A phase I/II pharmacodynamic study of hydroxychloroquine in combination with gemcitabine/abraxane to inhibit autophagy in pancreatic cancer.

Principal Investigator: Peter J. O'Dwyer, MD
16 Penn Tower
3400 Spruce Street
Philadelphia, PA 19104
215-662-7606
peter.odwyer@uphs.upenn.edu
Fax: 215-349 8551

Sub-Investigators: Ravi Amaravadi, MD
Bruce Giantonio, MD
Ursina Teitelbaum, MD
Jeff Drebin, MD
Gregory Beatty, MD
Nuzhat Ahmad, MD
Michael D. Feldman, MD
William Stavropoulos, MD
Arturo Loaiza-Bonilla, MD
Nevena Damjanov, MD
Mark O'Hara, MD
Kim Reiss Binder, MD

All at: The Hospital of the University of Pennsylvania
34th and Spruce Streets
Philadelphia, PA 19104

Erkut Borazanci, MD
Daniel Von Hoff, MD
TGEN, Phoenix, AZ

Ana de Jesus Acosta, MD
Daniel Laheru, MD
Johns Hopkins Medical Institute
Baltimore, MD

Funding Sponsor: Stand Up to Cancer

Statistician: Antje Hoering, PhD

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Study Summary

Title	**A phase I/II pharmacodynamic study of hydroxychloroquine in combination with gemcitabine/abraxane to inhibit autophagy in pancreatic cancer.**
Short Title	Gemcitabine/abraxane/HCQ in pancreatic cancer
Protocol Number	UPCC#19211, IRB#814704
Phase	Phase I/II
Methodology	Randomized Phase II
Study Duration	1 year
Study Center(s)	Three institutions
Objectives	***1.1 Primary Objective – Phase I*** 1. To define the Phase II dose and to describe the dose-limiting toxicity of the combination of hydroxychloroquine (HCQ) with gemcitabine/abraxane in previously untreated patients with advanced pancreatic cancer. ***1.2 Secondary Objective - Phase I*** 1. To describe the toxicity associated with the regimen, and to determine pharmacokinetics of HCQ in selected patients. ***1.3 Primary Objectives – Phase II*** 1. To describe the one year overall survival of the combination of HCQ with gemcitabine/abraxane in previously untreated patients with advanced pancreatic cancer. ***1.4 Secondary Objectives – Phase II*** 1. To describe the toxicity associated with the regimen. 2. To describe the progression-free survival, response rates, median overall survival associated with this treatment. 3. To describe the pharmacokinetics of HCQ when administered in this combination, and relate average and peak blood concentrations to toxicity. 4. To determine the degree of autophagy inhibition in peripheral mononuclear cells as a surrogate tissue, through an analysis of changes in target gene expression, and of the induction of autophagosomes after treatment. 5. To analyze tumor tissue before and after treatment for genomic, metabolic and autophagy markers of treatment effect.
Number of Subjects	Approximately 110 total, 45 patients per arm in the Phase II part

Diagnosis and Main Inclusion/Exclusion Criteria	1. Patients must have measurable disease as defined by the RECIST criteria. 2. Patients must be age 18 years or older and have an ECOG performance status of 0-1. 3. Standard laboratory criteria for hematologic, biochemical, and urinary indices. 4. Patients must be able to understand and to sign a written informed consent document.
Study Product, Dose, Route, Regimen	Hydroxychloroquine
Duration of administration	Until disease progression on treatment.
Statistical Methodology	Randomized Phase I/II with Overall Survival at one year as primary endpoint

2 Objectives

2.1 Primary Objective – Phase I

1. To define the Phase II dose and to describe the dose-limiting toxicity of the combination of HCQ with gemcitabine/abraxane in previously untreated patients with advanced pancreatic cancer.

2.2 Secondary Objective - Phase I

1. To describe the toxicity associated with the regimen, and to determine pharmacokinetics of HCQ in selected patients.

2.3 Primary Objectives – Phase II

1. To describe the one year overall survival of the combination of HCQ with gemcitabine/abraxane in previously untreated patients with advanced pancreatic cancer.

2.4 Secondary Objectives – Phase II

1. To describe the toxicity associated with the regimen.
2. To describe the progression-free survival, response rates, median overall survival associated with this treatment.
3. To describe the pharmacokinetics of HCQ when administered in this combination, and relate average and peak blood concentrations to toxicity.
4. To determine the degree of autophagy inhibition in peripheral mononuclear cells as a surrogate tissue, through an analysis of changes in target gene expression, and of the induction of autophagosomes after treatment.
5. To analyze tumor tissue before and after treatment for genomic, metabolic and autophagy markers of treatment effect.

3 Background

3.1 Metastatic Pancreatic Cancer

Pancreatic cancer is one of the most lethal malignancies of the gastrointestinal tract, with a 5-year survival of less than 5%, and it remains the fourth leading cause of cancer related mortality. In the United States an estimated total of 42,470 pancreatic cancers occurred in 2009, with 35,240 estimated deaths among these patients [1]. The majority of patients with pancreatic adenocarcinoma have evidence of metastatic disease at presentation, and they have an expected median survival of 6 months [2]. Chemotherapy for pancreatic cancer has been largely ineffective, but responses in a proportion of patients have motivated a continuing search for new

therapies. Antimetabolites have shown the most reproducible activity: 5-fluorouracil (5-FU) was shown to have response rates of about 10% in patients with advanced disease, and the subsequent development of gemcitabine showed an improvement over 5-FU in a randomized trial (3). Subsequent trials in the adjuvant setting, in which gemcitabine was superior to 5-FU there too, support a continuing role for gemcitabine (4,5).

3.2 Clinical Experience with Gemcitabine/Abraxane in Pancreatic Cancer

Recent strategies have focused on improving the efficacy of gemcitabine either by improving the method of delivery, or by combining gemcitabine with other non-cross resistant agents. A sequence of Phase III combination studies of gemcitabine in combination (with oxaliplatin, and with the targeted therapies bevacizumab and cetuximab) have been negative, though based on strikingly positive Phase II data generated in cancer centers. Several studies suggest that taxanes are active in pancreatic cancer, but a randomized trial of gemcitabine with taxanes has not been preformed, probably on the basis that the differences in Phase II were insufficiently persuasive. The development of a novel taxane conjugate with albumin, abraxane, with established activity in breast cancer, prompted a Phase II trial of gemcitabine/abraxane by Von Hoff (6). Phase I/II data were highly promising, with response rates of the order of 40%, with tolerable toxicity, and a one-year survival of about 48%. A phase III trial of gemcitabine versus gemcitabine/abraxane is in progress, and based on these promising data has served as the control chemotherapy for previous SU2C trials. The development of a more intensive, but toxic regimen (FOLFIRINOX) in no way diminishes the enthusiasm for this chemotherapy backbone, given the activity in Phase II trials that appears comparable (7). Given the promise of this regimen, and the possibility of making a substantial improvement in outcome with additional targeted interventions, we propose to continue to use this regimen in the current study.

Abraxane (nab-paclitaxel) is a cremophor-free formulation of nanoparticle paclitaxel stabilized with human serum albumin (130 nm particles). The drug achieves enhanced tumor penetration through gp60 albumin receptor-mediated endothelial transcytosis, which enables transit across the vessel endothelium, and makes the active paclitaxel available to the tumor. An additional pharmacodynamic consideration is that the albumin scaffold also binds a tumor-related protein SPARC, which may further enhance localization of this molecule in the tumor tissue. Preliminary data suggest that SPARC-expressing tumors may have better responses to abraxane. In the phase II trial of gemcitabine/abraxane, among 58 evaluable patients there were 23 partial responses (40%) and equivalent number of patients with stable disease (SD) of 4 months or longer, and a median survival of 10.3 months.

In an on-going study of nab- paclitaxel in combination with gemcitabine administered weekly (Abraxis Protocol CA040), a total of 41 patients with advanced metastatic pancreatic cancer were treated with doses ranging from 100 to 150 mg/m2. Though results are preliminary and follow-up is on-going, 27 patients (66%) experienced at least 1 treatment-related adverse event (AE) and 11 (27%) patients experienced at least one treatment-emergent serious adverse event (SAE). With the exception of the “Gastrointestinal: Dehydration”, “not classified” and “pain:

other" categories (2 (5%) events each), no treatment-emergent SAE categories had more than a single event.

Dose delays were mainly due to blood/bone marrow treatment-related AEs (mostly neutrophils). There were no nab- paclitaxel dose interruptions due to treatment-related AEs, and 2 instances of dose interruptions for gemcitabine (dermatology/skin: injection site reaction). Also, 2 patients had a total of 3 treatment-related AEs involving blood/bone marrow (neutrophils and platelets) resulting in dose reductions and 2 treatment-related AE resulted in dose discontinuation (gastrointestinal: diarrhea and infection: systemic). There were 3 treatment-emergent AEs resulting in death (lung infection, systemic infection, and gastrointestinal obstruction), but only systemic infection was considered treatment-related. Patients treated with nab- paclitaxel in combination with gemcitabine had levels of myelosuppression consistent with those expected following treatment with taxanes.

