Protocol Cover Page

“High Dose Vitamin C Intravenous Infusion in Patients With Resectable or Metastatic Solid Tumor Malignancies”

NCT0 3146962

TITLE: A phase II study of high dose vitamin C intravenous infusion in patients with resectable or metastatic solid tumor malignancies.

IRB Protocol #: 1610017688
IND/IDE # (if applicable): 132719
Version 8 Date: 08 March 2022

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Confidentiality Statement

List of Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTSC	Clinical Translational Science Center
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBFA	Human Research Billing Analysis Form
HUD	Humanitarian Use Device
ICF	Informed Consent Form
IDE	Investigational Device Exemption
IND	Investigational New Drug
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAP	Unanticipated Problem
WCM	Weill Cornell Medicine

Protocol Summary

Full Title:	A phase II study of high dose Vitamin C intravenous infusion in patients with resectable or metastatic solid tumor malignancies
Short Title:	High dose Vitamin C infusion for solid tumor malignancies
Clinical Phase:	Phase II
Principal Investigator:	Manish A. Shah, MD
Sample Size:	Cohort A = 20 evaluable (max 22); Cohort B = 25 evaluable (max 28) Cohort C = 20 patients (max 28)
Accrual Ceiling:	This study will enroll at most 78 subjects.
Study Population:	Adult patients with a solid tumor malignancy who are eligible for resection (cohort A), or with extended RAS (e.g. KRAS or NRAS mutation) or BRAF mutation with metastatic cancer that have had at least 1 line of therapy (cohort B). We will target colorectal cancer which is known to have 40-50% rate of extended RAS (e.g. KRAS or NRAS) or BRAF mutations, as well as patients with other solid tumors that are enriched for extended RAS (e.g. KRAS or NRAS) or BRAF mutations including pancreatic cancer and lung cancer. Cohort C will include patients with metastatic colorectal cancer with a known mutation in extended RAS (e.g KRAS or NRAS) or BRAF mutation with liver metastases amenable to radioembolization (RAE) therapy. This will be a dose escalation cohort with patients receiving ascorbate at 0.5 g/kg, 0.75 g/kg, 1.0 g/kg, and 1.25 g/kg dosing. Treatment will commence the week of RAE, with the test dose administered prior to RAE, and the treatment dose of VIT C to be administered on the day of RAE. VIT C will continue for 1-2 weeks following RAE (for a total of up to 3 weeks of therapy). At the maximal tolerated dose, an additional 4 patients will be treated in a dose expansion phase. Patients who clinically require a 2nd Y90 treatment may be eligible for the study (Cohort C) again and treated at one dose level below the currently enrolling dose level if they have not experienced prior DLT with previous protocol therapy.
Accrual Period:	72 months

Study Design:	This is a multicenter, single arm, 3-cohort, open-label trial of high dose Vitamin C intravenous infusion in subjects with solid tumor malignancies who are eligible for resection (cohort A) or with extended RAS (e.g.KRAS or NRAS) or BRAF mutation metastatic cancer who have received prior systemic treatment (cohort B). Cohort C will involve patients with colorectal cancer having an extended RAS or BRAF mutation who are amenable for localregional therapy of hepatic metastases with Yttrium-90 radioembolization.
Study Duration:	Patients in cohort A receive a high dose vitamin C infusion for 4 days per week for 2-4 consecutive weeks prior to surgery. Patients in cohort B receive high dose vitamin C infusion for 4 days per week for up to 6 months or disease progression. Cohort C will receive high dose vitamin C for 1-3 weeks. During week 1 vitamin C infusion and Y90 radioembolization of hepatic metastases will occur same day. Follow up is required for up to 8 weeks after enrollment of the study for cohort A and C, and up to 12 months after enrollment for cohort B. It is estimated that the study will end on 06/30/2023.
Study Agent:	Vitamin C (ascorbic acid)
Intervention Description:	Vitamin C infusion will be administered intravenously at 1.25 g/kg for 4 days per week for 2-4 consecutive weeks (cohort A) or up to 6 months (cohort B). Cohort C is a dose escalation cohort combining Vitamin C infusion with radioembolization for patients with liver metastases. This will be a dose escalation cohort with colorectal cancer patients receiving ascorbate at 0.5 g/kg, 0.75 g/kg, 1.0 g/kg, and 1.25 g/kg dosing.
Primary Objective:	Preliminary antitumor activity measured by pathologic response based on tumor regression grading in cohort A patients. 3-month disease control rate (DCR) will be evaluated using RECIST v 1.1 in cohort B patients. For Cohort C, the primary objective is to determine that maximal tolerated dose of the combination of high dose vitamin C with Y90 radioembolization for patients solid tumor malignancies and liver metastases amenable to local-regional therapy.
Secondary Objectives:	A. Objective response rate (ORR) and progression-free survival (PFS) in cohort B and cohort C. B. Assessment of pharmacokinetics of high dose vitamin C C. Safety of high dose vitamin C administration using CTCAE 4.03.

Exploratory Objectives:	a. In vitro activity of vitamin C in tumor organoids from patients treated with high dose vitamin C b. Pharmacodynamic and exploratory biomarker samples will be collected at the time points specified in the protocol.
Endpoints:	The primary endpoint for Cohort A will be pathologic response as assessed by tumor regression grading. Efficacy will be evaluated from CT scans (or similar) using RECIST 1.1 criteria to classify objective response for cohort B and cohort C. Other endpoints will include progression-free survival (cohort B and C), vitamin C concentrations, and other biomarkers of interest.

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1. Study Objectives

The objective of the study is to investigate whether high dose vitamin C infusion leads to pathological tumor response in resectable colorectal, pancreatic, and lung cancer (cohort A) or objective tumor response in extended RAS (e.g. KRAS or NRAS mutantion) or BRAF mutant solid tumors (cohort B).

1.1 Primary Objectives

A. To assess preliminary antitumor activity (pathological tumor response) of high dose vitamin C infusion, measured by pathologic assessment of tumor cell death and fibrotic response in resectable colorectal, pancreatic, and lung cancer in Cohort A.
B. To assess disease control rate (DCR) at 3 months as measured from CT scans using RECIST v 1.1 in metastatic colon, pancreatic, and lung cancer (Cohort B). Disease control is defined as complete tumor response, partial tumor response or stable disease at 3 months.
C. To determine the maximal tolerated dose of high dose vitamin C in combination with Y90 radioembolization in patients with RAS or BRAF mutant metastatic cancer.

1.2 Secondary Objectives

A. To evaluate the objective response rate (ORR) and progression-free survival (PFS) in cohort B and cohort C.
B. To evaluate pharmacokinetics of high dose Vitamin C.
C. To evaluate safety of high dose vitamin C administration using CTCAE 4.03.

1.3 Exploratory Objectives

A. To examine the *in vitro* activity of vitamin C in tumor organoids from cohort A patients. Correlation of GLUT1 protein expression and baseline FDG uptake with pathologic (cohort A) or objective response (cohort B);
B. Pharmacodynamic and exploratory biomarker samples will be collected at the time points specified in the protocol.

2. Background

2.1 Diseases

Colon cancer
In 2015, a total of 132,700 new cases of colorectal cancer are expected to be diagnosed in the United States accounting for about 8% of all new cancer diagnoses. In this same year

49,700 patients will die of metastatic colorectal cancers (mCRC), which will contribute to 8.4% of all cancer related mortality [1]. Activating *KRAS* and *BRAF* mutations are found in approximately 40% and 10% of human colorectal cancers (CRCs), respectively [2]. BRAF is directly downstream of KRAS, and both activate the mitogen-activated protein kinase (MAPK) pathway [3]. Clinical studies indicate that activating mutations in *KRAS* and *BRAF* predict resistance to and lack of survival benefit from epidermal growth factor receptor (EGFR)–targeting agents [4-7]. Bevacizumab, a recombinant humanized IgG-1 antibody against soluble VEGF-A, moderately improved overall survival (OS) when added to chemotherapy regimen FOLFOX4 (12.9 mo compared with 10.8 mo for the group treated with FOLFOX4 alone (hazard ratio for death 0.75) [8]. BRAF mutation is an independent prognostic factor for decreased survival [7, 9]. However, the BRAF inhibitor vemurafenib failed to confer response in previously treated CRCs carrying BRAF mutation, likely due to reactivation of EGFR pathway [10-12]. Furthermore, the combination of a BRAF inhibitor dabrafenib and MEK inhibitor trametinib led to 2% complete response and 12% partial response in a study of 43 BRAF V600-mutant patients [13]. Thus, novel therapies for *KRAS* or *BRAF* mutant CRCs are urgently needed.

Most relapse of colon cancer occurs in the first 2 years after curative resection [14]. KRAS exon 2 mutations are independent predictors of shorter time to recurrence (TTR) in patients with resected stage III distal colon cancers receiving adjuvant therapy [15].

Pancreatic cancer

Pancreatic adenocarcinoma remains one of the most devastating solid tumor malignancies with a median survival measured in months and 5-year survival rate of less than 6%. It is the fourth leading cause of cancer-related death in Europe and the United States. Most pancreatic cancers are diagnosed at late stage, and only 15–20% of patients are eligible for upfront radical surgery. Despite adjuvant treatment with chemotherapy or chemoradiation, recurrent rate is still high. For example, the CONKO-001 randomized trial reported rates of 5-year disease-free survival were 16.6% vs. 14.3%, and median disease-free survival of 13.4 months vs. 6.7 months in the gemcitabine treatment group compared with the observation group [16]. In metastatic pancreatic cancer, few regimens significantly improved overall survival compared to traditional single agent gemcitabine. The median overall survival was 11.1 months in the FOLFIRINOX group as compared with 6.8 months in the gemcitabine group [17]. The recently FDA-approved nabpaclitaxel improved median survival from 6.7 to 8.5 months when added to gemcitabine in newly diagnosed metastatic patients [18]. Genomic analyses of pancreatic cancer revealed a complex mutational landscape with four common oncogenic events in wellknown cancer genes (*KRAS, TP53, SMAD4* and *CDKN2A*), with KRAS mutations found in 92% of 456 cases studied [19].

