NCI Protocol #: N/A

DF/HCC Protocol #: 16-264

TITLE: A Phase II study of cisplatin + AZD1775 in metastatic triple-negative breast cancer and evaluation of pCDC2 as a biomarker of target response

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Other Agent(s): Cisplatin

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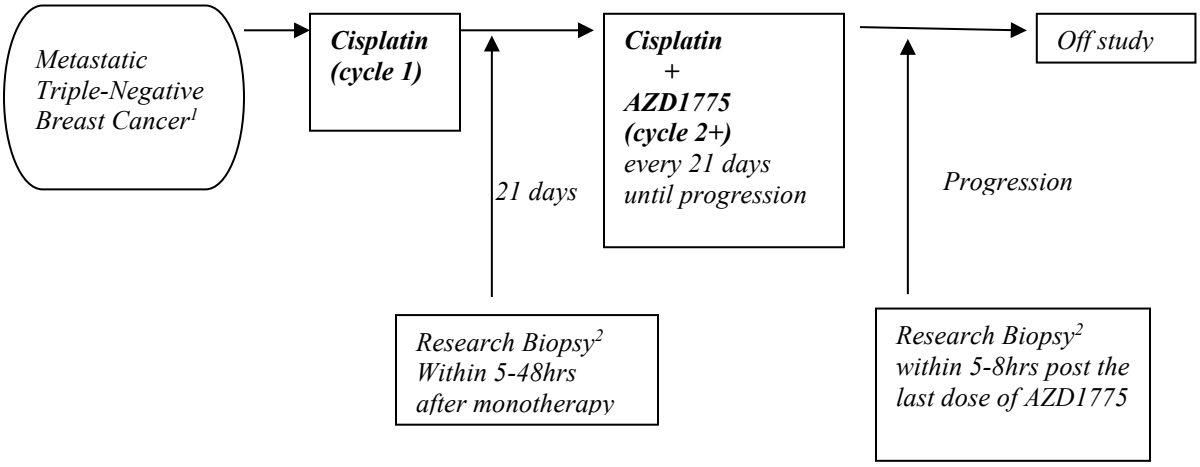
SCHEMA



1. Patients have received 0 (limited to n = 20 patients) to 1 line of chemotherapy in the metastatic setting.
2. All patients with tumor that is accessible to biopsy will undergo research biopsies.

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1. OBJECTIVES

1.1 Study Design

This is a single-arm, two-stage Phase 2 study assessing the efficacy of AZD1775, a Wee1 tyrosine kinase inhibitor, given in combination with cisplatin chemotherapy. The population to be studied will consist of approximately 35 patients with metastatic triple-negative breast cancer who have received 0 to 1 line of chemotherapy in the metastatic setting. All patients must have measurable disease at baseline and have archival tissue available. Treatment will consist of one cycle of cisplatin monotherapy (cisplatin 75 mg/m2 IV x1) followed by combination therapy of AZD1775 plus cisplatin starting 21 days later. AZD1775 will be administered as twice daily oral dosing (AZD1775 200 mg po bid) x 5 doses over 2.5 days, in combination with cisplatin 75 mg/m2 IV every 21 days. At least 10 patients will undergo a research biopsy within 5-48 hours after cisplatin monotherapy and then again within 5-8 hours after the last dose of AZD1775 in cycle 2 of cisplatin +AZD1775. Patients will undergo restaging scans every 6 weeks, those with complete responses, partial responses, and stable disease, will continue to receive study treatment. Patients with progressive disease will be taken off study.

1.2 Primary Objectives

The primary objective of the study is:

1. To evaluate the activity of AZD1775 in combination with cisplatin, as defined by objective response rate (ORR) in patients with triple-negative metastatic breast cancer

1.3 Secondary Objectives

The secondary objectives of the study are:

1) To evaluate Progression Free Survival (PFS).

2) To evaluate change in pCDC2 after therapy with AZD1775 in paired biopsies

3) Evaluate incidence of p53 mutations in tumor tissue and correlate mutational status with response

4) To evaluate change in markers for DNA damage (gamma-H2AX, phospho-Histone 3, and caspase 3) after exposure to cisplatin + AZD1775 compared to cisplatin therapy alone in paired biopsy samples

5) To evaluate the incidence BRCA1 and BRCA2 mutations, and correlate mutational status with response

6) To explore other possible gene mutations that may correlate with response.

7) To correlate next generation sequencing of tumors with participant outcomes (ORR and PFS) in DFCI participants only.

2. BACKGROUND

2.1 Study Disease(s)

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death in American women. Despite recent advances in breast cancer treatment, metastatic disease remains incurable. One possible limitation of our therapies has been an inability to select subsets of patients most likely to benefit from specific therapies. With the development of gene expression array technology, the heterogeneity of breast cancer has become clearer and the identification of novel cancer subtypes has reinvigorated the search for more specific and effective therapies. Hierarchical clustering of genomic expression data from breast cancer specimens have demonstrated several distinct tumor subgroups with unique expression profiles, including a HER2-positive subgroup, an estrogen-receptor (ER)positive group, and a "basal-like" group.[1] Hormonal therapies are only effective for ER or PR positive breast cancer, and the monoclonal antibody trastuzumab is effective only for tumors that have amplified HER2 genes.

The basal-like tumors have a poor prognosis relative to other subtypes[1], even with the best available chemotherapy. Approximately 15% of women are diagnosed with this form of breast cancer, with a disproportionate distribution in younger women or BRCA1 mutation carriers. Little progress has been made in identifying specific molecular pathways associated with these refractory cancers that may be effectively targeted for therapeutic purposes. Thus, no proven, specific therapy exists for this tumor subtype. A common feature of tumors in the basal-like subgroup is the lack of expression of ER, PgR, and HER2 (hence the term ''triple-negative'' breast cancer) as well as the expression of the Epidermal Growth Factor Receptor (EGFR) and certain cytokeratins (cytokeratin 5/6) that indicate a basal differentiation for these breast tumors[2]. The gene expression signature of the basal-like tumors shows high levels of expression of proliferation-associated genes and genes that are normally expressed in myoepithelial or basal cells of normal breast tissue, such as the basal keratins and smooth muscle actin. There are similarities between this subgroup and tumors with germline BRCA1 mutations, which also typically lack ER and PR receptors, are histologically high grade, and are reported to be positive for basal-like keratins[3,4].

2.2 AZD1775

Cytotoxic agents remain a key component of cancer therapy. Tumor cells rely on cell cycle checkpoints for repair of DNA damage induced by cytotoxic agents. Cytotoxic agents that induce DNA damage activate cell cycle checkpoints in proliferating cells. These checkpoints cause transient arrest at the G1-, S-, and G2-phase of cycling cells, allowing time to repair the DNA or initiate apoptosis if the DNA damage is too extensive. Human tumor cells are most sensitive to

chemotherapy during the phase of DNA replication (S-Phase) which is variable in length and lasts up to ~60 hrs in human tumors in-situ. While DNA checkpoints can protect normal cells from DNA damage, they also reduce the effectiveness of chemotherapy on tumor cells by allowing tumor cells to repair DNA damage induced by chemotherapy. Thus, selective inhibition of checkpoints in tumor cells is predicted to enhance the efficacy of DNA-damaging agents since mutations will not be repaired[5–7].

Wee1 is a tyrosine kinase that selectively phosphorylates the Tyr15 residue of cyclin-dependent kinase 1 (also known as CDC2) and inactivates its activity.[8–11] AZD1775 is a highly selective, small-molecule, inhibitor of Wee1 kinase activity. The protein p53 regulates the G1 checkpoint, while the Wee 1 tyrosine kinase regulates the G2 checkpoint by selectively phosphorylating CDC2. Phosphorylation of CDC2 inhibits its kinase activity, which abrogates G2 cell cycle arrest after DNA damage. Therefore, p53 deficient tumor cells lacking the G1 checkpoint are dependent on the G2 checkpoint for survival following DNA damage. G2 checkpoint abrogation is a therapeutic strategy designed to prevent cell cycle arrest in response to DNA damage, resulting in impaired DNA repair and increased tumor cell death[5,6]. Because wild type p53 cells retain both the G1 checkpoint and back-up p53-dependent pathways at the G2 checkpoint, G2 checkpoint abrogation in combination with DNA damage in p53 deficient cells is expected to selectively enhance cell death[12].

2.3 Cisplatin

Cisplatin and Breast Cancer

Platinum was studied in breast cancer in the 1970s and was shown to be active when given early in the course of the disease, but it was not adopted, perhaps because of the superior therapeutic index of other drugs then under development (the taxanes). The drug was initially tested in patients with advanced breast cancer, both as a single agent and in combination with other drugs. Small studies demonstrated objective response rates ranging from 42% to 54% with the use of cisplatin as a single agent, but response rates were lower in women who had received prior chemotherapy for metastatic disease[13–15]. When cisplatin was given after other chemotherapy, the response rate fell to 0-9%[16–20].

Notably, these studies used cisplatin in patients regardless of ER, PgR, and HER2 status. Several combination regimens were also explored, particularly cisplatin combined with taxanes, but there seemed little reason to continue these combinations when the taxanes were found to be so active and relatively nontoxic[21]. There was also interest in the evaluation of combined treatment with docetaxel and platinum. In several studies in unselected cases of metastatic disease, the overall response rate with combination regimen was around 50%, even with prior adjuvant anthracycline therapy[21].

There has recently been renewed interest in cisplatin for the treatment of breast cancer, in part because of improved strategies for managing its side effects, particularly nausea. A 2004 phase 2 study of preoperative paclitaxel and cisplatin demonstrated a 28% complete response rate and a 63% partial response rate in patients enrolled without regard to ER, PgR, and HER2 status[22]. These rates are comparable to those for other active regimens.

Published data on response rates with cisplatin used as a single agent in women with metastatic breast cancer unselected for breast cancer subtype are summarized in Table 2.3.1. Recent data from TBCRC009 demonstrated that platinum therapy (carboplatin or cisplatin) resulted in a response rate of 25.6% in the first or second line setting for metastatic triple-negative breast cancer, suggesting that these agents are active. The Triple Negative Breast Cancer Trial (TNT) study directly compared single-agent platinum chemotherapy with taxane therapy in the first line treatment of metastatic triple-negative breast cancer. Patients were randomized to carboplatin or docetaxel, and the objective response rate in the carboplatin arm was 31.4% compared to 35.6% in the docetaxel arm, which was not statistically different (p=0.44). However, among the 43 patients with a BRCA mutation, the objective response rate was 68% with carboplatin and 33% with docetaxel, which was statistically significant (p=0.03).