3.3 Autophagy as a Factor Protecting Tumor cells from Treatment

Autophagy is a multi-step process that involves the vesicular sequestration of cytoplasmic proteins and damaged organelles into a structure called the autophagic vesicle (AV). AVs fuse with lysosomes and AV contents are degraded by acid-dependent enzymes. This process is activated by numerous cell stresses including growth factor or nutrient limitation, hypoxia, chemotherapy, and radiation (reviewed in [8]). Stress-induced autophagy serves at least two pro-survival functions for cancer cells: 1) the disposal of damaged organelles and proteins and 2) catabolism during nutrient stress to generate energy for cell survival. Methodology to detect autophagy includes EM and LC3 (a protein which localizes to AV) immunoblotting. While autophagy is an evolutionarily conserved process that all eukaryotic cells rely upon, it may be suppressed by alterations of genes commonly dysregulated in pancreatic tumors, including tumor suppressor genes (*BECN1*, *TP53*, and *PTEN)*, or oncogenes (including Kras, and *EGFR)*. Preclinical investigations suggest that autophagy is likely activated early in tumor development. In vivo studies using an Akt-driven tumor model have demonstrated that autophagy is preferentially activated in tumor cells early in tumor growth when tumor vasculature is immature and nutrients, growth factors and oxygen are limited [9]. Autophagy activated in response to metabolic stress allows tumor cells to survive harsh conditions, establish tumor angiogenesis, and eventually resume exponential growth by downregulating autophagy. Multiple in vitro and in vivo studies have demonstrated that while persistent autophagy in vitro can lead to cell death, autophagy is a reversible process that allows cancer cells to escape cell death in vivo [9-11]. The anti-malarial drugs (chloroquine) CQ and HCQ induce the death of cells that rely on autophagy for survival by impairing lysosomal function, blocking the last step of autophagy. Amaravadi has reported studies in which p53 activation or alkylating chemotherapy activated apoptosis and autophagy in tumors. Inhibition of therapy-induced autophagy, either genetically or with CQ, enhanced therapy-induced apoptosis and tumor regression [11]. These results suggest that autophagy is a therapeutic target in a wide variety of cancers.

Abundant evidence from several cancer models supports the contention that autophagy is protective from cell death induced as a result of hypoxia and cytotoxic drugs [12]. In colon cancer models, we have found marked changes in the expression of autophagy genes in vivo

between untreated and bevacizumab-treated animals. Induced genes include ATG16, ATG2, ATG7, ATG5 and ATG10, as well as the Bcl-2 interacting gene BNIP3; several autophagy related genes are repressed also, including ATG3, Beclin1, ATG4 and ATG9 (unpublished data). In the HT29 model, we determined the effect of chloroquine on bevacizumab, oxaliplatin, and the combination, in mice bearing tumor xenografts. Treatment groups consisted of control, CQ alone, bevacizumab alone, bevacizumab and CQ combination, oxaliplatin alone, oxaliplatin and CQ combination, bevacizumab and oxaliplatin combinations, and bevacizumab, oxaliplatin and CQ combinations. In all treatments, the strategy of incorporating CQ increased the tumor growth delay significantly, but the most striking results were when chemotherapy, bevacizumab, and CQ were used in combination. These findings are consistent with other observations in pancreatic cancer recently published by Yang and colleagues (13).

Of particular interest in extending these studies to pancreatic cancer is the finding that autophagy inhibition is particularly deleterious to cell lines bearing a mutant Kras protein. Additional studies as part of the SU2C pancreatic cancer project reveal that an autophagy program is activated in the presence of mutant Kras, and thus prompts the testing of this strategy in a setting in which Kras is commonly (about 85%) mutated (SU2C, unpublished data).

3.4 Hydroxychloroquine Clinical Experience

Chloroquine (CQ) is a synthetic 4-aminoquinoline that has been used for 60 years in humans for malaria prophylaxis and treatment [14], rheumatoid arthritis [15], and human immunodeficiency virus (HIV) [16]. It is an inexpensive orally available drug that has CNS penetration. It has a large therapeutic index, and its most predictable cumulative toxicity is retinopathy, which can be prevented by discontinuation of the drug [17]. It is this toxicity and worldwide malarial resistance to CQ that lead to discontinuation of extensive research into CQ's non-malarial applications. Chloroquine derivatives such as HCQ are still used extensively in rheumatoid arthritis and lupus erythematosis and have a larger therapeutic index. The chemical structure of CQ derivatives allows them to serve as a weak base which is trapped in acidic cellular compartments [18]. Thus chloroquine deacidifies lysosomes, inhibiting the last step in autophagy. With this last step blocked, a cell reliant on autophagy will increase the generation of autophagosomes and will eventually undergo either apoptotic or non-apoptotic cell death. Evidence in mouse models and human cancer cell lines suggest CQ may have significant anti-tumor activity by inhibiting autophagy induced by cancer therapy [19].

Adding chloroquine to improve the efficacy of anticancer therapy has already been tested in a randomized clinical trial. A small single-institution placebo-controlled phase III trial testing the addition of CQ at an oral daily dose of 150 mg to RT and carmustine in patients with newly diagnosed glioblastoma multiforme (GBM) yielded surprising results [20]. Median overall survival was significantly longer in the CQ-treated patients (24 months) than in controls (11 months). At the end of the observation period, six patients (40%) treated with CQ were alive at 59, 45, 30, 20 (1 each) and 27 (2 patients) months after surgery. In contrast, patients in the control group survived 32, 25, and 22 months. Although not statistically significantly different, the rate of death over time was almost half as large in the CQ group compared to the placebo group (hazard ratio, 0.52 [95% CI, 0.21 to 1.26]; p= 0.139).

Based on excellent toxicity characteristics with other cytotoxics, there is no reason to think that there will be anything different with gem/abraxane.

Hydroxychloroquine (HCQ) is commonly prescribed for rheumatoid arthritis and lupus at doses of 400 mg po daily. A pharmacokinetic/pharmacodynamic study of escalating doses of HCQ at 400 mg/800 mg/1200 mg po daily in patients with rheumatoid arthritis followed by maintenance doses of 400 mg po daily found that doses of up to 1200 mg po daily were well tolerated [21]. Dose limiting toxicities of nausea, vomiting and abdominal pain were observed at 800 and 1200 mg po daily. This toxicity correlated with blood HCQ levels, but not to blood levels of the other active metabolites, desethylhydroxychloroquine (DHCQ), desethylchloroquine (DCQ), or bisdesethylchloroquine (BDCQ). Improvement of symptoms in rheumatoid arthritis correlated with blood DHCQ levels, suggesting a dose-response relationship. Chloroquine derivatives are metabolized through the p450 enzyme system and CQ may inhibit the metabolism of CYP2D6-metabolized drugs [22,23].

A predictable cumulative toxicity associated with CQ is retinopathy, and this is another reason why dose escalation with CQ would be limited. While a link between HCQ and retinopathy has also been made, it occurs infrequently and only after a prolonged exposure. A study using multifocal electroretinography to detect early pre-clinical retinal changes in long-term HCQ users, found that 10 out of 11 patients that developed early pre-clinical changes had been taking HCQ at doses of 400 mg po daily for greater than 5 years [24]. No overt retinopathy was noted in the 19 patients followed. This suggests that at a cumulative dose of 730 g, the risk of retinal changes increases, but techniques such as multifocal electroretinography can detect early changes and prevent overt visual loss.

We have initiated several trials of HCQ in combination with anticancer agents ranging from HCQ/alkylating therapy for glioblastoma, through HCQ/temsirolimus in Phase I, as well as FOLFOX/bevacizumab/HCQ in colorectal cancer. A CNS consortium trial (led from the University of Pennsylvania) to test the addition of HCQ at escalating doses to RT/temozolomide, found toxicity to be tolerable (25), and a dose of 800mg is recommended for further development. In this combination, there was more myelosuppression with the combination than one would expect from temozolomide alone, and this was dose-limiting. With temsirolimus, tolerable doses of HCQ have been higher, and a dose level of 1200 mg is currently being explored. Dermatologic effects and fatigue associated with mTOR inhibitors do not appear to be worsened by HCQ at doses up to 1000mg daily. In other combinations, dose definition is in progress.

The pharmacokinetics of hydroxychloroquine (HCQ) studied in patients with rheumatoid arthritis, malaria, and healthy volunteers demonstrate marked intra- and interpatient variability, with a two-fold range in total clearance (4-10 L/h) (26-8). The variability in the rate of absorption from oral dosing has been reported to be as high as 87%, contributing to differences in peak blood concentrations (Cmax) and time to peak concentration (Tmax) among patients receiving identical doses (26-8). Due to its long terminal elimination half-life of approximately 40 days, at least 120 days of continuous dosing are required before blood HCQ levels reach 90% of steady-state concentrations. Predicted blood concentrations are 898 ng/mL and 1796 ng/mL for patients receiving 200 mg and 400 mg daily of HCQ sulfate, respectively (28). A one-

compartment population pharmacokinetic model with a lag time was developed to estimate individual HCQ pharmacokinetic parameters in 36 patients who participated in a dose-escalation trial of HCQ in conjunction with radiation therapy and temozolomide for glioblastoma multiforme (25). Population values for apparent volume of distribution (Vd) and total clearance were 604 L and 10.7 L/hr, with CV% of 23% and 5%, respectively. Mean individual estimated HCQ pharmacokinetic values for Vd were 573 L (range, 205-1291 L) and clearance 10.5 L/hr (range, 6.8-13.7 L/hr). Mean estimated Cmax values were linear and proportional to total daily oral HCQ sulfate doses at 200, 400, 600, and 800 mg daily and consistent with predicted and observed blood concentrations from the published literature (26-8). The population pharmacokinetic estimates are comparable to data from a population pharmacokinetic study in patients with rheumatoid arthritis where the Vd was 605 L and total clearance was 9.9 L/h (29). Although pharmacokinetic analyses of HCQ sulfate at doses up to 1200 mg daily combined with other agents in phase I and II trials at the University of Pennsylvania have not been completed, dose-proportional HCQ concentrations and concentration-response relationships for daily doses between 400 and 1200 mg daily have been reported (26).