Lung cancer

Approximately 30% of lung adenocarcinoma harbor KRAS mutations, however it is rare in sqauamous lung cancer [20-22]. KRAS and EGFR mutations are mutually exclusive, and *KRAS* mutations has been proposed as a negative predictor of efficacy from EGFR

inhibitor erlotinib [23]. A maintenance basket trial demonstrated patients with EGFR mutation who were treated with erlotinib had longer median survival than patients with KRAS mutation who were treated with MEK inhibitor selumetinib (3.5 vs 2.3 years), following initial platinum doublet chemotherapy [24]. Thus, there is an unmet need to develop KRAS mutation-specific therapy.

2.2 Investigational Agent

Ascorbate (vitamin C) is formed in the course of the uronic pathway starting from UDP-glucuronate in animals, and its synthesis can be considered as an integral part of carbohydrate metabolism [25]. Humans lack the enzyme gulonolactone oxidase (GLO) which catalyses the last enzymic step in ascorbate synthesis, thus are unable to produce endogenous ascorbate [26]. Ascorbate plays a crucial role in various hydroxylation reactions [27]. At low ascorbate concentrations, ascorbate is prone to be a pro-oxidant, and at high concentrations, it will tend to be an antioxidant [28]. Since the 1950s, ascorbate has been proposed to have anticancer effects, although evidence for improved response rates or survival outcomes has been lacking [29]. It is postulated that generation of intracellular hydrogen peroxide H2O2 is responsible for preferential toxicity to tumor cells [30, 31] (Fig. 1).

The failure of earlier studies to observe anti-tumor activity might be due to insufficient dosing to maintain tumor cytotoxic plasma concentrations of ascorbic acid [32]. Extracellular concentrations as low 100-200 μM are toxic to some cell lines, but many types of malignant cells are killed only at concentrations approaching the mM range (Fig. 2). Plasma vitamin C concentrations that are cytotoxic to cancer cells in vitro can be achieved clinically by intravenous (but not oral) administration of ascorbate. [33].

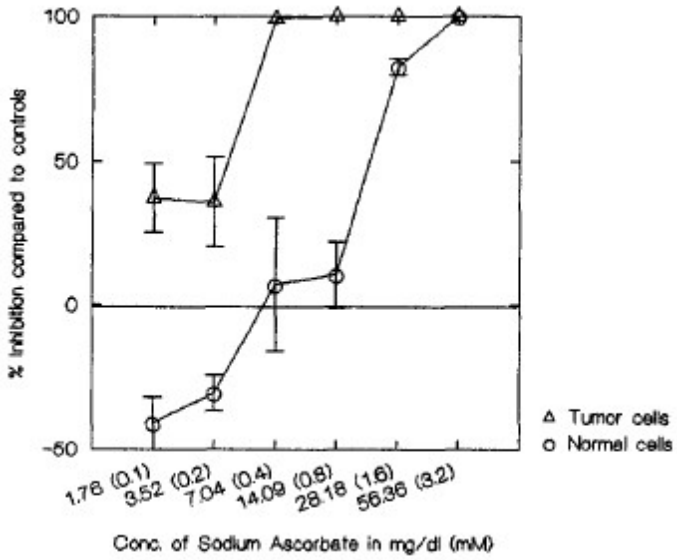

Fig. 1: Dose response of human colon fibroblast CCD-18 and human colon carcinoma cell line [32].

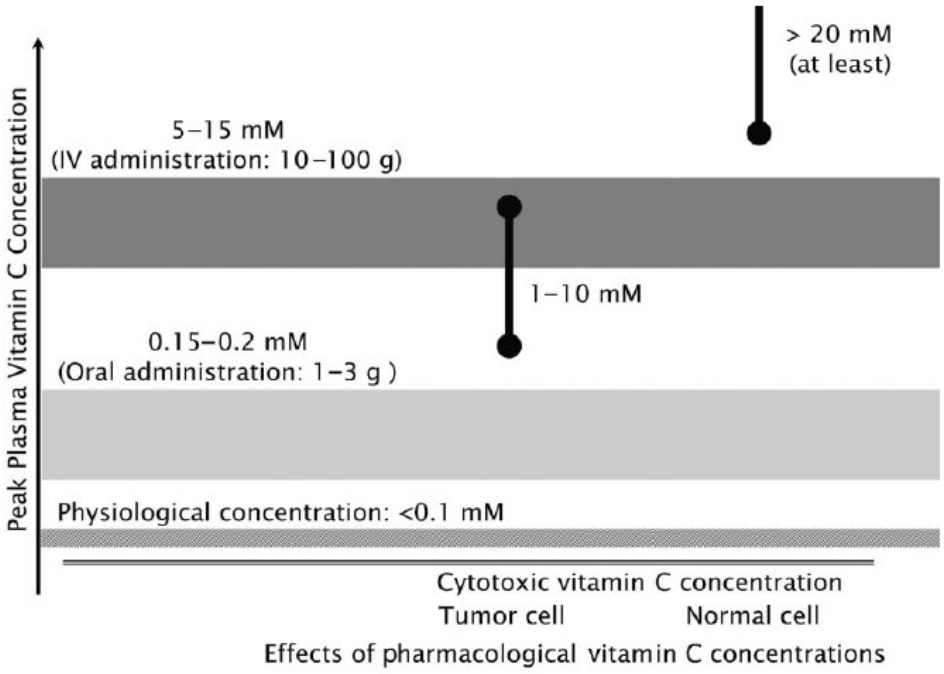

Fig. 2: Cytotoxic effects of pharmacological vitamin C concentrations in normal and tumor cells [33].

In a recent Phase I trial by Stephenson et al. in 2013, ascorbic acid was administered at 1 g/min for 4 consecutive days/week for 4 consecutive weeks [34]. They found maximum serum concentration (Cmax) correlates with area under the curve (AUC) well, and a dose of 50 g/m2 was able to achieve Cmax of 33 mM. Both 70 g/m2 and 90 g/m2 dose achieved Cmax of 49

mM, thus a dose higher than 70 g/m2 does not seem to increase Cmax further (Fig. 3). In addition, in each of the 3 highest dose groups (70 → 90 → 110 g/m2), plasma vitamin C levels were maintained between 10 and 20 mM for 5 to 6 hours. The infusion was generally well tolerated. Treatment-related nausea and headache (grade 1-2) were common in all cohorts. Dose limiting toxicity was hypernatremia and hypokalemia observed at 90 g/m2 and above. Other reported adverse events were hypertension, insomnia, abnormal urine color, loss of appetite, fatigue, chills, and hyperglycemia. Among 16 patients who completed study, there was no objective tumor response. Three patients had stable disease, while 13 had progressive disease. Of note, in a patient of 70 kg, dose of 70 g/m2 is equivalent to approximately 1.8 g/kg which is less than the 4 g/kg twice a day IP dosing used in the animal study described below [35].

In the preclinical study reported by Yun et al, 4 g/kg twice a day IP dosing achieved a Cmax of 32 mM at 10 min in xenograft mice with KRAS mutant colon cancer [35]. Treatment of at least 2 weeks was required to achieve at least 30% reduction of tumor volume compared to placebo group, with maximum reduction (approximately 50%) achieved at 3-4 weeks. Thus, it is reasonable to use 50 or 70 g/m2 for 4 consecutive days/week dosing, and a 4 week infusion schedule as described by Stephenson et al. to reach therapeutic concentration and maximize tumor response. In support of this, a Cmax of 26 mM was achieved by infusing 3 days a week even when the highest dose of 1.5 g/kg (approximately 56 g/m2) per infusion was given, as described by Hoffer et al. [36].

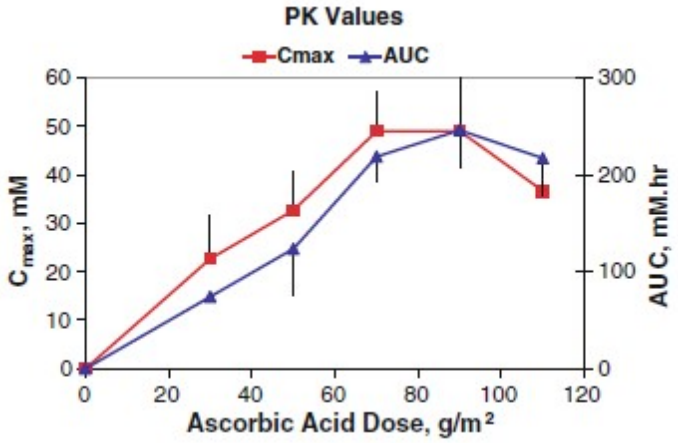

Fig. 3: Cmax and AUC values versus ascorbic acid dose [34].

A dose-escalating phase I study examined safety of combining Vitamin C with standard, fixed dose gemcitabine and erlotinib in previously untreated metastatic pancreatic patients [37]. Fourteen subjects received an 8 week cycle of intravenous ascorbic acid (three infusions per week, at doses of 50, 75, or 100 g per infusion). The treatment was well tolerated, and of the 23 total adverse events (8 being serious adverse events), most were likely attributable to progression of disease or concomitant treatment with gemcitabine and/or erlotinib. Plasma ascorbic levels were between 25.3 and 31.9 mM in patients who received 100 g per Vitamin C infusion. Among

9 patients who completed protocol treatment, 7 had stable disease and 2 had progressive disease per RECIST 1.0 criteria.

Based on these studies, we plan to treat patients with an initial test dose of 10 g vitamin C infusion on week 1 day 1, followed by daily 1.25 g/kg vitamin C infusion for week 1, days 2-4 and 4 days out of 5 weekdays in subsequent weeks, as long as no toxicities of grade 3 or higher occur. We favor this dosing regimen as it will achieve Cmax of approximately 40-50 mM and level above therapeutic concentration of above 10 mM for approximately 3-4 hours in a typical patient with very low likelihood of causing grade 3 or 4 toxicity based on the Stephenson[34], Hoffer [36], and Monti [37] phase I trials where responses were seen. Infusions will be given on a 4 times per week schedule for 2-4 weeks in cohort A to allow resection of primary tumor or recurrent/metastatic tumor as clinically indicated, and assess pathological response; or until toxicity or disease progression. In cohort B weekly infusions will be continued for up to 6 months to assess objective response and PFS. Infusions will be halted for toxicity of disease progression. The study drug vitamin C, will be provided by Bioniche Pharma USA as Ascorbic Acid, Injection, USP.