Table 2.3.1 Single-Agent Cisplatin in Metastatic Breast Cancer

Cisplatin Dose	Response Rate	Reference No.
No Prior chemotherapy		
30mg/m^2/d x 4 d every 3 wk	13/35	5
30mg/m^2/d x 4 d every 3 wk	5/12	6
30mg/m^2/d x 4 d every 3 wk	9/19	7
Total	33/66(60%)	
Prior chemotherapy		
35mg/m^2/d x 5 d every 4 wk	2/5	8
15mg/m^2/d x 5 d every 4 wk 100-120mg/m^2 every 4 wk	0/15 2/13	10
60mg/m^2 every 3-4 wk 120mg/m^2 every 3-4 wk	0/18 4/19	9
100mg/m^2 every 3-4 wk	2/17	11
20mg/m^2/d x 5 d every 4 wk 100mg/m^2 every 3-4 wk	0/14 0/12	12
Total	10/113(9%)	

Cisplatin and Basal-Like Breast Cancer

Basal-like breast cancers share many molecular features with BRCA1-associated breast cancer, a subset which have recently been shown to be more sensitive to cisplatin. Recent preclinical work has identified that platinum agents may be particularly active in a subset of triple-negative

breast cancer.[23] Cytotoxic agents, such as cisplatin, induce DNA damage that activates cell cycle checkpoints. These checkpoints results in transient arrest at the G1-, S-, and G2- phases of cycling cells, allowing time to repair the DNA damage or to initiate apoptosis, if the DNA damage is too extensive. While DNA checkpoints can protect normal cells from DNA damage, they also reduce the effectiveness of chemotherapy on tumor cells by allowing tumor cells to repair DNA damage induced by chemotherapy.

2.4 Rationale

While only about 20% of breast cancer is p53 deficient, approximately 60-80% of triple-negative breast cancers appear to have p53 pathway deficiency[24], suggesting that AZD1775 would be expected to have more selective chemosensitization effects for this tumor subtype. In addition, in both in vitro and in vivo xenograft tumor models, AZD1775 in combination with gemcitabine or platinum chemotherapy caused significantly greater efficacy than gemcitabine or platinum alone[12]. Additionally, we have treated 5 patients on protocol 001-07 (DF/HCC protocol 08-030) with metastatic triple-negative breast cancer with AZD1775+ cisplatin. Of these 5 heavily pretreated patients, 1 had a PR and 3 had SD as their best response. [25] Additionally, in a randomized phase 2 study in women with TP-53 mutant ovarian cancer, AZD1775 plus paclitaxel and carboplatin was associated with a significant improvement in progression-free survival compared with chemotherapy alone, suggesting activity for this agent when given in combination with platinum-based chemotherapy (REF: Oza et al ASCO 2015).

We propose a trial to look at cisplatin in combination with AZD1775 for metastatic triple-negative breast cancer.

2.5 Correlative Studies Background

We plan to demonstrate target engagement in tumor tissue by analysis of changes in pCDC2. Changes in pCDC2 have been seen in skin biopsies of patients receiving AZD1775, [26] and recently has also been demonstrated in 3 of 5 paired biopsies in patients receiving AZD1775 as monotherapy in a phase 1 trial (REF: Do et al., JCO 2015).

Patients will receive cisplatin monotherapy for 1 cycle upfront, followed by cisplatin + AZD1775 for subsequent cycles of therapy. Any patient with tumor that is accessible to biopsy will undergo research biopsies after one dose of cisplatin, and after the combination therapy. We anticipate that pCDC2 will be present after cisplatin monotherapy. After the combination, a reduction in pCDC2 relative to CDC2 is expected. This should be accompanied by evidence of checkpoint bypass and entrance of tumor cells into mitosis, which will be assessed by an increase in phospho-Histone 3 after combination treatment compared to cisplatin monotherapy. Checkpoint bypass will result in greater DNA damage, which should cause an increase in gamma-H2AX after combination treatment.[27] Finally, entrance of cells into mitosis without adequate DNA repair is frequently lethal and should result in increased apoptosis, which can be assessed with an increase in cleaved caspase 3 after combination treatment. [28]

Historically, it has been challenging to study the molecular evolution of tumors over time and through progression on serial treatment regimens. However, emerging technology now permits whole exome sequencing from cell-free DNA (cfDNA) isolated from fresh blood. We aim to examine the utility of this approach for detecting molecular changes in individual patients' tumor cell populations over time by performing serial cfDNA.

We will access DNA sequencing data (where possible) on tumors from all DFCI participants who previously had tumor sequencing performed under the Dana-Farber Cancer Institute's "Profile" targeted sequencing panel. MGH participants and any 16-264 participants who did not previously participate on or have sequencing performed under "Profile" will be excluded from any analysis. We will use these data to investigate candidate genomic correlates of response, including tumor mutation burden and alterations in genes involved in homologous recombination, DNA damage response, and cell cycle G1/S checkpoint signaling. Mutations in G1/S checkpoint genes, such as *TP53*, *MYC*, and *CCNE1*, may enhance reliance on the Wee1-mediated G2/M checkpoint for accurate DNA repair and therefore make these tumors more susceptible to the combination of AZD1775 and cisplatin. Altogether, these existing DNA sequencing data may reveal preliminary response biomarkers to guide future DNA and RNA sequencing studies.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

3.1.1 Participants must have histologically or cytologically confirmed invasive breast cancer, with either locally advanced or metastatic disease. Patients without pathologic or cytologic confirmation of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation.

3.1.2 Either the primary invasive tumor and/or the metastasis must be triple-negative, defined as:

- hormone-receptor poor, ER- and PR-negative, or staining present in <1% by immunohistochemistry (IHC)
- HER2-negative: 0 or 1+ by IHC, or FISH<2.0

3.1.3 Participants must have at least one lesion that is not within a previously radiated field that is measurable on computerized tomography (CT) or magnetic resonance imaging (MRI) scan per RECIST version 1.1. Bone lesions are not considered measurable by definition. See Section 11 for the evaluation of measurable disease.

3.1.4 Prior chemotherapy: Patients may have received 0-1 prior chemotherapeutic regimen for metastatic breast cancer and must have been off treatment with chemotherapy for at least 21 days before enrollment in the study. The number of patients with 0 prior chemotherapeutic regimen will be limited to a maximum of n = 20.

3.1.5 Prior biologic therapy: Patients must have discontinued all biologic therapy at least 21 days before participation.

3.1.6 Prior radiation therapy: Patients may have received prior radiation therapy in either the metastatic or early-stage setting. Radiation therapy must be completed at least 14 days prior to study participation and patients should have recovered from adverse effects of radiation to grade ≤1.

3.1.7 Age ≥18

3.1.8 ECOG performance status ≤1

3.1.9 Participants must have normal organ and marrow function as defined below:

- Absolute neutrophil count ≥1500/mm^3
- Platelets ≥100,000/mm^3
- Hemoglobin ≥9 g/dL
- Total Bilirubin ≤1.5 mg/dL
- Serum creatinine ≤1.5 mg/dL OR measured creatinine clearance (CrCl) ≥45 mL/min as calculated by the Cockcroft-Gault method OR 24-hour measured urine CrCl ≥45mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5 times the upper limit of normal. For patients with documented liver metastases, AST/ALT ≤ 5.0 times the upper limit of normal.

3.1.10 Patients on bisphosphonates may continue receiving bisphosphonate therapy during study treatment.

3.1.11 Availability of a tissue block from initial breast cancer diagnosis and/or metastatic recurrence. If a tissue block is not available, 10-20 unstained slides may be provided as an alternative. If unstained slides will be provided, they should not be sent until specifically requested by the DFCI study coordinator. If archival tumor tissue is not available, a fresh biopsy may be performed.

3.1.12 All patients with biopsy-accessible disease must be willing to undergo paired research biopsies. These biopsies will occur 5-48 hours after the C1D1 cisplatin dose (ie. C1D2or C1D3) and 5-8hrs (+/- 24hrs) after the last dose of AZD1775 on C2D3. The exact timing of the biopsy relative to receipt of study treatment should be accurately recorded.

- Biopsies may be done with local anesthesia or intravenous conscious sedation, according to standard institutional guidelines.
- Research biopsies requiring general anesthesia are not allowed on this protocol unless a biopsy is being obtained simultaneously for clinical reasons, in the judgment of the patients' treating physician.
- Patients who undergo an attempted on-treatment research biopsy and in whom inadequate tissue is obtained are still eligible to continue protocol therapy. They will not be required to undergo a repeat biopsy attempt**.**
- If dosing is delayed placing the biopsy outside of the allowable window, the biopsy should be rescheduled to be within the window. If not feasible, the biopsy should be obtained as close to within the window as possible.
- Fine needle aspirates (FNA) is not allowed

3.1.13 Female subjects of childbearing potential must have a negative serum pregnancy test at screening.

3.1.14 The effects of AZD1775 on the developing human fetus are unknown. Women of child-bearing potential and men must agree to use enhanced methods of contraception. All women are considered to be of childbearing potential unless they fulfill one of the following criteria at screening:

- Post-menopausal defined as age ≥50 and amenorrheic for at least 12 months
  OR
  Women age <50 if they have been amenorrheic for at least 12 months and have a serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level in the postmenopausal range (per institutional standards).
- If women have documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy, or bilateral salpingectomy, or bilateral tubal ligation, they are considered post-menopausal.

3.1.15 Appropriate contraception should be used from the time of screening, throughout the duration of study participation, and for four months after the last dose of AZD1775. Acceptable methods of contraception include abstinence, tubal ligation, intra-uterine devices, and vasectomised partner. All methods of contraception (with the exception of total abstinence) should be used in combination with the use of a condom by the male sexual partner for intercourse. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, the participants treating physician should be informed immediately. Additionally, male patients should refrain from donating sperm from the start of dosing until 6 months after discontinuing AZD1775. If male patients wish to father children they should be advised to arrange for freezing of sperm samples prior to the start of study treatment.

3.1.16 Participant must be able to swallow pills.

3.1.17 Participant may not have a percutaneous endoscopic gastrostomy (PEG) tube or be receiving total parenteral nutrition (TPN).

3.1.18 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Participants who are receiving any other investigational agents within 21 days of the first dose of study drug.

3.2.2 Major surgical procedures <28 days from beginning study treatment.

3.2.3 Participants who have received a prior inhibitor of Wee1 kinase activity

3.2.4 Participants who have received prior platinum chemotherapy

3.2.5 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Patients with a history of treated central nervous system (CNS) metastases are eligible. Treated brain metastases are defined as those having no evidence of progression for ≥ 1 month after treatment, or hemorrhage for >/= 2 weeks after treatment and no ongoing requirement for corticosteroids, as ascertained by clinical examination and brain imaging (magnetic resonance imaging or CT scan) during the screening period. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks before the first study drug. Treatment for brain metastases may include whole brain radiotherapy, radiosurgery, or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 1 month before day 1 of study treatment will be excluded.

3.2.6 Patients with grade >1 neuropathy or grade >1 toxicity (except alopecia or anorexia) from prior therapy

3.2.7 History of allergic reactions attributed to compounds of similar chemical or biologic composition to AZD1775 or Cisplatin.

