Pilot Study of a [^{14}C]Oxaliplatin Microdosing Assay to Predict Exposure and Sensitivity to Oxaliplatin-Based Chemotherapy

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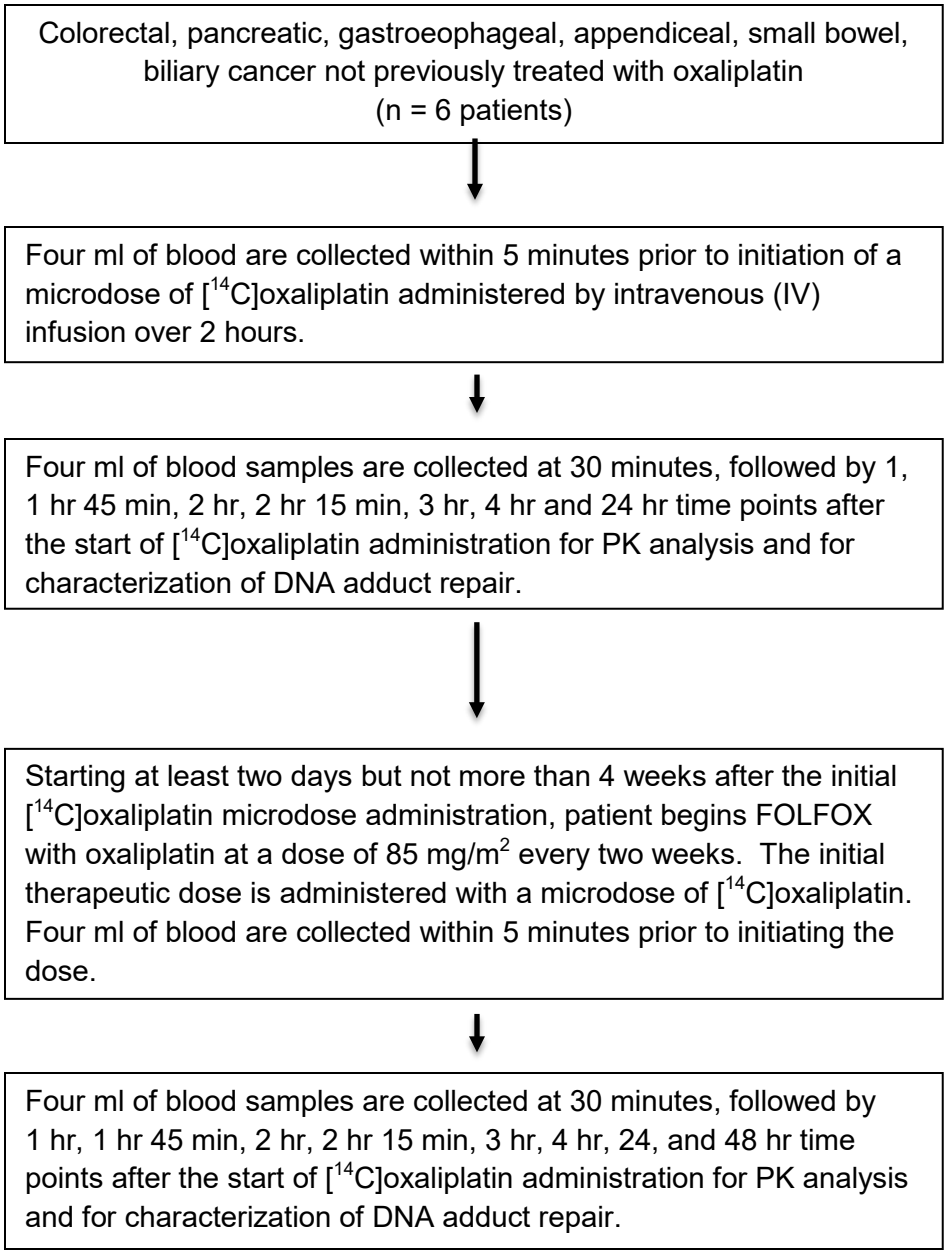
SCHEMA


Colorectal, pancreatic, gastroesophageal, appendiceal, small bowel, biliary cancer not previously treated with oxaliplatin
(n = 6 patients)
Four ml of blood are collected within 5 minutes prior to initiation of a microdose of [14C]oxaliplatin administered by intravenous (IV) infusion over 2 hours.
Four ml of blood samples are collected at 30 minutes, followed by 1, 1 hr 45 min, 2 hr, 2 hr 15 min, 3 hr, 4 hr and 24 hr time points after the start of [14C]oxaliplatin administration for PK analysis and for characterization of DNA adduct repair.
Starting at least two days but not more than 4 weeks after the initial [14C]oxaliplatin microdose administration, patient begins FOLFOX with oxaliplatin at a dose of 85 mg/m2 every two weeks. The initial therapeutic dose is administered with a microdose of [14C]oxaliplatin. Four ml of blood are collected within 5 minutes prior to initiating the dose.
Four ml of blood samples are collected at 30 minutes, followed by 1 hr, 1 hr 45 min, 2 hr, 2 hr 15 min, 3 hr, 4 hr, 24, and 48 hr time points after the start of [14C]oxaliplatin administration for PK analysis and for characterization of DNA adduct repair.

1 OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate the feasibility of [^{14}C]oxaliplatin microdose as a clinical assay to predict oxaliplatin exposure

1.2 Secondary Objectives

1.2.1 To estimate the degree to which a [^{14}C]oxaliplatin microdose predicts the observed pharmacokinetics of standard dose oxaliplatin

1.2.2 To validate that intrapatient variation of exposure to a [^{14}C]oxaliplatin microdose is less than 5%

1.2.3 To detect the levels of oxaliplatin-DNA adducts induced by oxaliplatin microdosing in peripheral blood mononuclear cells (PBMC), and correlate the results with patient response and progression free survival on oxaliplatin-based chemotherapy

1.2.4 To develop preliminary safety data of [^{14}C]oxaliplatin microdosing for future studies

2 BACKGROUND

The goal of this study is to test the feasibility of using microdose-based diagnostics in combination with oxaliplatin-based chemotherapy for personalized cancer treatment. The key hypotheses are that the intravenous infusion of a microdose of [^{14}C]oxaliplatin (~1% of the therapeutic dose) will 1) predict the pharmacokinetic exposure to treatment dose oxaliplatin and 2) that accumulation of the microdose-induced oxaliplatin-DNA damage in surrogate genomic DNA from peripheral blood mononuclear cells (PBMCs) will correlate with patient response to subsequent full-dose oxaliplatin-based chemotherapy. In this study, our main objective is to collect enough data to evaluate the feasibility of using a [^{14}C]oxaliplatin microdose as a clinical assay to predict oxaliplatin exposure. Key secondary objectives are to assess oxaliplatin-DNA adduct biomarkers in PBMCs and assess intrapatient variability of microdose pharmacokinetics. Since there will be less than a hundred oxaliplatin-DNA adducts per genome, an ultrasensitive measurement technology is required. Accelerator mass spectrometry (AMS) is the most sensitive method available for precise and accurate measurement of drug-DNA adducts. AMS requires a rare isotope label, such as radiocarbon (^{14}C). For this study we will be dosing patients with sub-therapeutic nontoxic doses of ^{14}C-labeled oxaliplatin ([^{14}C]oxaliplatin) that will result in minimal radiation exposure to patients (less than one chest X-ray of radiation exposure per microdose).

2.1 Overview of Cancer Treatment with Platinum Agents

Platinum (Pt) derivatives are among the most effective and commonly used chemotherapeutic drugs for lung, bladder, breast, gynecological, gastrointestinal, testicular, hematological and other malignancies. Three platinum-based drugs, cisplatin, carboplatin and oxaliplatin, are used for chemotherapy in the U.S. The pharmacodynamic mechanism of platinum chemotherapy is the formation of covalent platinum-DNA adducts (**Figure 1**).

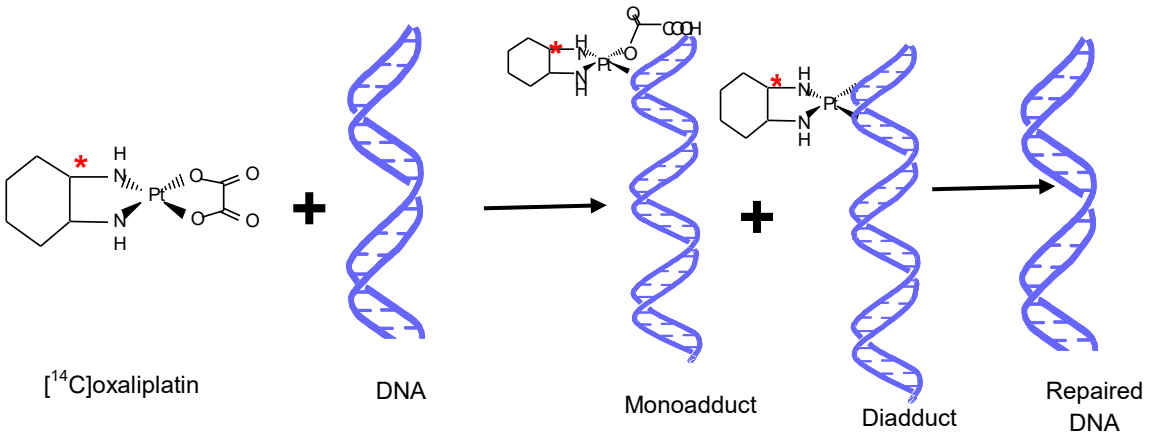


Figure 1. Structure of oxaliplatin and formation of oxaliplatin-DNA adducts. The ^{14}C label (*) is covalently linked to DNA in both oxaliplatin-DNA monoadducts and diadducts. Only when adducts are repaired is the ^{14}C label removed from DNA. AMS measures ^{14}C and, therefore, both monoadducts and diadducts in genomic DNA. By measuring the decrease of ^{14}C from genomic DNA, the rate of removal of DNA monoadducts and diadducts (DNA repair) can be measured.

Platinum-based drugs preferentially interact with the nucleophilic N7-site of purine bases of DNA to form a variety of monoadducts and diadducts (crosslinks) when Pt interacts with nucleotides through one or two bonds, respectively [1]. Both intrastrand and interstrand diadducts occur in DNA. The structures of these adducts are well characterized. These adducts induce local perturbations in the DNA double helical structure, which activate the proteins that recognize DNA damage [2-4]. The activation of these proteins also results in cell cycle arrest, induction of DNA repair processes, and the initiation of programmed cell death (**Figure 2**). Although many details of the mechanisms of action have yet to be elucidated, the binding of Pt agents to DNA is well known and is the underling basis for measuring Pt-DNA adducts in cells as a potential predictor of patient response to platinum-based chemotherapy. Moreover, the efficacy of platinum-based chemotherapy varies significantly depending on the types of cancer and individual molecular characteristics. For example, most testicular cancer patients respond to and can be cured with platinum-based chemotherapy regimens like BEP (bleomycin, etoposide and cisplatin), even in the presence of distant metastasis. Platinum-based chemotherapy for other types of cancer is less effective.

