STATUS PAGE
PROTOCOL **11-436**

Closed To New Accrual

Closure Effective Date: 11/09/2016
Reason: Study Accrual Goal Met

No new subjects may be enrolled in the study- as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

DFCI Protocol No: 11-436

Front Sheet

Report Generated: 12/12/2017 09:41 AM

Title: Randomized, double-blind, phase II trial of vitamin D supplementation in patients with previously untreated metastatic colorectal cancer

Overall Institution: Dana-Farber Cancer Institute

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Sponsor Name	Sponsor Protocol No	Roles	Grant Number(s)
DF/HCC Investigator		Regulatory	
Dana-Farber/Harvard Cancer Center		Funding	
National Cancer Institute/NIH/DHHS (NCI)		Funding	

Total Study-Wide Enrollment Goal: 140 **Total DF/HCC Estimated Enrollment Goal:** 100

Phase: II

Age: Adults

Age Ranges: Age-Adults (18-64); Age-Adults (65+)

Will all subjects be recruited from pediatric clinics?

CTEP Study: No

Management Group(s): BIDMC Gastrointestinal & Hepatobiliary
DF/HCC Affiliate Site
DF/HCC Gastrointestinal Malignancies (GI)
DF/HCC Satellite Site
DFCI/BWH Gastrointestinal Oncology
MGH Gastrointestinal Cancers
MGH Regulatory Coordinators
OTHER Registering Site

Primary Management Group: DF/HCC Gastrointestinal Malignancies (GI)

Investigational Drug? Yes

Drug(s), Biologic(s): FLUOROURACIL, LEUCOVORIN, OXALIPLATIN, RHUMAB VEGF, VITAMIN D3

IND #: 114200

IND Holder Type: DF/HCC Investigator

IND Holder Name: Kimmie Ng

Investigational Device? This study does not use an Investigational Device.

IRB of Record:

Report Generated: 12/12/2017 09:41 AM

Risk Category: Greater Than Minimal Risk

Protocol Involves: Chemotherapy; Genetic Studies; Human Material Banking; Human Material Collection; Medical Record Review; Radiological Exams
Other Reason: Vitamin D

Date Range: (Medical Record Review and Specimen Collection studies)

Participating Sites under the DFCI IRB

Institution: Beth Israel Deaconess Medical Center
Brigham and Women's Hospital
Dana-Farber Cancer Institute
Dana-Farber Cancer Institute at Faulkner
Dana-Farber Cancer Institute at Milford
Dana-Farber Cancer Institute at NHOH
Dana-Farber Cancer Institute at South Shore
Lowell General Hospital
Massachusetts General Hospital
New England Cancer Specialists
New Hampshire Oncology-Hematology, PA
Newton-Wellesley Hospital

Participating Institutions Under Other IRB

Institution:	Location:
Northwestern University School of Medicine	CHICAGO, IL
St Luke's Mountain States Tumor Institute	Boise
UCSF Comprehensive Cancer Center	SAN FRANCISCO, CA
Vanderbilt University Medical Center	NASHVILLE, TN

Protocol Number: 11-436

Approval Date: 12/27/11 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 03/29/12 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
04/10/12	Consent Form and Front Sheet replaced due to Amendment #2	04/09/12	-
04/11/12	Correction AM #2: Patient Emergency Card not included previously	N/A	-
06/08/12	Protocol, Consent Form (Am 2 most current approval date) and Front Sheet replaced due to Amendment #1	03/23/12	06/04/12
06/21/12	Delayed Activation: BIDMC now active (Note: Previously activated at DFCI on 03/29/12; MGH still pending)	N/A	N/A
06/21/12	Protocol, Vitamin D Diary, Consent Form and Front Sheet replaced due to Amendment #3	06/15/12	06/19/12
06/21/12	Correction: Emergency card replaced	N/A	N/A
08/07/12	Delayed Activation Alert Page removed: MGH now active (Note: Previously activated at DFCI on 03/29/12; BIDMC activated on 06/19/2012)	N/A	N/A
09/28/12	Front Sheet replaced due to Amendment #4	09/28/12	n/a
12/27/12	ON HOLD: All research must stop due to lapsed Continuing Review. Study approval expired 12/27/12.	N/A	N/A
12/27/12	Remove Hold. Study renewal/ Consent Form replaced due to Continuing Review #1	12/20/12	12/27/12
01/29/13	Protocol, Consent Form and Front Sheet replaced due to Amendment #5	01/29/13	01/29/13
07/09/13	Protocol/PES and Front Sheet replaced; Vitamin D Subsequent Cycle and Vitamin D Cycle 1 Diaries added due to Amendment #6	06/06/13	N/A
07/10/13	Correction: Local appendices/Drug diary document removed (has been replaced w/ 2 Vitamin D Diaries)	n/a	n/a
08/02/13	Protocol, Consent Form and Front Sheet replaced due to Amendment #7	07/09/13	07/24/13
09/16/13	Front Sheet replaced due to Amendment #8	08/28/13	n/a
09/26/13	Protocol replaced due to Amendment #9	09/25/13	N/A
11/05/13	Consent Form, Protocol, Front Sheet and Drug Diaries replaced due to Amendment #10 (Note: re-consent required)	10/29/13	11/04/13
11/15/13	Consent Form replaced/ Study renewal due to Continuing Review #2	11/14/13	11/14/13
12/16/13	Consent Form and Front Sheet replaced due to Amendment #12	12/11/13	12/12/13
01/21/14	Alert Page added due to Amendment #11	11/22/13	N/A
05/20/14	Front Sheet replaced due to Amendment #13 (outside sites added)	05/16/14	N/A
07/09/14	Protocol and Front Sheet replaced due to Amendment #14	07/08/14	n/a
10/27/14	Consent Form, Protocol and Front Sheet replaced due to Amendment #15	10/23/14	10/27/14

11/12/14	Study renewal/Consent Form footer replaced due to Continuing Review #3 (note: 3 month approval)	11/06/14	N/A
12/29/14	Amendment #16: no change to online documents	12/12/14	N/A
01/28/15	Study renewal/ Consent Form footer replaced due to Continuing Review #4	01/08/15	N/A
03/04/15	Protocol/PES and Front Sheet replaced; Optional Surgical Tissue Collection Consent Form added; Alert Page removed due to Amendment #17 (Note: CF footer approval date remains latest due to CR #4)	01/07/15	03/04/15
04/21/15	Consent Forms and Front Sheet replaced due to Amendment #18	04/06/15	04/21/15
04/27/15	Correction AM#18: Protocol replaced	N/A	N/A
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
11/10/15	Protocol, Consent Forms and Front Sheet replaced due to Amendment #19	08/14/15	11/10/15
12/15/15	Study Closed – Study Accrual Goal Met	12/15/15	N/A
12/22/15	Study renewal/ Consent Forms footer replaced due to Continuing Review #5	12/10/15	12/22/15
01/19/16	Study reopen; Main Consent Form, Protocol, Front Sheet and Nursing PES replaced due to Amendment #21	01/12/16	01/19/16
06/08/16	Protocol, Consent Forms and Front Sheet replaced due to Amendment #22	06/06/16	06/07/16
Date Posted	**Revised Sections**	**Approved Date**	**Version Date (OnCore)**
11/10/16	Study Closed – Study Accrual Goal Met	11/09/16	N/A
11/18/16	Protocol, Consent Forms and Front Sheet replaced due to Amendment #23	10/11/16	10/11/16
12/08/16	Study renewal/ Consent Forms footer replaced due to Continuing Review #6	12/07/2016	12/07/2016
02/02/17	No changes to online documents; Amendment #24	01/19/17	N/A
02/16/17	Protocol and Front Sheet replaced due to Amendment #25	02/08/17	n/a
10/19/17	Study renewal/Consent Form footers replaced due to Continuing Review #7	09/28/17	10/03/17
12/12/2017	Protocol, Front Sheet, Consent Form – Sub Study, Consent Form – Main replaced per Amendment #26	12/08/2017	12/12/2017
08/31/2018	Study renewal/Consent Form footers replaced due to Continuing Review #8	08/28/2018	08/30/2018

Protocol Version Date: October 11, 2017

NCI Protocol #: N/A

Local Protocol #: 11-436

Title:
Randomized, Double-Blind, Phase II Trial of Vitamin D Supplementation in Participants with Previously Untreated Metastatic Colorectal Cancer

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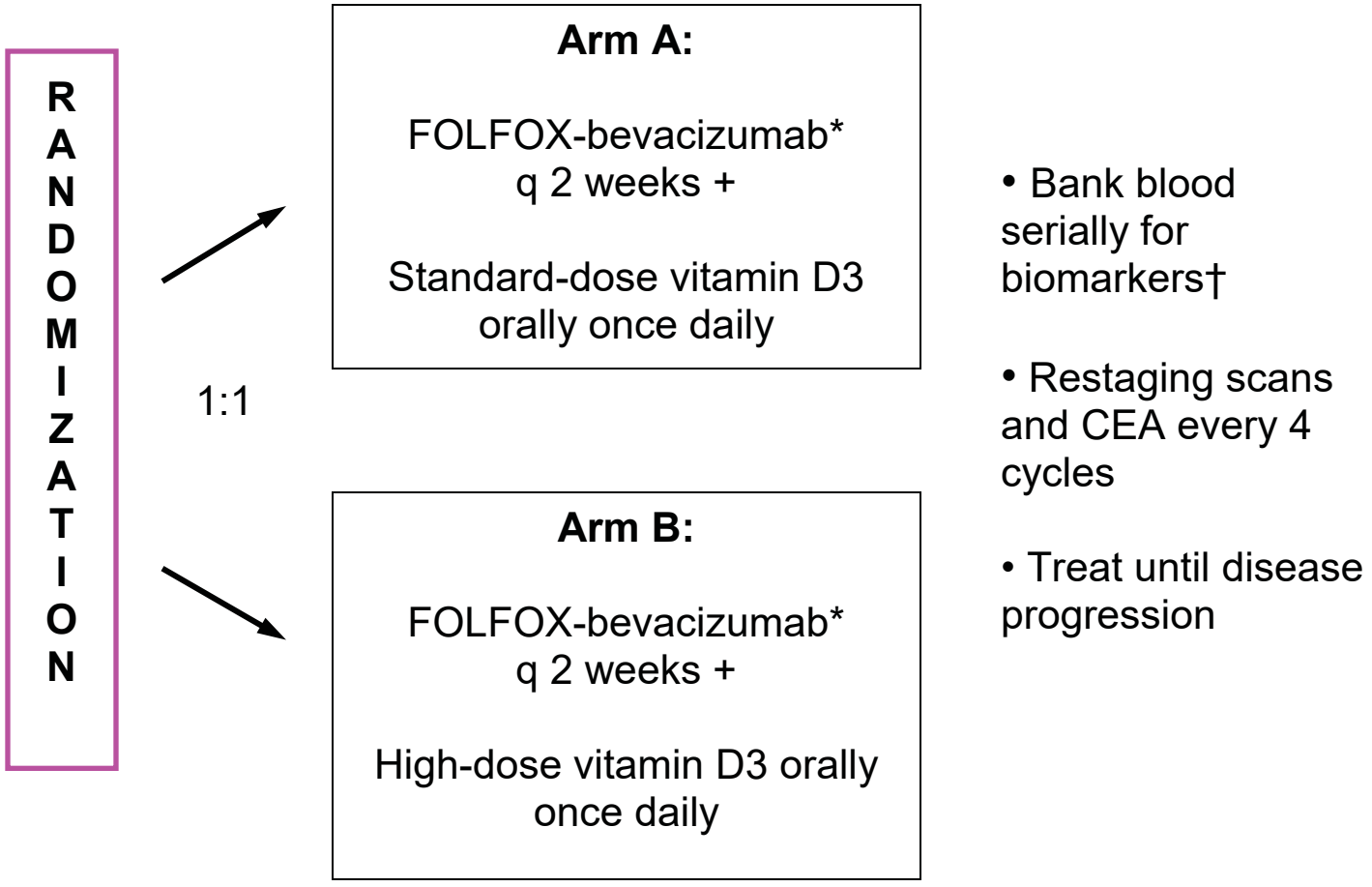
Agent(s):
Vitamin D3 (NSC 375571; IND# 114200) – Pharmavite, LLC
5-Fluorouracil (NSC 19893)
Leucovorin (NSC 3590)
Oxaliplatin (NSC 266046)
Bevacizumab (NSC 704865)

SCHEMA



*Bevacizumab may be held on cycle 1 day 1 at the investigator's discretion.

† In order to maintain blinding, plasma 25-hydroxyvitamin D [25(OH)D] levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed as part of the research blood samples collected during the study. Treating investigators should contact the lead Principal Investigator if questions arise about 25(OH)D levels.

FOLFOX = infusional 5-fluorouracil, leucovorin, and oxaliplatin
25(OH)D = 25-hydroxyvitamin D
CEA = carcinoembryonic antigen

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1. OBJECTIVES

1.1 Study Design

This is a prospective, randomized, double-blind phase II trial to evaluate the efficacy and safety of two doses of vitamin D supplementation in combination with standard chemotherapy in participants with previously-untreated metastatic colorectal adenocarcinoma. A total of 140 participants will be randomized in a 1:1 ratio to receive: A) standard dose D3 (400 IU daily) in combination with FOLFOX (infusional 5-fluorouracil [5-FU], leucovorin [LV], and oxaliplatin) chemotherapy plus bevacizumab, a standard first-line treatment for metastatic colorectal cancer; or B) higher-dose vitamin D3 (8000 IU daily x 2 weeks as loading dose, followed by 4000 IU daily as maintenance dose) in combination with FOLFOX-bevacizumab. Participants, providers, and investigators will be blinded to treatment assignment. Each cycle is 14 days in duration, and restaging scans and CEA tumor markers will be performed every 4 cycles to assess tumor response. Participants will be treated until disease progression, intolerable toxicity, or participant withdrawal of consent. Toxicity assessments will occur throughout the study, including serial measurements of serum calcium levels. Plasma will be banked serially throughout the trial for 25-hydroxyvitamin D [25(OH)D] assays and other relevant markers, and archival tumor tissue collected at study registration for future correlative studies. **In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.**

1.2 Primary Objectives

To compare the progression-free survival (PFS) of participants with previously untreated metastatic colorectal cancer randomized to FOLFOX-bevacizumab chemotherapy plus higher-dose vitamin D versus FOLFOX-bevacizumab chemotherapy plus standard-dose vitamin D

1.3 Secondary Objectives

- To compare the overall survival (OS) of participants with previously untreated metastatic colorectal cancer randomized to FOLFOX-bevacizumab chemotherapy plus higher-dose vitamin D versus FOLFOX-bevacizumab chemotherapy plus standard-dose vitamin D
- To compare the objective tumor response rate (RR) of participants with previously untreated metastatic colorectal cancer randomized to FOLFOX-bevacizumab chemotherapy plus higher-dose vitamin D versus FOLFOX-bevacizumab chemotherapy plus standard-dose vitamin D
- To evaluate and compare the toxicity of adding higher-dose vitamin D versus standard-dose vitamin D to FOLFOX-bevacizumab chemotherapy

- To evaluate the incidence of vitamin D deficiency in participants with previously untreated metastatic colorectal cancer
- To compare the proportion of participants who are able to achieve and maintain vitamin D sufficiency with higher-dose vitamin D versus standard-dose vitamin D
- To compare the time course of change in plasma 25-hydroxyvitamin D3 [25(OH)D] levels in participants randomized to higher-dose vitamin D versus standard-dose vitamin D
- To evaluate the association between plasma 25(OH)D levels and PFS and overall survival

2. BACKGROUND

2.1 Vitamin D

The hypothesis that vitamin D status is related to colorectal cancer has received strong experimental support over the past two decades, based on the almost ubiquitous expression in colon cancer cells of the vitamin D receptor (VDR)[1, 2] and 1-α-hydroxylase (CYP27B1),[3] which converts plasma 25(OH)D into 1,25-dihyroxycholecalciferol [1,25$(OH)_2$D], the active metabolite. Binding of VDR by 1,25$(OH)_2$D leads to transcriptional activation and repression of target genes, resulting in induction of differentiation and apoptosis,[4, 5] and inhibition of proliferation,[6] angiogenesis,[7, 8] and metastatic potential.[9, 10] *In vitro* and *in vivo* data have demonstrated growth inhibition and differentiation of colon carcinoma cell lines and xenografts by administration of 1,25$(OH)_2$D,[7, 11-14] and rat models of colorectal cancer maintained on a 1,25$(OH)_2$D diet developed fewer tumors and metastases compared to control animals.[10, 15] In Apc^{min} mice, treatment with vitamin D or its synthetic analogs significantly decreased tumor burden.[16]

Vitamin D and Risk of Colorectal Cancer

The best indicator of vitamin D status is plasma 25(OH)D, since it reflects not only skin exposure to ultraviolet-B (UV-B) light and total vitamin D intake, but also cholecalciferol production in the skin and hydroxylation of all sources of cholecalciferol in the liver.[17] Prospective studies have shown that individuals with higher plasma levels of 25(OH)D experience a significant reduction in risk of colorectal cancer when compared to those with low plasma levels.[18-23] In a recent meta-analysis of five epidemiologic studies, individuals with serum 25(OH)D level ≥33 ng/mL (1 ng/mL=2.496 nmol/L) had a 50% lower risk of colorectal cancer when compared to those with levels ≤12 ng/mL (P<0.01).[24]

Generally null findings for colorectal cancer incidence from the Women's Health Initiative (WHI), a randomized placebo-controlled trial of 400 IU vitamin D plus 1000 mg a day of calcium in post-menopausal women, appear to contrast with epidemiologic data.[22] However, relatively low-dose vitamin D supplementation (400 IU/day) may not confer a significant reduction in colorectal cancer risk, particularly with limited duration of follow-up. Indeed, the dose of 400 IU daily increased plasma 25(OH)D level by only

2-3 ng/ml, whereas in most epidemiologic studies, the contrast between the high and low quintiles was ≥20 ng/mL.[24] Although the WHI provides important data, benefits of calcium and vitamin D may exist at doses and durations not assessed in that study. Interestingly, WHI participants who had the highest baseline levels of plasma 25(OH)D did experience a significant 60% reduction in colorectal cancer risk (*P* for trend=0.02, see table below from Wactawski-Wende, et al.). Thus, consistent with other studies, participants who managed to achieve higher 25(OH)D levels (through means other than the assigned vitamin D supplement) did experience a substantial reduction in colorectal cancer risk.

