Protocol Number:	ZWI-ZW25-202
Version:	26-Sep-2023; Amendment 4
Protocol Title:	Phase 2a Study of ZW25 in Combination with Palbociclib Plus Fulvestrant
Investigational Drug(s):	ZW25 (zanidatamab, JZP598) Palbociclib Fulvestrant
Phase:	2a
IND Number:	144685
ClinicalTrials.gov Number	NCT04224272
EudraCT Number	2019-002956-18
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Sponsor:	Jazz Pharmaceuticals Ireland, Limited Fifth Floor, Waterloo Exchange Waterloo Road Dublin 4 Dublin, Ireland D04 E5W7
US Sponsor Representative	Jazz Pharmaceuticals, Inc 3170 Porter Drive Palo Alto, CA 94304
Medical Monitor:	[redacted] MD Medical Director Jazz Pharmaceuticals Contact information appears in trial site binder (or equivalent)

PROTOCOL SYNOPSIS

Protocol Number ZWI-ZW25-202 **Version** 26-Sep-2023; Amendment 4 **Phase** 2a	**Product Name** zanidatamab (ZW25, JZP598) **Sponsor** Jazz Pharmaceuticals Ireland, Limited Fifth Floor, Waterloo Exchange Waterloo Road Dublin 4 Dublin, Ireland D04 E5W7 **US Sponsor Representative** Jazz Pharmaceuticals, Inc 3170 Porter Drive Palo Alto, CA 94304

Protocol Title

Phase 2a Study of ZW25 in Combination with Palbociclib Plus Fulvestrant

Study Objectives and Endpoints	
Part 1	
Objectives	**Endpoints**
Primary:	
• To recommend a dose for ZW25 in combination with palbociclib plus fulvestrant for Part 2 by evaluating the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic human epidermal growth factor receptor 2 (HER2)-positive, hormone receptor (HR)-positive breast cancer	• Frequency of dose-limiting toxicities (DLTs) • Frequency and severity of adverse events (AEs) • Frequency of serious adverse events (SAEs) and deaths • Frequency and severity of clinical laboratory abnormalities • Frequency of electrocardiogram (ECG) and left ventricular ejection fraction (LVEF) abnormalities • Frequency and severity of adverse events of special interest (AESIs), including absolute decreases in LVEF ≥10 percentage points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis • Frequency of dose reductions of ZW25 • Frequency of dose reductions of components of combination therapy • Frequency of treatment discontinuations due to adverse events (AEs)
Secondary:	
• To evaluate the pharmacokinetics (PK) of ZW25 in combination with palbociclib plus fulvestrant	• Serum concentrations of ZW25 as a function of time post-dosing • PK parameters for single (first) dose and multiple doses of ZW25

• To evaluate the immunogenicity of ZW25 in combination with palbociclib plus fulvestrant	• Frequency, duration, and time of onset of anti-drug antibodies (ADA) and neutralizing antibodies, if applicable
Exploratory:	
• To explore the utility of potential serum and tumor biomarkers	• Exploratory biomarkers, including but not limited to circulating tumor DNA (ctDNA), Prosigna Breast Cancer Prognostic Gene Signature Assay (hereafter referred to as ProSigna; also, formerly called the PAM50 test) and HER2 extracellular domain (HER2 ECD)

Study Objectives and Endpoints	
Part 2	
Objectives	**Endpoints**
Primary:	
• To evaluate the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer	• Progression-free survival 6 (PFS6, defined as the % of modified intent to treat (mITT) subjects with PFS ≥ 24 weeks)
Secondary:	
• To evaluate additional measures of the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/ or metastatic HER2-positive, HR-positive breast cancer	• Objective response rate (ORR) • Duration of response (DOR) • Disease control rate (DCR) • Progression-free survival (PFS) • Overall Survival (OS) • Frequency and severity of AEs
• To evaluate the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant	• Frequency of SAEs and deaths • Frequency and severity of clinical laboratory abnormalities • Frequency of ECG and LVEF abnormalities • Frequency and severity of AESIs, including decreases in LVEF ≥ 10 percentage points from baseline, symptomatic heart failure, all ≥Grade 3 infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis • Frequency of dose reductions of ZW25 • Frequency of dose reductions of components of combination therapy • Frequency of treatment discontinuations due to AEs
• To evaluate the PK of ZW25 in combination with palbociclib plus fulvestrant	• Serum concentrations of ZW25 as a function of time post-dosing • PK parameters for single (first) dose and multiple doses of ZW25
• To evaluate the immunogenicity of ZW25 in combination with palbociclib plus fulvestrant	• Frequency, duration, and time of onset of ADA and neutralizing antibodies, if applicable
Exploratory:	

• To explore the utility of potential serum and tumor biomarkers	• Exploratory biomarkers, including but not limited to ctDNA, ProSigna, and HER2 ECD

Study Population

Subjects must meet all of the enrollment criteria to be eligible for this study. All eligibility criteria are applicable to both parts of the study unless otherwise specified. Eligibility criteria may not be waived by the investigator and are subject to review in the event of a Good Clinical Practice (GCP) audit and/or health regulatory authority inspection.

Inclusion Criteria

1. Pathologically-confirmed diagnosis of breast cancer with evidence of locally advanced (unresectable) and/or metastatic disease. All subjects in both Parts 1 and 2 must have HER2-positive and HR-positive disease as follows:
   - HER2-positive based on the HER2 Testing in Breast Cancer: American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) Clinical Practice Guidelines (Wolff 2018).
   - HR-positive defined as estrogen receptor-positive (ER-positive) and/or progesterone receptor-positive (PgR-positive) disease based on the ASCO/ CAP Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Hammond 2010).
2. Able to provide a new formalin-fixed, paraffin-embedded (FFPE) tumor sample (preferred) or archived tumor tissue (most recent sample available) for retrospective central review of HER2 status.

   Local assessments performed on a new tumor sample or archived tumor tissue in a Clinical Laboratory Improvements Amendments (CLIA)-certified lab using a combination of IHC and ISH/FISH methods may be used to determine HER2 and HR status for study eligibility. IHC must be used to determine HR status. Unless otherwise approved by the sponsor medical monitor, specimens should be provided for centralized retrospective review of HER2 status.
3. Received prior treatment with trastuzumab, pertuzumab, AND ado-trastuzumab emtansine (T-DM1); disease progression during or after the most recent prior therapy. Subjects in any part of the study who did not receive pertuzumab or T-DM1 because of lack of access (e.g., due to insurance coverage or because they were treated prior to regulatory agency approval of the agent in a relevant indication) or due to medical ineligibility for treatment with T-DM1 (e.g., history of severe infusion reactions to trastuzumab, ≥ Grade 2 peripheral neuropathy, or platelet count < 100 x 10^9/L) may be eligible for the study after discussion with and approval from the sponsor medical monitor. Prior treatment with endocrine therapy in the neoadjuvant, adjuvant, and/or metastatic setting is permitted.
4. Sites of disease assessable per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (both measurable and non-measurable disease allowed)
5. Male and female subjects aged 18 years or older
6. An Eastern Cooperative Oncology Group (ECOG) Performance Status score of 0 or 1
7. Life expectancy of at least 3 months in the opinion of the investigator
8. The following baseline laboratory data:
   a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
   b. Platelet count ≥ 75 x 10^9/L
   c. Hemoglobin ≥ 9 g/dL
   d. Prothrombin time (PT) and/or International Normalized Ratio (INR) and partial thromboplastin time (PTT)/ aPTT (activated partial thromboplastin time) ≤ 1.5 x upper limit of normal (ULN), unless on medication known to alter the INR or PTT
   e. Total bilirubin ≤ 1.5 x ULN per institutional values (subjects with known Gilbert's Syndrome may enroll with ≤ 2.5 x ULN provided the direct bilirubin is ≤ 1.5 mg/dL)

f. Alanine transaminase (ALT) ≤ 3.0 x ULN per institutional values (if liver metastases are present, ≤ 5.0 x ULN)
g. Aspartate transaminase (AST) ≤ 3.0 x ULN per institutional values (if liver metastases are present, ≤ 5.0 x ULN)
h. Serum creatinine ≤ 1.5 X ULN or calculated glomerular filtration rate ≥ 50 mL/min

9. Adequate cardiac left ventricular function, as defined by LVEF ≥ institutional standard of normal
10. All toxicity related to prior cancer therapies must have resolved to ≤ Grade 1, with the exception of alopecia or ≤ Grade 2 neuropathy
11. If female and of child-bearing potential, must have a negative pregnancy test ≤ 3 days prior to the first dose of ZW25
12. For female subjects who are not surgically sterile or post-menopausal and for male subjects with a partner of child-bearing potential, willingness to use 2 methods of birth control with a failure rate of less than 1% per year during the study and for 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant). These include, but are not limited to, established use of oral, implanted, or injected hormonal contraceptives; placement of intra-uterine device or intra-uterine system; or use of barrier methods, such as condom or diaphragm together with a spermicidal product.
13. Female subjects must agree not to breastfeed or donate ova starting at screening and throughout the study period, and for at least 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant)
14. Male subjects must not donate sperm starting at screening and throughout the study period, and for at least 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant)
15. Signed informed consent prior to any study procedures not considered standard of care

Exclusion Criteria

Subjects will be excluded from the study if 1 or more of the following criteria are applicable:

1. Prior treatment with trastuzumab, pertuzumab, lapatinib, T-DM1, or other anti-HER2-targeted therapy ≤ 3 weeks before the first dose of ZW25
2. Prior treatment with chemotherapy, other anti-cancer therapy not otherwise specified, or hormonal cancer therapy ≤ 3 weeks before the first dose of ZW25
3. Prior treatment with experimental biologic and non-biologic therapies ≤ 4 weeks before the first dose of ZW25
4. Prior treatment with radiation therapy other than for central nervous system (CNS) disease ≤ 3 weeks before the first dose of ZW25
5. Treatment with anthracyclines within 90 days before first dose of ZW25 and/or total lifetime load exceeding 360 mg/m^2 Adriamycin® or equivalent
6. Use of any medications or substances that are strong inhibitors or inducers of CYP3A isoenzymes within 7 days of first dose of any study drug
7. History of life-threatening hypersensitivity to monoclonal antibodies, recombinant proteins, or excipients in the drug formulation
8. Prior treatment with palbociclib or any other CDK4/6 inhibitors, including experimental agents
9. Use of corticosteroids administered at doses equivalent to > 15 mg per day of prednisone within 2 weeks of first ZW25 dosing unless otherwise approved by the sponsor medical monitor. Topical, ocular, intra-articular, intranasal, and/or inhalational corticosteroids are permitted.
10. History of myocardial infarction or unstable angina within 6 months prior to enrollment, troponin levels consistent with myocardial infarction, or clinically significant cardiac disease, such as ventricular arrhythmia requiring therapy, uncontrolled hypertension, or any history of symptomatic congestive heart failure (CHF)
11. QTc Fridericia (QTcF) >470 ms

12. Grade 2 or greater pneumonitis and/or interstitial lung disease, including pulmonary fibrosis, or other clinically significant infiltrative pulmonary disease not related to lung metastases
13. Active hepatitis B or hepatitis C infection
14. Acute or chronic uncontrolled renal disease, pancreatitis, or severe liver disease (Child-Pugh Class C)
15. Known infection with Human Immunodeficiency Virus (HIV)-1 or HIV-2 (Exception: patients with well-controlled HIV [e.g., cluster of differentiation 4 (CD4)-positive T cell count >350/mm^3 and undetectable viral load] are eligible.)
16. Major surgery ≤ 3 weeks prior to the first dose of ZW25
17. Prior or concurrent malignancy whose natural history or treatment has the potential to interfere with the safety or efficacy assessment of the investigational regimen
18. Any other medical, social, or psychosocial factors that, in the opinion of the investigator, could impact safety or compliance with study procedures
19. Females who are breastfeeding or pregnant, and females and males planning a pregnancy
20. Brain metastases: Untreated CNS metastases, symptomatic CNS metastases, or radiation treatment for CNS metastases within 4 weeks of start of study treatment. Stable, treated brain metastases are allowed (defined as subjects who are off steroids and anticonvulsants and are neurologically stable for at least 1 month at the time of screening).
21. Poorly-controlled seizures
22. History of or ongoing leptomeningeal disease (LMD). If LMD has been reported radiographically on baseline magnetic resonance imaging (MRI), but is not suspected clinically by the investigator, the subject must be free of neurological symptoms of LMD.
23. Grade 3 or greater peripheral neuropathy

Number of Planned Subjects

The total number of subjects enrolled in the study will depend upon the number of dose levels of each of the components of the study treatment evaluated during Part 1 of the study. In Part 1 of the study, the number of subjects to be enrolled in a dose cohort is 6 DLT evaluable subjects. Therefore, up to approximately 36 evaluable subjects may be enrolled in Part 1 if both dose levels of ZW25 are tested in combination with all 3 dose levels of palbociclib. In Part 2 of the study, approximately 50 subjects will be enrolled. Therefore, a total of up to approximately 86 subjects may be enrolled across the entire study.

This study will be conducted in approximately 20 study sites.

Study Design

This is a multicenter, Phase 2a, open-label, 2-part study to investigate the safety, tolerability, and anti-tumor activity of ZW25 in combination with palbociclib (IBRANCE®), an inhibitor of cyclin-dependent kinases 4 and 6 [CDK4 and CDK6] plus fulvestrant (FASLODEX®), an estrogen receptor antagonist. Premenopausal women and perimenopausal women will also be treated with a luteinizing-hormone releasing hormone (LHRH) analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.

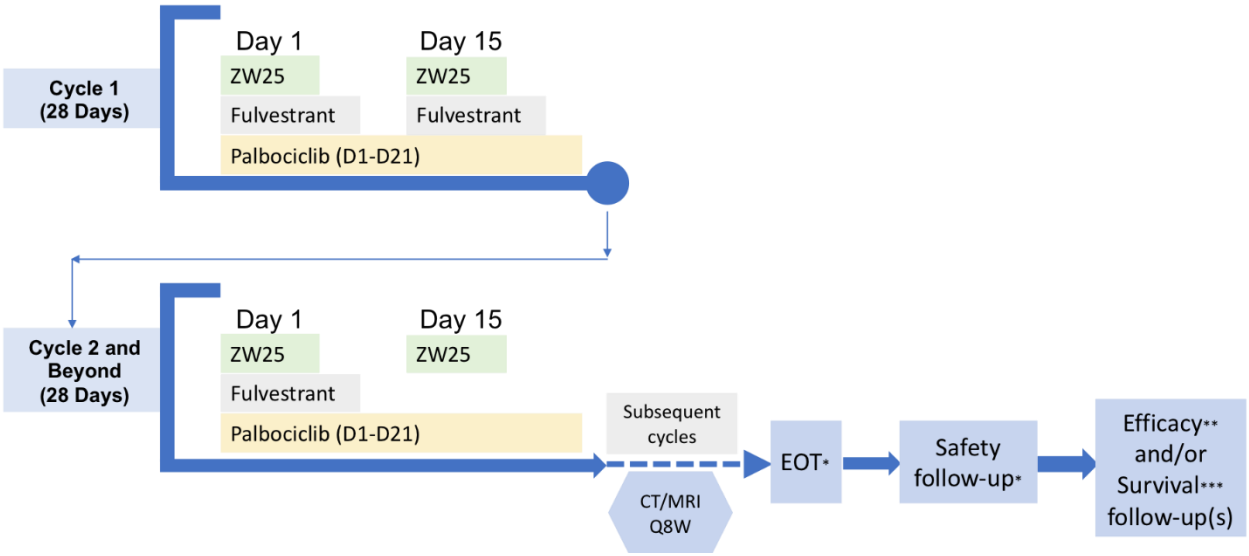
For both parts of the study, eligible subjects are those with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer. Subjects must have a diagnosis of locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive (ER-positive and/or PgR-positive) breast cancer based upon local assessment. Eligibility will be determined based on local (institutional) review of HER2 status, HR status, and disease pathology, with subsequent retrospective central review of HER2 status. HER2 expression will be determined based on the HER2 Testing in Breast Cancer: ASCO/CAP Clinical Practice Guidelines (Wolff 2018). HR status will be determined based on the ASCO/CAP Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Hammond 2010). If the local assessments at a

particular site cannot be done with these tests and to these standards, then central assessment will be required before a subject can be enrolled at that site.

Part 1 of the study will first evaluate the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant and will confirm the recommended doses of all the drugs in this combination. Then, Part 2 of the study will evaluate the anti-tumor activity of the recommended dose (RD) level of the combination of ZW25 with palbociclib plus fulvestrant in subjects with HER2-positive, HR-positive breast cancer.

The overall study design is presented in Figure 1. Each treatment cycle of ZW25 combined with palbociclib plus fulvestrant is 28 days. Following treatment discontinuation (see Treatment Duration section below), subjects will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and a safety follow-up visit at approximately 30 days after the last dose of study drug. Subjects who discontinue all study treatment on either part of the study for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks (± 7 days) after the previous scan until disease progression or start of subsequent anticancer therapy. Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued and subjects will enter long-term follow-up for survival status. Data on survival will be collected via clinic visits (if feasible) or via telephone calls every 3 months after the last follow-up visit. Subjects may be discontinued from the study due to death, lost to follow-up, consent withdrawal or study termination by sponsor. Reason for study discontinuation will be recorded.

Figure 1: Study Design



CT = computed tomography; D = day; EOT = end of treatment; MRI = magnetic resonance imaging; Q8W = every 8 weeks.
Note: The DLT-evaluation period will be the initial 28 days of treatment beginning Cycle 1 Day 1.
*, Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug and safety follow-up visit at approximately 30 days after the last dose of study drug.
**, Subjects who discontinue all study treatment for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks after the previous scan until disease progression or start of subsequent anticancer therapy.
***, Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued and subjects will enter long-term follow-up for survival status. Data on survival will be collected approximately every 3 months after the last follow-up visit.

Part 1: Safety Evaluation

The primary objective of Part 1 is to characterize the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant and to confirm the recommended doses for all the drugs in this combination.

On Days 1 and 15, ZW25 will be administered first followed by palbociclib and fulvestrant.

ZW25 will be administered intravenously (IV) at the initial dose of 20 mg/kg every 2 weeks (Q2W), which is the single-agent RD. Palbociclib will be administered orally (PO) with food at 125 mg once daily (QD) for the first 21 days of each 28-day cycle. Fulvestrant will be administered as an intramuscular (IM) injection at 500 mg Q2W for the first 3 doses, then once every 4 weeks (Q4W). A step-down dose level of ZW25 (e.g., 15 mg/kg Q2W or other dose not lower than 15 mg/kg Q2W) and/or palbociclib (e.g., 100 mg PO and/or 75 mg PO QD) may be allowed if recommended by the Safety Monitoring Committee (SMC). Subjects will also receive LHRH analogue treatment per institutional guidelines. The DLT-evaluation period will be the initial 28 days of treatment beginning Cycle 1 Day 1.

Initially, up to 6 evaluable subjects will be assessed at the ZW25 monotherapy recommended dose of 20 mg/kg Q2W in combination with palbociclib plus fulvestrant. A DLT evaluable subject is defined as one who has received ≥75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and was followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being followed was the occurrence of DLT. If DLTs are observed in ≥2 subjects, the SMC may recommend a step-down dose of ZW25 of 15 mg/kg Q2W or other dose not lower than 15 mg/kg Q2W for evaluation in up to 6 additional DLT evaluable subjects. Additionally, the SMC may recommend evaluation of step-down doses of palbociclib of 100 mg PO QD and/or 75 mg PO QD with or without recommending evaluation of a step-down dose of ZW25. There is no step-down dose for fulvestrant at a cohort or study population level; however, individual subjects who develop moderate hepatic impairment (Child-Pugh Class B) while on study treatment will have their fulvestrant dose reduced to 250 mg. Intermediate dose levels may also be recommended by the SMC. The SMC may recommend step-down doses for 1 or more of the drugs in the combination at any time based on the overall safety profile. The SMC may decide if more than 6 subjects may be enrolled in a given dose level to better characterize the safety profile for a particular dose level.

Prior to dosing with ZW25, all subjects must receive prophylactic treatment for infusion reactions. Additional premedications may be recommended for palbociclib, and fulvestrant per institutional guidelines and/or local prescribing information.

The study will advance to Part 2 based on SMC recommendation. Additional safety experiences in later cycles may be considered when confirming the recommended doses of all drugs in this combination.

Part 2: Anti-tumor Activity Evaluation

The primary objective of Part 2 of the study is to evaluate the potential anti-tumor activity of the recommended dose level of the combination of ZW25 with palbociclib plus fulvestrant. Enrollment for Part 2 will begin once the RD of ZW25 and the other drugs of the combination therapy have been confirmed in Part 1. The treatment and blood collection schedules, treatment cycle duration and imaging evaluation intervals are the same as in Part 1.

Part 2 of the study will evaluate the preliminary anti-tumor activity using the modified intent to treat (mITT) set. The mITT set includes all subjects who receive any amount of ZW25 with palbociclib and/or fulvestrant who had at least one identifiable (target and/or non-target) lesion at baseline and at least one post-baseline disease assessment or discontinued all study treatment and are no longer being followed for efficacy. All subjects will be assessed for safety and anti-tumor response. The primary efficacy endpoint will be PFS6 (defined as the % of mITT subjects with PFS of ≥ 24 weeks); secondary efficacy endpoints include ORR, DOR, DCR, PFS, and OS. Approximately 50 subjects are expected to be enrolled in Part 2.

If possible, an additional optional tumor biopsy may be obtained at the time of disease progression from an accessible site to allow for assessment of changes in HER2 expression as well as the presence of other exploratory biomarkers. Biomarkers of response may be evaluated.

Test Product, Dose, and Mode of Administration

Note that ketoconazole and other strong CYP3A4 inducers and inhibitors are prohibited.

ZW25 (investigational agent):

- Part 1:
  - 20 mg/kg IV ZW25 Q2W on Days 1 and 15 of each 28-day cycle. A step-down dose level to 15 mg/kg Q2W or another dose level (not lower than 15 mg/kg) of ZW25 may be evaluated if recommended by the SMC and approved by the sponsor
- Part 2:
  - RD of ZW25 as confirmed in Part 1 of the study

Combination Products (Dose and Mode of Administration)

Palbociclib (capsules for oral use):

- Parts 1 and 2:
  - 125 mg PO QD for the first 21 days of each 28-day cycle. Step-down dose levels of 100 and/or 75 mg PO QD may be evaluated if recommended by the SMC and approved by the sponsor.

Fulvestrant (injection for IM administration):

- Parts 1 and 2:
  - 500 mg IM Q2W for 3 doses (Cycle 1 Days 1 and 15 and Cycle 2 Day 1), and Q4W thereafter

LHRH analogue:

- Parts 1 and 2: Per institutional guidelines.

  Note: Concomitant norethindrone acetate is contraindicated for breast cancer patients.

Required Premedications:

- Required concomitant premedication for potential infusion reactions:
  - Prior to each ZW25 infusion:
    - Acetaminophen orally (650 mg) and diphenhydramine orally (50 mg) or equivalents of each; approximately 30 to 60 minutes prior to infusion of ZW25
    - Corticosteroids (hydrocortisone 100 mg IV or dexamethasone 10 mg IV); approximately 30 to 60 minutes prior to infusion of ZW25
  - If an alternative premedication regimen is thought to be required, sponsor approval is required before implementation.
  - For subjects who experience an infusion reaction despite the above premedication, other medication as needed per the Investigator or per institutional standards including H2 blockers may be given in addition to the mandatory premedications.
- Premedications may also be given for palbociclib and fulvestrant per institutional guidelines and/or local prescribing information.

Duration of Treatment

Subjects may continue on study treatment until radiographically-confirmed disease progression as defined by RECIST version 1.1, clinical disease progression, unacceptable toxicity, consent withdrawal, physician decision, pregnancy, protocol violation, start of a subsequent anticancer therapy, or study termination by the sponsor.

Note: Trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy.

Clinical progression is defined as worsening or re-emergence of pre-existing symptoms relating to underlying cancers, or emergence of new symptoms that cannot be attributed to drug toxicities or alternative causes. Every effort should be made to confirm disease progression radiographically.

If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy. Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and safety follow-up visit at approximately 30 days after the last dose of study drug.

Safety Assessments

Subjects' safety will be monitored at predetermined time points by recording the type, frequency, and severity of AEs graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0 (27 November 2017), including adverse events of special interest (AESIs), SAEs and deaths, clinical laboratory values (including hematology and serum chemistry), physical examination, vital signs (blood pressure, heart rate, respiratory rate, and temperature), body weight, and ECOG performance status. Cardiac function will be monitored via 12-lead ECG and echocardiogram or multiple gated acquisition scan (MUGA). AEs will be collected from the start of dosing of any study drug on Cycle 1 Day 1 through 30 days after last dose of study drug (ZW25, palbociclib, and/or fulvestrant). Study protocol-related AEs are to be collected from the time of informed consent. All investigators should follow up subjects with SAEs until the event returns to baseline, the event stabilizes (recovering/resolving) or is no longer considered clinically significant by the investigator, or the subject dies or withdraws consent. Treatment-emergent AESIs should continue to be followed until resolution or return to baseline and include absolute decreases in LVEF ≥ 10 percentage points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis.

Safety and study conduct will be monitored throughout the study by the SMC, consisting of the study investigators and the sponsor responsible medical expert and biostatistician. The committee is tasked with monitoring the safety of participants in this study through regular or ad hoc meetings. The roles, responsibilities and functioning of the SMC are described in more details in the SMC charter.

Efficacy Assessments

In both Parts 1 and 2 of the study, computed tomography (CT) and/or MRI scans will be performed at baseline and every 8 weeks during treatment (timed from Cycle 1 Day 1) and will be assessed according to the revised RECIST guideline version 1.1. Initial responses should be confirmed, if feasible, with a repeat scan 4 weeks (+1-week window) following initial documentation of objective response. The schedule of response assessments should not be adjusted after the confirmatory scan and should maintain the schedule of every 8 weeks timed from Cycle 1 Day 1. Determination of the radiographic restaging modality (CT and/or MRI) will be made at baseline for each subject by the investigator and should be used for all subsequent response assessments.

Pharmacokinetic (PK) and Immunogenicity Assessments

Serum concentrations of ZW25 will be measured as a function of time post-dosing. PK parameters to be estimated include the following: 1) for single (first) dose: maximum observed serum concentration ($C_{max,1}$), time to maximum observed serum concentration ($t_{max,1}$), area under the serum concentration-time curve from zero to the last measurable concentration (AUC_{0-t}), terminal elimination rate constant (λ_z), apparent elimination half-life ($t_{1/2}$), area under the serum concentration-time curve from zero to infinity ($AUC_{0-\infty}$), serum clearance (CL), volume of distribution in the terminal elimination phase (V_d), etc.; and 2) for multiple doses: average concentration over dosing interval (C_{ave}), maximum observed serum concentration (C_{max}) and minimum observed serum concentration (C_{min}) (trough), accumulation index (R_{Cmin}), trough concentration at steady state ($C_{trough,ss}$), and attainment of steady state. All subjects in Part 1 of the study will have PK assessments per the extensive PK schedule. In Part 2, at least 6 subjects will have PK assessments per the extensive PK schedule. Further subjects will then be assigned to the sparse PK schedule. A subject may be allowed to opt out of an extensive PK schedule with sponsor approval.

Anti-drug antibodies to ZW25 will be measured, including frequency, time of onset, and duration of immunogenicity response. Additionally, subjects with demonstrated ADA may be evaluated for possible neutralizing antibodies.

