15-1022

Quinacrine–Capecitabine Combinatorial Therapy for Advanced Stage Colorectal Adenocarcinoma: A Phase I/II Investigator-Initiated Clinical Trial

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Contents

1.0 INTRODUCTION

1.1 Background

Recently, in an attempt to rescue mutant forms of the tumor suppressor protein p53, our lab synthesized several small molecules using an acridine scaffold in order to mimic the effects of the structurally similar CP-31398, the first bioactive small molecule chemical compound found to restore wild-type activity of mutant p53 [1]. In addition to these small molecules, we screened quinacrine: an acridine derivative developed in the 1920s as an antimalarial and later used for decades in the clinic as an antiprotozoal, anti-rheumatic, anti-helminthic, and sclerosing agent [2]. We found that quinacrine was able to stabilize p53 and induce pro-apoptotic p53 targets, including DR5. We subsequently investigated quinacrine in the context of hepatocellular cancer (HCC) because of the drug's propensity to accumulate in liver tissues at high concentrations. We found that quinacrine was able to sensitize several HCC cell lines to a variety of anticancer therapies, including TRAIL and Sorafenib both in vitro and in vivo. Importantly, we found that quinacrine, in combination with Sorafenib, could synergistically diminish levels of Mcl-1 protein, an anti-apoptotic Bcl-2 family member that is essential for tumor survival in many types of human tumors [3].

Based on exciting finding in hepatocellular carcinoma, we continued to explore quinacrine in a variety of other malignancies. As shown in Figure 1, an ensuing series of dose-response experiments revealed that, the mean concentration of quinacrine required for 50% growth inhibition in CRC (1.56 µg/mL) was about half the concentration required for similar growth inhibition is HCC (3.02 µg/mL), suggesting this agent to be more active in CRC.

EFFECT OF QUINACRINE ON CANCER CELL VIABILITY 72 HOUR TREATMENT

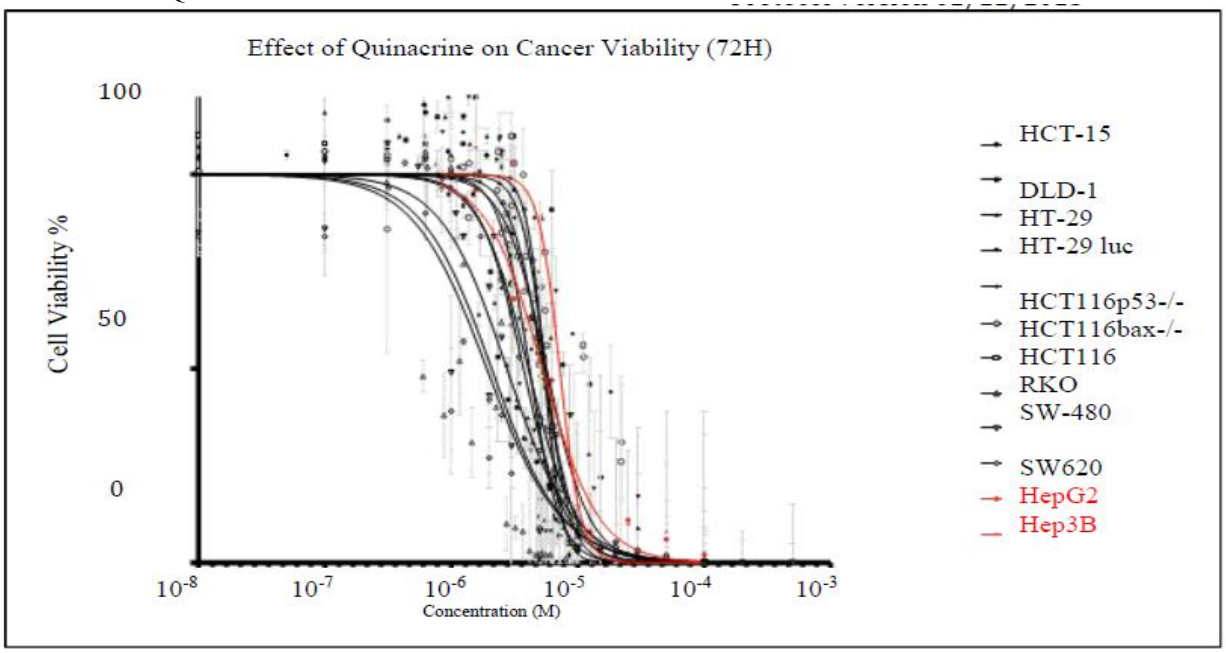


Figure 1: quinacrine inhibits the growth of a variety of CRC (black) and HCC (red) cell lines. Cells were treated with quinacrine for 72 hours and their viability assayed and resulted from four independent experiments.

Importantly, quinacrine in either dose concentration did not damage or induce the death of normal cells.

Based on this promising selective antitumor activity, we narrowed our investigation of quinacrine further, in the context of CRC. We found that quinacrine could enhance the activity of many anticancer therapies in CRC, including TRAIL, Sorafenib, Cisplatin, and 5-FU. Most importantly, we were able to demonstrate that quinacrine synergizes with 5-FU across a panel of ten genetically different human colorectal cancer cell lines in vitro (including those with KRAS and p53 mutations and wild types). Further studies in vivo showed that this combination synergizes to delay tumor growth in a mouse xenograft model. The combination of quinacrine and 5-FU was well tolerated at even high doses in mice, and effective across three different tumor xenografts [4]. Together these results suggest that quinacrine, which has been in Phase II clinical trials against prostate cancer, should be explored in combinatorial treatments with 5-FU against colorectal cancer in the clinic.

1.2 Metastatic Colorectal Cancer

CRC is the second leading cause of cancer-related deaths in industrialized nations [5]. In the United States, CRC is the third most commonly diagnosed malignancy and the third most frequent cause of cancer-related deaths, accounting for over 140,000 new cases and more than 50,000 deaths in 2010 [6]. The outcome for CRC patients is strongly correlated with the clinical and pathological stage of the cancer at diagnosis: 5-year survival rates are over 90% when the disease is diagnosed early and localized to the bowel mucosa, but survival rates drop to 70% as soon as neighboring organs and lymph nodes are involved [6]. The 5-year survival for patients with metastatic CRC (mCRC) is less than 10%, with the most common sites of CRC metastasis being the liver, where 30 to 60% of CRC patients develop metastatic lesions, and the lungs, where 20-30% of CRC patients develop metastatic lesions [7].

Although there has been noteworthy progress in the development of new therapies for mCRC— leading to targeted agents such as the monoclonal antibodies Cetuximab (targeted against EGFR) and bevacizumab (targeted against VEGF)—overall efficacy and tolerance of these agents pose significant problems, and the cytotoxic chemotherapeutic agent 5-FU remains an essential part of standard treatments. First line therapy regimen against mCRC—usually consists of a combination of 5-FU, Leucovorin, and Oxaliplatin, or the aforementioned targeted antibody therapies—only boost patient survival by 20-22 months [8]. New therapeutic approaches or combinations are therefore necessary to complement current therapies, and to offer additional options for patients with progressive metastatic disease.

Current research has highlighted the importance of modulating the function of commonly deregulated apoptotic pathways in cancer with targeted anticancer agents [9]. Indeed, the activation of inhibitors of apoptosis is frequently observed in cancers, and plays a key role in the resistance to chemotherapy (5-FU) and targeted agents (TRAIL). Because the constitutive activation of the antiapoptotic transcription factor NF-κB is frequently observed in several cancers, including breast, prostate, CRC, and HCC, much effort has been dedicated to modulating the effect of this protein complex [10-14]. Although NF-κB inhibitors are being explored in the clinical setting, it is unlikely that NF-κB inhibition alone will result in apoptosis and cancer regression, due to the transcription factor's redundant and entrenched signaling networks [15]. Thus targeting the effectors of NF-κB is likely to be more effective. For example, the expression of a particular NF-κB effector, Mcl-1, has been shown to be a key barrier to successful

therapy including NF-kB targeted therapy; blockade of Mcl-1 is a veritable gateway to the sensitization of a variety of cancers to TRAIL and other promising cytotoxic agents [16]. From our own lab's previous research data, effective elimination of Mcl-1, using variable methods (sorafenib, siRNA) was sufficient to induce cell death in some certain tumor types, including CRC [17]. Quinacrine, which is active against both NF-κB and Mcl-1, should be able to overcome such resistance of CRC to conventional chemotherapy.

1.3 Capecitabine

Overview: Capecitabine is an FDA-approved orally administered chemotherapeutic prodrug indicated for use in CRC and breast cancers. The prodrug is enzymatically converted to its active metabolite, 5-FU, in three sequential steps: it is first metabolized to 5'-DFCR, later converted to 5'-DFUR, and, finally, preferentially activated in tumor tissues to 5-FU [18]. In CRC, capecitabine is indicated for the adjuvant treatment of advanced or metastatic disease, or for the neoadjuvant treatment of rectal cancer. Beyond the fact that it is an easily administrable oral drug, capecitabine has several advantages over the traditional biweekly infusions of 5-FU and leucovorin, especially in patients with metastatic or unresectable CRC. A couple of Phase III clinical trials in CRC comparing capecitabine monotherapy to the Mayo Clinic regimen, combination of bolus 5-FU and leucovorin (IFL) demonstrated that patients treated with capecitabine had improved response rates as compared to the standard of care at the time of trial [19, 20]. A more recent meta-analysis comparing capecitabine-based treatments to 5-FU regimens in patients with mCRC showed a significant improvement of progression free survival among patients treated with capecitabine but that no significant improvement for overall survival could be shown for capecitabine-based treatments versus the standard of care [21]. The side effects of capecitabine therapy tend to be milder than standard therapy against mCRC, and patients receiving capecitabine treatment have lower rates of neutropenia, stomatitis, and alopecia as compared to the regimen of 5-FU and leucovorin [19]. Capecitabine therapy is therefore an appealing choice for potentially terminally ill patients with mCRC. The other intriguing fact of capecitabine is that when it is tried in patients with recurrent or metastatic CRC who had been treated with 5-FU, it still showed meaningful activity.

