RESEARCH ARTICLE

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A Phase I Trial to Determine the Safety and Tolerability of Autophagy Inhibition Using Chloroquine or Hydroxychloroquine in Combination with Carboplatin and Gemcitabine in Patients with Advanced Solid Tumors

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Abstract

Background: Autophagy is a catabolic process triggered in cells during periods of metabolic or hypoxic stress to enable their survival that may also impart advantages to tumors. Inhibition of early stage autophagy can rescue cancer cells, while inhibition of late stage autophagy leads to cell death due to accumulation of damaged organelles. Chloroquine (CQ) and hydroxychloroquine (HCQ) inhibit late phase autophagy. We assessed the safety, tolerability and activity of combining CQ/HCQ with carboplatin and gemcitabine (CG) in patients with advanced solid tumors. **Methods:** This single institution phase 1 dose-escalation study was designed to evaluate the maximum tolerated dose (MTD) of CQ/HCQ, in combination with CG in patients with advanced solid tumors. Secondary objectives were to determine ORR, PFS and OS. A starting dose of CQ/HCQ 50 mg was used in conjunction with CG, and increased in increments of 50 mg in each dose cohort. Grade 3 or greater toxicity that is treatment-related, and was not self-limited, or controlled in less than 7 days was considered dose limiting toxicity (DLT). **Results:** Twenty-two patients were enrolled. All patients had at least one prior treatment, and 11 of them had 3 prior regimens. HCQ 100 mg daily was found to be the MTD in combination with CG with thrombocytopenia and/or neutropenia dose-limiting. Median OS was 11 months, and the 1- and 3- year overall survivals were 30% and 7%, respectively. Median progression free survival was 5 months and the 6-, 12-, and 18-month progression-free survivals were 48%, 21% and 14%, respectively. **Conclusions:** The MTD identified for CQ/HCQ was lower than previously reported with concomitant use of chemotherapeutic regimes likely due to the myelosuppressive nature.

Keywords: HCQ- autophagy- advanced cancer

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Introduction

Autophagy, or 'self-eating' is a cellular process by which cytoplasmic organelles and proteins are sequestered into autophagic vesicles and delivered to lysosomes for "bulk" degradation and recycling [1, 2]. It is a housekeeping process that regulates organelle and cellular protein turnover [3]. Autophagy has been shown to become deregulated in certain pathological states including cancer. Under normal circumstances, autophagy is believed to suppress cellular transformation and tumor progression by limiting chromosomal instability. Alternatively, it has been demonstrated that established tumors utilize autophagy to survive periods of metabolic or hypoxic stress [4]. Thus, manipulation of autophagy has become a potential area for the development of novel antineoplastic strategies [5]. Aminoquinolines such as CQ have been shown to inhibit autophagy by mechanisms distinct from other inhibitors such as 3-methyladenine (3-MA). Whereas 3-MA inhibits early phase autophagy, consequently inhibiting formation of acidic vesicular organelles (AVO) that consist predominantly of autophagosomes and autolysosomes, CQ inhibits autophagy in its late phases after cytoplasmic AVOs have been formed. Therefore, CQ treated cells typically demonstrate accumulation of cytoplasmic AVOs [6]. CQ has been identified as a chemotherapy sensitizer when used in combination with certain antineoplastic drugs. [7, 8]. The lysosomotropic properties of CQ are likely responsible for many of its biological effects. Accumulating lines of evidence suggest that through its lysosomotropic effect, CQ can sensitize cancer cells to the killing effects of and various chemotherapeutic agents and ionizing radiation [9, 10].