Table 2. Odds Ratios for Invasive Colorectal Cancer According to the Quartile of Serum 25-Hydroxyvitamin D Level at Baseline and Treatment Groups in a Nested Case–Control Study.*

Baseline Serum 25-Hydroxyvitamin D	Main-Effect Odds Ratio (95% CI)†	Calcium + Vitamin D	Placebo	Intervention Odds Ratio (95% CI)‡
		No. with Colorectal Cancer/ No. of Controls		
≥58.4 nmol/liter	1.00	33/48	27/45	1.15 (0.58–2.27)
42.4–58.3 nmol/liter	1.96 (1.18–3.24)	44/41	34/32	1.12 (0.59–2.12)
31.0–42.3 nmol/liter	1.95 (1.18–3.24)	35/32	45/41	0.99 (0.51–1.91)
<31.0 nmol/liter	2.53 (1.49–4.32)	46/39	42/28	0.75 (0.39–1.48)

* To convert values for 25-hydroxyvitamin D to nanograms per milliliter, multiply by 0.401. CI denotes confidence interval.
† Odds ratios were derived from a logistic-regression model, conditioned on case–control pairs, estimating the main effect of the serum 25-hydroxyvitamin D level on the risk of invasive colorectal cancer (P for trend=0.02).
‡ P for interaction=0.54. The odds ratios were obtained from a logistic-regression model, conditioned on case–control pairs, and estimate the calcium with vitamin D intervention effect on the risk of colorectal cancer, according to serum 25-hydroxyvitamin D levels.

Further, in the WHI, although the dose of vitamin D was clearly suboptimal, supplemental vitamin D did confer non-statistically significant reductions for colorectal cancer mortality (relative risk [RR] 0.82; 95% confidence interval [CI], 0.52-1.29; P=0.39), total cancer mortality (RR 0.89; 95% CI, 0.77-1.03; P=0.12), and total mortality (RR 0.93; 95% CI, 0.83-1.01; P=0.07). In addition, in a re-analysis of the study, vitamin D and calcium supplementation was found to decrease the risk of colorectal cancer among women simultaneously randomized to the estrogen placebo arm of the trial (hazard ratio [HR] 071; 95% CI, 0.46-1.09).[25] More recently, a randomized placebo-controlled trial of vitamin D and calcium supplementation in postmenopausal women demonstrated a 60% decrease in all-cancer risk (including colorectal cancer) in the intervention arm (P<0.03).[26] Furthermore, a report from the Third National Health and Nutrition Examination Survey (in which 16,818 participants were enrolled from 1988-1994 and followed through 2000) demonstrated an inverse relationship between serum 25(OH)D levels and colorectal cancer mortality, with levels 32 ng/mL or higher associated with a 72% risk reduction (95% CI, 32%-89%), compared with levels <20 ng/mL (*P* trend=0.02).[27]

Vitamin D and Colorectal Cancer Survival

Provocative data are emerging for a protective effect of vitamin D on survival in patients with established colorectal cancer. A large observational study in Norway found that people diagnosed with colorectal cancer in the summer and autumn, when 25(OH)D concentrations are highest, had a significantly better survival than those diagnosed in the winter.[28, 29] The authors speculated that a high circulating 25(OH)D at the time of diagnosis, and possibly during initial treatment, may improve prognosis. Subsequently, a prospective study of 304 patients with stage I-IV colorectal cancer from the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS) showed that higher plasma 25(OH)D levels were associated with significantly improved OS (multivariate HR 0.52; 95% CI 0.29 to 0.94; P trend=0.02).[30] In subgroup analyses, the benefit of higher plasma 25(OH)D seemed greater in stage III and IV patients compared to stage I and II (adjusted HR 0.40 versus 0.90, respectively, comparing extreme quartiles). A second study showed similar results for a protective effect of vitamin D using a clinical score based on known determinants of vitamin D status, including geographical region of residence (surrogate for UV-B exposure), race (surrogate for skin pigmentation), dietary and supplement intake, body mass index (BMI), and leisure-time physical activity (surrogate for actual sunlight exposure).[31] Among 1,017 patients with colorectal cancer in NHS and HPFS, higher post-diagnosis vitamin D scores were significantly associated with improved cancer-specific (adjusted HR 0.50; 95% CI 0.26-0.95; P trend=0.02) and OS (HR 0.62; 95% CI, 0.42-0.93; P trend=0.002). A smaller third study conducted in Japan also corroborated the above results, with higher plasma 25(OH)D levels shown to be associated with better survival in 257 colorectal cancer patients undergoing surgery (*P* trend=0.03).[32]

To further characterize the vitamin D status of advanced stage colorectal cancer patients specifically, plasma 25(OH)D levels were measured among 515 stage IV colorectal cancer patients enrolled in a completed, NCI-sponsored clinical trial of palliative chemotherapy (North Central Cancer Treatment Group trial N9741). The median plasma 25(OH)D level in the cohort was 20.0 ng/mL and the mean was 21.0 ng/mL. At the start of chemotherapy, 50% of the study population were vitamin D deficient (<20 ng/mL) and 82% were vitamin D insufficient (<30 ng/mL). Only 10% of patients had plasma 25(OH)D levels ≥33 ng/mL, the threshold believed to be required for a potential protective effect on colorectal cancer risk.[24] Baseline plasma 25(OH)D levels were not associated with OS, although given the distribution of plasma levels in this cohort, statistical power for survival analyses was limited.[33] Interestingly, in subgroup analyses, a significant interaction was observed with treatment arm (*P* interaction=0.0005), with patients randomized to FOLFOX chemotherapy showing significantly improved survival with higher plasma 25(OH)D levels (multivariate HR 0.64 comparing extreme quartiles; 95% CI, 0.45-0.90; P trend=0.003). Although this finding may simply be due to chance, in vitro and in vivo analyses suggest that 1,25(OH)2D may potentiate the anti-neoplastic effects of platinum agents (see figure below from Light, et al.,1997).[34-36]

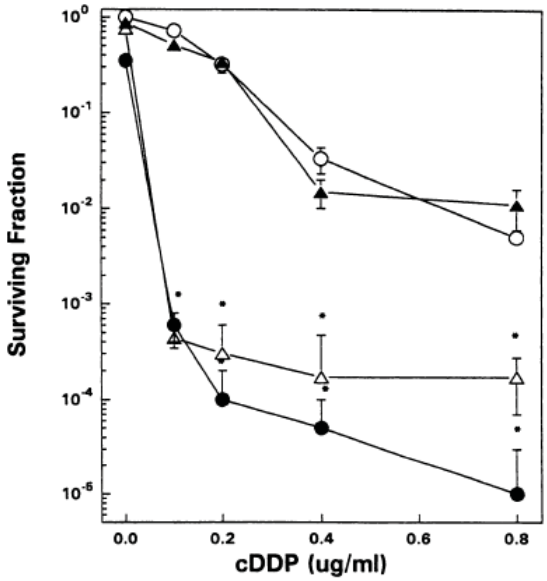

Fig. 3. Effect of Ro23-7553 on the *in vitro* cDDP-mediated tumor cell kill of murine SCCVII/SF cell line as determined by the *in vitro* clonogenic assay. Tumor cells were treated *in vitro* as described with varying concentrations of cDDP alone (○), concurrent cDDP and Ro23-7553 (4 nM) (▲), or pretreatment for 48 h with Ro23-7553 at 2 nM (△) or 4 nM (●) then cDDP. *Points* are means (*bars*, SD) of the surviving fraction of three replicates from a representative experiment that was replicated two to three times. Values significantly different from cDDP alone or concurrent cDDP and Ro23-7553 are shown: *, $P < 0.001$ (ANOVA).

Vitamin D and Chemotherapy

As mentioned above, preclinical experiments have demonstrated synergy between vitamin D and several chemotherapeutic agents in a variety of cancer types. Calcitriol and vitamin D analogues displayed additive antitumor activity with cisplatin and carboplatin in breast cancer,[37] prostate cancer,[36] retinoblastoma,[38] and squamous cancer cell lines and xenografts (see figure above).[35] Increased caspase-3 activation was seen when vitamin D was combined with platinums, suggesting induction of apoptosis as a potential mechanism for the enhanced cytotoxicity.[34] Increased arrest of cells in G_0-G_1 was also seen, therefore synergistic effects on the cell cycle may also play a role.[35] Vitamin D has also recently been shown to sensitize colon cancer cells to the antiproliferative activity of 5-FU. In an *in vitro* study of human colon carcinoma cells (HCT116), vitamin D stimulated expression of the calcium-sensing receptor, leading to downregulation of thymidylate synthase and survivin expression and enhanced response to 5-FU treatment.[39] Furthermore, vitamin D enhances the activity of taxanes, possibly due to decreased p21 expression, which sensitizes tumor cells to paclitaxel.[40] Finally, calcitriol was recently reported to enhance gemcitabine antitumor activity *in vitro* and *in vivo* by promoting apoptosis in a human pancreatic carcinoma model system, with an increase in caspase expression and decrease in Akt phosphorylation.[41]

Vitamin D and its analogues have also been tested *in vitro* and *in vivo* in combination with several targeted agents. Calcitriol and cetuximab administered together to a hormone-refractory prostate cancer cell line resulted in increased antiproliferative activity, considerable cell cycle arrest in G_0-G_1, and enhanced apoptosis compared to either agent alone.[42] Similarly, synergistic activity was seen with gefitinib.[43] Enhanced

antitumor activity and cell differentiation has also been demonstrated with the combination of vitamin D and non-steroidal anti-inflammatory drugs (NSAIDs).[44, 45]

In addition to preclinical data, several early clinical trials have demonstrated the safety and tolerability of administering vitamin D in combination with chemotherapy,[46-51] as well as targeted agents such as gefinitib.[52, 53] Three phase I studies of high-dose vitamin D in combination with carboplatin or paclitaxel in patients with advanced solid tumors reported no dose-limiting toxicities.[49, 54] Hints of efficacy were seen in several studies. In a phase II trial, Blanke et al. treated 25 patients with previously untreated metastatic or locally advanced pancreatic cancer with weekly oral calcitriol 0.5 mcg/kg and docetaxel 36 mg/m2.[51] The partial response (PR) rate was 12%, with 28% achieving stable disease (SD). Median time-to-progression (TTP) was 3.6 months (compared to a historical control of 1.5 months for single-agent docetaxel) and median OS 5.6 months. No toxicities attributable to calcitriol were seen. Another trial evaluated high-dose calcitriol in combination with docetaxel in androgen-independent prostate cancer, and saw an 81% PSA RR and 53% PR rate among patients with measurable disease. Treatment-related toxicity was similar to that expected from single-agent docetaxel.[50] Another phase II trial evaluated calcitriol in combination with naproxen, given vitamin D's purported anti-inflammatory effects, in hormone-refractory prostate cancer. The investigators saw a prolongation of PSA doubling time in 75% of patients treated for one year, and no significant toxicity was reported.[55]

Effect of Vitamin D Supplementation on Plasma 25(OH)D Levels

Multiple studies in healthy participants as well as cancer patients have examined the effects of oral supplementation on plasma 25(OH)D levels. In a landmark randomized clinical trial by Vieth et al., the efficacy and safety of relatively high intakes of vitamin D (1000 versus 4000 IU/day for 2-5 months) in 61 healthy adults was evaluated.[56] Baseline median plasma 25(OH)D among the population was approximately 16 ng/mL, comparable to the median among metastatic colorectal cancer patients. They found that vitamin D at a dose of 4000 IU/day was effective in elevating the serum 25(OH)D concentration to high-normal values of approximately 38 ng/mL in practically all adults, whereas 1000 IU/day raised 25(OH)D to only approximately 27 ng/mL. It is important to note that a steady-state of circulating 25(OH)D was achieved approximately 90 days following initiation of supplementation at the 4000 IU/day level, and that serum calcium and urinary calcium excretion did not change significantly at either dosage throughout the study. In a retrospective study of cancer patients receiving anti-cancer therapy and supplemented with 8000 IU daily for a mean of 14.7 weeks, baseline mean plasma 25(OH)D level increased from 19.1 ng/mL to 36.2 ng/mL, and no patients had any evidence of vitamin D toxicity.[57] We recently completed accrual to a randomized. placebo-controlled trial of vitamin D supplementation in healthy individuals as part of the Dana-Farber/Harvard Cancer Center (DF/HCC) SPORE in Gastrointestinal Cancer. The study randomized 328 participants from low-income housing sites in the Boston area to placebo, 1000, 2000, or 4000 IU daily of vitamin D3 for three months. The primary objective was to determine the dose necessary to achieve a plasma 25(OH)D level of 33 ng/mL or higher in 80% of compliant participants, and preliminary results indicate that the 4000 IU arm was the only dose that was able to meet this end point (manuscript in preparation).

Safety of High-Dose Vitamin D Supplementation

Toxicity from vitamin D supplementation has been observed only when there is consistent intake far in excess of 10,000 IU/day.[58, 59] Heany et al. supplemented 67 male Caucasian participants in Omaha during winter months with 1000, 5000, or 10,000 IU vitamin D/day for a period of four months.[60] Mean baseline 25(OH)D level was approximately 28 ng/mL. A steady-state of circulating 25(OH)D levels was observed following 90 days of supplementation. At the end of the study period, the average increase from baseline of circulating 25(OH)D was 4.8, 36.7 and 63.8 ng/mL for 1000, 5000, and 10,000 IU daily dose groups, respectively. The final circulating levels of 25(OH)D in these treatment groups were 33.6, 64.5 and 90.0 ng/mL, respectively, and not one incidence of hypercalcemia or hypercalcuria (the first indicator of hypervitaminosis D) was observed. In our DF/HCC randomized, placebo-controlled trial in healthy participants, no toxicity was seen in any of the supplementation arms (placebo, 1000 IU, 2000 IU, or 4000 IU daily).