Although most studies of high dose Vitamin C infusion showed favorable safety data, rare severe adverse events of hemorrhage, hemolysis have been reported [38], thus this study will exclude patients with known G6PD deficiency who are at higher risk of developing severe hemolysis.

2.3 Rationale

Recently, Yun et al demonstrated that cultured human CRC cells harboring *KRAS* or *BRAF* mutations are selectively killed when exposed to high levels of vitamin C [35]. This effect is due to increased uptake of the oxidized form of vitamin C, dehydroascorbate (DHA), via the GLUT1 glucose transporter. Increased DHA uptake causes oxidative stress as intracellular DHA is reduced to vitamin C, depleting glutathione. Thus, reactive oxygen species accumulate and inactivate glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Fig. 4). Inhibition of GAPDH in highly glycolytic *KRAS* or *BRAF* mutant cells leads to an energetic crisis and cell death not seen in *KRAS* and *BRAF* wild-type cells. Furthermore, high-dose vitamin C impairs tumor growth in *Apc/Kras*^{G12D} mutant mice. These findings suggest highdose vitamin C infusion may have anti-tumor activity in *KRAS* or *BRAF* mutant colon cancer patients.

In cohort A (pre-operative therapy), we will enroll patients with a high likelihood of extended RAS (e.g. KRAS or NRAS) or BRAF mutations(eg pancreas, colon, and nonsquamous lung cancer). Patients in this cohort will start treatment with vitamin C prior to the mutation status being known (given the ~10-14 day turnaround time for extended RAS (e.g. KRAS or NRAS) or BRAF mutation testing), and we will estimate the effect of high dose vitamin C in all patients. Outcome differences between *KRAS* and *BRAF* mutated cancers and wild-types will be important secondary analyses.

In cohort B (salvage therapy for metastatic disease), patients will be pre-screened for extended RAS (e.g. KRAS or NRAS) or BRAF mutation status, and those patients with mutations will be eligible.

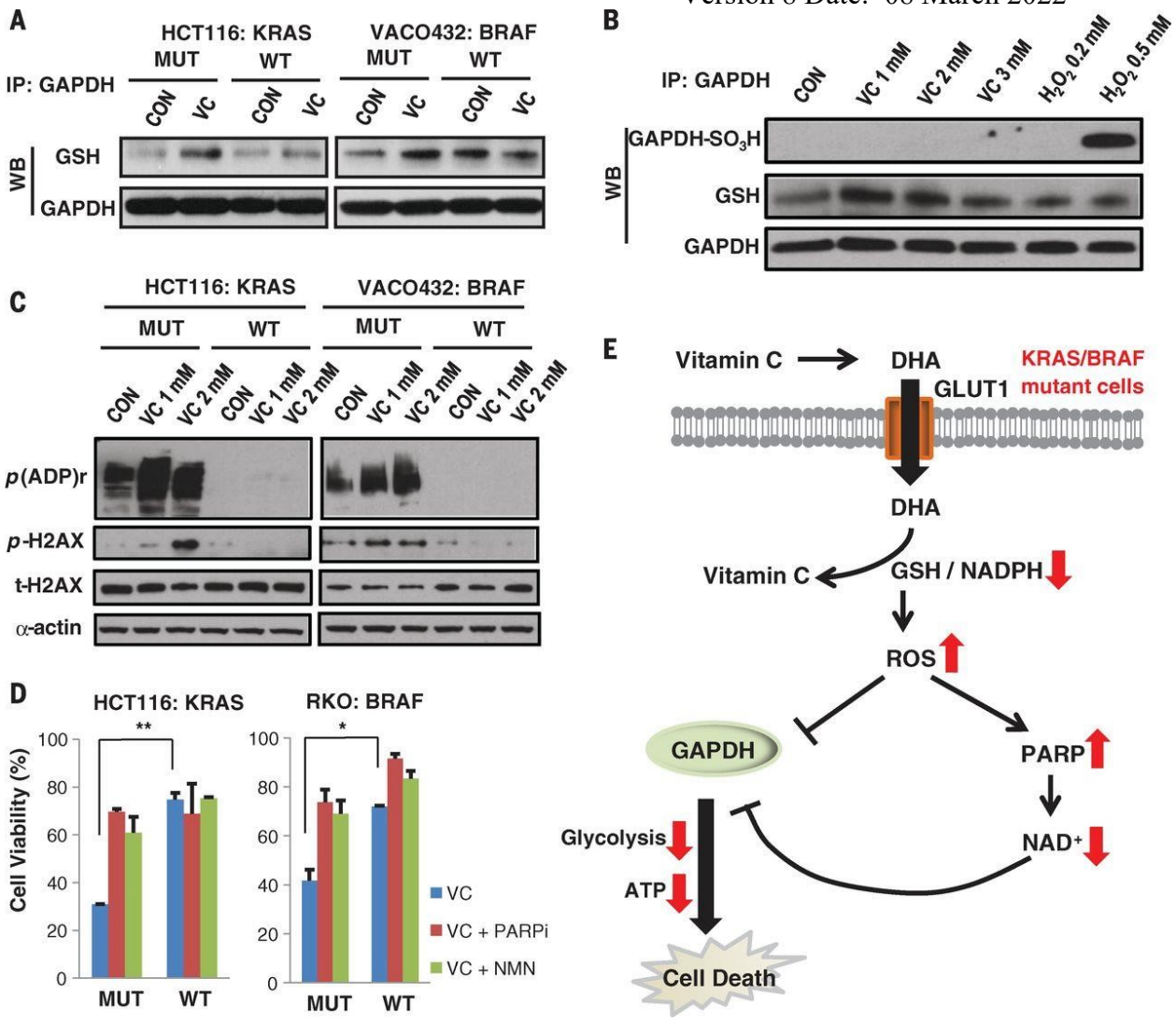

Fig. 4: Vitamin C–induced ROS inhibits GAPDH by cysteine S-glutathionylation and depletion of NAD+ [35].

2.3.1 March 2019 Update

Preliminary data of of RNA Seq analysis and metabolomics suggest that high dose vitamin C is demonstrating evidence of increased DNA damage and ROS increases, with compensatory activation of DNA repair and ROS regulation cellular pathways.

Specifically, 10 patients have enrolled on cohort B. Overall the treatment was well tolerated, and the first 6 patients were able to enroll and receive treatment without experiencing dose limiting toxicity. This prompted opening cohort A in June 2018. Thus far, a total of 10 patients have enrolled on cohort B and 3 patients in cohort A.

Metabolomics and RNA Analysis

At the cellular level, ascorbate has two forms: reduced form of ascorbate and oxidized form of dehydroascorbate (DHA). Tumor cells uptake ascorbate by sodium vitamin C cotransporters (SVCTs) via ascorbate form or by glucose transporters (GLUTs) via DHA form. Recent studies have shown that Kras mutant human colorectal cancers (CRC) cells up-regulate GLUT1 expression. Mice studies have shown that ascorbate has a selective accumulation effect on CRC cells with Kras mutation, which is known to be mostly refractory to approved targeted therapies.

By use of LC-MS, we found that both ascorbate and DHA accumulate in the Kras-mutated colon tumors after patients treated with intravenous ascorbate. DHA seems to be a better marker to assess the ascorbate treatment under our conditions. The high levels of threonate, tartarate and other degradation products of DHA further demonstrated the uptake of ascorbate/DHA by the Krasmutated tumors.

Intracellular DHA is reduced back to ascorbate at the expense of reduced glutathione (GSH) and NADPH. Increased DHA uptake causes ROS accumulated and oxidative stress due to the depletion of cellular GSH. The DNA damage induced by ROS activates Poly (ADP-ribose) polymerase, which, in turn, decreases NAD+ levels. The low NAD+ level decreases GAPDH activity. Then glycolysis is inhibited in cells that are highly dependent on energy, leading to an energy crisis and cell death. With ascorbate treatment, there is no obviously difference of ATP level observed although glycolysis decreases. Some potential oxidative stress biomarkers, such as uric acid, ADPribose, and 8-oxoGuo/8-oxoGuo, have been found to increase following ascorbate treatment. The pentose phosphate pathway (PPP) metabolite accumulation indicates PPP flux increase, which leads to NADPH formation. The increased flux is to restore cytosolic NADPH back to mitigate oxidative stress. Interestingly, the level of GSH does not decrease, while the level of its oxidized form, glutathione disulfide (GSSG) decreases with ascorbate treatment, which indicates that NADPH formed impair the potential oxidative stress.

The level of 2-hydroxyglutarte increases to 2.7-fold following ascorbate treatment, which might be related to the changes of phospholipid in the tumor.

Ascorbate treatment also induces other changes in the tumor metabolome, such as polyamine, proline, sulfur containing amino acid, branched chain amino acids, aromatic amino acids and carnitine.

Additionally, we found evidence of activation of pathways involving ROS handling and DNA damage repair. Specifically, in 6 matched pre- and post- tumor biopsies for whom RNA sequencing has been completed, 92 / 123 pathways involving ROS handling were significantly upregulated by gene set enrichment analysis. Similarly, 43/49 pathways involved in oxidative stress were also significantly upregulated, as well as 84/ 102 pathways involved in DNA repair.