3.2.8 Participants receiving any medications, substances, or foods (ie, grapefruit juice) listed below are ineligible (Please refer to Section 5.4 for list of restricted co-medications):

- prescription or non-prescription drugs or other products known to be sensitive CYP3A4 substrates or CYP3A4 substrates with a narrow therapeutic index, or to be moderate to strong inhibitors/inducers of CYP3A4 which cannot be discontinued prior to study start and withheld throughout the study until 2 weeks after the last dose of study drug. sensitive substrates of CYP2C8, CYP2C9, CYP2C19, or substrates of these enzymes with narrow therapeutic range
- inhibitors or substrates of P-gp

Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as http://medicine.iupui.edu/clinpharm/ddis/table.aspx; medical reference texts such as the Physicians' Desk Reference may also provide this information. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.9 Participants who have an uncontrolled intercurrent illness, including, but not limited to, ongoing or active infection, uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmia, congestive heart failure-New York Heart Association Class III or IV (Appendix B), active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, gastric or duodenal ulceration diagnosed within the previous 6 months, chronic liver or renal disease, or severe malnutrition. In addition, patients are ineligible if they have a psychiatric illness or a social situation that could limit their ability to comply with the study requirements.

3.2.10 Participants who have refractory nausea and vomiting, chronic gastrointestinal diseases, or previous significant bowel resection that would preclude adequate absorption of AZD1775.

3.2.11 Pregnant women are excluded from this study because AZD1775 is a Wee1 inhibitor agent with the potential for teratogenic or abortifacient effects.

3.2.12 Lactating or breastfeeding women are excluded because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with AZD1775, breastfeeding should be discontinued prior to being treated with AZD1775. These potential risks may also apply to other agents used in this study.

3.2.13 Known HIV-positive participants are ineligible because these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.2.14 Participant with mean resting corrected QT interval (specifically QTc calculated using the Fridericia formula [QTcF]) > 450 msec for males and > 470 msec for females, from 3 electrocardiograms (ECGs) performed within 2-5 minutes apart at study entry, or congenital long QT syndrome.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

Treatment will consist of one cycle (21 days) of cisplatin monotherapy (cisplatin 75 mg/m2 IV) followed by combination therapy of AZD1775 plus cisplatin starting 21 days later. AZD1775 will be administered as twice daily oral dosing (AZD1775 200mg po bid) x 5 doses for 2.5 days, in combination with cisplatin 75 mg/m2 IV every 21 days. Cycle length is 21 days long. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route****	***Schedule***	***Cycle Length***
AZD1775*	See Section 5.4	200 mg	PO	Twice daily x 5 doses From Cycle 2	21 days (3 weeks)
Cisplatin	See Section 5.3.2	75 mg/m2	IV 60 minutes	Days 1 From Cycle 1	

* The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

- ANC must be ≥ 1500/mm^3
- Hemoglobin must be ≥ 9mg/dl
- Platelets must be ≥100,000/mm^3
- Serum creatinine < 1.5mg/dl OR measured creatinine clearance (CrCl) ≥45 mL/min as calculated by the Cockcroft-Gault method OR 24-hour measured urine CrCl ≥45mL/min
- SGOT/SGPT must be ≤2.5 X ULN (≤5 X ULN if known liver metastases)
- Total Bilirubin ≤1.5 mg/dL

5.2.2 Subsequent Cycles

- ANC must be ≥ 1000/mm^3
- Hemoglobin must be ≥9mg/dl
- Platelets must be ≥75,000/mm^3
- Serum creatinine ≤1.5 x ULN, or measured creatinine clearance (CrCl) ≥45 mL/min as calculated by the Cockcroft-Gault method or 24-hour measured urine CrCl ≥45mL/min
- Transaminitis must return to ≤ grade 2
- Diarrhea must be resolved ≤ grade 1.
- Nausea and Vomiting must return to ≤ grade 1
- Neurosensory must be ≤ grade 2
- Other Treatment Related non-Hematologic Toxicity must return to ≤ grade 1

5.3 Agent Administration

5.3.1 *AZD1775*

Dose: 200mg po bid x 5 doses

AZD1775 will be administered bid for two and a half days starting concomitantly with the administration of cisplatin with cycle 2. The patient will take the first dose of AZD1775 in clinic on cycle 2 day 1, and subsequent doses that cycle can be taken at home. On cycle 2 day 1 and subsequent day 1 visits, AZD1775 and cisplatin may be administered concurrently. There is no preferential order of administration. The subsequent 4 additional doses of AZD1775 are to be taken in approximately 12 hour increments. Participants should take AZD1775on an empty stomach and should not have food 2 hours before or 2 hours after the dose. If the participant misses a dose by < 6 hours from scheduled dose, the dose should be taken as soon as possible. If the participant misses a dose by ≥ 6 hours from scheduled dose, the dose should not be made up. If the participant vomits after the administration of AZD1775, the dose should not be readministered. AZD1775 should not be crushed, chewed or dissolved in water.

Premedication:

All patients must receive a 5-HT3 antagonistprior to each dose of AZD1775. In addition, dexamethasone 4 mg PO will be given with each AZD1775 dose as a minimum on the first day of dosing AZD1775 of every 3-5 days dosing period, unless contraindicated or not well-tolerated. Dexamethasone or the 5-HT3 antagonist may be given by IV.

5.3.2 *Cisplatin*

Administration

- **Dose**: Cisplatin 75mg/m^2 IVB q3 weeks (every 21 days, plus minus 3 days for scheduling issues). Cisplatin may be administered according to institutional guidelines at participating sites. The preferred guidelines are as described below.
- **Infusion time**: 60 minutes.
- **Pretreatment hydration**: Hydration may be achieved by IV infusion of 1 liter of sodium chloride 0.9% (±potassium 20 meq) over 1-2 hours prior to Cisplatin infusion.
- **Pretreatment Magnesium Supplementation**: As needed, give prior to Cisplatin, Magnesium Sulfate 2 GM IV×1.
- **Treatment**: Ensure there is adequate urine output averaging at least 100cc/hr prior to Cisplatin infusion. As needed, give 12.5mg IVB of MANNITOL 25% **30 minutes prior** to Cisplatin infusion. Mix Cisplatin in 100-500ml sodium chloride 0.9% and infuse IVB over 60 minutes. **Post-treatment hydration**: Participants should receive 1 liter of sodium chloride 0.9% (±potassium 20 meq) IV over 1-2 hours after Cisplatin administration.
- **Post-treatment hydration:** Participants should receive 1 liter of sodium chloride 0.9% (±potassium 20 meq) IV over 1-2 hours after Cisplatin administration.

Caregiver Precautions:

- Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to environment should undertake the preparation, handling, and safe disposal of chemotherapeutic in a self-contained, protective environment.
- Discard unused portions of infectable chemotherapeutic agents that do not contain a bacteriostatic agent or that are prepared with unpreserved diluents (i.e., sterile water for injection, USP, or 0.9% sodium chloride for injection, USP) within 8 hours of vital entry to minimize the risk of bacterial contamination.
- The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

5.4 General Concomitant Medication and Supportive Care Guidelines

The major pathway for metabolism of AZD1775 involves CYP3A4. Therefore AZD1775 may be prone to drug-drug interactions when co-administered with drugs that are known to be moderate or potent inhibitors/inducers or substrates of CYP3A4.

Prohibited Medication

Patients may receive other medications that the investigator deems to be medically necessary, with the specific exception of non-protocol specified chemotherapy, radiotherapy, anti-neoplastic biological therapy or other investigational agents. Patients who require the use of any of the aforementioned treatments for clinical management should be removed from the study.

Drugs known to be moderate or potent inhibitors/inducers of CYP3A4 or substrates of CYP3A4 with narrow therapeutic windows are generally prohibited. However, some agents (e.g. midazolam, fentanyl, or any other agent used for anesthesia purposes) with which participants will have short term exposure are permissible. List of agents known to be moderate or strong inhibitors/inducers of CYP3A4 or substrates of CYP3A4 with narrow therapeutic widows includes:

- Inhibitors of CYP3A4: azole antifungals (ketoconazole, iraconazole, fluconazole and voriconazole), macrolide antibiotics (erythromycin, clarithromysin), cimetidine, aprepitant, HIV protease inhibitors, and nefazodone

- Inducers if CYP3A4: phenytoin, barbiturates and rifampicin

- Substrates of CYP3A4: statins (lovastain, simvastain [Atorvastatin is also prohibited because of BCRP effect]), midazolam, terfenadine, astemizole, and cisapride

Please refer to Table 5.5-1 for other concomitant medications including a list of inhibitors, inducers and substrates of CYP3A4. This list is not all inclusive, and, the Principal Investigator will determine if they are known to significantly influence CYP3A4. Supportive care with a CYP3A4 inhibitor / inducer / substrate according to institutional guidelines in the context of standard of care chemotherapy will be exempt in some cases provided it is administered within the guidance of the protocol (Please refer to Supportive Care below). Concomitant treatment with aprepitant is not allowable per protocol.

Table 5.5-1
DRUG INTERACTIONS
TABLE OF CYP3A INHIBITORS, INDUCERS, AND SUBSTRATES

EXAMPLES OF CYTOCHROME P450 ISOENZYME INHIBITORS AND INDUCERS			
Inhibitors		**Inducers**	
amiodarone	indinavir	barbiturates	phenobarbital
aprepitant	itraconazole	carbamazepine	phenytoin
cimetidine	ketoconazole	efavirenz	rifampin
ciprofloxacin	mibefradil	glucocorticoids	St. John's Wort
clarithromycin	mifepristone	modafinil	troglitazone
delabiridine	nefazodone	nevirapine	
dilitiazem	nelfinavir		
erythromycin	norfloxacin		
fluconazole	ritonavir		
fluvoxamine	saquinavir		
gestodene	troleandmycin		
grapefruit juice			

http://medicine.iupui.edu/flockhart/

Classification of Substrates

Examples of sensitive CYP3A substrates or CYP3A substrates with narrow therapeutic range			
Sensitive CYP3A substrates[1]		**CYP3A Substrates with Narrow therapeutic range[2]**	
budesonide	midazolam	alfentanil	pimozide
buspirone	saquinavir	astemizole(a)	quinidine
eplerenone	sildenafil	cisapride(a)	sirolimus
eletriptan	simvastatin	cyclospotine	tacrolimus
felodipine	triazolam	diergotamine	terfenadine(a)
fluticasone	vardenafil	ergotamine	
lovastatin		fentanyl	

Note that this is not an exhaustive list.

1. Sensitive CYP3A4 substrates refers to drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a known CYP2A inhibitor.
2. CYP3A substrates with narrow therapeutic range refers to drugs whose exposure-response indicates that increases in their exposure levels by the concomitant use of CYP3A inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).
(a) Not available in the United States.