Pharmacodynamic assessments of HCQ action have been performed in peripheral mononuclear cells and occasional tumor biopsies as available. The marker that has been studied to date has been accumulation of autophagocytic vesicles (AV) as quantitated by electron microscopy. Sporadic patients have shown an effect on this marker, but most patients do not show an increase in the PMN. A patient with melanoma, treated at 1000 mg, had striking increases in AV in serial tumor biopsies. Analyses that are still pending include western blots of PMN extracts for changes in autophagy-associated proteins.

3.5 Imaging Metabolism in Pancreatic Cancer

Pancreatic tumors have been studied for the activity of metabolic pathways. Some 70% utilize glucose, and thus may be imaged by FDG-PET. The remaining 30% also utilize glutamine, which enters directly into the citric acid cycle, for metabolism by oxidative phosphorylation. Hence in a proportion of pancreatic cancers, altered metabolism may provide targets for inhibition of tumor growth, initially by inhibiting oxidative phosphorylation. These tumors may also be more sensitive to autophagy inhibition, and their identification will be important for later analyses. We have developed an imaging probe based on glutamine (30), and this is soon to be evaluated in human subjects. As soon as is feasible, this imaging modality will be incorporated into this study to better understand the context of responses that may be observed.

3.6 Phase I Interim results

The initial Phase I escalation portion of this trial has been completed and has identified a suitable Phase II dose (to be re-evaluated as the study progresses). Accrual of 21 patients occurred between January 2012 and January 2013. Two dose levels of hydroxychloroquine were investigated: 800 and 1200 mg daily in two divided doses. Among the six evaluable (of seven entered) at 800 mg, there were no dose-limiting toxicities. At the 1200 mg level, there was no DLT among 7 evaluable patients (14 entered). The toxicity profile was as expected with gemcitabine/abraxane, and there did not appear to be cumulative toxicity. A conference call of

the principal investigators was held to evaluate these effects, and a re-evaluation of the toxicity profile will be undertaken after the accrual of 20 patients on each arm of the randomized trial.

3.7 Summary

In this Phase I/II clinical trial, we seek to pilot the addition of HCQ to a commonly-used front-line therapy of pancreatic cancer, gemcitabine/nab-paclitaxel. We plan a run-in to define tolerable doses, and will explore doses of 800 and 1200 mg/day in successive cohorts of 6 and up to 10 patients. We will assess toxicity continuously, and determine the dose for the Phase II trial based on standard toxicity criteria.

The correlative endpoints of this trial are directed to the pharmacokinetics of HCQ, and pharmacokinetic model of HCQ based on data from several ongoing trials, and the data from these patients will contribute to refining the model. We will analyze both measured and model-predicted indices for their relationship to autophagy induction. Autophagy will be assessed as the accumulation of autophagocytic vesicles in the PMN's of treated patients, together with the induction of the expression of autophagy-related proteins on western analysis, quantitated by densitometry. We will document the rates of metabolic response as a consequence of treatment, as a therapeutic marker that may be related to the degree of autophagy inhibition. Since we have previously demonstrated a key role of JNK1 in the induction of autophagy by chemotherapy, we will analyze archival tumor materials to determine variability in this marker, as a baseline for potential future trials. Finally, this study will incorporate metabolic profiling by mass spectrometry, which will be related to mutations (including Kras) in pretreatment tumor specimens. Mutational analysis will be accomplished by targeted sequencing or by next-generation sequencing, and the need for fresh tissue for all these endpoints will require selected patients to have a biopsy performed before treatment at at 6-8 weeks after beginning treatment. In our previous study of the Hh inhibitor GDC-0973 with the same chemotherapy, we were able to obtain repeat biopsies successfully on all patients. The importance of these biopsies, to move the science forward in an era in which the tools now exist to provide meaningful correlative science, cannot be overstated.

4 Patient Eligibility

4.1 *Inclusion Criteria*

4.1.1 Patients must have histologically or cytologically documented advanced or metastatic adenocarcinoma of the pancreas.

4.1.2 Patients must have measurable disease as defined by the RECIST criteria as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10mm with conventional techniques on either CT or MRI. Marker (CA19-9 or CEA) elevation alone is insufficient for entry.

4.1.3 Patients may have had prior adjuvant treatment for pancreatic cancer. The last dose of chemotherapy must have been > 4 months prior to study entry. Patients with prior radiotherapy are acceptable. It must be at least 4 months since administration of radiation therapy and all signs of toxicity must have abated.

4.1.4 Patients must be age 18 years or older.

4.1.5 Patients must have an ECOG performance status of 0-1.

4.1.6 The following required Initial Laboratory Values should be obtained within 4 weeks of the start of treatment:

Granulocytes	≥ 1,500/ml
Platelet Count	≥ 100,000/ml
Creatinine	≤ 1.5 x upper limit of normal
Bilirubin	≤ 1.5 x upper limit of normal
AST	≤ 5 x upper limit of normal
Hemoglobin	≥ 9.0g/dL

4.1.7 Patients must not be pregnant or lactating as chemotherapy is thought to present substantial risk to the fetus/infant. Women of childbearing potential and sexually active males must use an effective contraception method during treatment and for three months after completing treatment and negative serum or urine β-hCG pregnancy test at screening for patients of childbearing potential.

4.1.8 Patients with an accessible primary tumor or metastasis, will be asked to have a pre-treatment and post-treatment tumor biopsy (at 6 to 8 weeks after beginning). *Excluded from this requirement are a) patients on anticoagulants which can not be held for a biopsy and/or; b) patients deemed poor candidates for a biopsy as determined by the PI in consultation with the treating physician.*

4.1.9 Patients must have a life expectancy of greater than three months.

4.1.10 Patients must have the ability to understand and the willingness to sign a written informed consent document.

4.1.11 < Grade 2 pre-existing peripheral neuropathy per CTCAE

4.2 Exclusion Criteria

4.2.1 Patients may not be receiving any other investigational agents

4.2.2 Known allergy to HCQ

4.2.3 Patients with previous treatment with abraxane.

4.2.4 Patients on therapeutic doses of Coumadin (> 1 mg daily). The use of therapeutic or prophylactic low molecular weight heparin or fragmin is permitted.

4.2.5 Patients with known G6PD deficiency, severe psoriasis, porphyria, macular degeneration or severe diabetic retinopathy are ineligible because of the potential for greater HCQ toxicity.

5 Treatment Plan

5.1 Study Design

This is a phase I/II, three-institution trial to study the addition of the autophagy inhibitor HCQ to gemcitabine/abraxane in the treatment of advanced pancreatic cancer.

5.2 Randomization

Patients entering the Phase I portion of the trial will not be randomized.

Patients entering the Phase II part of the trial will be randomized to receive HCQ (specified number of tablets from the Phase I) or not. This study will use a dynamic allocation procedure to allocate an equal number of patients to each of the treatment regimens. The procedure will balance the marginal distribution of the stratification factors between these treatment groups. Randomization will begin after the Phase I portion of the trial, and the allocation will be made at registration.

5.3 Treatment Plan

5.3.1 Therapy will be administered as follows. In the Phase II portion, patients will be randomly assigned to receive HCQ or not (no placebo, no blinding).

Agent	Dose	Route	Treatment Administration
Abraxane	125mg/m2	IV infusion over 30 minutes	Day 1, 8, 15
Gemcitabine	1000mg/m2	IV infusion over 30-100 minutes	Day 1, 8, 15
Hydroxychloroquine	1200 mg/day	PO daily (600mg BID)	Daily from d1

NOTE: Abraxane should be given first.
Gemcitabine administration may be either over 30 minutes (as in label) or at a rate of 10 mg/m2/minute as recommended by Plunkett and colleagues (31). Dose modifications may therefore lead to changes in the infusion time if the latter approach is used. Investigators may choose either approach, and this should be documented in the case report form.

This cycle will be repeated every 4 weeks.

Patients will receive an appropriate anti-emetic regimen usually including dexamethasone 10-20mg IV and a 5-HT3 agent of choice, (i.e. ondansetron or granisetron) prior to administration of chemotherapy to decrease the incidence and severity of chemotherapy-associated nausea and vomiting. Drugs such as lorazepam may also be used if clinically indicated.

5.3.2 Hydroxychloroquine Dose

Patients will begin treatment with daily oral HCQ at 1200 mg daily (600 mg bid), based on our previous Phase I/II trials of HCQ with chemotherapy, and the favorable results of this regimen in the dose-escaltion portion of the study. .. Interim analysis to confirm tolerability of this dose will be performed after the accrual of 20 patients in each arm (without interrupting accrual).