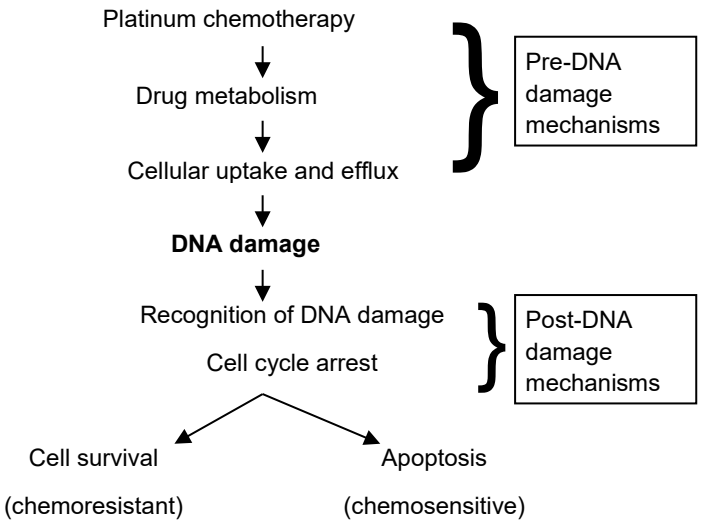


Figure 2. Simplified diagram of body/cellular response to platinum chemotherapy. DNA damage is the critical step in cellular response to platinum chemotherapy. In this study we will measure DNA damage and repair with a highly sensitive technology (AMS) and correlate with cellular responsiveness to platinum chemotherapy.

2.2 Oxaliplatin

Oxaliplatin is the third generation of platinum compounds approved for use in cancer therapy. It differs from cisplatin and carboplatin in two significant ways. First, oxaliplatin's toxicity profile shows a reduction in renal and auditory toxicities, and minimal myelosupression. And second, it does not share the same resistance spectrum as of cisplatin and carboplatin. Oxaliplatin is active in many solid tumors, including colorectal, pancreatic, ovarian, lung, and head and neck carcinomas. Oxaliplatin is presently approved for use in combination with 5-fluorouracil (5-FU) and leucovorin (folinic acid). This treatment regimen is indicated for (1) adjuvant treatment of stage III colon cancer in patients who have undergone complete resection of the primary tumor and (2) advanced colorectal cancer, (3) gastroeophageal adenocarcinoma, (4) pancreatic adenocarcinoma, (5) biliary adenocarcinoma, (6) appendiceal adenocarcinoma, (7) duodenal adenocarcinoma. At the present time, the most commonly used combination (modified FOLFOX6) combines oxaliplatin with bolus leucovorin and 5-FU followed by a 46-hour infusion of 5-FU [5, 6].

Oxaliplatin-associated toxicities are cumulative with dose received and are the most frequent cause of withdrawal from therapy for those patients that respond well to FOLFOX. The major dose limiting toxicity of oxaliplatin is neurotoxicity, which results in treatment interruption and discontinuation prior to disease progression in the majority of patients [7]. The predictability of neurotoxicity associated with oxaliplatin-based therapy should allow the development of strategies to manage this side effect in view of the individual patient's clinical situation. Variation of oxaliplatin in individual patients could affect the accumulation

of oxaliplatin with the nervous system as well as the disposition of oxalate. Thus, oxaliplatin dose optimization has the potential to reduce the development of neurotoxicity in patients treated with FOLFOX.

In phase I studies, the calculated exposure or area under the curve (AUC) of oxaliplatin administered at 85 mg/m^2 averaged 4.68 µg/mL/hr; however, there was significant inter-patient variation in AUC (SD 1.40 µg/mL/hr). The metabolism of oxaliplatin occurs in a triphasic manner. The initial ($T_{1/2\alpha}$), second ($T_{1/2\beta}$) and terminal ($T_{1/2\gamma}$) phase half-lives of oxaliplatin are reported to be 25.8 minutes, 16.8 hours and 391 hours, respectively [8, 9]. Approximately half of oxaliplatin is excreted in urine within the first 6 hours. Only a small fraction of oxaliplatin is excreted at the terminal phase. Because the formation of Pt-DNA adducts correlated to the concentration and incubation time of cells with Pt [10], and because there is a linear correlation between gross levels of platinum bound to DNA and the extent of cytotoxicity [11], it is conceivable that those patients with longer serum half-life of Pt may have more cytotoxic effects. We hypothesize that increased metabolism (short half-life) may be responsible for some cases of resistance to Pt agents. Therefore, a PK study may identify those patients with a short half-life of Pt and help design personalized therapy studies altering the dose of oxaliplatin to overcome resistance.

2.3 Resistance to Platinum-based chemotherapy

Except for testicular cancer, most malignancies have either intrinsic or acquire resistance to platinum-based chemotherapy. Individual aspects of patient and tumor genetic make-ups contribute to these drug resistance phenotypes. Numerous studies have been performed to explore the mechanisms of resistance to platinum [12]. The chemoresistance mechanisms are very complicated and involve more than 700 genes from multiple signaling pathways [13]. Studies exploring individual gene alterations have essentially failed to identify clinically applicable markers for chemoresistance. Chemoresistance mechanisms to platinum agents can be classified into two major groups (**Figure 2**): (1) those responsible for the formation of Pt-DNA adducts (pre-DNA damage mechanisms), and (2) those involved in cellular responses to Pt-DNA adducts (post-damage mechanisms).

2.3.1 Chemoresistance mechanisms related to the formation of Pt-DNA adducts

<u>Modulation of uptake and efflux of platinum compounds by cancer cells</u>

Several studies showed that there is high correlation between decreased Pt accumulation and resistance to Pt [14, 15]. Cellular uptake and efflux of Pt drugs appear to be mediated by transporters and carriers that are shared with copper [16, 17]. However, other studies suggested that passive diffusion plays an important role as well [18-21]. Multiple studies showed that resistance to Pt is associated with active efflux of drugs. For example members of the multi-drug resistance protein (MRP)-related transport proteins are associated with resistance to Pt drugs [22-24]. Cell lines sensitive to cisplatin have lower expression of CTR1, a drug efflux transporter [25]. We found different carboplatin efflux rates between NSCLC cell lines relative to carboplatin IC_{50} values [26].

Intracellular inactivation of Platinum Agents

Glutathione and other thiols can quench the reactivity of platinum-based drugs with DNA. In vitro, there is high correlation between intracellular glutathione levels and resistance to cisplatin [14, 15, 22]. Overexpression of glutathione S-transferase π confers increased resistance to cisplatin in CHO cells [27]. Metallothionein (MT) proteins are a family of sulfhydryl-rich proteins that participate in heavy metal binding and detoxification. Overexpression of MT-IIA increases resistance to cisplatin [28], while MT-deficient mice show hypersensitivity to cisplatin [29]. Increased MT was also found in bladder tumors from patients who were unsuccessfully treated with cisplatin chemotherapy [30]. However, the feasibility of these antioxidant proteins as predictive biomarkers of clinical response remains to be demonstrated [31-33].

2.3.2 Chemoresistance mechanisms related to Cellular Responses to Pt-DNA Adducts

Increased DNA Repair

Increased DNA repair capacity has been well documented to correlate with resistance to Pt-based drugs. Testicular cancer is unusually sensitive to Pt, and is deficient in its ability to repair DNA [34]. In several Pt-resistant cancer cell lines, high DNA repair capacity has been observed [15, 32, 35-37].

The mechanisms of DNA repair and its regulation are highly complex. More than a dozen proteins participate in the repair of platinum-DNA damage [38]. For example, the nucleotide excision repair (NER) complex that forms at a site of DNA damage contains at least 15 proteins [39, 40]. The excision repair cross-complementing (ERCC) gene family is an important component of NER complex. Increased ERCC1, ERCC2 and XPA are associated with resistance to Pt [24, 41], and shortened survival in lung cancer patients receiving adjuvant chemotherapy [42]. Similar associations were also observed in breast cancer patients who received carboplatin-based chemotherapy [43]. Although numerous proteins have been evaluated as biomarkers of response to therapy, only ERCC1 has thus far been used as a clinical marker with some success [24].

Increased Tolerance of DNA Damage

There is a significant correlation between increased tolerance of DNA damage and resistance to Pt-based drugs [14, 15]. Several mechanisms contribute to increased tolerance of DNA damage. Some of these mechanisms involve loss of mismatch repair [44], increased DNA replication that bypasses Pt-DNA adducts [45, 46], single nucleotide polymorphisms of cell cycle regulators [43], or decreased apoptosis. Decreased pro-apoptotic, or increased anti-apoptotic gene expression have been shown to participate in resistance to Pt and inhibition of apoptosis [47-49].

2.3.3 Summary of Resistance to Platinum-based Chemotherapy

Owing to the complexity of the above outlined platinum-based drug resistance mechanisms, scoring mutations or gene expression changes in a few individual pathways is likely to have limited impact on developing biomarker tests for predicting

efficacy. The microdosing approach of this feasibility study provides a strategy for obtaining a comprehensive measurement of major resistance factors at play in an individual tumor, since the phenotypic in vivo capacity for Pt-DNA adduct formation and repair can be directly measured in the patient prior to initiation of platinum-based chemotherapy.

2.4 Accelerator Mass Spectrometry (AMS)

AMS was initially developed for geochronology and archaeological research [50, 51]. An AMS instrument is an analytical tool that directly quantifies a very rare isotope (e.g. <1 ^{14}C atom per 109 carbon atoms) in a uniform graphite sample prepared from a biological matrix [52, 53]. The extreme sensitivity of AMS is the result of counting ^{14}C atoms directly instead of counting ^{14}C radioactive decay events (half-life = 5730 years; 6 x 1011 molecules are required for 100 dpm of radioactive decay). Specificity for ^{14}C atoms occurs by instrument design and operation. AMS has been used in the drug research by our co-investigator Paul Henderson, Ph.D. of UC Davis, and his colleagues at Lawrence Livermore National Laboratory for the last 15 years. AMS has been used to demonstrate metabolism and pharmacokinetics of several dietary and environmental carcinogens [54-56]. Recently, it has been used to study the metabolism of cancer drugs such as tamoxifen, doxorubicin and carboplatin [57-59]. Compared with the other technologies, the advantage of AMS to detect DNA adducts/damage is its high sensitivity and precision. AMS can detect ^{14}C at the zeptomole (zmole, 10-21 mole; a few hundred thousand molecules per sample) level per mg of carbon with the precision as low as 0.25% [60]. In the application of AMS to cancer research, the chemical dose to be administered to patients for AMS analysis can be minimized to sub-physiological and sub-toxic doses that are not compatible with other less sensitive measurement technologies that require higher doses of chemotherapeutic drugs. Furthermore, the specimen size can be reduced to the amounts that can be obtained from minimally or non-invasive procedures.