In light of *in vitro* data which suggests that AZD1775 may be a CYP2C8, CYP2C9, and CYP2C19 inhibitor, caution should be exercised with concomitant administration of AZD1775 and agents that are sensitive substrates of CYP2C8, CYP2C9, CYP2C19, or substrates of these enzymes with narrow therapeutic range. Please refer to Table 5.5-2 for a list of sensitive substrates of CYP2C8, CYP2C9, and CYP2C19, or substrates of these enzymes with narrow therapeutic range. This list is not all inclusive, and for other concomitant medications, the Principal Investigator will determine if they are known to significantly influence CYP2C8, CYP2C9, and/or CYP2C19.

Table 5.5-2
DRUG INTERACTIONS
TABLE OF CYP2C8, CYP2C9, AND CYP2C19 SENSITIVE SUBSTRATES

Sensitive substrates of CYP2C8, CYP2C9, and CYP2C19 and substrates with a narrow therapeutic range for these enzymes
Repaglinide, paclitaxel, warfarin, phenytoin, omeprazole, s-mephenytoin

Note that this is not an exhaustive list.

In light of *in vitro* data which suggests that AZD1775 may be a substrate for human P-glycoprotein (P-gp) caution should be exercised when agents that are inhibitors or substrates of P-gp are administered concomitantly with AZD1775. Please refer to Table 5.5-3 for a list of inhibitors or substrates of P-gp. This list is not all inclusive, and for other concomitant medications, the Principal Investigator will determine if they are known to significantly

influence P-gp.

Table 5.5-3
DRUG INTERACTIONS
TABLE OF INHIBITORS OR SUBSTRATES OF P-GP

Inhibitor of P-gp	Substrates of P-gp
ritonavir, cyclosporine, verapamil, erythromycin, ketoconazole, itraconazole, quinidine	digoxin, fexodenadine, indinavir, vinctistine, colchicines, topotecan, paclitaxel

Note that this is not an exhaustive list.

Supportive Care
Patients should receive optimal supportive care throughout the study, including transfusions of blood and blood products, antibiotics, antiemetics, when appropriate. The reason(s) for treatment, dosage, and dates of treatment should be recorded.

- **Nausea/vomiting**:
  All patients must receive a 5-HT3 antagonist prior to each dose of AZD1775. Additional doses of 5-HT3 antagonist may be used if needed. In addition, dexamethasone 4 mg PO will be given with each AZD1775 dose as a minimum on the first day of dosing AZD1775 of every 3-5 days dosing period, unless contraindicated or not well-tolerated. Dexamethasone may be continued on further days of dosing, potentially at a lower dose. Dexamethasone or the 5-HT3 antagonist may be given by IV.
  Promethazine (Phenergan), prochlorperazine (Compazine), and benzodiazepine may still be used as additional adjunctive treatments during AZD1775 therapy.

  Please note: aprepitant [Emend] and fosaprepitant are not permitted due to known DDIs.

  Patients should be strongly encouraged to maintain liberal oral fluid intake.

  Suitable alternative medications may be used, with adequate justification, where the use of any of the above medications might interfere with other study procedures or are deemed insufficient.

- **Diarrhea**: Diarrhea should be treated promptly with appropriate supportive care, including administration of an anti-diarrheal agent according to standard practice guidelines. Anti-diarrheal agents should not be taken prophylactically. Patients should be instructed to begin taking anti-diarrheal medication at the first sign of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day or 3) unusually high volume of stool. Anti-diarrheal agents should be deferred if blood or mucus is present in the stool or if diarrhea is accompanied by fever. In this setting, appropriate diagnostic microbiologic specimens should be obtained to exclude an infectious etiology. Patients should also be advised to drink liberal quantities of clear fluids to help prevent dehydration. Octreotide can also be considered to aid with management of diarrhea. If diarrhea is severe (ie. requiring IV hydration) and/or associated with fever or grade 3 or 4 neutropenia, broad spectrum

antibiotics should be considered. Additionally, hospitalization for IV hydration and eletrolyte correction should be considered.
Patients should be instructed to notify the Investigator or research staff for the occurrence of blood or black stools, symptoms of dehydration, fever, inability to take liquids by mouth, and inability to control diarrhea within 24 hours of using loperamide or other prescribed antidiarrheal medications.

- **Anemia**: Transfusions and/or erythropoietin may be utilized as clinically indicated for the treatment of anemia, but should be clearly noted as concurrent medications.
- **Neutropenia**: Colony-stimulating factors including G-CSF, pegylated G-CSF or GM-CSF according to Institutional Standards.
- **Febrile Neutropenia:** Patients experiencing febrile neutropenia with significant symptoms should be managed in a hospital setting according to standard procedures, with the urgent initiation of IV antibiotic therapy. Patients with febrile neutropenia without symptoms should be managed according to standard guidelines.
- **Thrombocytopenia**: Transfusion of platelets may be used if clinically indicated.
- Patients requiring radiation or surgery for palliation of a non-target lesion that have not measurably increased in size according to RECIST will be considered to have progressed.
- Participants may receive intravenous fluids on study.
- Bisphosphonates are allowed while on study.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Treatment delay of > 21 days due to toxicity
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF) and entered in the Clinical Trial Management System (OnCore). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify Sara Tolaney, MD MPH at 617-632-3800.

5.6 Duration of Follow Up

Participants will be followed on-study until progression or death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

The participant's treatment status will be updated in OnCore at the time treatment with cisplatin and AZD1775 is discontinued.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Disease Progression (in patients who come off treatment for reasons other than progressive disease)
- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF) and Clinical Trial Management System (OnCore).

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Dose Delays and Modifications for AZD1775

Participants can have a maximum of 2 dose modifications (if applicable) throughout the course of the study for toxicities. Participants who require more than 2 dose modifications may remain on study and continue with cisplatin monotherapy per investigator discretion. In the event that a participant is not willing to dose reduce AZD1775, they may continue with cisplatin monotherapy per investigator discretion.

In the event of toxicity, it is recommended that the investigator first modify the dose of AZD1775; however the investigator may choose to first modify the chemotherapy dose instead, in consultation with the Principal Investigator. If a second dose reduction is necessary during combination therapy, the dose of chemotherapy or AZD1775 may be modified. This decision should be made jointly by the treating physician and the Principal Investigator. Participants who experience toxicity that the investigator finds attributable to AZD1775 will have their AZD1775 dose reduced by 1 dose level as shown in Table 6-1. A participant's dose may be re-escalated at the discretion of the principal investigator.

If Day 1 dosing is delayed, all assessments will be delayed to accordingly.

Table 6-1
DOSE MODIFICATION OF AZD1775

Dose Level	AZD1775 **Dose**
0	200mg, twice daily x 5 doses
-1	175mg, twice daily x 5 doses
-2	150mg, twice daily x 5 doses
-3	125mg, twice daily x 5 doses

Nausea	**Management/Next Dose for AZD1775**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Discontinue treatment and follow for disease progression
*Participants requiring a delay of >3 weeks should discontinue AZD1775. **Participants requiring > two dose reductions should discontinue AZD1775	
All patients must receive a 5-HT3 antagonist prior to each dose of AZD1775. Additional doses of 5-HT3 antagonist may be used if needed. In addition, dexamethasone 4 mg PO will be given with each AZD1775 dose as a minimum on the first day of dosing AZD1775 of every 3-5 days dosing period, unless contraindicated or not well-tolerated. Dexamethasone may be continued on further days of dosing, potentially at a lower dose. Dexamethasone or the 5-HT3 antagonist may be given by IV. Promethazine (Phenergan), prochlorperazine (Compazine), and benzodiazepine may still be used as additional adjunctive treatments during AZD1775 therapy. Please note: aprepitant [Emend] and fosaprepitant are not permitted due to known DDIs. Patients should be strongly encouraged to maintain liberal oral fluid intake. Suitable alternative medications may be used, with adequate justification, where the use of any of the above medications might interfere with other study procedures or are deemed insufficient.	

Vomiting	**Management/Next Dose for AZD1775**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Discontinue treatment and follow for disease progression
*Participants requiring a delay of >3 weeks should discontinue AZD1775. **Participants requiring > two dose reductions should discontinue AZD1775.	
All patients must receive a 5-HT3 antagonist prior to each dose of AZD1775. Additional doses of 5-HT3 antagonist may be used if needed. In addition, dexamethasone 4 mg PO will be given with each AZD1775 dose as a minimum on the first day of dosing AZD1775 of every 3-5 days dosing period, unless contraindicated or not well-tolerated. Dexamethasone may be continued on further days of dosing, potentially at a lower dose. Dexamethasone or the 5-HT3 antagonist may be given by IV. Promethazine (Phenergan), prochlorperazine (Compazine), and benzodiazepine may still be used as additional adjunctive treatments during AZD1775 therapy. Please note: aprepitant [Emend] and fosaprepitant are not permitted due to known DDIs. Patients should be strongly encouraged to maintain liberal oral fluid intake. Suitable alternative medications may be used, with adequate justification, where the use of any of the above medications might interfere with other study procedures or are deemed insufficient. .	

Diarrhea	**Management/Next Dose for AZD1775**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Discontinue treatment and follow for disease progression
*Participants requiring a delay of >3 weeks should discontinue AZD1775. **Participants requiring > two dose reductions should discontinue AZD1775.	
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used. Participants should be instructed to notify their research doctor for the occurrence of blood or black stools, symptoms of dehydration, fever, inability to take liquids by mouth, and inability to control diarrhea within 24 hours of using loperamide or other prescribed antidiarrheal medications. If associated with severe (Grade 3 or 4) neutropenia, broad-spectrum antibiotics must be prescribed. Patients should be considered for hospitalization for IV hydration and correction of electrolytes.	

Neutropenia	**Management/Next Dose for AZD1775**
≤ Grade 1	No change in dose
First occurrence Grade 2	Hold* until ≤ Grade 1. Resume at same dose level.
Second or Third Occurrence Grade 2;	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
First Occurrence Grade 3 or Second Occurrence Grade 3	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
Grade 3 Febrile Neutropenia or Grade 3 Neutropenia with documented infection.	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
Grade 4 or Second Occurrence Grade 4	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
Grade 4 Febrile Neutropenia or Grade 4 Neutropenia with documented infection.	Discontinue.
*Participants requiring a delay of >3 weeks should discontinue AZD1775. **Participants requiring > two dose reductions should discontinue AZD1775.	

Note: Consider using G-CSF per institutional standards for neutropenia or febrile neutropenia.

Thrombocytopenia	**Management/Next Dose for AZD1775**
≤ Grade 1	No change in dose
First Occurrence Grade 2	Hold* until ≤ Grade 1. Resume at same dose level.
Second or Third Occurrence Grade 2	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
First Occurrence Grade 3 or Second Occurrence Grade 3	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
First or Second Occurrence Grade 4 without any evidence of bleeding	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated**
Thrombocytopenia haemorrhage (gross occult bleeding) associated with platelet count <50,000 uL	Discontinue.