Biomarker Assessments

Blood and tumor tissue samples will be collected at the specified timepoints for assessment of biomarkers. Biomarkers may include but are not limited to ctDNA, ProSigna (formerly called the PAM50 test), and HER2 ECD. Biomarkers will be investigated as an exploratory endpoint and will not be used to guide subject selection for the study.

Statistical Methods

Summaries of subject disposition, demographics, disease characteristics, safety, disease response, and exposure will be provided. Safety and efficacy endpoints will be summarized using descriptive statistics (e.g., mean, median, standard deviation, minimum, maximum) for continuous variables, and frequencies and percentages for categorical variables. In addition, the PK parameters of ZW25 will be estimated and summarized with descriptive statistics.

Sample Size Determination

No formal sample size calculations were performed for Part 1 of the study. In Part 1, approximately 6 evaluable subjects will be enrolled in each dose cohort with up to six ZW25 and palbociclib dose level combinations evaluated; therefore, the approximate minimum and maximum sample sizes for Part 1 are 6 and 36 evaluable subjects, respectively.

Details regarding sample size are presented in Table 1.

Table 1: Sample Size in Each Part of the Study

Study Part	Cohort or Stage	ZW25 Dose (mg/kg)	Palbociclib Dose (mg)	Sample Size[b]
1	1A	20	125	6
	1B	20	100	6 (if palbociclib de-escalation from cohort 1A recommended)
	1C	20	75	6 (if palbociclib de-escalation from cohort 1B recommended)
	1D	15	125	6 (if ZW25 de-escalation from cohort 1A recommended)
	1E	15	100	6 (if ZW25 de-escalation from cohort 1B or both ZW25 and palbociclib de-escalation from cohort 1A recommended)
	1F	15	75	6 (if ZW25 de-escalation from cohort 1C, palbociclib de-escalation from cohort 1E, or both ZW25 and palbociclib de-escalation from cohort 1B recommended)
2	2	RD[a]	RD[a]	Approximately 50

IV = intravenous; PO = orally (per oral); RD = recommended dose; Q2W = every 2 weeks.

[a] RD established in Part 1

[b] Part 1 sample sizes are based on the number of DLT evaluable subjects. Part 2 sample size is based on the approximate number of subjects planned for enrollment.

Notes: Cohorts 1A through 1F are not necessarily sequential. ZW25 is given IV Q2W. Step-down dose level to 15 mg/kg Q2W or another dose level (not lower than 15 mg/kg) of ZW25 may be evaluated if recommended by the SMC and approved by the sponsor. Palbociclib is taken PO QD on Days 1 through 21 of each 28-day cycle. There are no step-down doses for toxicity for fulvestrant.

A patient will be considered DLT evaluable if he or she has received ≥ 75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and was followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being followed was the occurrence of a DLT. If the reason for not receiving the required doses is a DLT, a patient will still be considered evaluable. Subjects who are considered non-evaluable may be replaced. The probabilities of observing DLTs in a cohort of 6 subjects for Part 1 are summarized in Table 2.

Table 2: Probabilities of Observing DLTs in Part 1

N = 6 Subjects	True DLT Incidence				
	5%	10%	20%	30%	40%
Probability of 0 DLTs	0.74	0.53	0.26	0.12	0.05
Probability of ≤1 DLTs	0.97	0.89	0.66	0.42	0.23
Probability of De-escalation (≥2 DLTs)	0.03	0.11	0.34	0.58	0.77

DLT = dose-limiting toxicity

In Part 2 of the study, approximately 50 subjects are expected to be enrolled. Assuming the observed PFS6 rate is between 40% and 70%, the corresponding 95% binomial exact confidence intervals (CI) are summarized in Table 3.

Table 3: PFS6 Rate and Corresponding 95% Confidence Intervals

PFS6 Rate	Binomial exact 95% CI (N=50)
40%	(26%, 55%)
50%	(36%, 64%)
60%	(45%, 74%)
70%	(55%, 82%)

Therefore, a total of up to approximately 86 subjects may be enrolled across the entire study.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
λ_z	terminal elimination rate constant
5-HT3	5-hydroxytryptamine
ADA	anti-drug antibody
ADC	antibody-drug conjugate
ADCC	antibody-dependent cellular cytotoxicity
AE	adverse event
AESI	adverse event of special interest
ALT	alanine transaminase
ALP	alkaline phosphatase
ANC	absolute neutrophil count
ANOVA	analysis of variance
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	aspartate transaminase
$AUC_{0-\infty}$	area under the serum concentration-time curve from zero to infinity
AUC_{0-t}	area under the serum concentration-time curve from zero to the last measurable concentration
BUN	blood urea nitrogen
CAP	College of American Pathologists
C_{ave}	average concentration over dosing interval
CBC	complete blood count
CD4	cluster of differentiation 4
CDK	cyclin-dependent kinases
CFR	Code of Federal Regulations
CH1	constant heavy chain 1
CHF	congestive heart failure
CI	confidence interval
CL	serum clearance
CLIA	Clinical Laboratory Improvements Amendment
C_{max}	maximum concentration
C_{min}	minimum concentration
CNS	central nervous system
CR	complete response
CRF	case report form
CT	computed tomography
ctDNA	circulating tumor DNA
C_{trough}	trough concentration
$C_{trough,ss}$	trough concentration at steady state
CYP	cytochrome P-450
DCR	disease control rate
DLT	dose-limiting toxicity
DOR	duration of response
ECD	extracellular domain
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor

Abbreviation	Definition
EOT	end of treatment
ER	estrogen receptor
EU	European Union
Fab	fragment antigen-binding
Fc	fragment crystallizable
FDA	Food and Drug Administration
FFPE	formalin-fixed, paraffin-embedded
FISH	fluorescence in situ hybridization
GCP	Good Clinical Practice
GEA	gastroesophageal adenocarcinoma
GLP	Good Laboratory Practice
HER	human epidermal growth factor receptor
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HR	hormone receptor
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IgG1	immunoglobulin G1
IHC	immunohistochemistry
IM	intramuscular
IND	investigational new drug
INR	international normalized ratio
IRB	institutional review board
IV	intravenous(ly)
LHRH	luteinizing-hormone releasing hormone
LMD	leptomeningeal disease
LVEF	left ventricular ejection fraction
MCH	mean corpuscular hemoglobin
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MUGA	multiple gated acquisition scan
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK-1	neurokinin-1
NSCLC	non-small cell lung cancer
ORR	objective response rate
OS	overall survival
PALOMA	Palbociclib Ongoing Trials in the Management of Breast Cancer
PD	progressive disease
PFS	progression-free survival
PFS6	percentage of evaluable subjects with PFS ≥ 24 weeks
PgR	progesterone receptor
PK	pharmacokinetic(s)
PO	orally (per oral)
PR	partial response

Abbreviation	Definition
PS	performance status
PT	prothrombin time
PTT	partial thromboplastin time
Q2H	every 2 hours
Q2W	every 2 weeks
Q4H	every 4 hours
Q8H	every 8 hours
Q8W	every 8 weeks
Q3M	every 3 months
QD	once daily
QTcF	QT interval corrected for heart rate using Fridericia's formula
QW	every week or weekly
R_{Cmin}	accumulation ratio, calculated as C_{min} (last dose)/C_{min} (first dose)
RD	recommended dose
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SAP	statistical analysis plan
scFv	single-chain variable fragment
SD	stable disease
SMC	Safety Monitoring Committee
SOC	system organ class
SULT	sulfotransferase
T-DM1	ado-trastuzumab emtansine
TEAE	treatment-emergent adverse event
TK	toxicokinetic(s)
T_{max}	time at which the maximum concentration occurs
ULN	upper limit of normal
US	United States
Vd	volume of distribution
VH	variable heavy
VL	variable light

1 INTRODUCTION

1.1 Breast Cancer

Breast cancer presents a significant health burden worldwide. Globally, in 2020, over 2 million new cases were reported with over 650,000 deaths (GLOBOCAN 2020). The lifetime incidence rate of female invasive breast cancer in the United States (US) is approximately 13% (one in eight women), and it is estimated that over 297,790 new cases of invasive breast cancer will be diagnosed in 2023 in women. The 5- and 10-year relative survival rates for women with invasive breast cancer in the US are 90% and 83%, respectively. However, 5-year relative survival rates drop to 30% when metastases are present (American Cancer Society 2023). The estimated number of breast cancer cases in both sexes and all ages in the European Union (EU) (27 countries) for 2022 is 355,457 with an estimated mortality of 91,826 cases (European Cancer Information System (ECIS) 2023).

1.2 HER2 as a Target in Breast Cancer

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor (EGFR/ErbB) family comprising 4 structurally related receptors: HER1 (EGFR), HER2, HER3, and HER4. HER receptors are normally activated by binding to specific ligands, resulting in a conformational change that allows formation of receptor homodimers and heterodimers. Receptor dimerization triggers autophosphorylation of specific tyrosine residues and activation of intracellular signaling pathways (Moasser 2007). HER2 is unique among HER family members in that it has no known ligand and maintains a dimerization-ready conformation. HER2 is the preferred dimerization partner for other HER family members. HER2-containing heterodimers, particularly HER2/HER3, deliver the most potent growth signals (Pohlmann 2009).

The oncogenic role of HER2 is best defined for breast cancers with HER2 gene amplification and high levels of HER2 protein expression, which historically have been associated with aggressive tumor growth and poor clinical outcomes. However, because HER2 is the preferred dimerization partner for all other HER family receptors and can interact synergistically with other receptor tyrosine kinase cell growth pathways (Moasser 2007), HER2-targeted therapy may be important even in the absence of gene amplification and/or in the setting of lower levels of expression.

Approximately 15% of patients with breast cancer have tumors that overexpress the HER2 protein, and these patients can benefit from HER2-targeted therapies. Approximately half of all HER2-positive breast cancers are also hormone receptor (HR) positive (Giordano 2014). Identification of receptor expression offers options for individualized targeted therapies (Schramm 2015).

1.3 Approved HER2-Targeted Therapies

The introduction of HER2-targeted therapy into the clinic has led to marked improvements in disease-free survival, progression-free survival (PFS), and overall survival (OS) for patients with HER2-high breast cancer (Schramm 2015). In the US, there are multiple approved HER2-targeted agents, all of which are indicated for the treatment of HER2-high breast cancer. These include the antibody-based therapies trastuzumab (HERCEPTIN®) and pertuzumab (PERJETA®), the antibody-drug conjugates (ADCs) ado-trastuzumab emtansine (KADCYLA®;

also referred to as T-DM1) and fam-trastuzumab deruxtecan-nxki (ENHERTU®; also referred to as T-DXd) (ENHERTU®); oral small molecule dual EGFR/HER2 inhibitor lapatinib (TYKERB®); the irreversible oral small molecule EGFR/HER2/HER4 inhibitor neratinib (NERLYNX®); and tucatinib (Tukysa™), a kinase inhibitor indicated in combination with trastuzumab and capecitabine.

Despite the gains obtained with current HER2-directed therapy, an unmet medical need remains for patients with all HER2-expressing cancers, particularly recurrent or metastatic disease that has progressed after standard of care therapy. This includes patients with HER2-overexpressing breast cancer that may have primary or secondary resistance to current HER2-targeted treatments. Resistance may be due to a number of factors including increased heterodimerization with other EGFR/ErbB family members as well as heterogeneity in levels of HER2 expression (Rye 2018). HER2 expression levels can vary within a tumor and/or be discordant between the site of primary and metastatic disease. Increased heterogeneity and/or decreased levels of HER2 expression may be particularly important in development of resistance to T-DM1, which relies on receptor binding and internalization for its cytotoxic effect (Barok 2014). In addition to a need for new targeted HER2 therapy that can overcome resistant disease, there is a need to develop less toxic treatment regimens that may help select patients avoid the use of chemotherapy, particularly in the adjuvant setting.

In summary, despite new therapeutic options, HER2-positive metastatic breast cancer still remains an incurable disease (Barok 2014).

1.4 ZW25

ZW25 is a novel humanized, bispecific antibody directed against 2 distinct HER2 epitopes, extracellular domains 2 and 4 (ECD2 and ECD4), the epitopes bound by pertuzumab and trastuzumab, respectively. Therefore, ZW25 combines both pertuzumab- and trastuzumab-like activities in a single antibody. ZW25 has the potential to have greater activity than either pertuzumab or trastuzumab in both HER2 overexpressing and HER2 lower expressing tumors. The sponsor is developing ZW25 as a treatment for locally advanced (unresectable) and/or metastatic HER2-expressing cancers.

Pharmacology studies demonstrate that ZW25 binds HER2 with subnanomolar affinity leading to enhanced tumor cell binding and inhibition of growth factor induced tumor cell growth (likely by blocking HER2 heterodimerization with other EGFR/ErbB family members). ZW25-induced cross-linking of HER2 also results in HER2 clustering and likely enhanced receptor internalization and downregulation as well as antibody-dependent cellular cytotoxicity (ADCC). In vivo studies in nude mice demonstrate anti-tumor activity and/or improved host survival against xenografts of human breast, head and neck squamous cell (HNSCC), non-small cell lung (NSCLC), pancreatic, gastric, and ovarian cancers.

ZW25 has been evaluated in a comprehensive toxicology program (non-Good Laboratory Practice [GLP] and GLP studies) for intravenous (IV) administration in clinical trials. The toxicology program was carried out in cynomolgus macaque monkeys because ZW25 binds to both human and monkey HER2 with similar subnanomolar affinity but does not bind to rodent

HER2. Safety pharmacology, including an assessment of its effect on cardiovascular and respiratory function, was conducted as part of the GLP toxicology study as outlined in International Congress on Harmonisation (ICH) S9[1]. ZW25 showed no effect on safety pharmacology parameters. In addition, in vitro studies demonstrated that ZW25 has a similar effect on human cardiomyocyte viability as trastuzumab (both alone and in combination with an anthracycline).

In non-GLP pharmacokinetic (PK) and GLP TK studies, ZW25 serum exposure increased with dose (5 mg/kg through 150 mg/kg IV) in cynomolgus monkeys. ZW25 was mostly distributed to the serum compartment and was cleared slowly from the serum. Mean half-life of ZW25 after either a single dose or repeat doses ranged from 74.3 to 215 hours (2.73 to 8.95 days) and was similar to the reported half-life (3 to 14 days) of trastuzumab from repeat-dose studies in rhesus and cynomolgus monkeys (EMEA 2005). Cohorts from all dosing levels of the GLP toxicology study of ZW25 were screened for antidrug antibodies (ADA). Only 2 of the 292 samples tested were confirmed positive; however, the majority of ZW25-treated animals had serum concentrations that exceeded the specific drug tolerance level of the assay. Thus, ADA may be present but undetectable due to high drug concentrations.

The GLP toxicology study evaluated the toxicity and toxicokinetics (TK) of weekly ZW25 at doses of 5, 50, and 150 mg/kg for 8 and 13 weeks. ZW25 was well tolerated and no adverse effects were observed at weekly doses up to 150 mg/kg IV for up to 13 weeks.

The nonclinical program supported evaluation of ZW25 as an IV infusion in the clinic. Therefore, a first-in-human clinical study was initiated in September 2016. This ongoing, multi-part, Phase 1 study (ZWI-ZW25-101) is evaluating the safety, pharmacokinetics (PK), immunogenicity, and anti-tumor activity of ZW25 as a single agent and in combination with selected chemotherapy agents in subjects with locally advanced (unresectable) and/or metastatic HER2-expressing tumors.

Part 1 of Study ZWI-ZW25-101 is using a standard 3+3 dose-escalation design to determine the maximum-tolerated dose (MTD), optimal biological dose, or recommended dose (RD) of ZW25 monotherapy administered weekly (QW), every 2 weeks (Q2W), or every 3 weeks (Q3W) in subjects with any HER2-expressing cancer that had progressed after receipt of all therapies known to confer clinical benefit. Part 2 of the study is characterizing the safety, tolerability, and preliminary anti-tumor activity of ZW25 monotherapy administered at Part 1 RDs in subjects with selected HER2-expressing locally advanced (unresectable) and/or metastatic cancers in up to 5 disease specific cohorts including HER2-high breast cancer (immunohistochemistry [IHC] 3+ or IHC 2+/fluorescent in situ hybridization [FISH]+), HER2-intermediate breast cancer (IHC 2+/FISH-negative [FISH-]), HER2-high gastroesophageal adenocarcinoma (GEA), HER2-intermediate GEA, and other HER2-high cancers. One recommended ZW25 monotherapy dose identified for further study was 20 mg/kg Q2W. Part 3 of the study is evaluating the safety, tolerability, and preliminary anti-tumor activity of ZW25 administered in combination with

[1] ICH S9 Guideline: Nonclinical Evaluation for Anticancer Pharmaceuticals. Step 4 version. 29 October 2009. Available from: https://database.ich.org/sites/default/files/S9_Guideline.pdf

selected chemotherapy agents including paclitaxel, capecitabine, and vinorelbine in subjects with HER2-expressing breast and gastric cancers.

A detailed summary of the safety and efficacy results of ZW25 as monotherapy or in combination with chemotherapy from Study ZWI-ZW25-101 is included in the Investigator's Brochure.

A Phase 2 study (ZWI-ZW25-201) of ZW25 plus physician's choice of first-line combination chemotherapy in HER2-expressing GEA is underway. However, no results are available at this time.

This study (ZWI-ZW25-202) is a multicenter, Phase 2a, open-label, 2-part study to investigate the safety, tolerability, and anti-tumor activity of ZW25 in combination with palbociclib (IBRANCE®), an inhibitor of cyclin-dependent kinases 4 and 6 [CDK4 and CDK6] plus fulvestrant (FASLODEX®), an estrogen receptor [ER] antagonist) in subjects with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer that has progressed on or been refractory to prior treatment with trastuzumab, pertuzumab, and T-DM1.

The primary objective of Part 1 is to characterize the safety and tolerability of ZW25 at a monotherapy RD derived from Phase 1 Study ZWI-ZW25-201 (20 mg/kg Q2W) when administered with palbociclib (125 mg PO QD for the first 21 days of each 4-week cycle) plus fulvestrant (500 mg intramuscularly [IM] Q2W for the first 3 doses, then Q4W thereafter) and to confirm the RD of ZW25 in combination with palbociclib plus fulvestrant. Part 2 of the study will evaluate the anti-tumor activity of the recommended dose level of the combination of ZW25 with palbociclib plus fulvestrant in this population (subjects with HER2-positive, HR-positive breast cancer).

The clinical trial will be conducted in compliance with the protocol, GCP and all the applicable regulatory requirements. A complete summary of the clinical and nonclinical data relevant to the investigational product and its study in humans is provided in the Investigator's Brochure.

1.5 Palbociclib

Palbociclib is an inhibitor of CDK4 and CDK6. Cyclin D1 and CDK4/6 are downstream of signaling pathways which lead to cellular proliferation. In vitro, palbociclib reduced cellular proliferation of ER-positive breast cancer cell lines by blocking progression of the cell from G1 into S phase of the cell cycle. Palbociclib is approved for the treatment of HR-positive, HER2-negative advanced or metastatic breast cancer in combination with fulvestrant in patients with disease progression following endocrine therapy. The recommended dose of palbociclib is a 125 mg capsule taken orally (PO) with food once daily (QD) for 21 consecutive days followed by 7 days off treatment during a 28-day treatment cycle . The addition of a CDK4/6 inhibitor to endocrine therapy has demonstrated improved clinical outcomes, with delayed onset of tumor progression. The combination of endocrine therapy and a CDK4/6 inhibitor is now included in the treatment guidelines for advanced HR-positive breast cancer and is being widely prescribed .

1.6 Fulvestrant

Fulvestrant is an ER antagonist that binds to the ER in a competitive manner with affinity comparable to that of estradiol and downregulates the ER protein in human breast cancer cells. Fulvestrant is approved for the treatment of HR-positive, HER2-negative advanced or metastatic breast cancer in combination with palbociclib in patients with disease progression after endocrine therapy. The recommended dose of fulvestrant is 500 mg to be administered IM into the buttocks (gluteal area) slowly (1 to 2 minutes per injection) as two 5-mL injections, one in each buttock, on Days 1, 15, and 29 and once monthly thereafter (FASLODEX®).

1.7 Rationale for Treatment with ZW25, Palbociclib and Fulvestrant in Breast Cancer

In HER2-positive breast cancer, cyclin D1 and its partner kinase CDK4 are critical drivers of cell proliferation. It has been demonstrated that the cyclin D1-CDK4 pathway can mediate resistance to HER2-targeting therapies in vitro and in vivo and that targeting resistant tumor cells with CDK4/6 inhibitors re-sensitizes them to anti-HER2 therapy and delays tumor recurrence in HER2-driven breast cancers in vivo in patient-derived xenograft tumors (Goel 2016).

CKD4/6 inhibition combined with anti-HER2 therapy is currently being explored in Phase 2 and Phase 3 trials in subjects with HER2-positive/HR-positive breast cancer (Corona 2017), (Pernas 2018). PATRICIA is an ongoing open-label Phase 2 trial exploring the safety and efficacy of the combination of trastuzumab plus palbociclib ± letrozole in subjects with pre-treated (2 to 4 prior lines) HER2-positive metastatic breast cancer. Preliminary results from 45 subjects show that palbociclib in combination is safe and may be effective in HER2-positive breast cancer, especially with HR-positive disease (Ciruelos 2018).

Developing a single multifunctional antibody with improved capacity and efficiency for binding HER2 compared with available HER2 inhibitors and the ability to activate ADCC, block ligand induced heterodimerization of HER2 with other EGFR/ErbB receptors, and down-regulate HER2 may be the key to creating effective HER2-targeted therapies for HER2-expressing tumors. Because ZW25 has potential for greater activity than either trastuzumab or pertuzumab, combining ZW25 with palbociclib and fulvestrant may offer an advantage over the combinations of palbociclib and other anti-HER2 therapy in the other currently ongoing clinical trials.

Based upon the activity of ZW25 as a single agent, as well as the safety profile observed to date, ZW25 has the potential to provide clinical benefit to patients with advanced breast cancer. This study (ZWI-ZW25-202) will evaluate the safety and preliminary anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in the treatment of subjects with advanced HER2-positive/HR-positive breast cancer that has progressed on or been refractory to prior treatment with trastuzumab, pertuzumab, and T-DM1. Premenopausal women and perimenopausal women will also be treated with a luteinizing hormone-releasing hormone (LHRH) analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.

1.8 Potential Safety Risk

ZW25 is being developed as a potential new treatment for patients with locally advanced (unresectable) and/or metastatic HER2-expressing cancers. Potential risks to subjects are based upon findings from the preliminary clinical trial data, non-GLP and GLP toxicology studies, and toxicities associated with drugs of similar mechanisms of action, including trastuzumab, pertuzumab, T-DM1, other HER2 inhibitors, and monoclonal antibodies in general.

For a summary of the clinical and nonclinical ZW25 safety data to date, please refer to the Investigator's Brochure. For additional information regarding detailed safety risks associated with palbociclib and fulvestrant, please refer to the country specific prescribing information.

In addition to the potential toxicities described below, other adverse events (AEs), including life-threatening or fatal events, may occur.

The safety of palbociclib (125 mg/day) plus fulvestrant (500 mg) versus placebo plus fulvestrant was evaluated in the PALOMA-3 study (McShane 2018). The data described below reflect exposure to palbociclib in 345 out of 517 subjects with HR-positive, HER2-negative advanced or metastatic breast cancer who received at least 1 dose of palbociclib plus fulvestrant. The median duration of treatment for palbociclib plus fulvestrant was 10.8 months. Dose reductions due to an AE of any grade occurred in 36% of subjects receiving palbociclib plus fulvestrant. No dose reduction was allowed for fulvestrant. Permanent discontinuation associated with an AE occurred in 19 (6%) of 345 subjects receiving palbociclib plus fulvestrant and in 6 (3%) of 172 subjects receiving placebo plus fulvestrant. Adverse reactions leading to discontinuation for subjects receiving palbociclib plus fulvestrant included fatigue (0.6%), infections (0.6%), and thrombocytopenia (0.6%). The most common adverse reactions (≥10%) of any grade reported in subjects in the palbociclib plus fulvestrant arm by descending frequency were neutropenia, leukopenia, infections, fatigue, nausea, anemia, stomatitis, diarrhea, thrombocytopenia, vomiting, alopecia, rash, decreased appetite, and pyrexia (IBRANCE®). Rare cases of severe interstitial lung disease/non-infectious pneumonitis, including fatal events, have been reported in subjects treated with palbociclib (Ahsan 2017), (Ahsan 2017), (IBRANCE®), and (Levy 2019). Subjects in the current study ZWI-ZW25-202 will be monitored for potential pulmonary toxicity by routine AE surveillance. Subjects who develop new or worsening respiratory signs or symptoms (e.g., cough, dyspnea, hypoxia, or interstitial infiltrate) will have further palbociclib dosing immediately put on hold and will be urgently evaluated for interstitial lung disease/non-infectious pneumonitis.

The oxidative metabolism of palbociclib is primarily mediated by cytochrome P-450 (CYP). Therefore, a number of medications and foods, including grapefruit products, may interact with palbociclib (McShane 2018). Additional information about prohibited concomitant medications and drug-drug interactions is included in Section 5.3.6 and Section 5.3.7.

Recommended dose modifications are provided in Section 5.3.3.

1.8.1 Cardiotoxicity

Although no cardiac toxicities were observed in the GLP toxicology study, other HER2 inhibitors have been associated with decreases in LVEF, particularly when given in combination with anthracyclines. Therefore, only subjects who have baseline normal LVEF and no history of significant cardiac disease will be eligible for treatment with ZW25. Subjects with a lifetime cumulative dose of anthracycline >360 mg/m^2 or who have received anthracycline treatment within 90 days of the expected first dose of ZW25 are not eligible for treatment.

Subjects in the current study ZWI-ZW25-202 will be monitored per protocol for changes in cardiac function. Cardiac monitoring will include 12-lead electrocardiograms (ECGs) and echocardiogram/multiple gated acquisition scan (MUGA) for estimation of LVEF.

1.8.2 Gastrointestinal Toxicity

Diarrhea has been recognized with other anti-HER2 antibodies. Per the Investigator's Brochure, diarrhea has been commonly observed when ZW25 is given as monotherapy.

As noted above, in the palbociclib plus fulvestrant arm in the PALOMA-3 study, gastrointestinal AEs occurring in ≥10% of subjects of any grade included nausea (34%), stomatitis 28%), diarrhea (24%), and vomiting (19%). None of these gastrointestinal events were Grade 4 in severity; and, in 1% of subjects, stomatitis and vomiting were recorded as a Grade 3 event (IBRANCE®).

Subjects in the current study ZWI-ZW25-202 will be monitored for potential gastrointestinal toxicity by routine AE surveillance. If gastrointestinal toxicity develops during the study, prophylactic or symptomatic treatment may be considered.

1.8.3 Hematologic Toxicity

Management of hematologic toxicities includes collection of samples for hematology tests at regular intervals per the protocol. Subjects with evidence of hematologic complications should receive prompt treatment and supportive care.