In a small randomized study, Sotelo et al, reported

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improved survivals in patients with glioblastoma treated with 4 cycles of carmustine with radiation and CQ versus placebo beginning 5 days after surgery [11]. Amaravadi et al. [12] demonstrated that targeting autophagy with CQ derivatives enhanced the efficacy of chemotherapy. HCQ has been extensively studied in combination with several chemotherapeutic agents to assess its clinical safety and activity. A number of phase I trials studying HCQ in combination with various antineoplastic agents determined the maximum tolerated dose (MTD) to be 200 to 1200 mg daily. HCQ has been studied combined with temozolomide 150 mg of temozolomide in patients with advanced solid tumors [11]. Wolpin et al. [13] reported the safety and antineoplastic activity of HCQ in 20 patients with metastatic pancreatic cancer that did not respond to conventional chemotherapy. In this phase II trial, patients received 400 (n = 10) or 600 (n = 10) mg of HCQ twice daily as a single agent. Though these dosages where generally well tolerated, two patients experienced treatment-related serious adverse events. Five other phase I trials of HCQ involved combination with various chemotherapeutic agents including temozolomide, bortezomib, temsirolimus, vorinostat or doxorubicin [14-16]. A number of patients with melanoma, colorectal cancer, myeloma and renal cell carcinoma demonstrated partial responses or stable disease, suggesting antitumor activity. In a phase II study in advanced pancreatic cancer, Karasic, et al showed that HCQ 600 mg daily in combination with gemcitabine and nab-paclitaxel resulted in an improved response rate making some tumors resectable. Based on this rationale and the importance of gemcitabine and carboplatin in treating many types of cancer, our study was designed to investigate if CQ will re-sensitize the use of chemotherapy again in heavily pretreated patients. Patients enrolled in our phase I study, were mostly heavily pre-treated and were candidates for the systemic therapy of carboplatin and gemcitabine and thus the choice of starting with lower doses of HCQ.

Study Objectives

Primary Objective

To determine the maximum tolerated dose (MTD) of chloroquine (CQ) or (HCQ) in combination with carboplatin and gemcitabine (CG) in patients with advanced solid tumors.

Secondary Objectives

1. To estimate the overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) of patients with advanced solid tumors treated chloroquine (CQ) or (HCQ) in combination with carboplatin and gemcitabine (CG).

2. To determine the pharmacokinetics of CQ/HCQ in combination with CG

3. To detect effects on autophagy through exosomal microtubule-associated protein 1A/1B light chain 3B (LC3) levels in peripheral blood.

Materials and Methods

Human Subjects Protections

Eligible patients were enrolled in this IRB-approved study through the University of Cincinnati Cancer Institute Clinical Trials Office (CTO). To register a patient, all of the following were obtained: Written informed consent form, Health Insurance Portability and Accountability Act (HIPAA) Authorization form, eligibility screening worksheet and registration form. The trial was listed on https://clinicaltrials.gov (NCT02071537) [16].

Study Design

This is a single institution phase I dose-escalation study using a 3+3 schema. Patients with advanced solid tumors with either no other available standard of care treatment, or where carboplatin and gemcitabine is considered an acceptable treatment option, and ECOG performance status 0-1 were eligible. Sequential CQ/HCQ dose cohorts of 3-6 patients were treated. The starting dose of CQ was 50 mg daily in addition to carboplatin and gemcitabine (Table 1). Patients in cohort 1 were treated with CQ; however, CQ became unavailable due to a national shortage, so the study continued using HCQ in cohorts 2, 3 and the expansion cohort with IRB-approval.

Eligibility Criteria

Subjects were required to have: Histologically or cytologically confirmed metastatic or unresectable cancer for which either standard curative measures do not exist, are no longer effective, or for which the combination of carboplatin and gemcitabine is considered a reasonable treatment option; no other than active malignancy, or chronic systemic immune therapy, and no known G6PD deficiency, age ≥18 years; ECOG performance status <2 (Karnofsky >60%), acceptable organ and bone marrow function defined as an absolute neutrophil count ≥1,500/μL, platelet count ≥100,000/μL, total bilirubin <1.5X upper limit of normal (ULN), AST (SGOT) or ALT(SGPT) <3X ULN, adequate baseline renal function with serum creatinine <1.5X ULN, a life expectancy >3 months, and at least one measurable lesion by RECIST 1.1. Patients

Table 1. Planned Dose Escalation and MTD Cohort Expansion.

