Abbreviated Title: ONC201 in advanced cancers

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Title: A Phase 2 Study of ONC201 in recurrent/refractory metastatic breast cancer and advanced endometrial carcinoma

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Investigational Agents:

Drug Name:	ONC201
IND Number:	136939
Sponsor:	Center for Cancer Research
Manufacturer:	Oncoceutics, Inc

PRÉCIS

Background:

- Advanced breast cancer and endometrial cancer have limited treatment options. Current treatments provide a modest improvement in progression free survival but no treatments improve survival.
- ONC201 is the founding member of a novel class of anticancer drugs called imipridones. The exact mechanism of ONC201 is unknown at this time, but preclinical data suggests that it causes global downregulation of mitochondrial genes leading to mitochondrial damage and ultimately non-apoptotic cell death.
- Preclinical studies have demonstrated that ONC201 selectively kills various cancer cells, including breast cancer cells (hormone-receptor positive cell lines, HER2+ cell line as well as triple negative breast cancer cell lines) and endometrial cancer cells, while having little effect on normal cells.
- An on-going phase I study of ONC201 has demonstrated clinical benefit in some solid tumors, including endometrial cancer.

Objectives:

- Cohort 1: To determine the progression free survival (PFS) at 8 months of ONC201 in metastatic hormone receptor positive breast cancer (HR+BC)
- Cohort 2: To determine the overall response rate (ORR) of ONC201 in metastatic triple negative breast cancer (TNBC)
- Cohort 3: To determine the ORR of ONC201 in advanced endometrial cancer (EC)

Eligibility:

Selected Inclusion Criteria

- Histologically confirmed metastatic breast cancer or endometrial cancer with appropriate IHC testing and confirmation of HER2 non-amplification required for the breast cancer cohorts (cohorts 1 and 2)
- Age 18 years or older
- Female and male breast cancer patients are eligible for the breast cancer cohorts
- ECOG 0 or 1
- Measurable metastatic disease with $\geq$1 biopsiable lesion with willingness to undergo a biopsy
- Cohort 1 (HR+BC) requires prior treatment with $\geq$2 lines of hormonal treatment. No prior treatment required for cohorts 2/3 (TNBC and EC).
- Adequate hematopoietic, hepatic and renal function

Selected Exclusion Criteria

- Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents within 3 weeks or a PD1/PDL1 agent within 4 weeks prior to first dose of study treatment.

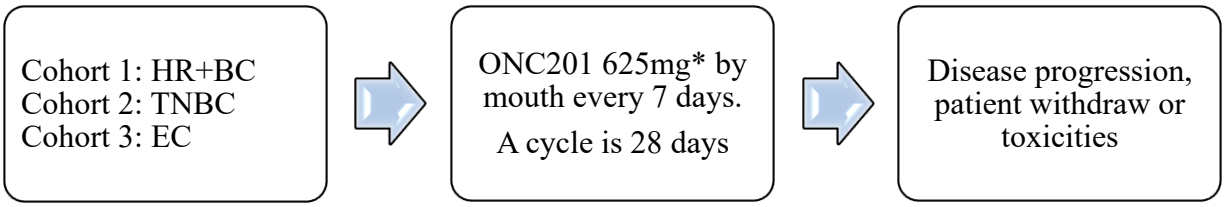
- Radiotherapy ≤ 4 weeks before first dose of study treatment
- Symptomatic CNS metastases. Asymptomatic or brain metastases treated > 4 weeks from first dose of study treatment are allowed.
- History of invasive malignancy ≤ 3 years
- Known history of cardiac arrhythmias including uncontrolled atrial fibrillation, tachyarrhythmias or bradycardia.
- History of CHF, or MI or stroke in the previous 3 months will be excluded.
- Started denosumab or bisphosphonate therapy within 28 days prior to Cycle 1 Day 1
- HIV, Hepatitis B, or Hepatitis C infection

Design:

- This is a phase II single arm study of ONC201 divided in three cohorts, each cohort with a different type of metastatic, advanced disease:
    - Cohort 1: HR+ breast cancer (male and female)
    - Cohort 2: Triple negative breast cancer (male and female)
    - Cohort 3: Endometrial cancer (female only)
- All patients will receive ONC201 at the recommended phase 2 dose (RP2D) of 625mg by mouth every 7 days with each cycle being 28 days long. Patients will receive ONC201 as long as they derive clinical benefit or toxicity becomes impeditive
- Patients will be evaluated for toxicity every 4 weeks by CTCAE v5.0 and for response every two cycles (8 weeks) by RECIST 1.1.

STUDY SCHEMA

Study Schema Figure



*See **Table 1** for dose reductions

Table 1: ONC201 Dose Reduction Schedule

Dose Level (DL)	**ONC201 (by mouth)**
1 (Starting Dose)	625mg every 7 days
-1	500mg every 7 days
-2	375mg every 7 days
-3	250mg every 7 days

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Cohort 1 HR+BC - To determine the progression free survival at 8 months (PFS by RECIST) of ONC201 in patients with refractory, metastatic hormone receptor positive breast cancer
- Cohort 2 TNBC - To determine the overall response rate (ORR; CR + PR by RECIST) of ONC201 in patients with metastatic triple negative breast cancer
- Cohort 3 Endometrial Cancer - To determine the ORR of ONC201 in patients with advanced or metastatic endometrial cancer

1.1.2 Secondary Objectives

- Cohort 1 HR+BC – Safety, ORR, clinical benefit rate (CBR; CR + PR + SD by RECIST)
- Cohort 2 TNBC – Safety, CBR, PFS
- Cohort 3 Endometrial Cancer – Safety, CBR, PFS

1.1.3 Exporatory Studies:

The primary goal of the exploratory studies is to identify potential predictive biomarkers of response as well as additional evidence of the mechanism of action of and resistance to ONC201. These will be performed in an exploratory fashion on select patients if there are adequate samples for analysis.

1.2 BACKGROUND AND RATIONALE

This is a phase II study of ONC201 for the treatment of metastatic hormone receptor positive breast cancer, metastatic triple negative breast cancer, and endometrial cancer. At this time, there is no clear benefit to the combination of ONC201 plus anti-HER2 therapies. However, we plan to develop preclinical data for ONC201 in combination with HER2 targeted drugs (*e.g.*, trastuzumab, trastuzumab emtansine, and lapatinib) and we anticipate initiating a Phase I run-in study at a later date.

1.2.1 Current Standards of Care for Recurrent/Refractory Invasive Breast Cancer

Invasive breast cancer remains the most common malignancy in women in the United States with an annual incidence of 123.1 cases per 100,000 women (5). Treatment of invasive breast cancer involves local control with surgery and/or radiation where appropriate, as well as systemic control with endocrine therapy, HER2 targeted therapy, and chemotherapy. Despite advances in early detection and effective treatments, breast cancer is the second most common cause of US cancer deaths in women with an annual rate of 21.9 deaths per 100,000 women (5). It is estimated that in 2017, there will be 252,710 new cases of breast cancer with 40,610 deaths (6).

1.2.1.1 Hormone Receptor Positive Breast Cancer

Hormone receptor positive breast cancer (HR+BC) accounts for approximately 75% of all breast cancers and is defined by immunohistochemical (IHC) staining of at least ≥ 1% of tumor cells with estrogen receptors and/or ≥ 1% with progesterone receptors. Despite optimal treatment, up to 30% of women with node-negative breast cancer recur after their initial diagnosis. While the risk is greatest within the first two years of diagnosis, women with HR+BC have an increased risk for recurrence up to 25 years after diagnosis (7, 8). Recurrence can be loco-regional or metastatic. Once metastatic, there are limited effective treatments and overall prognosis is poor.

Generally, patients with metastatic or recurred disease are first treated with endocrine therapy alone (e.g. tamoxifen, aromatase inhibitors, or fulvestrant) or, more recently, endocrine therapy in combination with CDK 4/6 inhibitors or mTOR inhibitors (9). However, once the cancer is determined to be hormone refractory, chemotherapy is the next treatment option.

Combination chemotherapy regimens have higher response rates and longer progression free survival (PFS), but with higher toxicity profiles and no added survival benefit when compared to single agent chemotherapy regimens (10). Therefore, the use of sequential single agents is the favored regimen once the cancer is refractory to endocrine therapy. Preferred single agents, like paclitaxel or capecitabine, carry a PFS of 5.7 to 8.0 months (11, 12). Both endocrine therapy and chemotherapy in this setting have been shown to have palliative benefit but no curative therapies exist.

1.2.1.2 Triple Negative Breast Cancer

Triple-negative breast cancer (TNBC) accounts for 10-20% of all breast cancers, and is defined by the absence of estrogen and progesterone receptors (ER, PR) and the absence of HER2 amplification or over-expression (13). Thus, patients with TNBC do not benefit from known targeted therapies to ER or HER2 (14). Standard therapy for metastatic TNBC is single agent chemotherapy and, in metastatic patients, ORRs are around 30-35%, with median PFS 4.5-6 months, and median OS around 12 months (15). Clearly, new approaches to the treatment of TNBC are needed to improve outcomes.

1.2.2 Current Standards of Care for Endometrial Cancer

Endometrial adenocarcinoma is the most common malignancy of the female genital tract with an estimated 61,380 new cases and 10,920 deaths in 2017 (6). While endometrial cancer is often found in early stages due to irregular vaginal bleeding in a predominantly postmenopausal population, there has been a recent increase in cancer-related mortality (16). This is suspected to be due to an increase in advanced-stage cancers, high risk histologies (e.g., serous carcinomas or clear cell carcinomas) and patients being diagnosed at an older age (16). In addition, there is an increased risk of endometrial cancer in patients with Lynch Syndrome (17).

Multi-agent chemotherapy regimens are preferred in the relapsed or advanced/metastatic setting. Current first line chemotherapy regimens have response rates ranging from 21% to 81%. These

responses are of short duration with median progression free survival of approximately one year (18, 19). Due to a relatively low toxicity profile and multiple studies demonstrating improved survival, carboplatin and paclitaxel is the preferred regimen for advanced/metastatic or recurrent endometrial cancer with response rates of 40 to 62% and overall survival from 13 to 29 months (20). If a multi-drug regimen cannot be tolerated, single agent treatment with paclitaxel (most active), cisplatin, carboplatin, doxorubicin, liposomal doxorubicin, topotecan, or docetaxel can be used. However, response rates with single agents are lower (first-line treatment response rate 21% to 36%; second line treatment response rates 4% to 27%) (21). None of these therapies are curative and second line therapies have not been shown to improve survival (21). New therapies are needed to improve outcomes in patients with advanced stage endometrial cancers.

1.2.3 Development of ONC201

1.2.3.1 Preclinical Development of ONC201

ONC201 (also known as TIC10 and NSC350625) is the founding member of a novel class of anti-cancer compounds called imipridones (22). It is an orally active small molecule that was initially identified by a screen for small molecules that could induce tumor necrosis factor (TNF) –related apoptosis-inducing ligand (TRAIL) on colon cancer cells, and activate the TRAIL death receptors on those cells inducing an autocrine apoptotic death.

TRAIL is a type II transmembrane protein that is expressed in various tissues and cells including the surface of Natural Killer (NK) and T cells, macrophages and dendritic cells (23, 24). On immune cells, the expression of TRAIL plays a role in immune surveillance in the prevention of tumors and metastasis (25). Humans have five distinct TRAIL receptors (26, 27). Death receptor 4 (DR4) and DR5 contain intracellular death domains and are capable of signaling apoptosis. There are two membrane-bound decoy receptors called DcR1 and DcR2 which lack a functional death domain and while unable to activate apoptosis, are able to inhibit TRAIL signaling. Osteoprotogerin is the fifth TRAIL-binding receptor which also functions as a decoy/inhibitor by sequestering TRAIL extracellularly. TRAIL is attractive as an anti-tumor agent because of its capability to induce apoptosis in cancer cells by activating DR4 and DR5 with little toxicity against normal cells (23, 28). In our preclinical work, we have shown that TRAIL receptor agonists are most active against triple negative breast cancer and in particular those with mesenchymal features (29, 30).

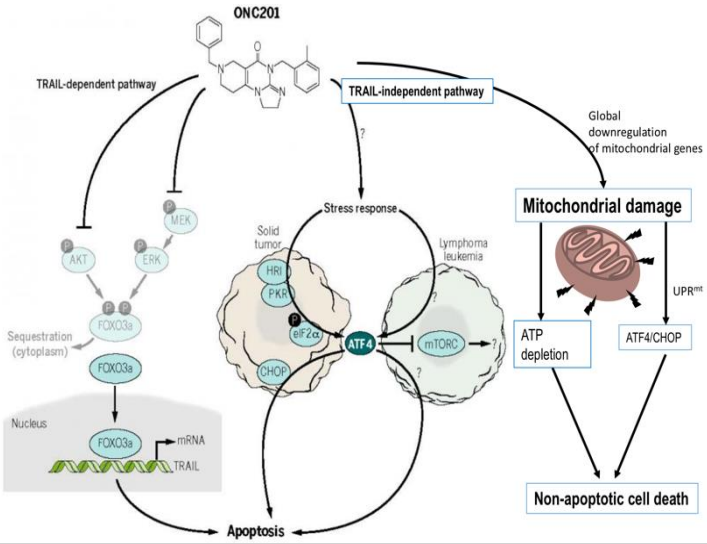

Figure 1: Structure and mechanism of action of ONC201. In the TRAIL-dependent pathway, ONC201 inhibits both AKT and MEK, resulting in the dephosphorylation of the transcription factor FOXO3a. This enables its translocation from the cytoplasm into the nucleus where it binds to the TRAIL promoter and upregulates gene transcription of TRAIL. Apoptosis occurs through activation of TRAIL-DR receptors. In the TRAIL-independent pathway, ONC201 induces a stress response through an unknown mechanism which differs between solid tumors and hematologic malignancies. The mechanism of ONC201 appears to be different in breast cancer cell lines, where ONC201 demonstrates caspase-independent, DR4/5 independent mechanisms distinct from TRAIL-induced apoptosis. Instead it induces mitochondrial dysfunction and ATP depletion. *Image adapted from Greer and Lipkowitz, 2016*: (3).

Despite their robust cell killing of tumor cells *in vitro* and in animal models *in vivo,* efficacy of TRAIL receptor agonists has been limited in clinical trials. The limited activity is thought to be due to lack of reliable predictive biomarkers of sensitivity and drug properties that limit efficacy such as short serum half-life, stability, and bio-distribution (25, 28, 31). There is a need to find alternative approaches to activating the DR pathways. ONC201 induces sustained up-regulation of TRAIL in tumor cell lines, which activates DR through autocrine and paracrine mechanisms.

Several alternative mechanisms have also been proposed for the anticancer activity of ONC201, including inhibition of the dopamine receptor D2 (DRD2), and activation of an atypical integrated stress response in tumor cells (32, 33).