No hematologic effects were observed in toxicology studies for ZW25. Per the Investigator's Brochure, anemia has been commonly reported when ZW25 is given as monotherapy. Neutropenia, the most common any-grade and Grade 3 or higher AE associated with palbociclib, is consistent with the drug's mechanism of action and can be effectively managed with dose interruption, dose reduction, or dose delay (McShane 2018). A Grade ≥3 decrease in neutrophil counts was reported in 66% of subjects receiving palbociclib plus fulvestrant in PALOMA-3. The median time to first episode of any grade neutropenia was 15 days and the median duration of Grade ≥3 neutropenia was 7 days. Febrile neutropenia has been reported in 1.8% of subjects exposed to palbociclib across PALOMA-2 (palbociclib plus letrozole) and PALOMA-3. One death due to neutropenic sepsis was observed in PALOMA-2. The most frequently reported Grade ≥3 adverse reactions (≥5%) in subjects receiving palbociclib plus fulvestrant in PALOMA-3 in order of descending frequency were neutropenia and leukopenia (IBRANCE®).

In addition, because fulvestrant is administered IM, it should be used with caution in subjects with bleeding diathesis, thrombocytopenia and anticoagulant use.

1.8.4 Infusion-related Reactions

While ZW25 is a fully humanized antibody, infusion reactions may occur. Infusion reactions consist of a symptom complex characterized by fever and chills and may include nausea, vomiting, pain, headache, dizziness, dyspnea, hypotension, rash, and asthenia. Severe and potentially fatal reactions may include bronchospasm, anaphylaxis, angioedema, hypoxia, and severe hypotension. Infusion reactions may occur during or immediately following the initial infusion or at a later time point. While severe reactions usually occur during or immediately after infusion, onset and clinical course can be variable.

Per the Investigator's Brochure, infusion related reaction has been commonly reported when ZW25 is given as monotherapy.

A description of safety data is presented separately in the Investigator's Brochure.

Prophylaxis for and management of ZW25-related infusion reactions are provided in Section 5.2.7.1 and Section 5.2.3.2, respectively.

2 OBJECTIVES AND ENDPOINTS

2.1 Part 1 Objectives and Endpoints

Objectives	Endpoints
Primary:	
• To recommend a dose for ZW25 in combination with palbociclib plus fulvestrant for Part 2 by evaluating the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer	• Frequency of dose-limiting toxicities (DLTs) • Frequency and severity of adverse events (AEs) • Frequency of serious adverse events (SAEs) and deaths • Frequency and severity of clinical laboratory abnormalities • Frequency of electrocardiogram (ECG) and left ventricular ejection fraction (LVEF) abnormalities • Frequency and severity of adverse events of special interest (AESIs), including absolute decreases in LVEF ≥ 10% points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis • Frequency of dose reductions of ZW25 • Frequency of dose reductions of components of combination therapy • Frequency of treatment discontinuations due to AEs
Secondary:	
• To evaluate the pharmacokinetics (PK) of ZW25 in combination with palbociclib plus fulvestrant	• Serum concentrations of ZW25 as a function of time post-dosing

Objectives	Endpoints
• To evaluate the immunogenicity of ZW25 in combination with palbociclib plus fulvestrant	• PK parameters for single (first) dose and multiple doses of ZW25 • Frequency, duration, and time of onset of anti-drug antibodies (ADA) and neutralizing antibodies, if applicable
Exploratory:	
• To explore the utility of potential serum and tumor biomarkers	• Exploratory biomarkers, including but not limited to circulating tumor DNA (ctDNA), Prosigna Breast Cancer Prognostic Gene Signature Assay (hereafter referred to as ProSigna; also, formerly called the PAM50 test) and HER2 extracellular domain (HER2 ECD)

2.2 Part 2 Objectives and Endpoints

Objectives	Endpoints
Primary:	
• To evaluate the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer	• Progression-free survival 6 (PFS6, defined as the % of mITT subjects with PFS ≥ 24 weeks)
Secondary:	
• To evaluate additional measures of the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive breast cancer	• Objective response rate (ORR) • Duration of response (DOR) • Disease control rate (DCR) • Progression-free survival (PFS) • Overall survival (OS)
• To evaluate the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant	• Frequency and severity of AEs • Frequency of SAEs and deaths • Frequency and severity of clinical laboratory abnormalities • Frequency of ECG and LVEF abnormalities • Frequency and severity of AESIs, including absolute decreases in LVEF ≥ 10% points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis • Frequency of dose reductions of ZW25 • Frequency of dose reductions of components of combination therapy • Frequency of treatment discontinuations due to AEs
• To evaluate the PK of ZW25 in combination with palbociclib plus fulvestrant	• Serum concentrations of ZW25 as a function of time post-dosing • PK parameters for single (first) dose and multiple doses of ZW25
• To evaluate the immunogenicity of ZW25 in combination with palbociclib plus fulvestrant	• Frequency, duration, and time of onset of ADA and neutralizing antibodies, if applicable
Exploratory:	
• To explore the utility of potential serum and tumor biomarkers	• Exploratory biomarkers, including but not limited to ctDNA, ProSigna, and HER2 ECD

3 INVESTIGATIONAL PLAN

3.1 Summary of Study Design

This is a multicenter, Phase 2a, open-label, 2-part study to investigate the safety, tolerability, and anti-tumor activity of ZW25 in combination with palbociclib (IBRANCE®), an inhibitor of

CDK4 and CDK6) plus fulvestrant (FASLODEX®), an ER antagonist). Premenopausal women and perimenopausal women will also be treated with a LHRH analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines. Eligible subjects include those with locally advanced (unresectable) or metastatic HER2-positive, HR-positive breast cancer.

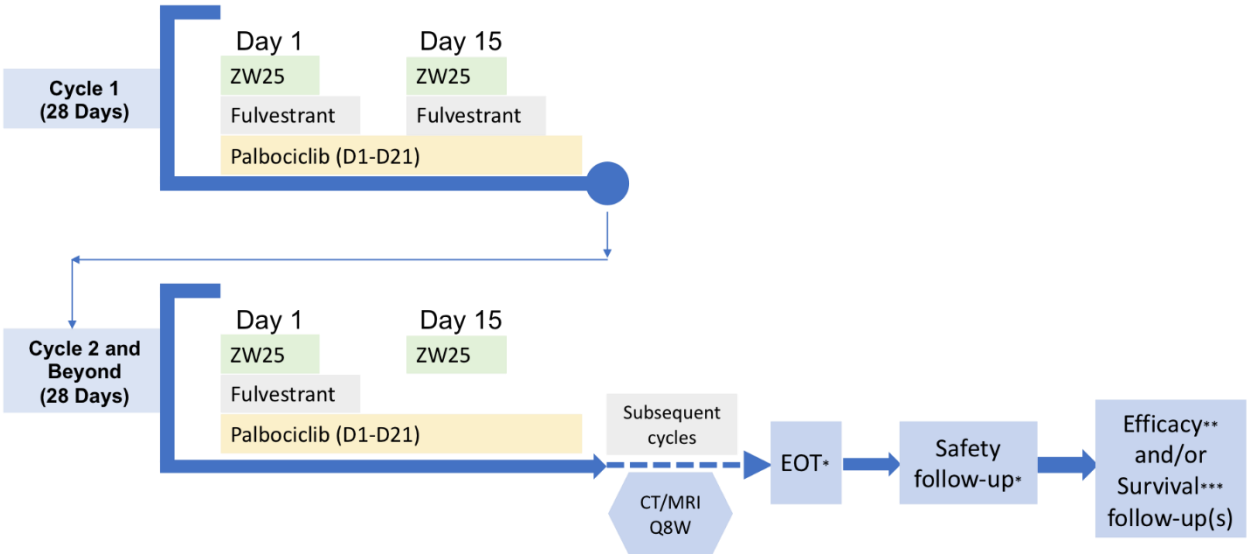
For both parts of the study, subjects must have a diagnosis of locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive (ER-positive and/or progesterone receptor-positive [PgR-positive]) breast cancer. Eligibility will be determined based on local (institutional) review of HER2 status, HR status, and disease pathology, with subsequent retrospective central review for HER2 status. HER2 expression will be determined based on the HER2 Testing in Breast Cancer: American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) Clinical Practice Guidelines (Wolff 2018). Hormone receptor status will be determined based on the ASCO/CAP Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Hammond 2010). If the local assessments at a particular site cannot be done with these tests and to these standards, then central assessment will be required before a subject can be enrolled.

Part 1 of the study will first evaluate the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant and will confirm the recommended doses of ZW25, palbociclib, and fulvestrant when given in combination. Then, Part 2 of the study will evaluate the anti-tumor activity of the recommended dose level of the combination of ZW25 with palbociclib plus fulvestrant in subjects with HER2-positive, HR-positive breast cancer.

The overall study design is presented in Figure 2. Each treatment cycle of ZW25 combined with palbociclib plus fulvestrant is 28 days. If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy. (Note: trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy). Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and safety follow-up visit at approximately 30 days after the last dose of study drug. Subjects who discontinue all study treatment on either part of the study for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks after the previous scan until disease progression or start of subsequent anticancer therapy. If subjects enter this follow-up period before 24-weeks have elapsed since the start of study treatment, a disease assessment must be done at 24-weeks from the start of treatment (not required if ≤ 4 weeks since last disease assessment or if disease progression has already been documented). Responses that are initially observed during this follow-up period should be confirmed with disease assessment 4 weeks (+1

week) later. Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued and subjects will enter long-term follow-up for survival status. Data on survival will be collected via clinic visits (if feasible) or via telephone calls every 3 months after the last follow-up visit. Subjects may be discontinued from the study due to death, lost to follow-up, consent withdrawal or study termination by sponsor. Reason for study discontinuation will be recorded. The same assessment schedule (Appendix A) will be used for subjects in both parts of the study. All subjects in Part 1 will have blood samples taken per the extensive sampling schedule (Appendix B). In Part 2, at least 6 subjects will have blood samples taken per the extensive sampling schedule (Appendix B) while the remaining subjects will have samples taken based on the sparse sampling schedule (Appendix C).

Figure 2: Overall Study Design



CT = computed tomography; D = day; MRI = magnetic resonance imaging; Q8W = every 8 weeks.
Note: On Days 1 and 15 of each cycle, ZW25 will be administered first followed by palbociclib and fulvestrant.
Note: The DLT-evaluation period will be the initial 28 days of treatment beginning Cycle 1 Day 1.
*, Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug and safety follow-up visit at approximately 30 days after the last dose of study drug.
**, Subjects who discontinue all study treatment for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks after the previous scan until disease progression or start of subsequent anticancer therapy.
***, Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued and subjects will enter long-term follow-up for survival status. Data on survival will be collected approximately every 3 months after the last follow-up visit.

For all subjects, computed tomography (CT) and/or magnetic resonance imaging (MRI) scans will be performed for tumor restaging every 8 weeks timed from Cycle 1 Day 1 during treatment and will be assessed according to the revised RECIST guideline version 1.1 (Eisenhauer 2009). Initial responses should be confirmed, if feasible, with repeat scans 4 weeks (+1-week window) following initial documentation of objective response. The schedule of response assessments should not be adjusted after the confirmatory scan and should maintain the schedule of every 8 weeks timed from Cycle 1 Day 1. The same imaging modality should be used throughout the

study as was used at baseline. Data on breast cancer serum tumor markers, if monitored, should also be collected at the time of disease assessment.

Subjects' safety will be monitored at predetermined time points by recording the type, frequency, and severity of AEs graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0 (27 November 2017), including AESIs, SAEs and deaths, clinical laboratory values (including hematology and serum chemistry), physical examination, vital signs (blood pressure, heart rate, respiratory rate, and temperature), body weight, and ECOG performance status. Cardiac function will be monitored via 12-lead ECG and echocardiogram or multiple gated acquisition scan (MUGA). AEs will be collected from the start of dosing of any study drug on Cycle 1 Day 1time of consent through 30 days after last dose of study drug (ZW25, palbociclib, and/or fulvestrant). Study protocol-related AEs are to be collected from the time of informed consent. All investigators should follow subjects with SAEs until the event returns to baseline, the event stabilizes (recovering/resolving) or is no longer considered clinically significant by the investigator, or the subject dies or withdraws consent. Treatment-emergent AESIs should continue to be followed until resolution or return to baseline and include absolute decreases in LVEF ≥ 10 percentage points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis.

Safety and study conduct will be monitored throughout the study by the Safety Monitoring Committee (SMC) (Section 3.1.3).

In both parts of the study, subjects may continue on study treatment until radiographically -confirmed disease progression as defined by RECIST version 1.1, clinical disease progression, unacceptable toxicity, consent withdrawal, physician decision, pregnancy, protocol violation, start of a subsequent anticancer therapy (Note: trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy) or study termination by the sponsor. Clinical progression is defined as worsening or re-emergence of pre-existing symptoms relating to underlying cancers, or emergence of new symptoms that cannot be attributed to drug toxicities or alternative causes. Every effort should be made to confirm disease progression radiographically.

If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjectsZW25 on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy. Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and safety follow-up visit at approximately 30 days after the last dose of study drug.

3.1.1 Part 1 Safety Evaluation

The primary objective of Part 1 is to characterize the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant. In addition, Part 1 will confirm the recommended doses for all the drugs in this combination.

ZW25 will be administered IV at the initial dose of 20 mg/kg Q2W, which is a single-agent RD identified in Study ZWI-ZW25-101. Palbociclib will be administered PO with food at 125 mg QD for the first 21 days of each 28-day cycle. Fulvestrant will be administered as an IM injection at 500 mg Q2W for the first 3 doses, then Q4W thereafter. A step-down dose level of ZW25 (e.g., 15 mg/kg [or other dose level not lower than 15 mg/kg] Q2W) and/or palbociclib (e.g., 100 mg PO and/or 75 mg PO QD) may be allowed if recommended by the SMC. Subjects will also receive LHRH analogue treatment per institutional guidelines. The DLT-evaluation period will be the initial 28 days of treatment beginning on Cycle 1 Day 1.

Initially, up to 6 evaluable subjects will be assessed at a ZW25 monotherapy RD of 20 mg/kg Q2W in combination with palbociclib plus fulvestrant; therefore, at least 6 evaluable subjects will be enrolled in Part 1 of the study and up to approximately 36 evaluable subjects if all step-down doses of ZW25 and palbociclib are evaluated (see Section 9.1 for possible dose combinations). The SMC may decide if more than 6 subjects may be enrolled in a given dose level to better characterize the safety profile of a particular dose level.

Prior to dosing with ZW25, all subjects should receive prophylactic treatment for infusion reactions (Section 5.2.7.1). Additional premedications may be given for palbociclib and fulvestrant per institutional standards and/or the local prescribing information.

The recommended doses for all drugs in this combination (ZW25 in combination with palbociclib plus fulvestrant) will be confirmed based on the evaluation in Section 3.1.2.

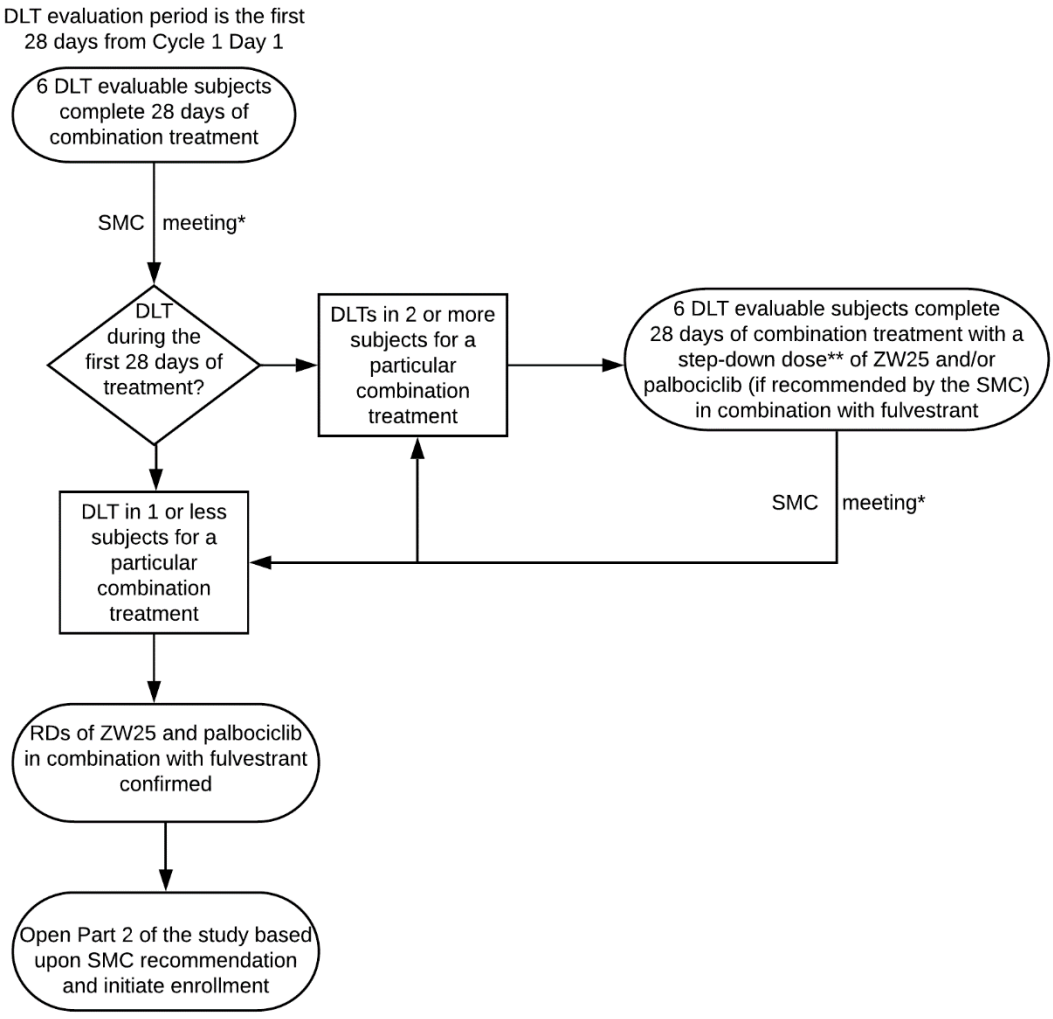
3.1.2 Dose-Limiting Toxicity Evaluation and Determination of Recommended Dose Level (Part 1 only)

The DLT-evaluation period will be the first 28 days of treatment.

The dose level (evaluated dose of ZW25 plus evaluated dose of palbociclib and fulvestrant) will be considered not tolerated if ≥ 2 of 6 DLT evaluable subjects experience a DLT. If DLTs are observed in ≥ 2 subjects, the SMC may recommend a step-down dose level of 15 mg/kg Q2W or other dose not lower than 15 mg/kg Q2W for evaluation in up to 6 additional DLT evaluable subjects. Additionally, the SMC may recommend evaluation of step-down doses of palbociclib of 100 mg PO QD and/or 75 mg PO QD with or without recommending evaluation of a step-down dose of ZW25. There is no step-down dose for fulvestrant at a cohort or study population level; however, individual subjects who develop moderate hepatic impairment (Child-Pugh Class B) while on study treatment will have their fulvestrant dose reduced to 250 mg. Intermediate dose levels of ZW25 and/or palbociclib may also be recommended by the SMC. The SMC may recommend step-down doses for 1 or more of the drugs in the combination regimen at any time based on the overall safety profile. The dose of ZW25 is allowed to be de-escalated by a maximum of 1 step-down dose as recommended by the SMC. The dose of

palbociclib is allowed to be de-escalated by a maximum of 2 step-down doses as recommended by the SMC. At a reduced dose level, if 1 or fewer of the 6 subjects experience a DLT, then the dose level may be confirmed as recommended for Part 2. When necessary, more than 6 subjects may be enrolled in a given dose level to better characterize the safety profile of a particular dose level. The study will advance to Part 2 based on SMC recommendation. The DLT evaluation schema is presented in Figure 3. Additional safety experiences in later cycles may be considered when confirming the recommended dose level (ZW25 in combination with palbociclib plus fulvestrant) for Part 2.

Figure 3: DLT Evaluation Schema (Part 1 Only)



DLT= dose-limiting toxicity; RD=recommended dose; SMC=Safety Monitoring Committee
*The roles, responsibilities and functioning of the SMC are described in more details in the SMC charter.
** Step-down dose levels of ZW25 and/ or palbociclib as recommended by the SMC.

A subject will be considered DLT-evaluable if he/she has received ≥ 75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and was followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being followed was the occurrence of a DLT. If the reason for not receiving required doses is a DLT, a subject will still be considered evaluable. To account for potential discontinuations prior to being evaluable for DLT (for example, progressive disease [PD] or subject preference), enrollment of replacement subjects will be allowed to permit assessment of DLTs and recommended dose level for Part 2.

Dose-limiting toxicities, defined using the (NCI CTCAE) version 5.0, are events that 1) occur following administration of ZW25, palbociclib, and fulvestrant, or any combination of ZW25 and 1 or more of these drugs; and 2) meet the criteria described below. Events for which there is an alternative clinical explanation (e.g., clearly related to an intercurrent illness, disease progression, or extraneous causes) will not be considered DLTs. The relationship of AEs to each component of study treatment will be determined by the investigator; however, the Sponsor and/or the SMC may query the relationship assessments and/or determine that the event was related to additional components of the regimen.

DLT Criteria Non-Hematologic

Any non-hematologic AE ≥ Grade 3 in severity that is not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) is considered a DLT, with the following exceptions:

- Grade 3 fatigue lasting ≤ 3 days
- Grade 3 diarrhea, nausea, or vomiting that resolves to ≤ Grade 1 or baseline within 3 days with adequate supportive care
- Grade 3 rash without maximal use of corticosteroids or anti-infectives
- Infusion reaction ≤ Grade 3 (for management of infusion reactions, see Section 5.2.3.2)

Grade 2 or greater events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis not clearly and incontrovertibly related to disease progression or extraneous causes is considered a DLT.

Any laboratory abnormality ≥ Grade 4 not clearly and incontrovertibly due to disease progression or extraneous causes is considered a DLT. Any asymptomatic ≥ Grade 3 electrolyte abnormality that lasts > 72 hours or any symptomatic ≥ Grade 3 electrolyte abnormality of any duration is considered a DLT. Amylase and lipase elevations not associated with symptoms or clinical manifestations of pancreatitis are not considered DLTs.

Any clinically relevant toxicities not clearly and incontrovertibly due to disease progression or extraneous causes and that do not resolve to ≤ Grade 1 or baseline within 2 weeks may be considered a DLT based upon review by the study SMC.

Greater than a 2-week delay in the start of Cycle 2 due to unresolved toxicity not clearly and incontrovertibly due to disease progression or extraneous causes will be considered a DLT.

Any death not clearly due to the underlying disease or extraneous causes is considered a DLT.

Hematologic DLT Criteria

The following hematologic AEs that are not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs:

- Absolute neutrophil count (ANC) of Grade 3 or 4 with fever (fever must be present for the Grade 3 or 4 ANC to be considered a DLT, and is defined as a temperature of ≥ 38.5°C)
- ANC of < 500/μL that persists for >7 days or growth factor support for ANC < 500/μL
- Grade 3 thrombocytopenia associated with significant bleeding (requiring blood and/or platelet transfusion)
- Grade 4 thrombocytopenia
- Grade 4 anemia

Hepatic DLT Criteria

The following hepatic AEs that are not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs:

- Grade ≥ 3 elevation of transaminases (alanine transaminase [ALT] or aspartate transaminase [AST]) that is NOT thought to be due to disease progression or other medical illness
- For subjects with documented hepatic metastases and a baseline AST or ALT > 3x upper limit of normal (ULN) an increase of AST or ALT > 8x ULN or AST or ALT > 5x ULN for ≥ 14 days that is NOT thought to be due to disease progression or other medical illness
- Grade 3 or 4 elevation of bilirubin irrespective of transaminases that is NOT thought to be due to disease progression or other medical illness
- Any single instance of AST or ALT > 3 × ULN AND total bilirubin > 2 × ULN that is NOT thought to be due to disease progression or other medical illness
- For subjects with documented hepatic metastases and a baseline AST or ALT > 3x upper limit of normal (ULN) any single instance of AST or ALT > 5 × ULN AND total bilirubin > 2 × ULN that is NOT thought to be due to disease progression or other medical illness

The guidelines for assessment of Drug-Induced Liver Injury are described in Appendix F.

Cardiac DLT Criteria

The following cardiac AEs that are not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs:

- LVEF below institutional limits and ≥ 10% points below pre-treatment baseline

- Grade 2 symptomatic heart failure

The first DLT will result in withholding of the drug until recovery of the ejection fraction to prior levels. If subjects remain off ZW25 for more than 4 weeks they will be removed from the study. For the management of left ventricular dysfunction, see Section 5.2.3.1.

3.1.3 Safety Monitoring Committee (SMC)

Safety and study conduct will be monitored throughout the study by the SMC, consisting of the study investigators and the sponsor responsible medical expert and biostatistician. The committee is tasked with monitoring the safety of participants in this study through regular or ad hoc meetings.

SMC activities related to evaluation of DLTs, recommending step-down dose levels for ZW25 and palbociclib, and recommending the doses for all the drugs in this combination to be used in Part 2 are described in Section 3.1.2.

The SMC activities will be described in and governed by the SMC Guidelines.

3.1.4 Part 2 Anti-tumor Activity

The primary objective of Part 2 of the study is to evaluate the potential anti-tumor activity of the recommended dose level of the combination of ZW25 with palbociclib plus fulvestrant in subjects with a diagnosis of HER2-positive, HR-positive breast cancer that is locally advanced (unresectable) and/or metastatic.

Enrollment for Part 2 will begin once the recommended doses of ZW25 and the other drugs of the combination therapy have been confirmed in Part 1. The treatment and blood collection schedules, treatment cycle duration and imaging evaluation intervals are the same as in Part 1.

Part 2 of the study will evaluate the preliminary anti-tumor activity. All subjects will be assessed for safety and anti-tumor response. The mITT set includes all subjects who receive any amount of ZW25 with palbociclib and/or fulvestrant who had at least one identifiable (target and/or non-target) lesion at baseline and at least one post-baseline disease assessment or discontinued all study treatment and are no longer being followed for efficacy. The primary efficacy endpoint will be PFS6 (defined as the % of mITT subjects with PFS of ≥24 weeks); secondary efficacy endpoints include ORR, DOR, DCR, PFS, and OS. Approximately 50 subjects are expected to be enrolled in Part 2. Assuming the observed PFS6 rate is between 40% and 70%, the corresponding 95% binomial exact confidence intervals (CI) are summarized in Table 4 below.

Table 4: PFS6 Rate and Corresponding 95% Confidence Intervals

PFS6 Rate	Binomial exact 95% CI (N=50)
40%	(26%, 55%)
50%	(36%, 64%)
60%	(45%, 74%)
70%	(55%, 82%)

Therefore, a total of up to approximately 86 subjects may be enrolled across the entire study.

If possible, an additional optional tumor biopsy may be obtained at the time of disease progression from an accessible site to allow for assessment of changes in HER2 expression as well as the presence of other exploratory biomarkers. Biomarkers of response may be evaluated.

3.1.5 Stopping Criteria

If the SMC recommends that the dose of ZW25 needs to be dropped below 15 mg/kg Q2W; the palbociclib dose needs to be reduced below 75 mg; and/or the fulvestrant dose needs to be reduced, then the study may be terminated.

The Sponsor reserves the right to terminate the study at any time for any reason.

For individual subjects, information on study treatment and study discontinuation is provided in Section 4.4. Also, individual subjects may have to discontinue study treatment in case the dose modifications for ZW25 and palbociclib are not sufficient for managing toxicities (Section 5.2.3 and Section 5.3.3).

3.2 Discussion and Rationale for Study Design

Although ZW25 has been evaluated as a single agent in a Phase 1 study and a single agent RD (20 mg/kg Q2W) has been confirmed, its tolerability when combined with palbociclib and fulvestrant in advanced or metastatic breast cancer has not been evaluated previously.