Dose level	Patients	CQ (first cohort) HCQ (all subsequent patients) (mg/day) Day -7 to day 21	Carboplatin (AUC) Day 1	Gemcitabine (mg/m^2) Day 1 and Day 8 out of 21 day cycle
1	3-6	CQ 50 mg daily	5	1,250-1,000
2	3-6	HCQ 100 mg daily	5	1000
3	3-6	HCQ	5	1000
4	3-6	200	5	1000
Expansion cohort	10-12	100	5	1000

with treated and asymptomatic brain metastases were eligible. Women and men of child-bearing potential must have agreed to use adequate contraception for the duration of study, and participants must have the ability to understand and willingness to sign a written informed consent document. Patients receiving other investigational agents, those with untreated brain metastases, history of allergic reactions to CQ/HCQ, or other agents used in study, and an uncontrolled intercurrent illness or infection were ineligible.

Treatment

CQ or HCQ was administered at the dose levels as indicated in Table 1 for a total of four 21 day treatment cycles(initially HCQ was used , then due to unavailability of HCQ patients switched to CQ). CQ was administered orally daily starting one week prior to the start of carboplatin and gemcitabine (CG) chemotherapy (Day -7 until Day 1), then throughout the 21-day cycle for a total of 4 treatment cycles of CG. Additional 5th and 6th cycles of carboplatin and gemcitabine was allowed without the addition of CQ or HCQ in case of continued response, or benefit per the decision of the treating investigator. The lower and higher dose groups (N = 6 and 3, respectively) received 50 mg, or 150 mg of CQ or HCQ as a fixed daily oral dose. The first seven patients received CQ 50 mg; 50 mg was given in a suspension form then 100 mg was given through splitting the 200 mg tablet. (where the first patient received only one dose of 50 mg of CQ and was found to be ineligible on Day 1 and was excluded and replaced), and the next 3 patients received 100 mg of HCQ due to the worldwide shortage and unavailability of CQ. The third cohort received 150 mg of HCQ and the expansion cohort of 10 patients received 100 mg of HCQ. HCQ tablets were splitted in to Half to provide the 100mg dose. This was done by an experienced clinical pharmacist to ensure all patients are getting the same dose.

Dose Limiting Toxicity definition and Dose Escalation

The dose limiting toxicity (DLT) of HCQ was 150 mg when given in combination with carboplatin and gemcitabine. We believe that the major toxicity though occurred due to the cytotoxic chemotherapy in heavily pre-treated patients.

The maximum tolerated dose (MTD) of HCQ was 100 mg when given in combination with carboplatin and gemcitabine.

Evaluation of Safety and Outcome

Adverse event descriptions and grading were as in the revised National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 were utilized for AE reporting.(CTCAE 4.0 was the available criteria used during the evaluation of our studied patients) . Primary outcome measures were: CTCAE grade >3 adverse events clearly linked to treatment and was not self-limited, or resolved in less than 7 days. Secondary outcome measures were RECIST 1.1 response criteria: Complete response (CR), partial response (PR), stable disease, stable disease (SD) and progressive disease (PD). The duration of overall response was 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. Duration of 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. Progression free survival (PFS) is defined as the duration of time from start of treatment to time of progression.

Ocular exam

Due to the potential ocular toxicity of CQ/HCQ, all subjects underwent a baseline ocular/fundus exam before the start of CQ/HCQ treatment and a repeat exam at the end of the study to ensure that there was no ocular toxicity.

Statistical Considerations

The primary endpoint was dose-limited toxicity (DLT), and they were defined as dichotomous variables in the study. At each dose level DLT have been summarized using frequency (%).

Secondary endpoints are a dichotomous variable of treatment response (CR or PR), events of progression free (PF) and overall survival (OS) are both censored at 12 months after treatment. The dichotomous variables of response have been summarized in frequency at each follow up visit. Kaplan-Meier curves were used to summarize the PFS and OS over time. In addition, as exploratory analyses, logistical and Cox proportional hazard models have been used to assess associations of secondary variables to baseline characteristics such as patient's demographics, cancer types and stages, and therapy plans.

Sample size justification

Determination of MTD was followed using an algorithm of a maximum of 6 patients in each cohort. No power analysis was needed as only descriptive statistics are provided for the primary variables. The analyses of secondary variables were based upon a total of 10 patients in the MDT cohort. Tiered enrollment for each cohort as each cohort was included according to the standard 3-6 patients and it takes up to 28 days to ensure no serious adverse events before moving to the next cohort.

Data and safety monitoring

Review of data and patients' outcome was discussed at the time of the initiation of the study, before expanding or moving to the following cohort, and at the end of the study by.