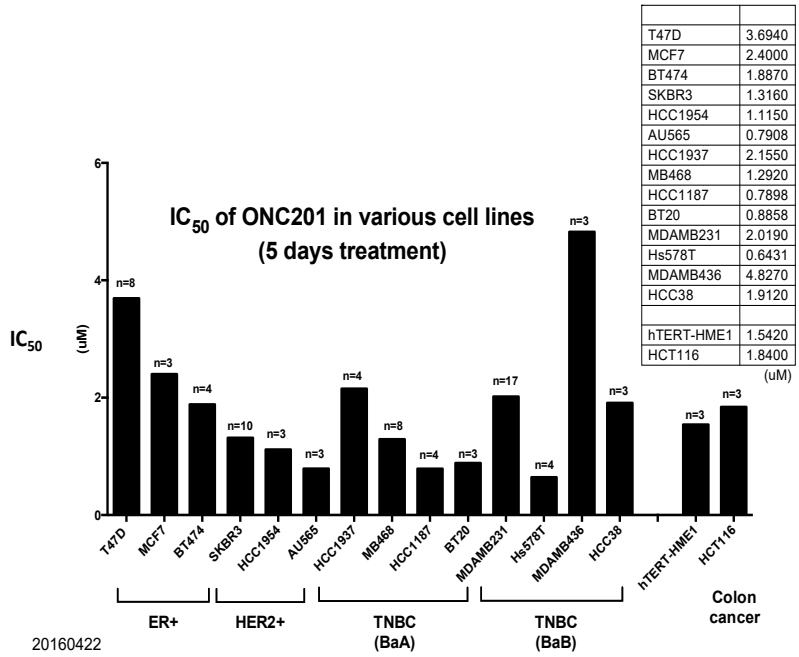

Figure 2: IC50 for breast cancer cell lines treated with ONC201. Cells were treated with a dose curve of ONC201 for 5 days and viability measured by MTS assay. The data represent the mean IC50 for each cell line (Greer and Lipkowitz, unpublished observations).

ONC201 has been extensively investigated in the Lipkowitz lab as a promising means to kill tumor cells. Rather than a simple induction of TRAIL and induction or DR receptor-dependent apoptosis, both the Lipkowitz lab and independent labs have shown that ONC201 has both TRAIL-dependent and TRAIL-independent pathways of inducing death (**Figure 1**– two right sided pathways).

We have investigated the mechanism by which ONC201 kills breast cancer cells. Unexpectedly, ONC201 did not induce caspase 3 or PARP cleavage, and its toxicity was not inhibited by Z-VAD-FMK, nor by siRNA knockdown of DR4 or DR5. By contrast GST-TRAIL induced caspase 3 and PARP cleavage and GST-TRAIL-induced cell death was inhibited by Z-VAD- FMK and by siRNA knockdown of DR5. Live cell imaging revealed ONC201 induces cell membrane ballooning followed by rupture consistent with necrosis, whereas GST-TRAIL induced classic apoptosis morphology. Together these results suggest that ONC201 kills breast cancer cells via a caspase-independent, DR4/5-independent mechanism distinct from TRAIL-induced apoptosis. ONC201 does induce ATF4 and CHOP, consistent with the recently published observations of another group (32, 33).

Further mechanistic studies in the Lipkowitz lab showed that ONC201 induced transcriptional down regulation of multiple nuclear encoded mitochondrial genes, resulting in decreased mitochondrial oxidative phosphorylation and ATP production (**Figure 1**– right pathway). Our data suggest that in addition to the published mechanisms, ONC201 can kill breast cancer cells by a novel mechanism involving inhibition of mitochondrial respiration. In breast cancer cells this appears to be the dominant mechanism. One of the reasons for conducting this trial in the Intramural program is to validate and expand our understanding of ONC201's mechanism of action in the human. Such data will translate back to the bench, enabling the development of combination therapies and plans for overcoming resistance.

We have tested ONC201 across a range of breast cancer cell lines and have found that all subtypes are inhibited by ONC201, and the IC50s for the cell lines range from 0.8-5μM (**Figure 2**). This is within the achievable concentrations that were seen in pharmacokinetic studies from phase I studies (2).

ONC201 has been evaluated as a therapeutic agent *in vitro* as well as *in vivo* in various cell lines. Single-dose experiments in HCT116 wild-type (human colon cancer) xenograft–bearing mice corroborated the antitumor activity of ONC201 (TIC10) (**Figure 3**). *In vivo* experiments have shown promising anti-tumor effects in 12 subcutaneous xenographs (including triple negative breast cancer, non-small cell lung cancer, hepatocellular carcinoma, glioblastoma, colon cancer and head and neck squamous cell carcinoma), one orthotopic xenograph (glioblastoma) and one transgenic (lymphoma) model. Dr. El-Deiry's group has demonstrated that ONC201 inhibits xenografts of the triple negative breast cancer cell line MDA-MB-231 (1).

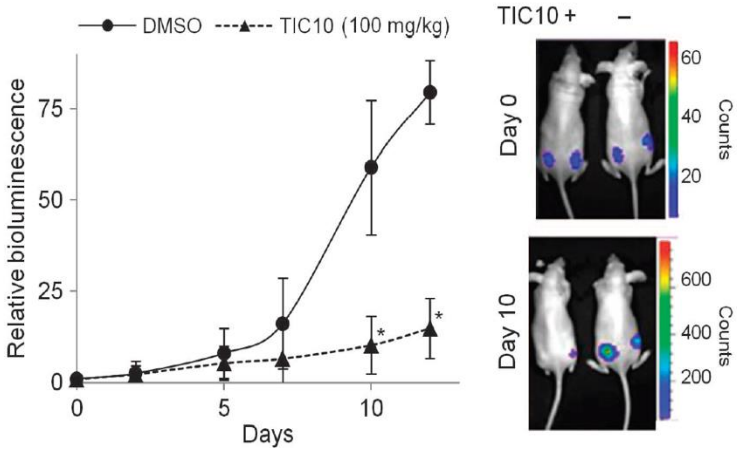

Figure 3: Bioluminescence imaging of luciferase-expressing HCT116 p53−/− xenografts that received a single intraperitoneal injection of ONC201 (TIC10) or vehicle (n = 6). Image from *Allen et al, 2013* (1).

1.2.3.2 Clinical Development of ONC201

Due to preclinical results, ONC201 is currently being investigated in phase I and II trials in patients with refractory solid tumors including glioblastoma as well as in patients with relapsed/refractory non-Hodgkin's lymphoma, relapsed/refractory acute leukemias and high-risk myelodysplastic syndromes. Many of these trials are ongoing, but results from the first-in-human phase I trial of ONC201 in patients with refractory solid tumors are promising (2, 4). The trial enrolled 28 patients with 15 different types of tumors including prostate cancer, endometrial cancer, ovarian cancer, colon cancer, gallbladder cancer and sarcoma. In this heavily treated population (average of 5-6 prior treatments), researchers found prolonged stable disease in many patients, as well as disease reduction in others patients, most notably in those with endometrial or prostate cancer. This trial also evaluated the safety and tolerability of ONC201. The recommended phase 2 dose (RP2D) was determined to be 625mg by mouth q3 weeks and dosing was subsequently adjusted to weekly dosing due to pharmacokinetic studies.

There have been no deaths attributed to study drug. There have been no SAEs assessed by the investigators as "probably" or "definitely" related to study drug, and no discontinuation of ONC201 due to toxicity has been reported.The most common AEs reported as possibly related to ONC201 were mild/moderate nausea, vomiting, anorexia, pyrexia and fatigue.

More than 70 patients with advanced solid tumors and hematological malignancies were evaluated in phase I clinical trials and no serious side effects were found attributable to ONC201. Grade I adverse events that have been observed and reported include fever, nausea, vomiting, fatigue, and elevated serum amylase that were attributed as possibly-related to ONC201. One Grade II allergic reaction occurred that was managed with standard

medications. One Grade III event neutropenia was transiently observed. No other Grade III or IV events were reported so far.

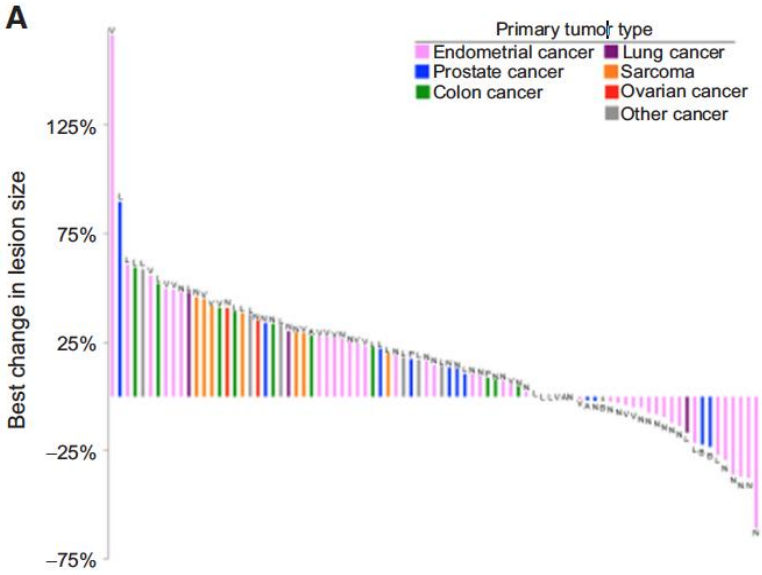

Figure 4: Waterfall plot of best fractional change in tumor size relative to baseline on a lesion-by-lesion basis. Best change in lesion size is defined as maximal reduction or minimal increase in sum of longest dimensions of target lesions relatively to pretreatment assessment. *Image from Stein et al, 2017(4).*

The lesion-by-lesion results of this phase 1 study were recently reported (**Figure 4**) (2, 4). In this phase I study, 3 of 5 patients with endometrial cancer had stable disease by RECIST criteria (> 4 months in 2 and >10 months in one). The Lipkowitz lab has also confirmed that ONC201 had a similar effect on the killing of various endometrial cancer cell lines (data not shown) as it did on breast cancer cell lines. Based on these result we propose to include a cohort of patients with endometrial cancer in our trial.

Only one triple negative breast cancer patient was recruited for the phase 1 study, and unfortunately had disease progression while on ONC201. Nevertheless, the preclinical data on breast cancer cell lines performed by the drug developer and confirmed by the Lipkowitz lab is encouraging that women with breast cancer may derive clinical benefit from ONC201.

1.2.4 Pharmaceutical Manufacturer Updates

1.2.4.1 ONC201 Investigator Brochure v6 November 2019

As of October 31, 2019, the majority of adverse events (AEs) experienced by participants on clinical trials who received ONC201 were mild or moderate in severity. The most frequently reported AEs of any grade (in greater than 25% of patients) were fatigue, nausea, headache and vomiting. These AEs were generally Grade 1/2, with fatigue being the only Grade 3/4 AE that occurred in more than one patient on the ONC013 study (17.4%).

Eleven patients experienced serious adverse events (SAEs) that were considered possibly related to ONC201. The list of SAEs reported by investigators that were attributed as possibly-related to ONC201 by the investigator are:

- Grade 1: Fatigue, vomiting
- Grade 3: Encephalopathy, cognitive impairment, neutropenia, upper respiratory infection, respiratory distress, pneumonia, stroke, tremor, fatigue, bone pain, anorexia, weakness
- Grade 4 Dysphagia, dyspnea

Two suspected unexpected serious adverse (SUSAR) events occurred at a dose of 625mg daily. Both events were encephalitis that occurred on the investigator-initiated trial NCT02392572 in patients with MDS or AML and are considered dose-limiting toxicities(DLT). No other DLTs occurred in other indications or at lower doses both as monotherapy or in combination with other agents. Only one dose reduction (625mg to 500mg) occurred in one patient with a Grade 3 neutropenia assessed by the PI as possibly related to ONC201. However, upon re-challenge neutropenia did not recur.

Based on the clinical safety data summarized above, ONC201 administered at the doses and schedules below 625mg daily appears to be well tolerated in adults and children.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA FOR COHORT 1: HORMONE RECEPTOR POSITIVE BREAST CANCER

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically confirmed persistent or recurrent invasive metastatic hormone receptor positive, HER2 normal breast cancer for which standard curative measures do not exist or are no longer effective. Hormone receptor positive is defined as estrogen receptor (ER) positive ≥ 10% by immunohistochemistry (IHC) and/or progesterone receptor (PR) positive ≥ 10% by IHC.HER2 will be considered negative per ASCO-CAP guidelines (HER2 test result as negative if a single test (or both tests) performed show: 1) IHC 1+ as defined by incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells; 2) IHC 0 as defined by no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells; or 3) ISH negative based on: a) Single-probe average HER2 copy number <4.0 signals/cell or b) Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell)and HER2 testing must have been performed in a laboratory accredited by the College of American Pathologists (CAP) or another accrediting entity.

2.1.1.2 Patients must have measurable disease, per RECIST 1.1. See Section **6.3.1** for the evaluation of measurable disease.

2.1.1.3 Patients must have at least one lesion deemed safe to biopsy and be willing to undergo mandatory biopsies.

2.1.1.4 HR+BC patients must have received prior treatment with at least 2 lines of hormonal treatment (SERM, AI, or fulvestrant) and deemed ineligible for further hormonal

therapy . Patients may have received prior chemotherapy and there is no limit to the number of prior chemotherapy.

2.1.1.5 Age ≥18 years.

2.1.1.6 ECOG performance status 0 or 1

2.1.1.7 Adequate renal function, defined as serum creatinine ≤ 1.5 X upper limit of normal (ULN), or measured creatinine clearance ≥ 60 mL/min/1.

2.1.1.8 Adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dL will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dL, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study.

2.1.1.9 Adequate bone marrow function, defined as absolute neutrophil (ANC) ≥ 1,500/mm^3 (≥1.5 $X10^6$/L), platelet count ≥ 75,000/mm3 (≥ 75 $X10^6$/L), and hemoglobin ≥ 9 mg/dL (transfusion to obtain hemoglobin ≥ 9 mg/dL within 24 hours prior to dosing is allowed).

2.1.1.10 Patients must be able to swallow oral medications (capsules) without chewing, breaking, crushing, opening or otherwise altering the product formulation.

2.1.1.11 The effects of *ONC201* on the developing human fetus are unknown. For this reason and because *imipridone* agents are known to be teratogenic, female patients must either be of non-reproductive potential (i.e., post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry and agree to use contraception or abstinence during the study and for and for at least 4 weeks after the final dose of any study-related medications. Male patients must use at least two forms of contraception during the study and for at least 4 weeks after the final dose of any study-related medications or have a partner who is not of reproductive potential. (See Section **4.2**)

2.1.1.12 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents within 3 weeks or a PD1/PDL1 agent within 4 weeks prior to first dose of study treatment.

2.1.2.2 Patients who have undergone radiotherapy within 4 weeks of first dose of study treatment.

2.1.2.3 Patients with a history of another invasive malignancy within the last 3 years.

2.1.2.4 Patients with symptomatic brain metastases or leptomeningeal involvement. Patients with asymptomatic or brain metastases that have been treated with radiation at least 4 weeks prior to first dose of study treatment are allowed.

2.1.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to *imipridones* or other agents used in study.

2.1.2.6 Patients with a mean QTcF interval of > 500 msec or receiving therapeutic agents known to prolong the QT interval

2.1.2.7 Known history of cardiac arrhythmias including uncontrolled atrial fibrillation, tachyarrhythmias or bradycardia, history of congestive heart failure, or myocardial infarction or stroke in the previous 3 months will be excluded.

2.1.2.8 Known history of gastrointestinal illnesses that would preclude the absorption of ONC201, which is an oral agent

2.1.2.9 Patients with bone metastases who have initiated denosumab or bisphosphonate therapy within 28 days prior to Cycle 1 Day 1.

2.1.2.10 Pregnant women are excluded from this study because ONC201 has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ONC201, breastfeeding should be discontinued if the mother is treated with ONC201. These potential risks may also apply to other agents used in this study.

2.1.2.11 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ONC201.