Table 1: Hydroxychloroquine Phase I: Dose Escalation Schema

Dose Level	Dose hydroxychloroquine (mg/day)	Number of Patients

-2	400 mg (200 mg bid)	
-1	600 mg (300 mg bid)	
1	800 mg (400 mg bid)	6-12
2	1200 mg(600 mg bid)	6-12

*Based on our current and ongoing Phase I/II trials in patients with other types of cancer, no further dose expansion is planned beyond 1200 mg/day, and this dose will be used for expansion cohort.

5.3.3 DLT Definition and Escalation Decision Process

Dose Limiting Toxicities will be defined by toxicity occurring during the first 4 weeks of this study. A DLT will be considered as any non-hematologic AE of Grade 3 or higher that is judged by the investigators to be probably treatment-related with the exception of nausea and vomiting which have not been treated with optimal anti-emetic therapy.

The following hematologic toxicities will be considered a DLT if any occurs in the first cycle:

1) grade 4 neutropenia lasting more than 7 days,
2) febrile neutropenia,
3) platelet count less than 10,000/mm^3.

Any HCQ-related toxicity that causes a patient to miss > 4 days of HCQ treatment in the first cycle will be counted a DLT. Toxicity-related interruption of HCQ for >28 consecutive days will result in the patient being taken off HCQ treatment.

If a DLT is observed in one or fewer patients in the initial cohort of 6 patients at 800mg/day, the dose will be increased to 1200 mg/day for the next up to 10 patients. If a DLT occurs in 3 patients per cohort, then this dose will be expanded to accrue 12 patients, and if fewer than 6 DLT's, utilized for the Phase II goals. If DLT's occur in more than 6/12 patients per cohort, the next lowest, tolerable dose will be used for Phase II goals, and 12 evaluable patients will be treated at this dose (or another dose, to be defined by amendment if needed) to ensure suitability for Phase II. The rules for dose escalation and cohort size are outlined in Table 2. No intra-patient dose escalation is planned. Patients will be evaluable for toxicity if they have taken one dose of HCQ and will be evaluable for response if they have completed 24/28 of their expected doses of HCQ in the initial 4 weeks. Should it become apparent that with expanded accrual the selected dose of HCQ is too high, an intermediate downward dose adjustment may be made in the interest of safety.

Table 2: Hydroxychloroquine Phase I: Criteria for Dose Escalation and Cohort Size

Number of patients with DLT	Rule
<3/6 DLT	Increase dose for next 6-10 patients
3/6 DLT	Expand to 12 patients total
>3/6 DLT	Phase II dose is next lower dose

The dose of HCQ will remain the same for each patient throughout treatment unless there is a need for a dose decrease for toxicity and potential subsequent escalation upon event resolution.

Dose escalation decisions for the phase I portion of the study will be made by teleconference on an ad hoc schedule, at which each of the three participating institutions will be represented.

5.3.4 Duration of Treatment

Patients will continue gemcitabine/abraxane with or without HCQ until disease progression. Significant toxicity related to treatment will be reason for chemotherapy modification, interruption or discontinuation – gemcitabine and abraxane dosing will be handled in a standard clinical fashion (see below). Most patients require dose decreases (by successive 20-25% increments we suggest) after 6-8 cycles of therapy, and patients in whom the abraxane must be discontinued may continue to receive gemcitabine/HCQ. All patients in whom protocol therapy has been discontinued will be followed for progression of disease and survival.

5.4 Tumor Analysis

Patients treated on this study will have tumor sampling before and after initiation of therapy. Core samples will be obtained from accessible metastatic sites (eg, liver, peritoneum, by US or CT-directed biopsy), or by laparoscopic sampling only if the patient is scheduled to undergo a non-research laparscopy for some reason and material can be obtained, or if needed, by EUS-guided biopsy. Three cores will be obtained at each sampling. Initial sample processing will be directed to flash-freezing of the specimens (2 cores) and preparation of the third for EM and IHC (autophagy markers).

i. Laboratory Assessment

In addition to standard laboratory assessments prior to administration of chemotherapy, patients will have blood drawn for pharmacokinetic and pharmacodynamic endpoints All laboratory measures for routine testing obtained within 2 weeks of the start of each cycle of chemotherapy will be acceptable.

5.5 Assessment of Disease Progression

Patients will be assessed on regularly prescribed intervals in order to determine if there is evidence of disease progression. A CT or MRI of the abdomen/pelvis and chest (if thoracic

disease is present) will be obtained at 2 month intervals after initiating chemotherapy treatment for the first year, and at 3 month intervals thereafter.

5.6 Pharmacokinetics

A population pharmacokinetic (PK) model of HCQ will be developed based on limited sampling protocol. Whole blood will be collected in one green top tube which is drawn at four clinic visits spanning the first 12 weeks of treatment. The sampling strategy will not be fixed, but will be devised to ensure that a total of up to 5 samples are collected randomly within a 6 hour block of time prior and subsequent to HCQ dosing from each patient in order to characterize minimum and maximum HCQ blood concentrations among the patient population. A randomization scheme for blood sampling will be developed for each treatment center. The heparinized blood will be aliquotted into 2 cryovials and immediately frozen. The date and time of last HCQ ingestion will be noted in PK logs.

Blood samples will be drawn for pharmacokinetic analysis at each of the time-points listed in the table below.

PK Sample number	Treatment Period
1	Within -14 days of starting HCQ (baseline)
2	Cycle 1, week 3, post-dose day 1 ± 7 days
3	Cycle 2, Day 1, pre-dose, ± 7 days
4	Cycle 3, Day 1, pre-dose, ± 7 days
5	

A block randomization scheme for blood sampling times will be developed for each treatment center using SAS® statistical software. Patients will be asked to take their last dose of HCQ within 12 hours of their scheduled clinic visit. A blood sample will be obtained either prior to their next HCQ dose taken during the clinic visit or following the dose, based on the randomized sampling schedule and as determined by physician and patient convenience. Blood HCQ concentrations will be analyzed at the University of Sciences.

Ship samples on **DRY ICE in batches** to:

Dr. Lisa Davis
University of the Sciences
Griffith Hall Room 365
600 South 43rd Street
Philadelphia, PA 19104

Investigators should call 215-596-8831 or make arrangements via email (l.davis@usciences.edu) before shipping samples.

Please do not ship samples on Fridays or the day before a national or federal holiday.

5.6.1 PMN Isolation

PBMCs will be separated from whole blood using the Ficoll Hypaque density gradient centrifugation method (or LeukoPrep tubes). The residual serum will be stored at -80◦C, and used for measurement of circulating markers. Three tubes should be drawn during PBMC collection. Samples will be shipped to in batches to:

Ravi Amaravadi, MD
Clinical Research Building 138
415 Curie Blvd
University of Pennsylvania
Philadelphia, PA 19104

Please do not ship samples on Fridays or the day before a national or federal holiday.

*

5.6.2 Plasma Sampling for PD Markers

Blood samples (8 ml) will be drawn into green-top tubes, centrifuged for 20 minutes at room temperature, and the plasma layer aspirated and transferred to a 5ml polypropylene tube, labeled and stored at -80°C.

5.6.3 Biopsy Specimen collection techniques

Core biopsy of tumor tissue – guidelines for study team, can be modified according to institutional standards.

5.6.3.1 Patient preparation instructions for core biopsy procedure
Detailed instructions may be provided to patients who are planning to have a tumor biopsy and their health care providers.

Medication and Supplement Restrictions:
• Must be off Plavix, Coumadin, aspirin and aspirin-containing medications, herbal, fish oil based (omega 3), and vitamin E supplements for 7 days prior to procedure
• Must be off all non-steroidal anti-inflammatory drugs (NSAIDs) for 4 days prior to procedure (this includes but is not limited to ibuprofen, naproxen, celecoxib, indomethacin, etodolac)
• Must be off Heparin for 6 hours prior to procedure.

Labwork:
• PT/PTT, and the platelet level must be within normal ranges within 7 days of the procedure.

Day of Procedure Restrictions:
• If procedure is in the morning, NPO after 12 midnight,
• If procedure is in the afternoon, may have a liquid breakfast only

5.6.3.2. Biopsy Procedure

Biopsies will be done by routine U/S or CT-guided procedures depending on the imaging characteristics of the area to be biopsied. 16-18 G core biopsy needles will be used, and 2-4 samples will be obtained from the lesion. Prior to biopsy, potential biopsy "targets" will be evaluated by a radiologist, to assess whether the lesion can be safely biopsied.

Analgesia and Anesthesia;

Local anesthesia will be achieved with lidocaine 2% injection. Conscious sedation may be administered per institutional standard practice for biopsies. Post-procedure analgesia, which is rarely needed, will consist of acetaminophen (<3 gm total in any 24-hour period), NSAID's (for patients with platelet count > 50,000 mm3 without overt bleeding), or oxycodone as needed. Unexpectedly severe post-procedure pain will be immediately evaluated by the study subject's primary oncologist or research personnel.

Anatomical sites to be biopsied:

Biopsies will be obtained only from sites that constitute minimal risk to the subject. It is not possible to designate every conceivable site, but acceptable areas include:

• liver metastasis (not immediately adjacent to major vessels)
• peripheral accessible lymph nodes (cervical, axillary, inguinal, extremities)
• Primary pancreatic mass by Endoscopic ultrasound and core biopsy
- Intraperitoneal or abdominal wall lesions

Of these, the liver is the most commonly biopsied site in patients with advanced pancreatic cancer, in our experience. Sites that will not be biopsied for research purposes only include:

- lung
- brain
- mediastinum
- intra-pelvic viscera
- any site deemed by the patient's primary oncologist, study investigator, or radiologist to represent greater than minimal risk to the subject.