Pharmacokinetics: Capecitabine is nearly 100% bioavailable after being rapidly absorbed from the GI tract [22]. Capecitabine and its metabolites are quickly distributed into tumors, intestinal mucosa, plasma, liver, and other tissues. Peak plasma concentrations of the prodrug occur within 1.5 hours, and peak plasma concentrations of 5-FU occur 2 hours after intake [22, 23]. Although capecitabine and its metabolites do not readily penetrate the blood-brain barrier, it is unknown whether the drug and its metabolites distribute into the cerebrospinal fluid, brain tissue, placenta, or breast milk [23]. There is no evidence of drug accumulation across a range of doses [24]. Plasma protein binding of capecitabine and its metabolites is lower than 60% and does not depend on concentration [23]. The half-life of capecitabine is between 0.49 -0.89 hours, while that of 5-FU is 0.67 to 1.15 hours [22, 24]. Because presence of food in the GI tract favorably decreases the rate and extent of absorption of capecitabine and decreases the peak plasma concentration and AUC of its metabolites, capecitabine should be dosed within 30 minutes of food [25]. Capecitabine, mostly in the form of its metabolites, is excreted in urine and almost 75% of a single dose of capecitabine is excreted within 24 hours [23].

1.4 Quinacrine

Overview: Quinacrine is an acridine derivative that was developed in the early 1920s and used extensively as an antimalarial during the Second World War, which was previously approved by FDA. It was used by over 3 million soldiers for up to 4 years in a controlled setting, which makes it as the best-studied drugs [26]. Although it has been superseded by chloroquine in the treatment of malaria, quinacrine is still used for the treatment of giardiasis and a host of autoimmune disorders including systemic lupus erythematous and rheumatoid arthritis [27-31], and available by prescription. Quinacrine has also been used as an intra-pleural sclerosing agent to prevent recurrence of pleural effusion or pneumothorax as well as for regional cancer therapy in cases of pericardial and abdominal effusions [32-37]. Due to its effectiveness as a sclerosing agent, quinacrine has also been used for non-surgical female sterilization, especially in developing countries [38, 39]. Quinacrine is under current clinical investigation for the treatment of Creutzfeldt-Jakob disease, because it binds to the C-terminal helix of cellular prion protein, and for the treatment of a variety of cancers, because of its dual abilities to suppress NF-κB and activate p53 [40-42]. It should be noted that for some of these conditions quinacrine has been superseded by other agents, however the use of quinacrine has to date not become contraindicated due to safety concerns. Its activity and favorable safety profile make quinacrine useful in a wide variety of contexts, including as an anticancer agent.
Pharmacokinetics: Quinacrine is usually administered orally with a full glass of water after a meal [26, 32, 33, 35, 43-45]. It is absorbed quickly after oral administration with plasma levels increasing 2-4 hours after administration and reaching a peak in 8-12 hours [26, 46, 47]. Plasma concentration increases fast during the first week and equilibrates by the fourth week [48, 49]. The highest concentrations of quinacrine are found in the liver, spleen, lungs, and adrenal glands, lowest are found in the brain, heart, and skeletal muscle [47]. Concentration in the liver may be 20,000 times that in the plasma [50]. Quinacrine is deposited in the skin, fingernails and hair [26]. The drug crosses the placental barrier and, concentrations in fetal tissue are similar to maternal concentrations [51, 52]. Cerebrospinal fluid concentrations are 1 to 5% of corresponding plasma level [47]. 80-90% of the drug is bound to plasma proteins when given at therapeutic doses. Half-life of the drug is 5-14 days depending on the dosing regimen [48, 53]. Excretion of quinacrine occurs principally through the urine where less than 11% is eliminated daily. Small amounts are excreted in bile, sweat, saliva and breast milk [47]. Elimination via the renal system may be enhanced by acidification and reduced by alkalization [26, 46, 47]. Quinacrine may be detected in the urine up to 2 months after therapy has been discontinued.

1.5 Pre-Clinical Data & Rationale

The scientific rationale and preclinical data for this study mainly stems from research in our lab. This work has been published:

Wenge Wang, Jean-Nicolas Gallant, Sharyn I. Katz, Nathan G. Dolloff, Charles D. Smith, Junaid Abdulghani, Joshua E. Allen, David T. Dicker, Bo Hong, Arunasalam Navaraj and Wafik S. El- Deiry. Quinacrine sensitizes hepatocellular carcinoma cells to TRAIL and chemotherapeutic agents. Cancer Biology & Therapy, 12:3. 229-238. 01 August 2011.

Jean-Nicolas Gallant, Joshua E. Allen, Charles D. Smith, David T. Dicker, Wenge Wang, Nathan

G. Dolloff, Arunasalam Navaraj and Wafik S. El-Deiry. Quinacrine synergizes with 5- fluorouracil and other therapies in colorectal cancer. Cancer Biology & Therapy, 12:3. 239-251. 01 August 2011.

Since its discovery as a potent antimalarial compound nearly one hundred years ago, quinacrine has been used effectively in the clinic as a medication for a wide range of disorders. Due to its anti-inflammatory activity, it has been used to treat lupus erythematous, rheumatoid arthritis, bronchial asthma, and other inflammatory diseases. Its multimodal mechanisms of action have also led to its use as an approved antihelmintic, antiprotozoal, and, most recently, anticancer agent. Indeed, quinacrine's antitumor efficacy was noted in the early 1950s, its use as an anticancer agent against malignant pleural effusions developed in the 1970s, and its molecular mechanisms justly expounded—quinacrine was recently shown to be a potent inhibitor of the antiapoptotic protein complex NF-κB and a simultaneously strong inducer of apoptosis and the tumor suppressor protein p53 [1, 42, 54, 55].

Studies conducted by our lab have demonstrated some of quinacrine's unique anticancer properties and its potential in novel combinatorial anticancer therapies. We have shown that quinacrine is able to rescue p53 from proteasomal degradation and induce p53 targets, including the pro-apoptotic cell death receptor, DR5 [1]. We also have demonstrated that quinacrine is able to sensitize HCC cells to TRAIL and other chemotherapies, in part due to quinacrine's ability to stabilize p53, both in vitro and in vivo via a mouse xenograft model of HCC [3]. Combinatorial high-dose quinacrine-Sorafenib therapy was well tolerated in several cohorts of mice and resulted in notable tumor growth inhibition [3]. In the context of HCC, quinacrine is remarkable in its ability to synergize with Sorafenib to effectively ablate levels of the antiapoptotic protein Mcl-1.

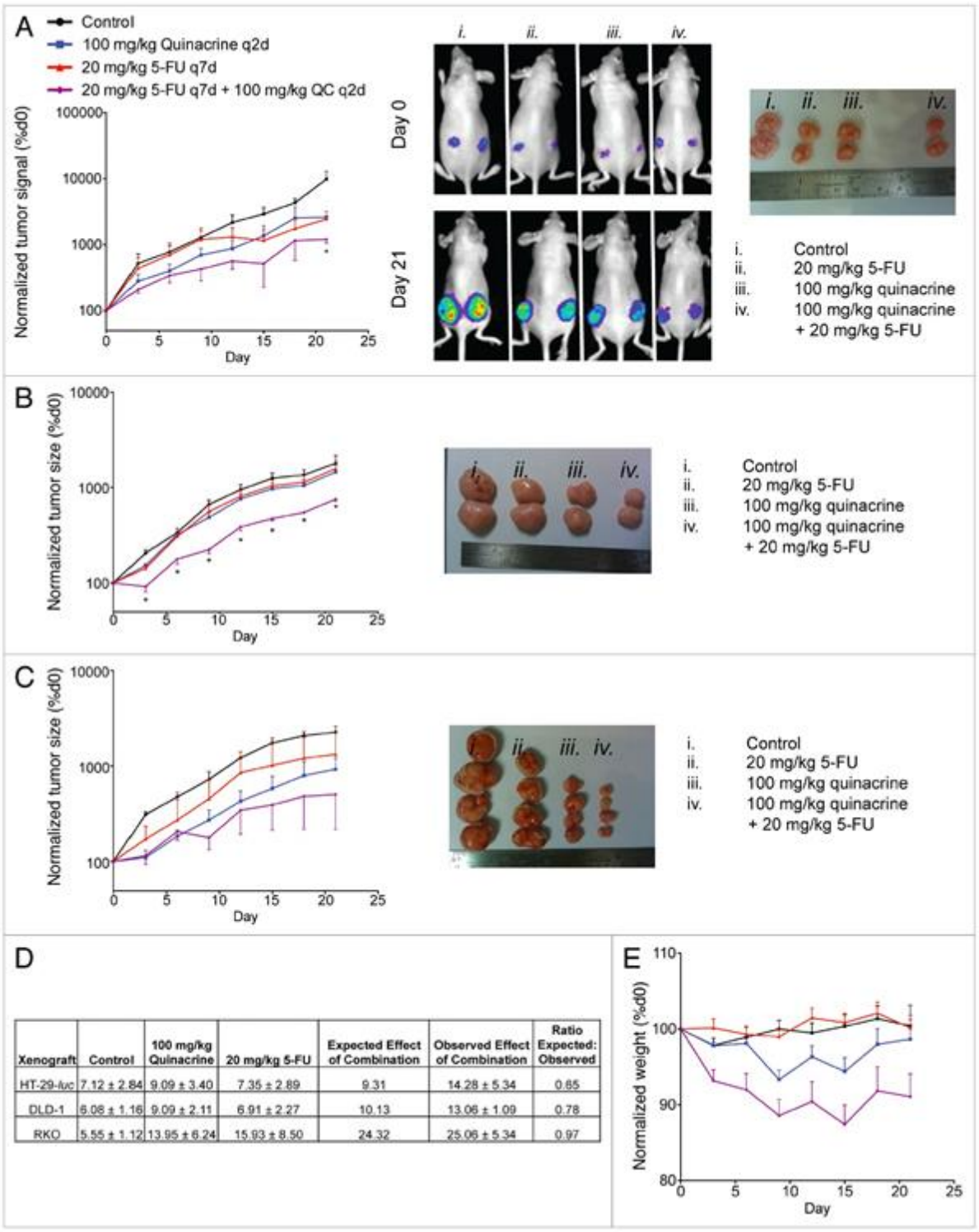
After discovery of quinacrine activity in HCC, our lab further found it to be particularly active in CRC. As previously shown in Figure 1, we established quinacrine as a potent cytostatic agent in CRC. Furthermore, we confirmed that quinacrine is a potent inhibitor of NF-κB and inducer of p53 in this malignancy [4]. Most importantly, we were able to show that quinacrine synergizes with 5-FU both in vitro and in vivo against CRC, augmenting each agent's antiproliferative ability independently of p53 and KRAS mutational status. This key result, recapitulated in Figure 2 on the following page, is the rationale undergirding this clinical protocol. Quinacrine and 5-FU synergize to delay tumor growth in three different CRC mouse xenografts, while having minimal toxicity at 100 mg/kg quinacrine. We hypothesize that quinacrine and capecitabine will similarly synergize in humans to maximize each agent's anticancer activity, providing patients with an attractive option in their fight against mCRC.