2.1.2.12 Patients who have known active Hepatitis B, or Hepatitis C infections.

2.2 Eligibility Criteria for Cohort 2: Triple Negative Breast Cancer

2.2.1 Inclusion Criteria

1.1.1.1 Patients must have histologically or cytologically confirmed persistent or recurrent invasive, metastatic triple negative breast cancer (TNBC) for which standard curative measures do not exist or are no longer effective. TNBC, defined as ER negative (ER < 10%), PR negative (PR <10%). HER2 will be considered negative per ASCO-CAP guidelines (HER2 test result as negative if a single test (or both tests) performed show: 1) IHC 1+ as defined by incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells; 2) IHC 0 as defined by no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells; or 3) ISH negative based on: a) Single-probe average HER2 copy number <4.0 signals/cell or b) Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell) and HER2 testing must have

been performed in a laboratory accredited by the College of American Pathologists (CAP) or another accrediting entity.

1.1.1.2 Patients must have received at least one line of prior chemotherapy in the metastatic setting.

2.2.1.1 Patients must have measurable disease, per RECIST 1.1. See Section **6.3.1** for the evaluation of measurable disease.

2.2.1.2 Patients must have at least one lesion deemed safe to biopsy and be willing to undergo mandatory biopsies.

2.2.1.3 Eligible patients may or may not have received prior chemotherapy and there is no limit to the number of prior chemotherapy. Patients are also eligible if they have received treatment with immunotherapy, such PD-1 inhibitors, PD-L1 inhibitors or CTLA4 inhibitors.

2.2.1.4 Age ≥18 years.

2.2.1.5 ECOG performance status 0 or 1

2.2.1.6 Adequate renal function, defined as serum creatinine ≤ 1.5 X upper limit of normal (ULN), or measured creatinine clearance ≥ 60 mL/min/1.

2.2.1.7 Adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dL will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dL, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study.

2.2.1.8 Adequate bone marrow function, defined as absolute neutrophil (ANC) ≥ 1,500/mm^3 (≥1.5 $X10^6$/L), platelet count ≥ 75,000/mm3 (≥ 75 $X10^6$/L), and hemoglobin ≥ 9 mg/dL (transfusion to obtain hemoglobin ≥ 9 mg/dL within 24 hours prior to dosing is allowed).

2.2.1.9 Patients must be able to swallow oral medications (capsules) without chewing, breaking, crushing, opening or otherwise altering the product formulation.

2.2.1.10 The effects of *ONC201* on the developing human fetus are unknown. For this reason and because *imipridone* agents as well as other therapeutic agents used in this trial are known to be teratogenic. Female patients must either be of non-reproductive potential (i.e., post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry and agree to use contraception or abstinence during the study and for and for at least 4 weeks after the final dose of any study-related medications. Male patients must use at least two forms of contraception during the

study and for and for at least 4 weeks after the final dose of any study-related medications or have a partner who is not of reproductive potential. (See Section **4.2**).

2.2.1.11 Ability of subject to understand and the willingness to sign a written informed consent document.

2.2.2 Exclusion Criteria

2.2.2.1 Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents within 3 weeks or a PD1/PDL1 agent within 4 weeks prior to study enrollment.

2.2.2.2 Patients who have undergone radiotherapy within 4 weeks of first dose of study treatment.

2.2.2.3 Patients with a history of another invasive malignancy within the last 3 years.

2.2.2.4 Patients with symptomatic brain metastases or leptomeningeal involvement. Patients with asymptomatic or brain metastases that have been treated with radiation at least 4 weeks prior to first dose of study treatment are allowed.

2.2.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to *imipridones* or other agents used in study.

2.2.2.6 Patients with a mean QTcF interval of > 500 msec or receiving therapeutic agents known to prolong the QT interval

2.2.2.7 Known history of cardiac arrhythmias including uncontrolled atrial fibrillation, tachyarrhythmias or bradycardia, history of congestive heart failure, or myocardial infarction or stroke in the previous 3 months will be excluded.

2.2.2.8 Known history of gastrointestinal illnesses that would preclude the absorption of ONC201, which is an oral agent

2.2.2.9 Patients with bone metastases who have initiated denosumab or bisphosphonate therapy within 28 days prior to Cycle 1 Day 1.

2.2.2.10 Pregnant women are excluded from this study because ONC201 has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ONC201, breastfeeding should be discontinued if the mother is treated with ONC201. These potential risks may also apply to other agents used in this study.

2.2.2.11 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ONC201.

2.2.2.12 Patients who have known active Hepatitis B, or Hepatitis C infections.

2.3 ELIGIBILITY CRITERIA FOR COHORT 3: ENDOMETRIAL CANCER

2.3.1 Inclusion Criteria

2.3.1.1 Patients must have histologically or cytologically confirmed persistent or recurrent advanced or metastatic invasive endometrial cancer (EC) for which standard curative measures do not exist or are no longer effective.

2.3.1.2 Patients must have measurable disease, per RECIST 1.1.. See Section **6.3.1** for the evaluation of measurable disease.

2.3.1.3 Patients must have at least one lesion deemed safe to biopsy and be willing to undergo mandatory biopsies.

2.3.1.4 Women with endometrial cancer must have had at least one prior line of therapy in the metastatic/recurrent setting but there is no limit to the number of prior chemotherapy lines. Patients are eligible if they have received treatment with immunotherapy, such PD-1 inhibitors, PD-L1 inhibitors or CTLA4 inhibitors.

2.3.1.5 Age ≥18 years.

2.3.1.6 ECOG performance status 0 or 1

2.3.1.7 Adequate renal function, defined as serum creatinine ≤ 1.5 X upper limit of normal (ULN), or measured creatinine clearance ≥ 60 mL/min/1.

2.3.1.8 Adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dl will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dL, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study.

2.3.1.9 Adequate bone marrow function, defined as absolute neutrophil (ANC) ≥ 1,500/mm^3 (≥1.5 $X10^6$/L), platelet count ≥ 75,000/mm^3 (≥ 75 $X10^6$/L), and hemoglobin ≥ 9 mg/dL (transfusion to obtain hemoglobin ≥ 9 mg/dL within 24 hours prior to dosing is allowed).

2.3.1.10 Patients must be able to swallow oral medications (capsules) without chewing, breaking, crushing, opening or otherwise altering the product formulation.

2.3.1.11 The effects of *ONC201* on the developing human fetus are unknown. For this reason and because *imipridone* agents as well as other therapeutic agents used in this trial are known to be teratogenic. Female patients must either be of non-reproductive potential (i.e., post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry and agree to use contraception or abstinence during the study and for and for at least 4 weeks after the final dose of any study-related medications. Male patients must use at least two forms of contraception during the

study and for and for at least 4 weeks after the final dose of any study-related medications or have a partner who is not of reproductive potential. (See Section **4.2**).

2.3.1.12 Ability of subject to understand and the willingness to sign a written informed consent document.

2.3.2 Exclusion Criteria

2.3.2.1 Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents within 3 weeks or a PD1/PDL1 agent within 4 weeks prior to study enrollment.

2.3.2.2 Patients who have undergone radiotherapy within 4 weeks of first dose of study treatment.

2.3.2.3 Patients with a history of another invasive malignancy within the last 3 years.

2.3.2.4 Patients with symptomatic brain metastases or leptomeningeal involvement. Patients with asymptomatic or brain metastases that have been treated with radiation at least 4 weeks prior to first dose of study treatment are allowed.

2.3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to *imipridones* or other agents used in study.

2.3.2.6 Patients with a mean QTc interval of > 500 msec or receiving therapeutic agents known to prolong the QT interval

2.3.2.7 Known history of cardiac arrhythmias including uncontrolled atrial fibrillation, tachyarrhythmias or bradycardia, history of congestive heart failure, or myocardial infarction or stroke in the previous 3 months will be excluded.

2.3.2.8 Known history of gastrointestinal illnesses that would preclude the absorption of ONC201, which is an oral agent

2.3.2.9 Patients with bone metastases who have initiated denosumab or bisphosphonate therapy within 28 days prior to Cycle 1 Day 1.

2.3.2.10 Pregnant women are excluded from this study because ONC201 has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ONC201, breastfeeding should be discontinued if the mother is treated with ONC201. These potential risks may also apply to other agents used in this study.

2.3.2.11 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ONC201.

2.3.2.12 Patients who have known active Hepatitis B, or Hepatitis C infections.

2.4 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites (e.g. clinicaltrials.gov) and on NIH social media platforms. Participants may also be identified through referrals from physicians, or from populations in NIH Clinics.

2.5 SCREENING EVALUATION

2.5.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects regarding their medical issues and possible eligibility to the clinical trial
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **12.6.1**.

2.5.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

The screening evaluations or tests will be conducted as described below and in the Study Calendar (Section **3.4**) after the participant has signed the consent document.

For baseline evaluations, please see section **2.9**.

2.5.2.1 Pathologic Confirmation

Confirmation of tumor histology in NCI Laboratory of Pathology (archival tumor samples will be requested; if unavailable, the subject may choose to undergo fresh biopsy). Pathological confirmation of diagnosis of breast cancer or endometrial cancer in the Laboratory of Pathology at NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda. However, if no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of HR+BC, TNBC or Endometrial Cancer in a College of American Pathologists (CAP) accredited laboratory and a clinical course consistent with the disease.

2.5.2.2 History and Physical, Vital Signs, Height, Weight, and Concomitant Medications

Medical history and physical examination, including vitals signs and concomitant medications, will be conducted at screening and at subsequent visits as indicated in the schedule of assessments. Results of the physical examination including any abnormalities will be documented and can be used for the baseline examination if conducted within 17 days prior to enrolling on trial. Abnormal findings will be reassessed at subsequent visits. On subsequent visits, any newly diagnosed or worsening conditions, signs and symptoms, whether related or unrelated to the trial, will be reported as adverse events.

2.5.2.3 Performance Status

The ECOG performance status (Appendix A) will be assessed at screening and at subsequent visits as indicated in the schedule of assessments and documented in the clinical record.

2.5.2.4 Scans for disease evaluation at screening.

- CT of chest/abdomen /pelvis (MRI may be substituted at investigator's discretion)
- Bone scan may be done at the investigator's discretion is there is concern for bone disease
- Brain MRI (preferred) or head CT with contrast may be done at the investigator's discretion if there is concern for CNS metastasis

If the screening scan has been obtained within 17 days prior to first dose of study treatment , it may be used for baseline evaluation.

2.5.2.5 Laboratory Studies

Screening laboratory studies to include:

- CBC with differential, PT/INR/PTT, Biochemical profile for Screening: 14 comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST (SGOT), ALT (SGPT), total bilirubin, serum creatinine. If the labs have been obtained within 10 days prior to initiation of therapy, they may be used for baseline evaluation.
- Anti-HIV, anti-HCV, Hepatitis B surface Ag: viral titers will be obtained within 28 days prior to enrollment.

2.5.2.6 Pregnancy Testing

For female subjects of childbearing potential, urine or serum HCG will be performed on initial screening.

- A urine beta-HCG will be performed before each administration of ONC201 during the treatment phase, at the end-of-treatment visit, and at the post-treatment follow-up visit.
- Patients who are postmenopausal (age-related amenorrhea for 12 or more consecutive months, or documented FSH > 40 mIU/mL) / Estradiol < 20mIU/mL), or who had undergone hysterectomy or bilateral oophorectomy are exempt from pregnancy testing.

2.5.2.7 Tumor Block

A primary tumor paraffin block will be requested to perform comparative genomic analysis as part of correlative studies planned for this clinical trial.

2.5.2.8 Tumor Biopsy

A biopsy of fresh frozen tissue will be planned after consent but prior to the first dose of ONC201.

2.5.2.9 Research Blood Samples

Peripheral blood samples for the correlative studies will be collected after consent but prior to the first dose of ONC201, per Section **5** Biospecimen Collection.

2.6 Participant Registration and Status Update Procedures

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found **here**.

2.7 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

2.8 Treatment Assignment and Randomization/Stratification Procedures

Cohorts

Number	Name	Description
1	HR+ breast cancer	HR+ breast cancer (male and female)
2	TNBC	Triple negative breast cancer (male and female)
3	Endometrial cancer	Endometrial cancer (female only)

Arms

Number	Name	Description
1	ONC201	All patients from all 3 cohorts will receive ONC201 at the recommended phase 2 dose (RP2D) of 625mg by mouth every 7 days with each cycle being 28 days long.

Randomization/Stratification Procedures

Randomization and stratification procedures are not applicable to this study.

- All patients from Cohort 1 will be assigned to Arm 1.
- All patients from Cohort 2 will be assigned to Arm 1.
- All patients from Cohort 3 will be assigned to Arm 1.

2.9 BASELINE EVALUATION

Scans and x-rays must be done within 17 days prior to the initiation of study therapy. All other studies should be performed within 10 days prior to initiation of study therapy, unless otherwise specified. If the tests were performed within these timeframes at screening, they do not need to be repeated at start of treatment.

- Start the process for documentation of germline BRCA mutation status (Cohort 1 and 2).
- Start the process for documentation of Lynch syndrome (germline evaluation) or MSI status of tumor (somatic evaluation by genomics, IHC or demonstration of Microsatellite instability of the tumor). (Cohort 3 only)
- History and physical examination with ECOG evaluation (Appendix A), history of prior therapy, review of concurrent medications, and review of baseline symptoms.
- Assessment of disease by imaging (may include CT chest, abdomen and pelvis or MRI)
- EKG obtained within 7 days of initiating therapy
- Laboratory evaluations
    - CBC with differential and platelet count
    - Sodium, potassium, chloride, CO2, creatinine or measured creatinine clearance, glucose, BUN
    - Mineral panel (magnesium, calcium, albumin, phosphorus), lactic acid and a lipid panel
    - Hepatic panel (alkaline phosphatase, AST/ALT/total bilirubin) with total protein
    - PT/INR/PTT
    - β-HCG in women of childbearing potential
    - Hormone panel: Prolactin, Estrogen
    - Lymphocyte phenotyping – TBNK
    - Tumor Markers: Breast cancer (cohorts 1 and 2) – CEA, CA15-3, CA 27.29; Endometrial cancer (cohort 3) – CA-125
    - Urinalysis
    - Germline mutation analysis of BRCA or Lynch Syndrome (germline evaluation) or MSI status of tumor (somatic evaluation by genomics, IHC or demonstration of Microsatellite instability of the tumor) (if not previously documented)

- Correlative Tests (Refer to the tables in section **5.5** for additional information)
    - Tumor biopsy
    - Blood sampling

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label, phase II single arm study of ONC201 divided in three cohorts, each cohort with a different type of metastatic, advanced disease: ER+ BC, TNBC and endometrial cancer. All proposed cohorts require measurable disease based on RECIST criteria and disease that can be biopsied safely. The study will specify a required biopsy prior to treatment and a biopsy during the second cycle after drug treatment. There will also be an optional biopsy at the time of progression. Participants will receive ONC201 starting at the RP2D of 625mg q7 days. Each cycle is 28 days long. ONC201 can be given +/- 1 day due to holidays, inclement weather, conflicts, or similar reasons.

During Cycle 1, patients will receive weekly phone calls by research staff to ask about changes to health and any potential side effects.

While the drug has activity against HER2 amplified breast cancer cells in vitro, we would not initiate a single agent HER2+ cohort at this time because of the clear benefit to HER2 targeted therapies in this subset of breast cancer. We will develop preclinical data for ONC201 in combination with HER2 targeted drugs (*e.g.*, trastuzumab, trastuzumab emtansine, and lapatinib) and plan to initiate a phase I run-in trial at a later date.

3.2 DRUG ADMINISTRATION

At the start of each cycle, enough study drug for one complete cycle will be dispensed by the pharmacy. Patients will be given a medication diary (see **Appendix E**) to record dates of administration as well as side effects. Patients in all cohorts will self-administer five capsules (each capsule 125mg x 5 = 625mg) of ONC201 by mouth every 7 days with each cycle being 28 days in duration. The capsules may be taken one hour before or two hours after eating, according to patient preference. If patients are unable to tolerate this dose, they can have three dose reductions before being removed from the study (see **Table 2**). Patients will receive ONC201 as long as they derive clinical benefit or toxicity becomes impeditive.