Results

Patients

Twenty-three patients with advanced solid tumors were enrolled between 2014-2018. The patient demographic is shown in Table 2. Among the 22 eligible treated patients, there were 15 males (68%) and 7 females (32%) with median age of 58 years (range, XX-YY). There 15 White (68%), 6 African-American (27%) and 1 Asian patient (5%). Regarding ECOG performance status (PS), 5

Table 2. Patient Characteristics

Variable	Number (N)	Percentage %
	Median	Range
Age in years (median-Range)	58	41-84
Gender		
Male	15	68
Female	7	32
Race		
White (W)	15	68
African American (AA)	6	27
Asian (A)	1	5
ECOG PS		
0	5	22
1	14	64
2	3	14
Histology		
Non small cell lung cancer, Adenocarcinoma	5	23
Non small cell lung cancer, Squamous cell carcinoma	4	18
Other (small cell, urothelial, hepatocellular, and cholangiocarcinoma)	13	59
Number of prior regimens		
0	3	
1	5	14
2	3	22
≥3	11	14

patients had PS of 0 (22%), 14 had PS of 1 (64%) and 3 had PS of 2 (14%). Different histological types were included, 5 patients had adenocarcinoma (23%), 4 had squamous cell carcinoma (18%) while 13 had different types (59 %) including small cell, urothelial, hepatocellular, and cholangiocarcinoma. The number of regimens received prior inclusion in this trial was 0 for 3 patients (14%), 1 for 5 patients (22%), 2 for 3 patients (14%) and 3 or more regimens for 11 patients (50%).

Table 3. Clinical Outcome

Outcome	Number of patients (N)	Percentage (%)
Response Rate		
PR	1	5
SD	15	68
PD	6	27
Disease control Rate		
>6 months		48
>12 months		21
>18 months		14

Dose escalation

The first cohort constituted of 7 patients as the first patient was excluded on Day 1, as he did not meet eligibility criteria having a baseline platelet count less than 100,000/μL. Cohort 1 was expanded to include 6 patients due to neutropenia and thrombocytopenia related to chemotherapy. It was recommended by the Data Safety Monitoring Board) to decrease the dose of gemcitabine from 1250 mg/m^2 to 1,000 mg/m^2. The next 3 patients who were enrolled tolerated carboplatin AUC = 5 and gemcitabine 1000 mg/m^2 days 1 and 8 in addition to CQ 50 mg with no DLT. HCQ replaced CQ due to an international shortage of CQ at that time. The second cohort included 3 patients who were treated with HCQ 100 mg daily with no DLT. The first 2 cohorts of that study thus showed no DLTs at doses of 50 mg and 100 mg of CQ and HCQ subsequently. The third patient cohort included 3 patients treated with HCQ 150 mg daily, and 2 of them experienced DLT mainly due to grade 4 thrombocytopenia and grade 3 neutropenia of more than 7 days duration. The patient with the neutropenia did not receive growth factor support. There were no protocol-related deaths.

One DLT occurred in X patients treated with HCQ 150 mg qd group and the MTD for this combination was determined to be HCQ 100 mg daily. Subsequently 10 patients were enrolled in the expansion cohort at HCQ 100 mg with carboplatin and gemcitabine.