Quinacrine-capecitabine combinatorial therapy for mCRC is particularly appealing for several reasons. Namely: (1) the combination of quinacrine and 5-FU synergizes to delay tumor growth at low doses as shown in mouse model, (2) quinacrine is an extremely well-studied pharmaceutical agent with unique upside as an anticancer therapy, and (3) the combination of two oral medications would be well tolerated, and affect the QOL of potentially terminally ill patients with CRC less compared to toxic chemotherapeutic agents.

The pharmacological model of synergy (Chou-Talalay) used in our research is that, the synergy observed with quinacrine and 5-FU in vitro and in our mouse models should equally be observed in people [56]. In vitro synergy described using the Choi-Talalay model has led to several successful Phase III clinical trials, most notably of paclitaxel and cisplatin, in the treatment of ovarian cancer [57]. We further expect to see synergy between quinacrine and capecitabine because the doses at which synergy

was observed in vitro, are comparable to achievable plasma concentrations of both quinacrine and 5-FU [4]. Although we were unable to describe the precise mechanism of quinacrine's synergy with 5-FU, quinacrine's multimodal anticancer mechanisms mean that the potential for synergy to be translated to humans is rather high. In other words, quinacrine's polypharmacology—its ability to have effects on the p53, NF- κB, mTOR, AKT, and arachidonic acid pathways—means that its effects on multiple key signaling pathways make the drug a promising candidate for combination therapies for patients with CRC.

The safety and bioavailability of quinacrine make it an attractive agent for use in combinatorial therapy. Quinacrine's long-term safety has been demonstrated in patients with chronic diseases, such as rheumatoid arthritis and malaria; patients have taken daily quinacrine for months at a time to control their symptoms, with few side effects. As previously mentioned, the pharmacokinetics and safety of quinacrine have been extensively studied as it was administered as a protective measure to millions of US soldiers in the Pacific region during World War II. Some of the more serious side effects of quinacrine are mild in comparison to other anti-cancer chemotherapeutics and most of the conditions can be easily reversed after treatment cessation or dose reduction. Many of quinacrine's side effects develop gradually, starting from minor lesions in the case of dermatitis or a slight decrease in blood counts in the development of anemia, and have been found to be completely and easily reversible, if quinacrine use is discontinued at this early stage [2]. In our lab work, the minimal weight loss caused by the quinacrine combinatorial therapies was reversed upon cessation of treatment. Some of the side effects exhibited due to quinacrine treatment can be used in the clinical setting to confirm proper dosing of the drug in the treatment of cancer patients. The yellow discoloration of the skin due to the accumulation of the bright yellow compound would indicate to the clinician that the drug has reached the equilibrium and as in the case of squamous cell carcinomas, has potentially reached areas where tumor has developed [58].



Xenograft	Control	100 mg/kg Quinacrine	20 mg/kg 5-FU	Expected Effect of Combination	Observed Effect of Combination	Ratio Expected: Observed
HT-29-*luc*	7.12 ± 2.84	9.09 ± 3.40	7.35 ± 2.89	9.31	14.28 ± 5.34	0.65
DLD-1	6.08 ± 1.16	9.09 ± 2.11	6.91 ± 2.27	10.13	13.06 ± 1.09	0.78
RKO	5.55 ± 1.12	13.95 ± 6.24	15.93 ± 8.50	24.32	25.06 ± 5.34	0.97

Figure 2: Quinacrine delays tumor growth and enhances the effects of 5-FU in vivo. Effect of quinacrine/5-FU combinatorial therapy on HT-29-luc (A), DLD-1 (B) and RKO (C) xenografts in nu/nu mice (n = 3–5/group ± s.d.). Quinacrine was administered via oral gavage every 48 h, 5-FU was given intraperitoneally once a week. (D) Tumor delay in days necessary for a tumor to reach a volume 5 times greater than the initial volume (or 10 times the bioluminescent signal). (E) Effect of combinatorial therapy on the body weight of the mice of all 3 xenograft experiments (n = 13–15/group ± s.d.) [4].

We plan to use capecitabine instead of intravenous 5-FU for this study because of the pro-drug's favorable safety profile and ease of administration. While some intravenous medications may be more bioavailable and have greater effects, as previously discussed, capecitabine produces serum levels of 5-FU comparable to the continuous infusion while having similar activity [19, 20]. We thus expect Capecitabine to act in the same manner in this study as 5-FU did in our preclinical work. The fact that Capecitabine is an oral drug makes it convenient for both the patient and study center in this trial. The typical administration of intravenous 5-FU usually requires a hospital visit where the patient begins receiving the medication over the course of 48 hours via a central line infusion. Conversely, patients can take Capecitabine in the comfort of their own homes, adding to their QOL. This mode of administration will also reduce the lengths of hospitalizations and costs associated with this study.

Based on the extensive clinical history of quinacrine, the proven efficacy of capecitabine, and our preclinical data, we plan to conduct a Phase I/II study to evaluate the safety and efficacy of quinacrine in combination with Capecitabine in patients with histologically confirmed adenocarcinoma of the colon or rectum, that is metastatic, recurrent and unresectable and for which standard curative or palliative measures do not exist or are no longer effective.

1.6 Correlative Studies Background

Blood draws for pharmacokinetics and banking for future research will be collected. Archival tissue, if available, will be procured for molecular studies (Caris Life Sciences®).

2.0 STUDY OBJECTIVES

2.1 Phase I Primary objectives:

To determine the MTD of quinacrine in combination with a fixed dose of capecitabine and describe the tolerability and safety of the combination in patients with advanced mCRC.

2.2 Phase I Secondary objectives:

To determine toxicities/adverse reactions of quinacrine treatment in combination with capecitabine in patients with advanced mCRC

To evaluate rate of response (CR, PR, SD) and time to tumor progression in patients with advanced mCRC receiving quinacrine treatment in combination with capecitabine.

2.3 Phase II Primary objectives:

To evaluate the rate of response (CR, PR, SD) and time to tumor progression in patients with advanced mCRC receiving quinacrine treatment in combination with capecitabine.

2.4 Phase II Secondary objectives:

To determine the duration of response in patients receiving quinacrine treatment in combination with capecitabine for advanced mCRC.

To estimate the overall and progression free survival distribution for patients receiving quinacrine treatment in combination with capecitabine for advanced mCRC.

To determine 1-year survival and safety in patients receiving quinacrine treatment in combination with capecitabine for advanced mCRC.

2.5 Phase I/II Exploratory Objectives

To investigate the pharmacokinetics of quinacrine and capecitabine, perform genetic analysis on tumor tissue, and evaluate/follow biomarkers and CEA levels in patients with advanced mCRC quinacrine.

3.0 STUDY DESIGN

3.1 Phase I

Because of the well published safety profiles of both quinacrine and capecitabine, the Phase I portion of our study will aim to determine the tolerability of both agents in combinations when used at established clinical doses. Given that capecitabine is considered as standard treatment for mCRC, a full dose of 1000mg/m^2 orally bid of capecitabine will be administered with different doses of quinacrine. The maximum dose of quinacrine to be tested is 200 mg bid. The first patient in this study will be treated with 1000 mg/m^2 orally bid of capecitabine on days 1- 14, and a starting dose of 100 mg orally daily of quinacrine on days 1-21 of a 21 day cycle (dose level -2 in table below). All patients will be evaluated for baseline toxicity. Toxicity will be evaluated according to the National Cancer Institute Common Toxicity Criteria (current version 4.0).

A modified version 4B of Simon's accelerated titration design (Figure 3) will be used in the Phase I portion of our study to limit the number of patients treated at potentially sub therapeutic doses [60]. In this design, a cohort has at least one patient treated at a dose level (designated as "i"). If no DLT is observed (i.e. no toxicity, T, >2) at cycle 1, another single-patient cohort is treated with the next higher dose level (i.e., "Add 1 to i"). On the other hand, if a DLT is observed in a single- patient cohort in the first cycle, an additional two patients are treated at the same dose level (i.e. Dose i 2 patients). If another instance of toxicity > grade 2 is observed, three additional patients will be treated at that dose level. If at most 2 of the 6 patients treated at a dose level experience a DLT, that dose level is identified as the MTD.