Thrombocytopenia	Management/Next Dose for AZD1775
*Participants requiring a delay of >3weeks should discontinue AZD1775. **Participants requiring > two dose reductions should discontinue AZD1775.	

Other Non-Hematologic Toxicities	Management/Next Dose for AZD1775
Grade 1	No change in dose
Grade 2^	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until < Grade 2. Resume at one dose level lower, if indicated.
Grade 4	Hold until < Grade 2. Resume at one dose level lower, if indicated.

- Dose modifications for AZD1775 non-hematologic toxicities should be based on toxicities occurring during the previous cycle.
- For toxicities that lead to a dose reduction, the dose will not be re-escalated during subsequent cycles.
- Any patient who develops a Grade 3 or 4 nonhematologic toxicity that does not resolve to ≤ Grade 1 within 3 weeks should discontinue AZD1775, unless approved by the Principle Investigator.
- Participants requiring > two dose reductions, should discontinue AZD1775.
- ^intolerable Grade 2

6.2 Dose Delays and Modifications for Cisplatin

Cisplatin will be initially administered at a dosage of 75mg/m^2 as an IV infusion given every 3 weeks. Patients who experience a Grade 3 or 4 toxicity attributable to cisplatin will have their cisplatin dose reduced to per Table 6-2. Toxicities resulting in myelosuppression, renal insufficiency, ototoxicity, and marked nausea and vomiting are most commonly seen with cisplatin monotherapy. Assessments should be made immediately prior to the scheduled administration of the chemotherapy. If patient needs to discontinue cisplatin therapy, patient can be treated on protocol with AZD1775 monotherapy.

Table 6-2
DOSE MODIFICATION OF CISPLATIN

Dose Level	Cisplatin Dose
0	75mg/m^2
-1	60mg/m^2
-2	50mg/m^2

Dose delays and modifications are summarized in the toxicity table in Table 6-6.

No dose re-escalations are allowed on study, except where noted below.

Expected toxicities for Cisplatin:

Nephrotoxicity
The major dose-limiting toxicity of cisplatin is cumulative nephrotoxicity. Tubular necrosis of both proximal and distal renal tubules has been noted in 28% to 36% of subjects treated with a single dose of 50mg/m^2. It is first noted during the second week after a dose and is manifested by elevations in BUN and creatinine, serum uric acid and/or a decrease in creatinine clearance. Renal toxicity becomes more prolonged and severe with repeated courses of the drug. Renal function must return to normal before another dose of cisplatin can be given. Nephrotoxicity can be reduced by IV hydration and mannitol diuresis.

Nausea and Vomiting
Cisplatin causes moderate to severe nausea and vomiting in almost all subjects treated. Nausea and vomiting usually begin within 1 to 4 hours after treatment and last up to 24 hours. Various degrees of vomiting, nausea, and/or anorexia may persist for up to 1 week after the treatment. Delayed nausea and vomiting (beginning 24 hours or after chemotherapy) has occurred with complete emetic control on the day of cisplatin therapy. The use of prophylactic and continuing antiemetic medication reduces these adverse effects.

Hypomagnesemia
Hypomagnesemia has been reported in subjects treated with cisplatin and is probably related to renal tubular damage. It may become severe enough to cause tetany. Generally, serum electrolytes return to normal levels when cisplatin is discontinued and supplemental electrolytes are administered.

Ototoxicity
Ototoxicity has been observed in up to 31% of subjects treated with a single dose of cisplatin (50mg/m^2) and is manifested by tinnitus and/or hearing loss in the high frequency range (4000 to 8000 Hz). Decreased ability to hear normal conversational tones may occur occasionally. Ototoxicity can be more severe in children than in adults and more frequent and severe with repeated administration. Hearing loss can be unilateral or bilateral and is usually not reversible. During treatment with cisplatin, it is necessary to monitor hearing at each visit as part of a standard physical exam.

Myelosuppression
Myelosuppression occurs in 25 to 30% of subjects treated with cisplatin. The nadirs in circulating platelets and leukocytes occur between days 18 and 23, with most subjects recovering by day 39. Leukopenia and thrombocytopenia are more pronounced at higher doses (>50 mg/m^2). Anemia (a decrease in hemoglobin of 2 gm/100ml) occurs at approximately the same frequency and with the same timing as leukopenia and thrombocytopenia.

Neurotoxicity
Cisplatin neurotoxicity is characterized by peripheral neuropathies, which are sensory in nature but can also include motor difficulties such as reduced deep-tendon reflexes and leg weakness. The symptoms usually occur after prolonged therapy (4 to 7 months). Cisplatin therapy should be discontinued when serious neuropathy develops. The neuropathy, however, may progress

further even after discontinuation of treatment.

Hematologic Toxicity
Dose adjustment at the start of a subsequent cycle of therapy will be based on the neutrophil and platelet counts on day 1 of the cycle. ANC must be $\geq 1.0\times10^9$/L and platelets $\geq 75\times10^9$/L before the start of each cycle. Treatment should be delayed to allow sufficient time for recovery. Upon recovery, if treatment is resumed, it should be accounting to the guidelines in Table 6-2 with respect to nadir platelet and ANC values for subsequent cycles, except as noted below. A delay in treatment due to hematologic toxicity if more than 3 weeks will result in withdrawal from the study.

The use of colony stimulating factors (filgrastim or pegfilgrastim) is permissible if recommended by the primary treating physician but should be clearly recorded in the medical record and case report form. Prophylactic use of GCSF is not recommended with the first cycle.

If GCSF is initiated, then the required dose modification will be adjusted according to the guidelines in footnote of Table 6-3.

Table 6-3
Dose Adjustments for Cisplatin Based on Hematologic Values for Preceding Cycle

Platelets ($\times10^9$/L)		ANC ($\times10^9$/L)	Dose of Cisplatin
≥75	and	≥1.0	75mg/m^{2*}
≥50	or	≥ 0.5	60mg/m$^{2*,\#}$
< 50	or	<0.5	50mg/m$^{2*,@}$
Recurrence of grade 3 or 4 thrombocytopenia after 2 dose reductions or alone			Withdraw subject from study continue on ADZ1775
Recurrence of grade 3 or 4 neutropenia after 2 dose reductions or alone			Withdraw subject from study continue on ADZ1775

***** Treatment should be delayed until recovery of platelets to $\geq100\times10^9$/L and ANC to $\geq1.0\times10^9$/L.
$^{\#}$ For ANC ≥0.5 treat at 75mg/m^2 dose if initiating GCSF
$^{@}$ For ANC<0.5 treat at 60mg/m^2 dose if initiating GCSF

Non-Hematologic Toxicity
For other grade 3 or higher non-hematologic effects (with the exception of grade 3 transaminase elevations and nausea or vomiting), treatment should be delayed until resolution (less than or equal to baseline). Treatment can be resumed at 60mg/m^2, if deemed appropriate by the treating physician, for all subsequent cycles.

Neurosensory Toxicity

Adjustments of the cisplatin dose in subjects with neurosensory toxicity are shown in Table 6-4. Cisplatin therapy should be held for grade 2 or higher neurosensory toxicity and should resume with a dose reduction once it has resolved to grade 1.

Table 6-4
Dose Adjustments for Cisplatin Based on Neurosensory Toxicity

CTCAE Grade	Dose of Cisplatin
0-1	100% of previous dose
2	60mg/m^2
3-4	Withdraw participant from study or continue on AZD1775 alone

Tinnitus or Significant Hearing Loss
In cases of tinnitus or significant hearing loss, cisplatin therapy should be reduced or stopped, at the discretion of the investigator.

Nephrotoxicity
Cisplatin dose adjustments in subjects with renal toxicity are shown in Table 6-5. Either serum creatinine or estimated creatinine clearance may be used. If a dose delay or dose reduction occurs due to nephrotoxicity, subsequent doses of cisplatin may be escalated based on the renal function on the day of treatment (+/- 3 days) and should be determined based on Table 6-5.

Table 6-5
Cisplatin Dose Adjustments for Renal Toxicity

Creatinine(mg/dl)	OR	Creatine Clearance(cc/min)	Dose of Cisplatin
<1.6		>50	75mg/m^2
1.6-2		10-50	60mg/m^2
>2		<10	Delay one week

Other Toxicity
Patients who develop treatment-related grade 3 non-hematological toxicity (except grade 3 transaminase elevations and nausea or vomiting) should have cisplatin therapy delayed until toxicity has resolved to less than or equal to grade 1 or baseline. Cisplatin will be continued at 60mg/m^2 for subsequent cycles. For grade 3 nausea, vomiting, or transaminase elevation, treatment will be delayed until toxicity resolves to grade 1 or less, and treatment may resume at 75 mg/m^2 dose. If any toxicity, including those described above, results in a three week treatment delay, patients will be taken off protocol. Patients with grade 4 non-hematological toxicity attributed to cisplatin will come off study unless exception is given by the Protocol Chair. Prior to retreatment, toxicity must resolve to less than or equal to grade 1 or baseline. For ≥ grade 2 Hgb toxicity, at the treating physician's discretion, dose may be reduced by one dose level in addition to transfusions and/or growth factor support as clinically indicated, with the option to re-escalate at physician's discretion. This should be documented in the medical record.

Table 6-6: Cisplatin Toxicity Management and Dose Modification Summary Table

Toxicity Event	Dose Modification
Hematologic	
ANC > 1000/mm^3	None
ANC ≥ 500/mm^3	Delay until >1000/mm^3 and reduce to 60mg/m^2 (treat at 75mg/mm^2 if initiating GCSF)
ANC < 500/mm^3	Delay until >1000/mm^3 and reduce to 50mg/m^2 (treat at 60mg/mm^2 if initiating GCSF)
Grade 3 or 4 toxicity after 2 dose reduction	Withdrawal from study or continue
Plt >100,000/mm^3	None
Plt >50,000/mm^3	Delay until >75,000/mm^3 and reduce to 60mg/m^2
Plt <50,000/mm^3	Delay until >75,000/mm^3 and reduce to 50mg/m^2
Grade 3 or 4 toxicity after dose reduction	Withdrawal from study
Gastrointestinal	
Diarrhea	
Grade 3/4 or requiring hospitalization	Delay until resolution and reduce to 60mg/m^2
Vomiting,Nausea	
Grade 3/4	Delay until ≤grade 1 or has returned to baseline and resume at 75mg/m^2 or with one dose reduction (Per investigator discretion)
Transaminases	
Grade 3/4	Delay until ≤grade 2 and with one dose reduction
Neurological	
Neurosensory	
Grade 0-1	None
Grade 2	Delay until resolves to ≤grade 1 and resume with one dose reduction
Grade 3-4	Withdrawal from study
Ototoxicity	
Tinnitus or significant hearing loss	Reduce dose or withdraw from study at investigator discretion
Nephrotoxicity	
Cr <1.6 mg/dl or CrCl >50 cc/min	None
Cr 1.6-2 mg/dl or CrCl 10-50 cc/min	50mg/m^2
Cr>2 mg/dl or CrCl <10-cc/min	Delay one week
Other Toxicity	
Grade 3	Delay until ≤ grade 1 or has returned to baseline and resume at 60mg/m^2

Grade 4	Withdraw from study, unless exception granted by the Principal Investigator

7. ADVERSE EVENTS

7.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.2 Expedited Adverse Event Reporting

7.2.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.2.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.4 Expedited Reporting to AstraZeneca

A serious adverse event is any adverse event occurring at any dose or during any use of AZD1775 that:

- Results in death;
- Is life-threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Serious adverse events that are ultimately related to disease progression do not need to be reported.