2.5 Prediction of Oxaliplatin Exposure

Optimizing the dose of chemotherapy is essential: If the dose is too low it will be ineffective, whereas the toxicity (side-effects) will be intolerable to the patient at excessive doses [61]. The standard method of individualizing chemotherapy dose is based on calculated body surface area (BSA). However, drug absorption and clearance are influenced by multiple other factors, including age, gender, metabolism, disease state, organ function, drug-to-drug interactions, genetics, and obesity [62-66]. As a result, there is high variability in the systemic chemotherapy drug concentration among patients dosed by BSA, and this variability has been demonstrated to be more than 10-fold for many drugs [61, 67]. There is a clear unmet medical need to optimize the dosing of chemotherapy drugs compared to BSA-based calculations.

Of the individual drugs in the FOLFOX regimen, 5-FU has been dose optimized in several clinical studies, and results in significantly better response and survival rates versus BSA dosing [68-70]. For example, in a study involving colorectal cancer patients treated with the FOLFOX regimen, the incidence of serious diarrhea was reduced from 12% in the BSA-dosed group of patients to 1.7% in the dose-adjusted group, and the incidence of severe

mucositis was reduced from 15% to 0.8% [68]. The FOLFOX study also demonstrated an improvement in treatment outcomes. Positive response increased from 46% in the BSA-dosed patients to 70% in the dose-adjusted group. Median progression free survival (PFS) and overall survival (OS) both improved by six months in the dose adjusted group.

The current method (ELISA) for optimizing 5-FU dose is not applicable to oxaliplatin, which is in its free form is a chemically reactive compound that is not amenable to antibody-based quantitation. Other common methods for measuring oxaliplatin (ICP-MS and LC-MS) are complex and lack the throughput needed for clinical testing. In this study, we will use liquid scintillation counting to assess [^{14}C]oxaliplatin from the plasma ultrafiltrate after administration of a microdose. If successful, we propose to use of a highly sensitive accelerator mass spectrometry (AMS) platform to perform assessment of free drug in plasma ultrafiltrate from clinical samples after administration of a nanocurie dose of [^{14}C]oxaliplatin, which is amenable to high-throughput analysis. Our goal is to develop a [^{14}C]oxaliplatin microdosing assay to optimize the use oxaliplatin, such that the delivered AUC is within 10% of the targeted value. The reduction in variation in achieved dose is expected to improve treatment outcome (i.e. response) amongst those who are underdosed, and reduce the development of toxicity (especially neurotoxicity) in those who are overdosed. This proposal is the first step in that project, with the aim of demonstrating the feasibility of using a microdose of [^{14}C]oxaliplatin to predict the pharmacokinetics of standard BSA-dosed oxaliplatin.

2.6 Assessment of Microdose-induced Oxaliplatin-DNA damage

Several studies have confirmed that the levels of drug-DNA adducts directly correlate with the outcomes of platinum-based chemotherapy, particularly in peripheral blood mononuclear cells (PBMC). After Pt-based chemotherapy, DNA adducts can be detected in multiple tissues [10, 71, 72]. The concentration of Pt-DNA adducts correlates to the drug concentration and incubation time in cell culture [10]. Decreased Pt-DNA adducts are associated with resistance to Pt chemotherapy in cell lines [73]. The levels of DNA adducts, as detected by immunohistochemical staining, correlated with response to Pt chemotherapy [71, 72]. Those patients with low drug-DNA adducts or increased repair of drug-DNA adducts had worse outcomes [74]. Oxaliplatin-DNA adducts have been measured by Pieck et al [75] in peripheral blood lymphocytes from 37 patients with a variety of malignancies. In patients showing tumor response, adduct levels after 24 and 48 h were significantly higher than in nonresponders, indicating that drug-DNA adduct levels in surrogate tissues may serve as biomarkers of response. However, the commonly used drug-DNA adduct detection methods such as atomic absorption spectroscopy, Southern blot, or immunological detection with antibodies, are not sufficiently sensitive for detection of drug-DNA adducts induced by microdose concentrations of platinum drugs. Cell lines and patients need to be exposed to chemotherapeutic doses (high, toxic concentrations) for a sufficient number of drug-DNA adducts to be created for the common adduct detection methods above to be informative [76]. Therefore, these traditional approaches can ONLY identify chemoresistance in patients AFTER full-dose chemotherapy.

In this study, we will adopt the highly sensitive technology of AMS for detection of microdose-induced platinum-DNA adducts. We have shown that AMS is about 1000 times

more sensitive than the traditional approaches for measuring Pt-DNA adducts [58]. Using AMS, we can detect DNA adducts after exposing cell cultures to one microdose of ^{14}C-oxaliplatin. Unique to this AMS-based diagnostic assay is that the biomarker is created de-novo as part of the assay. Consequently, this biomarker assay represents the ultimate pharmacodynamic measurement of all aspects of the alkylating drug in the individual patient's tumor. Given the high concordance of adduct formation in PBMCs with tumor cells, we will perform initial analysis through this surrogate tissue.

As a consequence of administration of a microdose of [^{14}C]oxaliplatin, radiolabelled drug molecules interact with DNA to form oxaliplatin-DNA adducts, creating a transient biomarker. With prolonged time, free [^{14}C]oxaliplatin is eliminated from serum and cells. Additionally, cells have the capacity to repair oxaliplatin-DNA adducts. In this study, repeated isolation of PBMCs at defined time points will allow for assessment of the kinetics of adduct formation and elimination over time. Using standard techniques for the isolation of genomic DNA and processing steps that minimize the loss of label by DNA degradation, [^{14}C]oxaliplatin-DNA adducts can be stably isolated. Using AMS, the oxaliplatin-adduct to DNA mass ratio is calculated as the sample specific ^{14}C/C ratio minus the background ^{14}C/C ratio. Using the mass and carbon content of the carrier, the mass of the DNA sample, and the specific activity of the [^{14}C]oxaliplatin, an absolute value for the number of ^{14}C atoms per DNA base-pair can be calculated.

2.7 Rationale for the Current Study

While oxaliplatin-based chemotherapy forms the basis of treatment for patients with various gastrointestinal cancers, methods to individualize this treatment based on expected response or observed exposure are urgently needed. For the reasons described in the preceding sections, our ***overall goal*** is to develop a [^{14}C]oxaliplatin microdosing assay. The assay will simultaneously 1) individualize oxaliplatin dosing and 2) predict response (or lack thereof) to oxaliplatin-based chemotherapy in individual patients. As an intermediate step toward that goal, the primary aim of this study to test the hypothesis that there is a correlation between the oxaliplatin exposure (AUC in plasma) of patients to a microdose and exposure of patients to a treatment dose. If feasible, such an assay would consistently and accurately predict treatment dose exposure to oxaliplatin. Furthermore, that assessment of DNA adducts formed via the microdose would predict sensitivity and resistance to oxaliplatin-based therapy. Preliminary estimates of these parameters are secondary endpoints of this pilot study.

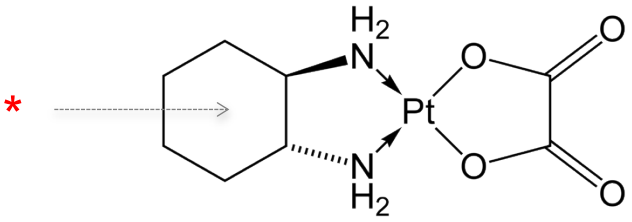
3 DRUG INFORMATION

3.1 Investigational Drug Information

[**^{14}C]Oxaliplatin Solution** is an investigational drug synthesized with a ^{14}C label and formulated specifically for this study. The **"microdose"** consists of a combination of the [^{14}C]Oxaliplatin Solution and unlabeled Oxaliplatin for Chemotherapy. Prior to administration as a microdose, the correct dose of [^{14}C]Oxaliplatin Solution and the correct dose of unlabeled Oxaliplatin for Chemotherapy must be calculated and prepared to achieve the correct patient total oxaliplatin dose and specific radioactivity for this study (see **section 6.3.1** and **section 6.4.1**).

3.1.1 Structural Information

Oxaliplatin



$^{14}C(*)$ is positioned at all carbons in the cyclohexane ring in ^{14}C-oxaliplatin

Chemical name	[(1*R*,2*R*)-cyclohexane-1,2-diamine](ethanedioato-*O*,*O*')platinum(II)
Chemical abstracts registry number	63121-00-6
Formula	$C_8H_{14}N_2O_4Pt$
Molecular weight	397.2858
NCS Number	266046

3.1.2 Mechanism of Action

Oxaliplatin undergoes nonenzymatic conversion in physiologic solutions to active derivatives via displacement of the labile oxalate ligand. Several transient reactive species are formed, including monoaquo and diaquo DACH platinum, which covalently bind with macromolecules. Both inter- and intrastrand Pt-DNA crosslinks are formed. Crosslinks are formed between the *N7* positions of two adjacent guanines (GG), adjacent adenine-guanines (AG), and guanines separated by an intervening nucleotide (GNG). These crosslinks inhibit DNA replication and transcription. Cytotoxicity is cell-cycle nonspecific.

3.1.3 Dosage

The dose of [^{14}C]oxaliplatin to be administered as a microdose may need to be modified (see **section 6.3.2**) based on the ^{14}C signal in genomic DNA isolated from patients after receiving one of the dose levels identified in the dose titration table (**Table 1**). The dose levels being evaluated were identified using the following rationale: Based on our ongoing clinical microdosing trial with [^{14}C]carboplatin, the radioactive dose of [^{14}C]carboplatin is 10^7 dpm/kg of patient body weight with a total carboplatin of 1% of therapeutic carboplatin with AUC of 6. Our pre-clinical studies with oxaliplatin dosing of cell lines indicate that the requirement for [^{14}C]oxaliplatin is about 10 times less than [^{14}C]carboplatin. We will begin this study at dose level 1, which we have already

demonstrated to be adequate and safe in a single breast cancer patient. Therefore, the start dose (Dose Level 1) of [^{14}C]oxaliplatin will be 2 X 10^6 dpm/kg of body weight with the total oxaliplatin at 1% of therapeutic dose (**Table 1**). Based on the ^{14}C signal in genomic DNA of peripheral blood mononuclear cells (PBMC) in patients after dosing with this dose level 1, the dose will be titrated up or down, if needed, and administered to separate patients with the goal of achieving a ^{14}C signal at 10-100 times the background. Instructions for preparing the microdose formulation are presented in **section 6.3.1**.

Table 1. Dose titration table

Dose levels	[^{14}C]oxaliplatin (dpm/kg of body weight)	Total oxaliplatin (% of therapeutic dose)
-1	1 X 10^6	1%
1	2 X 10^6	1%
2	2 X 10^6	3%
3	10 X 10^6	3%

3.1.4 Human Toxicity

3.1.4.1 Oxaliplatin Chemical Toxicity.

In this diagnostic feasibility study, patients will receive a small microdose of ^{14}C-labelled oxaliplatin (1% of therapeutic dose, or less than 3 mg). We do not expect to see any of the clinical side effects associated with standard dose oxaliplatin chemotherapy to occur at this low dose. The most common side effects of standard dose oxaliplatin chemotherapy are presented in **section 3.2.3**.