Dose Escalation		
Dose Level	Dose *	
	Quinacrine (mg)	Capecitabine (mg/m2)
Level -2	100, qd	1000 bid

Level -1	100, bid	1000 bid
Level 0	200, bid	1000 bid

Adopting the modified version 4B of Simon's accelerated titration design, it is possible that only three patients are enrolled in Phase I. This situation can occur in the event that the first patient that received dose level -2, the first patient that received dose level -1 and the first patient that received dose level 0 do not experience toxicity>2.

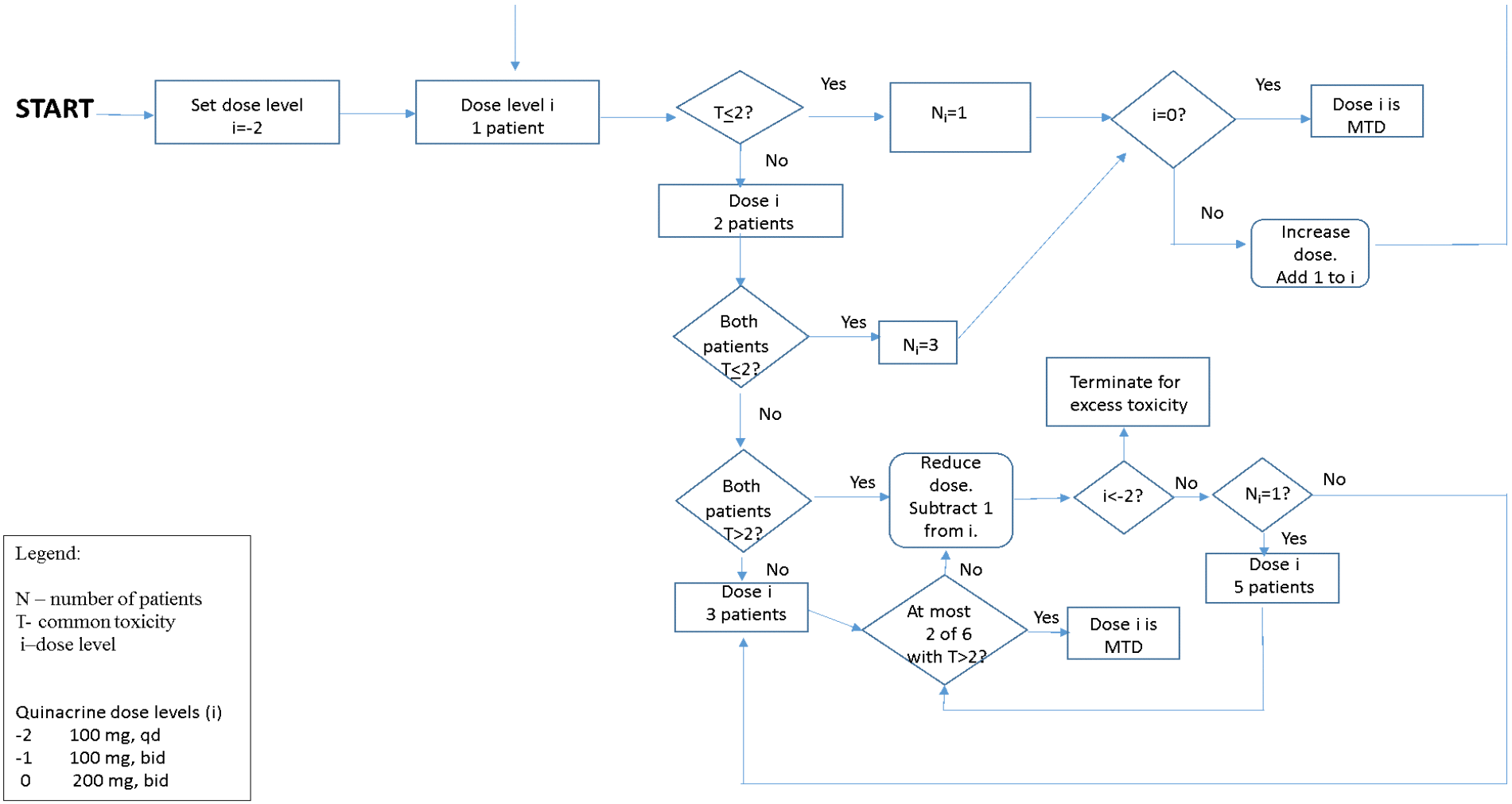


Figure 3: Accelerated Titration Design to be adopted in Phase I. At each dose level i, one patient will be initially treated. If that patient's common toxicity criteria score, T, is at most 2 the next patient is dosed at the next higher level (i.e. add 1 to i), unless dose i is dose 0 (the highest dose tested in this study). On the other hand, if the patient's score exceeds 2, two more patients are treated at the same dose level i. If these additional two patients' scores are both at most 2, the next patient is treated at the next higher dose level (i.e. add 1 to i), unless dose i is dose 0 (the highest dose tested in this study). On the other hand, if both patients have scores higher than 2, the next patient is dosed at the next lower level (i.e. subtract 1 from i), unless dose i is dose -2 (the lowest dose tested in this study). If only one of the two has a score higher than 2, then 3 additional patients are treated at the current dose level. If at most 2 of the 6 patients treated at this dose level have scores higher than 2, this dose is declared the MTD.

The chart below presents the different scenarios that can occur at a particular dose level, given the number of patients treated and number of patients that experience DLT, and the actions to be taken in the phase I dose escalation part of this study. The chart, therefore, is another means of understanding the research design of the Phase I portion of this study. Based on the research design, either 1, 3 or 6 patients can be treated with a particular dose level. The action will be either to escalate to the next dose with a single patient, add new patients to the current dose level (either 2, 3 or 5), de-escalate to the next lower dose level, declare the current dose level the MTD, or finally, declare all dose levels too toxic. For example, suppose dose level -2 does not result in a DLT, then dose level -1 is given to the next patient. If the patient treated with dose level -1 has a DLT, two more patients are treated with dose level -1. If all three patients treated with dose level -1 have DLTs, the study will be continued with the reduced dose, dose level -2. Because one patient was already treated at dose level -2 and no DLT's were observed, and there were patients previously treated at a higher dose (dose level -1), 5 more patients will be treated at dose level -2. If at most 2 of the six patients treated with dose level -2 have DLTs, dose level -2 will be declared MTD.

Number of patients treated at dose level	Numb er of DLT's	Were there patients previously treated at higher dose level? (Y/N)	Action required
1	0	N	If level<0, escalate and treat 1 patient on higher dose level. If level = 0, declare level 0 as MTD
		Y	Treat 5 more patients with this dose level
1	1	NA*	Treat 2 more patients with this dose level
3	1	N	If level<0, escalate and treat 1 patient on higher dose level. If level = 0, declare level 0 as MTD
		Y	Treat 3 more patients with this dose level
3	2	NA	Treat 3 more patients with this dose level
3	3	NA	Reduce dose to next lower dose level. Continue study, taking note of the total number of patients treated with the lower dose level and number of DLT's and taking corresponding action required. If current dose level is -2, terminate study for excess toxicity
6	1 or 2	NA	Declare this dose the MTD
6	3 or more		Reduce dose to next lower dose level. Continue study, taking note of the total number of patients treated with the lower dose level and number of DLT's and taking corresponding action required. If current dose level is -2, terminate study for excess toxicity

*NA indicates that the action taken is the same regardless of whether there were patients previously treated at a higher dose

The MTD will be used at recommended phase 2 dose (RP2D) unless there are any safety concerns. Patients who needed a dose reduction after being treated with RP2D in phase I will receive the reduced dose in phase II.

For the purposes of quinacrine dose escalation, the dose-limiting toxicities (DLT) that will be taken into account include toxicity grade >2 hematological, GI/diarrhea, skin, LFT abnormality or otherwise unexpected grade 3 toxicity or higher, associated with the combination therapy. Quinacrine dose modifications will not be made in the event of toxicities that are primarily attributed to capecitabine; namely, > grade 2 alopecia and hand and foot syndrome (HFS). Patients experiencing DLT will be permitted to continue their treatment after a single step dose reduction.

Intra-patient dose escalation will occur only for patients experiencing Common Toxicity Criteria < grade 2 toxicity on a previous course. The intra-patient dose escalation for a patient in lower level dose cohort will be on hold until the highest dose cohort patient completes first cycle of therapy. For purposes of toxicity assessment, patient response to the initial dose received will be considered. Total time in therapy, however, will be adjudged to include the whole duration the patient received treatment, regardless of the doses received.

General concomitant medications, dose adjustment, drug warnings and supportive care guidelines, duration of therapy, duration of follow up, and criteria for removal from treatment, as described throughout the protocol will be applied to both Phase I and II.

The endpoint of the Phase I portion of this study will occur when the safety and tolerability of the quinacrine-capecitabine combination is evaluated. Based on our preclinical data and the history of use of both capecitabine and quinacrine, we expect that the following regimen will be safely tolerated by the majority of patients.

Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Quinacrine	After a meal with a glass of water	200 mg tablets Orally twice a day	Orally: at least 8 hours between each dose	Daily on Days 1-21	21 days (3 weeks)
Capecitabine*	Within 30 minutes after a meal with water	1000 mg/m² Orally twice a day	Orally: at least 8 hours between each dose	Daily on Days 1-14	

*The calculated total daily capecitabine dose by body surface area (BSA) will be rounded down to allow doses using 500mg tablets

After finishing cycle #1, start cycle #2 on day 22, and continue every 21 days.