Published literature on the complications of tumor biopsies is typically organ-specific. For study subjects with colorectal cancer, it is expected that the liver will be the most common site of tumor biopsies. For liver biopsies, published reviews quote a complication rate of 0.06 – 0.32%.The complication rate does not appear to be affected by peri-hepatic ascites. Extrapolation to an oncology study population is somewhat problematic given that the indication for such biopsies in the general population is often severe liver disease or dysfunction; such patients would not be candidates for oncology clinical trials.

If a core biopsy is not feasible, a needle biopsy may be performed by endoscopic ultrasound (EUS). The reported yield of EUS-FNA is about 90-95%, with an overall sensitivity and specificity of 90% and 100%, respectively. The technique is highly sensitive for detection of Pancreatic Cancer (90-95% sensitivity) particularly in patients who are suspected to have a mass or present with jaundice and is appropriate for biopsing metastatic disease. EUS will be performed per institutional guidelines and under sedation.

Pharmacodynamics

Variation in toxic and therapeutic responses to therapy is universal, understanding of which can refine therapy. The basis of the anti-autophagy action of HCQ will depend on specific cellular

changes, some understood, some in development. In this trial it will be important to understand the variability in these markers after therapy, and to collect samples that can be tested using newer markers, the better to design and target these interventions to appropriate populations. Three sources of tissue will be used for pharmacodynamic markers: peripheral mononuclear cells (PMN's) obtained before and after initiation of therapy; plasma samples for the same purpose; and tumor samples to be obtained pre-treatment for the determination of genomic markers (DNA, RNA, protein, metabolomics) to relate to outcome. Some of the methodologies to be applied to these specimens will be applied after securing funding: however, it would be a very serious loss to fail to collect such tissues in a study that tests an important hypothesis in cancer treatment.

6 Dosing Delays/Dose Modifications

Toxicity will be graded using the NCI Common Toxicity Criteria which is available on the NCI website www.ctep.cancer.gov. The main toxicities reported with the combination gemcitabine/abraxane are neutropenia, nausea/vomiting, reversible elevations of liver enzymes, and neuropathy. The main toxicities reported with hydroxychloroquine include nausea, fatigue, visual changes, and possible potentiation of chemotherapy-induced myelosuppression (from our previous combination trial).

Laboratory abnormalities on the day of treatment for second and subsequent courses will be considered in treatment decisions. Values that deviate from eligibility criteria for the study may cause a delay of up to three weeks for recovery to occur. If the abnormalities have not resolved by then, the patient should ordinarily be taken off study. Exceptions may be made after discussion with the Principal Investigator. Regular meetings of the investigators will take place by phone to ensure that tolerability at later time points is maintained, and changes in dosing strategies that may be needed as a consequence of any possible cumulative toxicity (not currently known to occur) will be accomplished by amendment as needed.

6.1 Dose Modifications

As specified in the following tables, dose modification should be based upon the worst grade of toxicity experienced. Dose reductions should be continued for subsequent cycles, unless dose re-escalation may be performed at the time the event resolves to grade 1.

6.1.1 Dose Modifications for Gemcitabine and Abraxane Toxicity

NOTE: Patients who require discontinuation of both gemcitabine and abraxane for toxicity will discontinue protocol therapy but will continue to be followed for disease progression and survival. It is not planned to maintain patients on HCQ alone.

NOTE: Patients who require discontinuation of one may continue to receive the second chemotherapy agent along with HCQ. This is expected to be common since the cumulative neurotoxicity of Abraxane often requires its cessation after 4-6 months.

6.2 Gemcitabine and nab-Paclitaxel

6.2.1 Rules for Dose Omissions and Modified Schedules

Day 1 dose missed:
If the dose held or missed was to be given on Day 1 of the next cycle, that next cycle will not be considered to start until the day the first dose is actually administered to the patient (i.e., 1-2-3-Rest, X-1-2-3-Rest, etc.)

Day 8 dose is missed:
Cycle continues per protocol, with one dose not given (i.e., 1-2-3-Rest, 1-X-3-Rest, 1-2-3-Rest, etc.). Day 15 is administered as per cycle calendar if counts and chemistries permit.

Day 15 dose missed:
That week becomes the week of rest. Next dose (if counts and chemistries permit) becomes Day 1 of a new cycle, and the patient is considered to have had a x2q3 (21-day) cycle (i.e., 1-2-3-Rest, 1-2-X, 1-2-3-Rest, etc). More than 1 week of rest is permissible at the discretion of the investigator.

Doses will be reduced for hematologic and other non-hematologic toxicities. Dose adjustments are to be made according to the system showing the greatest degree of toxicity. Toxicities will be graded using the NCI CTCAE Version 4.0.

Table 3. Dose Levels of Gemcitabine and Abraxane

Dose Level	nab- Paclitaxel (mg/m^2)		Gemcitabine (mg/m^2)[a]
Study Dose	125		1000
-1	100		800
- 2 [b]	75		600

[a]Dose reductions may or may not be concomitant. Please refer to Tables 4-6 for specific recommendations regarding dose reductions
[b]Additional 25% dose modifications are permissible to establish the tolerable dose for an individual patient

Patients who experience study drug-related toxicities that require a delay in scheduled nab-paclitaxel and gemcitabine dosing for ≥ 28 days will be discontinued from further

participation in this study. When a dose reduction is required for Day 1 of any cycle, no dose re-escalation will be permitted for the duration of study treatment.

DOSE MODIFICATIONS AT DAY 1

In the event dose modifications are required at the beginning of a cycle due to AEs or hematologic toxicities, the doses and schedule of nab-paclitaxel and/or gemcitabine may be adjusted as detailed in Tables 4 and 5 below:

Table 4. Dose Modifications for Hematologic Toxicities (Day 1 of Each Cycle)

For counts on Day 1:

Absolute Granulocytes		Platelets	Timing
$\geq 1.5 \times 10^9$/L	AND	$\geq 100 \times 10^9$/L	Treat on time
$< 1.5 \times 10^9$/L	OR	$<100 \times 10^9$/L	Delay by one week intervals until recovery

In the case of dose delays to allow chemotherapy-related toxicity to resolve, HCQ dosing should continue as scheduled.

Table 5. Dose Modifications for Non-Hematologic Toxicity (Day 1 of Each Cycle)

Non-hematologic toxicity (except neuropathy, alopecia) ordinarily should have resolved to Grade 0 or 1 before initiating the next cycle. If such toxicity resulted in a dose hold in the previous cycle, the following will determine dosing for the current cycle:

Non Hematologic Toxicity (except neuropathy) and/ or Dose Hold with Previous Cycle	
Toxicity in previous cycle causing dose to be held	Gemcitabine + nab-paclitaxel dose this cycle [a]
Grade 0, 1 or 2	Same as day 1 previous cycle
Grade 3 toxicity	Decrease gemcitabine by one level
Grade 4 toxicity	Decrease gemcitabine two levels[b]
Dose held in 2 previous consecutive cycles	Decrease gemcitabine by one level

[a]If the toxicity only affects neuropathy, then only nab-paclitaxel should be reduced
[b]Pulmonary embolism (a Grade 4 toxicity in CTCAE tables) if mild or asymptomatic, will be exempt from this requirement.

DOSE ADJUSTMENTS WITHIN A TREATMENT CYCLE

In the event that patients must have treatment delayed within a treatment cycle due to toxicities, those doses held during a cycle will not be made up. Dose modifications due to hematologic toxicity (as represented by the blood counts and toxicities, below) within a treatment cycle should be adjusted as outlined in Table 6.

Table 6. Dose Modifications for Hematologic Toxicity within a Cycle (days 8, 15)

ANC		Platelets	Nab- paclitaxel	Gemcitabine
≥1500	AND	≥100,000	100%	100%
1000-1499	OR	75,000-99,000	100%	100%
500-999	OR	50,000-74,000	Decrease one dose level [a]	Decrease one dose level
<500	OR	<50,000	hold	hold
Febrile Neutropenia (Grade 3 or 4)			Hold. Upon resuming dosing, decrease to next lower level and do not re-escalate throughout the rest of treatment	Hold. Upon resuming dosing, decrease to next lower level and do not re-escalate throughout the rest of treatment
Recurrent Febrile neutropenia (Grade 3 or 4)			Decrease to next lower dose level and do not re- escalate throughout the rest of treatment	Decrease 2 dose levels and continue throughout the rest of treatment

[a]GCSF may be used (at dose and schedule per institutional guidelines) at the discretion of the investigator to maintain 'nab-paclitaxel dose intensity

Note: the combination of gemcitabine and nab'-paclitaxel is a standard treatment for this disease, but clinical circumstances vary considerably, and can not always be predicted. So as to serve the patients' needs best (balancing toxicity and benefit) there may be occasions in which the treating physician takes an approach different to that outlined, as is common in clinical practice.

6.2.2 Provisions for Fever/Infection

Because of significant risk of non-neutropenic sepsis, at the first occurrence of fever > 38.5 degrees Celsius, regardless of the neutrophil count, either ciprofloxacin (500mg orally bid) or amoxicillin/clavulanate (Augmentin, 500mg orally bid or tid) should be instituted. At initiation of the study treatment, patients should be provided prescriptions for one or other antibiotic, and instructed to begin treatment at the first observation of a fever or 38.5C or more, or if they feel they are developing a fever and a thermometer is not available. They should follow a clear plan for blood count evaluation, and clinical assessment for infection, and/or the need for hospitalization.