3.1.4.2 Radiation Toxicity Due to ^{14}C-label.

Patients will receive minimal radiation exposure (<0.02 mSv) with no expected toxicity.

Drug metabolism with ^{14}C-labeled drugs using AMS has been studied in healthy volunteers without significant toxicities [77, 78]. We have calculated the radiation dose each patient will receive if he/she participates in this trial. Most oxaliplatin is excreted in urine. The decline of ultrafilterable platinum levels following oxaliplatin administration is triphasic, characterized by two relatively short distribution phases ($t_{1/2\alpha}$ of 0.43 hours and $t_{1/2\beta}$ of 16.8 hours) and a long terminal elimination phase($t_{1/2\gamma}$ of 391 hours). We use 16.8 hours as the half-life for calculation.

For the starting dose, each patient will receive [^{14}C]oxaliplatin at 2 X 10^6 dpm/kg of body weight.

1 μCi = 2.22 x 10^6 dpm

If the patient's body weight is 75 kg, the total radiation dose *per microdose* will be:

2 X 10^6 dpm/kg x 75 kg = 1.5 X 10^8 dpm = 67.6 µCi *per microdose.*

1 μCi = 2.22 x 10^6 dpm x 0.049MeV/disintegration = 108,780 MeV/min = 108,780 MeV/min X (1.6 x 10^{-13} joules/Mev) = 1.74 x 10^{-8} joules/min

Therefore, each patient will receive:

67.6 μCi X (1.74 X 10^{-8} joules/min·μCi) = 1.2 x 10^{-6} joules/min *per microdose.*

If the average body weight is 75 kg, the radiation dose for each microdose will be:

1.2 x 10^{-6} joules/min person x person / 75 kg body weight = 1.6 x 10^{-8} joule/min/kg

We propose that, after 5 half-lives (84 hours), the [^{14}C]oxaliplatin remaining in the body will be negligible. If the patient is continuously exposed to ^{14}C radiation without metabolism of [^{14}C]oxaliplatin, the radiation dose will be:

1.6 x 10^{-8} joule/min/kg x 60 min/hr x 84 hr (five half-lives) = 7.9 x 10^{-5} joules/kg

1 joule/kg = 1 Sν

The radiation exposure for each microdose will be 7.9 x 10^{-5} Sν or 0.079 mSν. The radiation exposure for each patient completing both microdoses) will be 0.016 mSν.

If this study ends up at Dose Level 3 (10 X 10^6 dpm/kg, or 5 times the radiation dose used to calculate the above radiation exposure. **Table 1**), and patients undergo both microdose administrations, the radiation exposure will be 0.079 mSν x 5 x 2 = 0.79 mSν.

The annual effective radiation dose equivalent from natural internal sources is 1.6 mSν per person. The radiation exposure for an abdominal CT scan is 10 mSv. **The radiation exposure to ^{14}C for a patient completing both microdoses on this study is 0.016 mSv ÷ 10 mSv = 0.16% of an abdominal CT scan.**

Here, we assume that patients are exposed to [^{14}C]oxaliplatin radiation at the initial dose for 84 hours without correcting the calculation for reduction of the *in vivo* drug concentration drug by excretion, which makes this a very conservative calculation.

3.1.5 Pharmaceutical Data

See **section 3.2.3** for oxaliplatin pharmaceutical data.

3.1.6 Administration

cGMP-certified [^{14}C]oxaliplatin drug product, already in aqueous solution (0.5 mg/mL), is in sterile, single-use vials that will be stored at the UC Davis Investigational Drug Service. This [^{14}C]oxaliplatin is ready to use, and no reconstitution is needed. Nuclear medicine and oncology and physicians will calculate the [^{14}C]oxaliplatin dose and the unlabeled oxaliplatin dose to obtain a patient specific microdose of [^{14}C]oxaliplatin. When the two drugs are combined during infusion, a microdose is created at the desired radiochemical specific activity and chemical dose (see **section 6.3.1**). The [^{14}C]oxaliplatin infusion mixture will be prepared by a nuclear medicine technician by adding the [^{14}C]oxaliplatin to an infusion bag containing D5W solution. The unlabeled

oxaliplatin infusion mixture will be prepared by the infusion pharmacy at UC Davis Cancer Center by adding the unlabeled oxaliplatin to a separate infusion bag containing D5W solution. Both unlabeled and ^{14}C-labeled oxaliplatin will be concurrently infused intravenously over 2 hours at the UC Davis Cancer Center Infusion Center. A chemotherapy nurse will administer the unlabeled oxaliplatin. A Nuclear Medicine Technologist (CNMT) will administer the ^{14}C-labeled oxaliplatin via Y-site connection to the unlabeled oxaliplatin.

3.1.7 Storage and Stability

[^{14}C]oxaliplatin Solution (0.5 mg/mL in water for injection) is packaged as a sterile, pyrogen-free, 1.0 mL solution in single use, clear 2 mL glass vials with a rubber cap. It will be stored at the UC Davis Investigational Drug Service in a -20°C freezer. It is stable when stored at -20°C. High performance liquid chromatography (HPLC) will be performed with the [^{14}C]oxaliplatin at 6-month intervals to confirm its stability throughout this trial.

3.1.8 Manufacture and Formulation

[^{14}C]oxaliplatin drug substance was manufactured at GE Healthcare using organic chemical synthesis, therefore, free of viral or prion contamination. The specific activity of the stock [^{14}C]oxaliplatin is 54 mCi/mmol. The purity is 97.7% as determined by HPLC. The investigational [^{14}C]oxaliplatin solution (the drug product) will be formulated in water for injection and processed at the UC Davis cGMP facility for sterile filtration, bacteriostasis/ fungistasis tests, endotoxin testing, following cGMP manufacturing procedures. The cGMP [^{14}C]oxaliplatin for injection solution is stored at UC Davis Investigational Drug Service and ready for the clinical trial. The identity, strength, purity, and stability analyses are performed at Moravek Radiochemical Inc (Brea, CA). Upon thawing, vials will be visually inspected for the presence of particulate matter. Vials with observable particulates will be rejected.

3.2 Standard of Care Drug Information

3.2.1 5-Fluorouracil (5-FU)

a. Description

1. 5-FU is a fluorinated pyrimidine, which has been modified from the naturally occurring product uracil by the addition of a fluoride at position 5.

2. Molecular Formula: $C_4H_3FN_2O_2$

3. Molecular Weight: 130.1g/mol

b. Pharmacology

Mechanism of Action: 5-FU's primary mode of action is the inhibition of thymidylate synthase which is necessary for both the synthesis and repair of DNA. The agent exhibits activity in different phases of the cell cycle based on mode of administration. After bolus exposure of 5-FU s-phase cytotoxicity is noted. After 24 hours of continuous intravenous infusion G-1 phase cytotoxicity has been demonstrated.

c. Pharmacokinetics

1. Absorption: Oral 5-FU is incompletely absorbed from the GI tract with highly variable rates of bioavailability reported between 0 and 80%. Topical absorption

is also minimal with only 2.4% of active drug being absorbed from the 5% commercial cream.

2. Distribution: 5-Fluorouracil has wide ranging distribution to both tissue and extracellular fluid. Volume of distribution has been reported between 13-27 liters after IV bolus administration.

3. Metabolism: The metabolism of 5-FU is well known and occurs primarily in the liver via non-linear kinetics. The rate limiting step of hepatic breakdown is the conversion of 5-fluorouracil to 5-6 dihydrofluorouracil via dihydropyrimidine dehydrogenase (DPD). Patients with known DPD deficiency should not be given 5-FU.

4. Elimination: the elimination half-life of 5-FU after IV bolus administration has been reported to occur within 6-22 minutes. Small amounts of unchanged 5-FU are eliminated via the kidney and biliary systems.

d. Adverse Effects

1. Human:

• CNS: confusion, disorientation, euphoria, nystagmus, headache, encephalopathy, and peripheral neuropathy

• Opthalmic: lacrimal duct stenosis, visual changes, lacrimation, and photophobia, excessive nasal discharge, reddening of the eyes, blurring of the vision

• Gastrointestinal: stomatitis, esophagopharyngitis, nausea, vomiting, anorexia, diarrhea, gastrointestinal ulceration and bleeding

• Cardiovascular: cardiomyopathy, ischemic chest pain, myocardial ischemia, diaphoresis, thrombophlebitis, electrocardiogram changes.

• Dermatologic: maculopapular rash, alopecia, dry skin, fissuring, hand-foot syndrome, nail changes, rash, photosensitivity and vein pigmentation. onycholisis, dystrophy, pain and thickening of the nail bed, transverse striations, half and half nail changes, loss of nail, paronychial inflammation, and hyperpigmentation

• Hematologic: leucopenia, agranulocytosis, anemia, epistaxis, pancytopenia, and thrombocytopenia

• Immunologic: allergic reactions, anaphylaxis

2. Pregnancy and lactation: 5-FU is classified as pregnancy category D.

Teratogenic effects have been demonstrated in animals, however no well controlled studies in humans have been performed. In addition, no adequate data is available on the use of 5-FU while breast feeding. Women are advised to use formula if remaining on treatment.

e. Administration (Dosing): See treatment plan, **section 6.5**

f. Storage/Stability

1. Compatibility: Store unopen vials at room temperature and protect from light. May be diluted in NS or D5W.

2. 5-FU is a cytotoxic drug and appropriate procedures for handling, preparing and administering the drug should be followed.

3. When diluted in NS or D5W to a concentration of 1.5 mg/ml in either glass or polyvinyl chloride containers 5-FU is stable for 8 weeks at room temperature. In ethylene vinyl chloride pumps, 5-FU 10 mg/ml in NS or D5W is stable for 28 days at 4—35 degrees C.

g. How Supplied

5-FU is available as an IV solution for injection and will be obtained from commercial supply.

3.2.2 Leucovorin Calcium

a. Description

1. Leucovorin is a mixture of the diastereoisomers of 5-formyl derivative of tetrahydrofolic acid. The active component is the (-)-L-isomer known as Citrovorum factor.

2. Molecular Formula: $C_{20}H_{21}CaN_7O_7$

3. Molecular Weight: 511.51 g/mol

b. Pharmacology

Mechanism of Action: During normal processes, thymidylate synthetase forms a noncovalent ternary complex with deoxyuridylate (dUMP) and the reduced folate cofactor of leucovorin 5,10-methylenetetrahydrofolate (mTHF). The reduced folate facilitates the association and disassociation of the complex and the formation of thymidylate (dTMP) and dihydrofolate. Fluorouracil inhibits thymidylate synthetase through the covalent binding of 5-fluorodeoxyuridine monophosphate (FdUMP) and mTHF. The binding of FdUMP is dependent upon the intracellular concentration of mTHF. Since l-leucovorin is metabolized to mTHF, it increases and stabilizes the binding of FdUMP to thymidylate synthetase, thus increasing the cytotoxic effects of fluorouracil.

c. Pharmacokinetics

1. Absorption: Oral bioavailability of leucovorin is concentration dependent and severely reduced with doses greater than 25 mg. Studies have produced bioavailabilities of 97% 75%, and 37% for doses of 25 mg, 50mg and 100mg respectively.