Rationale for Cohort C

These data cumulatively suggest that high dose ascorbate is entering the cell and leading to increased ROS and resultant DNA damage, but that the cancer cells have upregulated compensatory mechanisms to handle this additional stress. This suggests that adding another direct cytotoxic insult to high dose ascorbate treated tissues may have significantly greater activity. In Sept 2018, Alexander and colleagues published their initial report of the safety of high dose ascorbate in combination with radiation for pancreatic cancer, in combination with gemcitabine[39]. In this study, patients with locally advanced or metastatic pancreatic cancer received weekly gemcitabine, along with standard radiation to 50.4 Gy in standard fractionation or 50 Gy by intensity modulated RT (IMRT), along with high dose ascorbate given in escalating doses (50 g → 75 g → 100 g) daily during radiation. This study found that 75 g ascorbate given intravenously daily during radiotherapy and weekly gemcitabine was safe and tolerable. There was preliminary evidence of improved efficacy as well, with median overall survival of 21.7 months, which compared favorably to a similar historical control population[39].

Recent PK analysis demonstrates that Vitamin C is no longer measurable in the serum after 24 hours from injection. We therefore believe that we can greatly increase the synergy between Y90 therapy and high dose vitamin C by concurrent administration. For this reason, the study schema for Cohort C has been updated to allow Vitamin C to be administered same-day, prior to or within 24 hours of a Y90 treatment by an expansion of the phase I study.

Additionally, it is apparent that Y90 therapy is now commonly performed with super-selective Y90 delivery to the specific tumor being treated. This limits toxicity and allows for an ablative dose to be delivered safely to the target lesion. For this reason, patients may require a 2nd Y90 therapy to target remaining hepatic metastases. Subjects who clinically require an additional Y90 treatment may be eligible for second treatment with high dose Vitamin C after reconsideration of eligibility for Cohort C. During the dose escalation phase, eligible patients will be treated at one dose level below the currently enrolling dose level if they have not experienced prior DLT with previous protocol therapy.

2.4 Risk/Benefit Assessment

This is a Phase II study to study the effect of vitamin C in patients with solid tumor malignancies. The preclinical data suggest that the activity of vitamin C is specific for tumors that harbor the prevalent KRAS or BRAF cancer mutation so we will preferentially treat such tumors (e.g. pancreas, colon, and lung cancer). The pre-operative setting is chosen because it offers an opportunity to obtain tissue following a short course of medical therapy to specifically identify the effect of that therapy on the tumor. The risk to subjects is minimal as there is often a 3-4 week time period from initial evaluation to resection and thus the trial will not extend the time to surgery. For cohort B, the risks are also minimal. There are no standard therapies for KRAS or BRAF mutant colon, pancreas, or lung cancers. We will monitor for efficacy and toxicity closely. Patients who are not benefiting from therapy may go on to receive other palliative therapy for their disease, as appropriate. Risks of large doses

of vitamin C are rare, and include electrolyte changes (hypokalemia, hypernatremia), headaches, nausea, hypertension, insomnia, abnormal urine color, loss of appetite, fatigue, chills, and hyperglycemia. For Cohort C, high dose vitamin C has not previously been administered to patients who also receive Yttrium-90 radioembolisation for liver metastases. However, high dose vitamin C has been previously safely been given to patients receiving external beam radiation to primary pancreatic tumors. We believe the risks of combining vitamin C with radioembolization are minimal, but will carefully examine the safety and tolerability of this combination in a standard dose escalation phase I cohort design. The starting dose of Vitamin C will be 0.5 g/kg given 3-4 x/week for 1week prior to and following standard dose Y-90 radioembolization.

Malignancies with *KRAS* or *BRAF* mutations tend to be more aggressive and offer a worse prognosis than wild-type tumors. Therefore, there may a benefit if we can demonstrate antitumor activity with vitamin C therapy.

2.5 Correlative Studies Background

1. Yun and colleagues showed that the oxidized form of vitamin C (dehydroascorbic acid (DHA)) is shuttled intracellularly via GLUT1 in KRAS or BRAF mutated colorectal cancer (CRC) cell lines and mouse models which resulted in growth inhibition and cell death [35]. We expect higher baseline tumor GLUT1 protein expression predict tumor response to Vitamin C infusion.

2. In a preclinical study, AMP-activated protein kinase (AMPK) was activated within 1 hour of Vitamin C treatment, suggesting Vitamin C infusion exerts anti-tumor effect by inhibiting glycolysis and depleting ATP [35]. We plan to assess tumor energy stress by measuring ROS level or phosphor-AMPK IHC in resected tumor samples from cohort A patients.

3. We will examine tissue specimens for molecular and metabolic changes associated with vitamin C use. This will include RNA sequencing (both bulk and single cell RNA sequencing depending on tissue availability), and whole exome sequencing. Additionally, we will grow organoids from tissue specimens and treat with vitamin C *in vitro*.

4. Patients with solid tumors demonstrate elevated rates of clonal hematopoiesis (CH) thus placing them at elevated risk of subsequent cardiovascular disease and hematologic cancer[40, 41]. Thus, development of a management strategy to mitigate this risk represents an important unmet need. Environmental stimuli can impact the expansion, attrition, and disease impact of CH clones including high glucose, metformin, and vitamin C. Our objective is therefore to assess the impact of high dose vitamin C on existing CH clones in cancer patients under study. We will isolate peripheral blood and buccal swabs from patients. Genomic DNA will be isolated and

we will perform DNA and RNA sequencing to detect and characterize clonal hematopoiesis.

3. Subject Selection

3.1 Study Population

Adult patients with solid tumor malignancy who are eligible for resection and meet the inclusion and exclusion criteria will be considered for participation in cohort A. Patients with metastatic tumors who meet the inclusion and exclusion criteria will be eligible for participation in cohort B. Patients eligible for cohort C will be patients with colorectal cancer with hepatic metastases amenable to Y90 radioembolization.

3.2 Inclusion Criteria

1. Male or female ≥ 18 years of age.

2. Patients with histologically proven early stage or locally advanced colorectal adenocarcinoma, lung cancer or pancreatic cancer, who are eligible for resection (cohort A).

3. Patients with inoperable, metastatic extended RAS (e.g. KRAS or NRAS) or BRAF mutant colorectal adenocarcinoma,lung cancer and pancreatic cancer, or other solid tumor, who have received at least 1 line of treatment for metastatic disease (cohort B).

Note: If subject refuses chemotherapy and therefore has no prior chemotherapy, they can be eligible for the trial with approval from the primary investigator.

3.1 Patients with metastatic cancer with an extended RAS (e.g. KRAS or NRAS) or BRAF mutation with liver metastases amenable to Y90 radioembolization (cohort C).

4. ECOG performance status 0-1.

5. Life expectancy of at least 6 months.

6. All women of child-bearing potential and all sexually active male patients must agree to use effective contraception.

7. Patient with adequate organ and marrow function as follows:

- ANC ≥ 1000 mm3,
- Platelets ≥ 100,000/mm3
- Hemoglobin ≥ 9 g/dL,
- Serum creatinine ≤1.8 mg/dL or creatinine clearance > 50 mL/min (Appendix C: Estimating Creatinine Clearance) ○ Note: Patients with a serum creatinine > 1.8 mg/dl will be excluded. For patients with a serum creatinine less than 1.8 mg/dl, an estimated calculated GFR of > 50 ml/min will be required.
- bilirubin ≤ 1.5 x ULN mg/dL ○ note: patients with Gilbert Syndrome must have a serum bilirubin < 3.0 x ULN
- Alanine aminotransferase (ALT), aspartate transaminase (AST) ≤ 2.5 times the upper limit of normal if no liver involvement or ≤ 5 times the upper limit of normal with liver metastases.

8. Patients with serum electrolytes (including calcium, magnesium, phosphorous, sodium and potassium) within normal limits (supplementation to maintain normal electrolytes is allowed).

9. Patients taking Vitamin C will have stopped taking oral vitamin C more than 1 week before planned study treatment.

10. Patients capable of understanding and complying with the protocol and who have signed the informed consent document.

3.3 Exclusion Criteria

1. Patients with uncontrolled intercurrent illness including, but not limited to uncontrolled infection, symptomatic congestive heart failure (NYHA class III and IV), uncontrolled cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements (Appendix B: New York Heart Association (NYHA) Classifications).

2. Patients with active heart disease including myocardial infarction within previous 3 months, symptomatic coronary artery disease, arrhythmias not controlled by medication, unstable angina pectoris, or uncontrolled congestive heart failure (NYHA class III and IV) (Appendix B: New York Heart Association (NYHA) Classifications).

3. Patients who have received systemic chemotherapy or targeted therapy for metastatic disease within 2 weeks from start of study drug treatment (cohort B).

4. Patients who have received an investigational drug within 21 days of the first dose of study drug.

5. Patient who have not recovered to grade ≤ 1 from adverse events (AEs) due to investigational drugs or other medications, which were administered more than 4 weeks prior to the first dose of study drug.

6. Patients who are pregnant or lactating.

7. Patients who are known to be positive for the human immunodeficiency virus (HIV). The effect of Vitamin C on HIV medications is unknown. Note: HIV testing is not required for eligibility, but if performed previously and was positive, the patient is ineligible for the study.

8. Patients who have the inability or unwillingness to abide by the study protocol or cooperate fully with the investigator or designee.

9. Patient who are receiving drugs which are known to interact with Vitamin C, potential risk and eligibility will be evaluated individually by the investigator. a. Most of the known interactions with vitamin C are from oral use and acidification of the stomach lining. There are few known interactions with high dose intravenous vitamin C. We recommend not using deferoxamine as there may be an association with ventricular dysfunction (unknown mechanism).

10. Patients who have uncontrolled or severe hyponatremia, hypernatremia, SIADH, hypokalemia, hyperkalemia, hypomagnesemia, or hypermagnesemia

11. Patients who have uncontrolled or severe coagulopathies or a history of clinically significant bleeding within the past 6 months, such as hemoptysis, epistaxis, hematochezia, hematuria, or gastrointestinal bleeding.

12. Patients who require therapeutic doses of warfarin is prohibited.

13. Patients who have uncontrolled seizure disorder, ascites, iron overload, edema, or dehydration.

14. Patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency, hereditary spherocytosis, or other conditions predisposing patient to hemolysis.