Among cancer patients specifically, multiple clinical trials have been performed in breast cancer patients that demonstrate the safety of high doses of vitamin D supplementation. In a phase II trial, 40 breast cancer patients with bone metastases, the majority of whom were receiving endocrine therapy and/or chemotherapy, received supplementation with 10,000 IU vitamin D3 daily for four months.[61] Baseline median plasma 25(OH)D was 27.8 ng/mL, and increased to 46.4 ng/mL at 1 month, 53.6 ng/mL at 2 months, 58 ng/mL at 3 months, and 64.8 ng/mL at 4 months. Median corrected serum calcium was 9.1 mEq/dL at baseline, and increased to only 9.3 mEq/dL after 4 months of supplementation. There was no significant change in urinary calcium excretion. In another study of breast cancer patients starting adjuvant letrozole therapy, treatment with 50,000 IU weekly for 12 weeks resulted in an increase in median plasma 25(OH)D from 29 ng/mL at baseline to 66 ng/mL without any occurrences of hypercalcemia or renal stones.[62] Finally, multiple phase I and II studies have evaluated calcitriol or synthetic vitamin D analogues in combination with chemotherapy in a variety of cancers, with no dose-limiting toxicities (see above).

Type of Vitamin D Supplementation

Vitamin D will be given in the form of vitamin D3 supplements in this trial. Although humans can get vitamin D from sunlight and dietary sources, ultraviolet radiation exposure is geographically variable and associated with skin cancer, and dietary sources of vitamin D are minimal. Supplements come as vitamin D2 or vitamin D3; both forms are believed to be equipotent, but some data suggest greater effectiveness of vitamin D3 at higher doses.[63] Calcitriol is also available as an oral supplement, however the doses needed for potential antitumor activity result in unacceptably high rates of hypercalcemia.

2.2 Colorectal Cancer

Colorectal cancer is the third most common cancer and the third leading cause of cancer death in the U.S.[64] In 2010, an estimated 142,570 cases of colorectal cancer will be diagnosed and 51,370 people will die from the disease. Fifteen to 25% of patients with

colorectal cancer have metastatic disease at the time of diagnosis, and a significant proportion of patients who are initially diagnosed with localized disease ultimately develop metastases.[65] While there have been substantive advances in the treatment of metastatic colorectal cancer over the past few years,[66] median survival for these patients remains at two years, and less than 6% survive for more than five years.[67] Clearly, there is a critical need for new effective treatments to further prolong survival.

5-FU-Based Chemotherapy

Historically, treatment for colorectal cancer was limited to 5-FU and LV, with RRs of 23% and median survival time of approximately 10 to 12 months.[68] 5-FU is incorporated into RNA and DNA and inhibits the enzyme thymidylate synthase (TS), thus interfering with the formation of new strands of DNA during replication. Leucovorin is a reduced folate which, when combined with 5-FU, augments 5-FU cytotoxicity by increasing the inhibition of TS by the 5-FU active metabolite FdUMP. Because 5-FU has a short half-life, continuous infusion regimens appear to offer a pharmacological benefit, with the maximal dose intensity of 5-FU delivery being achieved when the drug is administered over 24-48 hours.

Oxaliplatin

Oxaliplatin, a platinum analog, forms cross-linking adducts and blocks deoxyribonucleic acid replication. Oxaliplatin-derived platinum is distributed extensively in the plasma, although the elimination of total platinum through the urine is slow, with approximately 33% eliminated within 48 hours. Fecal elimination accounts for a trivial amount of clearance. Oxaliplatin and 5-FU appear to be at least additive in their anti-tumor activity, although the mechanism for this interaction is unclear. Oxaliplatin has been shown to be effective and well-tolerated when administered with both bolus and infusional forms of 5-FU, however rates of neutropenia and sensory neuropathies are higher with the combination.[69, 70] In first-line advanced colorectal cancer therapy, the FOLFOX4 regimen was shown to be superior to the combination of irinotecan, bolus 5-FU, leucovorin (IFL regimen) and to the combination of irinotecan and oxaliplatin (IROX regimen), with median OS times of 19.5 months, 15.0 months, and 17.4 months, respectively (N9741). The RRs and median TTP were 45%, 8.7 months; 31%, 6.9 months; and 35%, 6.5 months, respectively.[71] Tournigand et al. compared the efficacy of FOLFIRI (infusional 5-FU, LV, and irinotecan) followed by FOLFOX6 at progression to that of FOLFOX6 followed by FOLFIRI at progression in patients with previously untreated metastatic colorectal cancer, and found a similar median survival (21.5 versus 20.6 months, respectively; P=0.99). In this study, National Cancer Institute Common Terminology Criteria (NCI-CTC) grade 3/4 mucositis, nausea, and vomiting, and grade 2 alopecia were more frequent with FOLFIRI, while grade 3/4 neutropenia and neurosensory toxicity were more frequent with FOLFOX6.[72] Multiple variations of FOLFOX have been devised to enhance safety and efficacy of the combination. Modified FOLFOX6 displays comparable efficacy to FOLFOX4 with improved convenience of administration and is a standard FOLFOX regimen in the United States.

Bevacizumab

Bevacizumab is a monoclonal antibody that binds and inhibits the vascular endothelial growth factor A (VEGF-A), a protein that plays a critical role in tumor angiogenesis. The addition of bevacizumab to IFL compared to IFL alone significantly improved survival (20.3 months versus 15.6 months, respectively; $P<0.0001$) in a randomized study.[73] Bevacizumab is used in combination with intravenous 5-FU-based chemotherapy and is licensed in the United States for first-line and second-line treatment of patients with metastatic colorectal cancer. A recent international phase III trial demonstrated improved median PFS (9.4 months versus 8.0 months, $P=0.0023$, HR 0.83, 95% CI, 0.72-0.95) with the addition of bevacizumab to the oxaliplatin-containing regimens, FOLFOX4 and XELOX (capecitabine and oxaliplatin), for first-line treatment of metastatic colorectal cancer. In the FOLFOX arms, the median PFS for FOLFOX4-bevacizumab was 9.4 months versus 8.6 months for FOLFOX4-placebo ($P=0.19$, HR 0.89, 97.5% CI, 0.73-1.08). Although bevacizumab did not significantly improve PFS when added to FOLFOX in this study, many participants did not continue 5-FU/bevacizumab after experiencing oxaliplatin toxicity, confounding the interpretation of the data.[74] FOLFOX-bevacizumab remains a standard of care for metastatic colorectal cancer in the United States.

2.3 Rationale

Given the observational, preclinical, and early clinical data above, we propose a randomized blinded phase II trial of vitamin D supplementation in combination with chemotherapy with FOLFOX-bevacizumab in 140 previously untreated, metastatic colorectal cancer participants. We chose the metastatic patient population to study based on data mentioned above that suggests a greater benefit of vitamin D in advanced-stage disease. FOLFOX-bevacizumab was selected as the chemotherapy backbone for the trial based on its documented efficacy and widespread use as first-line therapy for metastatic disease, as well as on data from the N9741 cohort suggesting a survival benefit of higher vitamin D in patients receiving FOLFOX. Moreover, this regimen is supported by preclinical data demonstrating additive anti-tumor activity when 5-FU and platinum agents are administered with vitamin D.

Consequently, we plan to compare two doses of vitamin D3 in combination with FOLFOX-bevacizumab: a standard dose of 400 IU daily in the control arm versus a higher dose of 8000 IU daily for two weeks as loading dose followed by 4000 IU daily as maintenance dose. The regimen chosen for the investigational arm is supported by previous clinical trials that have demonstrated the safety and efficacy of these higher doses of vitamin D in raising plasma 25(OH)D levels into the high-normal range.[56] Participants and providers will be blinded to treatment arm. There are several reasons for not using a placebo control arm, including: 1) potential unwillingness of colorectal cancer participants to be randomized to a vitamin D placebo; 2) known prevalence of vitamin D deficiency and insufficiency in the metastatic population; 3) fact that vitamin D3 400 IU daily increases serum 25(OH)D levels by approximately 2-3 ng/mL only;[75] and 4) previous research indicating a lack of benefit of multivitamin supplements (which typically contain 400 IU of vitamin D) on outcomes in stage III colon cancer patients.[76] Therefore, vitamin D3 400 IU daily is a suitable control arm, and is actually

the estimated average requirement for people of all ages according to the Institute of Medicine (IOM) 2011 report on vitamin D.[63] In the experimental arm, the higher doses of vitamin D planned are likely to raise the low median levels of vitamin D in this patient population into the sufficient range. Moreover, 4000 IU daily is the tolerable upper limit of intake according to the IOM report,[63] and is therefore extremely unlikely to be associated with toxicity. Plasma will be banked serially throughout the trial for relevant biomarker assays, including vitamin D levels. **In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.**

Feasibility of Proposed Study Design

Concerns exist regarding the willingness of participants to be randomized to a lower vitamin D dose, and the potential for additional supplement use beyond the protocol-prescribed treatment. We have previously established the feasibility of a randomized, blinded, placebo-controlled clinical trial of vitamin D supplementation in healthy individuals as part of the DF/HCC SPORE in Gastrointestinal Cancer (see above). Accrual to that trial was completed ahead of projected time estimates, with no barriers to randomization relating to the placebo design. Participants were followed with biweekly telephone calls and monthly visits, and electronic pill dispenser bottles were used to monitor frequency and time between bottle openings. Preliminarily, <1% of participants reported taking supplemental vitamin D beyond the protocol-prescribed therapy during the period of the study, and the median adherence rate was 96.6%.

In regards to concerns about the prevalence of baseline use of vitamin D supplements among metastatic colorectal cancer patients, which would potentially limit the eligible patient population, we analyzed questionnaire results regarding intake of vitamin D supplements from participants enrolled on CALGB 80405. This ongoing phase III study randomizes previously untreated, metastatic colorectal cancer patients to FOLFOX or FOLFIRI (provider choice) chemotherapy + bevacizumab versus FOLFOX or FOLFIRI chemotherapy + cetuximab, with dietary and lifestyle questionnaires administered to patients within one month of starting chemotherapy. We found that among 1,083 patients with available information, only 7.3% reported use of vitamin D supplements. Consequently, the vast majority of eligible patients are not, in fact, taking vitamin D at baseline.

Importance of Trial and Potential Impact on Patients

Vitamin D insufficiency is highly prevalent in the United States, particularly among metastatic colorectal cancer patients. These low levels of vitamin D are concerning in light of increasing evidence that vitamin D may have health benefits beyond skeletal outcomes, including reducing the risk of and mortality from colorectal cancer. Currently, prospective observational studies suggest a benefit of higher vitamin D levels on survival in patients with established colorectal cancer, and randomized controlled treatment trials are desperately needed to further characterize the role of vitamin D in

colorectal cancer pathogenesis and establish causality. This proposed trial, along with its associated correlative studies, would provide critical information on many unanswered questions and issues deemed by the IOM to be important research needs: 1) Effects of two doses of vitamin D on survival and tumor response in colorectal cancer; 2) safety of two doses of vitamin D when administered with chemotherapy; 3) dose-response relationship of oral supplementation on plasma 25(OH)D levels; 4) influence of related factors such as BMI and geographic location on plasma 25(OH)D levels and response to supplementation; 5) effect of germline genetic variation on plasma 25(OH)D levels, response to supplementation, and participant outcome; and 6) interaction of vitamin D pathway markers with markers in related pathways, such as the insulin-like growth factor pathway and inflammatory pathway.

Finally, there remains a great need to improve survival outcomes for patients with metastatic colorectal cancer. Investigating vitamin D as a potential therapeutic modality is a novel approach to cancer treatment with compelling supportive preclinical and epidemiological data. Moreover, in the era of expensive and often toxic anti-neoplastic agents, vitamin D represents an attractive option for patients and oncologists in regards to safety and cost. Randomized phase II studies, such as the one proposed, provide an excellent opportunity for multiple centers across the U.S. to collaborate to rapidly test hypotheses regarding novel treatment approaches. Promising signals can then be rapidly moved forward to phase III testing.

2.4 Correlative Studies Background

Vitamin D Pathway Polymorphisms

Hypothesis: Polymorphisms in genes involved in vitamin D metabolism are associated with circulating 25(OH)D levels and influence the effect of vitamin D on participant outcome.

The pathway through which vitamin D exerts transcriptional effects is complex. An editorial in the *Journal of the National Cancer Institute* recommended that future investigations consider the inter-relationships of vitamin D and genetic polymorphisms within the pathway.[77] Two recent genome-wide association studies (GWAS) identified several loci that were significantly associated with circulating 25(OH)D levels. In the study performed by the SUNLIGHT consortium, variants at three loci reached genome-wide significance and were confirmed in replication cohorts: 4p12 (*GC*), 11q12 (near *DHCR7*), and 11p15 (near *CYP2R1*). Participants with a high genotype score derived by combining the three variants had a significantly increased risk of vitamin D insufficiency.[78] These three loci were confirmed in a second GWAS comprising five cohorts and 4,501 participants, and again, the joint effect of variants at these loci for clinical vitamin D deficiency was significant.[79] Moreover, a recent analysis mapped VDR binding domains throughout the human genome using chromatin immuno-precipitation followed by parallel DNA sequencing.[80] Following calcitriol stimulation, VDR bound to 2,776 genomic positions, with four-fold enrichment within DNA intervals that were significantly associated with the risk of developing colorectal cancer in GWAS ($P<0.0001$). We therefore propose to evaluate the individual and joint effects of SNPs in vitamin D-related genes (some of which are listed below), including SNPs in

specific genomic sequences that bind VDR, on plasma 25(OH)D levels and clinical outcome.

- The cellular effects of $1,25(OH)_2D$ are principally mediated through the vitamin D receptor (*VDR*), which regulates the transcription of target genes involved in cellular differentiation and inhibition of proliferation. Several common polymorphisms (*Fok*I, *Apa*I, *Taq*I, and *Bsm*I) have been identified in the *VDR* gene, although their functional effects are uncertain,[81-88] and associations between these polymorphisms and colorectal cancer risk are inconsistent.[89-103]
- The vitamin D binding protein (*VDBP,GC*) transports vitamin D and its metabolites to target tissues and may be involved in its intracellular metabolism.[104, 105] Several polymorphisms in *GC* were recently shown to be significantly related to circulating 25(OH)D levels.[78, 79, 106]
- 1-α-hydroxylase (*CYP27B1*) catalyzes the hydroxylation of 25(OH)D to $1,25(OH)_2D$, and is present in both normal colon mucosa and colorectal adenocarcinomas.[107-111] *CYP27B1* variants have been associated with the risk of Addison's disease, Graves' disease, and type 1 diabetes.[112, 113]
- 24-hydroxylase (*CYP24A1*) initiates degradation of both 25(OH)D and $1,25(OH)_2D$. CYP24A1 mRNA and protein levels are significantly upregulated in cancers relative to normal tissues, suggesting that *CYP24A1* may be an oncogene.[114] Polymorphisms in the promoter region of *CYP24A1* enhance both basal and vitamin D_3-stimulated promoter activity,[115] and variants at this locus were recently found to be significantly associated with plasma 25(OH)D levels in a genome-wide association study.[78]
- 25-hydroxylase (*CYP2R1*) may be the enzyme underlying 25-hydroxylation of vitamin D3 in the liver, however many other enzymes have also been shown to have 25-hydroxylase activity *in vitro*. Two recent genome-wide association studies found this locus to be significantly associated with circulating 25(OH) levels, lending support to this enzyme's role in the crucial first step in vitamin D metabolism.[78, 79]
- The retinoic acid receptor (*RXR*) functions as a heterodimer with VDR, forming a VDR/RXR heterodimeric complex that regulates transcription of several target genes.[108, 116] Binding of VDR to RXR is required for VDR to function.
- 7-dehydrocholesterol reductase (*NADSYN1/DHCR7*) converts 7-dehydrocholesterol to cholesterol, thus removing the substrate from the synthetic pathway of vitamin D3. This locus was recently identified in two separate genome-wide association studies as being strongly associated with 25(OH)D levels, and may therefore have a larger role in vitamin D regulation than previously recognized.[78, 79]
- Dehydroepiandrosterone (DHEA) sulfotransferase (*SULT2A1*) catalyzes the sulfate conjugation of DHEA and other related hydroxysteroid hormones. Ligand-activated VDR has been shown to transcriptionally upregulate *SULT2A1* expression,[117] and a variant in this locus was recently found to be significantly associated with circulating 25(OH)D (unpublished data).

VDR and 1-α-Hydroxylase Expression, and KRAS Mutation Status

Hypothesis: The effects of vitamin D on colorectal cancer outcome are strongest in patients whose tumors overexpress VDR and 1-α-hydroxylase.