SAE information will be sent via secure e-mail connection or via fax within 24 hours of learning of the event. Medwatch 3500A report form with supporting relevant source documents (e.g. history and physical [H&P], hospital discharge summary, autopsy report when available, and results of relevant diagnostic tests completed to evaluate the event) will be attached and sent via:

- Secure email: AEMailboxClinicalTrialTCS@astrazeneca.comor by
- Fax: 1-302-886-4114

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

Follow-up information for SAEs and information on non-serious AEs that become serious should also be reported to AstraZeneca as soon as it is available; these reports should be submitted using the MedWatch 3500A Form. The detailed SAE reporting process will be provided to the sites in the SAE reporting guidelines contained in the trial reference manual.

Investigators must report SAEs and follow-up information to their responsible Institutional Review Board (IRB) according to the policies of the responsible IRB. For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately.

7.5 Routine Adverse Event Reporting

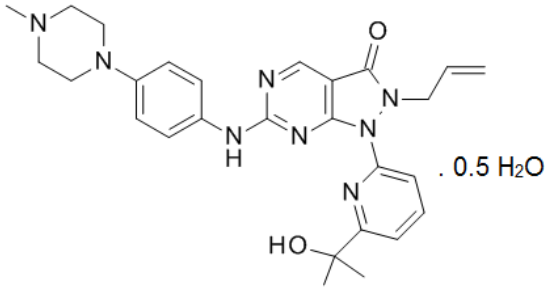
All Grade 2 or higher Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 AZD1775

8.1.1 Description



Systematic chemical name (IUPAC) is 2-Allyl-1-[6-(1-hydroxy-1-methyl-ethyl)-2-pyridyl]-6-[4-(4-methylpiperazin-1-yl)anilino]pyrazolo[3,4- d]pyrimidin-3-one hemihydrate. Other names include: MK-1775 hemihydrate, AZD1775 hemihydrate, and AZ13737568 hemihydrate. The molecular formula is $C_{27}H_{32}N_8O_2 \cdot 0.5H_2O$ and the relative molecular mass is 500.60.

8.1.2 Form

AZD1775 will be provided by AstraZeneca as summarized in Table 8-1.

Table 8-1

Product Descriptions

Product Name & Potency	Dosage Form
AZD1775 25mg	Capsule
AZD1775 100mg	Capsule

Supplies will be packaged in HDPE bottles.

8.1.3 Storage and Stability

Investigational clinical supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated assistants have access.

Storage condition: do not store above 30°C.

The clinical supplies storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the product label attached to the protocol. Documentation of temperature monitoring should be maintained.

8.1.4 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the agent in a self-contained and protective environment.

In accordance with Good Pharmacy Practices, gloves should always be worn by study personnel if directly handling capsules that are returned (i.e. when counting returns). The Clinical Monitor should be contacted with any questions concerning investigational products where special or protective handling is indicated.

8.1.5 **Availability**

AZD1775 will be provided by AstraZeneca.

8.1.6 **Ordering**

Investigative sites will order their own agent. It is necessary to ensure that the pharmacy will be able to receive and store the agent. The IRB should be kept informed of who will supply the agent so that any regulatory responsibilities can be met in a timely fashion.

8.1.7 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.8 **Destruction and Return**

Unused materials will not be returned and should be destroyed according to institutional policy.

8.2 **Cisplatin**

8.2.1 **Description**

The chemical name is cis-diamminedichloroplatinum(II). The Chemical structure is (SP-

4-2)-diamminedichloroplatinum platinum, diaminedichloro-, cis- (8CI). Cisplatin is also known as Platinol, CDDP. The molecular formula is $Cl_2H_6N_2Pt$. The molecular weight is 300.05.

Following cisplatin doses of 20 to 120 mg/m^2, the concentrations of platinum are highest in liver, prostate, and kidney; somewhat lower in bladder, muscle, testicle, pancreas, and spleen; and lowest in bowel, adrenal, heart, lung, cerebrum, and cerebellum. Platinum is present in tissues for as long as 180 days after the last administration.

Cisplatin does not undergo instantaneous and reversible binding to plasma protein that is characteristic of normal drug-protein binding. However, the platinum itself is capable of binding to plasma proteins, including albumin, transferrin, and gamma globulin. Three hours after a bolus injection and two hours after the end of a three-hour infusion, 90% of the plasma platinum is protein bound.

The parent compound, cisplatin, is excreted in the urine. Although small amounts of platinum are present in the bile and large intestine after administration of cisplatin, the fecal excretion of platinum appears to be insignificant.

Cisplatin decays monoexponentially with a half life of 20 to 30 minutes following administrations of 50 or 100 mg/m^2. Cisplatin has a plasma half-life of 30 minutes. The complexes between albumin and the platinum from cisplatin do not dissociate to a significant extent and are slowly eliminated with a minimum half-life of five days or more.

The renal clearance of free (ultrafilterable) platinum also exceeds the glomerular filtration rate indicating that cisplatin or other platinum-containing molecules are actively secreted by the kidneys. The renal clearance of free platinum is nonlinear and variable and is dependent on dose, urine flow rate, and individual variability in the extent of active secretion and possible tubular reabsorption.

8.2.2 **Form**

Commercially available cisplatin injection infusion (1mg/ml): Each ml of sterile, unpreserved solution contains: 1.0mg of cisplatin with 9 mg of sodium chloride and 1mg of mannitol in water for injection. Hydrochloric acid is added to adjust the pH. Single-dose glass vials of 10, 50, and 100mL contain 10, 50, and 100mg of cisplatin respectively.

8.2.3 **Storage and Stability**

Vials of cisplatin injection, USP, are stored at room temperature between 15°C and 25°C. Do not refrigerate or freeze cisplatin solutions, since a precipitate will form. IV needles, syringes, or sets having aluminum components should not be used in preparing or administering cisplatin solutions. An interaction will occur between aluminum and

platinum from cisplatin, causing a black precipitate that is visible in cisplatin solution.

8.2.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.2.5 Availability

Cisplatin is commercially available.

8.2.6 Preparation

Cisplatin should be prepared according to institutional guidelines.

8.2.7 Ordering

Cisplatin is commercially available. Check with the site Director of Pharmacy and/or the site research pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered **before** the protocol is activated.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

All biomarker analyses completed on blood and tissue samples for correlative data will be exploratory.

Wee1 kinase is a regulator of the G2/M checkpoint. As Wee1 phosphorylates CDC2 in response to genotoxic insults, inhibition of CDC2 phosphorylation by a Wee1 inhibitor is considered a direct measure of target engagement. Under normal conditions, pCDC2 levels in tissue is quite low. Changes in pCDC2 is contingent upon pCDC2 induction by DNA damage. Patients will therefore undergo tumor biopsies after one dose of cisplatin, and then again after a dose of cisplatin + AZD1775.

Formalin-fixed, paraffin-embedded (FFPE) biopsy specimens will be subjected to immunohistochemical analyses using standard procedures for: (1) Total CDC2; (2) phospho-CDC2; (3) gamma-H2AX; (4) phospho-Histone 3; and (5) cleaved caspase 3.

For total CDC2, phospho-CDC2, phospho-Histone 3 and cleaved caspase 3, immunohistochemical sections will be scored manually and the % positive cells will be annotated; a minimum of 200 cells will be counted. Additionally, stained slides will be scanned using an Aperio ScanScope XT workstation (Aperio Technology, Inc., Vista, CA). Images will be visualized and digitally annotated (as regions of interest, ROIs) using ImageScope software (version 10.0.35.1800, Aperio Technology). The ROIs will then be analyzed using a standard

analysis algorithm to quantitate the proportion of area that is positive for staining (color deconvolution v9.0, Aperio Technology). The expected result after cisplatin/AZD1775 compared to cisplatin alone is preserved total CDC2 staining, reduced phospho-CDC2 staining, increased phospho-Histone 3 staining and increased cleaved caspase 3 staining. In phase 1 studies, in which skin biopsies were analyzed, a decrease of phospho-CDC2 in the presence of AZD1775 of at least 50% was considered to represent evidence of target engagement.[1] In a study of AZD1775 monotherapy, increases in gamma-H2AX were seen in 3 of 5 paired biopsies.[2] We will analyze changes in phospho-CDC2 (using this parameter alone); additionally, the change in ratio of phospho-CDC/total CDC2 will also be assessed when biopsies post-cisplatin/AZD1775 and post-cisplatin alone are compared.

Immunohistochemistry for gamma-H2AX [pSer139] will be scored manually with a determination of the percentage of cells containing at least 5 nuclear foci.[3,4] It is expected that the percentage of foci-positive cells will be greater after cisplatin/AZD1775 compared to cisplatin alone. This work will be performed in collaboration with Dr. Geoffrey Shapiro, who was the DF/HCC PI on the Phase I trial of chemotherapy combinations with AZD1775, and Dr. Alan D'Andrea, who is internationally recognized expertise in the assessment of DNA damage pathways, and with Dr. Scott Rodig, a pathologist in the DF/HCC Pathology Core at Brigham and Women's Hospital, who has extensive experience with analysis of these markers.

The study will obtain paired tumor biopsies for all patients enrolled with accessible disease (after cisplatin monotherapy and after cisplatin + AZD1775 combination therapy). The analysis will treat the quantification of pCDC2, gamma-H2AX, phospho-Histone 3, and cleaved caspase 3 as continuous variables. To evaluate change in these marker levels after the addition of AZD1775, descriptive statistics for the change will be generated. Paired t-tests or Wilcoxon signed rank test, whichever is appropriate considering the data distribution, will also be used to describe change in marker levels.

Biopsy samples will also be subjected to whole exome sequencing (WES) to capture (1) BRCA reversion mutations as well as the mutation status of most genes involved with double strand break repair (2) evidence of telometric allelic imbalance, a predictor of cytotoxic therapy response[5], (3) overall mutational signature, and (4) cell cycle genes related to G1/S checkpoint, that may enhance reliance on Wee1-mediated G2/M checkpoint for proper DNA repair.[6]

The prevalence of BRCA 1 and BRCA2 mutations is approximately 10-15% among patients with triple-negative breast cancer.[37] Breast cancers with BRCA deficiency may be more susceptible to increases in DNA damage provoked by combination therapy with cisplatin and AZD1775. For patients in whom BRCA status is unknown, anonymized blood samples will be undergo testing for BRCA 1 and 2. We will determine whether there is an association between germline BRCA deficiency and response to therapy.