15. Patients who have a known history of recurrent oxalate renal calculi or multiple oxalate.

4. Registration Procedures

4.1 Patient Registration – Part 1

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- First and last page of signed informed consent form

Registration must be completed within 24 hours of the signing of informed consent.

4.2 Patient Registration – Part 2

Study participants will be centrally registered with the Weill Cornell Medicine Joint Clinical Trials Office (JCTO). To register a new study subject, email the following documents to JCTOIIT@med.cornell.edu :

- Completed WCM subject registration form
- First and last page of the fully executed informed consent form, plus additional pages if checkboxes for correlative studies are required
- Fully executed HIPAA research authorization form
- Eligibility checklist signed and dated by investigator and research nurse
- Documentation of any eligibility waivers granted
- Source documentation to verify eligibility

Note that attachments larger than 4.5 MB are not accepted, so larger attachments should be split into more than one email. Central registration information is reviewed and entered into the REDCap database.

5. Study Procedures

This section is a visit-by-visit listing of all the procedures that will take place at each visit. If the study will have multiple procedures in one day (e.g. blood draws for a PK study) then the timing of each of these should be included.

This is a single arm study, all eligible subjects will be given Vitamin C infusion. In cohort A, tumor sample from all patients will be tested for extended RAS (e.g. KRAS or NRAS), BRAF mutations, but all eligible patients will start study drug treatment before test result is available. For cohort A, tissue specimens will collected from an optional screening biopsy (screening biopsy is not mandatory, but highly encouraged) .and from the on study surgical resection. For cohort B patients a separate tissue biopsy will be required at screening and between weeks 3 and 6. This is to confirm extended RAS (e.g. KRAS or

NRAS) or BRAF mutational status and collection of tissue for correlative studies. For Cohort C patients, biopsies will be collected at screening and at approximately 6-8 weeks, and will be examined for evidence of increased oxidative stress (eg. RNASeq, metabolomics, and Organoid development if not already performed).

March 2021 Update:
Cohort C patients will receive vitamin C infusion and Yttrium-90 (Y90) during week 1 of therapy and again for 1-2 weeks following Y90 therapy, with a single dose of Vitamin C administered on the day of Y90 therapy, just prior to Y90 therapy, or within 24 hours of Y90 therapy.

Previously treated subjects from Cohort C, may be considered to participate in additional treatment if they have not experienced prior DLT with previous protocol therapy. Once eligibility is confirmed, subjects will be treated at one dose level below the currently enrolling dose level in the dose escalation phase.

Y-90 radioembolization is the selective internal radiation therapy of liver tumors utilizing SIR-Spheres Y-90 resin microspheres, which were approved by the FDA in 2002. SIR-Spheres Y-90 resin microspheres are microscopic resin beads that contain the radioactive isotope yttrium-90 (Y-90) which emits beta radiation. Due to their small size (average size 32.5 microns) and similar specific gravity to blood cells, the microspheres travel easily through the hepatic artery. The radioactive microspheres become lodged in the tumor microvasculature. Selective Internal Radiation Therapy (SIRT) with SIR-Spheres Y-90 resin microspheres is considered a safe and effective method of using radiation to treat colorectal liver metastases and are often used in conjunction with chemotherapy. SIRSpheres Y-90 resin microspheres are currently being offered at more than 1,230 medical centers around the world, including more than 300 centers in the U.S. Over 92,000 doses of SIR-Spheres Y-90 resin microspheres have been supplied worldwide.

Vitamin C protocol will allow for the use of samples collected through the biobank protocol #090810582 "Weill Cornell Digestive Disease Registry" PI: Manish Shah and the protocol # 1305013903 “Research for Precision Medicine” PI: Cora Sternberg, M.D

5.1 Schedule of Evaluations

After initial screening, patients will receive the agent infusion in four days per week (no more than 2 consecutive days of infusion)). Weekly blood testing will be performed once per week. Cohort A patients will receive infusions on weeks 1-4 or until surgery (minimum 2 weeks of vitamin C (8 infusions) are required to consider the patient evaluable). Cohort B patients will receive infusions for up to 6 months or until disease progression. On Day 1 of week 2 (Preferred; Day 1 or Day 3 of weeks 2 to 5 is also allowed) patients will remain in the clinic for up to 12 hours for vitamin C pharmacokinetic study. Patients need to receive at least 1 week (4 full doses, the test dose is not accounted) of Vit C to be considered evaluable.

For Cohort C, the vitamin C dose will be escalated in successive cohorts. Specifically, the first 3 patients were enrolled at a dose of 0.5 g/kg intravenously for 4 days /week for 1-2 weeks prior to and following Y90 therapy for a total of 4 weeks of vitamin C. No dose limiting toxicities were identified. The next group of 3 patients received a dose of 0.75 g/kg intravenously for 4 days/ week for 1-2 weeks prior to and following Y90 therapy for a total of 4 weeks of vitamin C. Subsequent dose escalations will involve including a single dose of Vitamin C on the day of Y90, administered prior to or within 24 hours of a Y90 treatment, as well as continued dose escalation to 1 g/kg and 1.25 g/kg intravenously (Dose levels 3-6). Patients will not be treated at a dose higher than 1.25 g/kg. Dose limiting toxicity will be defined as any grade 3-4 adverse event possibly, probably, or definitely attributed to vitamin C therapy in the 21 days of protocol therapy. In any group of 3 patients, if one patient experiences dose limiting toxicity, the group will be expanded by 3 additional patients (eg. 6 for that group). If, at any dose level, 2 or more patients experience a dose limiting toxicity, the maximal tolerated dose will be reached, and further dose escalation will not be pursued. The dose level may then be expanded up to 10 additional patients to confirm the safety and toxicity at that dose level. Additional dose levels may be included depending on toxicity observed. Patients who require additional Y90 treatment, clinically, may be considered for second treatment at the currently enrolling dose level if they have not experienced prior DLT with previous protocol therapy. Cohort C Dose levels are as follows:

Dose level	Vit C dose per infusion (weekly monotherapy)	Vit C dose on Day of Y90 Rx
Dose level 1	0.5 g/kg	0
Dose level 2	0.75 g/kg	0
Dose level 3	0.75 g/kg	0.5 g/kg
Dose level 4	1.0 g/kg	0.5 g/kg
Dose level 5	1.0 g/kg	0.75 g/kg
Dose level 6	1.25 g/kg	0.75 g/kg

January 2020 update: Dose level 1 has enrolled without dose limiting toxicity, and patients are currently being treated at dose level 2, also without dose limiting toxicity thus far.
March 2021 update: Subjects are currently being treated at dose level 4.

March 2022 update: Beginning with Dose level 5, we stopped administering Vitamin C in Weeks 1 and 2, prior to Y90 treatment. Additionally, we have not observed any dose limiting toxicity through dose level 6. As a result, we will continue our dose escalation with dose levels 7 and 8, as per the table below. We will also plan to treat up to 4 additional patients at the maximal tolerated dose, so the total accrual for Cohort C will remain 28 evaluable patients.

Dose level	Vit C wk 2-3	Day of Y90 Rx
Dose level 5	1.0 g/kg	0.75 g/kg
Dose level 6	1.25 g/kg	0.75 g/kg
Dose level 7	1.25 g/kg	1.0 g/kg
Dose level 8	1.25 g/kg	1.25 g/kg

Table 1. Schedule of Evaluations (Cohort A)

	Screening	Wk1[a]	Wk2[a]	Wk3[a]	Wk4[a]	Wk5	End of Study[h]
	Day -28 to -1					Surgery[i]	Wk 4-16
Agent Administration		X	X	X	X		
Informed consent	X						
Demographics	X						
Medical history	X						
Concurrent medication	X	X	X	X	X		X
Eligibility Criteria	X						
Physical exam with ECOG	X	X	X	X	X		X
Vital signs [b]	X	X	X	X	X		X
Height	X						
CBC w/diff	X	X	X[g]	X	X		X
Serum chemistry [c]	X	X	X[g]	X	X		X
PT/PTT	X						
EKG	X						
Adverse event evaluation		X	X	X	X		X
Fresh Tissue Collection	X d			X d-			
PET-CT (with contrast)	X						
Urinalysis	X						
G6PD enzyme	X						
Serum Pregnancy Test	X						
Vitamin C pharmacokinetics [f]			X				
Tumor markers: CEA, CA19-9	X				X		X

a: Agent administered on four days/week, other testing done on day 1
b: 5-minute sitting blood pressure, pulse, respiratory rate, oral temperature, weight c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
d. Baseline fresh biopsy is highly encouraged, but not mandatory. If not performed, archived tissue is requested. Pathologic review of surgical resection. Surgical Resection can occur at any time point between Week 3 and 5.
e: Women of childbearing potential f: PKs will be collected at Day 1 of Week 2 (Preferred; Day 1 or Day 3 of weeks 2 to 3 is also allowed) the following time points: prior to infusion, 1hr post start of infusion, end of infusion, 2hrs, 3hrs, 4hrs, 6hrs, 24hrs post end of infusion
g. CBC and chemistries can be collected within 3 days of treatment.; 1 day preferred
h. End of Study assessments are to be completed between week 4 and 16 after surgical resection
i. Vit C treatment is given for 2-4 weeks. Surgery is to occur as per clinical schedule.