The impact of vitamin D on patient outcome may be modified by molecular factors. VDR and 1-α-hydroxylase are frequently expressed in colon cancer cells. Well differentiated colon cancer cell lines have higher VDR expression,[118] and the antiproliferative effects of vitamin D may only occur in cell lines expressing high levels of VDR.[119] Expression of VDR and 1-α-hydroxylase increases in the early stages of colorectal tumorigenesis, but appears to decline in poorly-differentiated tumors and metastases.[120] In one study, overexpression of VDR was seen in approximately 38% of colorectal tumors.[121] Moreover, $APC^{min/+}$ mice lacking VDR developed increased tumor burden compared to $APC^{min/+}$ mice with wild-type VDR. The increased tumor burden in $APC^{min/+}$ $VDR^{2/2}$ supports the anticancer property of the VDR.[122]

Hypothesis: The effects of vitamin D on colorectal cancer outcome are strongest in tumors with wild-type KRAS.

Point mutations in the *KRAS* oncogene occur in approximately 40% of colorectal cancers.[123-125] Recent data indicate that *KRAS* mutations may be associated with lack of response to epidermal growth factor receptor (EGFR)-targeting agents.[126-129] Interestingly, the vitamin D pathway may interact with KRAS signaling. In a *RAS*-transformed cell line of human keratinocytes, malignant cells were found to be resistant to the growth-inhibitory effects of $1,25(OH)_2D$.[130, 131] Furthermore, VDR expression appears to be down-regulated in *KRAS*-mutated cell lines,[132] and vitamin D's ability to affect apoptosis may vary by *KRAS* status.[133] In another study, VDR overexpression was significantly associated with *KRAS* mutation (OR 1.55; 95% CI, 1.11-2.16; P=0.01) and *PIK3CA* mutation (OR 2.17; 95% CI, 1.36-3.47; P=0.001).[121]

Plasma Biomarkers of the Inflammatory and Insulin-Like Growth Factor Pathways

Hypothesis: Vitamin D supplementation decreases plasma markers of inflammation, and changes in these markers influence the effect of vitamin D on patient outcome.

Several mechanisms have been proposed to explain the anti-cancer properties of vitamin D. One hypothesis is inhibition of inflammation. Vitamin D exerts immunoregulatory activities, mediated by VDR, 1-α-hydroxylase, and 24-hydroxylase (CYP24A1), which are expressed in most immune cells.[134] Vitamin D down-regulates nuclear factor-κB (NF-κB) activity, increases production of anti-inflammatory IL-10, and decreases production of pro-inflammatory IL-6, IL-12, interferon-γ (IFN-γ), and TNF-α, leading to a profile that favors less inflammation.[134] Among patients with chronic renal failure, vitamin D supplementation has been associated with a lower mortality, attributed in part to its anti-inflammatory properties.[135, 136] In healthy women, a significant inverse relationship was seen between serum 25(OH)D and TNF-α after controlling for body fat, menopausal status, age, and hormone use.[137] Hypovitaminosis D has also been associated with autoimmune diseases, including type 1 diabetes, multiple sclerosis,[138] and inflammatory bowel disease.[139-149] Evidence of a causal link in humans derives from randomized controlled trials that show that vitamin D lowers CRP[150] and TNF-αR,[151] and increases IL-10.[151]

Hypothesis: Vitamin D supplementation will attenuate the impact of IGF and insulin on outcomes in patients with metastatic colorectal cancer.

The IGF axis influences cellular proliferation and apoptosis.[152] Supraphysiologic levels of insulin are required to activate the IGF receptor and stimulate cell division. IGF binding proteins (IGFBPs) oppose the actions of IGF-1, in part by sequestration,[153] but also by independent inhibitory effects mediated by specific IGFBP-3 membrane-associated receptors. Indeed, high IGF-1 and low IGFBP-3 levels were associated with risk of colorectal cancer in the Nurses' Health Study (NHS)[154] and Physicians' Health Study.[155] Moreover, among 373 non-metastatic colorectal cancer patients in NHS and HPFS, higher pre-diagnostic plasma levels of C-peptide increased overall mortality, whereas high IGFBP-1 was associated with decreased mortality.[156] Circulating IGF-1 and IGFBP-3 were not associated with mortality. In contrast, in a clinical trial of stage IV colorectal cancer patients (N9741), higher plasma IGFBP-3 was significantly associated with superior survival.[157] There is evidence that vitamin D interacts with the IGF pathway. Plasma 25(OH)D levels are significantly correlated with plasma IGF-1 levels.[158] Moreover, $1,25(OH)_2D$ influences mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) signaling through effects on IGF-1, and also induces apoptosis through the IGF receptor-1 (IGFR-1)-phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway.[159] In analyses of colorectal cancer risk, higher plasma 25(OH)D level appears to attenuate the increased risk of colorectal cancer associated with a higher IGF-1/IGFBP-3 ratio (unpublished data). Furthermore, treatment with $1,25(OH)_2D$ has been shown to inhibit cell growth, increase IGFBP-3 mRNA levels and stability, and lead to IGFBP-3 accumulation in prostate cancer cells.[160]

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Participants must have histologically confirmed adenocarcinoma of the colon or rectum that is metastatic or locally advanced (unresectable).

Patients with resected primary tumors who have documented metastases are eligible. Documentation of residual disease by CT scan or surgeon's notes is required for all patients, and histologic confirmation of metastases is strongly encouraged.

3.1.2 Patients with a history of colorectal cancer treated by surgical resection who develop radiological or clinical evidence of metastatic cancer do not require separate histological or cytological confirmation of metastatic disease unless:

- Either an interval of >5 years has elapsed between the primary surgery and the development of metastatic disease, OR

- The primary cancer was stage I.

Clinicians should consider biopsy of lesions to establish the diagnosis of metastatic colorectal cancer in each case if there is substantial clinical ambiguity regarding the nature or source of apparent metastases.

3.1.3 Patients must have measurable disease per RECIST 1.1.

3.1.4 Both *KRAS* wild type and *KRAS* mutant patients are eligible.

3.1.5 No prior systemic treatment for advanced or metastatic colorectal cancer is allowed. Prior regional chemotherapy (e.g., hepatic arterial infusion) is also not allowed.

Patients may have received previous neoadjuvant or adjuvant chemotherapy and/or chemoradiation per institutional standard of care. The last course of adjuvant therapy must have been concluded >12 months prior to colorectal cancer recurrence.

3.1.6 Patients may not have had prior radiotherapy to >25% of bone marrow. Standard rectal cancer chemoradiation will not exclude subject from study protocol.

Any radiation therapy must have concluded ≥4 weeks prior to start of protocol treatment.

3.1.7 Patients should have completed any major surgery or open biopsy ≥4 weeks from start date of chemotherapy.

Patients must have completed any minor surgery or core biopsy ≥1 week prior to first dose of bevacizumab. (Insertion of a vascular access device is not considered major or minor surgery.)

3.1.8 Age ≥18 years

3.1.9 ECOG performance status ≤1 (see Appendix A)

3.1.10 Paraffin-embedded and/or snap-frozen tumor tissue samples (from primary tumor or metastasis) taken as part of routine clinical care must be available for study-related correlative studies. If paraffin-embedded and/or snap-frozen tumor tissue samples are not available, at least 15 unstained tumor slides will be requested.

3.1.11 Participants must have normal organ and marrow function as defined below:

- Absolute neutrophil count (ANC) ≥ 1,500/mcL

- Platelets ≥ 100,000/mcL
- Hemoglobin ≥9 g/dL
- Total bilirubin ≤1.5x institutional upper limit of normal (ULN)
- AST (SGOT)/ALT (SGPT) ≤2.5 X institutional ULN, or <5x ULN if clearly attributable to liver metastases
- Serum calcium (corrected for albumin level) ≤1x institutional ULN
- Serum creatinine ≤1.5x ULN
- UPCR: <1.0
- Patients on full-dose anticoagulation are eligible if the following criteria are met:
    - Patient has an in-range INR (usually 2-3) on a stable dose of warfarin or is on a stable dose of low molecular weight heparin
    - Patient has no active bleeding or pathological condition that carries a high risk of bleeding (i.e., tumor involving major vessels or known varices)
    - Patients receiving anti-platelet agents are eligible. In addition, patients who are on daily prophylactic aspirin or anticoagulation for atrial fibrillation are eligible.

3.1.12 Non-pregnant and not nursing

Women of child-bearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 14 days prior to study entry. Women of child-bearing potential include any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥12 consecutive months; or women on hormone replacement therapy with documented serum follicle stimulating hormone level >35 mIU/mL). Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or who are practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child-bearing potential.

The effects of FOLFOX-bevacizumab and higher doses of vitamin D3 on the developing human fetus are unknown. For this reason and because DNA alkylating agents are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 30 days after the last dose of study drug. Should a woman

become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.13 Ability to understand and the willingness to sign a written informed consent document.

3.1.14 A vascular access device (port) or other central venous access for administration of chemotherapy is recommended.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Prior chemotherapy, other systemic therapy, or any investigational agent for treatment of advanced or metastatic colorectal cancer.

Patients who completed adjuvant or neoadjuvant chemotherapy >12 months prior to colorectal cancer recurrence are eligible.

3.2.2 Concurrent use of other anti-cancer therapy including chemotherapy agents, targeted agents, or biological agents not otherwise specified in this protocol.

3.2.3 Known or suspected brain or other CNS metastases.

Participants with known brain metastases are excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 No uncontrolled seizure disorders.

3.2.5 History of prior or synchronous malignancy except:

- A malignancy that was treated with curative intent and for which there has been no known active disease for >3 years prior to randomization
- Curatively treated non-melanoma skin malignancy, cervical cancer in situ, or prostatic intraepithelial neoplasia without evidence of prostate cancer

3.2.6 Regular use of vitamin D supplements ≥ 2,000 IU per day in the past year.

Use of supplemental vitamin D or supplements containing vitamin D beyond the protocol-prescribed study treatment is not allowed while enrolled on this clinical trial.

In order to maintain blinding, vitamin D levels should not be routinely checked at screening or during the study by the treating investigator.

Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.

3.2.7 History of allergic reactions attributed to compounds of similar chemical or biologic composition to 5-FU, capecitabine, oxaliplatin, leucovorin, bevacizumab, and/or vitamin D3.

3.2.8 Significant history of bleeding events or pre-existing bleeding diathesis, within 6 months of randomization (unless the source of bleeding has been resected)

3.2.9 History of gastrointestinal perforation within 12 months of randomization, except for GI perforation related to a primary colorectal tumor that has subsequently been fully resected. Subjects would be eligible if ≥4 weeks have elapsed from the time of surgery to start date of chemotherapy on protocol and ≥6 weeks have elapsed from time of surgery to first dose of bevacizumab. The subject must have recovered from the effects of the surgery (e.g. wound is healed, no active infection, no drains, etc.).

3.2.10 History of arterial thrombotic events within 6 months before randomization, including transient ischemic attack (TIA), cerebrovascular accident (CVA), unstable angina or angina requiring surgical or medical intervention in the past 6 months, or myocardial infarction (MI). Patients with clinically significant peripheral artery disease (e.g., claudication with <1 block) or any other arterial thrombotic event are also ineligible.

3.2.11 History of uncontrolled congestive heart failure defined as NYHA Class III or greater.

3.2.12 Serious or non-healing wound, ulcer, or bone fracture.

3.2.13 Patients with a history of hypertension must be well-controlled (<150/90) on a regimen of anti-hypertensive therapy.

3.2.14 Clinically significant peripheral neuropathy (defined as ≥ Common Terminology Criteria for Adverse Events [CTCAE] grade 2 [version 4.0]), neurosensory or neuromotor toxicity, regardless of causality.

3.2.15 Predisposing colonic or small bowel disorders in which the symptoms are uncontrolled, as indicated by a baseline pattern of >3 watery or soft stools daily in patients without a colostomy or ileostomy. Patients with a colostomy or ileostomy may be entered at investigator discretion.

3.2.16 Pre-existing hypercalcemia (defined as baseline serum calcium above the institutional ULN, corrected for albumin level if albumin is not within institutional limits of normal).

The use of supplemental calcium is prohibited while on study.

3.2.17 Known active hyperparathyroid disease or other serious disturbance of calcium metabolism in the past 5 years.

3.2.18 History of symptomatic genitourinary stones within the past year.

3.2.19 Regular use of thiazide diuretics (i.e., hydrochlorothiazide), which can lead to hypercalcemia, and unwillingness or inability to discontinue or switch to alternative anti-hypertensive agent.

3.2.20 Inability to swallow pills.

3.2.21 History of malabsorption or uncontrolled vomiting or diarrhea, or any other disease significantly affecting gastrointestinal function that could interfere with absorption of oral medications.

3.2.22 Use of chronic oral corticosteroid therapy, lithium, phenytoin, quinidine, isoniazid, and/or rifampin (all of which can cause vitamin D depletion). Short-term use of corticosteroids as anti-emetic therapy for chemotherapy is permitted.

3.2.23 Uncontrolled intercurrent illness, including, but not limited to, psychiatric illness/social situations, that in the opinion of the investgator may increase the risks associated with study participation or study treatment, or may interfere with the conduct of the study or the interpretation of the study results.

3.2.24 Pregnant or nursing women or men/women of child-bearing potential who are unwilling to employ adequate contraception

Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with FOLFOX-bevacizumab, breastfeeding should be discontinued if the mother is treated with FOLFOX-bevacizumab. These potential risks may also apply to other agents used in this study.

3.2.25 Known positive test for human immunodeficiency virus (HIV), hepatitis C virus, or acute or chronic hepatitis B infection

HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with FOLFOX-bevacizumab. In addition, these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Women and minorities will be eligible for this study without alteration in eligibility criteria. Outcome differences by gender and race/ethnicity will be explored using the proportional hazards model for PFS and OS, the logistic regression model for tumor RR, and the linear regression model for plasma level of 25(OH)D.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the QACT protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study may be cancelled. Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

The registration procedures are as follows:

1. Investigator will obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet all inclusion and exclusion criteria as described in the protocol and reflected on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

4. The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. An email confirmation of the registration will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration. A fax confirming randomization will be sent to the pharmacy.

4.3 General Guidelines for Other Participating Institutions

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute (DFCI) by the Study Coordinator. All sites should call the Study Coordinator at 617-632-6353to verify treatment availability.

Following registration, participants should begin protocol treatment within 14 days or as soon as possible. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be cancelled. The Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Participating Institutions

To register a participant, the following documents should be completed by the research nurse or data manager and emailed to the Study Coordinator(s) at Christine_Ganser@dfci.harvard.edu

- Copy of pathology report confirming cancer diagnosis, laboratory results (CBC with differential and platelets, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, phosphorous, albumin, total protein, total bilirubin, alkaline phosphatase, SGOT, SGPT, PT, INR, PTT, CEA, urinalysis [including urine protein] and pregnancy test in women of childbearing potential), and baseline CT or MRI report
- Signed study consent form
- HIPAA authorization form
- Eligibility Checklist

The research nurse or data manager at the participating site will then call 617 632 6353 or email the Study Coordinator at Christine_Ganser@dfci.harvard.edu to verify eligibility. To complete the registration process, the Coordinator will:

- Register the participant on the study with QACT
- Fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site

- Call the research nurse or data manager at the participating site and verbally confirm registration

Note: Registration and randomization with the QACT can only be conducted during the business hours of 8am – 5pm EST Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

5. TREATMENT PLAN

Protocol treatment is to begin within 14 days of randomization. One cycle will be defined as 14 days. Treatment will be administered on an outpatient basis. All laboratory and other assessments must be performed prior to administration of any study medication, however +/- 7 days will be allowed to change patient schedule without IRB approval at the discretion of the treating investigator.

The cycle 1 Day 1 dose of bevacizumab may be held at the investigator's discretion.

5.1 Expected toxicities and potential risks as well as dose modifications for vitamin D3 and FOLFOX-bevacizumab are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy. Pre-treatment Criteria:

5.1.1 Screening Assessments

Informed consent must be obtained before any study-specific screening evaluations are performed. Unless otherwise noted, the screening assessments outlined below must be performed within 14 days prior to Cycle 1 Day 1.

- Signed informed consent (within 4 weeks of randomization)

- Tumor tissue sample for correlative science will be requested for all study participants. A representative tumor specimen in paraffin blocks or at least 15 unstained slides with associated pathology report will be requested by the participating site. Sample does not need to be on-site to initiate study treatment, however every effort should be made to obtain sample prior to start of study. Once site has obtained sample, it will then be forwarded to DFCI study coordinator for storage as soon as possible.