Part 1 of the study will first evaluate the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant and will confirm the RDs of all the drugs in this combination. Then, Part 2 of the study will evaluate the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant (all drugs given at the RDs identified in Part 1) in subjects with HER2-positive, HR-positive breast cancer.

In summary, this study will evaluate the safety and preliminary anti-tumor activity of ZW25 combined with palbociclib plus fulvestrant in the treatment of subjects with advanced HER2-positive, HR-positive breast cancer that has progressed on or been refractory to prior treatment with trastuzumab, pertuzumab, and T-DM1. Premenopausal women and perimenopausal women will also be treated with an LHRH analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.

3.2.1 Method of Assigning Subjects to Treatment Groups

This is an open-label, non-randomized study. Subjects will be assigned to each cohort sequentially.

3.2.2 Rationale for Selection of Doses

To confirm the RD of ZW25 when combined with palbociclib and fulvestrant in subjects with breast cancer, Part 1 of the study is designed to address the question of whether the single-agent RD of ZW25 is tolerable when combined with palbociclib and fulvestrant. The initial doses to be explored will be a ZW25 monotherapy RD (20 mg/kg Q2W) from the Phase 1 study ZWI-ZW25-101 in combination with palbociclib per recommended dose (125 mg PO QD for the first 21 days of each 28-day cycle) in the package insert (IBRANCE®) and fulvestrant per recommended dose (500 mg IM Q2W for the first 3 doses, then Q4W thereafter) in the package insert (FASLODEX®). A step-down dose level of ZW25 (e.g., 15 mg/kg or other dose not lower

than 15 mg/kg Q2W) and/or palbociclib (e.g., 100 mg PO and/or 75 mg PO QD) may be allowed if recommended by the SMC. No step-down dose levels for fulvestrant are allowed; however, individual subjects with moderate hepatic impairment may receive a reduced dose per prescribing information. On Days 1 and 15 of each cycle, ZW25 will be administered first followed by palbociclib and fulvestrant. Premenopausal women and perimenopausal women will also be treated with a LHRH analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.

3.2.3 Blinding and Randomization

This is an open-label, non-randomized study.

4 STUDY POPULATION

Subjects must meet all of the enrollment criteria to be eligible for this study. All eligibility criteria are applicable to both parts of the study unless otherwise specified. Eligibility criteria may not be waived by the investigator and are subject to review in the event of a Good Clinical Practice (GCP) audit and/or health regulatory authority inspection.

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria.

1. Pathologically-confirmed diagnosis of breast cancer with evidence of locally advanced (unresectable) and/or metastatic disease. All subjects in both Parts 1 and 2 must have HER2-positive and HR-positive disease as follows:
   - HER2-positive based on the HER2 Testing in Breast Cancer: ASCO/CAP Clinical Practice Guidelines (Wolff 2018).
   - HR-positive defined as ER-positive and/or PgR-positive disease based on the ASCO/CAP Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Hammond 2010).
2. Able to provide a new formalin-fixed, paraffin-embedded (FFPE) tumor sample (preferred) or archived tumor tissue (most recent sample available) for retrospective central review of HER2 status.

   Local assessments performed on a new tumor sample or archived tumor tissue in a Clinical Laboratory Improvements Amendments (CLIA)-certified lab using a combination of IHC and ISH/FISH methods may be used to determine HER2 and HR status for study eligibility. IHC must be used to determine HR status. Unless otherwise approved by the sponsor medical monitor, specimens should be provided for centralized retrospective review of HER2 status.
3. Received prior treatment with trastuzumab, pertuzumab, AND ado-trastuzumab emtansine (T-DM1); disease progression during or after the most recent prior therapy. Subjects in any part of the study who did not receive pertuzumab or T-DM1 because of lack of access (e.g., due to insurance coverage or because they were treated prior to regulatory agency approval of the agent in a relevant indication) or due to medical ineligibility for treatment with T-DM1 (e.g., history of severe infusion reactions to trastuzumab, ≥ Grade 2 peripheral neuropathy, or

platelet count < 100 x 10^9/L) may be eligible for the study after discussion with and approval from the sponsor medical monitor. Prior treatment with endocrine therapy in the neoadjuvant, adjuvant, and/or metastatic setting is permitted.

4. Sites of disease assessable per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (both measurable and non-measurable disease allowed)
5. Male and female subjects aged 18 years or older
6. An Eastern Cooperative Oncology Group (ECOG) Performance Status score of 0 or 1
7. Life expectancy of at least 3 months in the opinion of the investigator
8. The following baseline laboratory data:
    a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
    b. Platelet count ≥ 75 x 10^9/L
    c. Hemoglobin ≥ 9 g/dL
    d. Prothrombin time (PT) and/or International Normalized Ratio (INR) and partial thromboplastin time (PTT)/ aPTT (activated partial thromboplastin time) ≤ 1.5 x ULN, unless on medication known to alter the INR or PTT
    e. Total bilirubin ≤ 1.5 x ULN per institutional values (subjects with known Gilbert's Syndrome may enroll with ≤ 2.5 x ULN provided the direct bilirubin is ≤ 1.5 mg/dL)
    f. ALT ≤ 3.0 x ULN per institutional values (if liver metastases are present, ≤ 5.0 x ULN)
    g. AST ≤ 3.0 x ULN per institutional values (if liver metastases are present, ≤ 5.0 x ULN)
    h. Serum creatinine ≤ 1.5 X ULN or calculated glomerular filtration rate ≥ 50 mL/min
9. Adequate cardiac left ventricular function, as defined by LVEF ≥ institutional standard of normal
10. All toxicity related to prior cancer therapies must have resolved to ≤ Grade 1, with the exception of alopecia or ≤ Grade 2 neuropathy
11. If female and of child-bearing potential, must have a negative pregnancy test ≤ 3 days prior to the first dose of ZW25
12. For female subjects who are not surgically sterile or post-menopausal and for male subjects with a partner of child-bearing potential, willingness to use 2 methods of birth control with a failure rate of less than 1% per year during the study and for 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant). These include, but are not limited to, established use of oral, implanted, or injected hormonal contraceptives; placement of intra-uterine device or intra-uterine system; or use of barrier methods, such as condom or diaphragm together with a spermicidal product.
13. Female subjects must agree not to breastfeed or donate ova starting at screening and throughout the study period, and for at least 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant)

14. Male subjects must not donate sperm starting at screening and throughout the study period, and for at least 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant)
15. Signed informed consent prior to any study procedures not considered standard of care

4.2 Exclusion Criteria

Subjects will be excluded from the study if 1 or more of the following criteria are applicable:

1. Prior treatment with trastuzumab, pertuzumab, lapatinib, T-DM1, or other anti-HER2-targeted therapy ≤ 3 weeks before the first dose of ZW25
2. Prior treatment with chemotherapy, other anti-cancer therapy not otherwise specified, or hormonal cancer therapy ≤ 3 weeks before the first dose of ZW25
3. Prior treatment with experimental biologic and non-biologic therapies ≤ 4 weeks before the first dose of ZW25
4. Prior treatment with radiation therapy other than for central nervous system (CNS) disease ≤ 3 weeks before the first dose of ZW25
5. Treatment with anthracyclines within 90 days before first dose of ZW25 and/or total lifetime load exceeding 360 mg/m^2 Adriamycin® or equivalent
6. Use of any medications or substances that are strong inhibitors or inducers of CYP3A isoenzymes within 7 days of first dose of any study drug
7. History of life-threatening hypersensitivity to monoclonal antibodies, recombinant proteins, or excipients in the drug formulation
8. Prior treatment with palbociclib or any other CDK4/6 inhibitors, including experimental agents
9. Use of corticosteroids administered at doses equivalent to > 15 mg per day of prednisone within 2 weeks of first ZW25 dosing unless otherwise approved by the sponsor medical monitor. Topical, ocular, intra-articular, intranasal, and/or inhalational corticosteroids are permitted.
10. History of myocardial infarction or unstable angina within 6 months prior to enrollment, troponin levels consistent with myocardial infarction, or clinically significant cardiac disease, such as ventricular arrhythmia requiring therapy, uncontrolled hypertension, or any history of symptomatic congestive heart failure (CHF)
11. QTc Fridericia (QTcF) >470 ms
12. Grade 2 or greater pneumonitis and/or interstitial lung disease, including pulmonary fibrosis, or other clinically significant infiltrative pulmonary disease not related to lung metastases
13. Active hepatitis B or hepatitis C infection

14. Acute or chronic uncontrolled renal disease, pancreatitis, or severe liver disease (Child-Pugh Class C)
15. Known infection with Human Immunodeficiency Virus (HIV)-1 or HIV-2 (Exception: Subjects with well-controlled HIV [e.g., cluster of differentiation 4 (CD4)-positive T cell count >350/mm^3 and undetectable viral load] are eligible.)
16. Major surgery ≤ 3 weeks prior to the first dose of ZW25
17. Prior or concurrent malignancy whose natural history or treatment has the potential to interfere with the safety or efficacy assessment of the investigational regimen
18. Any other medical, social, or psychosocial factors that, in the opinion of the investigator, could impact safety or compliance with study procedures
19. Females who are breastfeeding or pregnant, and females and males planning a pregnancy
20. Brain metastases: Untreated CNS metastases, symptomatic CNS metastases, or radiation treatment for CNS metastases within 4 weeks of start of study treatment. Stable, treated brain metastases are allowed (defined as subjects who are off steroids and anticonvulsants and are neurologically stable for at least 1 month at the time of screening).
21. Poorly-controlled seizures
22. History of or ongoing leptomeningeal disease (LMD). If LMD has been reported radiographically on baseline MRI, but is not suspected clinically by the investigator, the subject must be free of neurological symptoms of LMD.
23. Grade 3 or greater peripheral neuropathy

4.3 Childbearing Potential

A person of childbearing potential is anyone born female who has experienced menarche and who has not undergone surgical sterilization (e.g., hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or has not completed menopause. Menopause is defined clinically as 12 months of amenorrhea in a person over age 45 in the absence of other biological, physiological, or pharmacological causes.

A person who can father children is anyone born male who has testes and who has not undergone surgical sterilization (e.g., vasectomy followed by a clinical test proving that the procedure was effective).

According to the inclusion and exclusion criteria, pregnant or breast-feeding subjects cannot be included in the study, and adequate contraceptive measures should be taken to prevent female subjects of childbearing potential or female partners of male subjects (either of childbearing potential) from getting pregnant during study participation and for at least 12 months after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant). Subjects will be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the investigator. If a female subject is pregnant, the investigator will immediately discontinue the

subject from the study. Upon discontinuation, only those procedures that would not expose the subject to undue risk will be performed.

The investigator should also be notified of pregnancy occurring during the study or within 12 months following last study treatment administration but confirmed after completion of the study.

In the event that a female subject or female partner of a male subject is found to be pregnant after inclusion in the study, the pregnancy will be followed to term with subject/partner consent, and the status of mother and child will be reported to the sponsor after delivery and the status of the baby will be followed for a minimum of 6 months after a live birth.

Details of the pregnancy will be recorded on the withdrawal page of the case report form (CRF), and a Pregnancy Report Form will be completed.

4.4 Removal of Subjects from Therapy or Assessment

The sponsor or their designee must be notified if a subject is withdrawn from study treatment or from the study. The reason(s) for withdrawal must be documented in the subject's medical records and CRF.

4.4.1 Discontinuation of Study Treatment

If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy (Note: trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy). Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and safety follow-up visit at approximately 30 days after the last dose of study drug.

Study treatment may be discontinued for any of the following reasons:

- Adverse event (AE)
- Death
- Lost to follow-up
- Withdrawal of consent
- Physician decision (non-AE, non-progressive disease)
- Pregnancy
- Progressive disease (either radiographic or clinical progression)
- Protocol violation
- Study termination by sponsor
- Other, non-AE

Also, if a subject has not taken ZW25 for more than 4 weeks, he/she will be withdrawn from study treatment unless approval is obtained from the medical monitor to continue on study treatment.

Also, if a subject starts subsequent non-study anticancer therapy, then study treatment will be discontinued before the start of the new therapy

At a minimum, all efforts should be made for subjects to complete the EOT visit (Section 6.4) and the safety follow-up visit (Section 6.5), and efficacy (Section 6.6) and survival follow-ups (Section 6.7).

4.4.2 Subject Withdrawal from Study

Any subject may be discontinued from the study for any of the following reasons:

- Death
- Lost to follow-up
- Withdrawal of consent
- Study termination by sponsor

5 TREATMENTS

5.1 Treatments Administered

In this study, subjects will be treated with open-label ZW25 in combination with palbociclib plus fulvestrant. Although information for palbociclib and fulvestrant are included in this protocol, in case of conflict between these instructions and the most recent local prescribing information for these approved drugs, Investigators should follow the most recent local prescribing information.

5.2 ZW25

Detailed information describing the preparation, administration, and storage of ZW25 is located in the Pharmacy Manual. Study drug must be diluted as instructed in the Pharmacy Manual prior to administration.

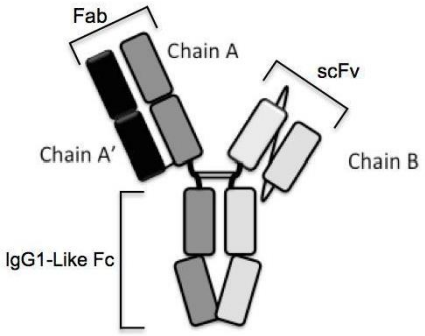
On Days 1 and 15 (i.e., ZW25 dosing days) of each treatment cycle, ZW25 will be administered first followed by palbociclib and fulvestrant.

5.2.1 Description of ZW25

ZW25 is a humanized bispecific antibody recognizing 2 non-overlapping epitopes of the ECD of the human HER2 antigen. A schematic representation of ZW25 is shown in Figure 4. The IgG1-like fragment crystallizable (Fc) region of ZW25 contains complementary mutations in each CH3 domain that impart preferential pairing to generate a heterodimeric molecule and correspondingly disfavor formation of homodimers. Chain A is otherwise a normal IgG1 heavy chain and forms a fragment antigen-binding (Fab) arm through pairing with IgG Kappa light Chain A'. Chain A/A' binds to ECD2 of HER2. Chain B has an IgG1-like hinge, CH2 and CH3 domains but contains a single-chain variable fragment (scFv) rather than a Fab arm. Chain B binds to ECD4 of HER2. In place of the constant heavy chain 1 (CH1) domain, it has a variable light (VL) domain, an unstructured 20 amino acid linker of glycine and serine residues, followed

by a variable heavy (VH) domain to form an scFv domain. ZW25 is being developed as a treatment for locally advanced (unresectable) and/or metastatic HER2-expressing cancers.

Figure 4: Structure of ZW25



Fab = fragment antigen-binding; Fc = fragment crystallizable; IgG1 = immunoglobulin G1; scFv = single-chain variable fragment.

5.2.2 Dose and Administration of ZW25

Dosing for ZW25 will be given as follows:

- Part 1:
  - 20 mg/kg IV ZW25; dosing Q2W on Days 1 and 15 of each 28-day cycle. Up to 1 step-down dose level (15 mg/kg Q2W or other dose not lower than 15 mg/kg Q2W) may be evaluated if recommended by the SMC and approved by the sponsor
- Part 2:
  - Recommended dose of ZW25 as confirmed in Part 1 for administration in combination with palbociclib and fulvestrant

Dosing is based on the subject's actual body weight taken at screening visit. Doses must be adjusted for subjects who experience a ≥ 10% change in weight from baseline/ screening visit on dosing day. Subject weight must be measured on all ZW25 dosing days as part of the physical exam (within a window of 3 days prior to dosing). Detailed instructions for dose preparation are provided in the Pharmacy Manual.

ZW25 should be administered prior to any component of the combination chemotherapy regimen. Please refer to the Pharmacy Manual for infusion times. Subjects who experience a Grade 1 or Grade 2 infusion reaction may resume infusion of drug at a reduced infusion rate (50% slower than initial rate). All subjects should receive prophylactic treatment for infusion reactions prior to ZW25 dosing as outlined in Section 5.2.7.1.

5.2.3 Dose Modifications of ZW25

An individual subject who experiences an event that meets the criteria for DLT (defined in Section 3.1.2) at any time on study treatment may continue at a step-down dose (or an intermediate dose level determined by the SMC) once the toxicity has returned to Grade 1 or baseline with approval of the medical monitor. If the subject then experiences another such event at the step-

down dose level, with approval from the medical monitor, the dose of ZW25 administered to that individual subject should be reduced again but should not go below 10 mg/kg Q2W.

For Part 2 of the study, dose modifications for ZW25-associated toxicity are presented in Table 5 below. Dose decreases or delays for other reasons may be permitted with approval from the medical monitor. The reason for dose decrease or delay must be recorded, and the sponsor must be notified. Up to 2 dose reductions per subject will be allowed for Q2W dosing, but the reduced dose cannot be lower than 10 mg/kg Q2W.

There must be a minimum of 10 days (±2 days) between doses. Day 15 doses not administered within 6 days after Day 15 must be skipped.

Note: Although only 1 step-down dose level (not lower than 15 mg/kg Q2W) of ZW25 is allowed in the study at a cohort level, individual subjects may have up to 2 dose reductions as long as the reduced dose is not lower than 10 mg/kg Q2W.

Table 5: Recommended Management and Potential Dose Modifications for ZW25-Associated Toxicity

Adverse Event Related to ZW25	Action for ZW25
Grade 1 or Grade 2 nausea and vomiting	• Suggest a 5-hydroxytryptamine (5-HT3) receptor antagonist until resolution of symptoms, or a prochlorperazine. • No dose modification of ZW25 is required. • For breakthrough nausea or vomiting, consider olanzapine 5 or 10 mg daily for three days. For subjects already receiving olanzapine, prochlorperazine may be used.
Grade 3 or Grade 4 nausea and vomiting	• Suggest a 5-HT3 receptor antagonist plus a glucocorticoid especially dexamethasone); consider adding a NK-1 receptor antagonist to a 5-HT3 receptor antagonist and glucocorticoids if the latter combination is not sufficient for symptom relief. Treatment until resolution of symptoms to ≤ Grade 1. • Do not administer ZW25 until severity ≤ Grade 1 or pretreatment level. • Optional dose reduction to next lowest dose level after symptoms resolved to ≤ Grade 1 or pretreatment level for Grade 3 nausea and vomiting. For Grade 4 symptoms or recurrent Grade 3 symptoms despite maximum use of 5-HT3 antagonists, glucocorticoids and NK-1 antagonist, dose reduction to the next lowest dose level is mandatory.
Grade 1 or Grade 2 diarrhea	• Oral hydration with fluid that contain water, salt and glucose, such as broth or Gatorade. • Suggest loperamide 4 mg PO followed by 2 mg Q4H until no diarrhea. • No dose modification of ZW25 is needed.

Adverse Event Related to ZW25	Action for ZW25
Grade 3 or Grade 4 diarrhea	• Aggressive fluid hydration and clear liquid diet. • Suggest loperamide 4 mg PO followed by 4 mg Q2H until resolution of diarrhea; consider octreotide 100 or 150 mcg subcutaneously Q8H for subjects with persistent diarrhea despite 48 hours of loperamide. If subjects are refractory to both loperamide and octreotide, gastroenterologist should be consulted. • For Grade 3 diarrhea, optional dose reduction of ZW25 to next lowest dose level after symptoms resolved to ≤ Grade 1 or pretreatment level. For Grade 4 symptoms or recurrent Grade 3 symptoms despite maximum use of loperamide and octreotide, dose reduction is mandatory.
Grade 1 or Grade 2 rash	• Suggest topical steroid as needed. • No dose modification of ZW25 is needed.
Grade 3 rash	• Suggest initiation with topical steroid; if insufficient, consider oral corticosteroid. Wound care for possible erosion and ulceration to prevent infection, and analgesics for pain control if necessary. • Do not administer ZW25 until severity ≤ Grade 1 or pretreatment level. • For Grade 3 rash, optional dose reduction of ZW25 to next lowest dose level after symptoms resolved to ≤ Grade 1 or pretreatment level. For Grade 4 symptom or recurrent grade 3 symptom despite maximum use of topic and oral corticosteroid, dose reduction is mandatory.

5-HT3 = 5-hydroxytryptamine; AE = adverse event; NK-1 = Neurokinin-1; PO = orally (per oral); Q2H = every 2 hours; Q4H = every 4 hours; Q8H = every 8 hours

For subjects who cannot receive or tolerate maximal treatment, including dose reduction of ZW25 or refused supportive treatment, study treatment should be permanently discontinued for recurrence of ≥ Grade 3 nausea, vomiting, diarrhea or rash.

5.2.3.1 Left Ventricular Dysfunction

Management of left ventricular dysfunction, regardless of relationship to study drug, is described in Table 6.

Table 6: Management of Left Ventricular Dysfunction

Left Ventricular Dysfunction (Regardless of Causality)	Action for ZW25
Symptomatic cardiac heart failure	• Discontinue ZW25
LVEF <40%	• Do not administer ZW25 • Repeat LVEF assessment within 4 weeks • If LVEF <40% is confirmed, discontinue ZW25
LVEF below institutional limits of normal and absolute decrease of ≥ 10% points below pre-treatment baseline	• Do not administer ZW25 • Repeat LVEF assessment within 4 weeks • If the LVEF has not recovered to within 10% points from baseline, discontinue ZW25
LVEF 40% to ≤ 45% and absolute decrease is < 10% points from baseline	• Continue treatment with ZW25 • Repeat LVEF assessment within 4 weeks
LVEF > 45%	• Continue treatment with ZW25, as applicable

LVEF = left ventricular ejection fraction

5.2.3.2 Infusion-Related Reactions

The symptoms of infusion-related reactions include fever, chills/rigor, nausea, pruritus, angioedema, hypotension, headache, bronchospasm, urticaria, rash, vomiting, myalgia, dizziness, or hypertension. Severe reactions may include acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. Subjects should be closely monitored for such reactions. Immediate access to an Intensive Care Unit or equivalent environment and appropriate medical therapy (including epinephrine, corticosteroids, intravenous antihistamines, bronchodilators, and oxygen) must be available to treat infusion-related reactions. Management of ZW25 related infusion reactions are based on the grade of symptoms as outlined in Table 7.

Table 7: ZW25 Treatment Modification for Symptoms of Infusion-Related Reactions

NCI CTCAE grade	Treatment modification for ZW25
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease infusion rate by 50%. Closely monitor for worsening symptoms. Medical management as needed. Subsequent infusions should be given after premedication and at the reduced infusion rate.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (eg, antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, intravenous fluids); prophylactic medications indicated for ≤ 24 hours.	Stop infusion. Once symptoms have resolved or decreased to Grade 1 in severity the infusion may be resumed at 50% of the previous rate. Closely monitor for worsening symptoms. Proper medical management should be instituted as described in the text accompanying this table. Subsequent infusions should be given after premedication and at the reduced infusion rate.
Grade 3 – severe Prolonged (eg, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae.	Immediately stop the infusion. Proper medical management should be instituted as described in the text accompanying this table. Infusion should not be restarted even if signs and symptoms completely resolve. With medical monitor approval the subject may be dosed at the next scheduled treatment.
Grade 4 – life-threatening Life-threatening consequences; urgent intervention indicated.	Immediately stop the infusion. Proper medical management should be instituted as described in the text following this table. The subject should be withdrawn from study drug treatment. Hospitalization is recommended.

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events

Once the ZW25 infusion rate has been decreased by 50% or suspended due to an infusion-related reaction, it must remain decreased for all subsequent infusions and premedication must be administered.

NCI CTCAE Grade 1 or 2 infusion reaction: Proper medical management should be instituted, as indicated per the type of reaction. This includes but is not limited to an

antihistamine (eg, diphenhydramine or equivalent), antipyretic (eg, acetaminophen or equivalent), and, if considered indicated, oral or intravenous glucocorticoids, bronchodilators, and oxygen.

NCI CTCAE Grade 3 or 4 infusion reaction: Proper medical management should be instituted immediately, as indicated per type and severity of the reaction. This includes but is not limited to oral or intravenous antihistamine, antipyretic, glucocorticoids, epinephrine, bronchodilators, and oxygen.

If feasible, blood samples for evaluation of PK and/or ADA may be drawn in the event of an infusion reaction.

5.2.3.3 Liver Toxicity

In the event of increased liver function tests (LFTs) without explanation such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the subject may be discontinued from the study treatment at the investigator's discretion. Discontinuation of treatment should be considered if:

- ALT or AST >8 × ULN
- ALT or AST >5 × ULN for more than 2 weeks
- ALT or AST >3 × ULN and total bilirubin >2 × ULN or international normalized ratio (INR) >1.5 (if INR testing is applicable/evaluated)
- ALT or AST >3 × ULN with the appearance of symptoms suggestive of liver injury, e.g., right upper quadrant pain or tenderness and/or eosinophilia (>5%)

These treatment discontinuation recommendations are based on the FDA Guidance for Industry (FDA Guidance for Industry 2009). The recommendations are a basic guide to the investigator based on accumulated clinical experience with drugs in development and are not specific to clinical experience with ZW25. See Appendix F for recommended liver safety monitoring and assessment criteria in patients with elevations in ALT, AST, or bilirubin.

5.2.4 Storage and Handling

Vials and solutions containing ZW25 should be stored at -20°C (±5 °C) and protected from light until ready for use. Drug must be stored in a controlled location, where access is limited to only designated site staff. For details regarding drug preparation and administration reference the Pharmacy Manual. Drug accountability procedures are also provided in the Pharmacy Manual.

5.2.5 Packaging and Labeling

Refer to the Pharmacy Manual for packaging and labeling.

5.2.6 Preparation

Detailed drug preparation instructions are provided in the Pharmacy Manual.

5.2.7 Concomitant Therapy

All medications being taken at the time of study entry should be continued as necessary and at a stable dose level and frequency, if possible unless prohibited for study entry.

5.2.7.1 Required Concomitant Therapy

All subjects must receive the following mandatory prophylactic treatment for infusion reactions at 30 to 60 minutes before the start of each ZW25 infusion:

- Acetaminophen orally (650 mg) and diphenhydramine orally (50 mg) or equivalents
- Corticosteroids (either hydrocortisone 100 mg IV or dexamethasone 10 mg IV)

If an alternative premedication regimen is thought to be required, the Investigator must seek sponsor approval. Sponsor approval is required before implementation.

For subjects who experience an infusion reaction despite the above premedication, other medication as needed per the Investigator or per institutional standards including H2 blockers may be given in addition to the mandatory premedications.

Premenopausal women and perimenopausal women will also be treated with an LHRH analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.
Note: Concomitant norethindrone acetate is contraindicated for breast cancer subjects.

5.2.7.2 Allowed Concomitant Therapy

Supportive therapy, including transfusions and bisphosphonates (e.g., Zometa®), is allowed on study. Supportive care treatments including supportive growth factors and colony-stimulating factors are also allowed. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy. Corticosteroid treatment is permitted only as follows:

- Topical, ocular, intra-articular, intranasal, and/or inhalational corticosteroids
- Physiologic replacement doses of systemic corticosteroids (i.e., < 15 mg/day)
- As part of pre-medication for ZW25 and/or chemotherapy components of study treatment
- For prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed hypersensitivity reaction caused by a contact allergen)
- For acute medical conditions (higher doses permitted, but not to exceed 4 weeks with medical monitor approval)

All other therapies not specifically listed in the exclusion criteria or in prohibited therapy are allowed.