Table 2. Schedule of Evaluations (Cohort B)

	Screening	Wk1[a]	Wk2a	Wk3[a]	Wk4[a]	Wk5[a]	Wk6[a]	Wk7-11[a]	Wk12[a]	Wk13-24[a]	End of Study
	Day -28 to -1										4-8 weeks post Vit C completion [k]
Agent Administration		X	X	X	X	X	X	X	X	X	
Informed consent	X										
Demographics	X										
Medical history	X										
Concurrent medication	X	X	X	X	X	X	X	X	X	X	X
Eligibility Criteria	X										
Physical exam with ECOG	X	X	X	X	X	X	X	X	X	X	X
Vital signs[b]	X	X	X	X	X	X	X	X	X	X	X
Height	X										
CBC w/diff [e]	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry [c, e]	X	X	X	X	X	X	X	X	X	X	X
PT/PTT	X										
EKG	X										
Adverse event evaluation		X	X	X	X	X	X	X	X	X	X
PET-CT (with contrast)	X[i]						X		X	Xh	
G6PD enzyme	X										
Urinalysis	X										
Serum Pregnancy Test [d]	X[d]										
Vitamin C pharmacokinetics [f]			Xg								
Tumor markers: CEA, CA19-9	X						X		X		X
Biopsy Collection	X			Xj							

a: Agent administered on three-four days/week, blood work (CBC/ Chemistry) performed once per week
b: Sitting blood pressure, pulse, respiratory rate, oral temperature, weight
c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
d: Applicable for women of childbearing potential
e: CBC and chemistries can be collected within 3 days of treatment; 1 day preferred.
f: PKs will be collected at the following time points on Day 1 week 2 prior to infusion, 1hr post start of infusion, end of infusion, 2hrs, 3hrs, 4hrs, 6hrs, 24hrs post end of infusion.
g: If PK sample collection is not possible on Week 2, Day 1 , the blood sample should then be collected on day 1 or Day 3 between subsequent weeks 2 to 5
h: PET-CT will occur at week 6, 12 and 24.
i. Screening PET-CT must be done within 30 days prior to enrollment
j. On treatment, biopsy can take place between week 3 and week 6.
k. End of study assessments can be done via telephone call (only adverse event & concurrent medication evaluation will be necessary with a telephone assessment.)

Table 3. Schedule of Evaluations (Cohort C)

	Screening	Wk1		Wk2	Wk3	Wk4	End of Study Week 8-16[i]
	Day -28 to -1	Day 1	Y90 Rx Day 2 (+2 day window)				
Agent Administration[a]		X	X[b] (single dose)	X	X		
Informed consent	X						
Demographics	X						
Medical history	X						X
Concurrent medication	X		X	X		X	
Eligibility Criteria	X						
Physical exam with ECOG	X		X	X		X	X
Vital signs[c]	X		X	X		X	X
Height	X						
CBC w/diff[e]	X		X	X		X	X
Serum chemistry[d,e]	X		X	X		X	X
PT/PTT	X						
EKG	X						
Adverse event evaluation			X	X		X	X
Biopsy Collection	X					X	
Correlative Bloods[j]	X					X	
Radioembolization (Y90)[b]			X[b](single dose)				
PET-CT (with contrast)	X[h]					X	
Urinalysis	X						
G6PD enzyme	X						
Serum Pregnancy Test [g]	X						
Vitamin C pharmacokinetics [f]							
Tumor markers: CEA, CA19-9	X				X		X

a: Agent administered on three-four days/week, blood work (CBC/ Chemistry) performed once per week
b: Single dose Y90 administered same day or within 24 hours of Vitamin C treatment on day 2 (+2 day window).
c: Sitting blood pressure, pulse, respiratory rate, oral temperature, weight
d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
e: CBC and chemistries can be collected within 3 days of treatment; 1 day preferred.
f: PK is performed for cohorts A and B only.
g: Applicable for women of childbearing potential.
h: Screening PET-CT must be done within 30 days prior to enrollment.
i: End of study assessments can be done via telephone call (only adverse event & concurrent medication evaluation will be necessary with a telephone assessment.)
j: Correlative bloods are for clonal hematopoiesis analysis

5.1.1 **Screening Visit**

(Within 4 weeks (28 days) of start of treatment, unless otherwise noted)

- Informed consent **(*within 30 days*)**
- Demographics
- Medical history
- Concomitant Medications
- Physical exam
- Height
- Vital signs (5-minute sitting blood pressure, pulse, respiratory rate, temperature, weight)
- ECOG performance status (Appendix A: ECOG Performance Status Criteria)
- Serum Pregnancy Test
- Complete Blood Count (CBC) with differential
- PT/PTT
- Urinalysis
- Serum chemistry (albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium)
- G6PD enzyme
- Tumor markers: (specific to the cancer type such as CEA, CA19-9)
- EKG
- PET-CT **(*within 30 days*)**
- Tissue Collection (screening biopsy is not required if tumor lesion is too small or per PI discretion. Non WCMC site should have a documented discussion with the principle investigator)
- *Correlative Bloods (Cohort C Only)*

5.1.2 **Treatment Phase**

Infusions will be given on 4 out of 5 days each week on week 1-4 in cohort A and weeks 1-24 in cohort B – or until disease progression. In Cohort C, infusions will be given on weeks 1-3 (up to 3 weeks total). Following an initial Vitamin C test dose on day 1 week 1, a single dose of Y90 will be administered on the same day or within 24 hours of the Vitamin C infusion. Radioembolization (Y90) should occur on day 2 (+2 day window) during week 1. No more than 2 consecutive days of infusions should be administered per week. VIT C will continue for 1-2 weeks following RAE (for a total of up to 3 weeks of therapy).

5.1.2.1 One day of each week when infusion given:

- Physical exam
- Vital signs (blood pressure, pulse, respiratory rate, oral temperature, weight) the vital signs are recorded pre-dose each infusion day.
- ECOG performance status (Appendix A: ECOG Performance Status Criteria)
- PKs will be collected at the following time points on Day 1 of Week 2 (Preferred; Day 1or Day 3 of weeks 2 to 5 is also allowed)): prior to infusion, 1hr post start of infusion, end of infusion, 2hrs, 3hrs, 4hrs, 6hrs, 24hrs post end of infusion (+/- 15 minutes).
- Complete blood count (CBC) with differential collected within 3 days of treatment; 1 day preferred
- Serum chemistry (albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium) collected within 3 days of treatment; 1 day preferred
- Tumor markers: CEA, CA19-9 (only at week 4 for Cohort A & C, at week 6 for Cohort B)
- Adverse event evaluation

5.1.2.2. Other On Study Evaluations

- PET CT (with contrast) are to be performed on weeks 6, 12 and 24 for Cohort B. They will be performed at screening for Cohort A. And at screening and between week 6-8 for Cohort C.
- Post treatment research biopsy to be performed between week 6-8 (cohort C)
- Surgical Resection (cohort A)
- On-study Biopsy between week 3-6 (Cohort B)
- *Correlative Bloods between week 6-8 (Cohort C Only)*

5.1.3 End of Study

For cohort A, end of study assessments can be done after surgical resection between weeks 4 and 16. For cohort B, end of study assessment will occur 4-8 weeks from end of treatment (+/- 4 weeks). For Cohort C, end of study assessment will occur between weeks 8-16. Note: end of study assessments for all cohorts can be done as a telephone assessment, in which only adverse & concurrent medication is reviewed with the patient.

- Vital signs (blood pressure, pulse, respiratory rate, oral temperature, weight)
- Physical Exam
- ECOG performance status
- Complete blood count (CBC) with differential
- Serum chemistry (albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, calcium, magnesium, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium)
- Adverse event evaluation
- Tumor markers- CEA, CA19-9

5.2 Treatment Administration

Treatment will be administered on an *outpatient* basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications for vitamin C are described in Section 6.

Patients will be treated with a test dose of 10 g vitamin C infusion on day 1 of week 1. If there is no grade 3 toxicity, patients will be given a proposed dose of on day 2 and subsequent infusions. Vitamin C will be administered over 2 hours for 4 days/week for 4 consecutive weeks in cohort A. In Cohort B, vitamin C will be administered over 2 hours for 4 days/week for up to 6 months, until progression or unacceptable adverse events. In cohort C, Vitamin C will be administered over 2 hours for 4 days/week. During week 1, Vitamin C will be administered on day 2 (+2 day window) prior to or within 24 hours of a Y90 treatment. Infusions will take place over a total of up to 3 weeks. Patients may receive Vitamin C for 3 days/week for logistical issues or patient preference after discussion with the PI or co-PI. Treatment cannot be given on 3 consecutive days.

For patients receiving doses of 100g or more of Vitamin C, infusion times may exceed 2 hours due to limitations of IV pump rates

5.3 General Concomitant Medication and Supportive Care Guidelines

Supportive measures including analgesics, blood transfusions, and antimicrobials are permitted throughout the trial. Concurrent chemotherapy or other anti-neoplastic agents are not allowed.

5.4 Duration of Therapy and Criteria for Removal from Study

In the absence of treatment delays due to adverse event(s), treatment may continue for 2-4 weeks in cohort A and up to 6 months in cohort B and up to 4 weeks for Cohort C, or until one of the following criteria applies:

- Treatment related toxicity which results in a delay of planned curative surgery,
- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

6. Dosing Delays/Dose Modifications

Table 4A . For Cohort A and B

AGENT(S)	DOSE	ROUTE	VISIT
Vitamin C	10 g	Intravenous	Week 1, Day1– Test Dose
Vitamin C	1.25 g/kg	Intravenous	Wk 1 D2 – Wk1 D4 Wk X D1 – WkX D4 (subsequent weeks)
Vitamin C	1.25 g/kg	Intravenous	3- 4 days/week until EOS

Patient who do not tolerate the test dose, should not continue with the study. There is no dosing cap for vitamin C therapy.

Patients may receive treatment if they have not experienced any grade 3-4 event attributable to IV vitamin C.

If a grade 3-4 attributable event occurs, it must improve to grade 2 or better prior to resuming therapy. Vitamin C would then be administered at a reduced dose of 1.0 g/kg IV for up to 4 days per week.

A delay in treatment of up to 2 weeks is permitted. If a treatment visit week does not occur, the following week should be the next treatment week

Table 4B. For Cohort C

The study design for cohort C is a traditional 3+3 dose escalation design. Sequential dose levels will accrue as per the following dosing schema.