3.2.1 Self-Administered Study Drugs:

The ONC201 used in this study is a self-administered investigational agent. Such agents are dispensed from the pharmacy to a participant or to a Patient Care Unit for self-medication and a record of the dispensed investigational agent is generated and kept by the dispensing pharmacy.

As indicated above, patients will be asked to keep a medication diary and bring it with them on each study visit (See **Appendix E**). Patients will also bring any remaining pills to each study visit. The research nurse will review and validate the completeness and accuracy of the participant's diary with the participant.

If a participant goes off study while at home, the research nurse will ensure and document the return of the unused oral investigational agents from the participant. Unused investigational agent will be destroyed per dispensing pharmacy procedure.

3.3 Dose Modifications/Delays and Management of Toxicities

- In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).
- Once a patient has a dose reduction for toxicity, the dose will not be increased.
- Participants continuing to experience toxicity at the off treatment visit will be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. Patients must remain on the study to have additional assessments completed.
- For AEs that are unrelated to the study drugs, study drug may be held for up to 14 days at the discretion of the PI.

3.3.1 Modifications and Toxicity Management

Table 2: ONC201 Dose Reduction Schedule

Dose Level (DL)	**ONC201 (by mouth)**	**Number of Capsules***
1 (Starting Dose)	625mg every 7 days	5 capsules
-1	500mg every 7 days	4 capsules
-2	375mg every 7 days	3 capsules
-3	250mg every 7 days	2 capsules

* Each ONC201 capsule is 125mg

Summary of Dose Modifications or Discontinuation for ONC201-Related Adverse Events

Worst Treatment-Related Non-Hematologic AE (except for controlled nausea and vomiting) During the Previous Cycles	
Grade	Dose modification
0-2	No dose modifications for non-hematologic AEs.
3	Reduce by one dose level (except controlled nausea, and vomiting).
4	Stop (except controlled nausea and vomiting).

Worst Treatment-Related Hematologic AE During the Previous Cycle

Worst AE	Platelets

		≥75 x 10^9 /L	50 – 75 x 10^9 /L	< 50 x 10^9 /L
ANC	≥ 1.5 x 10^9 /L	Dose unchanged	Dose unchanged	Reduce 1 dose level
	≥1 to 1.5 x 10^9 /L	Dose unchanged	Dose unchanged	Reduce 1 dose level
	< 1 x 10^9 /L	Reduce 1 dose level	Reduce 1 dose level	Reduce 1 dose level

Dose reductions: If any treatment related non-hematologic AE observed was grade > 2 (except controlled nausea and vomiting) and/or if platelets < 50 x 10^9 /L and/or ANC < 1 x 10^9 /L, then the dose should be reduced by one dose level. Patients who require more than three dose reductions will have treatment stopped. If any treatment-related non-hematologic AE observed was grade 4 (except controlled nausea and vomiting) then ONC201 treatment should be stopped.

Any dose reductions of ONC201 will be determined according to: (1) non-hematologic AE during the preceding treatment cycle, as well as (2) the lowest ANC and platelets observed. No dose escalation should be attempted.

Important: If the dose was reduced or delayed for AEs, there will be no dose re-escalation in subsequent treatment cycles.

3.3.2 **Medication Delay**

Hematologic AE on Day 1 of Each Cycle	
AE	Delay
ANC< 1.0 x 10^9 /L and/or Platelet count < 75 x 10^9 /L	Delay up to 4 weeks until all resolved. If not resolved after 4 weeks, then remove from study regimen.

Non-Hematologic AE (except for controlled nausea, and vomiting) on Day 1 of Each Cycle (within the prior 72 hours)	
Grade	Delay

2-4	Delay by 1 week up to 4 consecutive weeks until all resolved (to grade ≤ 1). If not resolved after 4 weeks, then remove from study regimen.

On day 1 of each cycle, patients must have adequate hematopoietic function (i.e., ANC ≥ 1.0 x 10^9 /L, platelet count ≥ 75 x 10^9 /L) and all grade 2, 3 or 4 non-hematologic AEs (except for controlled nausea, and vomiting) must have resolved to grade ≤ 1.

If AEs persists, treatment should be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all treatment related AEs have still not resolved (to grade ≤ 1): then any further treatment with ONC201 should be stopped.

3.3.3 Instructions on Missed Doses

ONC201 can be given **+/- 1 day** due to holidays, inclement weather, conflicts, or similar reasons. If the first dose of the 4-week cycle not taken within 1 day of day 1 of the cycle, the cycle will be delayed one week. If a mid-cycle dose (i.e., D8, D15, D22) is more than 1 day late, the weekly dose will be skipped and the next dose will be resumed in accordance with the planned administration day.

3.4 STUDY CALENDAR

Procedure	**Screening/ Baseline**	**Cycle 1**	**Cycle 2**	**Subsequent Odd numbered cycles**	**Subsequent Even numbered cycles**	**End of treatment visit (~ 30 days from last dose)**
		Day 1 (±3days)	**Day 1 (±3days)**	**Day 1 (±3days)**	**Day 1 (±3days)**	
Informed consent	X					
History and PE[2]	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X
Performance Score	X	X	X	X	X	X
Labs	X[3]	X[4] (≤ 10 days)	X[4]	X[4]	X[4]	X[4]
Pathology confirmation[5]	X					
Tumor block	X[6]					
Fresh frozen tissue (tumor biopsy)[7]	X		X			X (optional)
Correlative Research Studies (blood samples)	X	X	X	X	X	X
PK/PD		X				
NIH Advance Directives Form	X (optional)					
Radiological Assessments[8]		X (≤ 17 days)		X		
Response Evaluation				X		

Procedure	**Screening/ Baseline**	**Cycle 1**	**Cycle 2**	**Subsequent Odd numbered cycles**	**Subsequent Even numbered cycles**	**End of treatment visit (~ 30 days from last dose)**
		Day 1 (±3days)	**Day 1 (±3days)**	**Day 1 (±3days)**	**Day 1 (±3days)**	
Adverse Events		X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X
Electrocardiogram[9]		X[1]	X			

[1] Up to 7 days before enrolling the trial

[2] History including prior treatments and review of baseline adverse events and concurrent medications. Physical examination includes neurological exam. Height (at screening only) and weight are also included.

[3] CBC with differential, PT/INR/PTT, AST (SGOT), ALT (SGPT), total protein, total bilirubin, serum creatinine.Urine or serum HCG on day of screening for women of childbearing potential. Anti-HIV, anti-HCV, Hepatitis B Surface Ag (within 28 days prior to first dose of study treatment).

[4] Labs must be obtained $\leq$10 days of initiating treatment: CBC with differential, PT/INR/PTT, sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST, ALT, bilirubin, calcium, total protein, albumin, alkaline phosphatase, phosphorus, magnesium, urinalysis, prolactin, estrogen, lactic acid and lipid panel. Lymphocyte phenotyping (TBNK). Urine or serum HCG for women of childbearing potential. Tumor Markers (CEA, CA15-3, CA27.29 for breast cancer and CA125 for endometrial cancer). Evaluation of germline BRCA1/2 (cohorts 1 and 2) or Lynch syndrome (germline evaluation) or MSI status of tumor (somatic evaluation by genomics, IHC or demonstration of Microsatellite instability of the tumor) (cohort 3) if no documentation of mutational analysis provided.

[5] Pathological confirmation of diagnosis (plus ER, PR and HER2 expression for breast cancer patients) in the Laboratory of Pathology at NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda. However, if no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of HR+BC, TNBC or Endometrial Cancer in a College of American Pathologists (CAP) accredited laboratory and a clinical course consistent with the disease.

[6] Primary tumor paraffin block will be requested at enrollment to perform comparative genomic analysis as part of correlative studies planned for this clinical trial.

[7] Planned biopsy after consent but prior to first dose of ONC201 as well as a planned biopsy on C2D2 after the 5th dose of ONC201. There is also an optional biopsy at the time of progression.

[8]Baseline images within 17 days prior to initiation of therapy: CT of chest/abdomen /pelvis (MRI may be substituted at investigator's discretion), Bone scan only at baseline. Brain MRI may be ordered at the discretion of the investigator if there is concern for CNS metastasis. CT of chest/abdomen/pelvis to be repeated every 8 weeks +/- 4 days after start of treatment (odd numbered cycles). Additional imaging to be performed at the investigator's discretion.

[9] 12-lead EKG within 7 days prior to initiation of therapy and on C2D1, with monthly repeats only for abnormal baseline EKGs or if patient is symptomatic.

Note: Allowance for scheduling changes: Brief interruption and delay in the 28-day cycle may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts and government holidays, etc., for scheduling convenience. This can also extend to complications of disease not attributable to disease progression or protocol therapy. These delays will not be considered protocol deviation.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.5.1 Criteria for removal from protocol therapy

- Progressive disease
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in section **3.3**.
- Investigator discretion
- Positive pregnancy test

3.5.2 Off-Study Criteria

- Screen Failure
- Completed study follow-up period
- Study closure
- Participant requests to be withdrawn from study
- Death

4 CONCOMITANT MEDICATIONS/MEASURES

The investigator's brochure for ONC201 by Oncoceutics does not describe any known drug-drug interactions with ONC201. In vitro cytochrome P450 assays were conducted in human hepatocytes. In these studies, ONC201 is not an inducer of the CYP450 system (CYP1A2, 2B6, and 3A4). ONC201 was observed to be a mild inhibitor of the CYP450 enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) at 35-429 uM, i.e., at least 3.5-fold above the Cmax observed in the first-in-human trial.

Drugs that antagonize DRD2 are commonly used as antipsychotics. However, comparison between ONC201 and other typical antipsychotics (such as haloperidol and risperidone) reveal substantial differences in anti-tumor activity, therapeutic window, specificity and receptor pharmacology. Moreover, the current administration schedule of ONC201 dosing is atypical for antipsychotic drugs. Taken together, there is no clear expectation that side effects that are commonly observed with antipsychotic drugs will be observed with ONC201 in the current schedule. Nevertheless, it is possible that side effects associated with antipsychotics could be observed with ONC201including weight gain, diabetes, metabolic disorders, dystonia, akinesia, akathisia, tremors, muscle rigidity, insomnia, blurry vision, Parkinsonian symptoms, cognitive impairment, sexual dysfunction, osteoporosis, sedation, behavioral changes, and cardiac disorders.

4.1 RESTRICTIONS

As a general precaution, the use of metformin and similars are prohibited due to potential similarities in mechanisms and mitochondrial damage. Patients will also be prohibited from using herbs and magic potions.

Breast feeding

It is not known whether ONC201 is excreted in breast milk. It is recommended that women do not breastfeed during treatment with ONC201 and for at least 2 months after the last dose.

Blood Donation

Patients should not donate blood while participating in this study or for 90 days following the dose of ONC201.

4.2 CONTRACEPTION

The effects of ONC201 on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use two methods of adequate contraception (see **Table 3**) prior to study entry, for the duration of study participation and for at least 4 weeks after the final dose of any study-related medications. Cessation of birth control after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method and the withdrawal method are not acceptable methods of birth control.

Table 3: Effective methods of contraception (two methods must be used)

Barrier Methods	*Intrauterine Device Methods*	*Hormonal Methods*
Male condom plus spermicide	Copper T	Implants
Cap plus spermicide	Progesterone T[a]	Hormone shot or injection
Diaphragm plus spermicide	Levonorgestrel-releasing intrauterine system (e.g., Mirena)	Combined pill
		Minipill
		Patch

[a]This is also considered a hormonal method of birth control

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

The primary goal of the correlative studies is to identify potential predictive biomarkers as well as additional evidence of the mechanism of action of and resistance to ONC201. Other correlative study aims include investigation of proof-of-concept biomarkers. These will be

performed in an exploratory fashion on select patients if there are adequate samples for analysis. (**Table 4**; **Table 5**)

While the exact mechanism of ONC201 is unclear at this time, preclinical data as well as the phase I data have identified multiple potential mechanisms of action. As mentioned above, ONC201 is thought to have both TRAIL-dependent and TRAIL-independent mechanisms of killing. ONC201 has been identified as a selective antagonist of DRD2 that activates the integrated stress response in tumor cells leading to TRAIL activation. Serum prolactin will be evaluated as a surrogate marker for target engagement, which is induced by DRD2 antagonism in the pituitary gland (34). In the solid tumor phase I study, prolactin was found to increase in patients taking ONC201 and was loosely associated with response in the small trial. However, there is no evidence that DRD2 is present on tumors. In addition to serum prolactin levels, we will also evaluate select biopsy samples for the presence of DRD2.

ONC201 has shown TRAIL-dependent apoptosis as well as caspase-independent, DR4/5-independent, TRAIL-independent induction of the integrated stress response through inhibition of mitochondrial respiration. Due to these conflicting mechanisms, we will evaluate select tissue biopsy samples for markers of apoptosis including cleaved caspase-3, cleaved poly(ADP ribose) polymerase (PARP), cleaved terminal deoxynucleotidyl transferase dUPT nick end labeling (TUNEL), ATF4 expression, CHOP expression, phospho-AMPK levels, annexin-V, TRAIL receptor and TRAIL expression. We will also evaluate markers of apoptosis in the serum including TRAIL expression, M30 assays, and M65 assays. We also plan to evaluate serum cytokine levels including IL6, L24, IL32, IL1RAP, which were found to be increased after ONC201 administration on RNAseq.

Like Metformin, ONC201 induces inhibition of mitochondrial respiration. Metformin induces mitochondrial stress which in turn initiates the integrated stress response through AFT4 which increases fibroblast growth factor 21 (FGF21). Due to similarities between these two drugs, we will evaluate the lipid profile and serum lactic levels due to known effects of Metformin on these labs (38).

Recent data from the El-Deiry lab suggests that ONC201 increases NK cell numbers and that this may contribute to the anti-tumor activity.(39) If sufficient PBMCs are available, PBMCs will be processed and stored at Trepel Lab according to SOP (Table 5), and will be assessed using multi-parameter flow cytometry for immune subsets including but not necessarily limited to Tregs, myeloid-derived suppressor cells, effector CD4+ and CD8+ T-cells, NK cells and monocyte subsets. Assessment will include functional markers, i.e. PD-1, Tim-3, CTLA-4, CD40, HLA-DR and PD-L1. This study will be done in collaboration with Jane Trepel of Developmental Therapeutics Branch/CCR/NCI.

Peripheral blood will be collected at baseline and post-therapy to enumerate circulating tumor cell (CTC) levels at baseline and post-therapy, and analyze for correlation with clinical response or survival. CTCs will be assessed using ferrofluidic enrichment and multiparameter flow cytometric detection. CTCs will be identified as viable, nucleated cells, that positively express one or more epithelial or tumor markers and are negative for expression of hematopoietic markers. Cell free DNA (cfDNA) and mitochondrial DNA will also be evaluated.

Finally, due to impressive preclinical data involving mitochondrial stress following by ballooning after exposure to ONC201, tumor biopsy samples will be evaluated by electron microscopy to evaluate the impact of ONC201 on mitochondrial and cellular morphology.

All samples will be barcoded, with data entered and stored in Labmatrix, the patient sample data management system utilized by the NCI Clinical Pharmacology Core (CPC). This is a secure program, with access to the Labmatrix system limited to defined CPC personnel who are issued individual user accounts. The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, sample collection time, cycle time point, dose, material type, as well as box and freezer locations. Patient demographics associated with the clinical center patient number are provided in the system. Samples will be stored until requested by the PI. All requests are monitored and tracked in Labmatrix.