- Clinical evaluations:
  - Medical and surgical history, including demographics
  - Complete physical examination
  - Vital signs
  - Drug allergies

  - Height and weight
  - ECOG performance status (see Appendix A)
  - Concomitant medications

- Laboratory assessments:
  - Baseline hematologic (CBC with differential and platelets) and biochemical profiles, including calcium, albumin, total protein, total bilirubin, alkaline phosphatase, SGOT, SGPT, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, and phosphorous
  - Baseline carcinoembryonic antigen (CEA)
  - Baseline PT, INR, and PTT for subjects on anticoagulation
  - UPCR
  - Pregnancy test in all women of childbearing potential

- Tumor assessments:
  - Baseline CT scan of the chest and CT or MRI of the abdomen and pelvis with measurement of all measurable disease as defined by RECIST 1.1, within 4 weeks prior to Cycle 1 Day 1

- Correlative studies:
  - 3 blood samples for future research (may be obtained during screening period or on Cycle 1 Day 1 prior to dosing of study medication)

  In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.

Once eligibility is established based on the inclusion and exclusion criteria and screening procedures, all documentation must be submitted as described in Section 4. Participants must be treated within 14 days of registration.

5.1.2 Cycle 1, Day 1

Screening clinical evaluations and laboratory assessments may be used as the Cycle 1 Day 1 evaluations provided that the assessments are performed within 7 days of Cycle 1 Day 1, unless otherwise specified.

- Clinical evaluations:
  - Focused physical exam
  - Vital signs
  - Weight
  - Concomitant medications

- Laboratory assessments:
  - CBC with differential and platelets
  - Serum chemistries (see Study Calendar for complete list)

- Correlative studies:
  - 3 blood samples for future research (if not already obtained at screening)

  In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participants vitamin D status, the lead Principal Investigator should be contacted for further discussion.

- Adverse event assessment

- FOLFOX chemotherapy + bevacizumab administration (bevacizumab may be held on Cycle 1 Day 1 at investigator's discretion).

- Initiate vitamin D3/placebo (*two* capsules once daily for the first 14 days only; thereafter, participants will only take *one* capsule once daily)

5.1.3 All Subsequent Cycles

- Clinical evaluations:
  - Focused physical exam
  - Vital signs
  - Weight
  - Concomitant medications
  - Review of study drug diary and vitamin D3 pill counts

- Laboratory assessments:
  - CBC with differential and platelets
  - Serum chemistries (see Study Calendar for complete list)
  - Urinalysis (see Study Calendar in Section 9 for exact time points)
  - CEA (see Study Calendar in Section 9 for exact time points, which are around the time of each tumor assessment)

- Adverse event assessment

- Tumor assessment (see Section 5.1.4 below and Study Calendar in Section 9 for exact time points, which are approximately every 4 cycles)

- Two blood sample for correlative studies (see Section 5.1.5 below and Study Calendar in Section 9 for exact time points)

- FOLFOX chemotherapy + bevacizumab administration
- Review of previous cycle study drug diary
- Vitamin D3 (*one* capsule once daily, beginning on Cycle 2 Day 1)

5.1.4 Tumor Assessments

- CT scan of the chest and CT or MRI of the abdomen and pelvis with measurement of all measurable disease

  Radiographic tumor assessments will be performed after every 4 cycles of treatment, prior to Day 1 of the upcoming cycle (i.e., prior to Cycle 5, 9, 13, etc.).

  The same radiographic procedures used to define measurable disease sites at baseline must be used throughout the study (e.g., the same contrast protocol for CT scans). Any deviations to this will be reviewed and discussed with the Principal Investigator to determine if the participant may remain on study.

5.1.5 Correlative Studies

Archival tumor tissue for correlative studies and future research will be requested at screening. For participants who undergo surgical resection of metastatic disease or primary tumor during the study and have consented to the optional tissue collection, study teams should request tumor tissue for correlative studies and banking for future research. Once the site has obtained the sample, it will then be forwarded to DFCI study coordinator for storage as soon as possible.

Two blood samples for correlative studies will be drawn after Cycle 4 (prior to Cycle 5), after Cycle 8 (prior to Cycle 9), and at study discontinuation.

In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.

Please refer to Section 8.0 for further information on correlative studies.

5.1.6 Off-Treatment Assessments

A follow-up visit should occur 30 days (+/- 3 days) after the last dose of study treatment. The visit at which a response assessment shows disease progression may be used as the off-treatment visit.

- Clinical evaluations:
  - Limited physical exam
  - Vital signs
  - Weight
  - Concomitant medications
  - Review of study drug diary and vitamin D3 pill counts

- Laboratory assessments:
  - CBC with differential and platelets
  - Serum chemistries (see Study Calendar for complete list)
  - Urinalysis or spot urine protein and creatinine
  - CEA
- Correlative studies:
  - 2 blood samples for future research

  In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.

- Tumor assessment:
  - If no evidence of disease progression on scans when taken off study (e.g., off study for toxicity, clinical progression, or other reasons), perform CT scan of the chest and CT or MRI of the abdomen and pelvis with measurement of all measurable disease

- Adverse event assessment

5.1.7 Safety Follow-Up

After completion of or discontinuation from the study, participants will be followed for safety 30 days (+/- 3 days) after the last dose of study drug. Participants with an adverse event that is ongoing at the off-study visit should be contacted at least monthly until the event has resolved or until the participant begins treatment with a new anti-cancer therapy.

5.1.8 Survival Follow-Up

Participants will be asked to provide their contact information and/or a family member's contact information for long-term follow-up. All participants who discontinued study treatment for any reason other than disease progression and all participants whose tumor progression was not documented at the end-of-study visit will continue to have tumor assessments every 8-16 weeks (+/- 7 days) until documented disease progression or until the start of additional anti-tumor therapy. All participants whose disease has progressed will be followed by clinic visit or telephone call every three months for survival until 36 months from the date that the last participant was randomized or death, whichever occurs earlier.

5.2 Agent Administration

5.2.1 Vitamin D3 (Cholecalciferol)

Placebo, 400 IU, and 4000 IU supplements of vitamin D_3 will be manufactured by Pharmavite LLC, (Mission Hill, CA) in the form of liquid softgel capsules. Pharmavite produces 11.5 billion vitamin supplements annually in a facility that is compliant with United States Pharmacopeia's Good Manufacturing Practices (GMPs). All softgel capsules will be indistinguishable, regardless of dose or placebo, and packaged in bottles.

The designated pharmacist is responsible for dispensing the blinded study medication. All site study personnel (except pharmacist) and all study participants will be blinded to the treatment arm assignment.

Unblinding of the study medication (Vitamin D3) will occur only in the event of urgent or serious adverse event with the permission of the principal investigator Dr. Kimmie Ng (via email or telephone).

Vitamin D3 will be dispensed as a two week supply for Cycle 1, then as a monthly supply for all cycles thereafter.

All dosages prescribed and dispensed to the participant and all dose changes during the study must be recorded in the electronic case report form (eCRF). Medication labels will comply with institutional standards and U.S. legal requirements and be printed in English. The storage conditions for study drug will be described on the medication label.

Participants will self-administer vitamin D3 capsules by mouth, and will be instructed to keep a drug diary. The vitamin D3 should be kept in the bottle in which it was dispensed. The diary, empty bottle(s), or any extra vitamine D3 capsules will be returned to the designated study team member for reconciliation and review of the diary after the loading dose and then monthly.

Capsules should be taken at approximately the same time each day, with or without food. If vomiting occurs, no attempt should be made to replace the

vomited dose, unless the entire capsule can be seen intact in the vomitus. If a dose is missed (i.e., forgotten), the vitamin D3 dose should only be made up if it is within 12 hours of the regularly scheduled dose. On days in which both vitamin D3 and FOLFOX-bevacizumab are given, vitamin D3 can be taken either before or after FOLFOX-bevacizumab.

A corrected calcium should be calculated if the serum albumin is not within the institutional range of normal. Please refer to Section 6.2, Table 5 for dose modification guidelines for hyperclacemia. Up to a 4-week delay is allowed in the initiation of a new cycle of treatment for resolution of hypercalcemia to ≤ grade 1 (see Section 6.2, Table 5). At the discretion of the treating investigator, blood tests and/or clinical evaluations should occur at least weekly to monitor these parameters

In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.

In the event that vitamin D3 administration is held, FOLFOX-bevacizumab administration may continue.

If vitamin D3 interruption is ≤4 weeks from the previous cycle, and the participant has recovered from toxicity as specified above, and the participant's disease has not progressed, vitamin D3 should be restarted at the same dose, as outlined in Section 6. If vitamin D3 interruption is >4 weeks, but the participant has recovered from toxicity and the participant's disease has not progressed, the case should be reviewed by the lead Principal Investigator to determine the appropriateness of treatment resumption. In the event that vitamin D3 is permanently discontinued for any reason prior to disease progression, the participant will be discontinued from the study. Continued FOLFOX-bevacizumab administration off-protocol may be continued at the treating physician's discretion.

For the purposes of this protocol, 1-3 missed doses during the 28 day dispensation period will be considered a minor violation and more than 3 missed doses during this period will be reported as a major violation.

Control Arm A : (Dose is blinded to site study personnel and participant):

Dose: **Vitamin D3 400 IU daily**.

Cycle 1 only: The first cycle (14 days) is the loading dose period. The loading dose of vitamin D3 (400 IU) daily will be blinded to the participant and the study team and will be dispensed in two bottles. Each bottle will contain 14 capsules (one bottle will have placebo, the other 400 IU capsules). Participants will be

instructed to take one capsule from each bottle at the same time each day (total dose is 400 IU per day for 14 days). .

Cycle 2 and subsequent cycles: participants will take one capsule daily (Vitamin D3 400 IU). Pharmacy will dispense a one month supply of Vitamin D3. Treatment will continue until documented disease progression per RECIST 1.1 or treating investigator assessment, intolerable toxicity, or participant withdrawal.

Investigational Arm B: (Dose is blinded to site study personnel and participant)

Dose: **Vitamin D3 4000 IU daily**.

Cycle 1 only: The first cycle (14 days) is the loading dose period. The loading dose of vitamin D3 (8000 IU daily) will be blinded to the participant and the study team and will be dispensed in two bottles. Each bottle will contain 14 capsules of vitamin D3 (4000 IU capsules). Participants will be instructed to take one capsule from each bottle at the same time each day (total dose is 8000 IU per day for 14 days).

Cycle 2 and subsequent cycles: participants will take one capsule daily (Vitamin D3 4000 IU). Pharmacy will dispense a one month supply of Vitamin D3. Treatment will continue until documented disease progression per RECIST 1.1 or treating investigator assessment, intolerable toxicity, or participant withdrawal.

Table 1. Vitamin D3 Dosing by Treatment Arm. The dispensing pharmacist is the only member of the study team that is aware of the dose. All others are blinded to dose of vitamin D3.

ARM	CYCLE 1 (LOADING PERIOD, DAYS 1-14)	CYCLE 2 AND ALL SUBSEQUENT CYCLES
Control Arm A	400 IU + 1 placebo capsule once daily (at the same time)	400 IU once daily
Investigational Arm B	Two 4000 IU capsules once daily (at the same time)	4000 IU once daily

5.2.2 FOLFOX-bevacizumab

FOLFOX-bevacizumab chemotherapy will be administered intravenously on Day 1 (+/- 7 days) of every two-week cycle per institutional standard of care procedures to all participants on both the control and investigational arms. The modified FOLFOX6 schedule of administration found in Table 2 below is provided as a general guideline only; standard institutional procedures should be followed.

A new cycle of FOLFOX-bevacizumab may not be administered to the participant if the ANC is $<1.0 \times 10^3$ cells/L; if the platelet count is $<75 \times 10^9$/L; if stomatitis or diarrhea have not recovered to grade 1 or less; or if fatigue has not recovered to grade 2 or less.

Up to a 4-week delay is allowed in the initiation of a new cycle of treatment for resolution of clinically significant, drug-related toxicities. Any delay to the start of FOLFOX will result in the same delay to bevacizumab so that all treatments are given together. Study procedures associated with each cycle of therapy will also be delayed accordingly (including tumor assessments).

Table 2. Example of FOLFOX-bevacizumab Administration for All Treatment Arms

Each Cycle: FOLFOX	Bevacizumab	Oxaliplatin	Leucovorin (LV)*	5-FU
Day 1 (+/- 7 days)	5mg/kg IV Rate of administration per institutional standard	85mg/m^2 IV Administer after bevacizumab. Administer over 2 hours.	400 mg/m^2 IV Administer over 2 hours, either concurrently with oxaliplatin via separate line, or after oxaliplatin has been infused if second line is not available.	400mg/m^2 IV bolus Adminster after oxaliplatin and leucovorin, and follow with 2400 mg/m^2 continuously over 46-48 hours

* If racemic folinic acid/leucovorin is not available due to drug supply issues, discuss substitution or discontinuation with the Principal Investigator (e.g., intravenous levo-leucovorin 200 mg/m2, 50% of the protocol-specified dose of IV racemic leucovorin, lower dose of IV racemic leucovorin such as 20 mg/m2, or omission of leucovorin are possible options).

In the event that FOLFOX chemotherapy administration is permanently discontinued for any reason prior to disease progression, bevacizumab administration may continue on Day 1 of every 2-week cycle and vitamin D3 administration may continue orally once a day.

In the event that bevacizumab administration is permanently discontinued for any reason prior to disease progression, FOLFOX should be continued on Day 1 of every 2-week cycle and vitamin D3 should continue orally once daily until one of the following occurs: disease progression, unacceptable toxicity, death, or withdrawal of consent.

In the event that oxaliplatin administration is permanently discontinued for any reason prior to disease progression (e.g., Grade 3 progressive sensory or motor neuropathy), 5-FU and LV, bevacizumab, and vitamin D3 administration should continue until one of the following occurs: disease progression, unacceptable toxicity, death, or withdrawal of consent. Oxaliplatin may be resumed at investigator's discretion if neuropathy recovers to ≤Grade 1.

If FOLFOX chemotherapy interruption is ≤4 weeks from the previous cycle, and the participant has recovered from toxicity, as specified above, and the participant's disease has not progressed, FOLFOX-bevacizumab chemotherapy should be

restarted at doses as outlined in Section 6. If FOLFOX-bevacizumab chemotherapy interruption is >4 weeks, but the participant has recovered from toxicity and the participant's disease has not progressed, the case should be reviewed by the lead Principal Investigator to determine the appropriateness of treatment resumption.

Premedication

5-FU and oxaliplatin may be emetogenic. Prior to the administration of FOLFOX6, premedication with anti-emetics, such as serotonin (5HT3) antagonists (i.e., ondansetron, ganisetron) with or without dexamethasone may be used at the treating investigator's discretion or according to institutional standards.

Recommended Sequence of Administration of FOLFOX-Bevacizumab*

- Bevacizumab 5 mg/kg IV will be administered first, *preceding* FOLFOX (see Table 2 above). Bevacizumab should be administered at a rate of 0.5mg/kg/minute or per institutional standard rate.
- Oxaliplatin 85 mg/m^2 IV should then be administered over 2 hours. Oxaliplatin infusion time may be lengthened to 6 hours (maximum) at the discretion of treating investigator.
- Leucovorin (400mg/m2) may be administered concurrently (via separate infusion lines) with oxaliplatin or following infusion of oxaliplatin. Leucovorin is adminstered over 2 hours.
- 5-FU 400 mg/m^2 IV bolus, then 2400 mg/m^2 continuous IV infusion via portable infusion pump over 46-48 hours

**Please note that this sequence of administration is intended as a guideline. Participating sites may use their institutional standards of care and the sequence may be modified in cases where the treating investigator feels it is in the best interest of the patient.*

5.2.3 Participants Undergoing Surgery

During the study, participants who undergo interventional therapy for metastases (e.g., surgical resection, radiofrequency ablation, cryotherapy) will do so according to standard institutional practice. For participants for whom an elective surgery or procedure is contemplated, the lead Principal Investigator (Dr. Kimmie Ng) should be notified via email (preferred) or phone prior to any planned intervention. Participants may be allowed to continue on study post-intervention only if they have been off protocol therapy for ≤4 weeks, continue to have measurable disease per RECIST 1.1 outside of the treated metastatic lesion(s) post-intervention, and after discussion with the Principal Investigator.

Bevacizumab is to be discontinued for at least 6-8 weeks prior to surgery, and FOLFOX for at least 4 weeks prior to surgery. Vitamin D3 may continue up until the date of surgery at the treating physician's discretion. Tumor assessments by the

same modalities used previously in the study must be done prior to any scheduled intervention.