Dose level	Vit C dose per infusion (weekly monotherapy)	Vit C dose on Day of Y90 Rx
Dose level 1	0.5 g/kg	0
Dose level 2	0.75 g/kg	0
Dose level 3	0.75 g/kg	0.5 g/kg
Dose level 4	1.0 g/kg	0.5 g/kg
Dose level 5	1.0 g/kg	0.75 g/kg
Dose level 6	1.25 g/kg	0.75 g/kg

The DLT observation period will start with the first vitamin C infusion prior to radioembolization and end after the last vitamin C infusion post-embolization. A DLT is defined as a grade 3+ (latest CTCAE version) adverse event that is at least possibly related to the vitamin C infusion.

7. Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

7.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use

in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

7.1.1 Investigational Agent or Device Risks

The following adverse events were reported in previous clinical studies of Vitamin C infusion:

Proteinuria, hypertension, tumor fever, leg edema, hypoalbuminemia, hypokalemia, hypernatremia, hypercalcemia, increased creatinine, hyperglycemia, peripheral neuropathy, headache, hemolysis, hemorrhage, elevated aminotransferase, and kidney stones.

7.1.2 Adverse Event Characteristics and Related Attributions

NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for tracking adverse events during the conduct of this study.
CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.1.3 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log after Week 1, Day 1. The AE log will be maintained by the research staff and kept in the patient's research chart.

7.1.4 Reporting of AE to WCMC IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

7.2 Definition of SAE

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

- Death (NOTE: death due to disease progression will be recorded on the CRF and does not need to be reported as an SAE.
- A life-threatening adverse drug experience—any adverse experience that placed the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Requires at least a 24-hour inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.2.1 Reporting of SAE to IRB and DSMB

All SAEs occurring on this study will be reported to the IRB and DSMB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

For investigator-initiated studies where the WCM investigator holds the IND, the following reporting guidelines apply:

a. A Mandatory MedWatch Form 3500A must be completed and sent to the FDA. Specific instructions on how to complete the MedWatch Form 3500A can be found on the FDA website.
b. If the protocol requires SAEs to be reported to an outside agency (i.e., to the supplier of the study drug), specific instructions will be stated in the protocol.

c. If the study utilizes the WCM Data and Safety Monitoring Board (DSMB), a narrative of the event will be submitted to the Regulatory Coordinator on a modified SAE cover sheet. The completed form serves as acknowledgement of the SAE's occurrence and will be used to complete the DSMB periodic report. A copy should be filed in the regulatory binder.

d. For multicenter studies, the multicenter core within the Quality Assurance Unit (QAU) will distribute the IND Safety Report to participating sites. The MedWatch and SAE cover sheet must be emailed to JCTOIIT@med.cornell.edu within 7 days of the investigator notification.

7.3 AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required. AE/SAEs attributed to Vitamin C should be followed until resolution. This requirement indicates that follow-up may be required for some events after the patient discontinues participation from the study.

8. Pharmaceutical Information

A list of the adverse events and potential risks associated with vitamin C can be found in Section 7.1.

8.1 Investigational Agent

Vitamin C is supplied as Ascorbic Acid for Injection USP, dissolved in water, 500 mg/mL, packaged in a 50 mL sterile dispensing vial, each mL contains Ascorbic Acid 500 mg, Edetate Disodium 0.025%, Sodium Hydroxide 0.011%, Water for injection q.s. pH 5.5-7.0 adjusted with Sodium Bicarbonate. The vials should be stored in carton until time of use. Store between 2°-8° C (36°-46° F).

8.2 Availability

Vitamin C is available through commercial supply.

8.3 *Agent* Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents

received on a site Drug Accountability Record Form. Participating sites should follow their institutional standard for drug accountability.

9.0 Laboratory Correlative Studies

Using data from this trial, the relationship between molecular markers associated with Vitamin C transport and glycolysis, and the clinical outcomes with Vitamin C infusion will be explored. Tissue collections will be performed for for GLUT1, metabolomics, RNA sequencing, organoids and future correlative studies. Vitamin C pharmacokinetics will also be assessed.

9.1 Serum Vitamin C pharmacokinetics

Vitamin C pharmacokinetics will be measured on the week 2 day 1 of infusion, when proposed dose will be administered. If for any reason, PK sample collection is not possible on week 2 day 1, the blood sample should then be collected on day 1 or Day 3 between subsequent weeks 2 to 5. For Cohort C, the blood collection for PK analyses may occur during week 2, 4 or 5, on day 1 or 3 of that week.

9.1.1 Method of Assessment

Liquid-chromatography/mass spectrometry (LC/MS) will be performed to measure Vitamin C levels. Plasma samples will be incubated with 2.5 mM MBB in CH3OH:H2O (80:20) at room temperature for 30 min, then diluted with same or 10-fold volume of CH3CN:H2O (70:30) containing 0.025% formic acid. The diluted samples will be briefly vortexed and centrifuged for 20 min at 20,000g to pellet precipitated proteins. The supernatants will be transferred to autosampler vials with 3 μL solution injection for analysis by LC/MS. For absolute concentration of vitamin C measurements, MBB treated plasma will be diluted with 6-fold volume of 70% CH3CN containing 0.025% formic acid and 1 μL solution was injected for LC/MS.

Q-TOF LC/MS analysis will be performed as referenced [42]. Ascorbic acid will be detected in both negative mode ([M-H]- at m/z 175.0248) and positive mode ([M+H]- at m/z 177.0394) with retention times of 1.92 min and 0.99 min for ANP and RP columns, respectively. A vitamin C calibration curve will be established for free plasma in the 0-600 uM concentration range. Accuracy and precision will be evaluated by analyzing spiked vitamin C standards in plasma.

9.1.2 Collection of Specimen(s)

For Vitamin C pharmacokinetics, peripheral blood will be collected at the following time points: (+/- 15 minute window)

0 (prior to infusion start)
1 hour (during infusion)
2 hour (end of infusion)
4 hour (2 hours after end of infusion)
5 hour (3 hours after end of infusion)
6 hour (4 hours after end of infusion)
8 hour (6 hours after end of infusion)
24 hour (22 hours after end of infusion and prior to the start of the next dose)

9.1.3 Handling of Specimens

Refer to Vitamin C Lab Manual regarding handling of specimens.

9.2 Clonal Hematopoeisis

9.2.1 Assessment

20 cc of peripheral blood (purple cap tube) and 1 buccal swab. Buffy coat will be isolated. 10 cc will be used to generate DNA and RNA. 10 cc will be saved for single cell sequencing and/or flow cytometry.

9.2.2 Method of Assessment

We will isolate peripheral blood and buccal swabs from patients. Genomic DNA will be isolated and we will perform DNA and RNA sequencing to detect and characterize clonal hematopoiesis

9.2.3 Timing of Assessment

Baseline and week 6-8 (two time points only)

9.3 Gene expression profiling by RNA sequencing

9.3.1 Assessment

To explore potential correlation of gene expression pattern with anti-tumor activity of vitamin C, we plan to perform RNA sequencing using surgical sample in cohort A patients who will receive vitamin C infusion pre-operatively.

9.3.2 Method of Assessment

RNA extraction: RNA will be extracted from frozen tissue or FFPE using the RNeasy Mini Kit and purified with the RNeasy Min Elute Cleanup Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions. RNA quality was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). High quality RNA

will show a 28S rRNA band at 4.5 kb that should be twice the intensity of the 18S rRNA band at 1.9 kb.

RNA sequencing: mRNA will be purified from total RNA and fragmented following protocols from Illumina Inc. (San Diego, CA). cDNA library will be constructed by cDNA synthesis, end repair, addition of 'A' Bases to the 3' end, adapter ligation, and PCR amplification. The library will be denatured and hybridized onto a flow cell using cBot to generate clonal clusters of the DNA fragments. Paired end sequencing-by-synthesis will be performed on the HiSeq4000 platform. After the HiSeq generates the sequencing images, the data is analyzed in three steps: image analysis, base calling, and sequence analysis.

9.3.3 Timing of Assessment

RNA extraction and subsequent sequencing will be performed at the baseline tumor biopsy and after surgical resection of tumors in cohort A, or at baseline and the week 6-8 tumor biopsy for Cohort C.

10. Measurement of Effect

10.1 Response Criteria

10.1.1 Pathologic response

Pathologic response will be used in cohort A of this study. Surgical specimens will be evaluated for grading of tumor regression according to Mandard [40].

TRG 1: (complete regression): absence of residual cancer and fibrosis extending through the different layers of the colon wall in the cases of colon cancer, or pancreatic or lung tissue in the cases of pancreatic and lung cancer, respectively;
TRG 2: presence of rare residual cancer cells scattered through the fibrosis;
TRG 3: increase in the number of residual cancer cells, but fibrosis still predominated;
TRG 4: residual cancer outgrowing fibrosis;
TRG 5: absence of regressive changes.

10.1.2 Objective response

PET-CT will be performed at 6 weeks and 3 months from start of study treatment in cohort B patients or at early discontinuation of study. RECIST v 1.1 criteria to assess response for target lesions will be used to determine response.

Disease Control Rate (DCR) and Clinical Benefit Rate (CBR) are defined as the percentage of patients with advanced or metastatic cancer who have achieved complete response (CR), partial response (PR) and stable disease (SD) to the study agents.

Objective Response (ORR) is defined as the proportion of patients with a documented complete response or partial response (CR + PR) based on RECIST 1.1.

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 diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD): At least a 20% increase in the sum of 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 progression).
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.

Disease control (DC): this is defined as a CR, PR or SD.

10.2 Duration of Response

10.2.1 **Progress-free survival (PFS)**

PFS is defined as the time from study enrollment until the first objective observation of disease progression or death from any cause.

10.3 Other Response Parameters

N/A

11. Data Reporting / Regulatory Considerations

11.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

11.1.1 **REDCap**

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Webbased data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted

(SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

11.2 Regulatory Considerations

Once protocol amendments or consent form modifications are implemented at the lead site, Weill Cornell Medicine, updated documents will be provided to participating sites. Weill Cornell Medicine must approve all consent form changes prior to local IRB submission.

