an additional sample may be collected.

-Study Informed Consent (Visit 1):

In addition to the 2 previous scenarios, subjects may sign the main consent form if they have clonal *ESR1* Y537S mutation, as defined above, based on a local laboratory report, but may not be dosed on C1D1 until their mutational status has been confirmed by a sponsor-designated cfDNA test from a blood sample obtained during the screening period.

Informed consent (Visit 1) must be obtained ≤28 days prior to initiation of treatment and before any protocol-specific procedures are performed. The screening physical examination, medical history, ECOG PS, complete blood counts (CBCs), differential and platelets, comprehensive metabolic profile (CMP) including liver (alkaline phosphatase [ALP], AST, and ALT) and thyroid function (thyroid-stimulating hormone [TSH] and Free T4) tests, urinalysis, and prothrombin time/partial thromboplastin time (PT/PTT) using international normalized ratio (INR) should be done ≤7 days prior to initiation of treatment (Study Visit 2).

Whole blood and a pre-treatment biopsy will be collected at baseline for biomarker analysis. Subjects must be willing to undergo tumor biopsies prior to treatment and on Cycle 2 Day 1. In

the Phase 2 part of the trial, subjects with bone-only disease, or subjects for whom a biopsy is contra-indicated, may opt out of providing tumor biopsies following investigator concern and discussion and approval from the Sponsor. A urine or serum pregnancy test must be performed within 72 hours of Cycle 1 Day 1 for all pre-and peri-menopausal subjects. Computed tomography (CT) scans (Visit 1) should be performed ≤28 days prior to initiation of treatment (Cycle 1 Day 1). A bone scan (^{99}m-technetium-based scintigraphy, whole-body bone magnetic resonance imaging (MRI), or ^{18}F-sodium fluoride position emission tomography [NaF PET]) to establish a baseline should be performed prior to first dose of study drug (a historical bone scan performed within 6 weeks prior to the first dose is acceptable), and as clinically indicated. Lesions identified on bone scans should be followed with cross-sectional imaging.

The prescreening and screening assessments described in Appendix D and Appendix E, respectively, will be collected, reviewed, and determined to be acceptable by the site PI or designee after obtaining informed consent prior to the initiation of treatment.

A cycle of treatment is scheduled to last 4 weeks (28 calendar days).

Multiple procedures may be scheduled at the same time point relative to H3B-6545 dosing. Priority should be given to PK collection at the time specified. Vital signs and triplicate electrocardiogram (ECG) assessments should be performed prior to specimen collections.

7.2 Baseline Study Assessments (Visit 2)

The following information will be collected and procedures will be performed for each subject at screening ≤7 days prior to initiation of treatment, unless otherwise noted:

- Written ICF prior to any other study-related procedures (≤28 days prior to initiation of treatment).
- Assessment of inclusion/exclusion criteria.
- Medical history, including cancer history, eg, date and stage at diagnosis, hormone receptor status, extent of disease, and all previous anti-cancer treatments, response to such treatments, best overall response, and the Time to Progression (TTP) (per RECIST 1.1) for the most recent prior therapy.
- A bone scan (^{99}m-technetium-based scintigraphy, whole-body bone MRI, or NaF PET) will be performed to establish a baseline (a historical bone scan performed within 6 weeks prior to the first dose of study drug is acceptable), and as clinically indicated. Lesions identified on bone scans should be followed with cross-sectional imaging.
- Physical examination, measurements of height (first visit only), weight, and vital signs (resting HR, BP, respiratory rate [RR], and oral temperature).
- ECOG PS (Appendix A).
- 12-lead ECG in triplicate.
- Echocardiogram/MUGA assessment (≤28 days prior to initiation of treatment).
- Review and record concomitant medications (all concomitant medications taken within 28 days prior to Cycle 1 Day 1 should be documented in the eCRF).
- CBC including hemoglobin, hematocrit, white blood count (WBC) with 5-part differential, and platelets plus reticulocytes.

- CMP to include: glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, calcium, magnesium, phosphorus, lactate dehydrogenase, carbon dioxide (CO2), ALP, AST, ALT, TSH, free T4, total bilirubin, total protein, and albumin.
- PT/PTT.
- Urine testing dipstick.
- Serum or urine pregnancy test for all pre-and perimenopausal subjects (must be performed within 72 hours prior to the initiation of treatment).
- Archived tumor tissue if available (see Section 7.8.2). For subjects enrolled under Amendment 6 (or subsequent amendments), a recent archival tumor tissue obtained within 6 months prior to enrollment or a fresh tumor biopsy must be provided. A second biopsy after initiating trial therapy is not required.
- Whole blood for nucleic acids (cfDNA).
- Tumor biopsy (unless contraindicated)
- Transvaginal ultrasound (subjects in the Phase 2 endometrium sub-study only) (≤14 days prior to initiation of treatment).
- Disease assessment: CT scan of chest, abdomen, and pelvis (≤28 days prior to initiation of treatment). All study imaging for tumor assessments will be sent to an imaging core laboratory designated by the Sponsor for review of quality, and archived for potential independent review.

7.3 Study Treatment Assessments

7.3.1 Cycle 1 Days 1 (Visit 3), 8 (Visit 4), 15 (Visit 5), and 22 (Visit 6)

For all subjects and all visits, unless otherwise stated:

- Physical examination, measurements of weight, and vital signs (resting HR, BP, RR, and oral temperature) (Day 1 only). No need to repeat physical exams on Cycle 1 Day 1 if the baseline physical exam was performed ≤72 hours.
- ECOG PS (Day 1 only).
- 12-lead ECG or continuous Holter monitor:

  Phase 1: continuous Holter monitoring must begin at **least 1 hour prior** to dose on Cycle 1, Days 1 and 15, and continue through at least 24 hours post-dose. For convenience, Holter monitoring may begin up to 12 hours prior to dosing on Cycle 1, Day 1 or Day 15.

  Phase 2: in place of Holter monitoring, a 12-lead ECG will be performed in triplicate on Cycle 1, Days 1 and 15.

  - If sinus bradycardia is observed at any point during treatment (from ECG or other clinical assessment), it is recommended the subject undergo a stress test per institution policy. A stress test is recommended to be done only once for each subject who has under 60 beats per minute (while on treatment) and more than a

20 percent reduction from baseline in beats per minute. The stress test should be triggered by the previous two cardiac observations at rest.

- Assessment and recording of AEs (from the time informed consent was signed).
- Review and recording of concomitant medication (all concomitant medications taken within 28 days prior to Cycle 1 Day 1 should be documented in the eCRF)
- CBC including hemoglobin, hematocrit, WBC with 5-part differential, and platelets plus reticulocytes. Do not repeat on Cycle 1 Day 1 if it is within 72 hours of baseline.
- CMP. Do not repeat on Cycle 1 Day 1 if it is within 72 hours of baseline.
- PT/PTT.
- Urine testing dipstick.
- Phase 1 – PK samples for all subjects (Days 1 and 15: collected at time points 0 (pre-dose), 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, and 24 hr post-dose; Days 8 and 22: collected pre-dose only) (See Section 7.7).
- Phase 2 – PK samples for subjects in the food-effect sub-study: Days 15 and 22: collected at time points 0 (pre-dose), 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, and 24 hr post-dose) (See Section 7.7).
- Phase 2 – All other subjects: Sparse PK samples will be performed on Day 15 following one of three schedules, assigned sequentially:
    - Schedule 1: pre-dose (0 hr), 0.5 hr, and 6 hr post-dose
    - Schedule 2: pre-dose (0 hr), 2 hr, and 8 hr post-dose
    - Schedule 3: 1 hr, 4 hr, 24 hr post-dose (See Section 7.7). Plasma for cfDNA (nucleic acids) on Days 1 and 15.
    - For subjects enrolled under Amendment 6 (and subsequent amendments), PK samples will be collected on ***Days 1 and 15*** at pre-dose (0 hr) and at 1, 2, 4, and 6 hours post-dose.
- Whole blood for pharmacogenetics (PG) on Day 1 only.
- Blood sample for markers of bone resorption and formation markers (anytime from screening period up to Day 1 pre-dose).
- H3B-6545 administration.
- Schedule next visits for the cycle (Day 1 only).

7.3.2 Cycle 2 Days 1 (Visit 7) and 15 (Visit 8)

Both visits unless otherwise stated:

- Physical examination and vital signs (Day 1 only).
- ECOG PS (Day 1 only).
- 12-lead ECG in triplicate prior to dosing and 3 hours post-dose (Day 1 only). If sinus bradycardia is observed at any point during treatment (from ECG or other clinical assessment), it is recommended the subject undergo a stress test per institution policy. A stress test is recommended to be done only once for each subject who has under 60 beats

per minute (while on treatment) and more than a 20 percent reduction from baseline in beats per minute. The stress test should be triggered by the previous two cardiac observations at rest.

- AE assessment.
- Review and recording of concomitant medication.
- CBC including hemoglobin, hematocrit, WBC with 5-part differential, and platelets plus reticulocytes.
- CMP.
- PT/PTT.
- Urine testing dipstick.
- Serum or urine pregnancy test for all pre- and perimenopausal subjects (Day 1 only).
- Tumor biopsy (unless contraindicated)
    - The subject must have received at least 7 days of consecutive dosing prior to collection of the second biopsy. The window for collection of the Cycle 2 biopsy may be extended if needed in order to ensure 7 consecutive days of dosing prior to collection.
- Whole blood for nucleic acids (cfDNA) on Day 1 only.
- Blood sample for markers of bone resorption and formation on Day 15 only.
- H3B-6545 administration.
- Schedule next visit (Day 1 only).

7.3.3 Cycle 3 Days 1 (Visit 9) and 15 (Visit 10)

For all subjects, during both visits, unless otherwise stated:

- Physical examination and vital signs (Day 1 only).
- ECOG PS (Day 1 only).
- 12-lead ECG in triplicate prior to dosing and 3 hours post-dose (Day 1 only).
- AE assessment.
- Review and recording of concomitant medication.
- CBC including hemoglobin, hematocrit, WBC with 5-part differential, and platelets plus reticulocytes.
- CMP (Day 1 only).
- PT/PTT.
- Urine testing dipstick.
- Serum or urine pregnancy test for all pre-and perimenopausal subjects (Day 1 only).

- Disease assessment: CT scan of chest, abdomen, and pelvis. All study imaging for tumor assessments will be sent to an imaging core laboratory designated by the Sponsor for review of quality, and archived for potential independent review.
- Whole blood for nucleic acids (cfDNA) on Day 1 of Cycle 3 and every other cycle thereafter in conjunction with tumor imaging assessment.
- H3B-6545 administration.
- Schedule next visit (Day 1 only).

7.3.4 Cycle 4 and Beyond - Days 1 (Visits 11, 13, 15, and beyond) and 15 (Visit 12)

For all subjects, during s both visits, unless otherwise stated:

- Physical examination and vital signs (Day 1 only).
- ECOG PS (Day 1 only).
- 12-lead ECG in triplicate prior to dosing and 3 hours post-dose (Cycle 4 Day 1 only). If sinus bradycardia is observed at any point during treatment (from ECG or other clinical assessment), it is recommended the subject undergo a stress test per institution policy. A stress test is recommended to be done only once for each subject who has under 60 beats per minute (while on treatment) and more than a 20 percent reduction from baseline in beats per minute. The stress test should be triggered by the previous two cardiac observations at rest.
- AE assessment.
- Review and recording of concomitant medication.
- CBC including hemoglobin, hematocrit, WBC with 5-part differential, and platelets plus reticulocytes.
- CMP (Day 1 only).
- PT/PTT.
- Urine testing dipstick.
- Serum or urine pregnancy test for all pre-and peri-menopausal subjects (Day 1 only).
- Whole blood for nucleic acid (cfDNA) on Day 1 of Cycle 3 and every other cycle thereafter, in conjunction with tumor imaging assessment.
- Blood samples for markers of bone resorption and formation (Cycle 4 Day 1 only, before dosing).
- Transvaginal ultrasound (Cycle 4 Day 1 and then every 24 weeks until progression) (subjects in the Phase 2 endometrium sub-study only).
- H3B-6545 administration.
- Schedule next visit (Day 1 only)
- Day 15 visits can be discontinued after Cycle 4

7.4 Response Assessment Every 8 Weeks

Subjects will be evaluated for tumor response every 8 weeks (±1 week) with a CT scan of the chest, abdomen, and pelvis. Subjects with a partial response or CR will have a second confirmatory scan ≥ 4 weeks following the initial scan showing response.

Subjects with progressive disease or unacceptable toxicity should discontinue trial therapy; subjects with stable disease (SD) or response to therapy will continue treatment.

If a subject discontinues therapy for a reason other than radiological disease progression or withdrawal of consent, tumor assessment by CT and/or MRI scans should continue every 8 weeks (± 1 week) until radiological disease progression or starting a new anti-cancer therapy.

Refer to Section 7.6 and Appendix F for the tumor assessment schedule for subjects in the Extension Phase.

7.5 End-of-Treatment Visit

Subjects are permitted to continue treatment with H3B-6545 until disease progression, unacceptable toxicity, or decision to discontinue treatment by the subject or the study physician. Follow-up evaluations required after end of treatment are specified in Appendix E.