2. Distribution: Leucovorin is rapidly converted to mTHF and distributed widely to tissues including the CNS. Distribution is however slowed in the presence of methotrexate as this agent competes with bodily tissues for leucovorin, and a higher percentage of unchanged drug is excreted in the urine when methotrexate is present.

3. Metabolism: Through the intravenous route the active isomer of leucovorin, l-leucovorin, is primarily metabolized through hepatic means to mTHF. The d isomer is not metabolized nor biologically active. By the oral route leucovorin is converted to mTHF primarily by the intestinal mucosa.

4. Elimination: the d isomer is primarily excreted unchanged in the urine while the active form is extensively metabolized by intestinal and hepatic means. Leucovorin has a half life of approximately 6 hours.

d. Adverse Effects

1. Human toxicity:

• Gastrointestinal: Toxicity has been studied in combination with 5FU where diarrhea, dehydration and stomatitis are all more common with the combination.

• Immunologic: hypersensitivity and anaphylaxis have been reported

• Endocrine: hypocalcemia

2. Pregnancy and Lactation: Leucovorin is classified as FDA pregnancy risk category C. No adequate human studies have examined the effects of this drug on the fetus. It is not known if leucovorin is excreted into breast milk, and therefore mothers receiving the agent should consider alternative modes of feeding.

e. Administration (Dosing): See treatment plan, **section 6.5**

f. Storage/Stability

1. Compatibility Information: leucovorin may be dilute in D5W, D10W, NS, Ringer's, or lactated Ringer's solution.

2. Drug handling and compatibility: leucovorin is not considered as hazardous substance and as such may be prepared in a laminar flow hood using aseptic technique.

3. Store dry powder, reconstituted solution and tablets at controlled room temperature. Protect from light. When reconstituted with Bacteriostatic Water for Injection, the resulting solution must be used within seven days. If reconstituted with Sterile Water for Injection, use immediately and discard any unused portion. Diluted injections are stable for 24 hours.

g. How Supplied

Leucovorin is provided as both a powder and liquid for injection. Leucovorin is commercially available, and will be obtained from commercial supply for this study.

3.2.3 Oxaliplatin (Eloxatin®)

a. Description

1. Oxaliplatin is a third generation platinum analog. Oxaliplatin contains a bulky carrier ligand, 1,2-diaminocyclohexane (DACH), not present in either cisplatin or carboplatin.

2. Molecular Formula: $C_8H_{14}N_2O_4Pt$

3. Molecular Weight: 397.3 g/mol

b. Pharmacology

Mechanism of Action: Oxaliplatin is a non-cell cycle specific, alkylating antineoplastic agent that inhibits DNA synthesis through the formation of Crosslinks between the N7 positions of two adjacent guanines (GG), adjacent adenine-guanines (AG), and guanines separated by an intervening nucleotide (GNG). These crosslinks inhibit DNA replication and transcription.

c. Pharmacokinetics

1. Absorption: NA

2. Distribution: oxaliplatin has a protein binding of 70-95% with longer in vitro exposure leading to higher protein binding rates. In addition, it has been demonstrated approximately 37% of platinum is taken up by erythrocytes. Steady state volume of distribution is approximately 35L.

3. Metabolism: Oxaliplatin is rapidly metabolized in plasma primarily by biotransformation. No unchanged oxaliplatin is found 2 hours after administration. Oxaliplatin demonstrates no cytochrome P450 metabolism. 17 known metabolites of oxaliplatin have been identified, monochloro DACH platinum, dichloro DACH platinum, and mono and diaquo DACH platinum, are cytotoxic.

4. Elimination: Platinum is predominately excreted through the kidney with a clearance of 9-17 L/hour. Elimination kinetics are triphasic with alpha, beta, and gamma half-lives of approximately 25.8 minutes, 16.8 hours and 391 hours. A very small (2%) amount of platinum is excreted in the feces.

d. Adverse Effects:

1. Refer to package insert or manufacturer website for the most complete and up to date information on contraindications, warning and precautions, and adverse reactions.

• Cardiovascular: Dyspnea, coughing, hypertension, edema, chest pain

• Dermatology: injection site reaction

• Gastrointestinal: Stomatitis, nausea, vomiting, taste alteration, anorexia, diarrhea, gastrointestinal reflux

• Hematologic: leucopenia, agranulocytosis, anemia, epistaxis, pancytopenia, and thrombocytopenia, febrile neutopenia

• Immunologic: allergic reactions, anaphylaxis

• Hepatic/Metabolic/Laboratory/Renal: hypokalemia, dehydration

• Neurologic: peripheral neuropathy

2. Pregnancy and Lactation: Oxaliplatin may cause fetal harm when administered to a pregnant woman (FDA pregnancy risk category D). In animal studies, oxaliplatin at doses less than one-tenth the recommended human dose based on body surface area caused developmental mortality and adversely affected fetal growth (decreased fetal weight, delayed ossification). If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, the patient should be counseled regarding the potential risks to the fetus. Females of childbearing potential should avoid becoming pregnant while receiving treatment with oxaliplatin. It is unknown whether oxaliplatin is excreted in breast milk. Therefore alternative means to feeding, or delaying treatment should be considered.

e. Administration (Dosing): See treatment plan, **section 6.5**

f. Storage/Stability

1. Compatibility: Oxaliplatin is incompatible in a solution with alkaline medications or diluents (such as basic solutions of 5-FU) and must not be mixed with these or administered simultaneously through the same infusion line. The infusion line should be flushed with D5W prior to administration of any concomitant medications. Only D5W is acceptable for dilution. Aluminum needles or iv sets should not be used as degradation of platinum may occur.

2. Special Handling: oxaliplatin is a cytotoxic drug and appropriate procedures for handling, preparing and administering the drug should be followed.

3. Store the intact vials at controlled room temperature. Excursions permitted to 15°C to 30°C (59°F to 86°F), not exceeding 30°C. Reconstituted solution: in 5% Dextrose or Water for Injection in the original vial, the solution may be stored for up to 48 hours between 2°C to 8° C (36°F-46°F). Infusion solution: after dilution in 5% Dextrose in Water, the shelf life is 24 hours at 2°C to 8°C (36°F-46°F).

g. How Supplied

1. Oxaliplatin is commercially available and will be obtained from commercial supply.

4 ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

4.1.1 Histologically or cytologically confirmed colon, rectal, pancreatic, gastroesophageal, appendiceal, or small bowel adenocarcinoma.

4.1.2 Intent to treat the patient with a FOLFOX chemotherapy regimen containing 5-FU, leucovorin, and oxaliplatin according to clinical standard practice. The intent should be to dose oxaliplatin at 85 mg/m^2 on an every 2 week basis.

4.1.3 Treatment with any additional FDA-approved biologic agent (i.e. bevacizumab, cetuximab, or panitumumab) is allowed according to standard practice.

4.1.4 Prior radiation or surgery is allowed, but should be finished at least 2 weeks prior to study enrollment.

4.1.5 Any number of prior therapies other than oxaliplatin is allowed.

4.1.6 Participants must be 18 years or older.

4.1.7 Zubrod performance status equal to or less than 2 (Karnofsky equal to or greater than 50%). See **Appendix A**.

4.1.8 Life expectancy of at least 3 months.

4.1.9 Participants must have normal organ and marrow function as defined as:

-absolute neutrophil count greater than or equal to 1,500/microL

-Platelets greater than or equal to 100,000/microL

-total bilirubin less than 3 X institutional upper limit of normal (ULN)

-AST (SGOT) less than or equal to 5 X ULN

-creatinine less than 1.5 X ULN

4.1.10 Women of child bearing potential must not be pregnant. A pre-study pregnancy test must be negative.

4.1.11 Women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for 30 days after study participation.

4.1.12 Men must agree to use adequate contraception (barrier method or abstinence) prior to study entry and for 30 days after study participation.

4.1.13 Ability to understand and willing to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Prior treatment with oxaliplatin.

4.2.2 Patients must not receive concomitant radiation.

4.2.3 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.4 Participants who are pregnant or nursing.

4.2.5 Participants who are allergic to any platinum agent.

4.2.6 Participants who have more than Grade 1 peripheral neuropathy.

5 DESCRIPTIVE/STRATIFICATION FACTORS

There is no stratification for the primary analysis of this trial. However the following data will be collected for exploratory analysis of clinical, molecular and treatment factors on outcome.

- Number of prior treatments
- Time from diagnosis
- RAS and BRAF mutation status (if performed)
- ERCC1 expression (if performed)

6 STUDY DESIGN AND TREATMENT PLAN

6.1 Study Design

This is a prospective feasibility study of a [^{14}C]oxaliplatin microdose assay to predict exposure and sensitivity to oxaliplatin as part of standard-of-care FOLFOX chemotherapy. Consenting patients who will be receiving FOLFOX as their treatment for cancer will be enrolled. Patients will receive two [^{14}C]oxaliplatin microdose infusions over the course of the study. The first part of the study is a Phase 0 [^{14}C]oxaliplatin microdose infusion occurring over 2 hours and followed by serial blood draws over a 24 hour period for microdose pharmacokinetic and pharmacodynamic analysis (on surrogate peripheral blood mononuclear cells). Between 2 days and 4 weeks after the administration of this phase 0 microdose, patients will begin FOLFOX therapy according to usual care. The initial treatment oxaliplatin dose will be diluted with the same amount of [^{14}C]oxaliplatin microdose as previously administered. Repeat blood draws for pharmacokinetic assessment of both the microdose and therapeutic dose along with pharmacodynamic analysis on PBMCs will be performed over 46-48 hours (this last draw is timed for the disconnect visit of the 5-FU infusion).

6.2 Selection of Patients

Subjects will be selective from the Medical Oncology clinics at the University of California Davis Comprehensive Cancer Center, in-hospital or via outside referral. The study will be presented to potential subjects by the physician with possible assistance from a clinical research coordinator. The enrolling physician is responsible for proposing and explaining the study to the prospective subject for the consent process.