12. Statistical Considerations

12.1 Study Design/Endpoints

The study design for cohort A (patients who will be treated prior to tumor resection) is a two-stage trial with no stopping for the interim analysis. It will consist of a maximum of 20 total evaluable patients. The minimum pathologic tumor response rate that would warrant further study is 25%. The maximum pathologic tumor response rate (pRR) that would be uninteresting is 5%. The disease control rate is the rate not having progressive disease as defined by RECIST 1.1. criteria. The pathologic response rate is determined by a tumor regression grade (TRG). Both the RECIST radiology assessment (Eisenhauer et al. Eur J Cancer 2009;45: 228-247) and the TRG score (Thies et al. Front Oncol 2013;3:262) are standard criteria applied for oncologic studies. If after the first 13 patients, there has been 1 pRR the trial will continue to accrue an additional 7 patients. If after 20 patients are accrued, 3 or more patients have a pRR, the regimen will be recommended for further testing. If the regimen is inactive, there is a 51% chance that the trial will be stopped early.

The study design for cohort B (patients with metastatic tumors with extended RAS (e.g. KRAS or NRAS) or BRAF mutations) is a two-stage trial with no stopping for the interim analysis. A maximum of 25 evaluable patients will be accrued. The primary endpoint is the 3-month disease control rate (DCR). The minimum 3-month DCR that would warrant further study is 30%. The maximum 3-month DCR that would be uninteresting is 10%. If after the first 16 patients, 2 or more have disease control at 3-months, the trial will continue to accrue an additional 14 patients. If 5 or more patients out of 25 patients have disease control at 3 months, the regimen would be considered of interest for further testing. If the true 3-month DCR is 10% or less, the trial has a 51% chance of stopping early.

The study design for cohort C is a traditional 3+3 dose escalation design. Sequential dose levels will accrue as per the following dosing schema.

Dose level	Vit C dose per infusion (weekly monotherapy)	Vit C dose on Day of Y90 Rx
Dose level 1	0.5 g/kg	0
Dose level 2	0.75 g/kg	0
Dose level 3	0.75 g/kg	0.5 g/kg
Dose level 4	1.0 g/kg	0.5 g/kg
Dose level 5	1.0 g/kg	0.75 g/kg
Dose level 6	1.25 g/kg	0.75 g/kg

The DLT observation period will start with the first vitamin C infusion prior to radioembolization and end after the last vitamin C infusion post-embolization. A DLT is defined as a grade 3+ (latest CTCAE version) adverse event that is at least possibly related to the vitamin C infusion. Three patients will be enrolled at dose level 0.5 g/kg. if there are no DLTs observed for this cohort, the next cohort of three wil be treated with 0.75 g/kg. If there is 1 DLT at the 0.5g/kg level out of three patients, three more patients will be enrolled at this dose. If no additional DLTs are observed (1 of 6 patients with a DLT), the next three patients will be enrolled at the 0.75 g/kg level. If 2 or more out of 3 or 2 or more out of 6 experience a DLT at the 0.5 g/kg level, the trial will stop and it will be concluded the treatment has unacceptable toxicity. Dose escalations will continue if 0 of 3 or 1 of 6 patients experience a DLT. The recommended dose will be the dose immediately less that the dose that had 2 or more out of 3 or 6 patients with a DLT. An expansion cohort of at most 10 additional patients is planned at the recommended dose level (the dose immediate below the MTD, which is the dose level where 2 or more out of 3 or 6 patients experience a DLT). If the MTD is not reached, the 1.25 g/kg will be the recommended dose level.

12.2 Sample Size/Accrual Rate

The primary objective for cohort A is to evaluate the pathological response rate (pRR). Based on historical data for patients with extended RAS (e.g. KRAS or NRAS) or BRAF mutations and preclinical activity of Vitamin C in extended RAS (e.g. KRAS or NRAS) or BRAF mutant vs wild type colon cancer, the proposed null hypothesis (H0) is that the pRR for cohort A patients is $\leq 5\%$. The alternative hypothesis (Ha) is that the pRR is $\geq 25\%$ after treatment with Vitamin C supplementation in cohort A patients. This assumes a pRR of >40% extended RAS (e.g. KRAS or NRAS) or BRAF mutated cancers and 5% in wild type, and a 50% rate of extended RAS (e.g. KRAS or NRAS) or BRAF cohort. A sample size of 20 evaluable patients in Cohort A will have one-sided significance of 0.1 and 90% power with the indicated null and alternative hypotheses and the proposed interim analysis.

Patients in Cohort A will be considered evaluable if they received at least 8 vitamin C infusions prior to surgery, and TRG grading is available from surgery. Patients not fulfilling these requirements will be considered not evaluable and will be replaced. To account for patients who are not evaluable, the target accrual will be 22 patients.

The primary objective for cohort B is to determine the 3-month disease control rate (DCR). It is assumed that the 3-month DCR for this patient cohort without any further treatment is 10% (proposed null hypothesis, H0) and that the 3-month DCR for patients with extended RAS (e.g. KRAS or NRAS) or BRAF mutant tumors will be 30% or more when treated with high-dose vitamin C supplementation (alternative hypothesis Ha). A sample size of evaluable 25 patients will have a one-sided significance of 0.1 and power of 90%. Patients in Cohort B will be considered evaluable if they have 3-month scans available or have documented progression before 3-months, and they have received at least 4 vitamin C infusions. Patients not fulfilling these requirements will be considered not evaluable and will be replaced. To account for patients who are not evaluable, the target accrual will be 28 patients.

We project an accrual rate of 4.2 patients/months across the two cohorts over one year.

The minimum sample size for cohort C is 3 patients and will occur if 2 or more DLTs occur in the first 3 patients enrolled at dose level 0.5 g/kg. The maximum sample size is 34 patients and will occur if each dose level requires 6 patients be enrolled and there are 10 patients enrolled at the recommended dose (which would be 1.25 g/kg).

12.3 Analysis of Secondary Endpoints

In cohort A, Cmax and AUC of Vitamin C plasma levels and phosph-AMPK IHC will be analyzed for correlation with tumor response using a two-sample t-test (comparing values for patients with tumors that had a pRR and those who did not). Molecular signature of vitamin C efficacy will be determined using RNA sequencing and compared between extended RAS (e.g. KRAS or NRAS) or BRAF mutant vs wild type tumors. Organoids will be prepared from resected tumor samples and treated with vitamin C. Change of organoid growth from baseline will be compared in extended RAS (e.g. KRAS or NRAS) or BRAF mutant vs. wild type tumors by performing a two-sample t-test of the area under the growth curves. A comparisons of pRR between patients with extended RAS (e.g. KRAS or NRAS) or BRAF mutated tumors (cohort A) will be done using a Fisher's exact test. Finally there will be comparisons of the endpoints done for tumors with SUV < 10 versus tumors with SUV ≥ 10 and with GLUT1 IHC0/1 versus 2+/3+. The PFS of patients in cohirt B will be summarized with a Kaplan-Meier curve. For discrete endpoints (i.e. tumor response) the comparison will be done with Fisher's exact test and for time to event

endpoints (e.g. PFS) the comparison will be done with Kaplan-Meier curves and a log rank test.

The adverse events will be tabulated as the frequency and relative frequency by each dose level. Tumor responses will be evaluated using RECIST criteria. A patient will have a tumor response if they have a complete or partial response. The tumor response rate will be estimated for across all enrolled patients as well as by dose level. Tumor response will be reported a binomial point estimate and 95% confidence interval. In addition, we will access and report the disease control rate as defined for cohort B.

12.4 Reporting and Exclusions

12.4.1 **Evaluation of adverse events**

All patients will be evaluable for adverse events from the time of their first treatment with vitamin C.

12.4.2 **Evaluation of response**

All patients included in the study will be assessed for response to treatment if they have received at least 8 (Cohort A) 4 (Cohort B), or any (Cohort C)vitamin C infusions.

13. Data and Safety Monitoring Plan (DSMP)

This study will be monitored by the Weill Cornell Medicine DSMB. All adverse events will be summarized and reported to the DSMB. The study team will review AEs on a monthly basis via a report generated by the study statistician. Treatment will be modified for individual patients according to the dose-modifications described in section 6. Patients will discontinue protocol treatment for reasons described in Section 5, including treatmentrelated toxicities which result in a delay of planned curative surgery.

The study will begin with recruitment of six patients in cohort B. If after at least 4 weeks of treatment no more than 1 patient experienced a treatment-related toxicity requiring treatment interruption, recruitment may continue into cohort B and commence into cohort A.

Cohort A will recruit 5 patients initially. Once 5 patients have been accrued, the study will be temporarily suspended for cohort A patients if 20% or more of patients experience a grade 3+ AE that requires a delay of surgery for 1 week or longer. Upon review of the events and discussion with the IRB and DSMB, a decision will be made whether to reopen

the study as is, modify the study and reopen, or permanently close the study to cohort A patients.

The study will be temporarily suspended for cohort B patients if 2 of the first 6 patients or if 25% or more of patients thereafter experience a grade 3+ AE . Upon review of the events and discussion with the IRB and DSMB, a decision will be made whether to reopen the study as is, modify the study and reopen, or permanently close the study to cohort A patients.

There are not planned interim analyses for this trial. The primary endpoint data for each cohort will be summarized and presented to the DSMB for review.

For cohort C, the study team will determine whether doses can be escalated. All escalation decisions and observed AEs will be reported to the DSMB as part of the routine DSMB report.

13.1 DSMB Reporting

It is proposed that this study be reviewed by the DSMB every six months.

Appendix A

ECOG Performance Status Criteria

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

Appendix B

New York Heart Association (NYHA)

Classifications Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

Appendix C

Cockcroft-Gault Formula for Calculation of Creatinine Clearance

Males:

$$\text{Creatinine Clearance} = \frac{(140 - \text{age ⟨years⟩} \times \text{body weight ⟨kg⟩})}{(72) \times (\text{serum creatinine ⟨mg/dL⟩})}$$

Females:

$$\text{Creatinine Clearance} = \frac{(140 - \text{age ⟨years⟩} \times \text{body weight ⟨kg⟩})}{(72) \times (\text{serum creatinine ⟨mg/dL⟩})} \times 0.85$$

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