5.2.7.3 Prohibited Concomitant Therapy

Subjects may not receive cancer related surgery, radiotherapy (except as noted in Section 5.2.7.2 above), other investigational, or systemic anti-neoplastic therapy during the study. Use of alternative supplemental therapies is discouraged and use of any such product must be recorded.

Ketoconazole and other strong CYP3A4 inducers and inhibitors (see also Section 5.3.6) are prohibited during the study.

5.3 Palbociclib Plus Fulvestrant

5.3.1 Descriptions of Palbociclib and Fulvestrant

Palbociclib is an inhibitor of CDK4 and CDK6. Fulvestrant is an ER antagonist.

The combination of palbociclib plus fulvestrant is approved for the treatment of HR-positive, HER2-negative advanced or metastatic breast cancer in women with disease progression following endocrine therapy.

5.3.2 Dose and Administration of Palbociclib and Fulvestrant

The recommended dose of palbociclib is a 125 mg capsule taken orally with food once daily (QD) for 21 consecutive days (Days 1 through 21) followed by 7 days off treatment (Days 22 through 28) in a 28-day cycle.

The recommended dose of fulvestrant is a 500 mg IM injection into the buttocks (two 5-mL injections, one per buttock, with each injection being administered over 1 to 2 minutes) Q2W for 3 doses and Q4W thereafter. In a 28-day cycle, this means fulvestrant injections on Cycle 1 Day 1, Cycle 1 Day 15, Cycle 2 Day 1, and then Day 1 of all subsequent cycles.

5.3.3 Dose Modifications for Palbociclib and Fulvestrant

For palbociclib, the recommended dose and reduced dose levels for individual subjects (not to be confused with step-down dose levels for subject cohorts) are shown in Table 8.

Table 8: Dose Reduction Levels for Toxicities Associated with Palbociclib

Dose Level	Dose (mg/day on Days 1-21 of a 28-day cycle)
Recommended starting dose	125
First dose reduction	100
Second dose reduction	75

If dose reduction below 75 mg/day is required, then discontinue palbociclib.

Subjects should be monitored for pulmonary symptoms indicative of ILD/pneumonitis. Symptoms may include hypoxia, cough, dyspnea, or interstitial infiltrates on radiologic exams and in whom infectious, neoplastic, and other causes have been excluded by means of appropriate investigations. If new or worsening respiratory symptoms develop or there are other reasons to suspect ILD or pneumonitis, palbociclib dosing should be held while the subject undergoes urgent evaluation. In all subjects diagnosed with ≥ Grade 2 pneumonitis and/or interstitial lung disease, including pulmonary fibrosis, palbociclib should be permanently discontinued and the subject taken off of study treatment.

For specific instructions on how to manage other non-hematologic and hematologic toxicities associated with palbociclib, including dose reductions and dose delays, refer to the local prescribing information. On resumption of dosing after a dose delay, every attempt should be made to synchronize palbociclib and fulvestrant with Day 1 of the subsequent cycle.

The fulvestrant dose should be halved (250 mg injections) for subjects with moderate hepatic impairment (Child-Pugh class B). Fulvestrant has not been studied in subjects with severe hepatic impairment (Child-Pugh class C).

For fulvestrant, there are no reduced dose levels for toxicity. If fulvestrant is not tolerated at the recommended dose (or the modified dose for subjects with moderate hepatic impairment), it should be discontinued.

5.3.4 Warnings, Precautions, and Contraindications for Palbociclib and Fulvestrant

5.3.4.1 Palbociclib

Neutropenia: Monitor complete blood count (CBC) prior to start of palbociclib therapy and at the beginning of each cycle, as well as on Day 15 of the first 2 cycles, and as clinically indicated. For subjects who experience Grade 3 neutropenia, consider repeating CBC monitoring one week later.

Embryo-fetal toxicity: Palbociclib can cause fetal harm. Advise subjects of potential risk to a fetus and to use effective contraception.

5.3.4.2 Fulvestrant

Risk of bleeding: Use with caution in subjects with bleeding diatheses, thrombocytopenia, or anticoagulant use.

Increased exposure in subjects with hepatic impairment: Use a 250 mg dose for subjects with moderate hepatic impairment.

Injection site reaction: Use caution when administering fulvestrant at the dorsogluteal injection site due to the proximity of the underlying sciatic nerve.

Embryo-fetal toxicity: Fulvestrant can cause fetal harm. Advise female subjects of childbearing potential of the potential risk to a fetus and to use effective contraception.

Immunoassay measurement of serum estradiol: Fulvestrant can interfere with estradiol measurement by immunoassay, resulting in falsely elevated estradiol levels.

Fulvestrant is contraindicated in subjects with a known hypersensitivity to the drug or any of its components. Hypersensitivity reactions, including urticaria and angioedema, have been reported.

5.3.5 Storage, Handling, Packaging, Labeling, and Preparation for Palbociclib and Fulvestrant

For information on the storage, handling, packaging, labeling, and preparation of palbociclib and fulvestrant, refer to the local prescribing information.

5.3.6 Concomitant Therapy and Palbociclib and Fulvestrant

Premedications and supportive medications for palbociclib and fulvestrant should be administered per institutional standards as long as they are consistent with local prescribing information and not contraindicated by this study protocol.

Premenopausal women and perimenopausal women will also be treated with an LHRH analogue (also known as gonadotropin-releasing hormone analogue) per institutional guidelines.
Note: Concomitant norethindrone acetate is contraindicated for breast cancer subjects.

While taking palbociclib, avoid concomitant use of strong CYP3A inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, and voriconazole) and strong CYP3A4 inducers (e.g., phenytoin, rifampin, carbamazepine, enzalutamide, and St John's Wort). Also, avoid grapefruit or grapefruit juice during palbociclib treatment. If coadministration of palbociclib with a strong CYP3A inhibitor cannot be avoided, reduce the dose of palbociclib to 75 mg.

5.3.7 Drug Interactions of Palbociclib and Fulvestrant

Palbociclib is primarily metabolized by CYP3A and sulfotransferase (SULT) enzyme SULT2A1. In vivo, palbociclib is a time-dependent inhibitor of CYP3A. The dose of sensitive CYP3A4 substrates with narrow therapeutic indices may need to be reduced when given concurrently with palbociclib.

There are no known drug-drug interactions for fulvestrant. Although fulvestrant is metabolized by CYP3A4 in vitro, in drug interaction studies co-administration with ketoconazole (strong inhibitor) and rifampin (strong inducer) did not alter the PK of fulvestrant.

5.4 Duration of Study Treatment

In both parts of the study, subjects may continue on study treatment until radiographically-confirmed disease progression as defined by RECIST version 1.1, clinical disease progression, unacceptable toxicity, consent withdrawal, physician decision, pregnancy, protocol violation, start of a subsequent anticancer therapy, or study termination by the sponsor.

Note: Trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy.

If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy. Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and safety follow-up visit at approximately 30 days after the last dose of study drug.

5.5 Management of Adverse Reactions

Management of infusion reactions and other toxicities related to or associated with ZW25 is described in Section 5.2.3.2. Required premedications as prophylaxis for ZW25 infusion reactions are described in Section 5.2.7.1.

Management of toxicities related to palbociclib and/or fulvestrant is described in Section 5.3.3.

Initial management of adverse reactions should align with treatment measures recommended for the study drug that is most likely responsible for the observed toxicity. For hematological

toxicities (neutropenia, anemia, and thrombocytopenia), infections, and stomatitis palbociclib dose modifications as recommended in local prescribing information should be implemented. Initial management of diarrhea should utilize treatment measures listed for ZW25 in Table 5 (Section 5.2.3). For toxicities that are not common to either palbociclib or ZW25 treatment measures recommended for ZW25, the experimental agent, should be applied first. All study treatments should be permanently discontinued in any subject diagnosed with ≥ Grade 2 pneumonitis and/or interstitial lung disease, including pulmonary fibrosis.

5.6 Treatment Compliance

ZW25 administration will be performed by study site staff and documented in source documents and the CRF.

Compliance with the palbociclib regimen will be assessed by counting dispensed and returned capsules. Results will be recorded in source documents and the CRF.

Administration of fulvestrant will be performed by study site staff and documented in source documents and the CRF.

6 STUDY ACTIVITIES

6.1 Schedule of Events

Any study protocol-related AE (see Section 7.7.1.1) as well as any concomitant medications given for treatment of the AE, should be recorded from the time of informed consent. Otherwise, AEs and concomitant medications will be recorded from Day 1 (predose) start of dosing of any study drug on Cycle 1 Day 1 through the safety reporting period (see Section 7.7.1.3). A schedule of events is provided in Appendix A. Where applicable, the assessments shall follow the time windows described in Appendix A. Extensive and sparse PK sampling schedules are provided in Appendix B and Appendix C, respectively. Study activities are listed by visit in this section and descriptions of all study assessments are presented in Section 7.

6.2 Screening Visit (Days [-28] to 1)

Screening procedures (listed below) will be completed within 28 days before first administration of study treatment. In case a subject cannot receive their first treatment within the required time window for the screening assessments, rescreening should be performed. Procedures done as part of standard of care within the 28-day window and meeting study requirements may be used for study purposes.

- Informed consent
- Full medical history
- Eligibility (per the inclusion and exclusion criteria)
- Demographic data
- New tumor biopsy (archival tissue is allowed if new is unobtainable). Either an FFPE tissue block or freshly cut slides are required (see the Laboratory Manual for details).
- Disease assessment per RECIST 1.1 (computed tomography [CT]/ magnetic resonance imaging [MRI] scans, and breast cancer serum tumor markers [if being monitored]).

- Bone scan (scintigraphy)
- Brain scan (MRI)
- Physical examination, including height and body weight
- ECOG PS
- Vital signs
- Hematology, serum chemistry (plasma chemistry allowed if serum chemistry not available), and coagulation
- Urinalysis
- Pregnancy tests (only for women of childbearing potential)
- Single 12-lead ECG
- Echocardiogram/MUGA
- Hepatitis B surface antigen, hepatitis C antibody, and human immunodeficiency HIV tests (if indicated) (Note: subjects with well-controlled HIV [e.g., CD4 >350/mm3 and undetectable viral load] are eligible.)

Eligibility will be determined based on local (institutional) review of HER2 status, HR status, and disease pathology, with subsequent retrospective central review of HER2 status. Therefore, biopsy slides must be made available to the sponsor for central laboratory confirmation of disease diagnosis.

6.3 Treatment Period

Note: Throughout the treatment period, LHRH analogue will be administered to/taken by premenopausal women and perimenopausal women per institutional guidelines.

AEs and concomitant medications will be monitored and recorded throughout the treatment period.

6.3.1 Cycle 1

6.3.1.1 Cycle 1 Day 1

Prior to dosing:

- Confirm subject eligibility per inclusion/exclusion criteria
- Physical examination, including weight
- ECOG PS
- Vital signs
- Hematology, serum chemistry (plasma chemistry allowed if serum chemistry not available), and coagulation
- Pregnancy test (only for women of childbearing potential)
- Single 12-lead ECG
- ADA assessment sample
- Circulating tumor DNA (ctDNA) assessment sample
- HER2 ECD assessment sample

- PK assessment sample

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)
- Fulvestrant administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)
- Single 12-lead ECG (at 1 hour [±15 minutes] after the end of ZW25 infusion)
- PK assessment sample(s)
- Dispense palbociclib and drug diary card along with instructions for taking it on an out-patient basis and recording date/time of doses on Days 2 through 21, except for Day 15 when the subject should arrive at the clinic before taking palbociclib

Note: Physical examination, ECOG performance status, and samples for hematology, serum chemistry, coagulation, and pregnancy (if applicable) tests may be done within 3 days prior to administration of study treatment and do not need to be repeated if done within the prior 3 days.

6.3.1.2 Cycle 1 Day 2

Subjects will take palbociclib QD (with food) on an out-patient basis.

Subjects on the extensive PK sampling schedule will report to the clinic to have a blood sample drawn.

Subjects on the sparse PK sampling schedule will not have a clinic visit.

6.3.1.3 Cycle 1 Days 3 and 4

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.1.4 Cycle 1 Day 5

Subjects will take palbociclib QD (with food) on an out-patient basis.

Subjects on the extensive PK sampling schedule will report to the clinic to have a blood sample drawn.

Subjects on the sparse PK sampling schedule will not have a clinic visit.

6.3.1.5 Cycle 1 Days 6 through 14

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.1.6 Cycle 1 Day 15

Subjects will arrive at the clinic prior to taking palbociclib and bring their palbociclib supply.

Prior to dosing:

- Physical examination, including weight
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- ADA assessment sample
- HER2 ECD assessment sample
- PK assessment sample
- Count palbociclib capsules, review palbociclib diary card, and record compliance for Days 1-14

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)
- Fulvestrant administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)
- PK assessment sample

6.3.1.7 Cycle 1 Day 16 through 21

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.1.8 Cycle 1 Days 22 through 28

No clinic visits and no treatment.

6.3.2 Cycle 2

6.3.2.1 Cycle 2 Day 1

Prior to dosing:

- Physical examination, including weight
- ECOG PS
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- Pregnancy test (only for women of childbearing potential)
- ADA assessment sample
- PK assessment sample

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)

- Fulvestrant administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)
- PK assessment sample
- Palbociclib diary card review
- Dispense palbociclib and drug diary card along with instructions for taking it on an out-patient basis and recording date/time of doses on Days 2 through 21, except for Day 15 when the subject should arrive at the clinic before taking palbociclib

Note: Physical examination, ECOG performance status, and samples for hematology, serum chemistry, and pregnancy (if applicable) tests may be done within 3 days prior to administration of study treatment and do not need to be repeated if done within the prior 3 days.

6.3.2.2 Cycle 2 Days 2 through 14

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.2.3 Cycle 2 Day 15

Subjects will arrive at the clinic prior to taking palbociclib and bring their palbociclib supply.

Prior to dosing:

- Physical examination, including weight
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- ADA assessment sample
- HER2 ECD assessment sample
- PK assessment sample
- Count palbociclib capsules, review palbociclib diary card, and record compliance for Days 1-14

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)
- PK assessment sample

6.3.2.4 Cycle 2 Days 16 through 21

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.2.5 Cycle 2 Days 22 through 27

No clinic visits and no treatment.

6.3.2.6 Cycle 2 Day 28

- Disease assessment per RECIST 1.1 (CT/ MRI scans, and breast cancer serum tumor markers [if being monitored])
- Bone scan (scintigraphy)
- Brain scan (MRI)
- Circulating tumor DNA (ctDNA) assessment sample

6.3.3 Subsequent Cycles

6.3.3.1 Day 1

Prior to dosing:

- Physical examination, including weight
- ECOG PS
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- Pregnancy test (only for women of childbearing potential)
- Single 12-lead ECG (Cycle 3 and every 3 cycles thereafter plus Cycle 4)
- ADA assessment sample (Cycle 4 and every even-numbered cycle thereafter)[2]
- PK assessment sample (Cycle 4 and every even-numbered cycle thereafter)[2]
- Echocardiogram/MUGA (Cycle 3 and every 3 cycles thereafter [i.e., Cycles 3, 6, 9, 12, etc.] such that the assessment is done Q12W after the first post-baseline assessment at Cycle 3 Day 1)

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)
- Fulvestrant administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)
- Single 12-lead ECG (at 1 hour [±15 minutes] after the end of ZW25 infusion) (Cycle 4 only)
- PK assessment sample (Cycle 4 and every even-numbered cycle thereafter)[2]
- Palbociclib diary card review

[2] Per Memorandum MTF- 011 (dated 28 February 2023), PK and ADA sample collection should be stopped after completing Cycle 4, except for at the EOT visit and 2 additional collection time points for ADA at the Safety and Efficacy Follow-up visits.

- Dispense palbociclib and drug diary card along with instructions for taking it on an out-patient basis and recording date/time of doses on Days 2 through 21, except for Day 15 when the subject should arrive at the clinic before taking palbociclib

Note: Physical examination, ECOG performance status, and samples for hematology, serum chemistry, and pregnancy (if applicable) tests may be done within 3 days prior to administration of study treatment and do not need to be repeated if done within the prior 3 days.

6.3.3.2 Days 2 through 14

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.3.3 Day 15

Subjects will arrive at the clinic prior to taking palbociclib and bring their palbociclib supply.

Prior to dosing:

- Physical examination, including weight
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- Count palbociclib capsules, review palbociclib diary card, and record compliance for Days 1-14

Administer study treatment:

- ZW25 administration (Section 5.2.2)
- Palbociclib administration (Section 5.3.2)

Postdose activities:

- Vital signs (within 30 minutes of the end of the infusion)

6.3.3.4 Days 16 through 21

No clinic visits.

Subjects will take palbociclib QD (with food) on an out-patient basis.

6.3.3.5 Days 22 through 28

No clinic visits and no treatment.

6.3.4 Every 8 Weeks

- Disease assessment per RECIST 1.1 (CT/ MRI scans, and breast cancer serum tumor markers [if being monitored]; timed from Cycle 1 Day 1). Initial responses should be confirmed, if feasible, with a repeat scan 4 weeks (+1-week window) following initial documentation of objective response. The schedule of response assessments should not be adjusted after the confirmatory scan and should maintain the schedule of every 8 weeks timed from Cycle 1 Day 1.

- Bone scan (scintigraphy): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X ray, CT scan with bone windows, or MRI will be performed to confirm findings.
- Brain scan (MRI): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.

6.4 End of Treatment (EOT) Visit

The EOT visit will be performed for subjects who are withdrawn from treatment for any reason and will take place as soon as possible after a subject permanently stops all study treatment (within 14 days after the last dose of study drug [ZW25, palbociclib, and/or fulvestrant]) unless delayed due to an AE. However, EOT evaluations must be performed before initiation of subsequent therapy. The date the subject met criteria for treatment discontinuation and the reason for treatment discontinuation will be recorded.

- Optional tumor biopsy (and ProSigna assessment on tumor biopsy) at disease progression if the subject provides additional consent
- Disease assessment per RECIST 1.1 (CT/ MRI scans, and breast cancer serum tumor markers [if being monitored], not required if ≤ 4 weeks since previous scan)
- Bone scan (scintigraphy) (not required if ≤ 4 weeks since previous scan): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X-ray, CT scan with bone windows, or MRI will be performed to confirm findings.
- Brain scan (MRI) (not required if ≤ 4 weeks since previous scan): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.
- Physical examination, including weight
- ECOG PS
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- Pregnancy test (if applicable)
- Single 12-lead ECG
- Echocardiogram/MUGA (not required if ≤ 3 months since last scan)
- ADA sample
- ctDNA sample (only at time of disease progression, if applicable)
- PK assessment samples (only if the subject has completed < 6 months of treatment)

6.5 Safety Follow-up (Approximately 30 Days After the Last Dose of Study Drug [ZW25, Palbociclib, and/or Fulvestrant])

Approximately 30 days after the last dose of study drug, all subjects will have a follow-up visit, which will include the following assessments.

- Disease assessment per RECIST 1.1 (CT/MRI scans, and breast cancer serum tumor markers [if being monitored], not required if done at EOT visit or ≤ 4 weeks since previous scan)
- Bone scan (scintigraphy) (not required if done at EOT visit or ≤ 4 weeks since previous scan): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X ray, CT scan with bone windows, or MRI will be performed to confirm findings.
- Brain scan (MRI) (not required if done at EOT visit or ≤ 4 weeks since previous scan): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.
- Physical examination, including weight
- ECOG PS
- Vital signs
- Hematology and serum chemistry (plasma chemistry allowed if serum chemistry not available) panels
- Pregnancy test (if applicable)
- Single 12-lead ECG
- ADA sample (not required if ≤ 2 weeks since last sample)
- ctDNA samples (only at time of disease progression, if applicable, and not previously collected at EOT)

Additionally, AESIs (defined in Section 7.7.2) should continue to be followed until resolution or return to baseline.

6.6 Efficacy Follow-up (Every 8 weeks)

Subjects who have discontinued all study treatment on either part of the study for reasons other than progression or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks after the previous scan until disease progression or start of subsequent anticancer therapy. The efficacy follow-up will include the following assessments:

- Disease assessment per RECIST 1.1 (CT/MRI scans, and breast cancer serum tumor markers [if being monitored]
- Bone scan (scintigraphy): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X ray, CT scan with bone windows, or MRI will be performed to confirm findings.
- Brain scan (MRI): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.
- ADA sample (only if a subject had an ongoing anti-ZW25 ADA present at the safety follow-up visit): These will be discontinued after the resolution of ADA.

6.7 Survival Follow-Up (Every 3 Months)

Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued, and subjects will enter long-term follow-up for survival status. Data on survival will be collected via clinic visits (if feasible) or via telephone calls every 3 months after the last follow-up visit. . Subjects may be discontinued from the study due to death, lost to follow-up, consent withdrawal or study termination by sponsor. Reason for study discontinuation will be recorded.

7 STUDY ASSESSMENTS

A schedule of events is provided in Appendix A. Extensive and sparse PK sampling schedules are provided in Appendix B and Appendix C, respectively.

7.1 Screening/Baseline Assessments

Only subjects who meet all inclusion and exclusion criteria specified in Section 4 will be enrolled in this study.

Subject medical history includes a thorough review of significant past medical history, current conditions, any treatment for prior malignancies and response to prior treatment, and any current medications.

For both parts of the study, subjects must have a diagnosis of locally advanced (unresectable) and/or metastatic HER2-positive, HR-positive (ER-positive and/or PgR-positive) breast cancer. Eligibility will be determined based on local (institutional) review of HER2 status, HR status, and disease pathology, with subsequent retrospective central review of HER2 status. HER2 expression will be determined based on the HER2 Testing in Breast Cancer: ASCO/ CAP Clinical Practice Guidelines (Wolff 2018). Hormone receptor status will be determined based on the ASCO/CAP Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Hammond 2010). If the local assessments at a particular site cannot be done with these tests and to these standards, then central assessment will be required before a subject can be enrolled. A new formalin-fixed, paraffin-embedded (FFPE) tumor sample (preferred) or archived tumor tissue (most recent sample available) must be available for the retrospective central laboratory review of HER2 status.

Screening for hepatitis B surface antigen, hepatitis C antibody, and HIV will be performed at screening if indicated in the opinion of the treating investigator. For female subjects of childbearing potential, screening for pregnancy will be performed at screening. These tests will be done using the blood samples taken for clinical chemistry. A urine pregnancy test is also acceptable.

7.2 Response/Efficacy Assessments

Tumor response will be evaluated based on CT and/or MRI scans (using the same methodology [decided by the investigator at baseline] for each scan of the same subject throughout the study) of the chest, abdomen, and pelvis plus additional areas of known or suspected tumor involvement (e.g., brain [MRI] and/or bone scintigraphy with targeted assessment by X-ray, CT scan with

bone windows, or MRI to confirm findings as needed). Data on breast cancer serum tumor markers, if monitored, should also be collected at the time of disease assessment.

- Bone scan (scintigraphy): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X ray, CT scan with bone windows, or MRI will be performed to confirm findings.
- Brain scan (MRI): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.

The radiological assessment(s) will be performed at the visits according to the description provided in the assessment schedule (Appendix A).

Objective responses and tumor progression will be evaluated by the investigator using revised RECIST version 1.1 (Appendix D) (Eisenhauer 2009). Initial responses should be confirmed, if feasible, with a repeat scan 4 weeks (+1-week window) following initial documentation of objective response.

Scans from subjects will be collected and may undergo centralized review at the discretion of the sponsor. The investigator assessment will be used for all treatment-related decisions.

Subjects' clinical data must be available for CRF source verification. Copies of tumor images must be made available for review by the sponsor (or its designee), upon request.

7.3 Pharmacokinetic Assessments

Venous blood samples for measurement of serum concentrations of ZW25 will be drawn at selected time points specified in the PK assessment schedules. The actual date and time (24-h clock time) of each sampling will be recorded in the subject's source document at the site. The sampling window for each time point is presented in Appendix B (extensive sampling) and Appendix C (sparse sampling). Deviations from planned sampling windows will be assessed before database lock for the impact on PK and may be excluded from time point summaries of PK concentrations. Actual time will be used for derivation of PK parameters.

Complete instructions for sample collection, processing, handling, and shipment will be provided in the Laboratory Manual.

If a subject experiences an AE that results in an unscheduled visit or meets SAE criteria, a blood sample for the measurement of serum concentrations of ZW25 should be collected if less than 24 hours have elapsed since the last dose of ZW25, if possible. The sample will be recorded as unscheduled time point and may be also used in PK parameters derivation using actual time. An additional sample may also be drawn in the event of an infusion reaction.

All Part 1 subjects and at least six Part 2 subjects will be assigned to the extensive PK schedule. Further subjects will be then be assigned to the sparse PK schedule.

7.4 Biomarker Studies

The new formalin-fixed, paraffin-embedded (FFPE) tumor sample (preferred) or archived tumor tissue (most recent sample available) used for screening will also be used for a ProSigna assessment at screening.

If possible and additional consent is provided, an optional tumor biopsy may be obtained at the time of disease progression from an accessible site to assess changes in HER2 expression as well as the presence of other exploratory biomarkers. A ProSigna assessment will be done for this sample as well.

Blood samples will be taken at selected time points specified in the assessment schedule (Appendix A) for assessment of biomarkers including, but not limited to, ctDNA and HER2 ECD.

7.5 Immunogenicity Assessments

Blood samples to test for antibodies to ZW25 will be obtained at selected time points specified in the assessment schedule (Appendix A). Blood samples for ADAs will be taken before the start of the ZW25 infusion. All ADA blood sample collection time points during the first 2 treatment cycles coincide with a pre-dose PK sample time point. If a subject terminates early from the clinical trial, all efforts will be made to collect blood samples to test for antibodies to ZW25 unless consent has been withdrawn. Additional samples may be drawn in the event of an infusion reaction.

The immunogenicity testing will be performed in 3 steps as follows: screening assay (Tier 1), confirmation assay (Tier 2), and titration (Tier 3). Only samples positive in the screening assay will be tested in confirmation and further titrated to determine the titer of ADA.

For any samples that are confirmed positive for anti-ZW25 antibody, there may be additional testing done to characterize domain specificity and possibly the anti-ZW25 antibody potential for neutralizing activity on ZW25.

Additional sample handling, processing, storage, labeling, and shipping instructions will be provided to the site in the Laboratory Manual.

7.6 Biospecimen Repository

No genomic testing of germ-line DNA will performed in this study.

7.7 Safety Assessments

The assessment of safety during the course of this study will consist of the surveillance and recording of AEs including AESIs, SAEs, recording of concomitant medication, and measurements of protocol-specified physical examination findings, laboratory tests, and other assessments (ECOG PS, vital signs, ECGs, and echocardiogram/MUGA).

Safety and study conduct will be monitored throughout the study by the SMC, consisting of the study investigators and the sponsor responsible medical expert and biostatistician. The committee is tasked with monitoring the safety of participants in this study through regular or ad hoc meetings. The roles, responsibilities and functioning of the SMC are described in more detail in the SMC charter.