Efficacy

While assessing the response rate (RR) for various

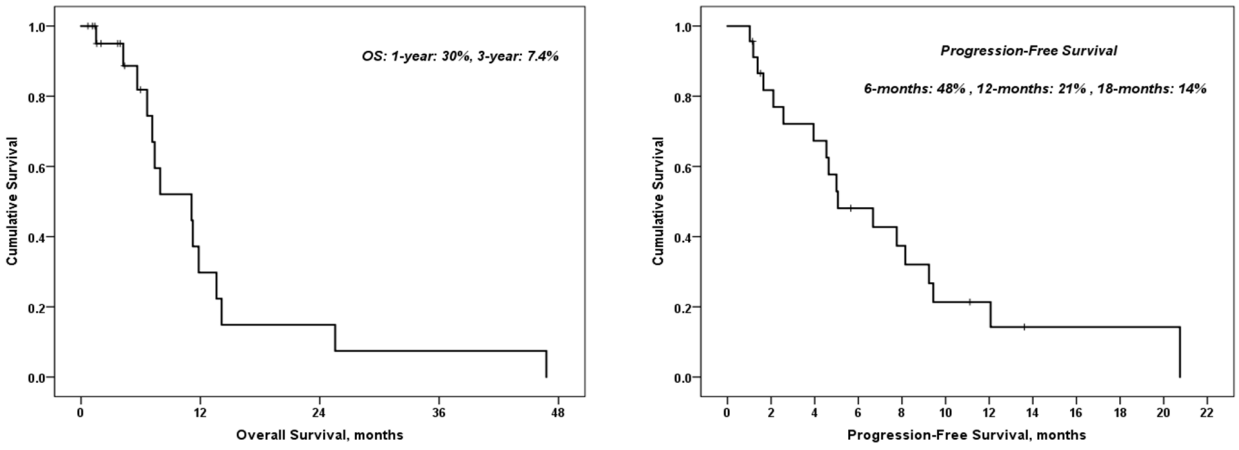

Figure 1. Progression-free Survival

Table 4. Table of Adverse Events

Event	Grade 2	Grade 3	Grade 4	Grade 5	Total all grades (N)
Fatigue	1	1			2
Rash	1				1
Dehydration		1			1
Leucopenia (Persistent > 7 days)	1 (Baseline)	3			4
Neutropenia (Persistent >7days)	1 (Baseline)	3	5		9
Anemia (Persistent >7 days)		3	1		4
Thrombocytopenia (Persistent > 7 days)		2	1		3
Elevated transaminases		2			2
Elevated serum Creatinine		1			1
Hyponatremia		1			1
Pain		4 (unrelated)			4
Weakness		1			1

patients included in this study, 1 patient achieved partial response (PR) (5%), 15 patients had stable disease (SD) (68%) while 6 patients had progressive disease (PD) (27%). Nevertheless, the disease control rate (DCR) was 48% for more than 6 months duration, 21% for more than 12 months and 14% for more than 18 months. In the univariate analysis of predictors of all-cause mortality and predictors of disease progression, neither age, gender, number of cycles was statistically significant. overall, the response rate was 71%. Progression-free survival (PFS) was 48% at 6 months. The disease control rate (DCR) was 68% at 6 months and median overall survival (OS) were 30% at 1 year (Figure 1).

Efficacy of subsequent therapies

Interestingly, we observed that patients receiving subsequent immunotherapy after progressing on this clinical trial had excellent clinical outcomes. One patient with squamous cell carcinoma of the lung (Cohort 1) had prolonged stable disease of 11 months on CA + HCQ. Similarly, prolonged stable disease was noted in a patient with small cell lung cancer in cohort 3 who experienced disease progression on this protocol who then benefited from subsequent nivolumab therapy with a partial remission and improvement of performance status from ECOG 2 to 0. This patient had an ongoing response following 15 cycles of the PD-1 inhibitor. Another elderly patient in cohort 3 with progressive urothelial cancer, tolerated the protocol treatment well with no serious adverse events. This patient achieved disease control on subsequent atezolizumab therapy.

Laboratory Correlates

To assess effects of treatment on the autophagy pathway we developed a panel of relevant assays.

Quantification of Autophagosomes

Study population

This study included 24 patients who were recruited in 4 cohorts, cohort 1 (n=6), cohort 2 (n=3), cohort 3 (n=3) and extension cohort (n=8). All patients were histologically diagnosed with advanced solid. All subjects provided a written informed consent before treatment in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the University of Cincinnati Hospital. The subjects enrolled have failed their previous lines of treatment and the proposed chemotherapy regimen (Carb/Gem) was considered a standard of care.