If further treatment on study is being considered post-intervention, the study participant must have measurable disease per RECIST 1.1 after the procedure. The first post-intervention radiological imaging will provide the "new baseline" tumor measurements, from which progression will be assessed and/or the "new nadir" from which progression will be assessed for the post-intervention RECIST tumor response assessments. Any remaining sites of disease must continue to be followed as target or non-target disease as designated at study screening.

For participants for whom non-elective (i.e., emergent or urgent) surgery is required, hold bevacizumab as long as possible prior to surgery, discontinue FOLFOX-bevacizumab immediately, and notify the lead Principal Investigator as soon as possible. Vitamin D3 may be continued at the treating physician's discretion after consultation with the lead Principal Investigator. A participant may be allowed to resume study treatment only if he/she has been off protocol therapy for ≤4 weeks, continues to have measurable disease per RECIST 1.1 post-surgery, and after discussion with the Principal Investigator regarding the appropriateness of treatment resumption. If protocol treatment is to be resumed, bevacizumab should be held for at least 6 weeks following surgery.

Any participant who discontinues study treatment prior to disease progression or death will continue to undergo tumor assessment by imaging every 8-16 weeks (+/- 7 days) to assess disease status until documented disease progression per RECIST or documented clinical progression, start of a new cancer treatment, death, withdrawal of consent, administrative decision, or the end of the study, whichever is earlier.

5.2.3.1 Optional Tissue Collection for Participants Undergoing Surgery

For participants who undergo surgical resection of primary tumor or metastatic disease during the study and have consented to the optional tissue collection, study teams should request tumor tissue for correlative studies and banking for future research. Once the site has obtained the sample, it will then be forwarded to the DFCI study coordinator for storage as soon as possible.

A representative tumor specimen in paraffin or a snap-frozen tumor block or at least 15 unstained slides with associated pathology report will be requested by the participating site. Once the site has obtained the sample, it will then be forwarded to the DFCI study coordinator for storage as soon as possible.

5.3 General Concomitant Medication

Throughout the study, treating investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care. However, participants may *not* receive:

- Other investigational agents
- Experimental or approved anti-tumor therapies other than therapy specified in this protocol
- Chemotherapy other than FOLFOX-bevacizumab
- Radiation therapy (except palliative radiation)
- Hormone-directed cancer therapy
- Tumor-directed antibody therapy other than therapy specified in this protocol (with the exception of use for pain control)
- Herbal medications or treatments
- Chronic corticosteroids, lithium, Phenobarbital, phenytoin, quinidine, isoniazid, and rifampin, all of which can cause vitamin D depletion. Short-term corticosteroids as anti-emetic therapy for chemotherapy is permitted.
- Any supplements that contain Vitamin D
- Calcium supplements

All prescribed and non-prescription concomitant medications that are ingested, applied, or injected on an ongoing basis from signing of the informed consent, as well as changes in such concomitant medications, and any new concomitant medication taken while the participant is on study, should be recorded in the clinical record. Concomitant medications should be recorded until 30 days after the last dose of protocol-specified therapy. Concomitant medications for medically significant adverse events which are ongoing at the end of study treatment and considered related to study treatment, should be followed until the adverse event is resolved or considered stable.

5.4 General Supportive Care Guidelines

Participants should be counseled to avoid cold drinks, chewing of ice chips, and exposure to cold water or air because the neurotoxicity often seen with oxaliplatin appears to be exacerbated by exposure to cold. The period of time during which the participant is at risk for these cold-induced sensory neuropathies is not well documented. Participants should exercise caution regarding cold exposure during the treatment period. Peripheral sensory neuropathies can occur at any time after receiving oxaliplatin therapy.

Loperamide

For symptoms of diarrhea and/or abdominal cramping that occur at any time during a treatment cycle, participants will be instructed to begin taking loperamide. Loperamide should be started at the earliest sign of 1) a poorly formed or loose stool, or 2) the occurrence of 1-2 more bowel movements than usual in one day, or 3) an increase in stool volume or liquidity. Loperamide should be taking in the following manner: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours around the clock until diarrhea-free for at least 12 hours. Participants may take loperamide 4 mg every 4 hours during the night. The maximum daily dose of loperamide is 16 mg/day. Participants should be provided with instructions for loperamide use at the initial treatment visit and instructed to purchase the medication over-the-counter so that they have sufficient supply on hand in case antidiarrheal support is required. Additional antidiarrheal measures may be used

at the discretion of the treating physician. Participant should be instructed to increase fluid intake to help maintain fluid and electrolyte balance during episodes of diarrhea. Please see Section 6.5 for dose modification guidelines for diarrhea.

Antibiotics

Oral fluoroquinolone treatment is strongly recommended and should be initiated at the discretion of the treating investigator for any of the following:

- Diarrhea persisting for >24 hours despite loperamide
- ANC<500 (even in the absence of diarrhea or fever)
- Fever with diarrhea (even in the absence of neutropenia)
- Antibiotic therapy should also be initiated in participants who are hospitalized with prolonged diarrhea (even in the absence of neutropenia)

Growth Factors

For low white blood cell counts, granulocyte colony-stimulating factor (GCSF) should be used in participants with serious neutropenic complications such as febrile neutropenia, tissue infections, sepsis syndrome, fungal infection, etc. GCSF should be used and administered according to the product label or applicable guidelines. GCSF should not be administered in the period between 24 hours before and 24 hours after the administration of cytotoxic chemotherapy.

For chemotherapy-induced anemia, erythropoiesis-stimulating agents should be used and administered according to the product label or applicable guidelines.

Oral Cryotherapy

Participants should not receive oral cryotherapy (i.e., ice for mucositis prophylaxis) on Day 1 of each treatment cycle, as this may exacerbate laryngopharyngeal dysesthesia caused by oxaliplatin.

Hypersensitivity

Platinum hypersensitivity can cause dyspnea, bronchospasm, itching, and hypoxia. Infusion of oxaliplatin should be suspended immediately. Appropriate treatment will be administered according to institutional standards and may include supplemental oxygen, standard epinephrine, corticosteroid, antihistamine therapy, bronchodilators, or vasopressors. Platinum hypersensitivity is a rare event and should be treated promptly. Please see Section 6.9 for dose modification guidelines for allergic reactions.

Laryngopharyngeal Dysesthesias

Oxaliplatin may cause discomfort in the larynx or pharynx associated with dyspnea, anxiety, and difficulty swallowing. This discomfort is exacerbated by cold. Infusion of oxaliplatin should be suspended or lengthened. Should a participant develop oxaliplatin-induced laryngopharyngeal dysesthesia, his/her oxygen saturation should be evaluated via pulse oximeter; if normal, an anxiolytic agent may be given and the participant observed in the clinic until the episode has resolved. Appropriate therapy may include the

use of antihistamine therapy, bronchodilators, cold avoidance, or monitoring. Increase the duration of oxaliplatin infusion to 6 hours for all subsequent treatments, as detailed in Section 6.4. Some overlap may exist between the manifestations of laryngopharyngeal dysesthesia and hypersensitivity reactions. A table comparing the two is presented below.

Table 3. Comparison of the Symptoms and Treatment of Laryngopharyngeal Dysesthesias and Platinum Hypersensitivity Reactions

CLINICAL SYMPTOMS	LARYNGOPHARYNGEAL DYSESTHESIA	PLATINUM HYPERSENSITIVITY
Dyspnea	Present	Present
Bronchospasm	Absent	Present
Laryngospasm	Absent	Present
Anxiety	Present	Present
O2 saturation	Normal	Decreased
Difficulty swallowing	Present (loss of sensation)	Absent
Pruritis	Absent	Present
Urticaria/rash	Absent	Present
Cold-induced symptoms	Yes	No
Blood pressure	Normal or increased	Normal or decreased
ACTION TO BE TAKEN	Anxiolytics, observation in a controlled clinical setting until symptoms abate or at the physician's discretion	Basic life support and ACLS as needed. Oxygen, steroids, epinephrine, bronchodilators, fluids, and vasopressors, if appropriate, and other supportive care as needed

5.5 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression, and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression per RECIST 1.1,
- Observed clinical progression of disease not measurable by RECIST,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Delay of 4 weeks or greater for recovery of toxicity,
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.6 Duration of Follow Up

Participants will be followed for 36 months from the date the last participant on the study was randomized or until death, whichever occurs first. Participants removed from

treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event, or until they start a new anti-cancer treatment. Please see Sections 5.1.7 and 5.1.8 for details.

5.7 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.6 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Kimmie Ng, MD, MPH, at 617-632-4150 or Kimmie_Ng@dfci.harvard.edu.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 which is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study, and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

A new cycle of FOLFOX-bevacizumab may not be administered to the participant if the ANC is $<1.0 \times 10^3$ cells/L; if the platelet count is $<75 \times 10^9$/L; if stomatitis or diarrhea have not recovered to grade 1 or less; or if fatigue has not recovered to grade 2 or less. All other study drug-related, clinically significant, non-hematological toxicity should resolve to grade 1 or baseline unless noted otherwise in the tables below. Dose modifications for hematologic toxicity should be based on laboratory values obtained as described in the Pre-Treatment Criteria section (Section 5.1). Interval counts (i.e., CBCs done on non-FOLFOX-bevacizumab days) are not determinants of dose reductions unless associated with other issues such as febrile neutropenia. For non hematological toxicities, assessment for dose modification or delay may occur at any time during the cycle.

Any delay to the start of FOLFOX will result in the same delay to bevacizumab so that all treatments are given together, although vitamin D3 should continue while FOLFOX is held. Study procedures associated with each cycle of therapy will also be delayed accordingly (including scans for tumor assessment). At the discretion of the treating investigator, blood tests and/or clinical evaluations should occur at least weekly to monitor these parameters.

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by actual weight without any modification unless explicitly described in the protocol. This

will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation. Physicians who are uncomfortable with calculating doses based on actual body weight should recognize that doing otherwise would be a protocol violation. The actual weight on the day of registration or the first day of treatment may be used for Cycle 1 unless a change in the weight results in a change in calculated dose ≥10%, in which case the weight on the day of treatment should be used. Over the course of treatment, it is not required to change the doses of 5-FU, LV, oxaliplatin, or bevacizumab due to changes in weight unless the calculated dose changes by ≥10%, but treating investigators should follow institutional standards for calculating doses according to BSA.

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1 Dose Levels

The table below indicates potential dose levels for each of the agents for which dose modifications will be allowed. It is recommended that treating investigators follow standard institutional practice guidelines for dose modification within the FOLFOX chemotherapy regimen. Dose adjustments of each agent may be made independently based on the specific types of toxicities observed. There will be no dose reduction of leucovorin, bevacizumab, or vitamin D3.

At the discretion of the treating investigator, doses of any agent may be re-escalated one dose level when the participant has recovered sufficiently from a study drug-related toxicity.

If FOLFOX chemotherapy is discontinued permanently due to toxicity, participants may continue on protocol therapy with bevacizumab and vitamin D3 only. If oxaliplatin is permanently discontinued due to toxicity, participants may continue on protocol therapy with 5-FU and LV, bevacizumab, and vitamin D3 only. If bevacizumab is permanently discontinued due to toxicity, participants may continue on protocol therapy with FOLFOX chemotherapy and vitamin D3 only.

Table 4. Dose Levels for Chemotherapeutic Agents

Dose Level	Oxaliplatin	Bolus 5-FU	Infusional 5-FU
Initial	85 mg/m2	400 mg/m2	2400 mg/m2 over 46-48 hours
-1	65 mg/m2	320 mg/m2	1920 mg/m2 over 46-48 hours
-2	50 mg/m2	270 mg/m2	1600 mg/m2 over 46-48 hours
-3	40 mg/m2	230 mg/m2	1360 mg/m2 over 46-48 hours

6.2 Vitamin D3 Toxicity Management and Dose Modifications/Delays

Vitamin D3 is usually well tolerated, and there are no known serious side effects at the doses used in this study. The placebo should not cause any side effects. Toxicity is usually associated with higher doses/excess intake and includes hypercalcemia, hypercalciuria, kidney stones, and decrease in bone mineralization. Constipation may occur. Less likely side effects include nausea, vomiting, anorexia, weakness, weight loss, confusion, polyuria, and polydipsia. Renal dysfunction may predispose to toxicity. There will be no dose reduction or escalation of vitamin D3 in this study. **In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant vitamin D status, the lead Principal Investigator should be contacted for further discussion.**

Please see Table 5 below for dose modification for hypercalcemia.

Table 5. Vitamin D3 Dose Modification Guidelines for Hypercalcemia

GRADE*	ACTION TO BE TAKEN
GRADE 1: Corrected serum calcium >ULN-11.5 mg/dL; >ULN-2.9 mmol/L; ionized calcium >ULN-1.5 mmol/L	Maintain dose
GRADE 2: Corrected serum calcium >11.5-12.5 mg/dL; >2.9-3.1 mmol/L; ionized calcium >1.5-1.6 mmol/L; symptomatic	Hold vitamin D3. Continue FOLFOX-bevacizumab at discretion of treating investigator. Restart at full dose upon resolution to ≤ grade 1. If second occurrence or treatment delayed by ≥ 4 weeks, discontinue permanently and take off study.
GRADE 3: Corrected serum calcium >12.5-13.5 mg/dL; >3.1-3.4 mmol/L; ionized calcium >1.6-1.8 mmol/L; hospitalization indicated	Discontinue permanently and take off study.
GRADE 4: Corrected serum calcium >13.5 mg/dL; >3.4 mmol/L; ionized calcium >1.8 mmol/L; life-threatening consequences	Discontinue permanently and take off study.

* Grade based on National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0

If vitamin D3 is being held for toxicity, treatment with FOLFOX-bevacizumab may continue at the discretion of the treating investigator.

Other non-hematologic toxicities thought to be clinically significant and related to vitamin D3 should be managed as described in the table below.

Table 6. Vitamin D3 Dose Modification Guidelines for Other Clinically Significant Non-Hematologic Toxicities Considered Related to Vitamin D3

GRADE*	VITAMIN D3 DOSE	OUTCOME	VITAMIN D3 DOSE MODIFICATION
1	Maintain dose	Resolution to ≤ grade 1	Maintain dose
2	Maintain dose If persistent grade 2 and participant finds unacceptable, hold vitamin D3 dose for up to 4 weeks. Continue FOLFOX-bevacizumab at discretion of treating investigator.	Resolution to ≤ grade 2 Resolution to ≤ grade 1	Maintain dose Restart at full dose If second occurrence, or treatment delayed by ≥ 4 weeks, discontinue vitamin D3 permanently and take off study.
3 or 4	Hold vitamin D3. Continue FOLFOX-bevacizumab at discretion of treating investigator.	Resolution to ≤ grade 1	Restart at full dose If second occurrence, or treatment delayed by ≥ 4 weeks, discontinue vitamin D3 permanently and take off study.

* Grade based on CTCAE version 4.0

Vitamin D3 will be permanently discontinued and the participant taken off study if any of the following occur:

- Development of genitourinary stones
- Treatment delay of ≥ 4 weeks due to any toxicity thought to be clinically significant and related to vitamin D3.

Otherwise, vitamin D3 administration should continue once daily as appropriate, even if other components of FOLFOX-bevacizumab are being held for toxicity. For example, if hematological toxicity is present, participants may continue with vitamin D3 at the treating investigator's discretion.

Unblinding for this study will be done only in the case of an emergency by contacting the Principal Investigator, Kimmie Ng, MD, MPH, at 617-632-4150 or Kimmie_Ng@dfci.harvard.edu. Examples of emergency include: 1) a life-threatening unexpected adverse event that is at least possibly related to treatment and for which unblinding would influence management decisions; or 2) medication error, such as

accidental overdose. If a treatment assignment is unblinded, the participant must discontinue protocol therapy.

6.3 FOLFOX-Associated Hematologic Toxicities

The following table describes the recommended dose modifications at the start of each course of therapy, based on the pretreatment labs drawn for that cycle.