Febrile patients will have their treatment interrupted until recovery (temperature below 100F for >3 days), and will be managed according to standard practice for this disorder. The HCQ dosing may continued at the discretion of the physician. Upon resolution of this condition, abraxane and gemcitabine therapy can resume at the next lowest dose. Dose modifications may also be made for non-hematological toxicity within a cycle as specified in Table 7.

Table 7. Dose Modifications for Non-Hematological Toxicity within a Cycle CTC Grade

CTC Grade	Percent of Day 1
0-2 (and Grade 3 nausea/ vomiting and alopecia)	100%
3 (except nausea/vomiting and alopecia)	50% or Hold[a]
4	Hold[a]
a This decision as to which drug should be modified will depend upon the type of non-hematologic toxicity seen and which course is medically most sound in the judgment of the physician/investigator. Treatment may be reinstated on Day 1 of the next cycle.	

6.2.3 Peripheral Neuropathy

Nab-Paclitaxel treatment should be withheld in patients who experience ≥ Grade 3 peripheral neuropathy. Gemcitabine administration can continue during this period at the discretion of the investigator. Nab-Paclitaxel treatment may be resumed at a lower dose level in subsequent cycles after the peripheral neuropathy improves to ≤ Grade 1 at the discretion of the investigator in alignment with standard of care. Patients experiencing peripheral neuropathy may require an extended delay in scheduled nab-Paclitaxel dosing, but can remain on gemcitabine/HCQ, and have 'nab-paclitaxel reintroduced at a subsequent cycle should the neuropathy improve as above. Patients receiving a reduced dose of nab-Paclitaxel who experience ≥ Grade 3 peripheral neuropathy at that dose level requiring a dose delay ≥ 21 days without resolving to ≤ Grade 1 should have 'nab-paclitaxel discontinued.

As observed in other clinical trials, ≥ Grade 3 neuropathy related to nab-Paclitaxel is usually seen in later phases of the treatment (cycle 6 and beyond). If ≥ Grade 3 neuropathy occurs in early treatment cycles, other factors predisposing the patient to neuropathy might be present (eg. diabetes, alcohol consumption, concomitant medications). To maintain dose intensity during the first 6 treatment cycles, careful consideration should be exercised when these predisposing factors are present.

6.2.4 Cutaneous Toxicity

Patients who develop Grade 2 or 3 cutaneous toxicity should have their dose reduced to the next lower dose level of both drugs. If the patient continues to experience these reactions,

treatment should be discontinued. Patients who develop Grade 4 cutaneous toxicity should have treatment discontinued.

6.2.5 Gastrointestinal Toxicity

If Grade 3 mucositis or diarrhea occurs, study drug should be withheld until resolution to ≤ Grade 1, then reinstituted at the next lower dose level of both drugs. Patients who develop Grade 4 mucositis or diarrhea should have treatment discontinued.

6.2.6 G-CSF

The use of growth factors to support neutrophil counts is permissible after cycle 1 if neutropenia would otherwise require a dose reduction to less than 100 mg/m2 of abraxane. GCSF may also be considered for therapeutic administration in the event of febrile neutropenia as noted above, according to institutional practice.

6.2.7 Dose Modification for Hydroxychloroquine

Any AE of ≥ Grade 3 and attributed as possibly, probably or definitely related to HCQ will result in the dose being held until the AE has resolved to ≤ grade 1 or baseline. If the AE resolves, reinstitution of treatment can occur at either the previous or reduced dose as described in Table 3 at the discretion of the investigator.

If the AE recurs at the reduced dose, treatment will be held until the AE has resolved to ≤ grade 1 and when resolved treatment can be reinstituted at the next lower dose level.

Table 8: Hydroxychloroquine Dose Reduction Schema

Dose Level	Dose mg/day	Reduce to
2	1200	1000
1	800	600
-1	600	400
-2	400	Discontinue

Toxicities that may be attributable to HCQ include nausea, vomiting, diarrhea, rash, and visual field deficit. If any of these AEs occur at grade ≤ 2, HCQ may be continued and the AE managed with supportive care. For any AE with a grade ≥ 3, the dose of HCQ should be held until the toxicity resolves to grade 1 (or is found to be unrelated to HCQ). Treatment following HCQ-attributable toxicity of this degree should be reinstituted at dose reduction as in Table 8.

With particular regard to visual field deficits patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. **These symptoms of peripheral retinal toxicity should prompt drug discontinuation and ophthalmologic evaluation.**

6.3 Supportive Care

All supportive measures consistent with optimal patient care will be given throughout treatment.

6.4 Duration of Therapy

Patients will receive protocol therapy unless the constraints of this therapy are detrimental to the patient's health. In this event, the protocol should be discontinued. Furthermore, the protocol will be discontinued should the patient withdraw consent.

6.5 Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for recurrence and for survival. All patients must also be followed through completion of all protocol therapy.

7 Pharmacologic Data

7.1 Gemcitabine

7.1.1 Generic Name

2'-Deoxy-2',2'-difluorocytidine monohydrochloride, Gemzar

7.1.2 Classification

Antimetabolite (nucleoside analog)

7.1.3 Mechanism of Action

Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S phase) and also blocking the progression of cells through the G1/S phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated intoDNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain

termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

7.1.4 Storage and Stability

Unreconstituted drug vials are stored at controlled room temperature. Reconstituted solution should be stored at controlled room temperature and used within 24 hours. Solutions of gemcitabine should not be refrigerated; crystallization may occur. The unused portion should be discarded.

7.1.5 Dose Specifics

For pancreatic cancer, a dose of 1000mg/m2 over 30 minutes once weekly for up to 7 weeks followed by a week of rest, then once weekly for three weeks of every four weeks is used. Other dosing schedules currently are being studied.

7.1.6 Preparation

Reconstitute the 200mg vial with 5ml and the 1Gm vial with 25ml preservative free normal saline to make a solution containing 38 mg/ml. Shake to dissolve.

7.1.7 Administration

The drug may be administered as prepared above or further diluted with normal saline to a minimum concentration of 0.1mg/ml. Gemcitabine is commonly diluted in 100 ml or 250ml of saline.

7.1.8 Availability

Gemcitabine is commercially available in 200mg and 1Gm vials.

7.1.9 Side Effects

1. Hematologic: Myelosuppression is usually mild to moderate and is more pronounced for the granulocyte count. Thrombocythemia is also commonly reported.
2. Dermatologic: A rash is seen in about 25% of patients and is associated with pruritus in about 10% of patients. The rash is usually mild, not dose-limiting, and responds to local therapy. Desquamation, vesiculation, and ulceration have been reported rarely. Alopecia is reported in <1% of patients.
3. Gastrointestinal: Nausea and vomiting are reported in about two-thirds of patients and requires therapy in about 20% of patients. It is rarely (<1%) dose-limiting, and is easily manageable with standard antiemetics. Diarrhea is reported in 8% of patients,

constipation in 6%, and oral toxicity in 7%.
4. Hepatic: Abnormalities of hepatic transaminase enzymes occur in two-thirds of patients, but they are usually mild, nonprogressive, and rarely necessitate stopping treatment. However, gemcitabine should be used with caution in patients with impaired hepatic function.
5. Pulmonary: Bronchospasm after injection has been reported in less than 1% of patients and is usually mild and transient, but parenteral therapy may be required. Dyspnea within a few hours of injection is reported in 10% of patients. It is usually mild, short-lived, rarely dose-limiting, and usually abates without any specific therapy. Cough and rhinitis are also commonly reported.
6. Neurologic: Somnolence has been reported in 10% of patients, and insomnia is common.
7. Cardiovascular: A few cases of hypotension were reported. Some cases of myocardial infarction, congestive heart failure, and arrhythmia have been reported, but there is no clear evidence that gemcitabine causes cardiac toxicity. Peripheral edema is reported in about 30% of patients. Some cases of facial edema have also been reported. Edema is usually mild to moderate, rarely dose-limiting, sometimes painful, and reversible after stopping gemcitabine treatment.
8. Other: Flu-like symptoms are reported for about 20% of patients. This includes fever, headache, back pain, chills, myalgia, asthenia, and anorexia. Malaise and sweating are also commonly reported.

7.1.10 Nursing Implications

1. Administer over 30 minutes.
2. If the patient reports burning at the injection site, slow down rate to allow the dose to run in over 1 hour.
3. Rash can be treated with topical therapy or the administration of diphenhydramine and dexamethasone prior to administration.
4. Flu-like symptoms can be treated with acetaminophen.

7.1.11 References

1. Anon. Clinical Brochure: Gemcitabine HCl (LY188011 HCl). Lilly Research Laboratories, Indianapolis, IN.
October 3, 1994.
2. Eli Lilly & Company. Package Insert. August 26, 1998.