3.2 Phase II

An open label, single stage design will be used in the Phase II portion of this study. The treatment outlined in the previous section, using RP2D derived from Phase I, will be administered on an outpatient basis. The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle. Reported adverse events and

potential risks of quinacrine and capecitabine are described in Section 6. Appropriate dose modifications for quinacrine and capecitabine are described in Section 5. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

3.3 General Quinacrine Information

Quinacrine:
Convulsive seizures have occurred in patients receiving quinacrine and corticosteroids concomitantly [62]. Concurrent use of primaquine with quinacrine may inhibit the metabolism of Primaquine or may displace it from tissue-binding sites, thereby increasing serum concentrations and potential toxicity of primaquine. These effects may last for up to 3 months after the last dose of quinacrine is administered. Therefore concurrent use of quinacrine and primaquine is contraindicated) [47, 51]. Since quinacrine concentrates in the liver, it should be used with caution in conjunction with hepatotoxic drugs and in patients with hepatic disease or alcohol dependence [51]. Alcohol, or alcohol-containing medication, is not recommended during quinacrine treatment (quinacrine has been reported to produce a mild Disulfram-like reaction when ingested with alcohol, which may be due to quinacrine's inhibition of the intermediary metabolism of alcohol; ingestion of alcohol or alcohol containing medication is not recommended during quinacrine treatment) [51].

Pregnancy/Lactation: FDA Pregnancy Risk Category C: Quinacrine should not be given to pregnant women because the drug readily crosses the placenta and reaches the fetal circulation system [52]. There is one case of possible renal agenesis and hydrocephalus in an infant, although normal pregnancies have been reported after quinacrine ingestion during the first 4 weeks of gestation [63]. Adequate, well-controlled human studies are lacking, and animal studies have shown a risk to the fetus or are lacking as well. There is a chance of fetal harm if the drug is given during pregnancy; but the potential benefits may outweigh the potential risk [63]. Quinacrine was embryo lethal but not teratogenic in rats administered intrauterine quinacrine and gestation day 8 or day 12 [64]. A small amount of quinacrine is excreted in breast milk. However, problems in humans have not been documented [26].

3.4 General Capecitabine Information

Capecitabine:
Capecitabine should be administered within 30 minutes after a meal. Capecitabine and/or its metabolites may inhibit cytochrome P-450 (CYP) 2C9 isoenzyme, and therefore increase levels of substrates of CYP2C9: Because the decreased rate of anticoagulant metabolism may increase patient response to coumarin and indandione derivatives, capecitabine and these agents should be used concomitantly with great caution [23]. If capecitabine is used concomitantly with a coumarin anticoagulant, prothrombin time (PT) or international normalized ratio (INR) should be monitored frequently, and the anticoagulant dose should be adjusted accordingly. Concomitant use of phenytoin and capecitabine may result in toxicity from increased serum phenytoin concentrations. In patients receiving capecitabine, serum concentrations of phenytoin must be monitored carefully, and reduction in the phenytoin dosage may be necessary [23]. Concomitant use of folic acid may affect the metabolism of capecitabine [23]. Leucovorin (LV) potentiates the antineoplastic activity of 5-FU and also may increase its toxicity. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in geriatric patients receiving a weekly regimen of combination therapy with LV and 5-FU [23].

Pregnancy/Lactation: FDA Pregnancy Risk category: Studies in humans, or investigational or post-marketing data, have demonstrated fetal risk. Nevertheless, potential benefits from the use of the drug may outweigh the potential risk. For example, the drug may be acceptable if needed in a life threatening. In lactating mice receiving a single dose of capecitabine, significant amounts of capecitabine metabolites were distributed into milk. Because of the potential for serious adverse reactions to capecitabine in nursing infants, nursing should be discontinued during capecitabine therapy.

Dose adjustments: For mild renal dysfunction (creatinine clearance 30-50 mL/min), it is recommended to reduce dose by 25%. For severe renal dysfunction (creatinine clearance <30 mL/min), treatment is not recommended. For elderly patients, lower doses may be required due to higher incidences of serious adverse reactions.

3.5 Duration of therapy

Treatment continues until at least one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (DLT)
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

3.6 Duration of follow-up

Patients will be followed for five years after removal from treatment or until death, whichever occurs first. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. We expect that most patients will not require a follow-up of 5 years due to their poor prognosis. However we want to include long time survivors due to the combination of quinacrine treatment with capecitabine. Patients will be followed every 3 months either in clinic or via the telephone. Patients will continue to require tumor measurements every 6-9 weeks per section 10.1 until disease progression.

3.7 Criteria for removal from treatment

Patients will be removed from treatment when any of the criteria listed in Section 3.5 applies.
The reason for removal from treatment and the date the patient was removed must be documented in eCRF system.

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria:

4.1.1 On primary diagnosis, patients must have had histologically confirmed adenocarcinoma of the colon or rectum. Metastasis or recurrence do not need to be histologically confirmed

4.1.2 Standard, curative or palliative measures do not exist or are no longer effective

4.1.3 Patients must have measurable recurrence or metastases, per RECIST 1.1

4.1.4 Patients must have prior chemotherapy for advanced CRC and have previously received both an oxaliplatin and an irinotecan based regimen. Patients who are not appropriate for second line therapy because of their KRAS mutational status or because they cannot tolerate second line therapy, will be included even after only one prior therapy regimen

4.1.5 All prior systemic cancer therapy (hormonal, chemotherapeutic, and immunotherapeutic) must be completed at least 4 weeks before the baseline visit

4.1.6 Patients must be at least 4 weeks from radiation therapy or major surgery and have recovered from prior toxicities

4.1.7 Age >18 years

4.1.8 Life expectancy greater than 3 months

4.1.9 ECOG performance status of 0, 1, or 2

4.1.10 Patients must have normal organ and marrow function as defined below:

Leukocytes	>1,500/mcL
Absolute neutrophil count	>1,500/mcL
Platelets	>100,000/mcL
Total bilirubin	<2.5 ULN
AST/ALT	<3.0 ULN
Creatinine	<2.0
Creatinine Clearance*	≥ 50

**Creatinine Clearance must be calculated using Cockroft-Gault formula*

4.1.11 Patient must be able to swallow capsules

4.1.12 Patients must be able to understand and willing to sign a written informed consent document

4.2 Exclusion Criteria

All candidates meeting any of the exclusion criteria at baseline will be excluded from study participation:

4.2.1 Patients may not be receiving any other investigational agents.

4.2.2 Patients who have had chemotherapy or radiotherapy 4 weeks or more prior to entering the study who's adverse events have not recovered to grade 1 or less with the exception of alopecia and neuropathy

4.2.3 Patients with known brain metastases

4.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to quinacrine, capecitabine or fluorouracil.

4.2.5 Patients receiving any medications or substances that are substrates, inducers, or inhibitors of CYP2C9 enzyme. For a list of common CYP2C9 substrates, inducers, and inhibitors, see Appendix B

4.2.6 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.

4.2.7 Pregnant or breast feeding women are excluded from this study

4.2.8 Other reasons:

- Patients currently being treated with quinacrine or drugs related to quinacrine
- Patients who require anti-arrhythmic treatment with Amiodarone or any drug with a quinidine-like effect on the heart or who have history of a malignant ventricular arrhythmia unless they have a functioning Automatic Implantable Cardio Defibrillator (AICD) implanted.
- Patients who have a history of noninfectious (toxic, autoimmune) hepatitis or alcoholism.
- Patients with a lifetime history of porphyria or psoriasis
- Patients with documented glucose-6-phosphate dehydrogenase deficiency.
- Patients with a history of seizure disorder (except infant febrile seizures).
- Patients with a lifetime history of dermatitis as an allergic/toxic reaction to any medication.
- Patients with known dihydropyrimidine dehydrogenase (DPD)deficiency.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. However, we will require that women of child bearing potential use adequate contraception during the administration of the study drugs.

4.4 Pregnancy

The effects of capecitabine and quinacrine on the developing human fetus at the recommended therapeutic dose are unknown. For this reason women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of treatment, and for at least 3 months after the completion of treatment. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

Prior to study enrollment, WOBCP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In additional, men enrolled on this study should understand the risks to any sexual partner of childbearing potential.

All WOCBP must have a negative pregnancy test within *72 hours* prior to receiving the first dose of the investigational agent(s). If the pregnancy test is positive, the patient must not receive protocol treatment and must not be enrolled in the study.

WOCBP is defined as follows: Any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or a bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea > 12 consecutive months, or women on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone (FSH) level > 35 mIU/ml). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g. vasectomy), should be considered to be WOCBP.

4.5 Patient Registration

Participants may be registered from 8:00 am to 4:00 pm EST excluding holidays by emailing the study monitor at: **FCCC.MONITOR@fccc.edu.** Eligible participants will be entered on study centrally once the following items have been received by email:

- Completed registration form
- Consent and HIPAA signature pages
- Eligibility checklist

Following registration, participants must begin protocol treatment within 7 days of registration. Issues that would cause treatment delays must be discussed with the Principal Investigator. If a registered participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. For additional registration questions, please email **FCCC.MONITOR@fccc.edu**

The study monitor or their designee will notify the site by email once registration is confirmed and the sequence number has been assigned. Participants must be registered and have received a sequence number prior to the initiation of treatment.

Exceptions to the current registration policies will not be permitted.

5.0 Treatment Modifications

5.1 Dose Reductions

In the event of alopecia, HFS, or stomatis, only capecitabine dose will be reduced according to the following table.