7.7.1 Adverse Events

7.7.1.1 Definitions

Adverse Event

According to the ICH E2A[3] guideline and Code of Federal Regulations (CFR) 312.32[4], an AE is any untoward medical occurrence in a subject or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

The following information should be considered when determining whether or not to record a test result, medical condition, or other incident on the Adverse Events or the Medical History and Pre-existing Conditions CRFs:

- From the time of informed consent to immediately before the first dose of any study drug on Cycle 1 Day 1, only study protocol-related AEs should be recorded. A protocol-related AE is defined as an untoward medical event occurring as a result of a protocol mandated procedure.
- All medical conditions present or ongoing predose on Cycle 1 Day 1 should be recorded on the Medical History and Pre-existing Conditions CRF page.
- All AEs (regardless of relationship to study drug) should be recorded from Cycle 1 Day 1 (during and post-dose) through the end of the safety reporting period (see Section 7.7.1.3). Complications that occur in association with any procedure (e.g., biopsy) should be recorded as AEs whether or not the procedure was protocol mandated.
- Changes in medical conditions and AEs, including changes in severity, frequency, or character, during the safety reporting period should be recorded.
- In general, an abnormal laboratory value should not be recorded as an AE unless it is associated with clinical signs or symptoms, requires an intervention, results in a SAE, or results in study termination or interruption/discontinuation of study treatment. When recording an AE resulting from a laboratory abnormality, the resulting medical condition rather than the abnormality itself should be recorded (e.g., record “anemia” rather than “low hemoglobin”).

Serious Adverse Events

An AE should be classified as an SAE if it meets one of the following criteria:

Fatal: AE resulted in death

[3] ICH E2A Guideline: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. March 1995. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/e2a-clinical-safety-data-management-definitions-and-standards-expedited-reporting.

[4] FDA 21CFR312.32: IND Safety Reporting. April 2019. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32

Life threatening:	The AEs placed the subject at immediate risk of death. This classification does not apply to an AE that hypothetically might cause death if it were more severe.
Hospitalization:	The AE resulted in hospitalization or prolonged an existing inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before the signing of informed consent in the study or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization. Hospitalizations or prolonged hospitalizations for scheduled therapy of the underlying cancer or study target disease need not be captured as SAEs.
Disabling/ incapacitating:	An AE that resulted in a persistent or significant incapacity or substantial disruption of the subject's ability to conduct normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or study treatment regimen before conception or during pregnancy.
Medically significant:	The AE did not meet any of the above criteria but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above or involves suspected transmission via a medicinal product of an infectious agent.

Adverse Event Severity

Adverse event severity should be graded using the (NCI CTCAE), version 5.0. These criteria are provided in the study manual.

Adverse event severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the sponsor for defining regulatory reporting obligations (see definition for SAEs, above).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to each of the study drugs (ZW25, palbociclib, and/or fulvestrant) should be evaluated by the investigator using the following criteria:

Related: There is evidence to suggest a causal relationship between the drug and the AE, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)

There is a reasonable possibility of a relationship based on facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

Unrelated: Another cause of the AE is more plausible (e.g., due to underlying disease or occurs commonly in the study population), or a temporal sequence cannot be established with the onset of the AE and administration of the study treatment, or a causal relationship is considered biologically implausible

Note: The Sponsor and/or the SMC may query the relationship assessments and/or determine that the event was related to additional components of the regimen.

7.7.1.2 Procedures for Eliciting and Recording Adverse Events

Investigator and study personnel will report all AEs and SAEs whether elicited during subject questioning, discovered during physical examination, laboratory testing and/or other means by recording them on the CRF and/or SAE form, as appropriate.

Eliciting Adverse Events

An open-ended or non-directed method of questioning should be used at each study visit to elicit the reporting of AEs.

Recording Adverse Events

The following information should be recorded on the Adverse Events and Medical History and Pre-existing Conditions CRFs:

- Description including onset and resolution dates
- Whether it met SAE criteria
- Severity
- Relationship to study treatment or other causality
- Outcome

Diagnosis vs. Signs or Symptoms

In general, the use of a unifying diagnosis is preferred to the listing out of individual symptoms. Grouping of symptoms into a diagnosis should only be done if each component sign and/or symptom is a medically confirmed component of a diagnosis as evidenced by standard medical textbooks. If any aspect of a sign or symptom does not fit into a classic pattern of the diagnosis, report the individual symptom as a separate AE.

Recording Serious Adverse Events

For SAEs, record the event(s) on both the CRF and an SAE form.

The following should be considered when recording SAEs:

- Death is an outcome of an event. The event that resulted in the death should be recorded and reported on both an SAE form and CRF.
- For hospitalizations, surgical, or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the narrative as part of the action taken in response to the illness.

Progression of the Underlying Cancer

Do not use the term 'disease progression' alone when reporting AEs, including SAEs, because it is too nonspecific. Symptoms of disease progression that meet the criteria for an SAE must be reported. When possible, report the specific disease (clinical) manifestation of the progression (e.g., 'malignant pleural effusion', 'spinal bone metastases', 'lymphadenopathy', 'brain metastases'). Otherwise, it is acceptable to report the specific disease (e.g., non-small cell lung cancer) as an SAE.

Pregnancy

Notification to Drug Safety: Complete a Pregnancy Report Form for all pregnancies that occur from the time of first dose of study drug (ZW25, palbociclib, and/or fulvestrant) until 12 months after the last dose of study drug including any pregnancies that occur in the partner of a male study subject. Only report pregnancies that occur in a male subject's partner if the estimated date of conception is after the male subject's first study drug dose. Email to the sponsor's Drug Safety Department within 24 hours of becoming aware of a pregnancy. All pregnancies will be monitored for the full duration; all perinatal and neonatal outcomes should be reported. Infants should be followed for a minimum of 6 months.

Collection of data on the CRF: All pregnancies (as described above) that occur within 30 days of the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) will also be recorded on the Adverse Events CRF.

Abortion, whether accidental, therapeutic, or spontaneous, should be reported as an SAE. Congenital anomalies or birth defects, as defined by the 'serious' criterion above (see definitions Section 7.7.1.1) should be reported as SAEs.

7.7.1.3 Reporting Periods for Adverse Events and Serious Adverse Events

The safety reporting period for all AEs and SAEs is from start of dosing of any study drug on Cycle 1 Day 1 to 30 days after last dose of study drug (ZW25, palbociclib, and/or fulvestrant). However, all study protocol-related AEs are to be recorded from the time of informed consent. All SAEs that occur after the safety reporting period and are considered study treatment-related in the opinion of the investigator should also be reported to the sponsor.

SAEs will be followed until significant changes return to baseline, the event stabilizes (recovering/resolving) or is no longer considered clinically significant by the investigator, or the subject dies or withdraws consent. All non-serious AEs will be followed through the safety reporting period. Certain non-serious AEs of interest may be followed until resolution or return to baseline.

Potential Hy's Law cases (Appendix F) should be reported as SAEs (even before all other possible causes of liver injury have been excluded).

7.7.1.4 Serious Adverse Events Require Immediate Reporting

Within 24 hours of observing or learning of an SAE, investigators are to report the event to the sponsor, regardless of the relationship of the event to the study treatment regimen.

For initial SAE reports, available case details are to be recorded on an SAE form. At a minimum, the following should be included:

- Subject number
- Date of event onset
- Description of the event
- Study treatment, if known

The completed SAE form is to be emailed to the sponsor's representative within 24 hours (see email specified on the SAE report form).

Relevant follow-up information is to be submitted to the sponsor as soon as it becomes available.

7.7.1.5 Sponsor Safety Reporting to Regulatory Authorities

Investigators are required to report all SAEs, including anticipated SAEs, to the sponsor (see Section 7.7.1.4).

The sponsor will report all SAEs to regulatory authorities as required per local regulatory reporting requirements.

7.7.2 Adverse Events of Special Interest

Adverse events of special interest include absolute decreases in LVEF ≥ 10 percentage points from baseline, symptomatic heart failure, infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis.

Adverse events of special interest should be recorded as AEs and reported as SAEs when appropriate. When an event does not meet the criteria for an SAE, a form for AESIs should be

completed and submitted to the sponsor or sponsor safety representative. AESIs should continue to be followed until resolution or return to baseline.

7.7.3 Clinical Laboratory Tests

Samples for clinical laboratory tests will be obtained at selected time points specified in the assessment schedule (Appendix A)

Clinical laboratory analyses may be performed at local laboratories. Any abnormalities in any of the laboratory parameters will be judged in relation to the reference ranges from the laboratory and to the clinical relevance assessed by the investigator.

The following parameters will be determined: hemoglobin, hematocrit, white blood cell count (total and differential), RBC count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and MCH concentration. Coagulation parameters such as PT, INR, and PTT/ aPTT will also be required at specified time points; also, additional coagulation tests may be done per institutional standards for subjects taking fulvestrant.

The following parameters will be measured: creatinine, urea (or blood urea nitrogen [BUN]), AST, ALT, ALP, lactate dehydrogenase, total bilirubin, albumin, total protein, sodium, potassium, chloride, glucose, uric acid, calcium, magnesium, and phosphorus.

Urine will be screened for pH, glucose, ketones, blood, protein, and microscopy (if indicated).

For female subjects of childbearing potential, screening for pregnancy will be performed at screening and at predetermined time points during the study. These tests will be done using the blood samples taken for clinical chemistry. A urine pregnancy test is also acceptable.

7.7.4 Vital Signs

Vital signs measures include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be recorded at selected time points specified in the assessment schedule (Appendix A) and done in a standardized manner (i.e., after the subject has rested in the sitting position for 5 minutes). Postdose assessments should be done within approximately 30 minutes of the end of the ZW25 infusion.

7.7.5 Physical Examination

Physical examinations should include assessments of the following body parts/systems: abdomen, extremities, head, heart, lungs, neck, and neurological. Height will be measured only at screening. Weight will be measured as part of all physical examinations.

7.7.6 ECOG Performance Status

ECOG PS will be assessed at selected time points specified in the assessment schedule (Appendix A). The ECOG PS scores are described in Appendix E.

7.7.7 Electrocardiogram

The 12-lead ECGs will be recorded at selected time points specified in the assessment schedule (Appendix A). The ECG will be recorded after at least a 10-minute rest. The date and an overall

interpretation of the ECG will be recorded in the CRF. The interpretation of the ECG will be assessed as normal or abnormal, and if abnormal as clinically significant or not. If the ECG is considered abnormal and clinically significant, the abnormality must be recorded in the CRF. At least the following parameters should be assessed: heart rate, PR interval, QRS complex, and QTcF.

7.7.8 Echocardiogram/MUGA

Echocardiograms or MUGAs are recorded at selected time points specified in the assessment schedule (Appendix A). Echocardiograms or MUGAs will be recorded locally and will be assessed for an estimate of the ejection fraction. The same method must be used throughout the study. Management of left ventricular dysfunction is described in Section 5.2.3.1

7.8 Appropriateness of Measurements

The efficacy planned for this study are widely used and generally recognized as reliable, accurate, and relevant to the disease condition.

The safety measures that will be used in this trial are considered standard procedures for evaluating the potential adverse effects of study medications.

Pharmacokinetic assessments are also common in clinical studies to help characterize dose-exposure-response relationships.

Immunogenicity is commonly assessed for biologics; therefore, standard tests will be performed to detect the possible presence of specific antibodies to ZW25.

8 DATA QUALITY CONTROL AND QUALITY ASSURANCE

8.1 Audit and Inspection

Study centers and study documentation may be subject to Quality Assurance audits during the course of the study by the sponsor or its nominated representative. In addition, inspections may be conducted by regulatory authorities or the Institutional Review Board (IRB) / Independent Ethics Committee (IEC) at their discretion.

The investigator must permit the IRB/IEC, the sponsor's auditors, and representatives from regulatory authorities to have direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the CRFs. Subject confidentiality will be protected at all times.

8.2 Monitoring

Data for each subject will be recorded on a CRF. Data collection must be completed for each subject who signs an ICF and undergoes any screening assessment.

In accordance with GCP and ICH guidelines, the study monitor will carry out source document verification at regular intervals to ensure that the data collected in the CRF are accurate and reliable. The frequency of monitoring visits will be as described in the Monitoring Plan but may be modified by the rate of subject recruitment.

A clinical monitoring plan will detail the tasks to be completed at each monitoring visit. This will at least include the following:

- Site monitoring procedures including review of the ICF, source document review, source data verification, and review of CRF data
- AE and SAE reporting
- Investigational product receipt, ordering, preparation and administration, storage, documentation, accountability, dosing compliance, and return/destruction

The monitoring visits also provide the sponsor with the opportunity to ensure the investigator's obligations and all applicable ICH or health authority regulation requirements are being fulfilled.

The investigator must permit the monitor direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the CRFs. Subject confidentiality will be protected at all times.

8.3 Data Management and Coding

Study centers will enter data directly into an electronic data capture system by completing the CRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study center. Data to be recorded directly on the eCRF will be identified. Any changes to the data entered into the electronic data capture system will be recorded in the audit trail and will be FDA 21 CFR Part 11[5] compliant and/or other region-specific electronic records regulatory requirements.

Medical coding will use Medical Dictionary for Regulatory Activities (MedDRA) for concomitant diseases and AEs and WHO Drug Dictionary for medications.

Missing or inconsistent data will be queried in the electronic data base to the investigator for clarification. Subsequent modifications to the database will be documented.

8.4 Drug Accountability

Each site will verify their inventory of study drug supplies throughout the study, and that study drug is received intact and in the correct amounts. The monitor may check the study supplies at each study center at any time during the study.

The study monitor will ensure that the site has correctly documented the amount of the study drug received, dispensed, and returned on the dispensing log. A full drug accountability log will be maintained at the study center at all times. All discrepancies must be accounted for and documented.

9 DATA ANALYSIS METHODS

9.1 Determination of Sample Size

No formal sample size calculations were performed for Part 1 of the study. Approximately 6 evaluable subjects will be enrolled in each dose cohort in Part 1 with up to six ZW25 and

[5] FDA 21CFR11: Electronic Records; Electronic Signatures. April 2019. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11.

palbociclib dose level combinations evaluated; therefore, the approximate minimum and maximum sample sizes for Part 1 are 6 and 36 evaluable subjects, respectively.

Details regarding sample size for Part 1 and Part 2 of the study are presented in Table 9.

Table 9: Sample Size Determination (Parts 1 and 2)

Study Part	Cohort or Stage	ZW25 Dose (mg/kg)	Palbociclib Dose (mg)	Sample Size[b]
1	1A	20	125	6
	1B	20	100	6 (if palbociclib de-escalation from cohort 1A recommended)
	1C	20	75	6 (if palbociclib de-escalation from cohort 1B recommended)
	1D	15	125	6 (if ZW25 de-escalation from cohort 1A recommended)
	1E	15	100	6 (if ZW25 de-escalation from cohort 1B or both ZW25 and palbociclib de-escalation from cohort 1A recommended)
	1F	15	75	6 (if ZW25 de-escalation from cohort 1C, palbociclib de-escalation from cohort 1E, or both ZW25 and palbociclib de-escalation from cohort 1B recommended)
2	2	RD[a]	RD[a]	Approximately 50

IV = intravenous; PO = orally (per oral); RD = recommended dose; Q2W = every 2 weeks.

[a] RD established in Part 1

[b] Part 1 sample sizes are based on the number of DLT evaluable subjects. Part 2 sample size is based on the approximate number of subjects planned for enrollment.

Notes: Cohorts 1A through 1F are not necessarily sequential. ZW25 is given IV Q2W. Step-down dose level to 15 mg/kg Q2W or another dose level (not lower than 15 mg/kg) of ZW25 may be evaluated if recommended by the SMC and approved by the sponsor. palbociclib is taken PO QD on Days 1 through 21 of each 28-day cycle. There are no step-down doses for toxicity for fulvestrant.

A subject will be considered DLT evaluable if he or she has received ≥ 75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and was followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being followed was the occurrence of a DLT. Subjects who are considered not evaluable for DLT may be replaced. The probabilities of observing DLTs in a cohort of 6 subjects for Part 1 are summarized in Table 10.

Table 10: Probabilities of observing DLTs in Part 1

	True DLT Incidence				
N = 6 Subjects	**5%**	**10%**	**20%**	**30%**	**40%**
Probability of 0 DLTs	0.74	0.53	0.26	0.12	0.05
Probability of ≤1 DLTs	0.97	0.89	0.66	0.42	0.23
Probability of De-escalation (≥2 DLTs)	0.03	0.11	0.34	0.58	0.77

DLT = dose-limiting toxicity

In Part 2 of the study, approximately 50 subjects are expected to be enrolled. Assuming the observed PFS6 rate is between 40% and 70%, the corresponding 95% binomial exact confidence intervals (CI) are summarized in Table 11 below.

Table 11: PFS6 Rate and Corresponding 95% Confidence Intervals

PFS6 Rate	Binomial exact 95% CI (N=50)
40%	(26%, 55%)
50%	(36%, 64%)
60%	(45%, 74%)
70%	(55%, 82%)

Therefore, a total of up to approximately 86 subjects may be enrolled across the entire study.

9.2 Analysis Sets

For the purposes of analysis, the subject analysis sets are defined in Table 12.

Table 12: Subject Analysis Sets

Analysis Set	Description
All Enrolled Subjects	All participants who sign the informed consent form and receive approval from the sponsor to be enrolled and treated.
Safety Analysis Set	All participants enrolled in the study who receive any amount of ZW25, palbociclib, and/or fulvestrant.
DLT Evaluable Set	All subjects who receive ≥75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and are followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being followed is the occurrence of DLT.
mITT Set	All subjects in the safety analysis set who had at least one identifiable (target and/or non-target) lesion at baseline and at least one post-baseline disease assessment or discontinued all study treatment and are no longer being followed for efficacy.
Measurable Disease	All subjects in the safety analysis set who had at least one measurable target lesion at baseline and at least one post-baseline disease assessment or discontinued the study due to death, clinical or radiologic progressive disease, or an AE.
Pharmacokinetics	All subjects in the safety analysis set who have sufficient post-dose samples collected to allow estimation of the PK. Subjects who have protocol deviations that could affect the PK evaluation, will be noted and may be excluded from the PK analysis set depending on the type of the deviation.

AE= adverse event; DLT = dose-limiting toxicity; mITT = modified intent to treat; PK= pharmacokinetics

9.3 Statistical and Analytical Plans

The statistical analysis plan (SAP) will be developed and finalized before database lock and will provide additional details regarding the statistical methods, endpoints, and analyses to be performed as well as the procedures for accounting for missing, unused, and spurious data, and deviations from the statistical analysis methods described in the protocol. This section is a summary of the planned statistical analyses of the primary, secondary, and exploratory endpoints. Any changes to the methods described in the final SAP will be described and justified in the clinical study report.

9.3.1 General Considerations

The primary efficacy analyses will be conducted after all subjects in Part 2 have either reached a progression-free survival time of at least 6 months or are considered to have a progression-free survival event (disease progression per RECIST v1.1, clinical progression or death) prior to 6 months, or are permanently censored for progression-free survival prior to 6 months. The final efficacy analyses will be conducted after all subjects have completed their treatment and the follow-up period or following study termination by the sponsor

9.3.1.1 Randomization and Blinding

This is an open-label, non-randomized study. Randomization and blinding will not be performed. Subjects will be assigned to each cohort sequentially.

9.3.1.2 Adjustments for Covariates

No adjustments for covariates will be performed.

9.3.1.3 Handling of Dropouts and Missing Data

Methods for handling of missing, unused, or spurious data will be detailed in the SAP.

9.3.1.4 Multicenter Studies

No comparisons between sites will be performed.

9.3.1.5 Multiple Comparisons and Multiplicity

No adjustments for multiple comparisons will be performed.

9.3.1.6 Data Transformations and Derivations

Data transformations, conventions, and derivations will be detailed in the SAP.

9.3.1.7 Examination of Subgroups

Potential subgroups to be evaluated and their associated analyses will be detailed in the SAP.

9.3.2 Subject Disposition

An accounting of study subjects by disposition will be tabulated and the number of subjects in each analysis set will be summarized. Subjects who discontinue study treatment and subjects who withdraw from the study will be summarized with reason for discontinuation or withdrawal.

9.3.3 Subject Characteristics

Baseline demographics and disease characteristics will be summarized using counts and percentages for categorical variables and summary statistics (e.g., mean, quartiles, standard deviation, and range) for continuous variables.

9.3.4 Treatment Compliance

The dose administered at each cycle for each treatment agent will be assessed and dose intensity will be summarized. Details will be provided in the SAP.

9.3.5 Efficacy Analyses

All efficacy analyses will be conducted on the Safety analysis set unless otherwise specified. Efficacy for Part 1 of the study will be summarized descriptively by dose cohort. For Part 2 of the study, subjects treated at the RD in Part 1 will be included in the analyses of efficacy.

9.3.5.1 Progression-free Survival (PFS)

Progression-free survival (PFS) time is defined as the time from first dose of ZW25, palbociclib, and/or fulvestrant to the date of first documented disease progression (per RECIST 1.1) or death from any cause, whichever occurs first. Subjects who are alive and have not progressed at the time of the analysis will be censored at the time of their last evaluable tumor assessment. Subjects who have received subsequent anticancer therapy excluding radiotherapy without a prior reported progression will be censored at the time of their last evaluable tumor assessment on or prior to the initiation of first subsequent anticancer therapy. Details of the censoring scheme for this analysis will be described in the SAP.

Kaplan-Meier plots and estimates of the quartiles and their corresponding 95% confidence intervals will be computed.

9.3.5.2 Progression-Free Survival at 6 Months (PFS6)

Progression-free survival at 6 months (PFS6) is defined as having a progression-free survival time ³ 24 weeks at the time of analysis where PFS time is defined as the time from the first dose of ZW25, palbociclib, and/or fulvestrant to the date of documented disease progression (per RECIST 1.1) or death from any cause. The percentage of subjects achieving PFS6 and the corresponding exact binomial 95% confidence interval (CI) will be calculated.

9.3.5.3 Objective Response Rate (ORR)

Objective response is defined as achieving a best overall response of CR or PR as determined per RECIST 1.1. The objective response rate (ORR) or proportion of subjects with an objective response and the corresponding exact binomial 95% CI will be calculated.

9.3.5.4 Disease Control Rate (DCR)

Disease control is defined as a best response of CR, PR, non-CR/non-PD (for subjects who have only non-target lesions), or SD per RECIST 1.1. The proportion of subjects who achieve a disease control response and the corresponding exact binomial 95% CI will be calculated.

9.3.5.5 Duration of Response (DOR)

DOR is defined as the time from the first confirmed objective response (CR or PR) to documented PD per RECIST 1.1 or death within 30 days of last dose of study drug (ZW25,

palbociclib, and/or fulvestrant) from any cause. Only subjects who achieve a confirmed objective response will be included in the analysis. Details of the censoring scheme for this analysis will be described in the SAP.

Kaplan-Meier plots and estimates of the quartiles and their corresponding 95% CIs will be computed.

9.3.5.6 Overall Survival (OS)

OS is defined as time from first dose of ZW25, palbociclib, and/or fulvestrant until death from any cause. Subjects who did not achieve the event (death) at the time of the analysis or are lost to follow-up will be censored at the date they were last known to be alive (i.e., right censored). Imputation for partial or missing dates of death or last contact and details of the censoring scheme will be described in the SAP.

Kaplan-Meier plots and estimates of the quartiles and their corresponding 95% CIs will be computed.

9.3.6 Pharmacokinetic Analyses

PK analyses will be performed based on PK analysis set as defined in Section 9.2.

PK concentrations and PK parameters will be summarized using descriptive statistics (i.e., n, arithmetic mean, geometric mean, median, standard deviation, range, coefficient of variance) by ZW25 dose and presented graphically as individual and mean plots.

The following PK parameters will be derived for single dose using extensive PK sampling data after dose 1:

- $C_{max,1}$: maximum observed serum concentration
- $t_{max,1}$: time to maximum observed serum concentration
- AUC_{0-t}: area under the serum concentration-time curve from zero to the last measurable concentration
- λ_z: terminal elimination rate constant
- $t_{½}$: apparent elimination half-life
- $AUC_{0-\infty}$: area under the serum concentration-time curve from zero to infinity
- CL: serum clearance
- V_d: volume of distribution in the terminal elimination phase

Elimination parameters will be calculated if data allow to meet standard PK acceptance criteria.

The following PK parameters will be derived for multiple dosing using PK sampling data after all doses for subjects in Part 1 and subjects with extensive PK sampling in Part 2 only:

- C_{ave}: average concentration over dosing interval

The following parameters will be derived and summarized for all subjects in all Parts using sparse sampling data:

- C_{max}: maximum observed serum concentration

- C_{min}: minimum observed serum concentration (trough)
- R_{Cmin}: accumulation index, calculated as C_{min} (last dose)/C_{min} (first dose)
- $C_{trough,ss}$: trough concentration at steady state

The attainment of steady state will be determined by repeated measures analysis of variance (ANOVA) using aggregate assessment of trough concentrations.

Dose proportionality will be assessed by appropriate statistical methods based on amount of available data. Single dose PK parameters as well as multiple dose parameters will be used in these analyses.

Further details of PK analysis will be described in the SAP.

9.3.7 Immunogenicity Analyses

All immunogenicity listings and analyses will be based on the safety analysis set as described in Section 9.2.

Antibody response will be listed by subject as negative or positive with a specific titer.

Tables summarizing the frequency of the occurrence of ADAs by ZW25 dose and by time point with overall summary per ZW25 dose will be produced. Titers for confirmed positive ADA will be summarized. The time of onset of immunogenicity, duration, and number of resolved ADA cases will be summarized. Summary statistics for domain specificity and neutralizing potential will be included, if available.

Effect of immunogenicity on PK and specifically clearance of ZW25 will be explored.

Further details of the immunogenicity analyses will be described in the SAP.

9.3.8 Biomarker Analyses

Potential biomarkers such as circulating tumor DNA (ctDNA), Prosigna (formerly called the PAM50 test), and HER2 ECD (but not limited to) will be summarized using descriptive statistics (i.e., n, arithmetic mean, geometric mean, median, standard deviation, range, coefficient of variance). Details will be described separately in the SAP.

9.3.9 Safety Analyses

All analyses of safety will be conducted on the safety analysis set unless otherwise specified. For Part 1 of the study, safety will be summarized separately by dose cohort. For Part 2 of the study, subjects treated at the RD in Part 1 will be included in all Part 2 analyses of safety.

9.3.9.1 Extent of Exposure

The frequency of dose reductions and discontinuations of ZW25, palbociclib, and fulvestrant will be summarized using counts and percentages. In addition, duration of exposure and total cumulative dose of ZW25, palbociclib, and fulvestrant as well as the total number of treatment cycles received will be summarized descriptively.

9.3.9.2 Dose-limiting Toxicities (DLTs)

The frequency of DLTs (as defined in Section 3.1.2) and the corresponding exact binomial 95% CI will be calculated for each dose cohort for Part 1 of the study only. This will be computed for the DLT Evaluable Set.

9.3.9.3 Adverse Events

An AE is defined as any untoward medical occurrence in a clinical study subject administered a medicinal product which does not necessarily have a causal relationship with this treatment. All AEs will be coded by the Sponsor to standard "preferred terms" and system organ classifications (SOC) using MedDRA. Severity will be graded by study investigators using (NCI CTCAE) v5.0.

The frequency of AEs will be summarized by preferred term and SOC using counts and percentages. In addition, treatment-related AEs and AEs which lead to premature discontinuation of study treatment will be summarized.

9.3.9.4 Deaths and Serious Adverse Events

The frequency of deaths will be summarized using counts and percentages. By-subject listings of deaths will also be produced.

The frequency of SAEs will be summarized by SOC and preferred term using counts and percentages. Treatment-related SAEs will also be summarized.

9.3.9.5 Clinical Laboratory Results

Summary statistics (mean, median, minimum, maximum, standard deviation) for actual value and change from baseline will be tabulated as appropriate for laboratory results.

Laboratory results will be graded using (NCI CTCAE) v5.0 and summarized by laboratory test and toxicity grade using counts and percentages.