Table 4**: Correlative Studies***

PMBC	Plasma/Whole Blood	Archived Tissue Samples	Tumor and/or Effusion Cells
Immune phenotyping Dendritic cell maturation	TRAIL levels Serum M30 assay Serum M65 assay Cytokines Circulating Tumor Cells Cell free DNA Cell free Mitochondrial DNA	Whole exome sequencing RNASeq Markers of Apoptosis TRAIL expression TRAIL receptor expression Dopamine Receptor D2	Whole exome sequencing RNASeq Dopamine Receptor D2 Electron Microscopy

* Correlative studies will include but are not limited to the studies listed above. Correlative studies will be performed if adequate samples are collected and on select patients.

5.2 Genomic Alterations in Breast and Endometrial Cancers

Patients with breast or endometrial cancer who are being evaluated for treatment may have tumor tissue obtained through the course of work-up, diagnosis or staging of their disease. This tissue may be submitted from outside (i.e. FFPE tissue) or obtained during tests at the Clinical Center. This tumor tissue will be submitted to Frederick for next-generation DNA sequencing using comprehensive cancer gene panel testing. This tumor sequencing is exploratory and will not be reported to the patient. Germline testing will be performed with the sole purpose to help identify the somatic mutations. As many as 20 patient tumor samples will be sent for molecular profiling per year of the protocol. No exploratory germline testing will be performed on this protocol.

Patients with endometrial cancer and associated malignant peritoneal cytology that is obtained at the time of staging laparoscopy will have that peritoneal fluid submitted for clinical diagnostic cytopathologic analysis in the Laboratory of Pathology. In addition, any peritoneal or pleural fluid collected during enrollment on the study will be examined for malignant cells in the Laboratory of Pathology's Cytopathology section.

5.3 TUMOR BIOPSIES

5.3.1 Timing*

Biopsies will be performed at the following times:

- Mandatory – after consent, prior to treatment on cycle 1 day 1
- Mandatory - cycle 2 day 2 (< 24 hours of ONC201 dose)
- Optional - at the time of progression

*Biopsies may be not performed on the specific dates and times due to the following reasons, including but not limited to, delayed recovery of hematologic toxicities, delayed clinic schedule, or national holidays.

5.3.2 Tissue Sampling and Handling

1. Attempts will be made to obtain up to four cores if safe and feasible. These tumor core biopsies will be obtained percutaneously or per vagina through interventional radiology (for endometrial cancer cohort) as long as considered minimal surgical risk. Two 3-millimeter punch biopsies of skin will be acceptable in lieu of 18-gauge core biopsies for patients with skin involvement. Inability to get tissue with a reasonable attempt will not preclude treatment and the patient will remain eligible for all other translational components.
    a. Core 1: Electron Microscopy
    b. Core 2: RNA later buffer
    c. Cores 3: FFPE
    d. Cores 4 and 5: OCT

2. The use of imaging to facilitate biopsies will be decided upon by members of the interventional radiology team. Should CT scans be needed for biopsy, a limit of 10 scans for each procedure will be observed to minimize radiation exposure to the patient.

3. The schedule for the biopsies will be made with Special Procedures (Dr. Elliott Levy). Members of the WMB lab will be on call to receive and embed biopsies: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.

4. All cores will be stored, barcoded, in the WMB lab at -80ºC on site until use according to our laboratory SOP. See **Table 5** for information about specimen collection and storage.

5.4 BLOOD SAMPLES

The following tests will be performed on selected patients' peripheral blood samples. If collected samples are not sufficient, the correlative studies will be performed in order of priority as detailed below. These correlative studies will be done in an exploratory fashion.

5.4.1 Timing

Research blood samples will be performed at the following times:

- C1D1: after consent, prior to treatment on cycle 1 day 1
- C1D1: 6 hours after ONC201 administration (blood for PK only)
- On Day 1 of each cycle starting with cycle 2: Prior to treatment on day 1
- Optional - at the time of progression

5.4.2 Sampling and Handling

5. We will collect no more than 40ml of research blood at each time point specified. If there is an issue obtaining blood from a participant, samples will be prioritized as follows:
    a. 8ml of blood for immune assays (citrate blue/black tiger top)
    b. 10 ml of blood for circulating tumor cells, cell free DNA and cell free mitochondrial DNA (cell save preservative)
    c. 10ml of blood for germline DNA analysis for correlative studies (Streck tube; C1D1 only)
    d. 4ml of blood for cytokine analysis (lavender top tube)
    e. 6ml of blood each for serum M30/M65 and Trail expression (red top tube)
    f. 6ml of blood for Pharmacokinetics (lavender top tube; C1D1 only but prior to drug ingestion and then 6 hours after)

6. Members of the WMB lab will be on call to receive patient samples: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.

7. See **Table 5** for information about specimen collection and storage.

8. As soon as possible after the patient is scheduled please send an email notification to the Trepel Lab, Developmental Therapeutics Branch, NCI: Jane Trepel at trepel@helix.nih.gov; Sunmin Lee at leesun@mail.nih.gov; Min-Jung Lee at min-

jung.lee@nih.gov and Akira Yuno at akira.yuno@nih.gov that the sample is scheduled. After the samples are drawn please call the Trepel lab at 240-760-6330 to communicate that the sample is ready. Keep the sample on the unit at room temperature. The sample will be picked up by a lab member, entered in a secure patient database and processed for CTC assessment and immune subset analysis.

5.5 TABLE 5: SPECIMEN COLLECTION AND STORAGE

Tumor Biopsy*

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
Electron Microscopy	C1D1 prior to treatment C2D2 *Optional at time of progression*	Preserve the biopsy tissue sample is to fix the tissue without delay 1. Prepare Eppendorf microfuge tube (~1.5ml) or conical centrifuge tube (15ml) fill with EM fixative (4% formaldehyde + 2% glutaraldehyde in 0.1M cacodylate buffer, ambient temperature) and drop the tissue into the fixative. 2. Fix for 2hrs at room temperature, then store in 4C. 3. Obtain barcode	Members of the WMB labs will be on call to receive and embed biopsies: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	Fix for 2hrs at room temperature, then store in 4C. Will be shipped in batches to Kunio Nagashima for processing/embedding for the EM analysis. Kunio Nagashima 8560 Progress Drive Wing D, Room D2018, 2nd floor Frederick Maryland 21701 Phone: 301-846-1594 FAX: 301-846-6716 EMAIL: nagashimak@mail.nih.gov
Dopamine Receptor D2 And Death Receptors	C1D1 prior to treatment C2D2 *Optional at time of progression*	a) Prepare two or three Eppendorf tubes which contain 1 ml of 4% formalin and leave tubes at room temperature. b) Quickly make your way to Interventional Radiology (IR) on the floor. Ask one of the techs or nurses where your patient is and prepare to receive the sample. c) Lay a paper towel next to your bucket. d) Take out the forceps and rinse/dry them thoroughly before placing them on the paper towel. e) Put on a mask, hair net and gloves.	Members of the WMB labs will be on call to receive and embed biopsies: ozakimk@nih.gov, Phone 213-703-2032 / 410-522-5661, beeper 102-11155..	Oncoceutics requests: 5 unstained slides (FFPE) from each time point Will later be shipped to Oncoceutics: John Coolidge Biorespiratory Manager- Boston SciSafe, Inc 35 Dunham Road Billerica, MA 01821 Tel. 404-242-1578

		f) When the radiologist is ready, take the slide with the sample on it while being **CAREFUL NOT TO TOUCH THE DOCTOR'S GLOVE.** g) Use the forceps to gently pickup and place the sample in the Eppendorf tube. h) Leave the Eppendorf tube at room temperature overnight. i) Next day carefully remove 4% formalin from the Eppendorf tube and replace 1 ml of 70% ethanol. Leave the tube at room temperature. j) Call AmericanHistolab (Tel: 301-330-1200) to pick up the sample. k) Cost for paraffin embedding and H&E staining: $7.95/block. Catalogue Number: 1001 l) The sample labelling with a random code and barcoding should be same as OTC preserved samples collected at the same day		Email: jfc@scisafe.com At the time of shipment, the laboratory must complete the Specimen Shipping Form (section **15.6.5**) and email it to the following: 1. Rohinton.tarapore@oncoceutics.com a) jfc@scisafe.com
Whole Exome Sequencing and RNAseq done	C1D1 prior to treatment C2D2 *Optional at time of progression*	• Perform core needle biopsy. • Pick the core from the biopsy needle onto a sterile glass slide. • Fill cryomold about 1/3 full with OCT. Place the cryomold in dry ice to partially freeze the optimal cutting temperature (OCT). The OCT should be jelly-like, not completely frozen. • Carefully lift the core biopsy by both ends with sterile forceps. Do not stretch the biopsy or it will break.	Members of the WMB labs will be on call to receive and embed biopsies: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	3 Frozen sections should be cut at 5-8um on plain, uncoated glass microscope slides. 4 The tissue section should be placed as close as possible to the center of the slide. Do not place the frozen section at the end of the slide. 5 Two tissue sections from the same biopsy may be placed on the same glass slide if space permits. 6 Do not allow the tissue section to air on the slide. Freeze immediately on dry ice or

		• Lay the biopsy as straight as possible in the OCT. Once the sample touches the OCT, you cannot reposition it or the sample will break apart. • Quickly add OCT on top of the biopsy, completely covering the sample. • Ensure the sample is level and freeze immediately in dry ice. • Obtain barcode Store wrapped in aluminum foil or in a 50ml Falcon tube at 70 degrees C.		at– 80 degrees C.

*Biopsies may be not performed on the specific dates and times due to the following reasons, including but not limited to, delayed recovery of hematologic toxicities, delayed clinic schedule, or national holidays.

Plasma/Serum

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
Pharmacokinetics	C1D1 prior to treatment C1D1 6hr after drug *Optional at time of progression*	1. Collect 6ml of blood into a Lavender top, vacutainer tube 2. Obtain barcode Blood must be centrifuged, separated and frozen within 2 hours of collection. Specimens must be stored frozen at -70ºC (preferred) or -20ºC (acceptable) at each study site until shipped on dry ice to the designated analytical laboratory.	Members of the WMB labs will be on call to receive study samples: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	1. Centrifuge the filled lavender top tube at 1500 g (3000 rpm) for 15 minutes until the plasma and the cells are well separated. 2. Label 2 blue cap transfer vials lengthwise with the appropriate barcode labels indicating “plasma (blue)”. 3. Clearly print the study number “Study XXX”, patient ID number, date and time of the blood draw. 4. Transfer ~1.5 mL of plasma into each of the 2 blue top transfer vials labeled “plasma (blue)” 5. Store sample in a freezer with a minimum -20ºC or preferable -70ºC

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
				Will later be shipped to Oncoceutics**:** Sample Receiving Officer XenoBiotic Laboratories, Inc 107 Morgan Lane Plainsboro, NJ 08536 Tel. 609-799-2295 x252 Email: sro@xbl.com At the time of shipment, the laboratory must complete the Specimen Shipping Form (**15.6.5** Appendix E) and email it to the following: m) Rohinton.tarapore@oncoceutics.com n) sro@xbl.com
Serum M30 and M65	C1D1 prior to treatment Day 1 of each subsequent cycle *Optional at time of progression*	4. Collect 6ml of blood into a Red top, vacutainer tube 5. Obtain barcode Blood must be centrifuged, separated and frozen within 2 hours of collection. Specimens must be stored frozen at -70ºC (preferred) or -20ºC (acceptable) at each study site until shipped on dry ice to the designated analytical laboratory.	Members of the WMB labs will be on call to receive study samples: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	1. Centrifuge the filled red top tube at 1500 g (3000 rpm) for 15 minutes until the plasma and the cells are well separated. 2. Label 2 yellow cap transfer vials lengthwise with the appropriate barcode labels indicating “serum (red)”. 3. Clearly print the study number “Study XXX”, patient ID number, date and time of the blood draw. 4. Transfer ~1.5 mL of serum into each of the 2 yelloq top transfer vials labeled “serum (red)” 5. Store sample in a freezer with a minimum -20ºC or preferable -70ºC

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
				See above Pharmocokinetics section for shipping information to Oncoceutics.
TRAIL Expression	C1D1 prior to treatment Day 1 of each subsequent cycle *Optional at time of progression*	1. Collect 6ml of blood into a Red top, vacutainer tube 2. Obtain barcode Blood must be centrifuged, separated and frozen within 2 hours of collection. Specimens must be stored frozen at -70ºC (preferred) or -20ºC (acceptable) at each study site until shipped on dry ice to the designated analytical laboratory.	Members of the WMB labs will be on call to receive study samples: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	2. Centrifuge the filled red top tube at 1500 g (3000 rpm) for 15 minutes until the plasma and the cells are well separated. 3. Label 2 yellow cap transfer vials lengthwise with the appropriate barcode labels indicating “serum (red)”. 4. Clearly print the study number “Study XXX”, patient ID number, date and time of the blood draw. 5. Transfer ~1.5 mL of serum into each of the 2 yelloq top transfer vials labeled “serum (red)” 6. Store sample in a freezer with a minimum -20ºC or preferable -70ºC See above Pharmocokinetics section for shipping information to Oncoceutics.
Germline gDNA	C1D1	1. Collect 10mL of blood into BCT Streck tubes (Label the tube with appropriate Study/Patient Number Identifiers. Record the time of collection and whether the blood was drawn using a peripheral venous access device (eg. cannula	Members of the WMB labs will be on call to receive study samples: ozakimk@nih.gov, Phone 410-522-5661, beeper 102-11155.	See **Appendix D** for full SOP

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
		or butterfly) or a central venous access device (CVAD). 2. After collection, gently invert tubes 8-10 times to mix and leave tubes upright prior to centrifugation. 3. Obtain barcode		
Circulating Tumor Cells, Cell free DNA, and Cell free mitochondrial DNA	C1D1 prior to treatment Day 1 of each subsequent cycle *Optional at time of progression*	1. Either one 10-mL CellSave Preservative Tube OR a 10-mL Streck tube, PLUS one 10-mL CPT tube for each time point will be collected from each patient. Please, make sure tubes are full. 2. Immediately after collection, invert the blood tubes 3-4 times. 3. Plasma from these samples will be stored at Trepel lab after CTCs are harvested. 4. Obtain barcode	As soon as possible after the patient is scheduled please send email notification to the Trepel lab: Jane Trepel at trepel@helix.nih.gov; Sunmin Lee at leesun@mail.nih.gov; Min-Jung Lee at min-jung.lee@nih.gov; Akira Yuno at akira.yuno@nih.gov that the sample is scheduled. As soon as the sample is drawn, please call the Trepel lab at 240-760-6330 to communicate that the sample is ready. Keep the sample on the unit at room temperature. The sample will be picked up by the lab and processed for CTC enumeration.	
Cytokine Analysis	C1D1 prior to treatment Day 1 of each subsequent cycle *Optional at time of progression*	3. Collect 4ml of venous blood in a venous blood BD Vacutainer spray-coated K2EDTA (lavender top) tube. 4. Gently invert 10 times to mix blood and anticoagulant. 5. Store tubes at room temperature until centrifugation.	As soon as possible after the patient is scheduled please send email notification to the Blood Processing Core for processing and stoage of the blood sampled (EDTA plasma) at NCIBloodcore@mail.nih.gov - at least 24 hours before transporting samples (the Friday before is preferred). For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov As soon as the sample is drawn, please call the BPC to communicate that the sample is ready. Keep the sample on the unit at room temperature.	