To evaluate the relationship between BRCA status and response, the data will be tabulated and Fisher's exact test will be applied, recognizing that only large differences are detectable if the overall response rate is 30% (78% power to detect response rate of 83% among BRCA+ and 20% among BRCA- assuming 15% of patients are deemed BRCA+ and data are available for all 35 patients).

9.2 Laboratory Correlative Studies

Table 9-1 Biomarker sample collection schedule

Sample	Specimen	Visit
FFPE Archival Tumor	Tumor	Screening
Research Biopsy	Tumor	Cycle 1 Day 1, 5-48hrs after Cisplatin Administration and Cycle 2 day 3, 5-8hrs (+/-24hrs) after AZD1775 administration
cfDNA	Blood (EDTA-purple top)	Cycle 1 Day 1 Restaging visits Time of Progression

9.2.1 Circulating cell free DNA Collection

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of cell-free DNA (cfDNA). The cfDNA will be processed and banked in the DF/HCC Clinical Trials Core laboratory for future research purposes. The banked samples will be used to analyze DNA, RNA and protein in future studies.

After shipment to and storage within the DF/HCC Breast Bank, cfDNA plasma samples will be sent to the Broad Institute of MIT by the DFCI study team, under the care of Dr. Nikhil Wagle, for sequencing. Using standard lab software, the Broad Institute will create randomly generated barcode labels with a sample number. The DFCI study team will label the plasma specimens with the labels provided and complete an Excel spreadsheet that correlates the barcode label with the patient's study ID and the timepoint of the sample. All samples will be de-identified and the Broad Institute, Dr. Wagle, and his staff, will not, in any way, be able to determine the identity of the patient.

9.2.1.1 Collection of Specimen(s)

20 ml of whole blood will be collected into 2 EDTA-containing purple top tubes. The blood sample will be collected and processed at baseline, restaging visits and time of progression for evaluation of cfDNA. Fill the tubes completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. The banked samples will be used to analyze DNA, RNA and protein in future

studies.

9.2.1.2 Handling of Specimens(s)

20 ml of whole blood will be collected into 2 EDTA-containing purple top tubes. The blood sample will be collected and processed at baseline, restaging visits and time of progression for evaluation of cfDNA. Fill the tubes completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results.

9.2.1.3 Shipping of Specimen(s)

Tubes must be delivered to DFCI for processing within 2 hours of collection at ambient temperature to:

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Tube precautions:
- Do not use tubes after expiration date.
- Fill the tube completely; overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytical results.

Shipping Note: Lavender tube samples are sent ambient.

9.2.2 **Archival Tissue Collection**

Formalin-fixed, paraffin-embedded (FFPE) biopsy specimens will be subjected to immunohistochemical analyses using standard procedures for: (1) Total CDC2; (2) phospho-CDC2; (3) gamma-H2AX; (4) phospho-Histone 3; and (5) cleaved caspase 3 and other relevant markers.

9.2.2.1 Collection of Specimen(s)

Formalin-fixed, paraffin-embedded (FFPE) or 15 unstained slides will be collected from participants archival tissue.

9.2.2.2 Shipping of Specimen(s)

Ship within 24 hours of collection at ambient temperature to:

Dana-Farber Cancer Institute
Attn: Arielle Brackett
Dana 157
450 Brookline Avenue
Boston, MA 02215
Abrackett@partners.org

Email the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

9.2.2.3 Site(s) Performing Correlative Study

DFCI/BWH

9.2.3 **Biopsy Collection**

The study will obtain paired tumor biopsies for all patients enrolled with accessible disease (after cisplatin monotherapy and after cisplatin + AZD1775 combination therapy). Paired biopsies will be used to evaluate changes in pCDC2, gamma-H2AX, phsopho-Histone 3, and cleaved caspase 3 and other relevant markers. Additionally, the biopsies will be subjected to whole exome sequencing (WES) to capture BRCA reversion mutations (and the mutation status of most genes involved with double strange break repair), evidence of telometric allelic imbalance, and overall mutational signature.

After shipment to and storage within the DF/HCC Breast Bank, a portion of the paired biopsy samples will be sent to the Broad Institute of MIT by the DFCI study team, under the care of Dr. Elie Van Allen. Using standard lab software, the Broad Institute will create randomly generated barcode labels with a sample number. The DFCI study team will label the biopsy specimens with the labels provided and complete an Excel spreadsheet that correlates the barcode label with the patient's study ID and the timepoint of the sample. All samples will be de-identified and the Broad Institute, Dr. Van Allen, and his staff, will not, in any way, be able to determine the identity of the patient.

9.2.3.1 Scheduling and Feasibility of Research Biopsies

The treating investigator will indicate the best lesion to target for potential biopsy, which will be ordered and scheduled per DFCI or BWH's institutional guidelines.

For research biopsies that are performed in the Cross-Sectional Interventional Radiology (CSIR) department, it will be expected that biopsy feasibility be reviewed within 24 hours of the scheduled procedure. If the radiologist determines that the selected area is unsafe or inappropriate in any way, the Radiologist will page the ordering provider to discuss the best course of action.

As biopsies for this trial may be occurring for research purposes only, with no additional clinical benefit to subjects, the threshold for determining safety and feasibility of the procedure must be lower than what might typically be used in determining safety and feasibility of a biopsy for clinical purposes. For this reason, the ordering provider will include information in the biopsy order comments that indicates that these biopsies are being performed for research purposes only and that the radiologist must use their best clinical judgement to determine if the procedure is unsafe. If at any point, a radiologist questions the safety of the procedure in question, they should not proceed.

9.2.3.2 Collection of Specimen(s)

Biopsies will be collected at C1D1 5-48hrs after Cisplatin adminsitration and at C2D3 within 5-8hrs (+/- 24hrs) after the last dose of AZD1775 administration. If dosing is delayed placing the biopsy outside of the allowable window, the biopsy should be rescheduled to be within the window. If not feasible, the biopsy should be obtained as close to within the window as possible.

Tissue specimens will be collected from recurrent or metastatic lesions using standard institutional procedures. The amount of tissue collected will follow the guidelines listed below. If a participant has more than one site of disease, only one site needs to be biopsied in order to go on to the study and the site is left to the discretion of the patient and their treating physician. Fine need aspirates (FNA) is not allowed. Participants who undergo a research biopsy procedure for the purpose of this protocol, and in whom inadequatetissue is obtained, are still eligible and are not required to undergo a repeat biopsy in order toenter the study.

Breast: A goal of 3-6 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass.

Skin/chest wall: A goal of 1-2 5-mm punch biopsies will be obtained.

Lymph node: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules will be performed on this protocol, unless they are clinically indicated.

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a participant has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13gauge needle.

Pleural Fluid: A goal of 500 cc of pleural fluid will be obtained with a standard thoracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Ascites fluid: A goal of 500 cc of ascites fluid will be obtained with a standard paracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Please note that the above are guidelines for the amount of tissue to be obtained at thebaseline biopsy, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure. If ascites or pleural fluid is to be used as the investigational biopsy specimen, consideration should be given to confirming the malignant nature of the ascites or pleural fluid prior to study entry.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue stored for research at the time of the procedure (provided that the tissue is processed as specified in Section 9.2.1.2), in which case, the participant would not be required to undergo a separate research biopsy for entry into this protocol.

9.2.3.3 Handling of Specimens(s)

Core biopsy specimens will be handled and processed at the time of biopsy collection. Ideally, sufficient core biopsy samples will be obtained to allow for some to be frozen (after embeding in OCT) and others to be fixed in formalin and subsequently embedded into paraffin blocks. The specific instructions for handling core biopsy material is provided in Appendix C.

9.2.3.4 Shipping of Specimen(s)

Ship within 24 hours of collection overnight on dry ice to:

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Genomic sequencing as described will be performed at the Broad Institute.

9.2.3.5 Site(s) Performing Correlative Study

Dana-Farber Cancer Institute

9.2.3.6 Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:

Breast (core biopsy):

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Skin/chest wall (punch biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, or infection

Lymph node, liver, or bone (core needle biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, damage to adjacent organs. Additional risks may be present if

intravenous conscious sedation is required

Pleural fluid (thoracentesis):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization

due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs

Ascites fluid (paracentesis):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs. In order to minimize the risk of a biopsy, only qualified personnel will perform these procedures.

Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain separate procedure consent. Patients will be evaluated for comorbidities or concomitant medications that may increase the risk of potential complications. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After lymph node biopsies, patients will be observed a minimum of 2 hours (range 2-4 hours) after the procedure, or according to standard institutional guidelines. After liver biopsies, patients will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

9.2.3.7 Risks of Anesthesia

Local Anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation

Certain biopsy procedures, such as lymph node, liver, or bone biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000[47]. The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia. In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation-the physician performing the biopsy procedure, and

the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, patients will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia

Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *arenot permitted* on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

9.2.3.8 Cyclic Immunofluorescence Analysis

Cyclic immunofluorescence (cyCIF) will be performed on paired biopsy specimens to characterize immune cell (lymphocyte and myeloid) populations and their spatial relationship. There are a total of eight participants who have these tissues available in whom this analysis will be performed. De-identified biopsy specimens will be shared with Harvard Medical School, specifically the laboratory of Peter Sorger, PhD. Any leftover specimens and all data generated from this will be returned to and become property of DF/HCC.

9.2.3.8.1. Waiver of Consent

When these participants initially consented to the trial, specimen and data sharing / future use was not a required part of the DF/HCC biomedical consent template and since that time has become a critical part of any external collaboration. Seven of eight participants whose tissue will be used for cyCIF analyses have passed away and cannot provide re-consent to these procedures. At this time, they are no longer human subjects. The eighth patient is currently lost to follow-up and the risk of privacy breach in trying to locate them is a greater risk that using their tissue for this analysis.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy unless otherwise specified. Informed consent, scans and x-rays must be done $\leq$4 weeks prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted. End of treatment assessments may be performed within 30 days of treatment discontinuation.