9.3.9.6 Cardiac Function

The frequency of ECG abnormalities (e.g., heart rate, PR interval, QRS complex, and QTcF) will be summarized using counts and percentages. In addition, the percentage of subjects with LVEF <50% and ≥50% and with an absolute decrease in LVEF ≥10 percentage points will be summarized.

9.3.9.7 Vital Signs

Vital signs (blood pressure, heart rate, respiratory rate, and temperature) will be summarized using descriptive statistics (i.e., mean, median, standard deviation, and range).

9.3.9.8 ECOG Status

ECOG status will be summarized for each visit. Shifts from baseline to the best and worst post-baseline score may be tabulated.

9.3.9.9 ECG

ECG status (normal, abnormal clinically significant, or abnormal not clinically significant) may be summarized for each scheduled ECG and shifts from baseline may be tabulated.

9.3.10 Interim Analyses

A SMC will be responsible for monitoring subject safety (see Section 3.1). Cumulative safety reviews will be conducted per the SMC Guidelines and real-time review of related SAEs will be conducted. Also, interim data from the study may be presented at scientific meetings or in manuscripts.

10 INFORMED CONSENT, ETHICAL REVIEW, AND REGULATORY CONSIDERATIONS

10.1 Institutional Review Board (IRB) / Independent Ethics Committee (IEC)

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the subjects, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval or favorable opinion of the study and all relevant study information must be obtained before the study center can be initiated. Any necessary extensions or renewals of IRB/IEC approval/favorable opinion must be obtained for changes to the study such as amendments to the protocol, the ICF or other study documentation. The written approval of the IRB/IEC together with the approved ICF must be filed in the study files.

The investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. The investigator will submit written summaries of the study status to the IRB/IEC as required. On completion of the study, the IRB/IEC will be notified that the study has ended.

10.2 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

10.3 Ethical Conduct of the Study

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines, and the applicable national and local laws and regulatory requirements.

10.3.1 Informed Consent

The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) and must adhere to GCP.

The investigator is responsible for ensuring that no subject undergoes any study-related examination or activity before that subject has given written informed consent to participate in the study.

The investigator or designated personnel will inform the subject of the objectives, methods, anticipated benefits and potential risks and inconveniences of the study. The subject should be given every opportunity to ask for clarification of any points he or she does not understand and, if necessary, ask for more information. At the end of the interview, the subject will be given ample time to consider the study. Subjects will be required to sign and date the ICF. After signatures are obtained, the ICF will be kept and archived by the investigator in the investigator's study file. A signed and dated copy of the subject ICF will be provided to the subject or their authorized representative.

It should be emphasized that the subject may refuse to enter the study or to withdraw from the study at any time, without consequences for their further care or penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or who withdraw written informed consent should not be included or continue in the study.

If new information becomes available that may be relevant to the subject's willingness to continue participation in the study, a new ICF will be submitted to the IRB(s)/IEC(s) (and regulatory authorities, if required). The study subjects will be informed about this new information and reconsent will be obtained.

10.3.2 Subject Confidentiality

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the IRB(s)/IEC(s) approving this research, and the US FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the Health Insurance Portability and Accountability Act (HIPAA 1996) and applicable national and/or local laws and regulations on personal data protection.

10.4 Study Documentation and Records Retention

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study (end of study defined as the date of the last visit of the last subject), all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the sponsor or its representatives. Essential documents should be retained for 2 years after the final marketing approval, for at least 2 years since the discontinuation of clinical development of the investigational product, or for the time period required by the applicable regulatory authorities. It is the responsibility of the sponsor to inform the study center when these documents no longer need to be retained. The investigator must contact the sponsor before

destroying any study-related documentation. In addition, all subject medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

10.5 Clinical Trial Agreement

Payments by the sponsor to investigators and institutions conducting the study, requirements for investigators' insurance, the publication policy for clinical trial data, and other requirements are specified in the clinical trial agreement.

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APPENDIX A: SCHEDULE OF EVENTS

Assessment	Screening Visit (≤ 28 days)	Cycle 1				Cycle 2			Additional 4-week cycles		Every 8 weeks	EOT[r]	Safety Follow-up (30 days post last dose)[v]	Efficacy Follow-up (Q8W)[v]	Survival Follow-up (Q3M)[v]
		D1	D2	D5	D15 (±2D)	D1 (±2D)	D15 (±2D)	D28 (±7D)	D1 (±2D)	D15 (±2D)	(±7D)	(+14D)	(+7D)	(+7D)	(±7D)
ZW25[a]		X			X	X	X		X	X					
Palbociclib[b]		Taken orally QD with food from Day 1 through 21 (off treatment on Days 22 through 28) in a 28-day cycle													
Fulvestrant[c]		X			X	X			X						
LHRH analogue[d]		Dependent on the LHRH selected for administration by the investigator													
Informed consent	X														
Medical history	X														
Eligibility	X	X[j]													
Demographics	X														
New tumor biopsy or archived tissue	X[l]											X[p]			
Disease assessment (CT/MRI, & tumor markers)[e]	X							X			X	X[s]	X[s]	X[s]	
Bone scan (scintigraphy)[e]	X							X			X	X[s]	X[s]	X[s]	
Brain scan (MRI)[e]	X							X			X	X[s]	X[s]	X[s]	
Physical exam[f, j]	X	X			X	X	X		X	X		X	X		
ECOG PS[f, j]	X	X				X			X			X	X		
Vital signs[k]	X	X			X	X	X		X	X		X	X		
Hematology[f, j]	X	X			X	X	X		X	X		X	X		
Coagulation[f, j]	X	X													
Serum chemistry[f, j,y]	X	X			X	X	X		X	X		X	X		
Urinalysis	X														
Pregnancy test [f, j] (WOCBP only)	X	X				X			X			X	X		
12-lead ECG[m]	X	X							X			X	X		
Echo/MUGA[j, q]	X	First post-baseline scan at Cycle 3 Day 1 (approximately 8 weeks from Cycle 1 Day 1), Q12W thereafter													
Hepatitis B, C and HIV[g]	X														
ADA[j, z]		X			X	X	X		X[o]			X	X[u]	X[w]	
ctDNA[j]		X						X				X[n]	X[n]		
HER2 ECD[j]		X			X		X								
PK samples[h, z]		X[k, l]	X	X	X[k, l]	X[k, l]	X[k,l]		X[k,l]			X[h]			

Assessment	Screening Visit (≤ 28 days)	Cycle 1				Cycle 2			Additional 4-week cycles		Every 8 weeks	EOT[r]	Safety Follow-up (30 days post last dose)[v]	Efficacy Follow-up (Q8W)[v]	Survival Follow-up (Q3M)[v]
		D1	D2	D5	D15 (±2D)	D1 (±2D)	D15 (±2D)	D28 (±7D)	D1 (±2D)	D15 (±2D)	(±7D)	(+14D)	(+7D)	(+7D)	(±7D)
AEs[t]	Study protocol-related AEs as well as any concomitant medications given for treatment of the AE, should be recorded from the time of informed consent. Otherwise, AEs and concomitant medications will be recorded from the start of dosing of any study drug on Cycle 1 Day through 30 days after last dose of study drug. Study drug (palbociclib only [other drugs administered at the clinic]) dispensing, collecting, and accountability will be conducted per Section 5.6														
Concomitant medications															
Drug accountability															
Survival status follow-up															X[x]

ADA = anti-drug antibody; AE = adverse event; AESI = AE of special interest; CT = computed tomography; ctDNA: circulating tumor DNA; D = day; ECD = extracellular domain; ECG = electrocardiogram; Echo = echocardiogram; ECOG PS = Eastern Cooperative Oncology Group performance status; EOT = End of Treatment; HER2 = human epidermal growth factor receptor 2; HIV = human immunodeficiency virus; LHRH = luteinizing hormone releasing hormone; mos = months; MRI = magnetic resonance imaging; MUGA = multiple gated acquisition scan; PK = pharmacokinetics; q8w = once every 8 weeks; q3mos = once every 3 months; RECIST v1.1 = Response Evaluation Criteria in Solid Tumors version 1.1; WOCBP = women of childbearing potential only.

[a] See Section 5.2.2 for ZW25 dosing and administration. There must be a minimum of 10 days (±2 days) between doses. Day 15 doses not administered within 6 days after Day 15 must be skipped.

[b] See Section 5.3.2 for palbociclib dosing and administration.

[c] See Section 5.3.2 for fulvestrant dosing and administration. Note that the third dose of fulvestrant must be on Day 29, which should be equivalent to Cycle 2 Day 1.

[d] LHRH analogue dosing and administration will be per institutional guidelines. Throughout the treatment period, LHRH analogue will be administered to/taken by premenopausal women and perimenopausal women per institutional guidelines

[e] After screening, all subsequent radiographic scans for disease assessments may be done within a ±7-day time window and based on 8-week intervals timed from Cycle 1 Day 1. Initial responses should be confirmed, if feasible, with a repeat scan 4 weeks (+1-week window) following initial documentation of objective response. The schedule of response assessments should not be adjusted after the confirmatory scan and should maintain the schedule of every 8 weeks timed from Cycle 1 Day 1. Data on breast cancer serum tumor markers, if monitored, should also be collected at the time of disease assessment. Screening bone scintigraphy should be done at the same time as screening disease assessment. For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by Xray, CT scan with bone windows, or MRI will be performed to confirm findings. After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care.

[f] May be done within 3 days before study drug administration; tests do not need to be repeated if done within the prior 3 days. Notes: Height will be measured only at screening. Weight will be measured as part of all physical examinations. Additional coagulation tests may be done per institutional standards for subjects taking fulvestrant.

[g] Only if indicated.

[h] See Appendix B for extensive sampling timepoints or Appendix C for sparse sampling timepoint. Note: Day 2 and Day 5 visits are ***not*** required for subjects on sparse sampling. The EOT PK sample is only required for subjects who have completed < 6 months of months of treatment.

[i] See Section 7.1 for details. Archived tumor tissue is allowed if new is unobtainable.

[j] On applicable dosing days, the assessment should be performed predose.

[k] On applicable dosing days, the assessment should be performed predose and postdose.

[l] On applicable dosing days, the assessment should include one performed within 15 minutes of end of infusion.

[m] On Cycle 1 Day 1 and Cycle 4 Day 1, a 12-lead ECG will be recorded predose and at 1 hour (±15 minutes) after the end of the ZW25 infusion. On Day 1 of Cycle 3 and every 3 cycles thereafter (Cycle 3, 6, 9, etc.), a 12-lead ECG will only be recorded predose. ECGs will also be recorded at screening, EOT, and 30-day follow-up visit.

[n] Only at disease progression and only at either EOT or follow-up, not both.

[o] Cycle 4 and every even-numbered cycle thereafter.

[p] Optional biopsy at disease progression if subject provides additional consent.

[q] After screening, an echocardiogram/MUGA should be done at Cycle 3 Day 1 (±7-day time window; approximately 8 weeks after Cycle 1 Day 1), then all subsequent scans should be done every 12 weeks thereafter (Day 1 of Cycle 6, 9, 12, etc.) within a ±7-day time window. An echocardiogram/MUGA is not required at EOT if ≤ 3 months since the previous scan.

[r] Should take place as soon as possible after a subject permanently stops all study treatment and definitely within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant).

[s] The scans for these assessments do not have to be performed at EOT if ≤ 4 weeks since the previous scans. If the scans were not done at the EOT visit or within the 4 weeks prior to the safety follow-up visit, these assessments should be performed at the time of the safety follow-up visit.

[t] AESIs should continue to be followed until resolution or return to baseline. Note: From the time of consent to immediately before the first dose of any study drug on Cycle 1 Day 1, only study protocol-related AEs should be recorded. A protocol-related AE is defined as an untoward medical event occurring as a result of a protocol mandated procedure.

[u] Not required if ≤ 2 weeks since last sample.

[v] Subjects who discontinue treatment for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks (± 7 days) after the previous scan until disease progression or start of subsequent anticancer therapy. If subjects enter this follow-up period before 24-weeks have elapsed since the start of study treatment, a disease assessment must be done at 24-weeks from the start of treatment (not required if ≤ 4 weeks since last disease assessment or if disease progression has already been documented). Responses that are initially observed during this follow-up period should be confirmed with disease assessment 4 weeks (+1 week) later. Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued, and subjects will enter long-term follow-up for survival status. Data on survival will be collected via clinic visits (if feasible) or via telephone calls every 3 months (± 7 days) after the last follow-up visit. Subjects may be discontinued from the study due to death, lost to follow-up, consent withdrawal or study termination by sponsor.

[w] Only if a subject had an ongoing anti-ZW25 ADA present at the safety follow-up visit. These will be discontinued after the resolution of ADA.

[x] Via clinic visits (if feasible) or via telephone calls every 3 months after the last follow-up visit.

[y] Plasma chemistry allowed if serum chemistry not available.

[z] Per Memorandum MTF- 011 (dated 28 February 2023), PK and ADA sample collection should be stopped after completing Cycle 4, except for at the EOT visit and 2 additional collection time points for ADA at the Safety and Efficacy Follow-up visits.

APPENDIX B: PHARMACOKINETIC EXTENSIVE SAMPLING TIMEPOINTS

	Cycle 1 (4 Weeks)									Cycle 2 (4 Weeks)		Every even-numbered cycle thereafter (4 Weeks)		
	D1					D2	D5	D15		D1 and D15		D1		
Time	**Pre-dose[a]**	**End of ZW25 infusion**	**2 h post-dose[b]**	**4 h post-dose[b]**	**8 h post-dose[b]**	**24 h post-dose[b]**	**96 h post-dose[b]**	**Pre-dose[a]**	**End of ZW25 infusion**	**Pre-dose[a]**	**End of ZW25 infusion**	**Pre-dose[a]**	**End of ZW25 infusion**	**EOT**
Allowed time window		+15 min	± 15 min	± 15 min	± 15 min	±4 h	± 4 h	– 4 h	+15 min	– 4 h	+15 min	– 4 h	+15 min	
PK serum sample	X	X	X	X	X	X	X	X	X	X	X	X	X	X[c]

D = day; EOT = End of Treatment; PK = pharmacokinetics
All subjects in Part 1 and at least 6 subjects in Part 2.
[a] Relative to the start of ZW25 infusion
[b] Relative to the end of ZW25 infusion
[c] Only required if the subject has completed less than 6 months of treatment

APPENDIX C: PHARMACOKINETIC SPARSE SAMPLING TIMEPOINTS

	Cycle 1 (4 Weeks)				**Cycle 2**		**Every even-numbered cycle thereafter (4 Weeks)**		
	D1		**D15**		**D1 and D15**		**D1**		
Time	**Pre-dose[a]**	**End of ZW25 infusion**	**Pre-dose[a]**	**End of ZW25 infusion**	**Pre-dose[a]**	**End of ZW25 infusion**	**Pre-dose[a]**	**End of ZW25 infusion**	**EOT**
Allowed time window		+15 min	– 4 h	+15 min	– 4 h	+15 min	– 4 h	+15 min	
PK serum sample	X	X	X	X	X	X	X	X	X[b]

D = day; EOT = End of Treatment; PK = pharmacokinetics
Subsequent subjects in Part 2.
a Relative to the start of ZW25 infusion
b Only required if the subject has completed less than 6 months of treatment

APPENDIX D: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS

MEASUREMENT OF EFFECT

Antitumor Effect – Solid Tumors

For the purposes of this study, subjects should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (Eisenhauer 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST version 1.1 criteria.

Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and at follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which

circumstance the next largest lesion which can be measured reproducibly should be selected. Previously irradiated lesions should not be chosen as target lesions unless there has been evidence of progression since radiotherapy treatment was given. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. Magnetic resonance imaging is also acceptable in certain situations (e.g., for body scans).

Response Criteria

Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the

smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or principal investigator).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Subjects with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response[a]
CR	CR	No	CR	>4 wks. Confirmation[b]
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation[b]
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once >4 wks. from Cycle 1 Day 1[b]
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD[c]	Yes or No	PD	
Any	Any	Yes	PD	

CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease; wks = weeks.

[a] See RECIST version 1.1 manuscript for further details on what is evidence of a new lesion.

[b] Only for non-randomized trials with response as primary endpoint.

[c] In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Subjects with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response; PD = progressive disease; SD = stable disease.

[a] Non-CR/non-PD" is preferred over "stable disease" for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

APPENDIX E: ECOG PERFORMANCE STATUS SCALE

ECOG	
Score	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office 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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX F: LIVER SAFETY MONITORING AND ASSESSMENT

The following recommendations are from the Food and Drug Administration (FDA) Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued July 2009 (FDA Guidance for Industry 2009).

Drug-induced hepatocellular injury sufficient to cause hyperbilirubinemia is associated with a high rate of poor outcomes (from 10 to 50% mortality or transplant) (Zimmerman 1999). The potential for drug-induced liver injury can be indicated by the following 2 observations (also known as Hy's Law):

1. Increase in transaminase levels of >3x ULN, with concurrent increase bilirubin of >2x ULN
2. No evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome (Temple 2006)

Subjects with normal baseline LFTs should undergo detailed testing for liver enzymes (including at least ALT, AST, ALP, and total bilirubin [TBL]) to confirm any increase of serum ALT and/or AST >3x ULN OR bilirubin >2 × ULN. Subjects with elevated baseline LFTs (i.e., those with liver metastases or Gilbert's Syndrome) should undergo detailed testing to confirm any elevation of AST and/or ALT >2x baseline. Testing should be repeated within 72 hours of notification of the test results. Symptoms suggestive of liver injury (e.g., right upper quadrant pain or tenderness and/or eosinophilia >5%) should also be assessed.

Confirmed abnormalities in liver function tests should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. Treatment discontinuation recommendations related to hepatic safety are described in Section 5.2.3. Recommended monitoring for subjects with confirmed hepatic abnormalities includes:

- Repeat liver function tests 2 to 3 times weekly. Frequency of testing can decrease to once a week or less if abnormalities stabilize and/ or a diagnosis has been made and does not require more frequent monitoring, or the trial drug has been discontinued and the subject is asymptomatic.
- Obtain a more detailed history of symptoms and prior or concurrent diseases.
- Obtain a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Rule out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Consider gastroenterology or hepatology consultations.

Additional testing to elucidate possible etiology may be performed as determined by the investigator.

Potential Hy's Law cases should be reported as SAEs (even before all other possible causes of liver injury have been excluded).

APPENDIX G: INVESTIGATOR SIGNATURE PAGE

Investigator Statement and Signature

I have read the attached protocol entitled "Phase 2a Study of ZW25 in Combination with Palbociclib Plus Fulvestrant."

I, the undersigned, have read and understood the protocol specified above and agree on its content. I agree to perform and conduct the study as described in the protocol and in accordance with the relevant laws/regulations and standards outlined in the Clinical Trial Agreement.

Investigator Signature	Date

Investigator Name, Printed
Investigative Site Name

APPENDIX H: DOCUMENT HISTORY

Original 12-Jul-2019

Amendment 1 03-Sep-2019

Amendment 2 10-Oct-2019

Amendment 3 07-May-2020

Amendment 4 26-Sep-2023

Summary of Changes in Amendment 4

Section(s)	Change	Rationale
Title page	• Added EU CT number. • Changed name and address of Sponsor due to a change in the Sponsor. • Changed name of Medical Monitor. • Added JZP598 as a synonym for zanidatamab in product names for completeness and clarity.	Administrative changes
Section 1.1	• Breast cancer incidence and mortality data were updated.	To reflect contemporaneous information.
Section 1.8	• Data from the zanidatamab Investigator's Brochure were removed and replaced with a cross reference to the current Investigator's Brochure.	To provide the most up-to-date information, which is found in the current Investigator's Brochure.
Section 6.3.3.1 Appendix A	• Added text to indicate stopping of PK and ADA sample collection after completing Cycle 4, except for 2 collection time points for ADA at the Safety and Efficacy Follow-up visits.	To reflect Memorandum MTF- 011 (dated 28 February 2023). Although not specifically stated in the memo, this change also indicates that collection should continue through Cycle 4.
Section 7.7.1.1 Section 7.7.1.2	• Updated the CRF name being used to collect AE and medical history information	To reflect Memorandum MTF-007 (dated 30 August 2022).
Section 9.3.5 Section 9.3.5.1 Section 9.3.5.2 Section 9.3.5.5	• Updated details of the statistical analysis related to the analysis set used for the efficacy analysis, definition of PFS, and definition of DOR.	To align with the statistical analysis plan.
Global	• Minor editorial and document formatting revisions.	Administrative changes.

Summary of Changes in Amendment 3

Section(s)	Change	Rationale
Title page	Added ClinicalTrials.gov number	To add the protocol registration number for ClinicalTrials.gov
Title page	Investigational Drug(s): ZW25 Palbociclib Fulvestrant	Added palbociclib and fulvestrant to the investigational drug list as the current label for these drugs does not include the current study's patient population.
Title page	Updated medical monitor information	Administrative change
Synopsis and Section 2.1	To recommend a dose for ZW25 in combination with palbociclib plus fulvestrant for Part 2 by evaluating ~~evaluate~~ the safety and tolerability of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/or metastatic human epidermal growth factor receptor 2 (HER2)-positive, hormone receptor (HR)-positive breast cancer	Updated the primary endpoint of Part 1 of the study to include identifying the recommended dose for ZW25 in combination with palbociclib and fulvestrant.
Synopsis, Section 2.1, Section 2.2, and Section 9.3.9.1	Added "Frequency of treatment discontinuations due to AEs" as a primary endpoint in Part 1 and Part 2	Added to assess tolerability
Synopsis, Section 2.1, Section 2.2, Section 3.1, Section 5.2.3.1, and Section 7.7.2	Updated the definition of AESIs to clarify that: • only absolute decreases in LVEF ≥ 10 percentage points from baseline and symptomatic heart failure, and not all cardiotoxicities, are considered AESI • all infusion-related reactions, regardless of Grade, are considered AESI AESIs include ~~all cardiotoxicities (e.g.,~~ absolute decreases in LVEF ≥ 10 percentage points from baseline~~)~~, symptomatic heart failure, ~~all ≥ Grade 3~~ infusion-related reactions, and all ≥ Grade 2 events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis.	Clarification

Section(s)	Change	Rationale
Synopsis and Section 2.2	Moved the following endpoints to secondary endpoints (currently incorrectly listed as primary endpoints in the tables) of Part 2 of the study. • Objective response rate (ORR) • Duration of response (DOR) • Disease control rate (DCR) • Progression-free survival (PFS)	The endpoints were correctly listed as secondary endpoints in the Synopsis Study Design and the body of the protocol (Section 3.1.4). This change is per ZW25-202 Admin Letter 1.
Synopsis and Section 2.2	Added the corresponding secondary efficacy objective for the corrected endpoints per Admin Letter 1in Part 2 of the study. To evaluate additional measures of the anti-tumor activity of ZW25 in combination with palbociclib plus fulvestrant in subjects with locally advanced (unresectable) and/ or metastatic HER2-positive, HR+ breast cancer	Clarification
Synopsis, Section 2.2, Section 3.1.4, and Section 9.3.5.6	Added "overall survival" as a secondary efficacy endpoint in the study.	To provide an assessment of long-term efficacy.
Synopsis, Section 2.2, Section 3.1.4, Section 9.1, and Section 9.3.5	Replaced the efficacy evaluable analysis set with the modified intent to treat (mITT) set for efficacy analysis: Primary endpoint (Part 2): • Progression-free survival 6 (PFS6, defined as the % of mITT ~~evaluable~~ subjects with PFS ≥ 24 weeks) Part 2 of the study will ~~utilize a Simon 2-stage Optimum design to~~ evaluate the preliminary anti-tumor activity using the modified intent to treat (mITT) set. The mITT set includes all subjects who receive any amount of ZW25 with palbociclib and/or fulvestrant who had at least one identifiable (target and/or non-target) lesion at baseline and at least one post-baseline disease assessment or discontinued all study treatment and are no longer being followed for efficacy.	Clarification
Synopsis, Section 3.1.2, and Section 9.1	Updated the definition of DLT evaluable subjects: A subject will be considered DLT-evaluable if he/she has received ≥ 75% of the planned total dose of each component of study treatment (ZW25, palbociclib, and fulvestrant) over the first 28 days of treatment, and was followed for the full DLT evaluation period, unless the reason for not receiving required doses or not being	Clarification

Section(s)	Change	Rationale
	followed was the occurrence of a DLT. If the reason for not receiving required doses is a DLT, a subject will still be considered evaluable.	
Synopsis, Section 3.1, Section 4.4.1, Section 5.2.7.2, and Section 5.4	If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy (Note: trastuzumab, alternate CDK4/6 inhibitors, or any other treatment not part of the study regimen will be considered subsequent anticancer therapy. Palliative radiotherapy to a non-target bone lesion that is not progressing is allowed after the second cycle of treatment and must be administered after the initial response assessment obtained per protocol. This will not be considered subsequent anticancer therapy but must not interfere with the assessment of tumor target lesions. Treatment with palbociclib should be interrupted during palliative radiotherapy).	Clarified which treatments are included or excluded from the definition of subsequent anticancer therapy
Synopsis, Section 3.1, Section 4.4.1, and Section 5.4	If any component(s) of the combination therapy is discontinued due to toxicity deemed related to the component(s), subjects on either part of the study may continue to receive the other component(s) of the study regimen until disease progression or start of subsequent anticancer therapy.	To allow continuation on palbociclib and fulvestrant when ZW25 is discontinued for reasons other than progression or start of subsequent anticancer therapy
Synopsis, Section 3.1, Section 6.1, Section 7.7.1.1, Section 7.7.1.3, and Appendix A	Clarified the timing of collection of AEs and protocol-related AEs. AEs will be collected from the ~~time of consent~~start of dosing of any study drug on Cycle 1 Day 1 through 30 days after last dose of study drug (ZW25, palbociclib, and/or fulvestrant) ~~or prior to beginning the next course of anti-cancer therapy, whichever occurs first~~. Study protocol-related AEs are to be collected from the time of informed consent.	Clarification
Synopsis, Section 3.1, Section 4.4.1, and Section 5.4	~~Following treatment discontinuation, patients~~Subjects who discontinue treatment with all 3 drugs for any reason (except death or withdrawal of consent) on either part of the study will have an end-of-treatment (EOT) visit within 14 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) and ~~a~~ safety ~~and efficacy~~ follow-up visit at approximately 30 days after the last dose of study drug.	Clarification
Synopsis, Section 3.1, Section 6.6, and Appendix A	Subjects who discontinue all study treatment on either part of the study for reasons other than progressive disease or start of subsequent anticancer therapy will continue in follow-up with disease assessments approximately every 8 weeks after the previous scan until disease progression or start of subsequent anticancer therapy. If subjects enter this follow-up period before 24-weeks have elapsed since the start of study treatment, a disease assessment must be done at 24-weeks from the start of treatment (not required if ≤ 4 weeks since last disease assessment or if disease progression has already been documented). Responses that are initially observed during this follow-up period should be confirmed with disease assessment 4 weeks (+1 week) later.	Added ongoing follow-ups (Q8wks) to support assessment of the efficacy endpoints, including the primary endpoint of PFS6

Section(s)	Change	Rationale
	The efficacy follow-up will include the following assessments: • Disease assessment per RECIST 1.1 (CT/MRI scans, and breast cancer serum tumor markers [if being monitored] • Bone scan (scintigraphy): For subjects with new lesions identified by post-baseline bone scintigraphy, targeted assessment by X ray, CT scan with bone windows, or MRI will be performed to confirm findings. • Brain scan (MRI): After screening, brain scans will be required per protocol only for subjects with findings on the screening brain scan; for subjects without findings on the screening brain scan, subsequent brain scans will be done per institutional standard of care. • ADA sample (only if a subject had an ongoing anti-ZW25 ADA present at safety follow-up visit): These will be discontinued after the resolution of ADA.	
Synopsis, Section 3.1, Section 6.7, and Appendix A	Following progression or start of subsequent anticancer therapy, disease assessments will be discontinued and subjects will enter long-term follow-up for survival status. Data on survival will be collected via clinic visits (if feasible) or via telephone calls every 3 months (± 7 days) after the last follow-up visit. Subjects may be discontinued from the study due to death, lost to follow-up, consent withdrawal or study termination by sponsor. Reason for study discontinuation will be recorded.	Added long-term survival follow-up to allow collection of survival data for the OS endpoint of the study
Synopsis, Section 3.1.4, and Section 9.1	Updated the sample size The total number of ~~patients~~subjects enrolled in the study will depend upon the number of dose levels of each of the components of the study treatment evaluated during Part 1 of the study~~; if Part 2 is initiated; and whether each of the 2 stages in Part 2 are fully enrolled.~~ In Part 1 of the study, the number of ~~patients~~subjects to be enrolled in a dose cohort is 6 DLT evaluable ~~patients~~subjects. Therefore, up to approximately 36 evaluable ~~patients~~subjects may be enrolled in Part 1 if both dose levels of ZW25 are tested in combination with all 3 dose levels of palbociclib. In Part 2 of the study, ~~15 efficacy evaluable patients (inclusive of efficacy evaluable Part 1 patients at the dose level used in Part 2) will be enrolled in Stage 1 (N1) and 31 additional efficacy evaluable patients may be enrolled in Stage 2 (N2). Therefore, a total of 46 efficacy evaluable patients~~approximately 50 subjects will be enrolled. Therefore, a total of up to approximately 86 subjects may be enrolled across the entire study.	Clarification
Synopsis, Section 3.1.4,	Replaced Simon 2-stage design which included interim and final statistical hypotheses and numbers with descriptive analyses including confidence intervals:	To allow a single robust analysis of PFS6 rate

<table>
<tr><th>Section(s)</th><th>Change</th><th>Rationale</th></tr>
<tr><td>Section 9.1,
Section 9.3.9</td><td>~~Assuming PFS6 of 30% and 50% as unacceptable and acceptable levels of anti-tumor activity, respectively, the null hypothesis will be rejected if 19 (41%) or more patients out of 46 achieve PFS6. The type I error rate and power for this design are 0.05 and 80%, respectively. The probability of early termination of the study in Stage 1 is 0.72 if the true percentage of patients with PFS6 is 30%.~~

Assuming the observed PFS6 rate is between 40% and 70%, the corresponding 95% binomial exact confidence intervals (CI) are summarized in Table 3 below.