Hand and Foot Syndrome/Stomatitis/Alopecia	Management/Next Dose for Capecitabine
Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until < Grade 1. Resume at one dose level lower, if indicated. *(Per Section 5.2 Dose Levels)*
Grade 4	Off protocol therapy

In the event of other toxicities that are specifically attributable to capecitabine (such as hand-foot syndrome, diarrhea), the dose of capecitabine will be delayed or reduced first. If toxicity progresses or does not improve within 72 hours after capecitabine delay or reduction, quinacrine will also be delayed/ dose-reduced. Dosing delays/modifications will be done according to the following schema:

Diarrhea	Management/Next Dose for quinacrine	Management/Next Dose for capecitabine
Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level. If it reoccurs, hold until < Grade 1 and resume at one dose level lower. *(Per Section 5.2 Dose Levels)*
Grade 3	Hold until < Grade 1. Resume at one dose level lower, if indicated.	Hold* until < Grade 1. Resume at one dose level lower, if indicated.** *(Per Section 5.2 Dose Levels)*
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: Loperamide antidiarrheal therapy Adjunct anti-diarrheal therapy is permitted and should be recorded when used.		

All other toxicities	Management/Next Dose for quinacrine	Management/Next Dose for capecitabine
Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until < Grade 1. Resume at one dose level lower, if indicated.	Hold until < Grade 1. Resume at one dose level lower, if indicated. *(Per Section 5.2 Dose Levels)*
Grade 4	Off protocol therapy	Off protocol therapy

Recommended management for nausea: antiemetics

If treatment needs to be held for more than 3 weeks due to severe treatment related toxicities that cannot be resolved despite maximum supportive care, the patient will be taken off protocol therapy.

5.2 Dose Levels for Phase II

Dose reductions will be as follows:

Dose Level	Quinacrine	Capecitabine
-2	50% reduction	500 mg/m2, schedule
-1	50% reduction	750 mg/m2, schedule
0	RP2D	1000 mg/m2, schedule

The number of dose de-escalations will depend upon the RP2D. For example if RP2D is 400mg/day of quinacrine and 1000mg/m^2 of capecitabine 2 dose reductions are possible but if RP2D is 200mg/day of quinacrine and 1000mg/m^2 of capecitabine of quinacrine only 1 dose reduction is possible or if RP2D is 100mg/day of quinacrine and 1000mg/m^2 of capecitabine no dose reductions are possible. In this study for Phase 2, dose re-escalations are allowed up to RP2D at the discretion of the treating Physician.

6.0 ADVERSE EVENTS

6.1. Adverse Event Definitions

6.1.1 **Adverse Events (AE)** is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, treatment or procedure regardless of whether it is considered related to the medical treatment or procedure *(NCI CTEP Guidelines March 28, 2011).*

6.1.2 **Serious Adverse Event (SAE)** is an AE that is fatal or life threatening, requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours), persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly/ birth defect, or results in any important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent any of the above outcomes. A "life-threatening" adverse event places the patient at immediate risk of death in the judgment of the investigator or sponsor.

6.1.3 **Severity Rating**

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.4.0) or study specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.4.0, severity is expressed in numerical grade using the following definitions:

1. Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2. Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.
3. Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
4. Grade 4: Life-threatening consequences; urgent intervention indicated.
5. Grade 5: Death related to AE

6.1.4 **Attribution/Relationship to study drug**

1. Definite – clearly related
2. Probable – likely related
3. Possible – may be related
4. Unlikely – doubtfully related
5. Unrelated – clearly not related

6.1.5 Expectedness

An Expected Adverse Event is one where the specificity or severity is consistent with the current information available from the resources.

An Unexpected Adverse Event is one where the nature, severity, or frequency of the event is related to participation in the research is not consistent with either:

1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts: or

2. The expected natural progression of any underlying disease, disorder, or condition of the subject (s) experiencing the adverse event and the subjects(s) predisposing risk factor profile for the adverse event.

6.2 Recording and Reporting Responsibilities

6.2.1 **Investigative Site Recording Responsibilities**:

1. Upon identification of an AE or SAE, the site investigator will utilize the above definitions to properly classify the event. Each category listed above must be recorded for each event.

2. All AEs and SAEs will be recorded in the "AE case report forms" (CRF) and in progress reports with details about the grade and attribution of each episode, action taken with respect to the study drug, and the patient's outcome will be recorded in the CRF. All events will be recorded on case report forms for the duration of the study until they resolve.

3. All SAEs will be recorded on the FDA MedWatch form 3500a. After submitting the initial report it may be necessary to submit follow up reports to the OCR, Sponsor and the FDA should the event require further investigation.

6.2.2 **Investigative Site Reporting Responsibilities**:

1. The investigator/ site is responsible to report all SAEs that occur on or after the first day of study treatment to the sponsor within 24 hours of becoming aware of the event. All subsequent SAEs must be reported for up to 30 days after the last

treatment.

Each investigator is responsible to report all AEs/SAEs to their local IRB following guidelines set by that IRB. The FCCC OCR reserves the right to request an event be reported to the IRB at their discretion. Copies of events reviewed by the IRB must be sent email to **SAE.FCCC@fccc.edu.**

2. If the investigator or IRB feels the event warrants a revision to the informed consent that was not already initiated by the OCR, draft revisions will be made in track changes and submitted to the OCR for consideration. Any consent revisions must receive OCR approval **prior** to submission to the IRB.

3. Any investigator who is in doubt of whether a particular AE needs to be reported is directed to call the Study Monitor for confirmation with the Sponsor Investigator

4. If the results of an investigator or OCR investigation show an adverse event not initially determined to be reportable is so reportable, the investigator will report the event following the above guidelines based on the date the determination is made.

5. Copies of all related correspondence and reporting documents must be submitted to the ISRU and will be maintained in the trial master file.

Participating sites should report events to:

Investigator-Sponsored Research Unit
Office of Clinical Research
Fox Chase Cancer Center
SAE.FCCC@fccc.edu

6.2.3 Sponsor Reporting Responsibilities:

1. Adverse events which meet all of the following criteria must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event.
   i. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
   ii. Possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
   iii. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.
2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the OCR for each site's IRB of record along with the report of the adverse event.

3. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study at OCR.
4. SAEs that are related, unexpected, fatal, or life threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions are as directed by FDA guidelines (http://www.fda.gov/medwatch/index.html). Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

Food and Drug Administration:
Telephone 1-800-332-1088
Fax 1-800-332-0178
http://www.fda.gov/medwatch/report.htm

6.3 Pregnancy

All WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

In the event of a confirmed pregnancy in a patient participating in the study, the Investigator must immediately notify the Study Monitor at 215-214-3704 who will notify Crystal Denlinger, MD.

7.0 CONCOMITANT MEDICATIONS, CAPECITABINE AND QUINACRINE

The concomitant use of quinacrine and primaquine is contraindicated. Since quinacrine concentrates in the liver, it should be used with caution in conjunction with hepatotoxic drugs and in patients with hepatic disease or alcohol dependence. Alcohol or alcohol-containing medications is not recommended during quinacrine treatment (quinacrine has been reported to produce a mild Disulfram-like reaction when ingested with alcohol, which may be due to quinacrine's inhibition of the intermediary metabolism of alcohol; ingestion of alcohol or alcohol containing medications is not recommended during quinacrine treatment). Patients receiving any medications or substances that are substrates of CYP2C9 enzyme are ineligible because Capecitabine inhibits CYP2C9.

7.1 Investigational Agents

Delivery of medications: Study drug – Quinacrine will be ordered and delivered to the investigational drug pharmacy, and then given to the patient on day 1 of each cycle. Capecitabine is available commercially.

	Quinacrine (NSC#: 14229)	Capecitabine (NSC#:71280)
Available dosage forms	100 mg capsules	150 mg and 500 mg tablets

Route of administration	Oral administration with a glass of water within 30 minutes after a meal	Oral administration within 30 minutes after a meal
Supplier	Philadelphia Professional Compounding Pharmacy	Commercial Supply

7.2 Capecitabine

NSC#: 712807

Supplier: Commercially Available

Hematologic
Hematologic side effects including lymphopenia (58% to 94%), anemia (44% to 74%), neutropenia (16% to 22%), and thrombocytopenia (15% to 21%) have been reported. Cases of severe hypertriglyceridemia induced by capecitabine have also been reported.

Gastrointestinal
Gastrointestinal side effects including diarrhea (50%), nausea (44%), vomiting (26%), stomatitis (23%), abdominal pain (17%), constipation (9%), and dyspepsia (6%) have been reported. A case of capecitabine-induced pancreatitis has also been reported.

Dermatologic
Dermatologic side effects including hand and foot syndrome (35% to 45%), dermatitis (23% to 31%), nail disorder (4%), alopecia, erythematous rash, pruritus, eruptive multiple lentigo maligna-like lesions, radiation recall dermatitis, pyogenic granulomas, photoeruption, hyperpigmentation, inflammatory responses in actinic keratosis have been reported. A case of leopard-like vitiligo and a case of subacute cutaneous lupus erythematosus have also been reported.

Hepatic
Hepatic side effects including hyperbilirubinemia (34%) and hepatic failure have been reported.

General
General side effects including fatigue (34%), pyrexia (10%), and limb pain (4%) have been reported.

Metabolic
Metabolic side effects including anorexia (20%) and dehydration (5%) have been reported.

Nervous system
Nervous system side effects including paresthesia (12%), headache (7%), dizziness (5%), and insomnia (3%) have been reported. A case of cerebellar ataxia has also been reported.

Ocular
Ocular side effects including eye irritation (10%) and lacrimal duct stenosis have been reported.

Cardiovascular
Cardiovascular side effects including edema (6%) have been reported. Three cases of severe

angina-like chest pain possibly or probably related to Capecitabine treatment have been reported. One case of Capecitabine-induced coronary vasospasm has also been reported.