If treatment is discontinued because of toxicity or any other reason(s) at a treatment visit and no study treatment is administered, that visit may fulfill the EOT visit.

If a subject withdraws from treatment during Cycle 1 (28 calendar days) due to any reason other than a DLT and does not meet the minimum requirements for inclusion in the DLT-evaluable population described in Section 10.10, that subject will be replaced.

After discontinuation of trial therapy, subjects must be followed for any new AEs for 28 calendar days after the last dose of H3B-6545.

The following EOT visit procedures will be performed:

- Physical examination, weight, and vital signs (resting HR, BP, RR, and oral temperature).
- ECOG PS (Appendix A).
- AE assessment.
- Review and recording of concomitant medication.
- CBC, including WBC with 3-part differential, and platelets.
- CMP.
- PT/PTT.
- Urine dipstick.
- 12-lead ECG in triplicate for all subjects. If sinus bradycardia is observed at the EOT visit, follow up ECGs should be performed weekly until HR returns 60 or more beats per minute or to pre-study baseline.
- Disease assessment: CT scan of chest, abdomen, and pelvis. All study imaging for tumor assessments will be sent to an imaging core laboratory designated by the Sponsor for review of quality, and archived for potential independent review.

- Whole blood sample for nucleic acids (cfDNA).
- Blood sample for markers of bone resorption and formation.

7.6 Follow-up after End-of-Treatment Visit

Subjects who discontinue treatment will be followed for safety for 28 days after the subject's last dose. Subjects must complete a safety follow-up visit on the 28th day (with up to + 7-day window). During the safety follow-up visit the following should be obtained: vital signs, hematological and CMP laboratory parameters, and triplicate ECGs on ERT provided study equipment. All AEs that occur during the safety follow-up period will be recorded.

If sinus bradycardia is observed at EOT visit, follow up ECGs should be performed weekly until HR returns 60 or more beats per minute or to pre-study baseline.

If a subject discontinues therapy for any reason other than radiological disease progression or withdrawal of consent, tumor assessments by CT and/or MRI scans should continue every 8 weeks (± 1 week) until radiological disease progression or until they start a new anti-cancer therapy.

There will be a data cutoff for the primary analysis at 6 months after the last subject receives their first dose of study treatment. All subjects who are still on study treatment at that time will enter the Extension Phase.

Following disease progression, all subjects will be followed for survival at least every 12 weeks and until the data cutoff for the primary analysis, when survival follow-up will end for all subjects. No survival follow-up will be done in the Extension Phase. Survival follow-up can be done by telephone call at the investigator's discretion. The first anti-cancer therapy following treatment discontinuation will be recorded up until the data cutoff for the primary analysis. Subjects may continue to receive treatment after progression only if, after a discussion with the medical monitor, it is determined that the subject continues to derive clinical benefit.

In the **Extension Phase**, subjects still on study treatment will continue to receive study drug in 28-day cycles until disease progression, development of unacceptable toxicity, subject request, withdrawal of consent, or discontinuation of study by the Sponsor. Tumor assessments will be performed according to the local standard of care, but no less than every 12 weeks. As per the Schedule of Assessments for the Extension Phase, only safety and dosing information will be collected (Appendix F). The End of Treatment Visit assessment will occur 28 (+ 7 days) days after the final dose of study treatment. All AEs/SAEs will be captured up to 28 days (+ 7 days) after the last dose of study drug.

The end of study is the last subject's last assessment (eg, the last assessment for the last subject in the Extension Phase).

7.7 Pharmacokinetics

During the Phase 1 part of the trial, plasma samples will be collected during Cycle 1 on Day 1 and Day 15 (pre-dose [0 hr], 0.5, 1, 2, 4, 6, 8, 10, and 24 hr post dose), for analysis of plasma concentration levels of H3B-6545 and potentially selected metabolite(s), at the specified time points. Additional pre-dose trough samples will be collected during Cycle 1 on Day 8 and Day 22.

For the subjects enrolled in the food effect cohort of Phase 2 of the trial, plasma samples will be collected during Cycle 1 on Day 15 and Day 22 (pre-dose [0 hr], 0.5, 1, 2, 4, 6, 8, 10, and 24 hr post dose prior to the next dose).

For all other Phase 2 subjects not enrolled in the food effect cohort, sparse PK samples will be collected on Day 15 following one of three schedules, assigned sequentially:

- Schedule 1: pre-dose (0 hr), 0.5 hr, and 6 hr post-dose.
- Schedule 2: pre-dose (0 hr), 2 hr, and 8 hr post-dose.
- Schedule 3: 1 hr, 4 hr, and 24 hr post-dose.

For subjects enrolled under Amendment 6 (or subsequent amendments), PK samples will be collected on Cycle 1 Days 1 and 15 at pre-dose (0 hr: -60 ~ 0 min) and at 1 (±10 min), 2 (±15 min), 4 (±20 min), and 6 (±30 min) hours post-dose.

Pharmacokinetic sample collection will end at the time of the data cutoff for the primary analysis (per Amendment 09).

In Phase 1 and Phase 2, a protocol deviation will be reported only when a PK time point is not collected (missing) or blood is drawn on the wrong study day.

Plasma concentrations of H3B-6545 will be tabulated and summarized by dose level, day, and time. Standard PK parameters including, but not limited to, the area under the plasma concentration-time curve from time point 0 through the last measurable point ($AUC_{0–t}$), C_{max}, time of maximum observed plasma concentration (t_{max}), and accumulation ratio (R_{acc}) will be determined. If data permit, area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\text{-}inf}$), $t_{½}$, and apparent total body clearance following oral administration (CL/F) will be calculated. Assessment of the effect of dose on C_{max} and AUC will also be performed. The primary assessment of PK will be done using non-compartmental methods; additional compartmental analysis may also be performed.

The effect of a high-fat meal on AUC, C_{max} and t_{max} of H3B-6545 will be evaluated using a mixed linear model of logarithmically transformed values of the primary PK parameters. Ratios of geometric means (fed/fasted comparison) and associated 2-sided 90% confidence intervals (CIs) will be presented.

Plasma samples collected for PK analysis may also be used for an exploratory assessment of the H3B-6545 metabolite profile. A report for metabolite identification will be provided in a separate document, and results for metabolites will be not included in the clinical study report.

Instructions for the processing, storage, and shipment of PK and metabolite identification samples will be provided in the Laboratory Manual. For PK, plasma concentrations of analyte(s) will be quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS) methodology using a validated assay.

7.8 Biomarkers

Instructions for the collection, processing, storage, and shipping of samples will be provided in the Laboratory Manual. Time points and minimum blood volume for biomarker specimens are presented in Table 5 . Biomarker sample collection will end at the time of the data cutoff for the primary analysis.

Table 5 Time Points and Minimum Blood Volume for Biomarker Specimens[g]

Biospecimen	Pre-Screen	Baseline (Screening)	Cycle 1		Cycle 2		C3	C4	EOT
			D1	D15	D1	D15	D1[e]	D1[f]	
CCI									

7.8.1 Prescreening Phase

CCI

7.8.2 Screening Phase Biomarkers

CCI

7.8.3 Baseline and Treatment Phase Biomarkers

CCI

[redacted]

7.8.4 Pharmacogenetics Assessment

CCI

[redacted]

CCI [redacted]

7.9 Biomarkers of Bone Resorption and Formation

CCI [redacted]

[redacted]

8. DRUG FORMULATION, AVAILABILITY, ADMINISTRATION, AND TOXICITY INFORMATION

8.1 H3B-6545

Investigational Product	Dosage Form and Strength	Manufacturer
H3B-6545	50 mg and 150 mg (Only 150 mg starting from Amendment 8)	Eisai Inc.

8.1.1 Labeling, Packaging, and Supply

H3B-6545 drug product for clinical studies is supplied as CCI [redacted]

At each drug dispensing visit, subjects will be dispensed sufficient supplies until the next drug dispensing visit. Study drug compliance will be assessed at each subject visit. The research staff will count and document the amount of study drug taken and returned by the subject. The batch number of the study drug dispensed to the subject should be entered on the case report form (CRF), if applicable.

H3B-6545 will be labeled in accordance with text that is in full regulatory compliance with each participating country and is translated into the required language(s) for each of those countries. Study drug labels will not bear any statement that is false or misleading in any manner or represents that the study drug is safe or effective for the purposes for which it is being investigated.

The immediate packaging will contain a statement to conform with FDA Investigational New Drug requirements as follows: "Caution: New Drug - Limited by federal (or United States) law to investigational use."

8.1.2 Administration of H3B-6545

H3B-6545 is administered orally. All subjects will take H3B-6545 at their assigned dose daily by mouth (PO) in an open-label fashion. H3B-6545 will be supplied as CCI [redacted]

Subjects will be instructed to take the dose on an empty stomach (no food 2 hours before or 2 hours after dose, with the exception of subjects taking the fed dose of the food-effect cohort) at approximately the same time each morning.

Subjects enrolled under Amendment 6 (and subsequent amendments) may take their dose of H3B-6545 irrespective of food conditions; under fasting or after a meal. CCI [redacted]

Capsules should be swallowed whole. Dosing should not be repeated if a subject vomits. A dose missed by greater than 8 hours should be skipped.

Trial therapy will continue until progression, unacceptable toxicity, or subject refusal. If subjects discontinue trial therapy for any reason other than progression or death, the investigator should continue tumor assessment as per protocol schedule until documented disease progression.

Detailed administration instructions will be provided in the Investigator's Brochure (IB).

8.1.3 Precautions and Risks Associated with H3B-6545

Because H3B-6545 is known to be metabolized by CYP3A4, and since grapefruit and Seville oranges are known to be strong inhibitors of CYP3A4 and Saint John's wort is known to be a strong inducer of CYP3A4 (FDA, 2012), subjects will be instructed to not consume grapefruit or Seville oranges, their juice, or Saint John's wort.

Further precautions and risks are located in the IB.

8.2 Storage Conditions

All study drugs must be kept in a secure place under appropriate storage conditions. Storage conditions for H3B-6545 are included on the investigational product label.

The Sponsor or its representatives must be granted access on reasonable request to check drug storage, dispensing procedures, and accountability records.

Study drug will be stored in accordance with the labeled storage conditions. Continuous temperature monitoring and recording is required at the storage location to ensure that the study drug is maintained within an established temperature range. The investigator or designee is responsible for ensuring that the temperature is recorded while the drug is at the clinic and that records are maintained; the temperature should be monitored continuously by using either an in-house validated data acquisition system, a mechanical recording device, such as a calibrated chart recorder, or by manual means, such that minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. When the H3B-6545 is dispensed to the subject, they will be asked to store the drug in the refrigerator.

8.3 Accountability for All Study Drugs

The PI (or designee) is responsible for accountability of all used and unused study drug supplies at the site. Under no circumstances will the investigator allow the study drug to be used other than as directed by this protocol. Study drug will not be dispensed to any individual who is not enrolled in the study.

All study drug inventories must be made available for inspection by the Sponsor or its representatives and regulatory agency inspectors upon request.

The site must maintain an accurate and timely record of the following: receipt of all study drug, dispensing of study drug to the subject, collection and reconciliation of unused study drug that are either returned by the subjects or shipped to site but not dispensed to subjects, and return of reconciled study drug to the Sponsor or (where applicable) destruction of reconciled study drug at the site.

This includes but may not be limited to: (a) documentation of receipt of study drug, (b) study drug dispensing/return reconciliation log, (c) study drug accountability log, (d) all shipping service receipts, (e) documentation of returns to the Sponsor, and (f) certificates of destruction for any destruction of study drugs/study supplies that occurs at the site. All forms will be provided by the Sponsor.

Any comparable forms that the site wishes to use must be approved by the Sponsor.

The study drug and inventory records must be made available, upon request, for inspection by a designated representative of the Sponsor or a representative of a health authority (eg, FDA, Medicines & Healthcare products Regulatory Agency). As applicable, all unused study drug and empty and partially empty containers from used study drug are to be returned to the investigator or designee by the subject and together with unused study drug that were shipped to the site but not dispensed to subjects are to be returned to the Sponsor's designated central or local depot(s) during the study or at the conclusion of the study, unless provision is made by the Sponsor for destruction of study drug and containers at the site.

Approval for destruction to occur at the site must be provided by the Sponsor in advance. Upon completion of drug accountability and reconciliation procedures by the site's personnel and documentation procedures by the Sponsor's personnel, study drug that are to be returned to the Sponsor's designated central or local depot(s) must be boxed and sealed and shipped back to the central or local depot(s) following all local regulatory requirements. In some regions, study drug may be removed from the site and hand delivered to the central or local depot by Sponsor representatives. Where study drugs are approved for destruction at the site, destruction will occur following the site's standard procedures and certificates of destruction will be provided to the Sponsor.

Drug accountability will be reviewed during site visits and at the completion of the study.

At the end of the study, all Drug Accountability Record Form(s) will be completed by the site and sent to the Sponsor CRA or designee. Please contact the Sponsor or CRA regarding disposal of any study drug.

9. RESPONSE EVALUATIONS AND MEASUREMENTS

Tumor assessments will be performed at baseline (within 28 days of starting trial therapy) and every 8 weeks after starting trial therapy until progression.