Table 7. FOLFOX6 Dose Modification Guidelines for Hematologic Toxicity

TOXICITY AND GRADE[1]	ACTION TO BE TAKEN[2]
Neutropenia (ANC)	
Grade 1 (ANC< LLN – 1.5 x 10^9/L)	Maintain dose level.
Grade 2 (ANC< 1.5 x 10^9/L - 1.0 x 10^9/L)	
Grade 3 (ANC<1.0 x 10^9/L – 0.5 x 10^9/L)	Hold FOLFOX-bevacizumab. When ANC has recovered to ≤ grade 2, resume treatment and decrease oxaliplatin by one dose level.
Grade 4 (ANC<0.5 x 10^9/L)	Hold FOLFOX-bevacizumab and check CBC weekly. When ANC has recovered to ≤ grade 2, resume treatment and decrease both 5-FU and oxaliplatin by one dose level. If ANC <1.0 x 10^9/L after 4 weeks, discontinue therapy.
Thrombocytopenia (PLT)	
Grade 1 (PLT<LLN – 75 x 10^9/L)	Maintain dose level.
Grade 2 (PLT<75 x 10^9/L – 50 x 10^9/L) Grade 3 (PLT<50 x 10^9/L – 25 x 10^9/L)	Hold FOLFOX-bevacizumab. When PLT has recovered to ≤ grade 1, resume treatment and decrease oxaliplatin by one dose level.
Grade 4 (PLT<25 x 10^9/L)	Hold FOLFOX-bevacizumab and check CBC weekly. When PLT has recovered to ≤ grade 1, resume treatment and decrease both 5-FU and oxaliplatin by one dose level. If PLT<75 x 10^9/L after 4 weeks, discontinue therapy.
Neutropenic Fever[3,4] ANC <1.0 x 10^9/L (i.e., grade 3 or 4) and fever ≥38.5ºC (101° F)	Hold FOLFOX-bevacizumab. When ANC has recovered to >1.0 x 10^9/L, fever resolved, and any infection under control, resume treatment and decrease both 5-FU and oxaliplatin by one dose level If ANC<1.0 x 10^9/L after 4 weeks, and/or fever or infection not resolved after 4 weeks, discontinue therapy

Other Hematologic Toxicities	Dose modifications for leukopenia at the start of subsequent courses of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.

[1] Grade based on CTCAE version 4.0

[2] Refers to initial dose used in previous cycle

[3] At the treating physician's discretion, GCSF should be used, and considered for use in subsequent cycles, according to standard of care, the most recent version of the product label, and all applicable guidelines. GCSF should not be administered in the period between 24 hours before and 24 hours after the administration of cytotoxic chemotherapy. Given the short interval between treatment cycles (≤14 days), pegylated GCSF should not be used.

[4] In case of febrile neutropenia or grade ≥2 infection at any time, GCSF should be used according to all applicable guidelines.

6.4 Oxaliplatin-Associated Neurotoxicity

The following table describes the recommended dose modifications for oxaliplatin based on the duration of oxaliplatin-associated neurotoxicity.

Table 8. Dose Modification Guidelines for Oxaliplatin-Associated Peripheral Motor or Sensory Neurotoxicity

GRADE[1]	DOSE LEVEL FOR SUBSEQUENT CYCLES BASED ON INTERVAL TOXICITY[3]	PERSISTENT (NOT RESOLVED BETWEEN CYCLES)
GRADE 1: Asymptomatic; clinical or diagnostic observations only; intervention not indicated	No change	No change
GRADE 2: Moderate symptoms; limiting instrumental ADL	No change	At treating investigator's discretion, consider decreasing oxaliplatin by one dose level.
GRADE 3: Severe symptoms; limiting self care ADL.	If resolution to ≤ grade 2, decrease oxaliplatin by one dose level.	Hold oxaliplatin until resolution to ≤ Grade 1. Continue 5-FU, LV, bevacizumab, and vitamin D3. Upon resolution to ≤ grade 1, oxaliplatin may be reintroduced at a lower dose level at the treating investigator's discretion.
GRADE 4: Life threatening consequences; urgent intervention indicated.	Discontinue oxaliplatin permanently. Continue 5-FU, LV, bevacizumab, and vitamin D3.	

Laryngopharyngeal dysesthesias (any grade)	Increase duration of oxaliplatin infusion to 6 hours
Cold Induced Dyesthesia	No change in dose

[1] Grade based on CTCAE version 4.0

Participants may also discontinue oxaliplatin following multiple cycles even in the absence of dose-limiting neurotoxicity if, in the physician's judgment, neurotoxicity is likely to become problematic. Participants should continue to receive other protocol therapy and the oxaliplatin may be reintroduced subsequently.

6.5 FOLFOX-Associated Gastrointestinal Toxicities

The following tables describe the recommended dose modifications at the start of each course of therapy, based on toxicity experienced at any time during the preceding cycle.

Table 9. Dose Modification Guidelines for Diarrhea and/or Mucositis

GRADE[1]	DOSE LEVEL FOR SUBSEQUENT CYCLES BASED ON INTERVAL TOXICITY[2]	AT TIME OF RETREATMENT
1	Maintain dose level	Maintain dose level
2	Maintain dose level if resolved to ≤ grade 1 by Day 1	If ≥ grade 2 diarrhea and/or mucositis present on Day 1 of cycle, hold FOLFOX-bevacizumab and check weekly, then treat based on interval toxicity. If ≥ grade 2 diarrhea and/or mucositis after 4 weeks, discontinue FOLFOX.
3, 4	Decrease 5-FU by one dose level if resolved to ≤ grade 1 by Day 1	

[1] Grade based on CTCAE version 4.0

[2] Refers to initial dose used in previous cycle

Table 10. Dose Modification Guidelines for Vomiting

GRADE[1]	DOSE LEVEL FOR SUBSEQUENT CYCLES BASED ON INTERVAL TOXICITY[2]	AT TIME OF RETREATMENT
1	Maintain dose level	No change
2	Maintain dose level if resolved to < grade 2 by Day 1	If ≥ grade 2 vomiting present on Day 1 of cycle despite optimal anti-emetic therapy, hold FOLFOX-bevacizumab and check weekly, then treat based on interval toxicity. If ≥ grade 2 vomiting after 4 weeks, discontinue FOLFOX.
3	Decrease oxaliplatin by one dose level if resolved to < grade 2 by Day 1	
4	Decrease 5-FU and oxaliplatin by one dose level if resolved to < grade 2 by Day 1	

[1] Based on CTCAE version 4.0
[2] Refers to initial dose used in previous cycle

6.6 Oxaliplatin-Associated Pulmonary Toxicities

For ≥ grade 3 cough, dyspnea, hypoxia, pneumoitis, or pulmonary infiltrates, hold oxaliplatin until interstitial lung disease is ruled out. Continue 5-FU, LV, bevacizumab, and vitamin D3. Discontinue all protocol therapy if interstitial lung disease is confirmed.

6.7 Oxaliplatin-Associated Thrombotic Microangiopathy

For ≥ grade 3 hemolytic uremic syndrome (HUS), discontinue oxaliplatin. Continue 5-FU, LV, bevacizumab, and vitamin D3.

6.8 Oxaliplatin Extravasations

Extravasation of oxaliplatin has been associated with necrosis. Administration through a central line is strongly recommended. If extravasation is suspected, the infusion should be stopped immediately and the extravasation site treated according to institutional guidelines. Oxaliplatin may be administered at another site.

6.9 FOLFOX-Associated Allergic Reactions

Table 11. Dose Modification Guidelines for Allergic Reactions

GRADE*	MANAGEMENT
1	Decrease infusion rate by 50% until symptoms resolve, then resume at initial planned rate
2	Stop infusion. Administer H1 and/or H2 blockers, and/or steroids according to institutional policy. Restart the infusion when symptoms resolve and pretreat before all subsequent doses. Treat according to institutional policy.
3 or 4	Stop the infusion. Discontinue the culprit agent permanently. Continue receiving other agents on study.

* Grade based on CTCAE version 4.0

6.10 Cardiovascular Toxicities

For grade 3 or 4 cardiac ischemia/infarction, discontinue all protocol therapy. For grade 3 or 4 cerebrovascular ischemia/infarction, discontinue all protocol therapy.

6.11 Other FOLFOX-Associated Non-Hematologic Toxicities

For other grade 3 or 4 non-hematologic, clinically significant toxicities considered related to FOLFOX, delay FOLFOX-bevacizumab until toxicity resolves to ≤ grade 1,

then resume treatment at one dose level reduction of 5-FU and oxaliplatin. Vitamin D3 may be continued while FOLFOX-bevacizumab is being held.

6.12 Bevacizumab-Associated Toxicities

General toxicities associated with bevacizumab therapy include bleeding, arterial clots (which could lead to stroke and heart attack), bowel perforation, wound healing difficulties, and hypertension. Gastrointestinal perforation complicated by intra-abdominal abscesses or fistula formation, and in some instances with fatal outcome, occurs at an increased incidence in participants receiving bevacizumab. If bevacizumab is held due to toxicities, FOLFOX and vitamin D3 should continue as planned and bevacizumab will be restarted at the next cycle of chemotherapy, so long as the toxicities have reduced in accordance with the protocol guidelines. Participants will then resume bevacizumab treatment at the same dose and schedule.

Bevacizumab may be held for the first cycle at the discretion of the treating investigator. The surgical incision should be fully healed prior to initiation of bevacizumab. The half life of bevacizumab is estimated to be 20 days. Treatment should be suspended prior to elective surgery.

Table 12. Dose Modification Guidelines for Allergic Reaction/Anaphylaxis

TOXICITY AND GRADE*	ACTION TO BE TAKEN
Allergic Reaction or Infusion-Related Reaction	
GRADE 1: Mild transient reaction (flushing, rash, drug fever <38° C or 100.4° F); infusion interruption not indicated; intervention not indicated	Continue bevacizumab infusion and monitor vital signs, administer supportive care per institutional standards and treating investigator's discretion. May reduce rate of infusion by 50% at investigator's discretion.
GRADE 2: Infusion interruption indicated but responds promptly to symptomatic treatment; prophylactic medications indicated for ≤ 24 hours	Immediately interrupt infusion and provide supportive care according to institutional standards. Upon recovery, may resume infusion on the same day at reduced rate with close monitoring at treating investigator's discretion. Premedication for subsequent infusions is recommended. If second occurrence, discontinue bevacizumab.
GRADE 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Immediately interrupt infusion and provide supportive care according to institutional standards. Discontinue bevacizumab.

GRADE 4: Life-threatening consequences; urgent intervention indicated	Immediately interrupt infusion and provide supportive care according to institutional standards. Discontinue bevacizumab.
Anaphylaxis GRADE 3: Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension GRADE 4: Life-threatening consequences; urgent intervention indicated	Immediately interrupt infusion and provide supportive care according to institutional standards. Discontinue bevacizumab. Immediately interrupt infusion and provide supportive care according to institutional standards. Discontinue bevacizumab.

* Based on CTCAE version 4.0

Table 13. Dose Modification Guidelines for Hypertension

GRADE*	ACTION TO BE TAKEN
GRADE 1: Prehypertension (systolic 120-139 mmHg or diastolic 80-89 mmHg)	No dose modification or delay
GRADE 2: Stage 1 hypertension (systolic 140-159 mmHg or diastolic 90-99 mmHg); medical intervention indicated; recurrent or persistent (≥24 hours); symptomatic increase by >20 mmHg (diastolic) or to >140/90 mmHg if previously within normal limits; monotherapy indicated	No dose modification or delay Initiate antihypertensive medication (Suggestion: DHP calcium channel blocker [amlodipine] at starting dose of 2.5 mg daily and increase as needed to a maximum of 10 mg daily)
GRADE 3: Stage 2 hypertension (systolic ≥160 mmHg or diastolic ≥100 mmHg); medical intervention indicated; more than one antihypertensive drug or more intensive therapy than previously used indicated	Hold bevacizumab Add additional antihypertensive medication (e.g., ACE inhibitor or angiotensin receptor blocker) When blood pressure is controlled to < 150/100, resume bevacizumab
GRADE 4: Life threatening consequences (e.g., malignant hypertension, transient or permanent neurological deficit, hypertensive crisis); urgent intervention indicated	Hold bevacizumab When BP is controlled to < 150/100 with antihypertensive medications and other supportive care, resume bevacizumab at treating investigator's discretion

* Based on CTCAE version 4.0

Table 14. Dose Modification Guidelines for Thromboembolic Events

GRADE*	ACTION TO BE TAKEN
GRADE 1: Venous thrombosis (e.g., superficial thrombosis)	Continue bevacizumab at treating investigator's discretion. Medical intervention as clinically indicated per institutional standards and treating investigator's discretion.
GRADE 2: Venous thrombosis (e.g., uncomplicated deep vein thrombosis), medical intervention indicated	
GRADE 3: Thrombosis (e.g., uncomplicated pulmonary embolism [venous], non-embolic cardiac mural [arterial] thrombosis), medical intervention indicated	Hold bevacizumab. Resume when medically stable and on stable dose of anticoagulation with the following parameters: INR: 2-3 if on stable dose of warfarin, or stable dose of low molecular weight heparin.
GRADE 4: Life threatening (e.g., pulmonary embolism, cerebrovascular event, arterial insufficiency); hemodynamic or neurologic instability; urgent intervention indicated	Discontinue bevacizumab. May continue FOLFOX and vitamin D3 on study upon recovery of acute event at treating investigator's discretion and discussion with lead Principal Investigator.

* Based on CTCAE version 4.0

Table 15. Dose Modification Guidelines for Proteinuria

TOXICITY AND GRADE[1]	ACTION TO BE TAKEN
Proteinuria GRADE 1: 1+ proteinuria (30 - < 100 mg/dL); urinary protein < 1 g/24 hours	Continue bevacizumab.
GRADE 2: 2+ proteinuria (≥100-< 500 mg/dL); urinary protein 1-3.4 g/24 hours	Continue bevacizumab. Obtain additional urine sample for urine protein: creatinine (UPC) ratio:[2] • UPC ratio ≤ 1.9: Continue bevacizumab. • UPC ratio ≥ 2: Hold bevacizumab and collect a 24-hour urine sample for urinary protein prior to next visit. May resume when urine protein is < 2.0 g/24 hours.
GRADE 3: Urinary protein ≥ 3.5 g/24 hours	Hold bevacizumab. Collect a 24-hour urine sample for urinary protein. May resume when urinary protein is < 2.0 g/24 hours. Consider nephrology consult.
Nephrotic Syndrome	Discontinue bevacizuamb. Consider nephrology consult. May continue FOLFOX and vitamin D3 on study upon recovery of acute event at treating investigator's discretion and discussion with lead Principal Investigator.

[1] Based on CTCAE version 4.0

[2] Obtain at least 4 ml of a random urine sample. Determine spot urine protein concentration (mg/dL) and spot urine creatinine concentration (mg/dL). Divide results of protein concentration by creatinine concentration to obtain the UPC ratio.

Table 16. Dose Modification Guidelines for Other Bevacizumab-Related, Non-Hematological Toxicities

TOXICITY AND GRADE	ACTION TO BE TAKEN
Hemorrhage (except intracranial hemorrhage) GRADE 1 OR 2: Mild or moderate; self limited; medical intervention may or may not be indicated (e.g., minor cauterization) GRADE 3: Requiring transfusion, radiologic, endoscopic, or elective operative intervention GRADE 4: Life threatening consequences; urgent intervention indicated	Medically manage per institutional standards and treating investigator's discretion. Continue bevacizumab at treating investigator's discretion. Hold bevacizumab for a maximum of 8 weeks. May permanently discontinue or treat with bevacizumab at investigator's discretion when there is no evidence of active bleeding. If any surgical intervention occurred, follow guidelines in Section 5.2.3. Discontinue bevaicuzmab. May continue FOLFOX and vitamin D3 on study upon recovery of acute event at treating investigator's discretion and discussion with lead Principal Investigator.
Abdominal Fistula	Discontinue bevacizumab. May continue FOLFOX and vitamin D3 on study upon recovery of acute event at treating investigator's discretion and discussion with lead Principal Investigator.
Intra-Abdominal Abscess	
Intestinal Perforation	
Wound Dehiscence (Grade 3 or 4)	
Intracranial Hemorrhage	

Reversible Posterior Leukoencephalopathy Syndrome (RPLS)

For signs and symptoms suggestive of RPLS (e.g., confusion, headache, seizures, cortical blindness), skip bevacizumab. Suspected RPLS should be investigated with MRI. If diagnosis of RPLS is confirmed, bevacizumab should be permanently discontinued.

If RPLS is ruled out via MRI, the decision on resuming bevacizumab should be based on the nature of the signs/symptoms. For grade 4 events with likely relationship to bevacizumab, discontinue bevacizumab; for grade 3 events, bevacizumab may be resumed if toxicities completely resolve within 4 weeks.