7.2 nab-paclitaxel [Abraxane]

7.2.1 Formulation

Nab- paclitaxel is a Cremophor EL-free, albumin-bound paclitaxel particle with a mean size of approximately 130 nm. Each 50 mL vial contains 100 mg of paclitaxel, and approximately 900 mg of human albumin, as a white to yellow sterile lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection. Protocol Amendment 4, date August 16, 2010 21

Nab- paclitaxel is a unique protein formulation of a non-crystalline, amorphous form of paclitaxel in an insoluble particle state. It has been developed to reduce the toxicities associated with Taxol (paclitaxel) Injection (in which paclitaxel - from the native crystalline form - is in solution with Cremophor EL/ethanol as the solvent) while maintaining or improving its chemotherapeutic effect. Nab- paclitaxel has been approved in the US, Canada, India, the EU, Korea and China (and is under review in a number of other countries) for the treatment of women with metastatic breast cancer (MBC). Nab- paclitaxel alone and in combination is being evaluated in a number of cancers including: metastatic melanoma, pancreatic cancer, cervical cancer and other solid tumors. Conditions which are responsive to paclitaxel such as non-hematological solid tumor malignancies are good clinical candidates for treatment with nab-paclitaxel.

7.2.2 Preclinical studies with nab- paclitaxel

A range of preclinical studies in the appropriate species have been completed with paclitaxel including single and repeat-dose toxicity studies, carcinogenicity evaluations, reproductive toxicity assessments, and mutagenicity and toxicity studies. A thorough discussion of these is included in the Investigator's Brochure (IB).
Preclinical studies comparing nab-paclitaxel to Taxol demonstrated lower toxicities, with a MTD approximately 50% higher for nab-paclitaxel compared to Taxol. At equitotoxic doses of paclitaxel, nab-paclitaxel was found to be markedly more efficacious in animal models than Taxol.

7.2.3 Potential Risks for nab- paclitaxel based on previous clinical studies

Nab- paclitaxel is not formulated in Cremophor and thus the risk of hypersensitivity reactions is much less than that of Taxol. The major risks of nab- paclitaxel have been assessed in clinical trials in patients with a variety of malignances and reflect the known toxicities of paclitaxel. See the IB for a complete description of all toxicities reported in conjunction with nab- paclitaxel administration.
The most common toxicities reported in previous clinical trials included:
• Myelosuppression, predominantly neutropenia. Grade 4 neutropenia was reported and typically resolved in < 7 days and did not require colony stimulating factor support.
• Peripheral neuropathy, predominantly sensory. Grade 3 peripheral neuropathy was reported and typically improved to Grade 1 or 2 within 21 days of interrupting the nab-paclitaxel dose. Following resolution of the peripheral neuropathy to acceptable levels, clinicians were able to restart nab-paclitaxel dosing at a lower dose levels.
• Nausea and vomiting. Nausea and vomiting were seen, typically at Grade 1 or 2 levels. This AE responded well to standard anti-emetic regimens.
• Myalgias and arthralgias. Myalgias and arthralgias were reported and typically were Grade 1 or 2; these were responsive to standard acetaminophen-containing medication.
• Mucositis. Mucositis was reported typically Grade 1 or 2. It was not dose limiting
• Alopecia. Alopecia was reported by most patients and was similar to that seen with Taxol.

7.2.4 Drug Supply

Nab-paclitaxel is commercially available, and may be available for this study through Celgene.

7.3 Hydroxychloroquine

For complete prescribing information, please refer to the approved package insert.

7.3.1 Other Names

Plaquenil, 7-Chloro-4-[4-[ethyl-(2-hydroxyethyl)amino]-1-methylbutylamino] quinoline

7.3.2 Classification

Antimalarial, autophagy inhibitor

7.3.3 Mode of Action

Disrupts formation of autophagocytic vesicles

7.3.4 Storage and Stability

All dosage forms are stored at room temperature.

7.3.5 Administration

Oral.

7.3.6 Side Effects

- Central nervous system: Irritability, nervousness, emotional changes, nightmares, psychosis, headache, dizziness, vertigo, seizure, ataxia, lassitude.
- Dermatologic: Bleaching of hair, alopecia, pigmentation changes (skin and mucosal; black-blue color), rash (urticarial, morbilliform, lichenoid, maculopapular, purpuric, erythema annulare centrifugum, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, and exfoliative dermatitis).
- Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, abdominal cramping.
- Hematologic: Aplastic anemia, agranulocytosis, leukopenia, thrombocytopenia, hemolysis (in patients with glucose-6-phosphate deficiency).
- Hepatic: Abnormal liver function/hepatic failure (isolated cases).
- Neuromuscular & skeletal: Myopathy leading to progressive weakness and atrophy of proximal muscle groups (may be associated with mild sensory changes, loss of deep tendon reflexes, and abnormal nerve conduction).
- Ocular: Disturbance in accommodation, keratopathy, corneal changes/deposits (visual disturbances, blurred vision, photophobia - reversible on discontinuation), macular edema, atrophy, abnormal pigmentation, retinopathy (early changes reversible - may progress despite discontinuation if advanced), optic disc

pallor/atrophy, attenuation of retinal arterioles, pigmentary retinopathy, scotoma, decreased visual acuity, nystagmus.

7.3.7 Drug Supply

HCQ is commercially available, and patients will be given prescriptions to be filled at their local pharmacy. Patients will be provided with a pill calendar.

7.4 Receiving and Return of Study Drug

7.4.1 Receipt of Drug Supplies

Upon receipt of the study treatment supplies (nabpaclitaxel only), an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

7.4.2 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

8 Statistical Plan

8.1 Sample Size Determination and Methods

The initial enrollment on this trial will be an unblinded dose escalation of HCQ in six-patient cohorts from 800 to 1200 mg daily. The maximum accrual in this portion of the study will be 24 patients, but the dose is expected to be defined with 12 patients (six in the highest dose level). The study will then enroll 90 evaluable patients at the dose deemed to be the Phase II dose of HCQ in combination with gemcitabine/abraxane using an unblinded randomized phase II design.

The primary objective of the phase I portion of the study will be to determine the optimal dose of HCQ in combination with gemcitabine and abraxane to be used in the phase II portion of this study. The algorithm for dose escalation and the definition of dose limiting toxicities (DLTs) are described in section 5.2.3 of this protocol.

The primary efficacy endpoint of the phase II portion of this study is overall survival at one year, with progression-free survival, response rates, median overall survival, and incidence of toxicity as secondary endpoints. We will compute exact 95% confidence intervals for the proportions of subjects experiencing each type of observed toxicity. With at least 12 subjects evaluated at the

defined MTD, any adverse event having a true occurrence rate of 20% or more is likely to be observed in at least one patient with a probability of 93%.

The samples size calculations for overall survival (OS) at a one-year landmark are based on an estimated median OS of 12.2 months for the gem/abraxane arm. 90 patients (45 per arm) yield 81% power (and a one-sided significance level alpha of 0.1) to detect an improvement in one-year overall survival of 23% (from 50% to 73%). These calculations are based on the assumption that events (deaths) at one year are binomially distributed.

Progression-free survival and overall survival will also be evaluated using the method of Kaplan Meier and compared between arms using the stratified log-rank test.

8.2 Pharmacodynamic Analyses

We have developed non-invasive pharmacodynamic surrogate measures to detect changes in autophagy in peripheral blood mononuclear cells (PBMC) of patients. Inhibition by HCQ of the last step of autophagy causes accumulation of autophagic vesicles (AV) visible on EM. EM analysis will be performed on all patients at baseline and 4 weeks. For each sample, digital images of 50 cells at 10,000X will be obtained. The number of double-membraned AV per cell will be scored, and the median number of AV/cell (mAV/cell) per sample defined. For each cohort, statistically significant differences between pre and post samples will be considered doses at which autophagy inhibition is effective, and the induction of autophagy related to outcome in the HCQ-treated patients.

We will confirm EM findings with LC3 performed on PMN's at baseline and 4 weeks. LC3 immunblotting analysis will be performed on all patients. Briefly, batched frozen PBMC pellets will be thawed, the pellet lysed with RIPA buffer and subjected to western blotting, and the autophagy marker LC3 detected using a specific anti-LC3 (QCB Biologicals 1:500) and anti-actin (Sigma 1:5000) control. Bands will be quantified densitometrically relative to actin. The mAV/cell will be correlated with the levels of LC3-II in each sample to determine the correlation between the assays. With n=50, there is >90% power to detect a correlation coefficient of $\geq$0.60, with a two-sided, 0.05-level test.

Metabolic Imaging and response. ^{18}F-FDG-PET analysis of pancreatic cancers has become a standard method of assessment of therapeutic response. We will perform exploratory analyses of SUV (sum of lesions) before and, and their relationship to CT measurements, and to autophagy induction.

Pharmacokinetic Modeling. To evaluate the pharmacokinetics and pharmacodyamics of HCQ, the nonlinear mixed –effects modeling approach will be used to perform a full population PK analysis. The population pharmacokinetic parameters that will be estimated using this approach are apparent clearance (Cl/F) and volume of distribution of the central compartment (Vd/F). Since the absolute bioavailability of HCQ (F) cannot be calculated for oral dosing alone, bioavailability will be represented by a fixed constant for each of the parameter estimates. The

results from the population analysis will be used as prior information to obtain Bayesian estimates of each individual patient's pharmacokinetic parameters (Cl and Vd) from his/her blood HCQ concentrations. The Bayesian approach incorporates data from a population pharmacokinetic model and each individual patient to estimate the patient's pharmacokinetic parameters. The a priori pharmacokinetic parameters of the population model are used as a starting estimate for the individual's parameters, which are then adjusted based on the patient's measured drug levels and with consideration of the variability of the parameters and drug concentration measurements. With this approach, the population model itself is incorporated into the estimation procedure. The Phoenix® NLME™ and WinNonlin® (Pharsight Corporation, St. Louis, MO) software platform will be used for pharmacokinetic analyses.