Response and progression will be evaluated in this study using the RECIST version 1.1 (see Appendix G). Lesions are either measurable or non-measurable according to the criteria. The term "evaluable" in reference to measurability will not be used, as it does not provide additional meaning or accuracy.

The data cutoff for the primary analysis will occur at 6 months after the last subject receives their first dose (see Section 7.6). All subjects who are still on study treatment at that time will enter the Extension Phase. Tumor assessments will be performed according to the local standard of care, but no less than every 12 weeks. The Schedule of Assessments for the Extension Phase is found in Appendix F.

Following progression, all subjects will be followed for survival at least every 12 weeks, and survival follow-up will end 6 months after the last subject receives their first dose of study treatment (at the time of data cutoff for the primary analysis) (as of Amendment 9), and no survival follow-up will be done in the Extension Phase. Survival updates may be obtained via phone call to the subject or her family and the information should be documented in the subject's file.

10. STATISTICAL CONSIDERATIONS

10.1 Statistical Design

This is a multi-center, open-label, dose-escalation followed by dose expansion Phase 1/2 study of H3B-6545.

The statistical analyses of study data are described in this section. Further details of the analytical plan will be provided in the Statistical Analysis Plan (SAP), which will be finalized before database lock.

10.2 Phase 1 Primary Objective

The primary objective of the Phase 1 portion of this study is to:

- Determine the MTD and/or the RP2D of H3B-6545 in women with locally advanced or metastatic ER-positive, HER2 negative breast cancer.

10.3 Phase 1 Secondary Objectives

The secondary objectives of the Phase 1 portion of this study are to:

- Evaluate the safety and tolerability of H3B-6545 in women with locally advanced or metastatic (ER)-positive, HER2-negative breast cancer
- Characterize the plasma PK of H3B-6545.
- Estimate the efficacy of H3B-6545 in terms of response rate, DoR, DCR, PFS, and OS.

10.4 Phase 1 Exploratory Objectives

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10.5 Phase 2 Primary Objective

The primary objective of the Phase 2 portion of this study is to:

- Estimate the efficacy of H3B-6545 in terms of best overall response rate, DoR, DCR, CBR, PFS, and OS in subjects with (ER)-positive, HER2-negative breast cancer and in those with and without $ER\alpha^{mut}$ (including a clonal ESR1 Y537S mutation).

10.6 Phase 2 Secondary Objectives

The secondary objectives of the Phase 2 portion of this study are to:

- Further characterize the safety of H3B-6545 in this subject population.
- Further characterize the PK of H3B-6545. At least sparse PK samples will be collected from all subjects on study.
- Evaluate the effect of a high-fat meal on the relative bioavailability of H3B-6545.
- Assess the effect of H3B-6545 on serum bone turn-over markers, namely BSAP (for osteoclast metabolism), PINP (for bone formation), and CTX (for bone resorption).
- Assess the effect of H3B-6545 on endometrial thickness and uterine volume. This objective will be assessed in a subgroup of women with intact uteri.

10.7 Phase 2 Exploratory Objectives

The exploratory objectives of the Phase 2 portion of this study are to:

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[redacted]

10.8 Study Endpoints

10.8.1.1 Phase 1

The primary study endpoint for Phase 1 is the occurrence of DLTs as a function of the dose of H3B-6545 for determination of the MTD and/or RP2D.

Secondary study endpoints for Phase 1 are:

- Safety/tolerability: the type and frequency of AEs and SAEs using NCI CTCAE v4.03, as well as changes in clinical laboratory values, vital sign measurements and ECG parameters.
- PK: standard primary PK parameters including, but not limited to, the area under the plasma concentration-time curve from time point 0 through the last measurable point (AUC_{0-t}), C_{max}, t_{max} and R_{acc}.
- Preliminary efficacy: Response will be determined by the investigator assessments. The following endpoints will be determined:
  - ORR, defined as the proportion of subjects achieving a best overall response of confirmed partial or complete response (partial response + CR)
  - DoR, defined as the time from the date of first documented CR/partial response until the first documentation of confirmed disease progression or death, whichever comes first.
  - DCR, defined as the proportion of subjects achieving a best response of CR or partial response, or SD
  - PFS, defined as the time from first dose date to the date of the first documentation of confirmed disease progression or death (whichever occurs first).
  - OS, defined as the time from first dose date to the date of death (event) or date last known alive (censored).

Exploratory study endpoints for Phase 1 and Phase 2 (except where noted) include:

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[redacted]

10.8.1.2 Phase 2

The primary study endpoint for Phase 2 is:

- ORR, defined as the proportion of subjects achieving a best overall response of confirmed partial response + CR. Response will be determined by the investigator assessment.
- Additional efficacy endpoints (determined by investigator assessment):
  - DoR, defined as the time from the date of first documented CR/partial response until the first documentation of confirmed disease progression or death, whichever comes first.
  - DCR, defined the proportion of subjects achieving a best response of CR, partial response, or SD
  - CBR: defined as the proportion of subjects with a best overall response of CR, partial response, or durable SD (duration of SD ≥23 weeks)
  - PFS, defined as the time from first dose date to the date of the first documentation of confirmed disease progression or death (whichever occurs first).
  - OS, defined as the time from first dose date to the date of death (event) or date last known alive (censored).

Secondary study endpoints for Phase 2 are:

- Safety/tolerability: same as Phase 1.
- Further characterize PK of H3B-6545.
- Evaluate the effect of a high-fat meal on the relative bioavailability of H3B-6545
- Endometrial thickness and uterine volume in a subgroup of subjects
- Effect of H3B-6545 on serum bone turn-over markers, namely BSAP (for osteoclast metabolism), PINP (for bone formation), and CTX (for bone resorption).

10.9 Sample Size Considerations

10.9.1 Sample Size Calculation for Phase 1

CCI [redacted]

10.9.2 Sample Size Calculation for Phase 2

CCI [redacted]

CCI

[redacted]

10.9.2.1 Sample Size Calculation for the Phase 2 Food-Effect Sub-Study

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[redacted]

10.9.2.2 Sample Size Calculation for the Phase 2 Endometrium Sub-Study

CCI

[redacted]

CCI [redacted]

10.10 Analysis Populations

Analyses will be performed using the following analysis sets:

- **Full Analysis Set (FAS),** which will consist of all subjects who receive at least 1 dose of study drug.
- **Subset analysis for ERα^{mut} and ERα^{WT}**, which will consist of all subjects who receive at least 1 dose of study drug and are either ERα^{mut} or ERα^{WT}.
- **Safety Analysis Set**, which will consist of all subjects who receive at least 1 dose of study drug. This will be the analysis set for all safety evaluations except DLT results.
- **Dose Evaluable Set**, which will consist of all subjects who were evaluable for DLT in Cycle 1 of Phase 1. This will be the analysis set for DLT results.
- **PK Analysis Set**, which will include all subjects who receive at least 1 dose of study drug and have at least 3 evaluable plasma concentrations
- **Food-Effect Analysis Set**, which will consist of all subjects who are assigned to the food-effect cohort and have at least 3 evaluable plasma concentrations at the fed and fasted time points.

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- **Response-Evaluable Set**, which will consist of those subjects who receive at least 1 dose of study drug and have measurable disease at baseline and at least 1 post-baseline evaluation. This will be the primary analysis set for efficacy evaluations.
- **Endometrium Safety Evaluable Set**, which will consist of those subjects with intact uteri at baseline who receive at least 1 dose of study drug and have ultrasound assessments at screening/baseline and three months after starting trial therapy.
- **Bone Turnover Markers-Evaluable Set**, which will consist of those subjects who have baseline/screening assessments of bone turnover markers and at least one additional assessment at 6 and/or 12 weeks after starting trial therapy. To be included in this set, subjects must have received trial therapy uninterrupted for at least 14 days prior to the 6-week or the 12-week assessment.

10.11 Data Analysis

Descriptive statistics, including mean, median, standard deviations and ranges for all continuous measures will be tabulated and reported. Percentages and frequencies for all categorical measures will also be presented. Time-to-event endpoints will be reported using Kaplan-Meier estimates. Baseline demographics and other characteristics will be reported.

In general, statistical analyses will be performed for the overall population and subgroups of population. Detailed presentation plan and definition of subgroups will be included in the SAP.

10.11.1 Subject Disposition

The number of subjects screened for participation and number enrolled overall and by study part will be tabulated along with the proportion included in each analysis population. The proportion of subjects who discontinue the study will be tabulated, along with the primary reason for discontinuation.

10.11.2 Demographic and Other Baseline Characteristics

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

10.11.3 Prior and Concomitant Therapy

All investigator terms for medications recorded in the CRF will be coded to an 11-digit code using the current version of the World Health Organization Drug Dictionary (WHO DD).

The number (percentage) of subjects who took prior and concomitant medications will be summarized on the FAS by study part, cohort and overall Anatomical Therapeutic Chemical class (ie, anatomical class, therapeutic class, pharmacologic class, chemical class), as well as WHO DD preferred term. Prior medications will be defined as medications that stopped before the first dose of study drug. Concomitant medications will be defined as medications that started after the date of the first dose of study drug up to 28 days after the subject's last dose. All medications will be presented in subject data listings.

10.11.4 Efficacy Analysis

The efficacy analyses will be based on data collected as of the data cutoff date for the primary analysis, ie, 6 months after the last subject receives their first dose of study treatment.

In Phase 2 portion of the study, CCI [redacted]

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ORR, DoR, PFS, OS, CBR and DCR will be listed and descriptively summarized as appropriate. All efficacy parameters will be summarized for the RES and FAS.

ORR will be reported in summary tables. For response duration, summary statistics (median, Q1, Q3, and range) will be generated on subjects achieving a best overall response of confirmed partial response + CR. For DoR, the summary statistics will be generated using Kaplan-Meier estimate.

For PFS, subjects who do not have disease progression or death information will be treated as right-censored observations at the time of the last response evaluation. The PFS censoring rules will follow the FDA guidance. PFS and OS will be reported in both summary tables and plotted with Kaplan-Meier curve.

For DCR and CBR, summary tables will be provided.

For endometrial thickness and uterine volume, descriptive statistics of the values at baseline and at three months post-baseline as well as the changes from baseline will be provided. Confidence intervals of the changes from baseline will be provided.

10.11.5 Safety Analysis

All safety analyses will be performed on the Safety Analysis Set. Safety data will be summarized on an "as treated" basis using descriptive statistics (eg, n, mean, standard deviation, median, minimum, maximum for continuous variables; n [%] for categorical variables). Safety variables include treatment-emergent adverse events (TEAEs), clinical laboratory parameters, vital signs, and 12-lead ECG results.

Safety will be assessed through the analysis of the reported incidence of TEAEs, those with an onset on or after consent and enrollment on study, which will be graded according to NCI CTCAE v4.03. The AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA,), and summarized using SOC and preferred term by treatment group/dose level for all subjects in the Safety Population. In addition, summaries of SAEs, AEs leading to treatment discontinuation, AEs by maximum NCI CTCAE v4.03 grade, and AEs related to study treatment will also be presented by dose level.

Other safety endpoints, including laboratory results, vital signs, and ECG findings, will be summarized for all subjects in the Safety Analysis Set.

Concomitant medications will be coded using the WHO DD and they will be listed and summarized by dose level.

Evaluation of safety will be performed on the Safety Analysis Set except for the determination of the MTD during Phase 1 (dose escalation). The determination of the MTD will be based on all DLT-evaluable subjects enrolled in Phase 1.

Subjects who do not receive study drug for at least 70% of the planned dose of H3B-6545 during the first cycle (28 calendar days) for reasons not considered to be a DLT by both the investigators and the Sponsor will be replaced. The subjects who are replaced will not be considered evaluable for DLT assessments.

Safety data to be evaluated include AEs, clinical laboratory results, vital sign measurements, and ECG parameters.

Safety parameters will be summarized using descriptive statistics (mean, standard deviation, median, Q1, Q3, and range for continuous variables; numbers and percentages for categorical measures).

The effects of H3B-6545 on cardiovascular re-polarization will be evaluated via a 12-lead continuous Holter. On Cycle 1, Day 1 and Day 15, monitoring via Holter will begin at least 1 hour prior to dose and continue through at least 24 hours postdose for Phase 1 subjects only.

For convenience, Holter monitoring may begin up to 12 hours prior to dosing on Cycle 1, Day 1 or Day 15. Individual ECGs will be extracted in triplicate from the Holter recordings at specified time points and will be evaluated by a central laboratory. QT intervals will be measured from Lead II and will be corrected for HR (QTc) using Fridericia's (QTcF) correction factors. The primary QTc parameter will be QTcF. Secondary parameters (QTc interval using Bazett's correction factor (QTcB), QT, QRS interval, and HR) and waveforms (T waves) will be evaluated.

In addition, the effects of H3B-6545 on the cardiovascular system will be evaluated via 12-lead ECG at screening and on Day 1 and Day 15 of Cycle 1, and Day 1 of Cycle 2, 3 and 4 as well as at the End of Treatment visit for all subjects (Phase 1 and Phase 2 subjects). If sinus bradycardia is observed at EOT visit, follow up ECGs should be performed weekly until HR has returned 60 or more beats per minute or pre-study baseline. QT intervals will be measured from Lead II and will be corrected for HR (QTc) using Fridericia's (QTcF) correction factor. The primary QTc parameter will be QTcF; secondary parameters (QTc corrected using Bazett's formula, QT, QRS interval, and HR) and waveforms (T waves) will be evaluated.