6.3 Phase 0 [^{14}C]Oxaliplatin Microdose

6.3.1 Preparation and Administration of [^{14}C]Oxaliplatin Microdose

The starting dose (Dose Level 1, **Table 1**) of [^{14}C]oxaliplatin microdose to be administered in this study is 2 X 10^6 dpm/kg of body weight, with the total dose of both unlabeled and ^{14}C-labeled oxaliplatin solution being 1/100th of the planned therapeutic dosage (85 mg/m^2). Calculations of the therapeutic dose and microdose of oxaliplatin will use the Du Bois and Du Bois formula for BSA:

BSA (m^2) = 0.20247 x Height(m)0.725 x Weight(kg)0.425

The dose of [^{14}C]oxaliplatin is calculated in the following formula using Dose Level 1 (2 X 10^6 dpm/kg, 1% therapeutic dose):

2 X 10^6 dpm/kg ÷ (2.22 X 10^9 dpm/mCi) ÷ 54 mCi/mmol X 397 mg/mmol = 0.0066 mg/kg

The patient specific dose of [^{14}C]oxaliplatin will be added to an infusion bag containing D5W. Unlabeled oxaliplatin will be added to a separate infusion bag also containing D5W. Both treatment dose of unlabeled oxaliplatin and ^{14}C-labeled oxaliplatin will be concurrently infused intravenously over 2 hours at the UC Davis Cancer Center Infusion Center. When these two infusion bags are concurrently administered to a patient, together they achieve a total final oxaliplatin microdose of 1% of the therapeutic dose. A chemotherapy nurse will administer the unlabeled oxaliplatin. A Nuclear Medicine Technologist (CNMT) will administer the ^{14}C-labeled oxaliplatin via Y-site connection to the unlabeled oxaliplatin.

As an example, a patient with a height of 1.7 m and a body weight of 75 kg will have a BSA of 1.86 m^2. This patient would be planned to receive a therapeutic oxaliplatin dose of 158 mg (85 x 1.86). This patients microdose would then be 1.58 mg of total oxaliplatin containing 1.08 mg of unlabeled oxaliplatin for injection and 0.50 mg [^{14}C]oxaliplatin solution. The above dosing calculation should be modified as appropriate for the other dose levels in **Table 1**. Dr. Kim, Dr. Pan or their designees are the only authorized prescribers for the microdosing formulation. If the designee signs the pharmacy order form in Dr. Kim or Dr. Pan's absence, the designee must notify Dr. Kim or Pan as soon as possible before dosing the patient.

After the oxaliplatin infusion mixtures are prepared, the shelf-life is 6 hours at room temperature [20-25^{o}C] or up to 24 hours under refrigeration [2-8^{o}C]. The oxaliplatin microdose infusion mixtures should be administered concurrently through a 120-minute intravenous infusion.

6.3.2 Modification of the radioactive microdose during the study

We will vary the radioactive dose of [^{14}C]oxaliplatin (dpm/kg) in the patient's microdose by combining the [^{14}C]oxaliplatin with unlabeled oxaliplatin until the desired radioactive dose and chemical microdose (total dose at 1/100th of the therapeutic dose) are obtained. This effectively varies the radiochemical specific activity of the microdose infusion. The dose of [^{14}C]oxaliplatin (dpm/kg) for this feasibility study will be determined by measuring the ^{14}C associated with genomic DNA isolated from patient's PBMCs after microdosing. A ^{14}C signal 10-100 times background is acceptable for this study. Of note, the sensitivity for pharmacokinetic analysis is much higher and we do not expect dose modification to have any impact on pharmacokinetic feasibility.

We will start [^{14}C]oxaliplatin at 2 X 10^6 dpm/kg of body weight, and add unlabeled oxaliplatin to 1% of the therapeutic oxaliplatin as calculated based on the dose of 85 mg/m^2. This dose level was demonstrated to be adequate and safe in a single breast cancer patient in a prior study. Two patients will be recruited to this dose level (Dose

Level 1). If the ^{14}C signal in PBMC genomic DNA is sufficient, dose level 1 will be used for the rest of the study. If the ^{14}C signal is insufficient or too high, the dose level will be titrated up or down, respectively, based on the ^{14}C signal as outlined in **Table 1**, and administered to two additional patients per dose level until a sufficient ^{14}C signal is obtained. If no ^{14}C signal can be detected in the genomic DNA at Level 3, no further escalation will be performed.

6.3.3 Sample Collection Schedule

Blood will be collected for pharmacokinetic and pharmacodynamic analysis according to the schedule in **Table 2**. See section 10.1.1 for sample processing.

Table 2. Time Points of Blood Collection for PK Assessment after Phase 0 Microdose

Sample Number	Scheduled time points relative to [^{14}C]oxaliplatin administration		
	Day	**Scheduled time**	**Description**
1	1	0-5 min predose	Pre-dose
2	1	30 minutes (±5 minutes)	Post-dose
3	1	1 hr (±10 minutes)	Post-dose
4	1	1 hr 45 min (±10 minutes)	Post-dose
5	1	2 hr (±10 minutes)	Post-dose
6	1	2 hr 15 min (±10 minutes)	Post-dose
7	1	3 hr (±10 minutes)	Post-dose
8	1	4 hr (±60 minutes)	Post-dose
9	1	24 hr (±60 minutes)	Post-dose

6.4 Cycle 1 [^{14}C]Oxaliplatin Microdose Concurrent with FOLFOX

6.4.1 Preparation and Administration of [^{14}C]Oxaliplatin Microdose with Therapeutic Oxaliplatin dose.

The [^{14}C]Oxaliplatin infusion mixture in D5W will be prepared as described in **Section 6.3.1**. After this infusion mixture is prepared, the shelf-life is 6 hours at room temperature [20-25 ^{o}C] or up to 24 hours under refrigeration [2-8 ^{o}C]. The treatment dose of oxaliplatin should also be prepared in a separate infusion bag containing D5W. Both treatment dose of unlabeled oxaliplatin and ^{14}C-labeled oxaliplatin will be concurrently infused intravenously over 2 hours at the UC Davis Cancer Center Infusion Center. A chemotherapy nurse will administer the unlabeled oxaliplatin. A Nuclear Medicine Technologist (CNMT) will administer the ^{14}C-labeled oxaliplatin via Y-site connection to the unlabeled oxaliplatin.

6.4.2 Sample Collection Schedule

Blood will be collected for pharmacokinetic and pharmacodynamic analysis according to the schedule in **Table 3**. See section 10.1.1 for sample processing.

Table 3. Time Points of Blood Collection for oxaliplatin PK Assessment after Therapeutic-Associated Microdose			
Sample Number	**Scheduled time points relative to [^{14}C]oxaliplatin administration**		
	Day	**Scheduled time**	**Description**
1	1	0-5 min predose	Pre-dose
2	1	30 min (±5 minutes)	Post-dose
3	1	1 hr (±10 minutes)	Post-dose
4	1	1 hr 45 minutes (±10 minutes)	Post-dose
5	1	2 hr (±10 minutes)	Post-dose
6	1	2 hr 15 min (±10 minutes)	Post-dose
7	1	3 hr (±10 minutes)	Post-dose
8	1	4 hr (±60 minutes)	Post-dose
9	1	24 hr (±60 minutes)	Post-dose
10	2	48 hr (± two hours)	Post-dose

6.5 FOLFOX Treatment

6.5.1 Premedication and Supportive Care

Premedication associated with standard drug administration and supportive care (including anti-diarrheals, antibiotics, diuretics or other medications) should be given according to institutional standards.

6.5.2 FOLFOX

Patients will be treated with modified FOLFOX6 according to the following initial schedule for cycle 1. Cycle length is defined as 14 days. Modifications to cycle 1 treatment (other than oxaliplatin dose) may be allowed, but discussion with the primary investigator is required. Modifications to dosing beyond cycle 1 are at the discretion of the treating investigator according to usual medical practice.

Table 4. Modified FOLFOX6 Chemotherapy Regimen

Agent	**Dose**	**Route**	**Day**	**Schedule**
Leucovorin Calcium*	400 mg/m2	IV	1	Every 14 days
5-FU	400 mg/m2	Bolus IV	1	Every 14 days
Oxaliplatin	85 mg/m2	IV over 2 hours	1	Every 14 days
5-FU	2400 mg/m2	IV over 46-48 hours via ambulatory infusion pump	1-2	Every 14 days

*In the event of a leucovorin calcium shortage, the dosage may be reduced to 20 mg/m2 or 200 mg/m2 racemic levoleucovorin may be substituted. Leucovorin may be infused concurrently with oxaliplatin (via separate infusion lines).
** Note during cycle 1 only, Oxaliplatin infusion will contain [^{14}C]Oxaliplatin Microdose as described in section 6.4.1

6.6 Follow Up Period

Patients will remain on study until progression of disease or until any of the criteria in **section 6.7** are met. If an intermittent oxaliplatin strategy is chosen, the time of progression will be defined as progression after the first reintroduction of oxaliplatin, as has previously been defined [79, 80].

6.7 Criteria for Discontinuation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Intercurrent illness
- Occurrence of an unacceptable adverse event
- A treatment delay between phase 0 microdose and FOLFOX treatment of greater than 28 days for any reason
- Patient request
- Protocol violations
- Non-compliance
- Administrative reasons
- Failure to return for follow-up
- General or specific changes in the patient's condition unacceptable for further treatment in the judgment of the investigator

7 EVENTS TO BE MONITORED AND DOSE MODIFICATIONS

7.1 Safety Assessments

Safety will be monitored by performing a physical examination and assessing vital signs, performance status, laboratory evaluations, and collecting the adverse events. Because the investigational intervention is not expected to result in any significant toxicity, study safety evaluations will be performed at baseline, following each microdose administration, and for 14 days following microdose administration or until all attributed adverse events from the microdose have resolved, whichever is later. The timing of safety assessments after this period is at the discretion of the treating investigator according to good medical practice. For details on adverse event collection and reporting, refer to Section 7.2.

7.1.1 Physical Examination

A baseline comprehensive physical examination will be performed which must comprise a total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, and extremity). A comprehensive physical examination will be performed at the Pre-Study visit and at the Cycle 2 - Day 1 study visit. A symptom-directed physical examination will be performed on the Cycle 1 - Day 1 study visit and at any time during the study, as clinically indicated.

7.1.2 Vital Signs

Vital sign assessment consists of height (pre-study visit only), pulse, blood pressure, respiration rate, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.

7.1.3 Performance Status

Zubrod (ECOG) Performance Status (**Appendix A**) will be determined by the treating Investigator at each physical examination visit.

7.1.4 Hematology

Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (neutrophils; lymphocytes; monocytes; eosinophils; basophils), and platelet count will be measured Pre-Study, on day 1 of Phase 0 Microdose, on Cycle 1 - Day 1, and Cycle 2 Day 1. Testing must be performed within 14 days of registration, and within 72 hours of subsequent time points.

7.1.5 Serum chemistry

Blood urea nitrogen (BUN), creatinine, total bilirubin, AST, ALT, alkaline phosphatase, sodium, potassium, chloride, calcium, glucose, total protein, and albumin, will be measured Pre-Study, on day 1 of Phase 0 Microdose, on Cycle 1 - Day 1, and Cycle 2 Day 1. Testing must be performed within 14 days of registration, and within 72 hours of subsequent time points.