Table 3: PFS6 Rate and Corresponding 95% Confidence Intervals

<table><tr><th>PFS6 Rate</th><th>Binomial exact 95% CI (N=50)</th></tr><tr><td>40%</td><td>(26%, 55%)</td></tr><tr><td>50%</td><td>(36%, 64%)</td></tr><tr><td>60%</td><td>(45%, 74%)</td></tr><tr><td>70%</td><td>(55%, 82%)</td></tr></table></td><td></td></tr>
<tr><td>Synopsis and
Section 4.1</td><td>Updated inclusion criteria #3

Received ~~Disease that has progressed on or been refractory to~~ prior treatment with trastuzumab, pertuzumab, AND ado-trastuzumab emtansine (T-DM1); disease progression during or after the most recent prior therapy.</td><td>Clarification</td></tr>
<tr><td>Synopsis and
Section 4.1</td><td>Updated inclusion criteria #8d

Prothrombin time (PT) and/or International Normalized Ratio (INR)and partial thromboplastin time (PTT)/ activated partial thromboplastin time (aPTT) ≤ 1.5 x upper limit of normal (ULN), unless on medication known to alter the INR or PTT</td><td>Clarification</td></tr>
<tr><td>Synopsis and
Section 4.1</td><td>The inclusion criteria #8e was missing </= (less than or equal to) symbol for caveat for allowing subjects with Gilbert’s syndrome who have total bilirubin ≤ 2.5 x ULN.

Total bilirubin ≤ 1.5 x ULN per institutional values (~~patient~~subjects with known Gilbert’s Syndrome may enroll with ≤ 2.5 x ULN provided the direct bilirubin is £ 1.5 mg/dL)</td><td>Correction</td></tr>
<tr><td>Synopsis and
Section 4.2</td><td>Updated exclusion criteria #11

QTc Fridericia (QTcF) >~~450~~470 ms</td><td>To align the exclusion criteria with the abnormal limit for women</td></tr>
</table>

Section(s)	Change	Rationale
Synopsis and Section 4.2	Updated exclusion criteria #22 History of or ~~known~~ ongoing leptomeningeal disease (LMD).	To clarify that patients with a history of LMD should also be excluded from the study.
Synopsis, Section 3.1.3 and Section 7.7	~~At a minimum, SMC meetings will be held every 2 weeks during Part 1 of the study (i.e., before a dose level of ZW25 in combination with palbociclib plus fulvestrant is recommended for Part 2). The SMC will also meet at least quarterly throughout the study for review of the safety data from multiple treatment cycles in order to identify safety concerns.~~ The roles, responsibilities and functioning of the SMC are described in more details in the SMC charter.	Added reference to SMC charter for additional information concerning SMC.
Synopsis, and Section 9.1	[b] Part 1 sample sizes are based on the number of DLT evaluable subjects. Part 2 sample size is based on the approximate number of subjects planned for enrollment.	Added a footnote to clarify the sample size in Part 1 and Part 2 of the study
Section 1.8, Section 1.8.1, Section 1.8.2, Section 1.8.3, and Section 1.8.4	Updated the safety data per the current ZW25 Investigator's Brochure V6	To align the safety data per ZW25 Investigator's Brochure V6 (Dec 2019)
Section 5.2.2	Dosing is based on the subject's actual body weight taken at screening visit. Doses must be adjusted for subjects who experience a ≥ 10% change in weight from baseline/ screening visit on dosing day. Subject weight must be measured on all ZW25 dosing days as part of the physical exam (within a window of 3 days prior to dosing).	Clarification
Section 5.2.3 and Appendix A	There must be a minimum of 10 days (±2 days) between doses. Day 15 doses not administered within 6 days after Day 15 must be skipped.	To prevent dosing ZW25 too frequently and to allow all the three drugs to be synchronized and cycles to remain on schedule if dosing is delayed
Section 5.2.3.3 and Appendix F	Added language to describe assessment for Drug Induced Liver Injury (DILI) Liver Toxicity	To provide guidance on evaluation of liver abnormalities during the study

Section(s)	Change	Rationale
	In the event of increased liver function tests (LFTs) without explanation such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the patient may be discontinued from the study treatment at the investigator's discretion. Discontinuation of treatment should be considered if: • ALT or AST >8 × ULN • ALT or AST >5 × ULN for more than 2 weeks • ALT or AST >3 × ULN and total bilirubin >2 × ULN or international normalized ratio (INR) >1.5 (if INR testing is applicable/evaluated) • ALT or AST >3 × ULN with the appearance of symptoms suggestive of liver injury (e.g., right upper quadrant pain or tenderness and/or eosinophilia (>5%) These treatment discontinuation recommendations are based on the FDA Guidance for Industry (FDA Guidance for Industry 2009). The recommendations are a basic guide to the investigator based on accumulated clinical experience with drugs in development and are not specific to clinical experience with ZW25. See Appendix F for recommended liver safety monitoring and assessment criteria in patients with elevations in ALT, AST, or bilirubin. Refer to Appendix F for full text	
Section 5.3.3	On resumption of dosing after a dose delay, every attempt should be made to synchronize palbociclib and fulvestrant with Day 1 of the subsequent cycle.	Clarification
Section 5.3.6	If coadministration of palbociclib with a strong CYP3A inhibitor cannot be avoided, reduce the dose of palbociclib ~~by one dose level~~to 75 mg.	Updated to align with information in Palbociclib label.
Throughout Section 6, and Appendix A	Added plasma chemistry panels in addition to serum chemistry panels • Hematology, serum chemistry (plasma chemistry allowed if serum chemistry not available), and coagulation	To allow flexibility to perform plasma chemistry tests for sites where serum chemistry is not available
Section 6.2, Section 6.3.2.6, Section 6.4, Section 6.5, Section 6.6, Section 7.2, and	Disease assessment per RECIST 1.1 (CT/ MRI scans, and breast cancer serum tumor markers [if being monitored], not required if ≤ 4 weeks since previous scan)	Added to allow collection of breast cancer serum tumor markers if being monitored by the investigator

Section(s)	Change	Rationale
Appendix A	Data on breast cancer serum tumor markers, if monitored, should also be collected at the time of disease assessment.	
Section 6.6, and Appendix A	Added sampling for ADA to the ongoing Q8wks efficacy follow-up visits ADA samples (only if a subject had an ongoing anti-ZW25 ADA present at the safety follow-up visit). These will be discontinued after the resolution of ADA.	To allow monitoring for ZW25 ADA after treatment discontinuation in case the subject had ZW25 ADA present at the safety follow-up visit
Section 7.7.1.2	The language has been revised as follows: Diagnosis vs. Signs or Symptoms In general, the use of a unifying diagnosis is preferred to the listing out of individual symptoms. Grouping of symptoms into a diagnosis should only be done if each component sign and/or symptom is a medically confirmed component of a diagnosis as evidenced by standard medical textbooks. If any aspect of a sign or symptom does not fit into a classic pattern of the diagnosis, report the individual symptom as a separate AE. ~~Important exceptions for this study are adverse reactions associated with the infusion of study drug. For infusion reactions, do not use the NCI-CTCAE terms of "cytokine release syndrome," "acute infusion reaction," or "allergic or hypersensitivity reaction." Instead, record each sign or symptom as an individual AE. If multiple signs or symptoms occur with a given infusion-related event, each sign or symptom should be recorded separately with its level of severity.~~	To correctly reflect the procedure for reporting IRRs as AEs. Similar to other AEs, IRRs should be reported as diagnoses and not individual symptoms.
Section 9.2	Updated the definitions of analysis sets in the study	Clarification
Section 9.3.1	Added the timing of primary efficacy analysis and clarified the timing of the final analyses ~~The final analyses will be conducted after all patients have either completed all study visits per protocol or discontinued the study.~~ The primary efficacy analyses will be conducted after all subjects in Part 2 have either reached a progression-free survival time of at least 6 months or are considered to have a progression-free survival event (disease progression per RECIST v1.1, clinical progression or death) prior to 6 months, or are permanently censored for progression-free survival prior to 6 months. The final efficacy analyses will be conducted after all subjects have completed their treatment and the follow-up period or following study termination by the sponsor.	Clarification
Section 9.3.5	All efficacy analyses will be conducted on the mITT set and may be conducted on the measurable disease analysis sets unless otherwise specified. Efficacy for Part 1 of the study will be summarized descriptively by dose cohort. For Part 2 of the study, mITT subjects treated at the RD in Part 1 will be included in the analyses of efficacy.	Updated for consistency with other clarifications

Section(s)	Change	Rationale
Section 9.3.9.2	The frequency of DLTs (as defined in Section 3.1.2) and the corresponding exact binomial 95% CI will be calculated for each dose cohort for Part 1 of the study only. This will be computed for the DLT Evaluable Set.	Clarification
Section 9.3.9.3	The frequency of AEs will be summarized by preferred term and SOC using counts and percentages. ~~Multiple occurrences of the same AE within a patient~~subject ~~will be summarized only once at the most severe grade level for the time frame under consideration. For summaries by severity, only the worst grade for an AE will be counted for a particular patient~~subject. ~~AEs occurring more than 30 days after the last dose of study drug (ZW25, palbociclib, and/or fulvestrant) will be excluded from summaries but included in data listings.~~	Removed for simplicity; analysis details to be provided in SAP.
Section 10.3.1	~~Prescreening of HER2 status, after the patient consents to allow the central laboratory retrospective review of HER2 status (or assessment of HER2 and/or HR status if the local assessments cannot be done to the required specifications [Section 7.1]), is allowed before written informed consent for all other study procedures is given.~~	Removed the optional HER2 prescreening step from the study to allow sites to perform HER2 testing only at the screening visit.
Appendix A	Corrected the palbociclib dosing schedule in Appendix A ~~Dependent on the LHRH selected for administration by the investigator~~ Taken orally QD with food from Day 1 through 21 (off treatment on Days 22 through 28) in a 28-day cycle	Correction
Appendix B	Changed the allowed time window for drawing blood samples for PK assessments at 24- and 96-hour post-dose timepoints from ± 1h to ± 4h.	To allow flexibility to sites for drawing blood samples for PK assessments
Appendix D	~~Definitions~~ ~~*Evaluable for toxicity:* All patients will be evaluable for toxicity from the time of their first treatment with ZW25.~~ ~~*Evaluable for objective response:* Only those patients who have measurable disease present at baseline, have received at least 1 cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)~~ ~~*Evaluable Non-Target Disease Response:* Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least 1 cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.~~	Alignment with Section 9.2
Appendix D	Added to the definition of target lesions	Clarification

Section(s)	Change	Rationale
	Previously irradiated lesions should not be chosen as target lesions unless there has been evidence of progression since radiotherapy treatment was given.	
Appendix D	Updated the Best Overall Response criteria for stable disease to clarify that confirmatory scan is to be done at least 4 weeks after the Cycle 1 Day 1, and not at least 4 weeks after baseline: documented at least once >4 wks. from ~~baseline~~ Cycle 1 Day 1[b]	Clarification
Throughout protocol	Various other administrative changes	For clarity or alignment of language where needed

Summary of Changes in Amendment 2

Section(s)	Change	Rationale
Title page	Added EudraCT number	To add the protocol registration number for European Union Drug Regulating Authorities Clinical Trials Database (EudraCT)
Synopsis, Section 2.1, and Section 2.2	Clarified secondary endpoint for PK parameters in Part 1 and Part 2 is for ZW25 only: PK parameters for single (first) dose and multiple doses of ZW25	Clarification
Synopsis, Section 3.1, Section 6.3.4, Section 7.2, and Appendix A	Added "if feasible" where confirmatory scans are mentioned in the body of the protocol. Also, added the time window (+1-week) for confirmatory scan and clarified that the schedule of response assessments should not be adjusted after confirmatory scan (Synopsis, Section 3.1, Section 6.3.4, and Appendix A): Initial responses should be confirmed, if feasible, with a repeat scan 4 weeks (+1-week window) following initial documentation of objective response. The schedule of response assessments should not be adjusted after the confirmatory scan and should maintain the schedule of every 8 weeks timed from Cycle 1 Day 1.	To allow flexibility in the requirement of confirmatory scans when not possible and the timing of confirmatory scans if done. Also, to ensure that the frequency of scanning patients' disease is consistent across all patients.
Synopsis and Section 4.2	Added exclusion criterion #8: 8. Prior treatment with palbociclib or any other CDK4/6 inhibitors, including experimental agents	Per Health Canada request
Synopsis and Section 4.2	Revised exclusion criteria as follows: ~~12.~~ 13. Active hepatitis B or hepatitis C infection ~~or other known chronic liver disease~~ ~~13.~~ 14. Acute or chronic uncontrolled renal disease, pancreatitis, or severe liver disease (Child-Pugh Class C) ~~(with exception of patients with Gilbert's Syndrome, asymptomatic gall stones, liver metastases, or stable chronic liver disease per investigator assessment)~~	Per Health Canada request
Synopsis and Section 4.2	Revised exclusion criterion as follows: 15. Known ~~I~~infection with Human Immunodeficiency Virus (HIV)-1 or HIV-2 (Exception: patients with well-controlled HIV [e.g., cluster of differentiation 4 (CD4)-positive T cell count >350/mm^3 and undetectable viral load] are eligible.)	Because screening for HIV is only performed if indicated in the opinion of the treating investigator, exclusion criterion #15 was revised to reflect "known infection with HIV".
Synopsis and Section 9.2	Corrected the condition of enrollment in Stage 2: Enrollment of patients in Stage 2 will not occur if ~~≤~~ < 6 efficacy evaluable patients achieve PFS6 in Stage 1.	Correction to align with already specified enrollment condition for Stage 2 that a minimum of 6 efficacy-evaluable patients must be alive and progression-free for ≥ 24 weeks in Stage 1 for additional enrollment to occur in Stage 2.

Section(s)	Change	Rationale
Section 2.1	Corrected the table header formatting, which was causing the third secondary objective regarding immunogenicity in Part 1 to be incorrectly being displayed as a primary objective.	Administrative correction
Section 5.3.4.1	Added the following for complete blood count monitoring per the IBRANCE Product Monograph: For patients who experience Grade 3 neutropenia, consider repeating CBC monitoring one week later.	Per Health Canada request
Section 5.2.3	Clarified that medical monitor approval is required for continued dosing at a second step-down dose level (not to go below 10 mg/kg) if a patient experiences another event that meets the criteria for DLT at the initial step-down dose level.	Per Health Canada request
Section 6.2 and Appendix A	Removed the Study Activity of the ProSigna assessment.	Removed this Study Activity as this is not an activity for the site as the biomarker will be evaluated by the central lab receiving the tissue.
Section 6.3.3.1	Aligned language in the body with the correct language in the schedule of events: Echocardiogram/MUGA (Cycle 3 and every 3 cycles thereafter [i.e., Cycles 3, 6, 9, 12, etc.] such that the assessment is done Q12W after the first post-baseline assessment at ~~the end of Cycle 2~~ Cycle 3 Day 1)	To align the timing of the first post-baseline Echo/MUGA in the body of the protocol with the schedule of events
Section 6.5	Removed "and" from the caveat not requiring CT/MRI, bone, and brain scans at the safety follow-up visit. The text now reads as follows for all the three assessments: "…(not required if done at EOT visit ~~and/~~or ≤ 4 weeks since previous scan)"	Clarification
Appendix A	Added ±7-day time window for Echo/ MUGA assessment to occur at Cycle 3 Day 1	Added the time window to the footnote to confirm that Echo/MUGA assessment can also be done within 7 days of Cycle 3 Day 1
Appendix A	Added the annotation for footnote "s" to the brain and bone scans at the EOT visit and revised the footnote text for the CT/MRI, brain, and bone scans to align with Section 6.4 and Section 6.5 as follows: "The ~~Radiographic~~ scans for these ~~disease~~ assessments do not have to be performed at EOT if ≤ 4 weeks since the previous scans. If the ~~radiographic~~ scans were not done at the EOT visit ~~nor~~ or within the 4 weeks prior to the safety follow-up visit, ~~disease~~ these assessments should be performed at the time of the safety follow-up visit."	For the annotation of footnote "s", added to align with already mentioned condition for rescanning in Section 6.4 and Section 6.5. For the revision to the footnote text, revised to clarify that if scans were not done at the EOT visit OR within the 4 weeks prior to the safety follow-up visit, then scans should be done at the safety follow-up visit.

Section(s)	Change	Rationale
Throughout protocol	Various other administrative changes	For clarity or alignment of language where needed

Summary of Changes in Amendment 1

Section(s)	Change	Rationale
Synopsis and Sections 2.1, 2.2, 3.1, and 7.7.2	Added ≥ Grade 2 events of pneumonitis and/or ILD, including pulmonary fibrosis to AESIs	Per FDA request
Synopsis and Section 4.1	Revised inclusion criterion #3 as follows: ~~Progressive~~ Disease that has progressed on or been refractory to prior treatment with trastuzumab, pertuzumab, AND ado-trastuzumab emtansine (T-DM1). Patients in any part of the study who did not receive ~~these 3 agents~~ pertuzumab or T-DM1 because of lack of access (e.g., due to insurance coverage or because they were ~~ineligible to receive such therapy (e.g., pre-existing~~ treated prior to regulatory agency approval of the agent in a relevant indication) or due to medical ~~contraindication~~ ineligibility for treatment with T-DM1 (e.g., history of severe infusion reactions to trastuzumab, ≥ Grade 2 peripheral neuropathy, or platelet count < 100 x 10^9/L) may be eligible for the study after discussion with and approval from the sponsor medical monitor. Prior treatment with endocrine therapy in the neoadjuvant, adjuvant, and/or metastatic setting is permitted.	Per FDA request
Synopsis and Section 4.2	Revised exclusion criterion #11 as follows: Grade 2 or greater pneumonitis and/or interstitial lung disease, including pulmonary fibrosis, or other clinically significant infiltrative pulmonary disease not related to lung metastases)	Per FDA request
Section 1.8	Clarified that the safety data presented for ZW25 are per the ZW25 Investigator's Brochure Version 5.	Clarification
Section 1.8	Added the following to the potential safety risks: Rare cases of severe interstitial lung disease/non-infectious pneumonitis, including fatal events, have been reported in patients treated with palbociclib (Ahsan 2017), (Ahsan 2017), (IBRANCE®), and (Levy 2019). Patients in the current study ZWI-ZW25-202 will be monitored for potential pulmonary toxicity by routine AE surveillance. Patients who develop new or worsening respiratory signs or symptoms (e.g., cough, dyspnea, hypoxia, or interstitial infiltrate) will have further palbociclib dosing immediately put on hold and will be urgently evaluated for interstitial lung disease/non-infectious pneumonitis.	Per FDA request
Synopsis and Section 3.1.2	Aligned DLT definition for step-down dosing and DLT evaluation	Per FDA request

Section(s)	Change	Rationale
Section 3.1.2	Revised the Non-Hematologic DLT Criteria as follows: Any non-hematologic AE ≥ Grade 3 in severity that is ~~related~~ not clearly and incontrovertibly due to ZW25 disease progression or ~~any combination of ZW25 and 1 or more of the drugs in the combination regimen is~~ extraneous causes (e.g., accidental injury or similar event) is considered a DLT, with the following exceptions: • Grade 3 fatigue lasting ≤3 days • Grade 3 diarrhea, nausea, or vomiting that resolves to ≤Grade 1 or baseline within 3 days with adequate supportive care • Grade 3 rash without maximal use of corticosteroids or anti-infectives • Infusion reaction ≤ Grade 3 (for management of infusion reactions, see Section 5.2.3) ~~Any laboratory abnormality ≥Grade 4 that is related to ZW25 or any combination of ZW25 and 1 or more of the drugs in the combination regimen is considered a DLT.~~ Grade 2 or greater events of pneumonitis and/or interstitial lung disease, including pulmonary fibrosis not clearly and incontrovertibly related to disease progression or extraneous causes are considered a DLT. Any laboratory abnormality ≥Grade 4 not clearly and incontrovertibly due to disease progression or extraneous causes is considered a DLT. Any asymptomatic ≥ Grade 3 electrolyte abnormality that lasts >72 hours or any symptomatic ≥ Grade 3 electrolyte abnormality of any duration is considered a DLT. Amylase and lipase elevations not associated with symptoms or clinical manifestations of pancreatitis are not considered DLTs. Any clinically relevant toxicities ~~that are related to ZW25 or any combination of ZW25 and 1 or more of the drugs in the combination regimen~~ not clearly and incontrovertibly due to disease progression or extraneous causes and that do not resolve to ≤ Grade 1 or baseline within 2 weeks may be considered a DLT based upon review by the study SMC. Greater than a 2-week delay in the start of Cycle 2 due to unresolved toxicity not clearly ~~related~~ and incontrovertibly due to ~~ZW25~~ disease progression or ~~any combination of ZW25 and 1 or more of the drugs in the combination regimen~~ extraneous causes will be considered a DLT. Any death not clearly due to the underlying disease or extraneous causes is considered a DLT.	Per FDA request

Section(s)	Change	Rationale
Section 3.1.2	Revised the Hematologic DLT Criteria as follows: The following hematologic AEs that are ~~related to ZW25 or any combination of ZW25 and 1 or more of the drugs in the combination regimen~~ not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs: • Absolute neutrophil count (ANC) of Grade 3 or 4 with fever (fever must be present for the Grade 3 or 4 ANC to be considered a DLT, and is defined as a temperature of ≥38.5°C) • ANC of <500/μL for >7 days • Grade 3 thrombocytopenia associated with significant bleeding (requiring blood and/or platelet transfusion) • Grade 4 thrombocytopenia Grade 4 anemia	Per FDA request

Section(s)	Change	Rationale
Section 3.1.2	Revised the Hepatic DLT Criteria as follows: The following hepatic AEs that are ~~related to ZW25 or any combination of ZW25 and 1 or more of the drugs in the combination regimen~~ not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs: • Grade ≥ 3 elevation of transaminases (alanine transaminase [ALT] or aspartate transaminase [AST]) that is NOT thought to be due to disease progression or other medical illness • For patients with documented hepatic metastases and a baseline AST or ALT > 3x upper limit of normal (ULN) an increase of AST or ALT > 8x ULN or AST or ALT > 5x ULN for ≥ 14 days that is NOT thought to be due to disease progression or other medical illness • Grade 3 or 4 elevation of bilirubin irrespective of transaminases that is NOT thought to be due to disease progression or other medical illness • Any single instance of AST~~/~~ or ALT > 3 × ~~upper limit of normal (~~ULN~~)~~ AND total bilirubin > 2 × ULN that is NOT thought to be due to disease progression or other medical illness • For patients with documented hepatic metastases and a baseline AST or ALT > 3x upper limit of normal (ULN) any single instance of AST or ALT > 5 × ULN AND total bilirubin > 2 × ULN that is NOT thought to be due to disease progression or other medical illness	Per FDA request
Section 3.1.2	Revised the Cardiac DLT Criteria as follows: The following cardiac AEs that are ~~related to ZW25 or any combination of ZW25 and 1 or more of the drugs in the combination regimen~~ not clearly and incontrovertibly due to disease progression or extraneous causes (e.g., accidental injury or similar event) are considered DLTs: • LVEF below institutional limits and ≥ 10% points below pre-treatment baseline • Grade 2 symptomatic heart failure The first DLT will result in withholding of the drug until recovery of the ejection fraction to prior levels. If patients remain off ZW25 for more than 4 weeks they will be removed from the study. For the management of left ventricular dysfunction, see Section 5.2.3.	Per FDA request
Section 4.4.2	Revised reasons a patient may withdraw from the study	To align with reasons for withdrawal across the ZW25 program

Section(s)	Change	Rationale
Sections 5.2.1, 5.2.2, and 5.2.3	Added 'of ZW25' to the headings of the description, dose and administration, and dose modifications sections	Clarification
Section 5.2.2	Revised the reduced infusion rate for patients who experience Grade 1 or 2 infusions reactions to 50% slower than the initial rate rather than giving a range of 30% through 50% for the reduced infusion rate	Per FDA request
Section 5.2.3	Specified detailed dose modifications of ZW25 for symptoms of infusion-related reactions	Per FDA request
Section 5.3.3	Specified dose modifications of palbociclib for pulmonary symptoms indicative of ILD/pneumonitis.	Per FDA request
Section 5.5	Specified management of adverse reactions when not directly attributable to a specific drug.	Per FDA request
Appendix A	Revised cross reference for palbociclib dispensing, collecting, and accountability	Correction

PROTOCOL APPROVAL SIGNATURE

Protocol Title: Phase 2a Study of ZW25 in Combination with Palbociclib Plus Fulvestrant

Protocol Number: ZWI-ZW25-202

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP) and applicable regulatory requirements.

Sponsor Signatory

[redacted] MD
Medical Director
Jazz Pharmaceuticals

See Document Approval page for eSignature and date of approval