10.11.5.1 Extent of Exposure

Descriptive statistics for subjects treated, including the duration of treatment, the number of cycles received, and the number of subjects requiring dose changes, will be presented. A by-subject listing of the date of study drug administration and the dose administered will be presented.

10.11.5.2 Adverse Events

AEs will be collected for each subject until 28 days after last trial therapy administration. The NCI CTCAE v4.03 will be used for AE reporting.

The AE verbatim descriptions (investigator terms from the CRF) will be classified into standardized medical terminology using MedDRA. Adverse events will be coded to the MedDRA (Version 19.1 or higher) lower level term closest to the verbatim term. The linked MedDRA preferred term and primary SOC are also captured in the database.

A TEAE is defined as:

- An AE that emerges during treatment, having been absent at pretreatment (baseline), or
- Re-emerges during treatment, having been present at pretreatment (baseline) but stopped before treatment, or

- Worsens in severity during treatment relative to the pretreatment state, when the AE is continuous.

Only those AEs that were treatment-emergent will be included in summary tables. All AEs, treatment-emergent or otherwise, will be presented in subject data listings. The TEAEs will be summarized for each study phase. The incidence of TEAEs will be reported as the number (percentage) of subjects with TEAEs by SOC and preferred term.

A subject will be counted only once within a SOC and preferred term, even if the subject experienced more than 1 TEAE within a specific SOC and preferred term. The number (percentage) of subjects with TEAEs will also be summarized by maximum CTCAE grade and by relationship to study drug (Yes [related] and No [not related]).

The number (percentage) of subjects with TEAEs leading to death will be summarized by MedDRA SOC and preferred term for each study phase. A subject data listing of all AEs leading to death will be provided. The number (percentage) of subjects with treatment-emergent SAEs will be summarized by MedDRA SOC and preferred term for each study phase. A subject data listing of all SAEs will be provided. The number (percentage) of subjects with TEAEs leading to discontinuation from study drug will be summarized by MedDRA SOC and preferred term for each study phase. A subject data listing of all AEs leading to discontinuation from study drug will be provided.

In the Dose-Evaluable Set, the number (percentage) of subjects with DLTs will be presented by dose level for Phase 1 of the study. A listing of subjects with DLTs will be provided.

AEs will be summarized using the Safety Analysis Set. The number of AEs and number and incidence (%) of subjects with AEs will be summarized by cohort/dose level and overall. To obtain the incidence (%), the number of subjects with at least 1 event and the percentage of subjects with AEs by SOC and by preferred term will be calculated. Incidence (%) by causal relationship with study drug and by severity (CTCAE v 4.03) will also be calculated. For clinically significant events, time of onset, and recovery will be reported.

10.11.5.3 Laboratory Values

Laboratory results will be summarized using Système International (SI) units, as appropriate.

For all quantitative parameters listed, the actual value and the change from baseline to each post-baseline visit and to the end of treatment (defined as the last on-treatment value) will be summarized by visit using descriptive statistics. Qualitative parameters listed will be summarized using frequencies (number and percentage of subjects), and changes from baseline to each post-baseline visit and to end of treatment will be reported using shift tables. Percentages will be based on the number of subjects with both non-missing baseline and relevant post-baseline results.

Furthermore, the frequency of laboratory abnormalities by maximum post-baseline CTCAE grade will be tabulated by cycle and overall for selected laboratory parameters to include at least, hemoglobin, WBC, absolute neutrophil count, lymphocytes, platelet count, ALP, AST, ALT, bilirubin, creatinine, and electrolytes. Shift tables will also be produced for these parameters based on the baseline CTCAE grade and the maximum CTCAE grade by cycle and overall.

10.11.5.4 Vital Signs

Changes in vital sign parameters (including systolic and diastolic BP, HR, RR, and temperature) and body weight will be summarized over time, and any abnormal values will be tabulated.

Descriptive statistics for vital signs parameters (ie, systolic and diastolic BP, pulse, RR, temperature, and weight) and changes from baseline will be presented by visit.

10.11.5.5 Electrocardiograms

ECG assessments will be performed throughout the study. Descriptive statistics for ECG parameters and changes from baseline will be presented by visit.

For all subjects, shift tables will present changes in ECG interpretation (categorized as normal; abnormal, not clinically significant; and abnormal, clinically significant) from baseline to end of treatment. In addition, summary tables and listings for the various intervals (eg, QT and PR) and changes from baseline will be presented.

In addition, the number (percentage) of subjects with at least 1 post-baseline abnormal ECG result in QTcF during the treatment period will be summarized. Clinically abnormal ECG results in QTcF will be categorized as follows:

Absolute QTc interval prolongation:

- QTc interval >450 msec
- QTc interval >480 msec
- QTc interval >500 msec

Change from baseline in QTc interval:

- QTc interval increases from baseline >30 msec
- QTc interval increases from baseline >60 msec

All ECG abnormalities will be listed on a per-subject basis.

10.11.6 Pharmacokinetic, Pharmacodynamic, Pharmacogenomic, and Other Biomarker Analyses

10.11.6.1 Pharmacokinetic Analyses

Plasma concentrations of H3B-6545 will be tabulated and summarized by dose level, day, and time.

H3B-6545 PK parameters will be derived from plasma concentrations by noncompartmental analysis using actual times. Minimally, the following PK parameters will be calculated: C_{max}, t_{max}, $AUC_{0\text{-}t}$, R_{acc}, and if data permit, area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\text{-}inf}$), $t_{½}$, CL/F, and V_z/F.

The effect of a high-fat meal on AUC and C_{max} of H3B-6545 will be evaluated using a mixed linear model of logarithmically transformed values of the primary PK parameters. Ratios of geometric means (fed/fasted comparison) and associated 2-sided 90% CIs will be presented.

10.11.6.2 Pharmacodynamic, Pharmacogenetic, and Other Biomarker Analyses

CCI [redacted]

10.12 Analysis Time Points

10.12.1 Primary Analysis

The data cutoff date for the primary analysis of the study will be 6 months after the last subject in the expansion cohort with clonal *ESR1* Y537S mutation (Protocol Amendment 6 and subsequent amendments) of the trial receives their first dose of study treatment.

10.12.2 Planned Interim Analysis

CCI [redacted]

In addition, under Amendment 6 and subsequent amendments, approximately CCI [redacted] subjects will be enrolled to further characterize the efficacy of H3B-6545 in terms of response rate in subjects who carry a clonal ESR1 Y537S mutation at AF ≥0.5% as determined at prescreening by a sponsor-designated central laboratory from a plasma cfDNA sample. Subjects may enter the screening phases based on an existing local cfDNA result. Confirmation of patient eligibility by a sponsor-designated central laboratory cfDNA test (ie, presence of ESR1 Y537S mutation at AF ≥0.5% and absence of ESR1 D538G at AF ≥0.5%) must be obtained prior to Cycle 1 Day 1.

10.12.3 Safety Review Committee

The SRC will consist of site investigators, the study medical monitor, and the study director. Investigator teleconferences will occur on a regular basis during the study to review ongoing safety data. Further, the Sponsor and the investigators will meet at the end of each treatment cohort to discuss and evaluate safety data. At the dose escalation teleconference, the clinical course and all available safety (DLTs, adverse event of special interest [AESI], and CTCAE Grade 2 or higher toxicity data) and PK/PD data for each subject in the cohort will be described in detail. Updated safety data on other ongoing subjects, including data in later cycles, will be discussed as well.

Dose escalation decisions will be based on a clinical synthesis of all available data and not solely on DLT information. Selection of the actual dose for the next cohort of subjects will be guided by a modified Fibonacci dose escalation model, and a medical review of relevant clinical, PK/PD and laboratory data. The Sponsor and the investigators must reach a consensus on whether to

declare an MTD and/or RP2D, escalate or de-escalate the dose, and/or recruit additional subjects at the current or previous dose levels. The SRC may be convened earlier at the discretion of the Sponsor if important safety issues arise requiring the attention of the committee.

During the Phase 2 part of the trial, the SRC will receive all available safety data approximately every 6 months.

11. SAFETY REPORTING

Safety assessments will consist of monitoring and recording all AEs, including all Common Terminology Criteria for Adverse Events (CTCAE) v 4.03 grades (for both increasing and decreasing severity), and SAEs; regular monitoring of hematology, blood chemistry, and urine values; periodic measurement of vital signs and ECGs; and performance of physical examinations as detailed in Appendix D, Appendix E, and Appendix F.

The PI is responsible for recognizing and reporting AEs to the Sponsor or designee. It is the Sponsor's responsibility to report relevant SAEs to the applicable local, national, or international regulatory bodies. In addition, investigators must report SAEs and follow-up information to their responsible IRB/EC according to the policies of that IRB/EC.

The PI is also responsible for ensuring that every staff member involved in the study is familiar with the content of this section.

11.1 Definitions

11.1.1 Adverse Events

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered an investigational product. An AE does not necessarily have a causal relationship with the medicinal product. For this study, the study drug is H3B-6545.

The criteria for identifying AEs in this study are:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product (Note: Every sign or symptom should not be listed as a separate AE if the applicable disease [diagnosis] is being reported as an AE).
- Any new disease or exacerbation of an existing disease. However, worsening of the primary disease should be captured under efficacy assessments as disease progression rather than as an AE, unless the event meets criteria for a serious AE as outlined in Section 11.1.2. In such cases, the event should be reported as an SAE.
- Any deterioration in non-protocol-required measurements of a laboratory value or other clinical test (eg, ECG or x-ray) that results in symptoms, a change in treatment, or discontinuation of study drug.
- Recurrence of an intermittent medical condition (eg, headache) not present pre-treatment (Baseline)

- An abnormal laboratory test result should be considered an AE if the identified laboratory abnormality leads to any type of intervention, withdrawal of study drug, or withholding of study drug, whether prescribed in the protocol or not.

All AEs, regardless of relationship to study drug or procedure, should be recorded beginning from the time the subject signs the study ICF through 28 days after the last dose of drug.

Abnormal laboratory values should not be listed as separate AEs if they are considered to be part of the clinical syndrome that is being reported as an AE. It is the responsibility of the investigator to review all laboratory findings in all subjects and determine if they constitute an AE. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE. Any laboratory abnormality considered to constitute an AE should be reported on the Adverse Event CRF.

Abnormal ECG (QTcF) results, if not otherwise considered part of a clinical symptom that is being reported as an AE, should be considered an AE if the QTcF interval is more than 450 msec and there is an increase of more than 60 msec from baseline. Any ECG abnormality that the investigator considers as an AE should be reported as such. *Note: If sinus bradycardia is observed at EOT visit, follow up ECGs should be performed weekly until HR has returned 60 or more beats per minute or pre-study baseline.*

All AEs must be followed for 28 days after the subject's last dose. All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization.

Every effort must be made by the investigator to categorize each AE according to its severity and its relationship to the study treatment.

Assessing Severity of Adverse Events

Adverse events will be graded on a 5-point scale according to CTCAE v 4.03. Investigators will report CTCAE grades for all AEs (for both increasing and decreasing severity).

Assessing Relationship to Study Treatment

Items to be considered when assessing the relationship of an AE to the study treatment are:

- Temporal relationship of the onset of the event to the initiation of the study treatment
- The course of the event, especially the effect of discontinuation of study treatment or reintroduction of study treatment, as applicable
- Whether the event is known to be associated with the study treatment or with other similar treatments
- The presence of risk factors in the study subject known to increase the occurrence of the event
- The presence of non-study treatment-related factors that are known to be associated with the occurrence of the event.

Classification of Causality

The relationship of each AE to the study drug will be recorded on the CRF in response to the following question:

Is there a reasonable possibility that the study drug caused the AE?

Yes (related) A causal relationship between the study drug and the AE is a reasonable possibility.

No (not related) A causal relationship between the study drug and the AE is not a reasonable possibility.

Adverse Events of Special Interest

Hy's Law is the only reportable AESI for this protocol and events of Hy's Law should be reported as outlined in Section 11.2.

11.1.2 Serious Adverse Event

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (ie, the subject was at immediate risk of death from the adverse event as it occurred; this does not include an event that, had it occurred in a more severe form or was allowed to continue, might have caused death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect (in the child of a subject who was exposed to the study drug).

Other important medical events that may not be immediately life-threatening or result in death or hospitalization but, when based on appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the outcomes in the definition of SAE listed above should also be considered SAEs.

For example, an isolated laboratory abnormality could be considered to meet the criteria for serious (eg, if the event results in hospitalization or is considered medically significant).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in such situations.

In addition to the above, events associated with special situations include pregnancy or exposure to study drug through breastfeeding; AEs associated with study drug overdose, misuse, abuse, or medication error. These events associated with special situations are to be captured using the SAE procedures but are to be considered as SAEs only if they meet one of the above criteria. All AEs associated with special situations are to be reported on the CRF whether or not they meet the criteria for SAEs.

All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (ie, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care.
- Planned hospitalizations required by the protocol.

- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed after study drug administration).
- Hospitalization for administration of study drug or insertion of access for administration of study drug.
- Hospitalization for routine maintenance of a device (eg, battery replacement) that was in place before study entry

It is important to distinguish between "serious" and "severe" AEs, as the terms are not synonymous. Severity is a measure of intensity; however, an AE of severe intensity need not necessarily be considered serious. Seriousness serves as the guide for defining regulatory reporting obligations. It is based on subject/event outcome or action usually associated with events that pose a threat to a subject's life or vital functions.

For example, nausea, which persists for several hours, may be considered severe nausea, but may not be considered an SAE. On the other hand, a stroke, which results in only a limited degree of disability, may be considered only a mild stroke, but it would be considered an SAE. Severity and seriousness should be independently assessed when recording AEs on the eCRF screen and SAEs on the SAE Report Form.

11.1.3 Adverse Reaction

An adverse reaction (AR) means any AE caused by a drug. Adverse reactions are a subset of all SARs where there is a reason to conclude that the drug caused the event.

11.1.4 Suspected Adverse Reaction

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the drug and the AE. An SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by a drug.

11.1.5 Recording and Reporting of Adverse Events

Recording of Adverse Events

All AEs of any subject during the course of the research study will be recorded in the eCRF, and the investigator will give his or her opinion as to the relationship of the AE to the study drug treatment (ie, whether the event is related or unrelated to study drug administration).

All AEs should be documented. A description of the event, including its date of onset and resolution, whether it constitutes an SAE or not, any action taken (eg, changes to study treatment), and outcome, should be provided, along with the investigator's assessment of causality (ie, the relationship to the study treatment[s]). For an AE to be a suspected treatment-related event, there should be at least a reasonable possibility of a causal relationship between the protocol treatment and the AE. Adverse events will be graded according to the NCI CTCAE v4.03, and changes will be documented.

If the AE is serious or of special interest (eg, a case of Hy's Law), it should be reported immediately to the Sponsor or designee. Other untoward events occurring in the framework of a clinical study are to be recorded as AEs (ie, AEs that occur prior to assignment of study treatment that are related to a protocol-mandated intervention, including invasive procedures such as biopsies, medication washout, or no treatment run-in).

Any clinically significant signs and symptoms, abnormal test findings, changes in physical examination, hypersensitivity, and other measurements that occur will be reported as AEs and recorded on the relevant eCRF screen.

Test findings will be reported as an AE if the test results require an adjustment in the study drug(s) or discontinuation of treatment, test findings require additional testing or surgical intervention; a test result or finding is associated with accompanying symptoms; and/or a test result is considered to be an AE by the investigator.

Reporting Period for Adverse Events

All AEs, regardless of relationship to study drug or procedure, should be recorded beginning from the time the subject signs the study ICF through 28 days after the last dose of drug. SAEs will be collected for 28 days after the last dose.

All AEs resulting in discontinuation from the study should be followed until resolution or stabilization. All new AEs occurring during this period must be reported and followed until resolution unless, in the opinion of the investigator, the AE or laboratory abnormality(ies) are not likely to improve because of the underlying disease. In this case, the investigators must record his or her reasoning for this decision in the subject's medical record and as a comment on the eCRF screen.

After 28 days of completion of protocol-specific treatment or discontinuation, only AEs, SAEs, or deaths assessed by the investigator as treatment related are to be reported.

11.1.5.1 Regulatory Reporting of Adverse Events

Adverse events will be reported by the Sponsor or a third party acting on behalf of the Sponsor to regulatory authorities in compliance with local and regional law and established guidance. The format of these reports will be dictated by the local and regional requirements.

11.2 Serious Adverse Event Reporting by Investigators

All SERIOUS ADVERSE EVENTS and adverse events of special interest (AESI), regardless of their relationship to study treatment, must be reported on a completed SAE form by email or fax as soon as possible but not later than 24 hours from when the investigator becomes aware of the event.

SAEs, regardless of causality assessment, must be collected through the last visit. SAEs will be collected for 28 days after the last dose. All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization. Any SAE judged by the investigator to be related to the study treatment or any protocol-required procedure should be reported to the Sponsor regardless of the length of time that has passed since study completion.

The detailed contact information for reporting of SAEs is provided in the Investigator Study File.

For urgent safety issues, please ensure all appropriate medical care is administered to the subject and contact the appropriate study team member listed in the Investigator Study File.

It is very important that the SAE report form be filled out as completely as possible at the time of the initial report. This includes the investigator's assessment of causality.

Any follow-up information received on SAEs should be forwarded within 24 hours of its receipt. If the follow-up information changes the investigator's assessment of causality, this should also be noted on the follow-up SAE form.

Preliminary SAE reports should be followed as soon as possible by detailed descriptions including copies of hospital case reports, autopsy reports, and other documents requested by the Sponsor.

Transmission of the SAE report should be confirmed by the site personnel submitting the report.

Follow-up information for SAEs and information on non-serious AEs that become serious should also be reported as soon as it is available; these reports should be submitted using the SAE Report Form. The detailed SAE reporting process will be provided to the sites in the SAE reporting guidelines contained in the study reference manual.

Investigators must report SAEs and follow-up information to their responsible IRB according to the policies of the responsible IRB.

11.2.1 Investigator Reporting After Study Discontinuation

Twenty-eight days after completing protocol-specific treatment or study discontinuation, treatment-related AEs, SAEs, or deaths determined by the investigator as treatment-related are to be reported to the Sponsor or designee.

11.3 Recording of Adverse Events and Serious Adverse Events

11.3.1 Diagnosis Versus Signs and Symptoms

All AEs should be recorded individually in the subject's own words (verbatim) unless, in the opinion of the PI or designated physician, the AEs constitute components of a recognized condition, disease, or syndrome. In the latter case, the condition, disease, or syndrome should be named rather than each individual sign or symptom. If a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE as appropriate on the relevant form(s) (SAE Report Form and/or AE eCRF screen).

If a diagnosis is subsequently established, it should be reported as follow-up information is available. If a diagnosis is determined subsequent to the reporting of the constellation of symptoms, the signs/symptoms should be updated to reflect the diagnosis.

11.3.2 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between subject evaluation time points. Such events should only be recorded once on the SAE Report Form and/or the AE eCRF screen. If a persistent AE becomes more severe or lessens in severity, it should be recorded on a separate SAE Report Form and/or AE eCRF screen.

A recurrent AE is one that occurs and resolves between subject evaluation time points, and subsequently recurs. All recurrent AEs should be recorded on an SAE Report Form and/or AE eCRF screen.

11.3.3 Abnormal Laboratory Values

If an abnormal laboratory value or vital sign is associated with clinical signs and/or symptoms, the sign or symptom should be reported as an AE or SAE, and the associated laboratory value or

vital sign should be considered additional information that must be collected on the relevant eCRF screen. If the laboratory abnormality is a sign of a disease or syndrome, only the diagnosis needs to be recorded on the SAE Report Form or AE eCRF screen.

Abnormal laboratory values will be reported as an AE if the laboratory result requires an adjustment in the study drug(s) or discontinuation of treatment; laboratory findings require additional testing or surgical intervention; a laboratory result or finding is associated with accompanying symptoms; and/or a laboratory result is considered to be an AE by the investigator.

11.3.4 Deaths

All on-study treatment deaths, regardless of attribution during the AE reporting period, will be recorded on an SAE Report Form and expeditiously reported.

When recording an SAE with an outcome of death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the SAE Report Form and Adverse Event screen of the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Death NOS" on the eCRF Adverse Event screen.

During post-study treatment survival follow-up (after the 28-day post-dose AE reporting period), deaths attributed to progression of disease will be recorded only on the "After Progressive Disease Follow-Up" eCRF screen.

11.3.5 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization of >24 hours or prolongation of pre-existing hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol. There are some hospitalizations that do not require reporting as an SAE.

Treatment within or admission to the following facilities is not considered to meet the criteria of "inpatient hospitalization" (although if any other SAE criteria are met, the event must still be treated as an SAE and immediately reported):

- Emergency department or emergency room.
- Outpatient or same-day surgery units.
- Observation or short-stay unit.
- Rehabilitation facility.
- Hospice or skilled nursing facility.
- Nursing homes, custodial care or respite care facility.

Hospitalization during the study for a pre-planned surgical or medical procedure (one which was planned prior to entry in the study), does not require reporting as a SAE.

11.3.6 Pre-Existing Medical Conditions

A pre-existing medical condition is one that is present at the start of the study. Such conditions should be recorded on the General Medical History of the eCRF screen. A pre-existing medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the

condition worsens during the study. When recording such events on an SAE Report Form and/or AE eCRF screen, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors.

11.3.7 New Cancers

The development of a new primary cancer should be regarded as an AE and will generally meet at least one of the seriousness criteria. New primary cancers are those that are not the primary reason for the administration of the study treatment and have developed after the inclusion of the subject into the study. They do not include metastases of the original cancer.

11.3.8 Pregnancy, Abortion, Birth Defects/Congenital Anomalies

Any pregnancy in a female subject in which the estimated date of conception is either before the last visit or within 28 days of last study treatment, or any exposure to study drug through breastfeeding during study treatment or within 28 days of last study treatment, must be reported.

If an adverse outcome of a pregnancy is suspected to be related to study drug exposure, this should be reported regardless of the length of time that has passed since the exposure to study treatment.

A congenital anomaly, death during perinatal period, an induced abortion, or a spontaneous abortion are considered to be an SAE and should be reported in the same time frame and in the same format as all other SAEs (see Reporting of Serious Adverse Events in Section 11.2).

Pregnancies or exposure to study drug through breastfeeding must be reported by fax or email as soon as possible but no later than 1 business day from the date the investigator becomes aware of the pregnancy. The contact information for the reporting of pregnancies and exposure to study drug through breastfeeding is provided in the Investigator Study File. The Pregnancy Report Form must be used for reporting. All pregnancies must be followed to outcome. The outcome of the pregnancy must be reported as soon as possible but no later than 1 business day from the date the investigator becomes aware of the outcome.

A subject who becomes pregnant must be withdrawn from the study.

The detailed contact information for reporting of pregnancies is provided in the Investigator Study File. If a subject becomes pregnant while enrolled in the study, a Pregnancy Form should be completed and submitted expeditiously, irrespective of whether or not it meets the criteria for expedited reporting. Abortions (spontaneous, accidental, or therapeutic) must also be reported.

Congenital anomalies/birth defects always meet SAE criteria, and should therefore be expeditiously reported as an SAE, using the previously described process for SAE reporting. A Pregnancy Form should also have been previously completed and will need to be updated to reflect the outcome of the pregnancy.

11.3.9 H3B-6545 Overdose

Adverse events associated with study drug overdose, misuse, abuse, and medication error refer to AEs associated with uses of the study drug outside of that specified by the protocol. Overdose, misuse, abuse, and medication error are defined as follows:

Overdose Accidental or intentional use of the study drug in an amount higher than the protocol-defined dose

Misuse	Intentional and inappropriate use of study drug not in accordance with the protocol.
Abuse	Sporadic or persistent intentional excessive use of study drug accompanied by harmful physical or psychological effects.
Medication error	Any unintentional event that causes or leads to inappropriate study drug use or subject harm while the study drug is in the control of site personnel or the subject.

All AEs associated with overdose, misuse, abuse, or medication error should be captured on the Adverse Event CRF and also reported using the procedures detailed in Reporting of Serious Adverse Events even if the AEs do not meet serious criteria. Abuse is always to be captured as an AE. If the AE associated with an overdose, misuse, abuse, or medication error does not meet serious criteria, it must still be reported using the SAE form and in an expedited manner but should be noted as nonserious on the SAE form and the Adverse Event CRF.

Symptomatic and non-symptomatic overdose must be reported in the eCRF system. Any accidental or intentional overdose with the study treatment that is symptomatic, even if not fulfilling a seriousness criterion, is to be reported no greater than 24 hours from first knowledge of the event using the corresponding screens in the eCRF and following the same process described for SAE reporting (see Section 11.2) if the overdose is symptomatic.

For information on how to manage an overdose of H3B-6545, refer to the IB.

11.4 Cardiovascular

Triplicate 12-lead ECGs will be collected approximately 5 minutes apart at the screening visit, and prior to dosing and 3-hour post dose on Day 1 and Day 15 of Cycle 1, and Day 1 of Cycle 2, Cycle 3, and Cycle 4, as well as at the end of treatment for all subjects (Phase 1 and Phase 2 subjects). 12-lead safety ECGs will be performed locally from Day 1 of each cycle starting Cycle 5 till the last cycle prior to the EOT visit, and at Unscheduled visits as clinically required. A single 12-lead safety ECG is required at a minimum. Data from local safety ECGs should be entered into the clinical database.

In addition, in the event of any alteration or if clinically indicated, additional ECGs and/or cardiac enzyme evaluations should be performed. If sinus bradycardia is observed at EOT visit, follow up ECGs should be performed weekly until heart rate has returned to 60 or more beats per minute or pre-study baseline.

An ECG abnormality may meet the criteria of an AE as described in this protocol (see Section 11.1.1) and the CRF Completion Guidelines. In these instances, the AE corresponding to the ECG abnormality will be recorded on the Adverse Events CRF.