Cell Culture

We used four cell lines in this study, human glioblastoma cell line (U251), human pancreatic carcinoma cell line (MiaPaCa2), adenocarcinoma human alveolar basal epithelial cells (A549) and human embryonic kidney cells (HEK293). The variety was intentional to insure matching results regardless of baseline autophagic activity of the cell line. These cells were maintained in DMEM media, supplemented with 10% fetal bovine serum (FBS), antimycotic, antibiotic and L-glutamine. At 40% confluency, the culture dishes were washed twice with PBS and the media replaced with 10% exosome depleted FBS (dFBS) DMEM media (50 ml EXO-FBSTM Exosome-Depleted FBS Media, System Biosciences #EXO-FBS-50A-1) and incubated to settle for 24 hours. After 24 hours, the culture dishes were washed with PBS and new dFBS DMEM media was added and treated with chloroquine diphosphate salt (Sigma-Aldrich, UK) and incubated. Incubation period was 48 hours for western blotting on HEK293 plates and 72 hours for western blotting on U251. The discrepancy in the incubation time was attributed to the confluency of the plates, CQ concentration used and the number of exosomes expected to be secreted by the

cell line. The media was collected and used for exosome extraction directly after the incubation time is over.

Exosome extraction
From Patients Plasma

Patients' blood samples were collected at the mentioned time points and span down at 1500 g for 15 minutes. The upper phase (plasma) was collected in new tubes and stored at -80 degrees till us. Exosomes extraction was done using an exosome extraction reagent (Total Exosomes Precipitation Reagent from plasma, Invitrogen by Thermo-Fisher Scientific Ref: 4484451) following manufacturer's instructions, then, suspended in PBS and stored at -80 C.

From Cell Culture Media

Exosomes were extracted from freshly collected cell culture media using exosome precipitation reagent (50ml Total Exosome Isolation from cell culture media, InvitrogenTm by Life TechnologiesTM, ref# 4478359) following manufacturer's instructions. Extracted exosomes were suspended in PBS and stored at - 80 C. The method for patient extraction is described in full details here[34].

Western blotting

Detection of LC3b expression in the isolated exosomes was done using western blotting following standard protocols. LI-COR detection was used to scan the membranes. LC3B protein detection was achieved by anti-LC3B rabbit monoclonal antibody (Cell Signaling Inc., catalogue #2775, USA). CD9 was used as a loading control was blotted using rabbit monoclonal antibody (#3700) from Cell Signaling Technology Inc. All western blots were run on 4-15% gradient gels after estimating and unifying samples protein content by BCA.

Flow cytometry

HEK293 cells were plated in 10 cm culture dishes in 10% FBS DMEM media. The media was replaced with dFBS DMEM media once a 40% confluency is reached and incubated for 24 hours. Drugs as single agents, Gem at 20uM concentration (Gem20), CQ at 10uM (CQ10) and 20uM (CQ20) concentrations, and in combinations: Gem+CQ10 and Gem+CQ20 were used to treat the plated HEK293 cells in fresh dFBS DMEM media for 16 hours. Both the cells and the media were collected, spinned down, then washed and finally stained with propedium iodide (PI) and annexin V before running the samples. Cell cycle analysis was performed using fresh cells on a FACS Calibur (Becton Dickinson) after incubation with 25 µg/ml of PI. Cell cycle phases were analyzed with the CellQuest-Pro software program (Becton Dickinson).

LC-3B conversion from LC-3B I to II has been used as an indicator for autophagy since it measures the dynamicity of the process by reflecting the turnover of autophagosome fusion with lysosomes[17]. However, increased expression of both isoforms is used to measure the activity of both autophagy inducers and inhibitors[18, 19]. Although this method is biologically explained, but there was no explanation on how to differentiate between both actions and unless other quantification methods are used, both actions can be interpreted similarly on western blotting. Our in vitro results had showed different picture for each when LC3-B was measured in exosomes instead of cells. This conclusion can serve as simple and cost-effective method to track stimulus effect on autophagy by interpretation of t western blotting compared to well-known controls.

Pharmacokinetics
Metabolism of Chloroquine/Hydroxychloroquine

CQ/HCQ is 60% bound to plasma proteins and cleared equally by the kidney and liver. Following administration of C, it is rapidly de-alkylated via cytochrome p450 (CYP) into active desethylchloroquine and bisdesethylchloroquine with elimination half lives of 20 to 60 das. Both parent drug and metabolite can be detected in urine months after a single dose.