This algorithm will determine the individual estimates of blood clearance (Cl/F) and volume of distribution (Vd/F) from which the following PK parameters of HCQ exposure in individual patients will be calculated: 1) area under the concentration-versus time curve (AUC); 2) peak (Cmax) blood concentrations; 3) trough (Cmin) blood concentrations; and 4) average drug concentration at steady-state (Css). AUC will be computed as follows: AUC (mcg * h/mL) = Dose (mg)/Cl (L/h). The Css, Cmax, and Cmin levels will be derived from model-predicted concentrations just prior to and following a dose of HCQ.

Archival Tumor Immunohistochemistry. Tissue sections (5μ) from tumor blocks will be processed and incubated with appropriate antibodies using imaging analysis software (Nuance Multispectral Imaging System, Cambridge Research & Instrumentation, Woburn, MA) and a microscope (Leica DMR82). Multispectral digital images will be acquired and signal quantified using NIH Image J software - described in detail in reference [34]. ATG5, beclin-1 and LC3 will be analyzed by immunofluorescence using a validated assay in the Abramson Cancer Center Core Pathology Facility. Archival tissue will only be obtained if the biopsy specimens are inadequate.

9.0 Patient Registration

9.1 General Guidelines

All sites should e-mail the coordinating site, that a patient is being screened. A signed informed consent document must be obtained prior to entry onto the study. This study will use a web based data entry system for data submission through Velos. Patient enrollment materials may be accessed online through the study website.

Eligibility criteria must be verified according to the eligibility checklist located in Appendix F and stored in the patient chart. The Principal investigator of each site is responsible to verify that all the eligibility criteria are met. Following completion of the eligibility checklist, subjects must be registered on the study website to be considered on study. Each patient will be automatically assigned a patient identification number following enrollment.

Signed and completed informed consent forms should be provided to the coordinating center accordingly. Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center
.

9.2 Data Submission Procedures

Data will be completed and submitted electronically using the study web based system. Electronic case report forms (eCRFs) can be accessed on-line through the study website. This website is used to register patients and submit data using eCRFs. In addition, study resource information (SAE submission forms, protocol documents and other study guidelines) can be found on the website.

9.3 Coordinating Center Guidelines

9.3.1 Responsibility of the Protocol Chair: University of Penn, Peter O'Dwyer, MD

• The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
• The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements are the responsibility of the Protocol Chair.
• The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
• The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

9.3.2 Responsibilities of the CRO:

ACC CRU :

• TGEN, Johns Hopkins Medical Institute, and UPenn will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. ACC CRU is responsible for assuring that TGEN, Johns Hopkins Medical Institute, and UPenn has an OHRP assurance and must maintain copies of IRB approvals from TGEN, Johns Hopkins Medical Institute, and UPenn.
• ACC CRU is responsible for collecting, compiling and disseminating SAEs to all sites.
• ACC CRU is responsible for providing an electronic data capture (EDC) system and maintaining the study web based system that is used for registration and data submission. ACC CRU is responsible for assuring IRB approval has been obtained at each participating site prior

to the first patient registration from that site. During the registration process, ACC CRU will collect the date each patient signs the informed consent form and HIPAA authorization form.

• ACC CRU is responsible for the preparation of all submitted data for review by the Protocol Chair.

• ACC CRU is responsible for conducting on-site monitoring as described in Section 11.d – Data and Safety Monitoring Plan for TGEN, Johns Hopkins Medical Institute, and UPenn.

10.0 Safety and Adverse Events

10.1 Definitions

Adverse Event

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. The CTC version 4.0 will be used to grade toxicity. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

.. results in study withdrawal
.. is associated with a serious adverse event
.. is associated with clinical signs or symptoms
.. leads to additional treatment or to further diagnostic tests
.. is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:

.. fatal
.. life-threatening
.. requires or prolongs hospital stay
.. results in persistent or significant disability or incapacity
.. a congenital anomaly or birth defect
.. an important medical event

Important Medical Events

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above. All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the patient is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each patient to report any subsequent event(s) that the patient, or the patient's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if the abnormality is of a degree, typically at least grade 2 and not present as grade 1 or better at baseline, that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

.. Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
.. Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
.. Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

10.2 Recording of Adverse Events

At each contact with the patient, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis. All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

10.3 Reporting of Serious Adverse Events

10.3.1 Study Sponsor Notification by Investigator

A serious adverse event must be reported to the study sponsor within 24 hours of the event.

Report serious adverse events by phone and facsimile to:

Peter O'Dwyer, MD, Phone 215-662-7606 or via the HUP page operator

At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Patient number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

10.3.2 IRB Notification by Investigator-sponsor

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 10 working days, according to IRB guidelines.

Reporting Process to IRB

Principal Investigators are required to submit reports of unanticipated problems posing risks to subjects or others that are probably or definitely study related via the HS-ERA system (Penn, or each site's reporting mechanism) within 10 working days of the event. Each site is responsible for ensuring that all IRB correspondence is forwarded to the PI within 10 working days.

For reportable deaths, the initial submission to the IRB may be made by contacting the appropriate IRB coordinator as soon as the death is known, with a report via HS-ERA (Penn, or each site's reporting mechanism) within 10 days if death is from underlying disease and within 24 hours if study related activity is considered contributory to the death.

10.3.3. FDA Notification by Investigator-sponsor, Voluntary

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information. If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the

FDA as soon as possible, but no later than 15 calendar days from the time the determination is made..

10.3.4 Internal Safety and Compliance Entities

Each site is responsible for following its own institutional guidelines and policies regarding reporting and auditing, deviations and exceptions. Deviations and exceptions should be maintained on a deviation log.

10.3.5 Expedited Reporting by Investigator to Celgene

Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required. The Celgene tracking number (AX-PANC-PI-0029) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

Celgene Drug Safety Contact Information:

Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr.
Suite 6000
Berkeley Heights, NJ 07922
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

Safety Reporting Requirements for IND Holders

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements. This study meets the criteria for IND exemption.

11. Data Handling and Record Keeping

a. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

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

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

b. Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

c. Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. Data will be collected using CRFs designed, stored and secured in Velos.

d. Study Monitoring, Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and the ACC CRU monitors of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12. Measurement of Effect

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

12.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment on study.

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.

12.1.2 Disease Parameters

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

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be $\geq$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 $\geq$ 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.

12.1.3 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. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

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

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.

12.1.4 Response Criteria

12.1.4.1 Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

12.1.4.2 Evaluation of Non-Target Lesions

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

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

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

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

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

12.1.4.3 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not	No	PR	

	evaluated			
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

12.1.5 Duration of Response

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

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.

13. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

14. References

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Schedule of Events

Please note: labs can be drawn within 3 days of scheduled date.

		Cycle 1			Cycle 2		Cycle 3 on
Test	Screening [a]	Day 1	Day 8	Day 15	Day 1	Day 15 +/- 7 days	Day 1 Subsequent Cycles
Informed Consent	X						
Medical History	X						
Physical Examination/ Performance Status/weight	X	X			X		X
Height	X						
Disease Assessment (routine PET[g], CT or MRI)	X						X (every 8 weeks)[f]
AE assessment		X			X		X
Serum Chemistry [b]	X	X			X		X
CBC, differential	X	X	X	X	X	X	X Every two weeks while patient is on treatment
Pregnancy test [c]	X						
HCQ PK[d]		X within 14 days of day 1		X ± 7 days	X ± 7 days		X ± 7 days (cycle 3 only)
PMN's for PD endpoints		X within 14 days of day 1		X ± 7 days	X ± 7 days		X ± 7 days (cycle 3 only)
Plasma for PD endpoints		X (within 14 days of day 1)		X ± 7 days	X ± 7 days		X ± 7 days (cycle 3 only)
Optional Fresh Tumor biopsy[e]	X						X – at 6-8 weeks after beginning therapy

[a] Screening evaluations are to be conducted within 28 days prior to start of protocol therapy (except Disease assessment and biopsy which can be conducted within 28 days prior to start of protocol therapy). Some screening procedures may occur on cycle 1 day 1.

[b] Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, CA19-9 (repeat only if abnormal)

[c] Pregnancy test (women of childbearing potential)., at screening, within 14 days and within 24 hrs prior to first dose protocol therapy

[d]A 5 mL venous blood sample for determination of HCQ in anticoagulated whole blood will be collected in a heparinized green-top tube and placed on ice until it can be aliquotted into two polypropylene tubes as detailed in Section 5.6. All specimens will be labeled with the patient's assigned study number, day on study, date and time. A separate log of patient samples will also record this information, and in addition, the time of last HCQ dose, and time of last meal, and a description of concomitant medications at the time of collection. All samples will be collected pre-dose except cycle 1 week 3 which is post dose.

[e] The optional fresh tumor biopsy will be obtained by CT- or US-guided biopsy (unless the target lesion is easily palpable) as described in section 5.6.3.

[f] A CT or MRI of the abdomen/pelvis and chest (if thoracic disease is present) will be obtained at 2 month intervals after initiating chemotherapy treatment for the first year, and at 3 month intervals thereafter

[g] A pre-treatment PET scan, in addition to CT or MRI should be performed, provided its costs is covered by insurance.