For ECG abnormalities meeting the criteria of an SAE, the site must fax or email the SAE report including the ECG report to the Sponsor using the SAE form.

11.5 Sponsor Serious Adverse Event Reporting Requirements

Adverse events will be reported by the Sponsor or a third party acting on behalf of the Sponsor to regulatory authorities in compliance with local and regional law and established guidance. The format of these reports will be dictated by the local and regional requirements.

All studies that are conducted within any European country will comply with European Good Clinical Practice Directive 2005/28/EC and Clinical Trial Directive 2001/20/EC. All suspected unexpected serious adverse reactions (SUSARs) will be reported, as required, to the competent authorities of all involved European member states.

11.5.1.1 Expedited Reporting

The Sponsor must inform investigators and regulatory authorities of reportable events, in compliance with applicable regulatory requirements, on an expedited basis (ie, within specific time frames). For this reason, it is imperative that sites provide complete SAE information in the manner described above.

12. QUALITY ASSURANCE AND QUALITY CONTROL

12.1 Monitoring

Site monitoring shall be conducted to ensure that subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet Sponsor, Good Clinical Practice (GCP)/International Conference on Harmonisation (ICH) and, when appropriate, regulatory guidelines.

12.2 Audits and Inspections

The investigator will permit study-related quality audits and inspections by the Sponsor or its representative(s), government regulatory authorities, and the IRB/EC of all study-related documents (eg, source documents, regulatory documents, data collection instruments, CRFs). The investigator will ensure the capability for review of applicable study-related facilities. The investigator will ensure that the auditor or inspector or any other compliance or quality assurance reviewer is given access to all study-related documents and study-related facilities.

Participation as an investigator in this study implies the acceptance of potential inspection by government regulatory authorities, the IRB/EC, and the Sponsor or its representative(s).

13. ETHICAL, FINANCIAL, AND REGULATORY CONSIDERATIONS

This research study will be conducted according to the standards of GCP outlined in the ICH E6 Tripartite Guideline and Code of Federal Regulations (CFR) Title 21 Part 312, applicable government regulations, institutional research policies and procedures and any other local applicable regulatory requirement(s).

13.1 Institutional Review Board Approval

The clinical study protocol, ICF, IB, available safety information, subject documents (eg, study diary), subject recruitment procedures (eg, advertisements), information about payments (ie, PI payments) and compensation available to the subjects and documentation evidencing the PI's qualifications should be submitted to the IRB for ethical review and approval if required by local regulations, prior to the study start.

The PI/Sponsor and/or designee will follow all necessary regulations to ensure appropriate, initial, and on-going IRB study review. The PI/Sponsor (as appropriate) must submit and, where

necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the ICF. Investigators will be advised by the Sponsor or designee whether an amendment is considered substantial or non-substantial and whether it requires submission for approval or notification only to an IRB.

Safety updates for H3B-6545 will be prepared by the Sponsor or its representative as required for distribution to the investigator(s) and submission to the relevant IRB.

13.2 Regulatory Approval

As required by local regulations, the Sponsor will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained, prior to study initiation. If required, the Sponsor will also ensure that the implementation of substantial amendments to the protocol and other relevant study documents happens only after approval by the relevant regulatory authorities.

13.3 Informed Consent

Informed consent is a process by which a subject voluntarily confirms his or her willingness to participate in a particular study after having been informed of all aspects of the study that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated ICF.

The ICF will be submitted for approval to the IRB that is responsible for review and approval of the study. Each ICF must include all of the relevant elements currently required by the FDA, as well as local county authority or state regulations and national requirements.

Before recruitment and enrollment into the study, each prospective candidate will be given a full explanation of the research study.

Once the essential information has been provided to the prospective candidate, and the investigator is sure that the individual candidate understands the implications of participating in this research study, the candidate will be asked to give consent to participate in the study by signing an ICF. A notation that a written ICF has been obtained will be made in the subject's medical record. A copy of the ICF, to include the subject's signature, will be provided by the investigator to the subject.

If an amendment to the protocol substantially alters the study design or the potential risks to the subject, the subject's consent to continue participation in the study should be obtained.

13.3.1 Confidentiality

13.3.1.1 Subject Confidentiality

The contents of this protocol and any amendments and results obtained during the study should be kept confidential by the investigator, the investigator's staff, and the IRB/IEC and will not be disclosed in whole or in part to others, or used for any purpose other than reviewing or performing the study, without the written consent of the Sponsor. No data collected as part of this study will be used in any written work, including publications, without the written consent of the Sponsor. These obligations of confidentiality and non-use shall in no way diminish such obligations as set forth in either the Confidentiality Agreement or Clinical Trial Agreement (CTA) executed between the Sponsor/Contract Research Organization (CRO) and the institution/investigator.

All persons assisting in the performance of this study must be bound by the obligations of confidentiality and non-use set forth in either the Confidentiality Agreement or CTA executed between the institution/investigator and the Sponsor/CRO.

Confidentiality of subject's personal data will be protected in accordance with all applicable global regulations. Regulations require that, in order to participate in the study, a subject must sign an authorization from the study that he or she has been informed of following:

- What protected health information (PHI) will be collected from subjects in this study.
- Who will have access to that information and why.
- Who will use or disclose that information.
- That health information may be further disclosed by the recipients of the information, and that if the information is disclosed the information may no longer be protected by federal or state privacy laws.
- The information collected about the research study will be kept separate from the subject's medical records, but the subject will be able to obtain the research records after the conclusion of the study.
- Whether the authorization contains an expiration date.
- The rights of a research subject to revoke his or her authorization.

In the event that a subject revokes authorization to collect or use his or her PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (ie, that the subject is alive) at the end of their scheduled study period.

In compliance with ICH GCP guidelines and applicable parts of 21 CFR it is a requirement that the investigator and institution permit authorized representatives of the Sponsor, the regulatory authorities and the IRB/EC direct access to review the subject's original medical records at the site for verification of study-related procedures and data.

Measures to protect confidentiality include that only a unique study number and initials will identify subjects in the eCRF system or other documents submitted to the Sponsor. This information, together with the subject's date of birth, will be used in the database for subject identification. Subject names or addresses will not be entered in the eCRF database system. No material bearing a subject's name will be kept on file by the Sponsor. Subjects will be informed of their rights within the ICF/patient information sheet.

13.3.1.2 Investigator and Staff Information

Personal data of the investigators and sub-investigators may be included in the database and shall be treated in compliance with all applicable laws and regulations. When archiving or processing personal data pertaining to the investigator or sub-investigator, all appropriate measures will be taken to safeguard and prevent access to this data by any unauthorized party.

The name and telephone and fax numbers of the Medical Monitor and other contact personnel at the Sponsor and of the CRO(s) are listed in the Investigator Study File provided to each site.

13.4 Financial Information

The finances for this clinical study will be subject to a separate written agreement between the Sponsor and applicable parties. Any investigator financial disclosures as applicable to 21CFR Part 54 shall be appropriately provided.

14. RESEARCH RETENTION AND DOCUMENTATION OF THE STUDY

14.1 Amendments to the Protocol

Amendments to the protocol shall be planned, documented and signature authorized prior to implementation.

If an amendment to the protocol is required, the amendment will be originated and documented by the Sponsor or its representative. All amendments require review and approval of all pharmaceutical companies and the PI supporting the study. The written amendment must be reviewed and approved by the Sponsor and submitted to the IRB at the investigator's facility for the board's approval.

Amendments specifically involving change to study design, risk to subject, increase to dosing or exposure, subject number increase, or addition or removal of new tests or procedures shall be reviewed and approved by the IRB of record for the investigator's facility.

The amendment will be submitted formally to the FDA or other regulatory authorities by the Sponsor as applicable and IRB/EC approval obtained, specifically when an increase to dosing or subject exposure and/or subject number has been proposed; or, when the addition or removal of an investigator is necessitated.

Items requiring a protocol amendment with IRB and/or FDA or other regulatory authorities' approval include, but are not limited to, the following:

- Change to study design.
- Risk to subject.
- Increase to dose or subject exposure to drug.
- Subject number increase
- Addition or removal of tests and/or procedures
- Addition/removal of a new investigator

It should be further noted that, if an amendment to the protocol substantially alters the study design or the potential risks to the subjects, their consent to continue participation in the study should be obtained.

14.2 Documentation Required to Initiate the Study

Before the study may begin certain documentation required by FDA regulations and ICH GCP must be provided by the investigator. Documents required to begin a study in the US include at a minimum, but are not limited to, the following:

- A signed and dated confidentiality agreement
- A signature-authorized protocol and contract

- A copy of the official IRB approval of the study and the IRB/EC members list
- Current curricula vitae for the PI and any associate investigator(s) who will be involved in the study
- Indication of appropriate accreditation for any laboratories to be used in the study and a copy of the normal ranges for tests to be performed by that laboratory
- Original Form FDA 1572 (Statement of Investigator), appropriately completed and signed
- A copy of the IRB/EC-approved ICF (and patient information sheet, if applicable) containing permission for audit by representatives of the CRO, the Sponsor, the IRB, and the FDA and other regulatory agencies (as applicable).
- Financial disclosure forms for all investigators listed on Form FDA 1572 (if applicable, ie, for covered trial).
- Site qualification reports, where applicable.
- Verification of PI acceptability from local and/or national debarment list(s)

14.3 Study Documentation and Storage

The PI must maintain a list of appropriately qualified persons to whom he/she has delegated study duties and should ensure that all persons assisting in the conduct of the study are informed of their obligations. All persons authorized to make entries and/or corrections on the eCRFs are to be included on this document. All entries in the subject's eCRF are to be supported by source documentation where appropriate.

Source documents are the original documents, data, records, and certified copies of original records of clinical findings, observations, and activities from which the subject's eCRF data are obtained. These can include, but are not limited to: hospital records and clinical and office charts; laboratory, medico-technical department and pharmacy records; diaries, microfiches, and ECG traces; copies or transcriptions certified after verification as being accurate and complete; photographic negatives, microfilm or magnetic media; X-rays; and correspondence.

The PI and each study staff member is responsible for maintaining a comprehensive and centralized filing system (eg, regulatory binder or investigator study file [ISF]) of all study-related (essential) documentation, suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. The ISF must consist of those documents that individually or collectively permit evaluation of the conduct of the study and the quality of the data produced.

The ISF should contain as a minimum all relevant documents and correspondence as outlined in ICH GCP Section 8 and 21 CFR Part 312.57, including key documents such as the IB and any amendments, protocol and any amendments, signed ICFs, copies of completed eCRFs, IRB approval documents, Financial Disclosure forms, subject identification lists, enrollment logs, delegation of authority log, staff qualification documents, laboratory normal ranges, and records relating to the study drug including accountability records.

Drug accountability records should, at a minimum, contain information regarding receipt, shipment, and disposition. Each form of drug accountability record, at a minimum, should contain PI name, date drug shipped/received, quantity, and batch/code or lot number for identity

of each shipment. In addition, all original source documents supporting entries in the eCRF must be maintained and be readily available.

The Sponsor shall maintain adequate investigational product records and financial interest records as per 21CFR Part 54.6 and Part 312.57 for no less than 2 years after the last marketing application has been approved by FDA; or, in the event that the marketing application has not been approved by FDA, for no less than 2 years after the last shipment/delivery of the drug for investigational use is discontinued and the FDA has been notified of the discontinuation.

The IRB shall maintain adequate documentation/records of IRB activities as per 21CFR Part 56.115 for at least 3 years after completion of the research.

The investigator shall maintain adequate records of drug disposition, case histories and any other study-related records as per 21 CFR Part 312.62 for no less than 2 years after the last marketing application has been approved by FDA; or, in the event that the marketing application has not been approved by FDA, for no less than 2 years after the last shipment/delivery of the drug for investigational use is discontinued and FDA has been notified of the discontinuation.

To enable evaluations and/or audits from regulatory authorities or from the Sponsor or its representative, the investigator additionally agrees to keep records, including the identity of all participating subjects (sufficient information to link records eg, eCRFs, medical records), all original signed ICFs, and copies of all eCRFs, SAE Reporting forms, source documents, detailed records of treatment disposition, and related essential regulatory documents. The documents listed above must be retained by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Sponsor or its representatives will notify the investigator(s)/institutions(s) when the study-related records are no longer required.

If the investigator relocates, retires, or for any reason withdraws from the study, both the Sponsor and its representative should be prospectively notified. The study records must be transferred to an acceptable designee, such as another investigator, another institution, or to the Sponsor. The investigator must obtain the Sponsor's written permission before disposing of any records, even if retention requirements have been met. All ISFs will be maintained by the Sponsor or its representative throughout the study and will be transferred to the Sponsor at the conclusion of the study.

14.4 Data Collection

The study eCRF is the primary data collection instrument for the study. Case report forms will be completed using the English language and should be kept current to enable the Sponsor to review the subjects' status throughout the course of the study.

In order to maintain confidentiality, only study number, subject number, initials and date of birth will identify the subject in the eCRF system if applicable by governing laws. If the subject's name appears on any other document (eg, laboratory report), it must be obliterated on the copy of the document and replaced instead with the subject number and subject's initials or allowable country specific subject identifying information. The investigator will maintain a personal subject identification list (subject numbers with corresponding subject identifiers) to enable records to be identified and verified as authentic. Subject data/information will be kept confidential and will be managed according to applicable local, state, and federal regulations.