CQ/HCQ has a rapid and almost complete absorption and peak plasma concentrations reached within 1-2 h following oral administration. CQ/HCQ has a long half-life of 3-5 days. For pharmacokinetic analysis, blood samples (5 mL per time point) was collected on Day -7 at baseline pre-dose, then at 1, 2, 4, 6, and 24 hours on Day 1. Trough levels were collected at Days 8 and 15. Blood samples will be collected at each subsequent cycle (cycle 2-4) on Day 1 at 1, 2, 4, 6, 24, 48 and 72 hrs. Trough levels will be collected on Days 8 and 15 for cycles 2-4. Blood was collected into B-D vacutainer tubes containing K3-EDTA mixed and centrifuged at 1500 g for 10 minutes at 4°C. Plasma will be transferred into a storage tube and maintained on dry ice until stored in a -20°C freezer. Post-dose trough levels for CQ/HCQ were measured on Days 8, 15 and 22.

Discussion

Our study determined a MTD for HCQ that was very close to the dose determined in a study using CQ in addition to standard therapy for patients with glioblstoma multiforme [5]. Our study used CQ or HCQ combined with carboplatin and gemcitabine (CG) in a heavily pre-treated patient population with various advanced solid tumors. As a resuly, the MTD appeared to be much lower than the MTD dose of CQ or HCQ in reported other studies [20]. The highest dose cohort in the current study included patients that were heavily pretreated and experienced >7 days of either neutropenia or thrombocytopenia. No on-study deaths occurred. Other trials that incorporated either CQ or HCQ were able to deliver higher doses of these agents given that the these studies included agents that are not usually myelosuppressive [20], or in chemotherapy naïve subjects [21].

Our observation that subsequent responses to immunotherapeutic agents such as PD-1 or PD-L1 inhibitors might be enhanced following autophagy inhibition is intriguing. Autophagy modifying agents combined with evolving immunotherapy as a potential new treatment option may offer an interesting area for addition studies, both in the laboratory and the clinic. Autophagy is involved in the processing of tumor antigens

and their presentation to the immune system and thus may be considered as a line of defense against cancer.

Autophagic pathways induced by hypoxia in the tumor microenvironment can impair antitumor immune responses mediated by cytotoxic T-lymphocytes (CTL) and natural killer (NK) cells, and has also been shown to enhance the immunosuppressive properties of myeloid-derived suppressor cells (MDSCs) [21]. In response to hypoxia, the hypoxia-inducible family of transcription factors (HIFs), do not become ubiquitinated, thus evading degradation by the ubiquitin-proteasome system. As a result, they accumulate in cytoplasm and are transported to the cell nucleus leading to the activation of about 300 genes involved in many biological processes, including angiogenesis, enhaced cell survival, metastasis, induction of a stem cell-like phenotype, and immune escape [21]. Targeting HIF-2α decreases PD-L1, while HIF-2α over-expression increased both PD-L1 mRNA and protein expression in renal cancer cells [22].

In his study, Wolpin et al. used HCQ as monotherapy with previously treated metastatic pancreatic cancer and he achieved much lower median PFS and OS (46.5 and 69.0 days respectively) while using higher doses or HCQ (400 mg and 600 mg twice daily dose). Also, Malhotra et al used chemotherapy (carboplatin, paclitaxel (and bevacizumab if meeting criteria))in addition to HCQ (twice daily dose of 200 to 600 mg) in newly diagnosed non-small cell lung cancer (NSCLC) patients achieving PFS of 3.7 months, thus demonstrating the improved response with addition of HCQ even with lower doses to CG chemotherapy regimen [13, 23] .

Although we only measured PDL-1 expression using in blood samples using RNA as no second biopsies were acquired, addition of HCQ to chemotherapy increased PLD-1 expression which is consistent with the study of Patel et al. in which they acquired on-study biopsies after 1st cycle of treatment from only 2 patients, which was also demonstrated by blood samples from other patients [24].

Results from this study demonstrate that HCQ opens a new era for heavily treated HCQ naïve patients to receive HCQ in addition to chemotherapy, thus improving both progression free and overall survival. Furthermore, anti PDL-1 resistant patients can be re-challenged with PDL-1 inhibitors after receiving HCQ +/- chemotherapy due to HCQ's ability to increase PDL-1 expression. These are still ambitious hypotheses that need further research, and that is why we need to expand our trial to phase II.

Author Contribution Statement

All authors contributed equally in this study.

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Author Disclosure Statement

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