Musculoskeletal
Musculoskeletal side effects including myalgia (4%) have been reported.

Other
Other side effects including a case of tumor lysis syndrome have been reported.

Over dosage of capecitabine would be expected to cause nausea, vomiting, diarrhea, GI irritation and bleeding, and bone marrow depression. Patients receiving capecitabine 1657 mg/sq m daily (as 2 divided doses) in a continuous daily regimen for at least 6 weeks experienced severe palmar-plantar erythrodysesthesia, mucositis, and diarrhea [25].

Patients experiencing any of the following adverse effects of capecitabine at the severity described should immediately discontinue capecitabine therapy and promptly notify their clinician nocturnal stools or an increase of 4-6 stools daily or greater, nausea with a substantial decrease in food intake, frequent vomiting (i.e., 2-5 or more episodes of vomiting in a 24-hour period), painful erythema and swelling of the hands and/or feet that results in discomfort, affecting activities of daily living, painful erythema, edema, or ulcers of the mouth or tongue. Patients who develop a fever of 100.5 degree Fahrenheit or greater or other evidence of potential infection should promptly notify their clinician [25]. Capecitabine should be avoided in elderly patients with renal dysfunction or overlapping toxicities.

7.3 Quinacrine

NSC #: 14229

Supplier: Philadelphia Professional Compounding Pharmacy
23 South York Road

Adverse reactions were shown to be mostly minor. Many of quinacrine's side effects develop gradually and have been found to be completely and easily reversible, if quinacrine use is discontinued at this early stage [28, 65-68].

Hematologic
Aplastic anemia - increased reports from 0.66/100,000 to 2.84 with using of quinacrine during World War II – in this report, 48 out of 57 patients were receiving quinacrine.

Gastrointestinal
Gastrointestinal side effects including abdominal cramping, diarrhea, reversible hepatitis reported in high dose (300mg daily), one case report of peritonitis on high dose. Mild anorexia, nausea has also been reported.

Dermatologic
Dermatologic side effects including rashes (1.67%) – eczematous rash is most common, rest can be lichenoid, and exfoliative. Lichen planus reported in 1/2000 soldiers who received 100mg daily, and 1/500 in ones received 200mg daily.

Quinacrine temporarily imparts a yellow color to the urine and skin; however the drug does not cause jaundice [55]. Patients should be counseled regarding urine discoloration and/or skin discoloration. Since quinacrine may precipitate severe attacks of psoriasis and may exacerbate porphyria, the drug should not be used in patients with these conditions unless the potential benefits justify the possible risks [55].

Hepatic
Long-term high dose use was occasionally associated with reversible hepatitis.

General
General side effects include mild transient headache, dizziness (33%).

Metabolic
Metabolic side effects including anorexia (20%) and dehydration (5%) have been reported.

Nervous system
Nervous system side effects including fast frequencies in EEG have been reported. High dose usage can rarely be associated with restlessness, vertigo, insomnia, nightmares, hyperirritability, psychosis and convulsion (0.1-0.4% reported).

Ocular
Only one retinotoxicity case has been reported. High dose use can be associated with hypersensitivity reaction as corneal edema, reported in 600mg daily dose.

Cardiovascular
No known cardiovascular side effects.
Musculoskeletal
No known musculoskeletal side effects.

Other
Quinacrine is an oxidizing agent and can cause methemoglobinemia or hemolytic anemia especially in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency [69]. It should be administered with caution to patients with glucose-6-phosphate dehydrogenase deficiency [55].

Toxicity: Although some adults have survived single quinacrine hydrochloride doses up to 25 g, a dose of 6.8 g has been fatal when given intraduodenally. Symptoms of acute quinacrine toxicity include CNS excitation with restlessness, insomnia, psychic stimulation, and seizures; GI disorders; vascular collapse with hypotension, shock, cardiac arrhythmias or arrest; and yellow skin pigmentation [55].

8. CORRELATIVE/SPECIAL STUDIES

8.1 Laboratory Correlative Studies

8.1.1 Pharmacokinetic (PK) study

Pharmacokinetic information including Cmax, T1/2(half-life) and AUC of investigational drugs capecitabine and quinacrine will be collected as exploratory objectives of phase I and II parts of the study. Given linear kinetics of quinacrine, and a short half-life (4hrs) from previous human study data, blood sample for pharmacokinetic (PK) analysis in this study will be collected on 5 different time points on day 1 of cycle 1, and on day 1 of cycle 2 and cycle 3. For pharmacokinetic analysis, 4.5mL of blood will be drawn into a lithium heparin tube at each time point. .

8.1.2 Sera / Plasma for Banking

Sera, plasma and red blood cells will be acquired and banked for possible exploratory studies for future testing to investigate toxicities or treatment outcomes.

8.1.3 Archival Tissue

If tissue is available and the patient has signed consent, samples will be submitted to Caris Life Sciences® for mutational studies and additional biomarker analysis.

T 25-30 (4-5μm) unstained sections placed on positively charged slides will be prepared and sent to Caris Life Sciences® under the patient's name for testing. Results will be returned for placement into the patient's medical records, clinical trial record and to the attending physician. Caris will provide data under the patient study number for informatics manipulation to be performed at Fox Chase Cancer Center.

If a biopsy is performed on the patient as standard of care, and the patient has signed consent, and if sufficient tumor tissue is collected, the tumor biopsy, in addition or in place of archival tissue, will be submitted to Caris as described above.

Correlative Special Studies.

Sample requirements:

Timepoints	PK (Quinacrine/ Capecitabine)	Sera/Plasma for banking	Tissue ++ (Archival or Biopsy)
Prior to treatment Screening (Day -14 to -1)			25-30 (4-5μm) unstained slides
Cycle 1 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA	1(5mL) red top 1(4mL) EDTA#	
1hr post dose	1(4mL) Li heparin 1(4mL) EDTA		
2hr post dose	1(4mL) Li heparin 1(4mL) EDTA		
4hr post dose	1(4mL) Li heparin 1(4mL) EDTA		
8hr post dose	1(4mL) Li heparin 1(4mL) EDTA		
Cycle 2 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA		
Cycle 3 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA		

 # And at end of treatment or at progression,whichever comes first.
++ If archival tissue is available, or if a standard of care biopsy is being performed

8.2 Collection of Specimens

Blood samples will be drawn at the same time as other blood for routine clinical tests. All samples will be processed, stored, and/or distributed by the Protocol Support Laboratory at Fox Chase Cancer Center. The Protocol Support Laboratory will be responsible for labeling of all specimens with the patient assigned study number only.

No additional tests or samples for the study will be requested following the final cycle of chemotherapy.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to administration of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	PRE-TX	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	C 11	C12-18	End of Treatment	Follow Up[f]
Quinacrine (Days 1-21 of each cycle)		X	X	X	X	X	X	X	X	X	X	X	X		
Capecitabine (Days 1-14 of each cycle)		X	X	X	X	X	X	X	X	X	X	X	X		
Informed consent	X														
Demographics	X														
Medical history	X														
Concurrent meds	X	X----------X													
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital signs[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Height	X														
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECOG P/S	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Serum chemistry[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
EKG (as indicated)	X														
CEA & CA 19-9[k]	X	X	X	X	X	X	X	X	X	X	X	X	X		
AE evaluation		X----------X												X	
Radiologic evaluation	X	Radiologic measurements should be performed after every 3 cycles. Documentation (radiologic) must be provided for patients removed from study for progressive disease.													X [e]
B-HCG	X[b]														
PKs Phase I & II		X	x	x											
Tissue Collection		x[j]													
Sera/Plasma banking		x[i]												x	
Overall Survival															x[d]

a:Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus,potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
b:Serum pregnancy test (women of childbearing potential) to be performed within 72 hours of beginning therapy
c:Vital Signs: Heart Rate, Blood Pressure, Respirations, Temperature.
d:Telephone call is appropriate if patient is unable to be seen in clinic.
e:Every 6 or 9 weeks per protocol until disease progression.
f: Until death, lost to follow up or off study due to 5 years passing from the removal of therapy
I: Sera/Plasma for banking C1D1 pre and end of treatment
J: If archival tissue is available (or if a standard of care biopsy is performed) unstained slides will be prepared for submission to Caris Life Sciences® for evaluation.
K: CEA and CA 19-9 are to be performed on day one of every cycle
C = cycle # and day 1 of that cycle.

10. MEASUREMENT OF ANTITUMOR EFFECT

For the purposes of this study, patients should be re-evaluated for response after every 3 cycles. In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). 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 treatment with quinacrine in combination with capecitabine. During the course of treatment, patients will be evaluated according to the National Cancer Institute CTCAE (current version 4.0).

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

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re- evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Disease Parameters
Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter for non-nodal lesions and short axis for nodal lesions 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 might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

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 ≥ 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.

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

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

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

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 measureable 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.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

The study is designed as a combined Phase I/II interventional non-randomized single-center study of the safety and efficacy of quinacrine in combination with capecitabine in the treatment of patients with advanced stage colorectal adenocarcinoma.

The primary aims in Phase I are to describe toxicities/adverse reactions of quinacrine treatment in combination with capecitabine in patients with advanced CRC. Simon's accelerated titration 4B design is used to determine the dose for Phase II part of the trial. This trial design will be utilized given that quinacrine has been used in the clinic for many decades. We are not anticipating additional toxicity due to the combination with capecitabine. Total Phase I enrollment will be between 3 and 18.