All data requested by the eCRF system must be supported by and be consistent with the subject's source documentation. All missing data must be explained. When a required laboratory test, assessment, or evaluation has not been done or an "Unknown" box is not an option on the eCRF, a note should be created verifying that the field was "Not Done" or "Unknown." For any entry errors made, the error(s) must be corrected, and a note explaining the reason for change should be provided.

The investigator will electronically sign and date the subject eCRF indicating that the data in the eCRF has been assessed. Each completed eCRF will be signed and dated by the PI, once all data for that subject is final.

14.5 Discontinuation of Study

The Sponsor reserves the right to discontinue the study for medical reasons or any other reason at any time. If a study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions and regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

The investigator reserves the right to discontinue the study should his/her judgment so dictate. If the investigator terminates or suspends a study without prior agreement of the Sponsor, the investigator should inform the institution where applicable, and the investigator/institution should promptly inform the Sponsor and the IRB/IEC and provide the Sponsor and the IRB/IEC with a detailed written explanation of the termination or suspension. Study records must be retained as noted above.

14.6 Disclosure and Publication Policy

All manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the Sponsor in advance of submission pursuant to the terms and conditions set forth in the executed CTA between the Sponsor/CRO and the institution/investigator. The review is aimed at protecting the Sponsor's proprietary information existing either at the date of the commencement of the study or generated during the study.

The detailed obligations regarding the publication of any data, material results, or other information generated or created in relation to the study shall be set out in the agreement between each investigator and the Sponsor or CRO, as appropriate.

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16. APPENDICES

APPENDIX A: ECOG PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated. Death no imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead	0	Dead

ECOG = Eastern Cooperative Oncology Group.

APPENDIX B: NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION OF CARDIAC DISEASE

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
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.	Objective evidence of minimal cardiovascular disease.
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.	Objective evidence of moderately severe cardiovascular disease.
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 is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

APPENDIX C: GUIDELINES FOR WOMEN OF CHILDBEARING POTENTIAL

Acceptable Contraception Methods:

Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for 28 days after stopping treatment.

Highly effective contraception is defined as either:

True Abstinence When this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Sterilization When a woman of childbearing potential has had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to study entry. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.

Male Partner Sterilization When the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate.

Intrauterine Device Placement of an intrauterine device (IUD) or intrauterine system (IUS).

A Double-Barrier Method [such as condom plus diaphragm with spermicide] Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.

Contraceptive Implant

Oral Contraceptive

Unacceptable Contraception Methods for women of childbearing potential include:

- IUD progesterone T
- Female condom
- Natural family planning (rhythm method) or breastfeeding
- Fertility awareness
- Withdrawal
- Cervical shield

Pregnancies

To ensure subject safety, each pregnancy in a subject on study treatment must be reported to within 24 hours of learning of its occurrence.

The pregnancy should be followed up for 3 months after the termination of the pregnancy to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Study Pregnancy Form and reported by the investigator. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

The detailed contact information for reporting of pregnancies is provided in the Investigator Study File.

Women Not of Childbearing Potential are Defined as Follows:

- Women are considered postmenopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (eg, age appropriate, history of vasomotor symptoms).
- Women who are permanently sterilized (ie, bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy, all with surgery at least 1 month before dosing).

APPENDIX D: PRESCREENING FOR ESR1 MUTATIONS (AMENDMENT 6 AND SUBSEQUENT AMENDMENTS)

Activity	Prescreening[a]
Informed consent for pre-screening for *ESR1* mutations	X
Plasma sample for clonal *ESR1* mutation testing[b]	X
ESR1 mutation conformation[c]	X

ESR1=estrogen receptor 1 gene

[a] Pre-screening for *ESR1* mutations can be done at any time prior to signature of the screening informed consent form. Subjects may enter the screening phase based on an existing local mutation results on a case by case basis after consultation with the Sponsor. The mutation results from a sponsor-designated cfDNA test will be used to determine patient eligibility prior to starting study drug. Patients may be pre-screened more than once. If a redraw is necessary due to quality concerns at the analysis laboratory (eg, rejected for analysis), an additional sample may be collected.

[b] Required for all subjects (either in pre-screening or screening visit), independently of availability of *ESR1* mutational status from a prior local test. Detailed plasma sample collection, storage and shipping procedures will be provided in a separate laboratory manual.

[c] Eligible subjects must have *ESR1* Y537S mutation at AF ≥0.5%, in the absence of D538G mutation at the same AF value.

APPENDIX E: SCHEDULE OF ASSESSMENTS

Procedures	Screening		STUDY TREATMENT One cycle = 28 days										EoT	Safety Follow up	Follow up
	Baseline		Cycle 1				Cycle 2		Cycle 3		Cycle 4+				
Day	-28	-7	1	8±1	15±1[r]	22±1	1±1	15±1	1±1	15±1	1±1	15±1[z]		28+7[x]	
Study Visit[y]	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11 ...*	*12 ...*			
TESTS & OBSERVATIONS															
Informed consent[a]	X														
Assess subject inclusion/ exclusion criteria		X													
Medical history		X													
Physical examination, vital signs, height, and weight[b]		X	X				X		X		X		X	X	
Bone scan[c]	X														
ECOG performance status		X	X				X		X		X		X		
Triplicate 12-lead ECG[d]		X	X		X		X		X		X		X[d]	X	
Holter monitor[e]			X		X										
Echocardiogram/MUGA	X														
Adverse event evaluation			X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant medication review[f]		X	X	X	X	X	X	X	X	X	X	X	X		
STUDY DRUG ADMINISTRATION															
H3B-6545 dosing			Daily												
LABORATORY TESTS															
CBC, including 5-part differential and platelets plus reticulocytes[g]		X	X	X	X	X	X	X	X	X	X	X	X	X	
Chemistry/CMP[h]		X	X	X	X	X	X	X	X		X		X	X	
PT/PTT[i]		X	X	X	X	X	X	X	X	X	X	X	X		

Procedures	Screening		STUDY TREATMENT One cycle = 28 days										EoT	Safety Follow up	Follow up
	Baseline		Cycle 1				Cycle 2		Cycle 3		Cycle 4+				
Day	-28	-7	1	8±1	15±1[r]	22±1	1±1	15±1	1±1	15±1	1±1	15±1[z]		28+7[x]	
Study Visit[y]	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11 ...*	*12 ...*			
Urine testing dipstick[j]		X	X	X	X	X	X	X	X	X	X	X	X		
Serum or urine pregnancy test[k]		X					X		X		X		X	X	
PK blood samples[l]			X	X	X	X									
Archived tumor tissue (if available)[m]		X													
DISEASE ASSESSMENT															
CT scan of the chest, abdomen, pelvis[n,o]	X								X[n]				X[o]		X[n]
Survival follow-up[w]															X[v]
BIOMARKERS															
CCI [redacted]															
ENDOMETRIUM ASSESSMENT															
Transvaginal ultrasound[u]		X									X				

CBC = complete blood count; CMP = comprehensive metabolic profile; CT = computed tomography; DNA = deoxyribonucleic acid; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = End-of-Treatment; ERαmut = estrogen receptor alpha mutant; ERαWT = estrogen receptor alpha wild-type; ESR1 = estrogen receptor 1 gene; MUGA = multiple-gated

acquisition; PD = pharmacodynamics; PG = pharamacogenetic; PK = pharmacokinetic; PT/PTT = prothrombin time/partial thromboplastin time; RECIST = Response Evaluation Criteria in Solid Tumors; TSH = thyroid-stimulating hormone

a. Informed consent must be obtained ≤28 days prior to the initiation of study treatment. Informed consent must be obtained for pre-screening for *ESR1* mutations, and the results from a sponsor-designated cfDNA test confirming subject eligibility must be obtained prior to signature of the informed consent form, unless prior authorization has been received by the Sponsor, based on local mutation analysis-.

b. Physical examinations will include measurements of weight and vital signs (resting heart rate [HR], blood pressure [BP], respiratory rate [RR], oral temperature). No need to repeat physical exams on Cycle 1 Day 1 if the baseline physical exam was performed ≤72 hours. Height will be recorded at the baseline visit only. Cycle 2 and beyond, physical exam will be obtained on Day 1 of each cycle.

c. A bone scan (^{99}m-technetium-based scintigraphy, whole-body bone magnetic resonance imaging, or ^{18}F-sodium fluoride position emission tomography) to establish a baseline should be performed prior to first dose of study drug (a historical bone scan performed within 6 weeks prior to the first dose is acceptable), and as clinically indicated. Lesions identified on bone scans should be followed with cross-sectional imaging.

d. Triplicate 12-lead ECGs will be collected approximately 5 minutes apart at the screening visit for all subjects. On Day 1 of Cycles 1, 2, 3, and 4, triplicate ECGs will be collected approximately 5 minutes apart pre-dose and 3 hours (± 1 hour) post-dose for all subjects. Mandatory collections will be taken for both Phase 1 and Phase 2 subjects on Day 1 and Day 15 of Cycle 1. 12-lead safety ECGs will be performed locally from Day 1 of each cycle starting Cycle 5 till the last cycle prior to the EOT visit, and at Unscheduled visits as clinically required. A single 12-lead safety ECG is required at a minimum. Data from local safety ECGs should be entered into the clinical database. At the end-of-treatment visit, 1 set of triplicate ECGs will be collected approximately 5 minutes apart. If sinus bradycardia is ongoing at the EOT visit, ECGs should be obtained at least once a week until confirmation obtained that subject has returned to 60 or more beats per minute or to pre-treatment baseline value. If sinus bradycardia is observed at any point during treatment (from ECG or other clinical assessment), it is recommended the subject undergo a stress test per institution policy and data is recorded in the clinical database. A stress test is recommended to be done only once for each subject who has under 60 beats per minute (while on treatment) and more than a 20 percent reduction from baseline in beats per minute. The stress test should be triggered by the previous two cardiac observations at rest**.** Baseline, Cycle 1- Cycle 4, EOT, and 28 Day Safety Follow-up visit ECGs should be collected on study specific equipment provided by ERT. Additional ECGs taken during the study period on local machines should also be reported in the clinical database.

e. A 12-lead continuous Holter will be used in Cycle 1, Days 1 and 15 [at least 1 hour prior to the first administration of the drug and continue through at least 24 hours after] for Phase 1 subjects only (ECG and Holter monitoring grouped together in Phase 1). For convenience, Holter monitoring may begin up to 12 hours prior to dosing on Cycle 1, Day 1 and Day 15. Holter monitoring will not apply to subjects enrolled under Amendment 6 (or subsequent amendments).

f. All concomitant medications taken within 28 days prior to Cycle 1 Day 1 and while on study treatment through 28 days post-last dose should be documented in the electronic case report form (eCRF).

g. Hematological parameters include the following laboratory tests: CBC consisting of hematocrit, hemoglobin, total white blood count (WBC) with 5-part differential, and platelet count plus reticulocyte count. Do not repeat on Cycle 1 Day 1 if it is within 72 hours of baseline. CBCs are collected every visit before treatment.

h. CMP panel includes the following: blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, chloride, carbon dioxide, magnesium, phosphorous, glucose, albumin, total protein, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, and thyroid function tests (TSH and Free T4). Do not repeat on Cycle 1 Day 1 if it is within 72 hours of baseline.

i. PT/PTT will be assessed at screening by standard method at a local laboratory. PT/PTT will be analyzed weekly during the first cycle and on Day 1 and Day 15 of all subsequent cycles and at the end-of-treatment visit. This must be obtained with a peripheral blood stick.

j. Urine testing dipstick will be done at baseline and weekly during Cycle 1. Thereafter urine dipstick will be done on Day 1 and Day 15 of each 28-day cycle. If abnormalities are present, microscopic testing should be done.

k. A serum or urine pregnancy test must be performed at baseline for all women of childbearing potential (ie, pre-and perimenopausal subjects) (no need to repeat on Cycle 1 Day 1 if within 72 hours). For women of childbearing potential, the test will be repeated on Day 1 of each additional cycle prior to administration, at end of treatment visit and safety follow up visit (28 days (with up to +7 day window). A negative result must be confirmed prior to administration.

l. PK blood samples will be collected for all Phase 1 subjects on Cycle 1 Day 1 and Day 15 (at time points 0 (pre-dose), 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr 8 hr, 10 hr and 24 hr post-dose) and at pre-dose on Cycle 1 Day 8 and Day 22. The ±1 day-window does not apply to the Day 1 and Day 15 visits. Subjects in the food-effect portion of Phase 2 will have PK blood samples collected on Cycle 1 Day 15 and Cycle 1 Day 22 at time points 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 10, and 24 hr post-dose. Subjects in the food-effect cohort will be randomly assigned to receive the Cycle 1 Day 15 dose of H3B-6545 in a fed or fasted state. Each subject will then receive the Cycle 1 Day 22 dose in the reverse/untried state. At least sparse sampling for PK (3 time points per subjects) will be performed on Day 15 for the Phase 2 subjects not enrolled in the food-effect sub-study following one of three schedules, assigned sequentially: Schedule 1: pre-dose (0 hr), 0.5 hr, and 6 hr, Schedule 2: pre-dose (0 hr), 2 hr, 8 hr, or Schedule 3: 1 hr, 4 hr, 24 hr. As this is a first-in-human clinical trial and no prior human pharmacokinetics data are available, it is essential that the clinical staff take the blood samples for PK analysis as close as possible to the scheduled time point. This should help improve the accuracy of the PK modeling and minimize the variability around the PK estimates for H3B-6545. The exact date and time of the trial therapy administration must be recorded. The exact time and date of the blood draw must be recorded. A protocol deviation will be reported only when a PK time point is not collected (missing) or blood is drawn on the wrong study day.
For subjects enrolled under Amendment 6 (or subsequent amendments), PK samples will be collected on Cycle 1 Days 1 and 15 at pre-dose (0 hr) and at 1, 2, 4, and 6 hr post-dose.