	Baseline	Cycle 1 Day 1	Cycle 2 Day 1	Cycle 2 Day 21	Cycle 3 Day 1	Cycle 4+ Day 1	Cycle 4+ Day 21	End of Treatment	Follow-Up[l]
AZD1775[a]			X		X	X			
Cisplatin[b]		X	X		X	X			
Informed consent	X[m]								
Demographics	X								
Medical history	X								
Physical exam	X	X	X		X	X		X	
Vital signs[c]	X	X	X		X	X		X	
Weight[d]	X	X	X		X	X		X	
Height	X								
Performance status	X	X	X		X	X		X	
Adverse Event Assessment		X	X		X	X		X	
CBC w/diff, plts	X	X	X		X	X		X	
Serum chemistry[e]	X	X	X		X	X		X	
B-HCG[f]	X								
EKG[g]	X							X	
Radiologic evaluation[h]	X			X			X		X
Archival tumor tissue	X								
Research biopsy[i]		X	X						
cfDNA Blood collection[j]	X				X[j]	X[j]		X[j]	
Blood for genomic sequencing[k]		X							

a. AZD1775: 200mg (PO) BID beginning with Cycle 2 Day 1. The patient will take 5 doses of AZD1775 each cycle. Day 1 dosing is to be done in the clinic; subsequent doses can be taken at home. The 4 additional doses of AZD1775 are to be taken in approximately 12 hour increments. Patients should take AZD1775 either two hours before or two hours after a meal.
b. Cisplatin: 75mg/m^2 (IV) given on Day 1 of each 21-day cycle (+/- 3 days)
c. Vital signs should be collected pre-dose on dosing days and are to include measurements of heart rate, respiratory rate, systolic and diastolic blood pressure while the patient is in a seated position, and oral or tympanic temperature.
d. Weight adjustments for cisplatin will be done according to institutional standards.
e. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, magnesium
f. Serum pregnancy test (women of childbearing potential as defined in the eligibility criteria).
g. A standard, triplicate12-lead EKG will be collected baseline. A standard, single EKG will be collected at the end of treatment. EKGs may be collected during protocol treatment if clinically indicated.
h. Radiologic measurements should be performed every 2 cycles/6 weeks (+/-7 days). Participants who have their treatment held or delayed will continue to have restaging scans that align with cycles of treatment received, rather than weeks on study. Participants taken off study for reasons other than progressive disease should continue to have radiologic assessments completed every 6-9 weeks until progression.
i. All patients with tumors that is accessible to biopsy will undergo research biopsies 5-48 hours after cisplatin monotherapy (Cycle 1 Day 1) and after the combination therapy (Cycle 2 Day 3, 5-8hrs (+/- 24hr) after AZD1775). If dosing is delayed placing the biopsy outside of the allowable window, the biopsy should be rescheduled to be within the window. If not feasible, the biopsy should be obtained as close to within the window as possible.
j. At baseline, restaging visits, and at time of progression, two 10 mL blood samples will be drawn into EDTA-containing "purple top" test tubes or Streck Tubes for cfDNA analysis. If sample collection is missed for any reason at baseline or at the time of progression then the sample should be drawn at a future appointment.
k. Blood sample for genomic sequencing will be collected at Cycle 1 Day 1
l. Participants will be contacted q 6 months for survival.
m. Informed consent must be provided before any study-specific procedures are performed and within 28 days of registration.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 2 cycles of treatment or approximately every 6 weeks. .

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 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 criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have initiated study treatment, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one 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.

11.1.2 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 or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might be considered measurable if the investigator thinks it appropriate to include them.

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

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, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered 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 participant, 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. 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.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. 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 in 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 thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches

may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine).The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For adults 3 drops t.i.d. Participants are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it

cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 Response Criteria

11.1.4.1 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.

11.1.4.2 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 patient 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).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 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 patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
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 baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants 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 Participants 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*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* '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

11.1.5 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, or

death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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.

11.1.6 Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7 Response Review

Central Review will be conducted by the DF/HCC Tumor Imaging Metric Core for DF/HCC Institutions.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a single-arm, two-stage phase 2 study to evaluate the response to AZD1775 in combination with cisplatin in patients with triple-negative breast cancer. The primary endpoint is to investigate objective response rate, and secondary endpoint will examine changes in pCDC2, as well as markers of checkpoint bypass, DNA damage and apoptosis in tumor tissue. Patients may have received 0-1 prior chemotherapeutic regimen for metastatic breast cancer. The number of patients with 0 prior chemotherapeutic regimen will be limited to a maximum of n = 20.

There is data to suggest that in patients with ≤1 prior chemotherapy for triple-negative disease, response to ranges from 10-37%. A recent study that looked at the activity of platinum therapy for first or second line treatment of metastatic triple-negative disease found an overall response rate of 25.6%. The response rate in the first line setting was 29% and in the second line setting was 12%.[39] Another study, found a response rate of 10.3% to cisplatin monotherapy in patients who had received ≤ 1 prior line of chemotherapy.[40]

Based on the maximum enrollment of patients receiving first line chemotherapy for metastatic triple-negative breast cancer, aa rate response of no more than 20% to cisplatin alone would be anticipated. A response rate of 40% would be of clinical interest for the combination of cisplatin with AZD1775.

The study uses a Simon optimal two-stage design with a one-sided type I error of 0.1 and type II error of 0.1 (90% power) to detect the difference between null (20%) and alternative (40%) objective response rates. In the first stage, 17 patients will be entered. If there are at least 4

responses, the study will continue to stage 2 where another 20 patients will be enrolled. If there are 10 or fewer responses among the 37 patients, the regimen will be declared not worthy of further study. If there are 11or more responses (>30%), the regimen will be declared worthy of further study in a randomized setting for patients with metastatic triple-negative breast cancer comparing cisplatin to cisplatin + AZD1775. If the true response rate is 20%, the chance the regimen is declared ineffective is approximately 90% (exact alpha=0.095). If the true response rate is 40%, the chance that the regimen is falsely declared ineffective is 10% (exact power=0.903).

13.2 Analysis of Primary Endpoints

For this phase II trial, the analysis population is all patients who initiate protocol regimen. All patients who receive at least one dose of protocol regimen will be evaluable for objective response every 6 weeks from the time of their first treatment. The primary analysis will evaluate the objective response rate per RECIST 1.1. We will estimate this percentage and a corresponding 95% confidence interval (CIs) will be calculated.

13.3 Analysis of Secondary Endpoints

PFS is defined as the time from enrollment until disease progression or death, whichever occurs first. Patients alive and free from disease progression will be considered censored. PFS will be estimated by the method of Kaplan and Meier.

The study requires paired biopsies from all patients with accessible tumor. With a sample size of 37 and assuming that 50% of them either without accessible lesions or assay failure, paired biopsy assessment are available for 18 patients. Assuming the percent of pCDC2 positive cells decreases 50% in the presence of AZD1775 and the standard deviation of pCDC2 equals to the difference, using t statistics with 1-sided alpha of 0.05, there will be 99% power to detect the change in pCDC2 pre- and post-treatment. The calculation was done using East v6.3 (Cytel Inc). Analysis plan for all biomarker data was written in section 9.1.

Difference of % CDC2 positive cells in paired samples	S.d. of paired difference	Power
0.5	0.5	99%
0.5	0.75	86%
0.5	1	65%

Descriptive statistics will be used to characterize the tumor genomic profiles available for DFCI participants by disease response to therapy (response assessed by RECIST 1.1). PFS and ORR will be estimated using Kaplan-Meier methods among patients with and without certain gene mutations, respectively. The Benjamini-Hochberg procedure will be used to control the false discovery rate and address multiple comparisons.

13.4 Reporting and Exclusions

13.4.1 Evaluation of Toxicity

All participants who have received at least one dose of study medication will be evaluable for toxicity from the time of their first treatment.

13.4.2 Evaluation of Response

All participants who have received at least one dose of study medication will be assessed for response.

Patients would be replaced if she/he withdraws study consent prior to initiation of protocol therapy.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA[41]

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

APPENDIX B New York Heart Association Heart Failure Classifications

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

APPENDIX C: Tissue Acquisition Guidelines

Core needle tumor biopsies (18-gauge) will be acquired with 3-6 cores each at the following timepoints:

- Cycle 1 Day 1 5-48hrs after Cisplatin administration
- Cycle 2 Day 3 5-8hrs (+/- 24hrs) after AZD1775 administration

The order of specimen collection should be:

- First core: Frozen OCT
- Second core: Frozen OCT
- Third core: Frozen OCT
- Fourth core: 10% neutral buffered formalin
- Fifth core: Frozen OCT
- Sixth core: Frozen OCT
- Seventh core: 10% neutral buffered formalin

Supplies:

- Tissue-Tek® Cryomold,
  - Standard size 25x20x5mm (#4557), Fisher Scientific Cat# NC9511236
    -OR-
  - Intermediate size 15x15x5mm (#4566), Fisher Scientific Cat# NC9542860
- Tissue-Tek® O.C.T. Compound, 4oz (#4583): Fisher Scientific Cat# NC9638938
- Small specimen bag: Fisher Cat# 01-002-37 (or equivalent)
- Forceps: Fisher Cat# NC9832137 (or equivalent)
- Dry Ice and Cooler
- Cryoware Pen: Fisher Cat# 13-382-88
- 10mL – 10% Formalin: Fisher Cat#032-065

Procedure for Freezing samples in OCT:

- Prepare cryomolds ahead of time: Squeeze small amount of OCT into cyromolds* – enough for there to be a thin layer covering the bottom let settle on a flat surface for a minute or two. It is *crucial* for future sectioning that these core biopsies are placed on a very flat OCT surface, so the best way to make sure these mold are flat is the *freeze the bottom layer on a -80 freezer shelf*.

  *If time permits, the cryomolds can be placed onto the dry-ice once a thin layer of OCT has been put it but before the tissue is put in. Once this is frozen or begins to freeze, the tissue can be placed on top of the now frozen OCT and then covered with more liquid OCT and then placed back onto the dry ice to freeze completely.
- Bring all supplies needed to the biopsy as the tissue needs to be frozen immediately.
- As soon as the specimen is ready, use disposable forceps to gently place only 1 core into each cryomold. Gently pick up the tissue with the forceps from one end of the core biopsy, being careful not to crush the tissue and immediately lay out the fresh biopsy tissue onto the center of the mold. Be sure to lay the tissue as flat to the mold, and as straight as possible. Keep molds on dry ice at all times.
- You should ideally collect between 3 and 6 separate cores.
- Once the specimen is in the cryomold, cover with more OCT making sure the tissue is

entirely submerged.

- Immediately place cryomold with OCT and tissue onto dry-ice making sure the cryomold is level and will not tip over.
- The OCT will freeze into a solid white block within 5-10 minutes
    - Once the blocks have completely frozen they can be put into a specimen bag and sealed. More than one block can be put into a bag.
    - The bag should be labeled with:
        - Patient name
        - DFCI Study #:
        - DFCI MRN #
        - Date of biopsy
        - Time Point
        - Number of blocks in the bag
    - Samples should be kept on dry ice at all times until they can be placed in a -80ºC freezer. Samples should remain frozen at -80ºC until shipment.

All biopsy samples should be brought to the Clinical Trials Core Lab:

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Procedure for Samples Placed in Formalin:

- Immediately place the specimen in a container of 10mL formalin.
- The container and/or bag should be labeled with:
    - Patient name
    - DFCI Study #:
    - DFCI MRN #
    - Date of biopsy
    - Time Point
    - Number of blocks in the bag

All biopsy samples should be brought to the Clinical Trials Core Lab to be processed into formalin fixed paraffin embedded blocks (FFPE):

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org