Figure 3 charts the accelerated titration phase I design. It indicates that at each dose level one patient will be initially treated. If that patient's common toxicity criteria score, T, is at most 2 the next patient is dosed at the next higher level. If the patient's score exceeds 2, two more patients are treated at the same dose level. If these two patients' scores are both at most 2, the next patient is treated at the next higher dose level. If both patients have scores higher than 2, the dose is de-escalated one level. If only one of

the two has a score higher than 2, then 3 additional patients are treated at the current dose level. Finally, if at most 2 patients among the 6 at this dose level have scores higher than 2, this dose is declared the MTD. When the dose is de-escalated, the number of patients treated at the lower dose level is enlarged to 6 by adding 5, if escalation from it was made using only one patient, or by adding 3, if 3 patients have been previously treated with the lower dose. The table below lists the chance of dose escalation, pesc, in terms of the underlying chance that this dose will result in a score, T > 2. For example, if the chance that T will exceed 2 is 30% the chance of escalation is 85%. The table also lists the chance of de-escalation, pdesc, in the same way, and the chance that the MTD will be decided, pstay, on first entering a dose level. The chance that a dose level will be declared MTD when being reached by de-escalation, mtddsc, is listed directly below line listing p (T>2).

	Chance of escalation, MTD in place or de-escalation								
pesc:	0.981	0.928	0.847	0.744	0.625	0.496	0.363	0.232	0.109
pstay:	0.013	0.033	0.043	0.041	0.031	0.018	0.008	0.002	0.000
pdesc:	0.006	0.039	0.110	0.215	0.344	0.486	0.629	0.766	0.891

p(T>2):	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800	0.900

mtddesc:	0.990	0.936	0.828	0.676	0.500	0.324	0.172	0.064	0.010

Chance of MTD on de-escalation

The primary efficacy endpoint for Phase II is the rate of response by RECIST (CR, PR, SD, PD). The null hypothesis to be tested is that the RP2D observed in Phase I is not efficacious. We will use the standard Simon's two-stage design with 80% power at alpha level 0.05. The response rate is assumed to be 5% for the standard treatment and 20% for quinacrine in combination with capecitabine. The Phase II portion will first enroll 10 patients. The trial will be stopped if no patient responds to treatment. Otherwise, 19 additional patients will be enrolled for a total of 29 patients. The drug will be declared to have sufficient response rate if 4 or more subjects respond to treatment.

The other primary end point for Phase II is time to tumor progression, which will be analyzed by the Kaplan and Meier method.

Toxicity endpoint: If ever 5 of the initial cohort of 10 patients experience DLTs, or if ever 8 of the 29 total evaluable phase II patients do, the trial will be halted for excess toxicity and either dose adjusted or terminated. If the true chance of DLT is 33.3% the trial will be halted in this way with power 80.5%. If the true toxicity is at most 15%, the chance of termination in error is at most 6.3%.

11.2 Sample Size/Accrual Rate

Sample size for Simon's two-stage Optimum design will be as large as 29 (if the study progresses to stage 2) as described in section 12.1. If the study requires a dose reduction after the first 6 patients have enrolled, the sample size may be as large as 35 (29 at a 100 mg daily dose of quinacrine plus 6 patients who were treated at a 100mg bid dose of quinacrine). The study is expected to accrue over two years.

Patient Replacement: If during the Phase I or phase II portion of the study a patient is lost to follow-up before the patient can be evaluated at least once clinically or radiologically, the patient will not be

evaluable for the primary endpoint and will be replaced. Patients that are taken off-treatment due to severe drug related toxicity will not be replaced. If a patient is lost to follow-up after at least one evaluation is completed the patient will not be replaced and will be considered a failure towards the primary endpoint.

11.3 Stratification factors

There are no stratification factors.

11.4 Analysis of Primary Endpoints

The intent-to-treat population will include all patients who have received at least one dose of both Capecitabine and quinacrine. The response rate and its 95% confidence interval will be estimated using the approach described by Koyama and Chen (71), which accounts for the interim futility analysis.

11.5 Analysis of Secondary Endpoints

- Progression-free survival will be displayed with a Kaplan-Meier curve and the median PFS will be reported with its 95% confidence interval.
- PK parameters (C max, T1/2, AUC of quinacrine) will be described using graphical display and summarized using summary statistics (e.g., mean, median, confidence intervals).
- Adverse events will be tabulated by type and grade.

12.0 DATA, SAFETY MONITORING PLAN

12.1 Monitoring Plan

FCCC ISRU will monitor the medical and study records of each participant accrued throughout the course of the study. In addition, the ISRU will collect and report data to the study Sponsor Investigator who will review these data on a regular basis at a rate dependent on subject accrual. All serious adverse events (SAEs) will be reviewed on a real time basis first by the study site PI and subsequently by the ISRU and Sponsor Investigator as applicable.

12.2 Data Safety Monitoring Board

Interim analysis of toxicity, outcome and ongoing scientific investigations may be performed at least every 3 months by the Fox Chase Cancer Center Data Safety Monitoring Board (FCCCDSMB). In this capacity the FCCCDSMB will serve as an advisory committee to the Sponsor Investigator. The FCCCDSMB will review those aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Plan (DSMP). If the committee decides that changes should be made to this trial, it will make recommendations in writing to the Study Principal Investigator, the Associate Director of Clinical Research, and the Protocol Management Executive Committee, which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Sponsor Investigator before they are implemented. These changes may

include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

13.0 ADMINISTRATIVE

This study will be conducted in accordance will local, state and Federal regulations and according to accepted good clinical practice guidelines.

13.1 Data Reporting

The FCCC Study Monitor will request case report forms to be completed within 2 weeks of the protocol visit. Participating sites are responsible to respond to queries prior to the next scheduled monitoring visit.

The ISRU is responsible for compiling and submitting data to the Sponsor Investigator and statistician on an ongoing basis for monitoring as described in the data safety monitoring plan and reporting to the Data and Safety Monitoring Board.

All patient information will be stored in an EDC system accessible only to the study team members for the purpose of entering, reviewing and analyzing data. Any paper records, such as case report files, produced will be stored in a secure location.

The ISRU is responsible for distributing and tracking review of all IND Action Letters, Safety Reports, study specific Serious Adverse Events.

13.2 Retention of Records

Time points for the retention of records are described in detail in the contract between the grantor and the OCR and passed on to the participating site. Please refer to the study specific terms for specific time points. In all cases the Study Monitor must be notified of any plans to move records to an offsite location prior to doing so.

13.3 Study Agents

Any study agent supplied through the OCR from the manufacturer or a third party distributor may not be used for any purpose outside the scope of this protocol. The agent may not be transferred to any party not participating in the clinical trial.

13.4 Informed Consent

The IRB approved informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative.

Original signed consent forms must be filed in each patient's study file or medical record with a copy in the study file.

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Appendix A: Sample Processing

PK – Quinacrine is "light sensitive" and PK sample tubes will be wrapped in foil before and after sample collection. Samples must be gently mixed by inversion and placed in ice following collection. There is no special handling for the capecitabine samples.

Samples will be transported to the Protocol Support Laboratory(PSL) by the PSL staff, remixed gently, centrifuged at 1500-2000 x g (3000rpm) 10-15min 4ºC, aliquots prepared (quinacrine samples placed in amber tubes, capecitabine in clear tubes) and frozen at -70ºC for batched storage.

PKs will be batched and analyzed at the end of the study.

Sera/Plasma samples for banking – Samples will be gently mixed by inversion at the time of collection and transported to the Protocol Support Laboratory (PSL) by the PSL staff, remixed gently, (red top tube will be allowed to clot – 30-60 min from time of collection), then both tubes centrifuged at 1500-2000 x g (3000rpm) 10-15min 4ºC, aliquots prepared and frozen at -70ºC for batched storage. In addition the red blood cells remaining in the EDTA tube will be divided into two aliquots and frozen at -70ºC for batched storage to be used as a normal patient control, if needed.

Archival tissue – Tumor tissue, if available and if the patient has signed consent, will be located during screening. Once treatment is initiated 25-30 (4-5 □m) unstained sections placed on positively charged slides will be prepared by the PSL. Slides will be sent to Caris Life Sciences® under the patient's name for testing. Results will be returned to the Clinical Pathology department for placement into the patient's medical records, to the attending physician and a copy to the PSL for incorporation into the clinical trial records. Caris will provide data under the patient study number for informatics manipulation to be performed at Fox Chase Cancer Center.

If a biopsy is performed on the patient as standard of care, and the patient has signed consent, and if sufficient tumor tissue is collected, the biopsy tumor, in addition or in place of archival tissue, will be submitted to Caris as described above.

Appendix A: Sample Processing:

Sample Collection Chart

Timepoints	PK (Quinacrine/ Capecitabine)		Sera/Plasma for banking	Tissue ++ (Archival or Biopsy)
Prior to treatment Screening (Day -14 to -1)				25-30 (4-5μm) unstained slides
Cycle 1 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA		1(5mL) red top 1(4mL) EDTA#	
1hr post dose	1(4mL) Li heparin 1(4mL) EDTA			
2hr post dose	1(4mL) Li heparin 1(4mL) EDTA			
4hr post dose	1(4mL) Li heparin 1(4mL) EDTA			
8hr post dose	1(4mL) Li heparin 1(4mL) EDTA			
Cycle 2 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA			
Cycle 3 Day 1 Pre 0hr	1(4mL) Li heparin 1(4mL) EDTA			

 # And at end of treatment or at progression, whichever comes first.
++ If archival tissue is available, or if a standard of care biopsy is being performed

Appendix B: Common CYP2C9 Substrates

CYP2C9 is the primary enzyme responsible for metabolizing NSAIDs, oral antidiabetic agents, and angiotensin II receptor blockers (ARBs).The following is a list of common CYP2CP substrates, inducers, and inhibitors.

<u>This list is not comprehensive</u>
All medications must be individually verified to not be an inducer, inhibitor, or substrate of CYP2CP