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
		It is generally preferred and often necessary to process the samples within 5 hours of collection.	Plasma will be separated and aliquoted according to the BPC SOPs and stored, barcoded, in the CPC repository until use (See Section **15.6.5**).	

Peripheral Blood Mononuclear Cells (PBMCs)

Correlative Study	Collection Point(s)	SOP for collection	Contact	SOP after collection
Immune Assays	C1D1 prior to treatment Day 1 of each subsequent cycle *Optional at time of progression*	6. One 8-ml BD Vacutainer Cell Preparation Tubes (CPT citrate blue/black tiger top) will be collected from each patient. 7. Immediately after collection, mix the blood sample by gentle inversion several times. The date and exact time of each blood draw should be recorded on the tube.	As soon as possible after the patient is scheduled please send email notification to the Trepel lab at trepel@helix.nih.gov; Sunmin Lee at leesun@mail.nih.gov; Min-Jung Lee at min-jung.lee@nih.gov; Akira Yuno at akira.yuno@nih.gov and call the Trepel lab at 240-760-6330 to arrange for immediate pick-up when the sample is drawn.	

5.6 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples collected at NCI will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

At the end of the protocol, samples will be stored for potential further analysis as new information becomes available (only for those subjects who consented to future optional studies). Any new use of identified or coded samples, specimens, or data will undergo prospective and continuing IRB review and approval.

5.6.1 Patient sample protections

Each patient sample set will be coded with a unique patient identifier. No patient specific information is encoded in this identifier. The protocol scientific investigators handling samples will be blinded as to as to the patient identification, patient data and outcome. Tissue and blood samples obtained by and stored through WMB will be labeled with unique barcodes.

Blood samples obtained by and stored through the Clinical Pharmacology Core and Trepel Lab will be labeled with unique barcodes obtained by Dr. Figg's laboratory barcode system.

The amount of blood that may be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

If a patient needs to have a malignant effusion or ascites tapped for diagnostic or therapeutic purposes, a sample will be collected for research.

5.6.2 Sample Storage, Tracking and Disposition (CPC)

Sample Data Collection:

All samples sent to the Clinical Pharmacology Core (CPC) will be barcoded, with data entered and stored in the Labmatrix system utilized by the CPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Sample Storage and Destruction:

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20° or -80° C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested) The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section **7.2**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.6.3 Laboratory of Jane Trepel

Samples will be processed immediately by the Trepel laboratory. Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. Specimen labels will indicate: protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

5.6.4 Procedures for storage of tissue specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissue that is not placed in paraffin blocks is stored in formalin for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.6.5 End of Study

At the end of the protocol, samples will be stored for potential further analysis as new information becomes available (only for those subjects who consented to future optional studies). Any new use of identified or coded samples, specimens, or data will undergo prospective and continuing IRB review and approval.

The study will remain open so long as sample or data analysis continues. Samples, and associated data, will be stored in the locations listed above until they are no longer of scientific value, or unless the patient withdraws consent for their continued use. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the WMB laboratory or to the BPC repository. Samples can only be saved at the completion of the study for future use if subjects consented. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

5.7 Samples for Genetic/Genomic Analysis

If not previously evaluated for germline mutations in BRCA1/2 or Lynch syndrome, patients will undergo evaluation upon enrollment on trial. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects will

be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory which has an FDA approved test for that mutation. If the research findings are verified in the CLIA certified lab, the subject will be referred to the NIH Genetics counseling service for the disclosure of the results.

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.7.1 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study will obtain a Certificate of Confidentiality which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the study treatment was last administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Source Documents

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, patients' diaries or evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, X-rays. The investigator will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s), providing direct access to source documents.

6.1.2 Case Report Forms

Data may be entered from the source documents directly into eCRFs in C3D for each patient enrolled in this study. The principal investigator or research nurse will review the eCRFs for completeness and accuracy. Independent audits may also be conducted by NCI personnel to ensure completeness and accuracy of data in C3D.

6.2 Data Sharing Plans

6.2.1 Human Data Sharing Plan

I will share human data generated in this research for future research as follows:

1. Coded, linked data in an NIH-funded or approved public repository.
2. Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
3. Identified or coded, linked data with approved outside collaborators under appropriate agreements.

Data will be shared through*:*

1. An NIH-funded or approved public repository. Insert name or names:_clinicaltrials.gov, dbGaP.
2. BTRIS (automatic for activities in the Clinical Center)
3. Approved outside collaborators under appropriate individual agreements.
4. Publication and/or public presentations.

Data will be shared:

a) Before publication.
b) At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks +/- 4 days with imaging modalities determined appropriate by the investigators. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks +/- 4 days following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).(40) 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.

6.3.1 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:

1. By chest x-ray: ≥20 mm;
2. By CT scan:
    1. Scan slice thickness 5 mm or under: as ≥10 mm
    2. Scan slic thickness > 5 mm: double the slice thickness
3. With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and 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, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, 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.

6.3.2 Methods for Evaluation of Measurable Disease

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

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

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

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. 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.

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.

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 date 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 at 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.

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.

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.

6.3.3 Response Criteria

6.3.3.1 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 of 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 of diameters while on study.

6.3.3.2 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).

6.3.3.3 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 Patients 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: Patients 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 Patients 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		

6.3.4 Duration of Response

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

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

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

6.3.5 Progression-Free Survival

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

6.4 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found **here**.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found **here**. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found **here**..

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7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the

investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of

death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version v5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

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8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Safety Reporting Criteria To The Pharmaceutical Collaborators

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

Serious Adverse Event and Suspected Unexpected Serious Adverse Reaction, Medical Device Event, Potential Incident, Device Deficiency or Incident Reporting**:** Principal Investigator shall forward to Oncoceutics Inc, any SAE, SUSAR, Medical Device Event, Device Deficiency or Incident information, including, but not limited to, all initial and follow-up information involving any Study subject in the Study. Notification shall be in the form of a completed CIOMS I/MedWatch (or other mutually agreed upon format) within two (2) business days of learning of the information. This information shall be transmitted to Oncoceutics using the contact information provided below or such other modified contact information as provided by Oncoceutics in writing. All information shall be transmitted in the English language and contain the reporter's name and the Study subject identifier code. SUSAR information will be reported unblinded if the Study Drug has been blinded in the Study. Randomization codes for all other SAEs will be provided to Oncoceutics at end of Study if the Study Drug has been blinded in the Study.

Oncoceutics may define certain Non-Serious Events of Interest. If any Non-Serious Events of Interest are defined, Oncoceutics will provide such information in writing to Principal Investigator at the time of Protocol approval, execution of the CTA or anytime thereafter. Reporting of any defined Non-Serious Events of Interest will be handled in the same manner as SAEs unless mutually agreed otherwise in writing by the parties.

All reports of Study Drug exposure during pregnancy or lactation (including a female partner of a male Study subject using the Study Drug), whether associated with an AE or not, must be reported to Oncoceutics in accordance with the timelines and contact information for an SAE. Principal Investigator shall follow pregnancies to term to obtain the outcome of the pregnancy. The outcome of the pregnancy shall be forwarded to Oncoceutics.

Institution and Principal Investigator shall fully comply with all of their respective reporting obligations to the applicable regulatory authorities with respect to any AE, SAE or SUSAR that arises from the Study.

SAE reports and any other relevant safety information are to be forwarded to Josh Allen, PhD of Oncoceutics via email (josh.allen@oncoceutics.com) and/or facsimile number: 1-844-245-7650.

A MedWatch form is available at http://www.fda.gov/medwatch/

8.5 REPORTING PREGNANCY

8.5.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the pregnancy becomes known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 28 days after the last dose of ONC201.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 28 days after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation

- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

The primary objectives of this trial are to determine whether ONC201 is able to be associated with 1) potential improvement in the PFS probability of patients with refractory/recurrent metastatic HR+ breast cancer, 2) an improvement in overall response rate in patients with metastatic TNBC, or 3) an improvement in overall response rate in patients with advanced endometrial cancer.

Primary Endpoints:

- Cohort 1: To determine the progression free survival (PFS) at 8 months of ONC201 in metastatic hormone receptor positive breast cancer (HR+BC)
- Cohort 2: To determine the overall response rate (ORR) of ONC201 in metastatic triple negative breast cancer (TNBC)
- Cohort 3: To determine the ORR of ONC201 in advanced endometrial cancer (EC)

Secondary Endpoints for all cohorts will include safety of ONC201, clinical benefit rate (defined as partial response + complete response + stable disease).

10.2 SAMPLE SIZE DETERMINATION

Cohort 1: Refractory/recurrent metastatic HR+ breast cancer

Data from published trials indicate that the median PFS in patients with refractory/recurrent metastatic HR+ breast cancer is approximately 4 months. In this cohort, the goal is to determine if use of ONC201 is able to be associated with an 8-month median PFS probability (7, 8). With 24 evaluable patients receiving the proposed therapy, assuming accrual would take place over approximately 2 years, and that there would be at least 1 year of additional potential follow-up after the last patient has begun the therapy, there would be 80% power to determine whether there is a difference between a 4 month median PFS and an improved 8-month median PFS, with a one sided 0.10 alpha level test, using the method of Brookmeyer and Crowley (41). In practice, Kaplan-Meier curves and appropriate confidence intervals at selected time points will be provided to help interpret results relative to the expected results. As an early stopping rule, if 0 of the first 5 patients is able to be progression-free at 8 months, then no further patients will be accrued into this cohort as soon as that is able to be determined since the upper 95% one-sided confidence bound on 0/5 is

45%, and thus this indicates inconsistency with an 8-month median PFS. The study may need to pause accrual to evaluate if PFS in any of the first 5 patients is ≥ 8 months.

Cohort 2: Metastatic TNBC

Data which have been published suggest that a response rate (PR+CR) for treatment of recurrent or metastatic TNBC is approximately 30-35% (13-15). In order to establish the efficacy of this treatment in patients with metastatic TNBC, the primary objective in this cohort would be to determine if using ONC201 would rule out a 25% response rate and result in a response rate consistent with 45%. As such, this cohort will be evaluated using a single stage design in order to rule out an unacceptably low PR+CR rate of 25% (p0=0.25) in favor of an improved response rate of 45% (p1=0.45). Based on a one-sided exact binomial test, 29 patients would have 82% power to rule out 25% and result in findings consistent with a 45% PR + CR rate. At the end of the study, we would form the two-sided 80% and 95% CI about the observed percentages of both response (PR+CR) and clinical benefit (SD+PR+CR). 11 responses (PR+CR) in 29 patients (37.9%) has a two-sided 80% CI of 25.7-51.5%, which would be sufficiently interesting for further evaluation. As an early stopping rule, after 10 evaluable patients have been enrolled, if there are 0 or 1 with clinical benefit (SD+PR+CR), then the cohort would end accrual as soon as this would be determined since the 90% one-sided upper CI bound on 1/10 is 33.7%, which would be marginally too low for further interest. Both the PR+CR and SD+PR+CR rates can be formed at the interim time for descriptive purposes as well as for evaluation for early stopping. The study may need to pause accrual to evaluate if clinical benefit in the first 10 patients exceeds the early stopping rule.

Cohort 3: Advanced endometrial cancer

Published data from several trials report response rates of 8 to 15% for second line treatment in patients with advanced endometrial cancer (18-21). As such, it would be desirable if new treatments are associated with at least a 12-15% response rate for further consideration. In patients with advanced endometrial cancer, the primary objective of this study would be to determine if using ONC201 would rule out a 5% response rate and result in a response rate consistent with 25%. As such, the trial will be conducted using a single stage design (42) in order to rule out an unacceptably low PR+CR rate of 5% (p0=0.05) in favor of an improved response rate of 25% (p1=0.25). Based on a one-sided exact binomial test, 25 patients would have 90% power to rule out 5% and result in findings consistent with a 25% PR + CR rate. At the end of the study, we would form the two-sided 80% and 95% CI about the observed percentages of both response (PR+CR) and clinical benefit (SD+PR+CR). 3 responses (PR + CR) in 25 patients (12.0%) has a two-sided 80% CI of 4.5-24.8%, which is potentially worthy of further consideration, while 4 responses in 25 (16.0%) has a two-sided 80% CI of 7.2-29.5%, which would be sufficiently interesting for further evaluation. As an early stopping rule, after 13 evaluable patients have been enrolled, if there are 0 patients with clinical benefit (SD+PR+CR), then the cohort would end accrual since the 90% one-sided upper CI bound on 0/13 is 16.2%, which would be marginally too low for further interest. Both the PR+CR and SD+PR+CR rates can be formed at the interim time for descriptive purposes as well as for evaluation for early stopping. The study may need to pause accrual to evaluate if clinical benefit in the first 13 patients exceeds the early stopping rule.

Any secondary evaluations performed will be done in an exploratory fashion, with results presented without any formal adjustment for multiple comparisons.

It is expected that approximately 1 patient per month may enroll onto this trial in the ER+ breast cancer cohort, and that 6 to 8 patients per year may enroll in each of the other two cohorts. Thus, it is expected that 3 to 4 years may be required in order to enroll up to 24+29+25=78 evaluable patients. In order to allow for a small number of unevaluable patients, the accrual ceiling will be set at 90 patients.

10.3 Populations for Analyses

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with ONC201.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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

Evaluable for Progression Free Survival: In all three cohorts, all participants who took at least one cycle of study drug and have adequate follow-up to be included in a PFS analysis (cohort 1) or to have response and clinical benefit evaluated (cohorts 2 and 3) will be included in the analyses.

10.4 Statistical Analyses

10.4.1 General Approach

Analyses performed will be descriptive, reporting the outcome measure indicated along with two-tailed 80% and 95% confidence intervals for PFS (cohort 1), and 80% and 95% confidence intervals about the response rate and clinical benefit rate in cohorts 2 and 3.

10.4.2 Analysis of the Primary Endpoints

Cohort 1: Kaplan-Meier curves for PFS using all patients who receive treatment and are eligible for evaluation will be included, and appropriate confidence intervals (80% and 95% two sided confidence intervals) at selected time points will be provided to help interpret results relative to the expected results.

Cohort 2 and 3: At the end of the trial we will form the two-sided 80% and 95% CI about the observed percentages of both response (PR+CR) and clinical benefit (SD+PR+CR).

10.4.3 Analysis of the Secondary Endpoints

- Cohort 1 HR+BC – Safety—AEs will be tabulated, ORR—response rate will be reported along with 95% two-sided confidence interval, clinical benefit rate (CBR; CR + PR + SD

by RECIST) clinical benefit rate will be reported along with 95% two-sided confidence interval

- Cohort 2 TNBC – Safety-AEs will be tabulated CBR, clinical benefit rate will be reported along with 95% two-sided confidence interval; PFS by Kaplan-Meier analysis
- Cohort 3 Endometrial Cancer – Safety, CBR clinical benefit rate will be reported along with 95% two-sided confidence interval, PFS by Kaplan-Meier analyses

10.4.4 Safety Analyses

The safety of ONC201 tabulations be evaluated by descriptive statistics of each adverse event type and grade.

10.4.5 Baseline Descriptive Statistics

No formal analysis of baseline statistics will be performed.

10.4.6 Planned Interim Analyses

The interim analyses are described for each cohort in the sample size determination paragraph.

10.4.7 Sub-Group Analyses

No subgroup analyses are planned.

10.4.8 Tabulation of individual Participant Data

No individual participant data will be provided.