7.1.6 Pregnancy Test

All pre-menopausal women who are not surgically sterile will have a urine pregnancy test at the Pre-Study visit and a urine pregnancy test as clinically indicated. A positive urine pregnancy test requires a serum β-hCG to be performed to confirm this result.

7.2 Adverse Events

Because the investigational intervention is not expected to result in any significant adverse events, adverse event evaluations will be performed at baseline, following each microdose administration, and for 14 days following microdose administration or until all attributed adverse events from the microdose have resolved, whichever is later. Adverse events will be graded accorded to the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0). If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, or grades 1 - 4, will be used. Upon completion of the adverse event assessment window, patients will be followed for efficacy only (see **section 7.3**) until the follow up criteria in **section 6.6** are met.

7.2.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An 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 it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

For this protocol an abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic

intervention, or is considered by the investigator to be a clinically significant change from baseline.

7.2.2 Serious Adverse Event Definition

Serious adverse event (SAE) means any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification below on planned hospitalizations in Section 6.3).
- Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a congenital anomaly/birth defect.
- Is a medically important event. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent.
    - Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
    - With respect to the suspected transmission via a medicinal product of an infectious agent; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), whether pathogenic or non-pathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criteria described above and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/microL to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

7.2.3 Reporting Requirements

In addition to completing appropriate demographic and suspect medication information, the report should include the following information within the Event Description of the Medwatch Form 3500A

- Treatment regimen (dosing, frequency, combination therapy)
- Protocol description (include number if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive diagnostic and laboratory results
- Investigator's assessment of the relationship of the SAE to each investigational product and suspect medication

7.2.3.1 Follow-up information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original Medwatch Form 3500A and submitting it as follow-up
- Adding supplementary summary information and submitting it as follow-up with the original Medwatch Form 3500A
- Summarizing new information and faxing it with a cover letter including subject identifiers (ie, DOB, initials, subject number), protocol description and number, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted

7.2.4 Safety Reporting Requirements for IND

For Investigator Sponsored IND Studies there are some additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 312.32. Sponsor-investigators of studies conducted under an IND must comply with the following safety reporting requirements:

7.2.4.1 Expedited IND Safety Reports

The Sponsor-Investigator is required to report all serious, unexpected and related adverse events directly to the FDA on a MEDWATCH Form 3500A within 7 (if fatal or life-threatening) or 15 calendar days of first awareness.

The Sponsor-Investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected (21 CFR 312.32). Before submitting this report, the sponsor needs to ensure that the event meets all three of the definitions contained in the requirement:

- Suspected adverse reaction
- Serious
- Unexpected

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report to the FDA.

The timeframe for submitting an IND safety report to FDA is no later than 15 calendar days after the investigator determines that the suspected adverse event or other information qualifies for reporting.

Any fatal or life-threatening serious, unexpected and related adverse event must be reported to no later than 7 calendar days after initial awareness. Sponsor-Investigators should initially notify FDA by telephone or facsimile transmission. Other means of rapid communication (e.g., email) may also be used, if prior to transmission, the Project Manager in the FDA review division that has responsibility for review of the IND confirms that other means of rapid transmission are acceptable.

Any serious, unexpected and related adverse event that is not fatal or life-threatening must be reported to FDA no later than 15 calendar days after initial awareness.

The Sponsor-Investigator must comply with any applicable requirements related to the reporting of SAEs involving his/her subjects to the IRB that approved the study.

7.2.4.2 IND Annual Reports

In accordance with the regulation 21 CFR 312.33, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR 312.33 for a list of the elements required for the annual report.

7.3 Efficacy Assessments

For the purposes of this study, screening tumor assessments using CT of the chest/abdomen/pelvis and other areas of known disease or newly suspected disease should be performed within 28 days prior to registration. Scans of the abdomen, pelvis and other areas of the body may be done with MRI instead of CT, but evaluation of the chest should be done with CT. CT scans should be performed with iodinated IV contrast and MRI scans with IV gadolinium chelate unless there is a medical contraindication to contrast. If iodinated IV contrast is contraindicated, chest CT should be done without IV contrast. The CT component of a combined PET/CT may be used as long as the slice thickness is < 5 mm.

For the purposes of this study, patients with radiographically measurable disease at baseline should be re-evaluated for response every 8 weeks (+/- 1 week) for 6 months and then every 12 weeks (+/- 2 weeks) thereafter. The interval in imaging may be changed after discussion with the principal investigator. Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. Please see **Appendix C** for RECIST 1.1 criteria.

7.4 Dose Modifications

After initiation of treatment in Cycle 1 all dose modifications and delays are at the discretion of the treating investigator according to best medical practices.

8 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

8.1 Criteria for Evaluation

Evaluable patients will include eligible subjects who receive both the phase 0 and FOLFOX-integrated microdose and have samples collected at least 80% of time points. All patients who receive any component of microdose will be followed for toxicity, but any patient not completing both microdose administrations and 80% of blood samples will be replaced.

8.2 Endpoint Definitions

8.2.1 Primary Endpoint

The primary endpoint is the feasibility of assessing therapeutic pharmacokinetic (PK) parameters of the therapeutic dose using the microdose in evaluable patients. For the purposes of this pilot study, this assessment will be considered feasible if the area under the curve extrapolated to infinity ($AUC_{0-\infty}$) of the microdose and the therapeutic dose are within 2-fold of each other when normalized for differences in dose with a 95% confidence interval.

8.2.1.1 Justification of the primary endpoint

Prior microdosing work suggests that a close relationship between multiple microdose and full dose pharmacokinetic parameters including $t_{1/2}$ and AUC can be observed [77]. In this study, we have chosen to focus on total exposure to treatment as total exposure is related to the primary dose limiting toxicity of oxaliplatin treatment (i.e. peripheral neuropathy) [81]. While a close relationship will be required for final development, a relationship within 2-fold will provide sufficient preliminary data to suggest that further development and optimization of the assay will be warranted.

8.2.2 Secondary Endpoints

Secondary endpoints include:

8.2.2.1 Assessment of other PK parameters (e.g. C_{max}, t_{max}, $t_{1/2}$, CL) of the phase 0 microdose compared to the therapeutic dose.

8.2.2.2 Comparison of the PK parameters of the phase 0 microdose to the FOLFOX-integrated microdose.

8.2.2.3 The association of oxaliplatin-DNA adducts (see **section 10.3**) and tumor response by RECIST 1.1 (see **Appendix C**).

8.2.2.4 The association of oxaliplatin-DNA adducts (see **section 10.3**) and duration of disease control (see **Appendix C**).

8.2.2.5 Safety according to the Common Terminology Criteria for Adverse Events version 4 (see **section 7.2**).

9 STUDY CALENDAR

PERIOD	Pre-Study[a]	Phase 0 Microdose		Cycle 1 + Microdose			Ongoing Treatment		Off Study
		Day 1	Day 2	Day 1	Day 2	Day 3	Cycle 2, Day 1	Ongoing Treatment	
Safety Assessments.[b] See Section 7.1									
History & Physical Exam	X			X			X	*	
Vital Signs	X	X		X			X	*	
Performance Status	X			X			X		
Hematology	X	X		X			X	*	
Serum Chemistries	X	X		X			X	*	
Pregnancy Test	X								
Adverse Events. See Section 7.2									
Adverse Events		X----------X							
Efficacy Assessments. See Section 7.3									
Imaging Studies	X							X[c]	X
Treatments. See Section 6.									
[^{14}C]Oxaliplatin Microdose		X		X					
FOLFOX				X----------X			X	*	
Biologic Agent, if appropriate						X		*	
Correlative Studies. See Sections 6.3.3, 6.4.2 and 10.1									
Plasma Pharmacokinetics		X	X	X	X	X			
PBMCs		X	X	X	X	X			

[a] Pre-study assessments within 14 days before registration, except for imaging studies within 28 days of registration
[b] All on study safety assessments may be performed +/- 72 hours of the indicated timepoint
[c] Every 8 weeks (+/- 1 week) for 6 months then every 12 weeks (+/- 2 weeks) until progression. Required only in patients with measurable disease at baseline.
* After Cycle 2, Day 1 and resolution of all microdose-associated toxicities, all safety assessments and treatments are at the discretion of the treating investigator according to good medical practice.

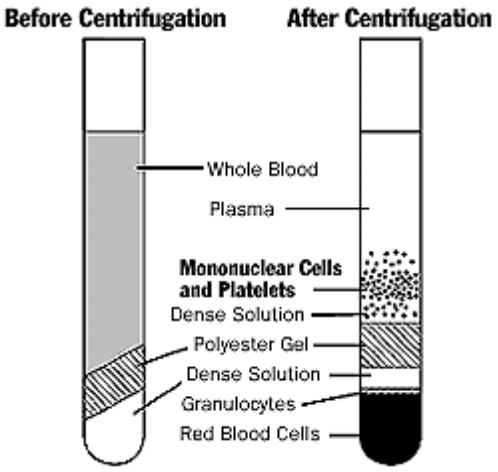
10 SPECIAL INSTRUCTIONS

10.1 Specimen Collection, Storage, Shipping and Submission Requirements

10.1.1 Collection Method and Storage

One 8-ml blood specimen will be collected into a BD Vacutainer CPTTM tube with sodium heparin (Becton Dickinson products # 362753) through an indwelling temporary venous catheter at the time points described in **Table 2** and **Table 3**. The tube will be gently inverted several times to ensure mixing with heparin anticoagulant. The tube will be immediately put on ice. To isolate plasma, the tubes are centrifuged at room temperature in a horizon rotor for 25 minutes at 1600 X g. After centrifugation, plasma is located at the top of the specimen (**Figure 3**), and will be transferred to two polypropylene cryogenic vials. The vials will be labeled with patient's identification information and stored at or below -70^{o}C until shipping. After collection of all of the specimens from the same patients, the ^{14}C content in plasma is determined by liquid scintillation count. The oxaliplatin concentration is calculated based on the ^{14}C content in the plasma specimens.

Figure 3. Centrifugation to separate Plasma and Mononuclear Cells in Peripheral Blood



After most of the plasma is removed for PK study, PBMC will be transferred to another tube, washed twice with ice-cold phosphate-buffered solution (PBS) with 2% fetal calf serum. DNA will be extracted from PBMC as described before [67], and sent to Accelerated Medical Diagnostics and LLNL for Pt-DNA adduct measurement.

10.1.2 Shipping and Submission Requirements

The peripheral blood specimens can be immediately kept at 4^{o}C or on ice up to four hours before the plasma and PBMC are separated. Plasma will then be stored at –70^{o}C freezer until PK studies. PBMC will be used for DNA extraction immediately after separation.

10.2 Pharmacokinetics

10.2.1 Specimens & Collection Frequency

Blood specimens will be collected at the time points outlined in **Table 2** and **Table 3**. Plasma will be used for PK assessment. Since a very small fraction of oxaliplatin is metabolized at the terminal phase, we will not determine the terminal half-lives if they are longer than 24 hours.