m. Archival tumor samples will be requested prior to the subject's first dose. Material should be provided as a tissue block or 15 unstained slides.

n. Subjects will be restaged according to RECIST 1.1 at 8-week intervals (± 1 week) until radiologic disease progression or starting a new cancer therapy. All study imaging for tumor assessments will be sent to an imaging core laboratory designated by the Sponsor for review of quality and archived for potential independent review.

o. Tumor assessments will only be done in subjects who have not previously demonstrated disease progression in the study unless completed within the previous 4 weeks.

p. In addition to prescreening, screening, whole blood for cell-free DNA (nucleic acids) will be collected at Cycle 1 Day 1 and Day 15 and at the same day (or as close as possible) to each tumor assessment, that is, approximately every 8 weeks after Cycle 1 Day 1. Only 1 tube of 10 mL of whole blood is needed at each time point, except at prescreening, screening, Cycle 1 Day1, and EoT, where 2 tubes of 10 mL of whole blood are required.

q. Blood for CTCs in Phase 1 only. All biomarkers should optimally be collected pre-dose.

r. The ±1 assessment window for Cycle 1 Day 15 does not apply to Phase 2 subjects participating in the food effect study.

s. Subject must have at least one biopsiable lesion in the Phase 1 portion. In Phase 2, subjects must also have either (a) at least 1 measurable lesion as per RECIST 1.1, or (b) at least one predominantly lytic bone lesion. Subjects must be willing to undergo tumor biopsies prior to treatment and on Cycle 2 Day 1. In the Phase 2 part of the trial, subjects with bone-only disease, or subjects for whom a biopsy is contra-indicated, may opt out of providing tumor biopsies following investigator concern and direct discussion and approval from Sponsor The second biopsy must be acquired during the first week of Cycle 2 (Cycle 2 Day 1-Cycle 2 Day 8). The subject must have received at least 7 days of consecutive dosing prior to collection of the second biopsy. The window for collection of the Cycle 2 biopsy may be extended if needed in order to ensure 7 consecutive days of dosing prior to collection.
For subjects enrolled under Amendment 6 (or subsequent amendments), a recent archival tumor tissue obtained within 6 months prior to enrollment or a fresh tumor biopsy must be provided. A second biopsy after initiating trial therapy is not required.

t. Biomarkers of bone turn-over will be collected at Cycle 1 Day 1 (any time during screening up to pre-dose), Cycle 2 Day 15 and Cycle 4, Day 1 and EOT. A total of 6 mL of blood will be collected in a serum separating tube at each visit. There can be an important circadian rhythm variation of these markers; samples should thus optimally be collected after fasting in the morning (between 8:00 and 10:00 am) and specimen collection should be consistent during study visits.

u. Transvaginal ultrasound will be performed during the screening period and at Cycle 4 Day 1 (±10 days), and every 24 weeks after that, as long as the subject is on study treatment; only for subjects who are part of the endometrium safety sub-study.

v. All subjects will undergo the end-of-treatment assessments listed within 28 days after treatment ends.

w. Following progression, all subjects will be followed for survival at least every 12 weeks from EOT visit. The follow-up can be done by telephone call at the investigator's discretion.

x. Subjects must be followed for AEs for 28 calendar days after the last dose and complete a safety follow-up visit on the 28th day (with up to + 7 day window). During the safety follow-up visit, the following should be obtained: vital signs, hematological and CMP laboratory parameters and triplicate ECGs on ERT provided study equipment.

y. Missed visits: If a scheduled protocol visit is missed or rescheduled for any reason, all clinic visits will be scheduled based on the subject's Cycle 1 Day 1 date. Missed safety assessments are to be performed at the subsequent visit when patient resumes study treatment.

Dose interruption: During or immediately following a dose interruption, the protocol requires that study visit take place in order for the completion of required safety assessments. PK/PD samples missed due to dose interruptions do not have to be retroactively collected.

Unscheduled visit: Safety assessments that are obtained outside of the routine visits are recorded in the eCRF under "Unscheduled Visit" using the actual visit date.

z. Subjects may discontinue Day 15 visits for all cycles beyond cycle 4.

APPENDIX F: EXTENSION PHASE

Extension Phase will begin for subjects still on treatment at the time of the data cutoff for primary analysis

Procedure	Treatment[A]	EoT[N]	Safety Follow-Up
Vital signs and weight[B]	X		
Physical examination[C]	X	X	
ECOG performance status	X	X	
12-Lead ECG[D]	X	X	
Echocardiogram or MUGA scan[E]	As clinically indicated	As clinically indicated	
Hematology and clinical chemistry (local lab)[F]	X	X	
Urine dipstick testing[G]	X	X	
Pregnancy test[H]	X	X	
Tumor assessments (CT/MRI)[I]	As per local standard of care but not less frequently than every 12 weeks or earlier if clinically indicated		
Bone Scan[J]	As clinically indicated		
Brain scan (CT/MRI)[K]	As clinically indicated		
Study Treatment	28 Day cycle		
Concomitant medications[L]	X	X	X
AEs/SAEs[M]	X	X	X
AE = adverse event; BP = blood pressure; CT = computed tomography; ECG = electrocardiogram; EoT=end of treatment assessment; HR = heart rate; LVEF = left ventricular ejection fraction; MRI=magnetic resonance imaging; MUGA = multiple-gated acquisition; RR = respiratory rate; SAE-serious adverse event.			

A. Efforts should be made to conduct study visits on the day scheduled (Cycle X Day 1 ±3 days). The study visit (and safety assessments) still needs to occur regardless of a study medication hold per the visit schedule.

B. Assessments will include vital signs (resting BP [including date and time of measurement], HR, RR and body temperature) and weight.

C. A symptom-directed physical examination during the study, as clinically indicated.

D. Single 12-lead ECG. Subjects are suggested to be in the recumbent position for a period of 5 minutes prior to obtaining ECG.

E. An echocardiogram or MUGA scan to assess LVEF will be performed as clinically indicated

F. Clinical laboratory assessments will be conducted at a local laboratory. Clinical chemistry and hematology results should be reviewed prior to administration of study drug for all cycles. Assessments may be performed within 72 hours prior to the visit.

G. Urine dipstick testing for subjects should be performed preferably at the investigational site (but may be performed locally by the primary care physician or a local laboratory if the subject does not have to come for a visit to the site).

H. A serum or urine pregnancy test will be performed at Day 1 of every cycle, and at the EoT assessment in women of childbearing potential (ie, premenopausal and perimenopausal women who have been amenorrheic for less than 12 months).

I. After the data cutoff for the primary analysis: Tumor assessments using contrast-enhanced CT of the chest and contrast-enhanced CT or MRI of the abdomen, pelvis and other areas of known disease at screening or newly suspected disease should be performed as per local standard of care but not less frequently than every 12 weeks or earlier if clinically indicated. The same methodology (CT or MRI) and scan acquisition techniques that were used for the assessment during the Study Treatment Phase should be used after the data cutoff for the primary analysis.

J. A bone scan to assess bone metastases should be performed as clinically indicated.

K. Brain scans should be performed as clinically indicated.

L. Concomitant medications will be recorded until 28 days after last dose

M. Subjects must be followed for AEs for 28 calendar days after the last dose and complete a safety follow-up visit on the 28th day (with up to + 7 day window).

N. Tumor assessments will only be done in subjects who have not previously demonstrated disease progression in the study unless completed within the previous 4 weeks.

APPENDIX G: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST) VERSION 1.1

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.

APPENDIX H: CONCOMITANT MEDICATIONS

Combination administration of study drugs could result in DDI that could potentially lead to reduced activity or enhanced toxicity of the concomitant medication and/or H3B-6545.

Use of antacids is allowed.

The use of palonosetron, prochlorperazine, promethazine, and cyclizine for management of nausea and vomiting is also allowed. The use of ondansetron and granisetron is not permitted because of their potential to prolong QT interval

Herbal preparations/medications are not allowed throughout the study; thus, the subject should stop using herbal medications 7 days prior to first dose of H3B-6545. These herbal medications include, but are not limited to: St. John's wort, kava, ephedra (ma huang), gingko biloba, DHEA, yohimbe, saw palmetto, and ginseng.

The following list of prohibited medications is based on the Oncology Clinical Pharmacology Drug-Drug Interaction Database (release date: 29 Oct 2012), which was compiled from the Indiana University School of Medicine's "Clinically Relevant" Table and supplemented with the FDA Draft Guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (February 2012). These lists are not comprehensive and are only meant to be used as a guide. Please contact the medical monitor with any questions.

List of prohibited medications (including but not limited to:)

Category	Drug Name
Strong CYP3A4 inhibitors and inducers	Carbamazepine, clarithromycin, conivaptan, itraconazole, ketoconazole, nefazodone, phenobarbital, phenytoin, posaconazole, rifabulin, rifampin, telithromycin, voriconazole
Strong CYP3A4 inhibitors	Clarithromycin, conivaptan, itraconazole, ketoconazole, nefazodone, posaconazole, telithromycin, voriconazole,
Strong CYP3A4 inducers	Carbamazepine phenobarbital, phenytoin, rifabulin, rifampin, St. John's wort
P-gp inhibitors[1]	Amiodarone, cyclosporine, dronedarone, ranolazine, verapamil
CYP2C9 substrates with narrow therapeutic index[2] (NTI)	Phenytoin, warfarin
CYP2C19 substrates with NTI	Diazepam, amitriptyline, phenytoin
CYP3A substrates with NTI	Alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus
P-gp substrate with NTI	Digoxin
BCRP substrate with NTI	Sulfasalazine, methotrexate
Medications with a ***known*** *risk* for QT prolongation	See Appendix I

BCRP = breast cancer resistance protein; CYP = cytochrome P450; P-gp = P-glycoprotein.

1. P-gp inducers already listed as CYP3A4 inducers.
2. Narrow therapeutic index drugs are drugs where small differences in dose or blood concentration may lead to serious therapeutic failures and/or adverse drug reactions that are life-threatening or result in persistent or significant disability or incapacity.

APPENDIX I: DRUGS THAT PROLONG QT INTERVAL AND/OR INDUCE TORSADES DE POINTES

This is not a comprehensive list. A complete list may be found in Woosley, RL and Romero, KA, QTdrugs List, Accession Date, AZCERT, Inc. 1822 Innovation Park Dr., Oro Valley, AZ 85755 and updated at the following Web address: http://crediblemeds.org.

Antiarrhythmics Amiodarone Disopyramide Dofetilide Ibutilide Procainamide Quinidine Sotalol
Antibiotics Clarithromycin Erythromycin Gatifloxacin Moxifloxacin Sparfloxacin
Antipsychotics Chlorpromazine Haloperidol Mesoridazine Pimozide Risperidone Thioridazine Ziprasidone
Antidepressants Amitriptyline Desipramine Doxepin Imipramine Maprotiline Venlafaxine
Antifungals (azoles) Ketoconazole Itraconazole
Antimalarials Chloroquine Halofantrine
Antiemetics Dolasetron Domperidone Droperidol Ondansetron Tropisetron

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Antihistamines
Astemizole
Terfenadine
Alimemazine
Hydroxyzine
Diphenhydramine
Miscellaneous
Arsenic trioxide
Bepridil Methadone
Pentamidine
Cisapride
Tacrolimus

APPENDIX J: SECURITY OF THE SAMPLES, USE OF THE SAMPLES, AND RETENTION OF THE SAMPLES

Sample processing, for example, DNA and/or RNA extraction, genotyping, sequencing, or other analysis, will be performed by a laboratory under the direction of the Sponsor. Processing, analysis, and storage will be performed at a secure laboratory facility to protect the validity of the data and maintain subject privacy. Samples will only be used for the purposes described in this protocol. Laboratories contracted to perform the analysis on behalf of the Sponsor will not retain rights to the samples beyond those necessary to perform the specified analysis and will not transfer or sell those samples. The Sponsor will not sell the samples to a third party.

Samples will be stored for up to 15 years after the completion of the study (defined as submission of the clinical study report to the appropriate regulatory agencies). At the end of the storage period, samples will be destroyed. Samples may be stored longer if a health authority (or medicinal product approval agency) has active questions about the study. In this special circumstance, the samples will be stored until the questions have been adequately addressed.

It is possible that future research and technological advances may identify genomic variants of interest or allow alternative types of genomic analysis not foreseen at this time. Because it is not possible to prospectively define every avenue of future testing, all samples collected will be single or double coded (according to the ICH E15 guidelines) in order to maintain subject privacy.