10.4.9 Exploratory Analyses

Blood samples at baseline and Day 1 of each subsequent cycle, as well as biopsy specimens at baseline and cycle 2 will yield a set of biomarkers, such as markers of apoptosis, indications of mitochondrial damage and immune subsets. The difference of the values at cycle 1 vs. baseline and cycle 2 vs. baseline will be determined, and these differences will be used as a covariate for analysis of PFS in each cohort to see if the level of change in biomarkers may be associated with PFS. These analyses will be done in an exploratory fashion using Kaplan-Meier curves and log rank tests. The changes in the biomarkers will also be compared between responders and non-responders in the three cohorts using an exact form of a Wilcoxon rank sum test. All of these explorations will be done only if adequate patient data are available. Results will be presented without adjustment for multiple comparisons.

11 COLLABORATIVE AGREEMENTS

11.1 CLINICAL TRIAL AGREEMENT

The study agent, ONC201 is provided by Oncoceutics., Inc. under a Clinical Trial Agreement (NCI CTA #1035). Results or study data may be communicated to CTA partner, according to the terms of the NIH- Advanced Accelerator Applications SA CTA.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial and ethnic groups are eligible for this trial if they meet the eligibility criteria. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to racial or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

12.2 PARTICIPATION OF CHILDREN

The age group for enrollment on this trial is 18 or more years of age. Because no dosing or adverse event data are currently available on the use of ONC201 in participants < 18 years of age, children are excluded from this study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

ONC201 is an investigational agent for use in metastatic breast cancer and in advanced endometrial cancer. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity. Toxicity data will be collected and reviewed to ensure that there were no severe toxicities that would preclude further patient enrollment. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored.

12.5 RISKS/BENEFITS ANALYSIS

12.5.1 Benefits

The potential benefit to a patient on this study is a reduction or stability in breast cancer or endometrial cancer, as well as potentially the prevention or delay in development of new metastatic lesions, which may or may not have favorable impact on symptoms and/or survival.

12.5.2 Risks

12.5.2.1 Study Drug Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or in section **13.1.2** of this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.5.2.2 Radiation Risks

The study will involve radiation from the following sources:

- Up to 3 CT scans for the collection of biopsies
- Up to 8 CT scans per year for disease assessment
- 1 technetium-99 bone scan for disease assessment

Subjects may be exposed to approximately 11.59 rem. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 1.2 out of 100 (1.2 %) and of getting a fatal cancer is 0.6 out of 100 (0.6%).

12.5.2.3 Risks from Blood Draws:

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.5.2.4 Risks of CT Scans:

In addition to the radiation risks discussed above, risks associated with CT scans are allergic reaction to and kidney damage from the contrast dye, nausea, vomiting, and anxiety.

12.5.2.5 Risks of Technetium-99 Bone Scans:

In addition to the radiation risks described above, risks associated with technetium-99 bone scans are pain, bruising, injection site infection, and allergic reaction to the contrast agent.

12.5.2.6 Specimen Collection Risks

Risks include those associated with specimen collection including pain, bleeding and the possibility of infection at the sampling site. All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsy, immediate medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully

evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5.3 Risks/Benefits Analysis

The potential benefits from this therapy are stabilization or shrinkage of the tumor and a reduction in chances of developing new lesions, with decrease of symptoms caused be progressive disease. Given the efforts to minimize risk with the administration of this combination, this protocol involves greater than minimal risk, but presents the potential for direct benefit to individual subjects.

12.6 Consent Process and Documentation

The informed consent document will be provided to the participant or consent designee(s) (e.g., the legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

For the optional biopsy in the protocol, the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.6.1 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.5.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be

provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 PHARMACEUTICAL INFORMATION

13.1 DRUG ONC201 (IND # 136939)

13.1.1 Source

ONC201 is an investigational drug supplied under a Clinical Trials Agreement by Oncoceutics INC.

13.1.2 Toxicity

Generally, ONC201 is well tolerated at all of the tested dose levels. To date, over 70 patients have taken at least one dose of ONC201 and no Grade IV events have been reported. One grade II allergic reaction occurred after the 5th dose of ONC201 625mg (q3 week schedule) that was managed by standard over the counter medications. One grade III neutropenia was transiently observed after the 5th dose of ONC201. The dose was reduced to 500mg q3weeks without recurrence of the neutropenia (**Table 6**).(2, 4)

Table 6: All Grade I AEs attributed (possibly related) to ONC201 on every 3-week dosing schedule for the Phase I trial.

ONC201 Dose	**125mg**	**250mg**	**375mg**	**500mg**	**625mg**
No. of Patients	1	1	1	1	24
Pyrexia	1	0	0	0	0
Fatigue	1	0	0	0	0
Elevated Amylase	0	0	0	0	2
Emesis	0	0	0	0	1
Nausea	0	0	0	0	1

* Note: No drug-related AE > Grade 1 reported on the Phase I solid tumor trial

While human exposure to ONC201 is still somewhat limited at this time, side effects seen in animals included the following:

- Nausea
- Salivation
- Vomiting
- Abnormal breathing
- Twitching

- Abnormal walking or standing

While side effects are minimal, based on pharmacokinetic data, it would be reasonable to dose reduce if more significant side effects did occur. Based on the mean AUC and Cmax data from the phase I study, a dose reduction to 500mg weekly with two further reduction to 375mg weekly and then 250mg weekly would be reasonable and would likely not significantly affect clinical benefit (**Figure 5**).

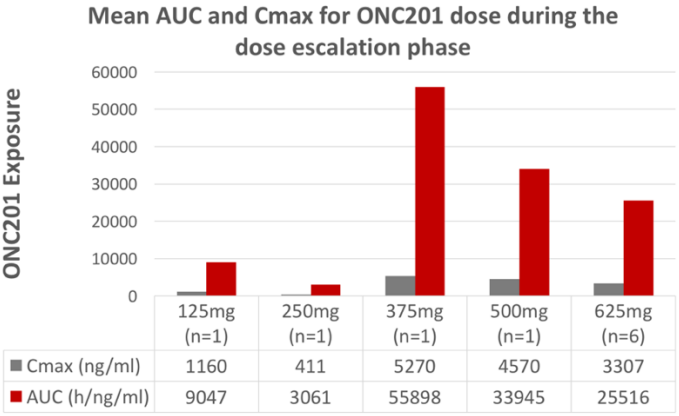


Figure 5: Mean AUC and Cmax for each dose cohort following first dose of ONC201. *Image adapted from Stein et al, 2015* (2).

The metabolism and excretion of ONC201 is still unknown at this time. However, it is suspected to be excreted by the kidneys. This is supported by preliminary observations in rats (unpublished data, communication with Oncoceutics, Inc).

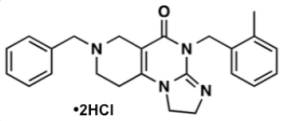
The effects of ONC201 on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. An assessment should be made to ensure the potential benefit would justify a potential risk to the fetus.

It is not known whether ONC201 is excreted in breast milk. It is recommended that women do not breastfeed during treatment with ONC201 and for 2 months after the last dose.

13.1.3 Formulation and preparation

The investigational drug product is a hydroxypropyl methylcellulose (HPMC) capsule filled with ONC201 dihydrochloride, intended for oral administration. Each capsule of drug product contains the equivalent 125mg of anhydrous ONC201 free base with or without microcrystalline cellulose. This corresponds to ~150mg of drug substance that corrects.

The ONC201 drug substance is a well characterized small molecule produced as a dihydrochloride salt. ONC201 dihydrochloride is a white to off-white solid, freely soluble in water.

Compound Code(s)	ONC201•2HCl
Alternative Name(s)	ONC201 TIC10 MLS003171082 NSC-350625 41276-02-2 AC1L7JLY NCIStruc1_00799 NCIStruc2_001940 CCG-37418 NCGC00014802 NCI350625 NCGC00014802-02 NCGC00097903-01 NCI60_003126 SMR001874991
Chemical Name(s)	7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(4H)-one•2HCl
Chemical Abstracts Service (CAS) number	41276-02-2
Molecular Formula	C24H26N4O (free base) C24H26N4O•2HCl (salt)
Molecular Weight	386.49 (free base) 459.41 (salt)
Molecular Structure	

13.1.4 Stability and Storage

The capsules are to be stored in a closed container at room temperature (15 to 30°C).
Based on the current stability data room temperature (25°C/60%RH) will be used for the drug product storage. No shelf life has been established for this product at this point. However, representative clinical trial batches will be placed on stability. Any batches that are out of specifications will be removed from use.

The product is stored in a multi-dose container. The capsules are packaged in high-density polyethylene (HDPE) white opaque bottles, closed with an induction seal and capped with a white ribbed SecuRx® polypropylene (PPE) cap. The capsules are to be stored in the original closed container at room temperature (15 to 30°C).

Administration procedures
ONC201 is taken by mouth every 7 days by the patient. Pharmacy can dispense the exact number of capsules needed for cycle, as prescribed by the physician, by transferring them to a white,

opaque, HDPE container with child-proof closures. This type of container is the same as the one the drug is initially supplied in. Remaining capsules will be kept in the original bottle and will be dispensed for the subsequent prescription/subject.

13.1.5 Incompatibilities

The investigator's brochure for ONC201 by Oncoceutics does not describe any known drug-drug interactions with ONC201. In vitro cytochrome P450 assays were conducted in human hepatocytes. In these studies, ONC201 is not an induced of the CYP450 system (CYP1A2, 2B6, 2B6, and 3A4). ONC201 was observed to be a mild inhibitor of the CYP450 enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 3A4) at 35-429 uM, i.e., at least 3.5 fold above the Cmax observed in the first-in-human trial.

Drugs that antagonize DRD2 are commonly used as antipsychotics. However, comparison between ONC201 and other typical antipsychotics (such as haloperidol and risperidone) reveal substantial differences in anti-tumor activity, therapeutic window, specificity and receptor pharmacology. Moreover, the current administration schedule of ONC201 dosing is atypical for antipsychotic drugs. Taken together, there is no clear expectation that side effects that are commonly observed with antipsychotic drugs will be observed with ONC201 in the current schedule. Nevertheless, it is possible that side effects associated with antipsychotics could be observed with ONC201; weight gain, diabetes, metabolic disorders, dystonia, akinesia, akathisia, tremors, muscle rigidity, insomnia, blurry vision, Parkinsonian symptoms, cognitive impairment, sexual dysfunction, osteoporosis, sedation, behavioral changes, and cardiac disorders.

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15 APPENDICES

15.1 APPENDIX A-PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (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.

15.2 APPENDIX B- METHOD FOR PREPARING PBMC SAMPLES FROM BLOOD FOR PHARMACODYNAMIC (PD) STUDIES

1. Using a BD Vacutainer CPT, draw the blood sample. Assure that the anticoagulant being used (sodium citrate or sodium heparin) is correct for the intended use of the specimen and that the tube size is correct for the blood draw volume required by the protocol.
2. Mix the sample by inverting the tube for 5-8 times and centrifuge the sample at 1500 g for 30min at 18-25°C, without break.
3. After centrifuge, remove two thirds of plasma and transfer whitish PBMC layer into the labeled 15-ml conical tube using a 3-ml Falcon transfer pipet.
4. Discard the Vacutainer CPT tube in biohazard waste container.
5. Add Plasma-Lyte A USP to the 15-ml tube to bring total volume to 14 ml, cap and mix by gentle inversion 5-8 times.
6. Centrifuge the sample at 430 g for 10 min at 18-25°C, without break.
7. Using a new transfer pipet, aspirate as much supernatant as possible without disturbing the cell pellet and discard the supernatant into biohazardous liquid waste.
8. Re-suspend the cell pellet in 2 ml of Plasma-Lyte A USP by gently flicking the bottom of the tube with the index finger and then gently pipet up and down a few times.
9. Immediately after resuspending the cell pellet, transfer 10 ul of sample into a cell count to count the number of cells.
10. The calculated volume required to make a suspension /ml.
    Transfer 1 mL aliquots of the PBMC cell suspension into individual 1.5-ml cryovials.
    Centrifuge the cryovials in Eppendorf centrifuge at 10000 rpm for 10 minutes at 4-10°C.
    Remove and discard as much supernatant as possible without disturbing the cell pellet.
11. For viable cells storage, the cell pellet should be suspended in FBS with 10% DMSO at this stage and then stored at -80°C until use.

15.3 APPENDIX C – SOP FOR CYTOKINE COLLECTION

Appendix C -

Time points of the sampling

- Venous blood should be collected from all patients enrolled in the protocols at the beginning of therapy, prior to the administration of the investigational agent. The pre-cycle 1 samples will serve as the baseline sample. Additional time points should be defined in the clinical protocol.

Blood collection

- Collect 4 mL of blood in a venous blood BD Vacutainer® spray-coated K2EDTA tube (example BD Cat# 367861; http://www.bd.com/us/contact/). Gently invert 10 times to mix blood and anticoagulant.
- Store the tubes at room temperature until centrifugation. It is generally preferred and often necessary to process the samples within 5 hours.

Blood sample processing

- Centrifuge at 1300 RCF for 10 min in a swing-head centrifuge, or 15 min in a fixed angle centrifuge (balance the tube in the centrifuge). Do not use brake to stop centrifuge.
- Carefully aspirate the supernatant (plasma) into a new centrifuge tube.
- Inspect serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove insoluble matter.

Sample storage

- Carefully aliquot each sample into two cryovials with printed labels with a sample identifier and store them at -80ºC.

Shipping information

- One of the two sets of plasma samples should be sent to Dr. Liang Cao at the National Cancer Institute at the end of the study on dry ice. A separate sample sheet with at least a patient number and a sample identifier for each sample should be provided via secured electronic mail. A separate email with tracking information should be sent to the recipient at the time of shipping to ensure the receiving of the samples package on time. See Storage **Instructions** in Section 15.6 Appendix F for information regarding the second set of plasma samples.

- Ship information is as follows:

  Liang Cao, Ph.D.

  Genetics Branch

Center for Cancer Research
National Cancer Institute
37 Convent Dr. MSC 4265
Bldg 37, Rm 6134
Bethesda, MD 20892-1906
Phone: (301) 435-9039
E-mail: caoli@mail.nih.gov

Data collection and transmission

- Data will be generated in a table format.
- Data will be transmitted via email to the PI of the studies in an Excel document.

15.4 APPENDIX D- STANDARD OPERATING PROCEDURE FOR PLASMA AND BUFFY COAT COLLECTION FOR CIRCULATING TUMOR DNA

Ref 1: Plasma protocol from Brenton Laboratory SOP at Cancer Research UK Cambridge Institute.

Ref2: Manual for cell free DNA BCT® tubes from Streck labs for buffy coat isolation.

Required consumables

- Streck tubes (Cell free DNA BCT tubes, Streck Inc) with freshly drawn patient blood..
- 2ml screw-capped micro-tubes (Sarstedt: 72.694.006), Dnase/Rnase free.
- 2ml non-screw-capped Non-Stick DNase/RNase-free Microfuge Tubes (Applied Biosystems: AM12475)
- Transfer pipette (Fisher Scientific: 2655116)

Procedure 1: Recommended procedure for the Plasma collection, processing, storage and transport of plasma for ctDNA and/or circulating nucleic acid studies

IMPORTANT: Blood samples should be centrifuged as soon as possible after collection to avoid fragmentation, degradation and leukocyte lysis. Samples should be spun within 1 hour of collection.

1. Collect blood into BCT Streck tubes (Label the tube with appropriate Study/Patient Number Identifiers. Record the time of collection and whether the blood was drawn using a peripheral venous access device (eg. cannula or butterfly) or a central venous access device (CVAD).
2. After collection, gently invert tubes 8-10 times to mix and leave tubes upright prior to centrifugation.
3. Within 1 hour of collection, centrifuge samples at 1600g for 10 minutes at room temperature using a “swing out” rotor. (NB. Centrifuge with the brake off). Record the time of centrifugation.