10.2.2 Analysis Method

The ^{14}C content in plasma is measured by liquid scintillation counting. The carboplatin concentration is calculated based on the ^{14}C content in the plasma specimens. C_{max}, $t_{1/2}$, and AUC of plasma carboplatin will be calculated for the carboplatin microdose setting and reported.

10.3 Assessment of Platinum-DNA Adducts in PBMCs

DNA containing [^{14}C]oxaliplatin-DNA adducts will be extracted from PBMCs (collected according to **section 10.1**) at Accelerated Medical Diagnostics as described previously [58, 82]. The DNA samples will be quantified by micro spectrophotometry, aliquoted and then delivered to Lawrence Livermore National Laboratory (LLNL) at 7000 E. Avenue, Livermore, CA 94550 for AMS analysis. Oxaliplatin-DNA adduct frequency levels (1-100 adducts per 10^8 nucleotides) will be reported. Comparison of oxaliplaton-DNA adduct levels in PBMC's to response data will be as described in **section 11.2.**

11 STATISTICAL CONSIDERATIONS

11.1 Statistical Design

This is a prospective feasibility diagnostic study of a phase 0 microdose of [^{14}C]oxaliplatin to ascertain whether there is sufficient promise to proceed for further study.

11.2 Analysis Plan

11.2.1 Primary Analyses

For the primary endpoint of feasibility is defined in **section 8.2.1.** We will correlate dose normalized $AUC_{0-\infty}$ parameters from phase 0 microdosing with parameters from the therapeutic dose of oxaliplatin for evaluable patients (the criteria for evaluation are listed in **section 8.1**). While assessment of PK feasibility is the primary endpoint of this study, we will use the totality of the data, including assessment of DNA adduct formation in PBMCs to determine the future development of this assay.

11.2.2 Secondary Analyses

Given the small number of patients included in this pilot study, all secondary analyses will be considered hypothesis generating for the design of future confirmatory study.

11.2.2.1 For secondary endpoint 8.2.2.1, we will correlate PK parameters from microdosing with parameters from therapeutic doses of oxaliplatin. All parameters will be normalized for dose. We will present descriptive summaries (scatterplots, tables, mean, SD,

correlation coefficient) of the relationship between the two sets of parameters.

11.2.2.2 For secondary endpoint 8.2.2.2, we will correlate PK parameters from phase 0 microdosing with parameters from FOLFOX-integrated microdosing of oxaliplatin. We will present descriptive summaries (scatterplots, tables, mean, SD, correlation coefficient) of the relationship between the two sets of parameters.

11.2.2.3 For secondary endpoint 8.2.2.3 and 8.2.2.4, we will characterize the repair of DNA adducts in PBMC, using descriptive statistics (graphical summaries, mean, SD, box plots). Response rate and duration of disease control (DDC) will be measured according to RECIST 1.1 (**Appendix C**). The DDC is equivalent to progression free survival if an intermittent oxaliplatin strategy is not employed. Progression free survival will be calculated from the date of enrollment to the date of first objective evidence of radiographic progression (soft tissue or bone lesion) or date of death due to any cause, whichever occurs first. If an intermittent oxaliplatin strategy is chosen, we will define DDC as the addition of the initial PFS and the PFS of the reintroduction, except in case of progression at the first evaluation after FOLFOX reintroduction. Radiological assessment by the treating Investigator will be the primary report in this analysis. We hypothesize that higher levels of DNA repair will be associated with non-response and shorter duration of disease control.

11.2.2.4 For secondary endpoint 8.2.2.5, we will assess toxicity to both micordoses of [^{14}C]oxaliplatin. Toxicities potentially related to [^{14}C]oxaliplatin will be assessed from initiation of the study to at least 14 days after the administration of the FOLFOX-integrated microdose or until full recover of toxicity (whichever is longer). Safety will be assessed through summaries of adverse events and laboratory evaluations. Descriptive statistics will be used rather than inferential statistics.

11.3 Sample Size

The number of subjects required to provide 80% probability that a 95% CI for the ratio of geometric means of full dose and micro dose PK parameters is within 2 fold (i.e., from 0.5 to 2) depends on the coefficient of variation (CV) of the ratio of the parameters, which depends on the CV of each parameter and the within-person correlation between the full dose and micro dose parameters (on a log scale). According to the Lappin et al. [77], CVs for $t_{1/2}$ were in the 0.3-0.5 range for diazepam and midazolam (which had good concordance between doses as well as adequate bioavailability). With these specifications, a sample size of 6 patients receiving both full- and micro-doses is sufficient.

12 REGISTRATION GUIDELINES

Once signed, informed consent has been obtained; patients will be entered on study. To register a patient, the data manager must complete the Eligibility Checklist (**Appendix B**). The data coordinator will register the patient onto the study and assign a unique patient number.

13 DATA SUBMISSION

13.1 Confidentiality of Records

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

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

The data collection system (eVELOS) for this study uses built-in security features to encrypt all data, preventing unauthorized access to confidential participant information. Access to the system is controlled by a sequence of individually assigned user identification codes and passwords, made available only to authorized personnel who have completed prerequisite training.

13.2 Patient Consent Form

At the time of registration, three signed and dated copies of the patient Informed Consent form with the Human Rights must be available (for patient, patient's medical chart and one for UC Davis).

13.3 Registration Eligibility Worksheet

At the time of registration, the information requested on Eligibility Checklist will be submitted to the Protocol Coordinator.

13.4 Data Collection Forms and Submission Schedule

All data will be collected using the UCD Database System (eVELOS) forms. All data forms will be completed, submitted and processed in accordance with UCD CTSU policies.

13.5 Database Management and Quality Control

Quality assurance audits of select patients and source documents may be conducted by the UC Davis Cancer Center Quality Assurance Committee as outlined in the UC Davis Cancer Center Data and Safety Monitoring plan.

Quality control will be maintained by the CTSU Quality Assurance team according to CTSU policy.

14 MINORITIES AND WOMEN STATEMENT

Recruitment is open to all minorities. Although distributions may vary by disease type, our recruitment procedures have been developed to enroll patients who are representative of the respective target population.

15 ETHICAL AND REGULATORY CONSIDERATIONS

15.1 Ethical Conduct of the Study

This study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) according to (International Conference on Harmonization) ICH guidelines. Specifically, the study will be conducted under a protocol reviewed by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject will give his or her written, informed consent before any protocol-driven tests or evaluations are performed.

Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

15.2 Informed Consent Procedures

Informed consent will be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent will be documented in the patient source documents.

All patients will have signed an informed consent for participation in research activities in accord with all institutional, NCI and Federal regulations, and will have been given a copy of the Experimental Subject's Bill of Rights.

16 DATA AND SAFETY MONITORING

This protocol is also subject to the UC Davis Cancer Center’s (UCDCC) Data and Safety Monitoring Plan. The UCDCC is committed to pursuing high-quality patient-oriented clinical research and has established mechanisms to ensure both scientific rigor and patient safety in the conduct of clinical research studies. The UCDCC relies on a multi-tiered committee system that reviews and monitors all cancer clinical trials and ensures the safety of its participants, in compliance with institutional and federal requirements on adverse event (AE) reporting, verification of data accuracy, and adherence to protocol eligibility requirements, treatment guidelines, and related matters. The Scientific Review Committee (SRC) assumes overall oversight of cancer studies, with assistance and input from two independent, but interacting, committees: the Quality Assurance Committee and the Data Safety Monitoring Committee. A multi-level review system strengthens the ability of the UCDCC to fulfill its mission in conducting high quality clinical cancer research.

As per University of California Davis Cancer Center (UCDCC) Clinical Trials Support Unit (CTSU) SOP AM 506: Protocol Specific Meetings, the principal investigator (PI), clinical research coordinator (CRC), and the clinical research nurse meet at least monthly for ongoing study information, to discuss patient data and adverse events and to determine if dose escalation is warranted, when applicable.

According to the UCDCC Data and Safety Monitoring Plan (DSMP), any new serious adverse events related to the drugs being used on this trial are reviewed monthly by the UCDCC Data and Safety Monitoring Committee (DSMC) and any applicable changes to the study are recommended to the PI, if necessary.

The UCDCC Scientific Review Committee (SRC) determines if a UCDCC Data and Safety Monitoring Board (DSMB) is required. If required, the DSMC will appoint a DSMB. The DSMB is responsible for reviewing study accrual logs, adverse event information and dose escalation meeting minutes (where applicable) to ensure subject safety and compliance with protocol defined guidelines.

17 PATHOLOGY REVIEW

All patients will have malignancy confirmed by review of their biopsy specimens by the Department of Pathology at the University of California at Davis.

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19 APPENDIX

Appendix A – ECOG/Zubrod Performance Status Scale

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

Appendix B – Eligibility Worksheet

Patient Name _____ MRN _____

Each question must be answered appropriately for a patient to be considered eligible for registration. Patients are eligible if they meet the following criteria (all answers must be "yes"). If the criteria is not applicable answer "yes."

YES	NO	CRITERIA
		Histologically or cytologically confirmed colon, rectal, pancreatic, gastroesophageal, appendiceal, or small bowel adenocarcinoma
		Intent to treat with a FOLFOX chemotherapy regimen containing oxaliplatin at 85 mg/m^2 on an every 2 week basis.
		Prior radiation or surgery is allowed, but should be finished at least 2 weeks prior to study enrollment.
		18 years or older.
		Zubrod performance status equal to or less than 2. See **Appendix A**
		Life expectancy of at least 3 months.
		Absolute neutrophil count greater than or equal to 1,500/microL
		Platelets greater than or equal to 100,000/microL
		Total bilirubin less than 3 X institutional upper limit of normal (ULN)
		AST (SGOT) less than or equal to 5 X ULN
		Creatinine less than 1.5 X ULN
		Women of child bearing potential must not be pregnant. A pre-study pregnancy test must be negative.
		Women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for 30 days after study participation.
		Men must agree to use adequate contraception (barrier method or abstinence) prior to study entry and for 30 days after study participation.
		Able to understand and willing to sign a written informed consent document.
		No prior oxaliplatin.
		No plans to receive concomitant radiation.
		No uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
		Not allergic to any platinum agent.
		No more than Grade 1 peripheral neuropathy.

Eligibility Checklist Completed By: _____ Date: _____

Appendix C - Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.1

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first administration of the [^{14}C]oxaliplatin microdose.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of FOLFOX 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.

Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable unless they have been demonstrated to progress prior to their selection as a measurable lesion for this study.

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.

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 $\geq$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.

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

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

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

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:

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

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.

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.

Response Criteria

Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

Evaluation of Non-Target Lesions

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a 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).

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

Duration of Response

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

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

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

Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Duration of Disease Control

If an intermittent oxaliplatin strategy is chosen, the duration of disease control will be measured. Duration of disease control (DDC) is defined as the addition of the initial PFS and the PFS of the reintroduction, except in case of progression at the first evaluation after FOLFOX reintroduction [79, 80].