Note: If adapters are not available, place the streck tubes into 50 mL conical tubes and then spin

4. Transfer 1ml aliquots of plasma to sterile 2ml Microfuge tubes (DNase/RNase-free tubes can be used at this stage) and leave about 0.5 ml over the buffy coat (Refer figure on next page).

Take care to avoid any buffy coat layer in this step. See Procedur 2 below for separate collection of buffy coat for genomic DNA

5. Centrifuge the plasma aliquots in a bench top centrifuge at 14,000rpm (10,000 xg) for 10 minutes to pellet any remaining cellular debris.
6. Carefully transfer 1ml aliquots of supernatant to sterile 2ml screw-capped micro-tubes, and discard the pellet.
7. Label tubes with appropriate barcode labels as required by the Study. Freeze aliquots in a -80^{0}C freezer in appropriately labeled storage boxes.

Procedure 2. Preparation of buffy coat isolate from blood sample for isolation of total genomic DNA (gDNA).

1. Following Step 4 in the plasma collection protocol 1 above, carefully transfer the buffy coat layer into a separate sterile screw capped tube using a transfer pipette or standard pipette. It may help to use a gentle swirling action with the tip to remove the buffy coat layer, taking care to avoid plasma and the red blood cell layer wherever possible. Volume should not exceed 0.5 -1 ml.
2. Label tubes with appropriate barcode labels as required by the Study. Freeze tubes in a -80^{0}C freezer in appropriately labelled storage boxes.

Note: The buffy coat is going to contain erythrocytes and some plasma. This may be frozen directly in -80C freezer for months before being used for gDNA preparation. Qiagen blood DNA mini kits may be used and this requires about 200 ul of buffy coat.

** Electronically record all appropriate collection details (study and anonymized patient identifier), collection date, sample volume, and sample/biorepository identifiers into study log records, as defined by study requirements. Record box number/row/column position, and if there were any sample handling or quality issues (e.g. if samples were hemolyzed).

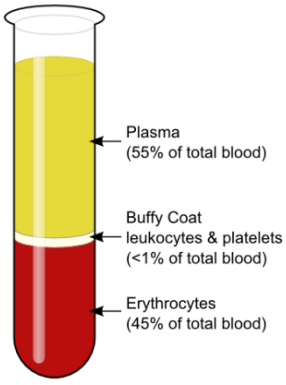

Figure 1. Streck tubes after centrifugation (step 3)

15.5 APPENDIX E- ORAL MEDICATION DIARY

Oral Medication Diary for ONC201

Patient's Name: _____ Date: _____

Patient Study ID: _____ Cycle #: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle (28 days).
2. You will take ___ (number) ____ mg (dosage) capsules each Tuesday Morning (white spaces) +/- 1 day (light gray space). You may take the capsules one hour before or two hours after eating, as you prefer. If you forget to take the pills on your assigned day, you can take the missed dose up to 1 day after (light gray space). Do not take the weekly dose if more than 1 day late after scheduled dose (dark gray spaces).
3. Record the date, the number of pills you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring your pill bottle and this form to your physician when you go for your next appointment.

General Comments:

_____

_____

Day	# pills and when taken: ONC201			Comments	Day	# pills and when taken: ONC201			Comments
	Date	# Capsules	Time			Date	# Capsules	Time	
1					15				
2					16				
3					17				
4					18				
5					19				
6					20				
7					21				
8					22				
9					23				
10					24				
11					25				
12					26				
13					27				
14					28				

Patient's Signature: _____Date: _____

Study Team will complete this section:

6. Date patient started protocol treatment_____
7. Date patient was removed from study _____
8. Patient's planned daily dose_____
9. Total number of pills taken this month _____

Physician/Nurse's Signature _____

15.6 APPENDIX F- ONCOCEUTICS SPECIMEN COLLECTION, PROCESSING, STORAGE AND SHIPPING

3624 MARKET STREET; SUITE 5E

UNIVERSITY CITY SCIENCE CENTER

PHILADELPHIA, PA 19104

SPONSOR CONTACT:

ROHINTON TARAPORE, PHD

TELEPHONE: 215-966-6115

E-MAIL: ROHINTON.TARAPORE@ONCOCEUTICS.COM

Summary of Laboratory Studies

Laboratory studies on specimens obtained from patients receiving ONC201 treatment will be performed to characterize ONC201 exposure and its biological activity in advanced cancer patients. Blood for laboratory analysis will be drawn to assess plasma ONC201 drug concentration and serum tumor biomarkers.

If available, as described in the protocol, archival tumor tissue will be accessed at the time of enrollment for subjects enrolled in this study to conduct future correlative assays to measure biomarkers of therapeutic response to ONC201 including molecular markers involved in the mechanism of action of ONC201.

All blood and tissue specimens will be collected and prepared for shipping by the clinical sites.

Blood Sample Collection and Processing

At the specified time points, approximately 6 mL of blood will be collected in the specified tube type(s), to be processed, aliquoted into duplicate transfer vials and frozen, to be shipped to the designated analytical laboratory. Refer to the study protocol for blood sample collection time points.

Blood must be centrifuged, separated and frozen within 2 hours of collection. Specimens must be stored frozen at -70ºC (preferred) or -20ºC (acceptable) at each study site until shipped on dry ice to the designated analytical laboratory.

Supplies for Collection of Blood Samples

Plasma Sample Supplies

Plasma samples are to be collected and aliquoted using the following commercially available tubes.

For plasma blood collection

Becton-Dickinson Plastic Whole Blood tube with spray-coated K2EDTA

Lavender top, Vacutainer

Catalogue 367525

Tube Size: 16x100

Draw Volume: 10mL

For plasma aliquots

Wheaton 2mL Ext FS CryoElite

Blue Cap Patch Sterile

Catalogue W985868

15.6.1 Serum Sample Supplies

Serum samples are to be collected and aliquoted using the following commercially available tubes.

For serum blood collection

Serum Tube, Increased Silica Act Clot Activator, Silicone-Coated Interior

Red Top Vacutainer

BD Catalogue 366430

Tube Size: 16 X 100

Draw Volume: 10mL

For serum aliquot storage

Wheaton 2mL Ext FS CryoElite

Yellow Cap Patch Sterile

Wheaton Catalogue W985866

Collection of Blood

Check the expiration dates on the vacutainer tubes before collecting blood. If the tubes have expired, discard them. Do not substitute another brand of collection tube. Prepare the blood collection tubes by affixing the barcode labels to each tube before collecting specimens from the subject.

Using standard aseptic venipuncture techniques, draw the blood sample into the specified collection tube. For time points requiring both plasma and serum blood draws, the plasma sample should be drawn first. Remove tourniquet as soon as blood flow into the first vacutainer tube is established; excessive use of tourniquets can lead to hemoconcentration and falsely elevated results. Ensure each vacutainer is full to provide accurate blood:additive ratios. Mix blood tubes by gently inverting each tube 5 times. On the barcode label, clearly print with a black permanent marking pen the study number "XXX", patient ID number, the date and time of the blood draw (hours and minutes). Enter the information on the Patient-Specific Specimen Collection Log and maintain the log in the Patient source records.

Place the full blood collection tube(s) into a resealable clear biohazard bag. Arrange prompt pick up by, or delivery to, the local laboratory since blood must be processed and frozen within 2 hours of collection.

Processing of Blood Specimens

15.6.2 Plasma Processing

Centrifuge the filled lavender top tube at 1500 g (3000 rpm) for 15 minutes until the plasma and the cells are well separated. Label 2 blue cap transfer vials lengthwise with the appropriate barcode labels indicating "plasma (blue)". Clearly print the study number "Study XXX", patient ID number, date and time of the blood draw. Transfer ~1.5 mL of plasma into each of the 2 blue top transfer vials labeled "plasma (blue)."

15.6.3 Serum Processing

After filling the tubes, let the tubes stand at room temperature until a clot has formed in the red top tube (approximately 30 minutes). Centrifuge the tube at 1500 g (3000 rpm) for 15 minutes until the serum and the cells are well separated. Label 2 yellow cap transfer vials lengthwise with the appropriate barcode labels indicating "serum (red)". Clearly print the study number "Study XXX", the patient ID number, date and time of the blood draw. Transfer ~1.5 mL of serum into each of the 2 yellow top transfer vials labeled "serum (red)".

Obtaining Archived Tumor Tissue Sections

Archived tumor blocks obtained prior to treatment with ONC201 are to be obtained as a baseline sample. If multiple blocks are available, preference will be given to the most recently obtained tissue. A total of 5 slides should be prepared from this archival tissue. The archival tissue should be returned to its original storage location.

Using black permanent marking pen, clearly print the study number "Study XXX", patient ID number and the date of collection (i.e., biopsy) on each slide.

Storage Instructions

The clinical site laboratory is responsible for proper storage of the samples. Do NOT store plasma/serum specimens in a frost-free refrigerator-top freezer--results may be affected by temperature variation during the freezer cycle. Store in a freezer with minimum -20ºC or preferably -70ºC. Take all precautions to ensure the specimens remain frozen at the study site.

Duplicate aliquots of plasma and serum specimens will be prepared. One set of aliquots is to be kept stored frozen at the clinical site laboratory, and the second set is to be shipped according to this procedures manual.

Shipping Instructions

All shipments must be authorized by the Sponsor. Before preparing the shipment, the clinical site personnel preparing the shipment must contact the Sponsor and e-mail a copy of the completed Specimen Shipping Form to the Sponsor Project Office: rohinton.tarapore@oncoceutics.com.

Preparation of the Dry Ice Shipments

The frozen plasma/serum aliquots must be shipped on dry ice to the designated analytical laboratory. Ensure that specimens remain frozen during preparation of the shipment.

Specimens must be shipped by overnight courier service on dry ice on a Monday, Tuesday or Wednesday only. Shipments are not to be initiated between Thursday and Sunday as it is not possible to ensure specimens will remain frozen if shipment is delayed, or upon receipt over weekends or on a holiday.

All packaging must conform to IATA Packaging Instruction 650 for shipment of non-infectious human specimens. Samples from participants known to be infectious (Hepatitis B virus, HIV-I and HIV-II non-cultured) are not to be shipped.

Ensure sufficient dry ice is used to keep the specimens solidly frozen until they arrive at the designated analytical laboratory. Place half of the dry ice in the bottom of the shipping box. Place the resealable bag containing the specimens in transfer vials, filter paper and absorbent on top of the dry ice. Do not put dry ice inside the plastic resealable bags. Cover and surround the specimens with the remaining dry ice. Place the lid on the Styrofoam portion of the shipper.

DO NOT TAPE BOX SHUT. Ensure the Hazardous Goods label for Dry Ice is affixed to the box (diamond with upper half stripped and '9' at bottom). Record the amount of dry ice (in Kg or pounds) added to the box on the small label with a permanent black marking pen. Prepare shipment documentation for over-night shipping of the package (FedEx is preferred).

Affix the clear, resealable plastic courier envelope to the side of the dry ice box. Please ask your local courier for a supply of these plastic courier envelopes. Prepare a Specimen Shipping Form and include it in the plastic courier envelope.

Ask your courier for a version of the airway bill that allows you to indicate the shipment is packed with dry ice. Meticulous attention to labeling will ensure prompt delivery of dry ice shipments. The "Nature and Quantity of Goods" box of the air waybill must show "UN 3373", the text "BIOLOGICAL SUBSTANCE, CATEGORY B" and the number of packages. In addition, since the package contains dry ice, you also need to write "Dry Ice - UN1845" and the number of packages and the net quantity of dry ice per package. Finally, these markings must be accompanied by the Class 9 label for Miscellaneous Dangerous Goods.

Preparing Tumor Sections for Shipping

The tumor section slides should be shipped at room temperature (RT) to the Tumor Specimen Repository. Samples on glass or plastic slides must be adequately cushioned. Place the slides in a primary receptacle such as a slide box. Place this primary receptacle into a secondary leak-proof sturdy outer container or flexible-envelope packaging. The shipment must be packaged in compliance with IATA Packaging Instruction 650 for shipment of non-infectious human specimens. Use of the FedEx Clinical Pak is an option. Indicate the # of boxes shipped, and their total weight. Select 'Overnight Service', or other appropriate service assuring delivery within 24 hours.

One aliquot of plasma should be shipped to XenoBiotic Laboratories while the duplicate aliquot is shipped to Scisafe. Both the serum aliquots and tumor slides should be shipped to Scisafe.

15.6.4 Arranging for Shipments

- In the Ship From section: Enter name and address of person responsible for the shipment from the clinical site.

- In the Ship To section: Enter name and address of designated laboratory as below:

Ship **PLASMA** Samples to:
Sample Receiving Officer
XenoBiotic Laboratories, Inc.
107 Morgan Lane
Plainsboro, NJ 08536
Tel. 609-799-2295 x252
Email: sro@xbl.com

Ship **SERUM** Samples to:
John Coolidge, Biorepository Manager - Boston
SciSafe, Inc.
35 Dunham Road
Billerica, MA 01821
Tel. 404-242-1578
Email: jfc@scisafe.com

Ship **TUMOR** Slides to:
John Coolidge
Biorepository Manager - Boston
SciSafe, Inc.
35 Dunham Road
Billerica, MA 01821
Tel. 404-242-1578
Email: jfc@scisafe.com

Sign and date the waybill. Tear off "Senders Copy" for retention. It is recommended that all shipments be tracked to ensure timely delivery.

Notification of Shipment

At the time of shipment, the laboratory must e-mail the completed Specimen Shipping Form to the following:

a) Sponsor Project Office at rohinton.tarapore@oncoceutics.com

b) **Plasma** Pharmacokinetics Laboratory at sro@xbl.com

OR

Serum Pharmacodynamic Laboratory at jfc@scisafe.com

OR

Tumor Tissue Repository at jfc@scisafe.com

c) Local study nurse or coordinator

Specimen Collection Log – Patient Specific

PATIENT ID#_____

	Type of Specimen and Protocol-Specified Time Point	Date / Time of Collection (mm/dd/yyyy) ; (mm:hh)	Notes
	□**Tumor Tissue** Archival sample at time of enrollment.		
	□**Plasma**: Cycle 1 = Baseline		
	□**Plasma**: Cycle 1 = 6 hrs (±15 minutes) post-administration of ONC201		
	□**Serum:** Cycle 1 = Baseline; pre-dose		
	□**Serum**: Cycle 1 = 6 hours after 1st dose		
	□**Serum**: Cycle 2 = Day 1		

Specimen Collection Log

Patient Specific, Continuation Page for Additional Treatment Cycles

PATIENT ID#_____

Cycle	Type of Specimen and Protocol-Specified Time Point	Date / Time of Collection (mm/dd/yyyy) ; (mm:hh)	Notes
2	□ **Serum** Pre-Dose		
3	□ **Serum** Pre-Dose		
4	□ **Serum** Pre-Dose		
5	□ **Serum** Pre-Dose		
6	□ **Serum** Pre-Dose		
7	□ **Serum** Pre-Dose		
8	□ **Serum**		

	Pre-Dose		
9	□ **Serum** Pre-Dose		
10	□ **Serum** Pre-Dose		

15.6.5 Oncoceutics Specimen Shipping Form

(Check a single applicable sample type; use additional forms as needed)

□ Plasma □ Serum □ Tissue

Date: _____

Total Number of Specimens in Shipment: _____

Courier and Tracking Number: _____

Contact Information for Person Preparing Shipment:

Name: _____

Institution: _____

Phone Number: _____

E-mail: _____

	Patient ID#	**Date / Time of Collection (mm/dd/yyyy) ; (mm:hh)**	**Packed By: Initials/Date**	**Received By: Initials/Date**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

12				
13				
14				
15				
16				
17				
18				
19				
20				