Other protocol therapy may be continued at the discretion of the treating physician.

Other Bevacizumab-Associated Non-Hematologic Toxicities

For other grade 3 or 4 non-hematologic, clinically significant toxicities considered related to bevacizumab, delay bevacizumab until toxicity resolves to ≤ grade 1, then resume treatment at the same dose and schedule. FOLFOX and vitamin D3 may be continued while bevacizumab is being held. For any clinically significant, bevacizumab-related toxicity that persists for >8 weeks or recurs after a dose delay, bevacizumab will be discontinued permanently.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Vitamin D3 (Cholecalciferol)

7.1.1 Description

Vitamin D3 is also known as cholecalciferol (NSC #375571). The chemical name is (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-trien-3-ol. The molecular formula is C27H44O. The molar mass is 384.64 g/mol. Vitamin D3 is practically soluble in water, soluble in the usual organic solvents, and slightly soluble in vegetable oils.

7.1.2 Form

Placebo, 400 IU, and 4000 IU supplements of vitamin D_3 will be manufactured by Pharmavite, LLC (Mission Hill, CA) in the form of liquid softgel capsules and provided free of charge to study participants. Pharmavite produces 11.5 billion vitamin supplements annually in a facility that is compliant with United States Pharmacopeia's Good Manufacturing Practices (GMPs). All softgel capsules will be indistinguishable, regardless of dose or placebo, and packaged in bottles. Medication labels will comply with local institutional guidelines and U.S. legal requirements and be printed in English. They will supply no information about the participant. All site personnel and participants will be blinded to the treatment arm assignment.

7.1.3 Storage and Stability

Vitamin D/placebo capsules should be stored at room temperature in the original prescription vial. When stored under these conditions, Pharmavite has indicated the capsules are stable for up to 180 days.

7.1.4 Compatibility

Steroids interfere with vitamin D metabolism, as do lithium, phenobarbital, phenytoin, quinidine, isoniazid, and rifampin. Patients taking these drugs are not eligible to participate in this study (see Exclusion Criteria, Section 3.2.22). There are no anticipated drug interactions with FOLFOX chemotherapy or bevacizumab.

7.1.5 Handling

N/A

7.1.6 Availability

Vitamin D3 is a commercially available agent, but will be supplied by Pharmavite, LLC (Mission Hill, CA) free of charge for this study. The research pharmacist will be responsible for dispensing the study medication to participants. Vitamin D3 will be dispensed as a two-week supply for Cycle 1 (loading period), and then as a monthly supply for all cycles thereafter.

7.1.7 Administration

Vitamin D3/placebo will be administered orally daily at approximately the same time of day.

For the purposes of this protocol, 1-3 missed doses during the 28 day dispensation period will be considered a minor violation and more than 3 missed doses during this period will be reported as a major violation.

7.1.8 Ordering

Vitamin D3 is a commercially available agent, but will be supplied by Pharmavite, LLC (Mission Hill, CA) for this study. Ordering of Vitamin D3 will be done through the lead site. Initial shipment of Vitamin D3 may be ordered only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the lead site. Vitamin D3 will be stored in the treating institution's research pharmacy per institutional guidelines. Only the unblinded research pharmacist will be responsible for dispensing the study medication to participants. The blinded study personnel may deliver the study medication to participants.

7.1.8.1 DF/HCC Satellite Sites

DF/HCC satellites will be shipped drug from the lead site.

7.1.8.2 External Sites and DF/HCC Affiliate Sites

External sites and DF/HCC affiliate sites will receive drug directly from Pharmavite, LLC. Ordering of Vitamin D3 will be done through the lead site.

For Drug Re-Supply Contact:
Christine Ganser
Telephone: 617-632-3773
Email: Christine_Ganser@dfci.harvard.edu
Fax: 617-582-7988

7.1.9 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

7.1.10 Destruction and Return

At the end of the study, unused supplies of vitamin D3 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 5-Fluorouracil (5-FU, Fluorouracil, Adrucil)

Please refer to the package insert for complete product information.

7.2.1 Storage and Stability

Intact vials should be stored at room temperature and protected from light. Slight yellow discolor does not usually indicate decomposition. Stability in ambulatory pumps varies according to the pump, manufacturer of drug, concentration, and diluent. Please refer to appropriate reference sources for additional information.

7.2.2 Compatibility

Leucovorin enhances the cytotoxicity of 5-FU by forming a more stable tertiary complex with thymidylate synthase. Concomitant administration of 5-FU with warfarin has been reported to result in increased INR/prolonged prothrombin time. Participants receiving both drugs should be followed with weekly INRs.

7.2.3 Availability

5-FU is commercially available as a 50 mg/mL solution for injection in 10 mL, 20 mL, 50 mL, and 100 mL vials. 5-FU will not be provided by the study as it is considered standard of care for treatment of colorectal cancer.

7.2.4 Preparation

Inspect for precipitate; if found, agitate or gently heat in water bath. Bolus injections are prepared using undiluted drug. 46-48 hour infusion of 5-FU should be prepared for administration via ambulatory infusion pump according to the individual institution's standards. These solutions may be prepared in D5W or 0.9% NaCl. 5-FU should not be mixed in the same solution with most parenteral anti-emetics.

7.2.5 Administration

In this study, 5-FU is administered as a 400 mg/m2 IV bolus followed by 2400 mg/m2 by IV infusion over 46-48 hours.

7.3 Leucovorin (Leucovorin Calcium, Folinic Acid, Citrovorum Factor, N 5-Formyltetrahydrofolate, 5-Formyl-FH4)

Please refer to the package insert for complete product information.

7.3.1 Storage and Stability

Intact vials should be stored at room termperature and protected from light. Solutions reconstituted with bacteriostatic water for injection (BWI) are stable for at least 7 days at room temperature.

7.3.2 Availability

Leucovorin calcium is commercially available in 50 mg, 100 mg, 350 mg vials for reconstitution. Leucovorin will not be provided by the study as it is considered standard of care for treatment of colorectal cancer.

7.3.3 Preparation

Leucovorin may be reconstituted with BWI or with sterile water for injection. Solutions should be further diluted in D5W, 0.9% NaCl, or Rungers solution for infusion over two hours.

7.3.4 Administration Guidelines

Leucovorin will be administered as a 400 mg/m2 IV infusion over 2 hours after oxaliplatin administration. Leucovorin may also be administered concurrently

with oxaliplatin as a separate IV infusion. Sites may administer Leucovorin per institutional standard of care procedures.

7.4 Oxaliplatin (Eloxatin)

Please refer to the package insert for complete product information.

7.4.1 Storage and Stability

Intact vials should be stored at room temperature. Solutions diluted in D5W are stable for 6 hours at room temperature or 24 hours under refrigeration.

7.4.2 Availability

Oxaliplatin is commercially available as an aqueous solution in vials containing 50 mg and 100 mg at a concentration of 5 mg/mL. The vials do not contain any preservative and they are intended for single use. Oxaliplatin will not be provided by the study as it is considered standard of care for treatment of colorectal cancer.

7.4.3 Preparation

The calculated dose of oxaliplatin should be diluted for infusion with 250 mL to 500 mL in D5W. Oxaliplatin should not be diluted with a sodium chloride solution. Needles, syringes, catheters, or IV administration sets containing aluminum should not be used with oxaliplatin. As with other platinum compounds, contact with aluminum may result in a black precipitate.

7.4.4 Administration Guidelines

Oxaliplatin will be administered by intravenous infusion over 120 minutes in participants receiving FOLFOX. Infusion time may be prolonged (up to 6 hours) in participants experiencing pharyngolaryngeal dysesthesia. Oxaliplatin is unstable in the presence of chloride or alkaline solutions. Do NOT mix or administer oxaliplatin with saline or other chloride-containing solutions. Do NOT administer other drugs or solutions in the same infusion line. Flush IV lines/catheters with Dextrose 5% in Water both before and after oxaliplatin administration. Sites may administer Oxaliplatin per institutional standard of care procedures.

7.5 Bevacizumab (Avastin, rhuMAb VEGF)

Please refer to the package insert for complete product information.

7.5.1 Description

Bevacizumab (NSC #704865) is a recombinant humanized anti-VEGF monoclonal antibody, consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-determining regions. Bevacizumab blocks the binding of VEGF to its receptors, resulting in inhibition of angiogenesis.

7.5.2 Storage and Stability

Intact vials of bevacizumab should be stored in a refrigerator (2º to 8º C) and should remain refrigerated until just prior to use. Do not freeze. Do not shake. The sterile single use vials contain no antibacterial preservatives; therefore, it is recommended that vials be discarded 8 hours after initial entry. Solutions diluted for infusion may be stored in a refrigerator for up to 8 hours.

7.5.3 Availability

Bevacizumab is available commercially in vials of 100 mg at a concentration of 25 mg/mL. Bevacizumab will not be provided by the study as it is considered standard of care for treatment of colorectal cancer.

7.5.4 Preparation

The calculated dose of bevacizumab should be diluted in 100 mL of 0.9% sodium chloride for injection.

7.5.5 Administration Guidelines

Bevacizumab is administered as an intravenous solution. Bevacizumab may be administered per your institution's standard rate.

Suggested bevacizumab administration lengths: The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur after the initial dose, the second dose should be administered over a minimum of 60 minutes. If no adverse reactions occur after the second dose, all subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated.

8. CORRELATIVE/SPECIAL STUDIES

Submission of tumor tissue and collection of blood samples for vitamin D assays are required for all participants. **In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.** All participating

institutions must ask patients for their consent to participate in the other correlative studies, although patient participation is optional. The rationale for the scientific components of these studies is described in Section 2.4.

For patients who consent to participate, samples will be collected at the following time points:

Table 17. Collection of Specimens for Correlative Studies

TYPE OF SPECIMEN	PRE-TREATMENT	AT 1ST & 2ND RESTAGING (AFTER CYCLES 4 & 8)	AT SURGICAL RESECTION (IF APPLICABLE)	TREATMENT DISCONTINUATION
Paraffin-embedded or snap-frozen tumor tissue block, or 15 unstained slides [a]	Required for all participants		Optional for participants who undergo surgical resection while on study	
Buffy coat (EDTA/purple top)[b]	1 x 6 mL			
Plasma (EDTA/purple top)[c]	2 x 6 mL	2 x 6 mL		2 x 6 mL

[a] To be used for KRAS sequencing, immunohistochemical studies, and future research
[b] To be used for vitamin D pathway genotyping studies and future research
[c] To be used for 25(OH)D assays, inflammatory and IGF pathway biomarker assays, and future research

8.1 Submission of Paraffin-Embedded Specimens

Submission of archival tumor specimens is required for all participants at baseline and optional for participants who undergo surgical resection of primary tumor or metastatic disease while on study. Specimens will be used to evaluate VDR and 1α-hydroxylase protein expression, and assess *KRAS* mutation status (see Section 2.4 for additional background). Tissue will also be banked for future research studies. A paraffin-embedded or snap-frozen tumor block, or 15 unstained slides will be requested, along with the associated operative and pathology reports. Tumor samples obtained for this study may come from a variety of sources, including primary tumor, metastatic lymph node deposits, and liver metastases. All specimens must be labeled with the participant's initials, participant's study ID, DFCI protocol number, date collected, and type of specimen, and mailed to:

Christine Ganser
Gastrointestinal Cancer Center
Dana-Farber Cancer Institute
450 Brookline Avenue, DA1B-16

Boston, MA 02215
Ph: 617-632-3773

Specimens may be shipped Monday-Thursday by overnight service to ensure receipt.

8.1.1 Tumor Tissue Samples and Genomic DNA Extraction

Tumor tissue (and normal tissue if applicable) will be reviewed and dissected from tissue sections obtained from the paraffin tissue blocks, and genomic DNA will be extracted using QIAmp DNA Mini Kit (Qiagen, Valencia, CA).[161] Tissue microarrays will be constructed and banked for future research, along with DNA.

8.1.2 PCR and Sequencing of *KRAS*

In a previous study, it was determined that a DNA sequencing assay using Pyrosequencing (nucleotide extension sequencing with an allele quantification capability) was more sensitive for detecting *KRAS* mutations in paraffin-embedded tissue than standard dideoxy sequencing.[161] PCR amplification primers for Pyrosequencing will be as follows: KRAS-F, forward, 5'-nnn ggc ctg ctg aaa atg act gaa-3'; and KRAS-R, reverse biotinylated primer, 5'-tta gct gta tcg tca agg cac tct-3'. Each PCR mix will contain the forward and reverse primers (each 20 pmol), 2.81 nmol each of dNTP, 3 mmol/L $MgCl_2$, 1x PCR buffer, 1.25 U of AmpliTaq Gold, and 5 µl of template whole genome amplification (WGA) product in a total volume of 50 µl. PCR conditions will consist of initial denaturing at 94°C for 1 minute; 50 cycles of 95°C for 20 seconds, 58°C for 20 seconds, and 72°C for 40 seconds; and final extension at 72°C for 1 minute. The PCR products will be electrophoresed in an agarose gel to confirm successful amplification of the 82-bp PCR product. The PCR products (each 10 µl) will be sequenced by Pyrosequencing PSQ96 HS System (Biotage AB) following the manufacturer's instructions, using all three Pyrosequencing primers (see below). Nucleotide dispensation order will be cyclic (CTAG from 5' to 3').

To increase sensitivity for the detection of all mutations in *KRAS* codons 12 and 13, three slightly different Pyrosequencing primers were designed, using software ADSW (Biotage AB). For further details, please refer to the report by Ogino et al. in the *Journal of Molecular Diagnostics* in 2005.[161]

For quality control analyses of *KRAS* mutations, pyrosequencing has been designed to confirm the presence of a mutation by artificial frameshifting in pyrograms (with extra fluorescence peaks), and/or by a second pyrosequencing primer.[161] We will also add approximately 5-10% repeated QC samples as blinded specimens; they will be randomly nested in the sample sets with coded IDs.

8.1.3 Immunohistochemical (IHC) Analyses

For IHC analyses, paraffin sections of colorectal tumor will be deparaffinized, incubated with 3% H_2O_2 (20 minutes) to block endogenous peroxidase, and incubated with pepsin at 37°C (10 minutes). Protein block (Vector Laboratories, Burlingame, CA; 20 minutes) will be followed by application of primary antibodies. The following antibodies will be applied: polyclonal rabbit anti-VDR (C-20; Santa Cruz, Cat# SC-1008; dilution 1:200) and monoclonal anti-CYP27B1 (clone H-90; The Binding Site # sc-67261; dilution 1:400). We will record intensity of staining (absent, weak, moderate/strong) in each relevant cellular compartment (nucleus, cytoplasm, and/or membrane), as well as fraction of tumor cells with staining. Because tumors may be heterogeneous, scoring will reflect the staining level of the most reactive tumor regions, which are most often located in the deepest portions of the tumor. All pathologists will be blinded to participant outcome data. In all IHC analyses, appropriate positive and negative controls will be included in each run of IHC assay. In addition, a random sample will be re-examined by a second pathologist in Dr. Ogino's laboratory to assess inter-rater agreement using a kappa measure of agreement (κ) proposed by Kraemer.[162] The concordance rate between two pathologists is currently 82% (κ=0.62; n=139) for VDR.

8.2 Submission of Buffy Coat Specimens

For patients who have consented, a buffy coat sample will be obtained prior to treatment for the vitamin D pathway genotyping studies described in Section 2.4, and remaining material banked for future research. This sample will be taken at the time the other registration blood work is collected. Venous blood of a quantity of 6 mL should be collected in a lavender top (EDTA coagulant) vacutainer. Blood samples will be soft-spun at room temperature in a centrifuge at 1000 x g for 10 minutes. After centrifugation, three different fractions are separated: 1) the upper clear layer is plasma; 2) the intermediate layer is buffy coat, a very thin whitish layer of platelets plus highly concentrated white blood cells (WBCs) below into the red cell layer; and 3) the bottom layer is concentrated erythrocytes. Unless there is a very high WBC count, you cannot distinguish between buffy coat and red cells. Collect 2 mL of buffy coat by "wagging" your pipette tip around as you aspirate just below the plasma/buffy coat line (will be red). For very low WBC counts, you can collect up to half of the "red" portion. Aliquot 500 microliters of buffy coat into a 1.8 mL cryogenic vial and store at -80ºC for vitamin D pathway genotyping studies. You can then return your tube to the centrifuge for a hard spin to salvage plasma if needed (see Section 8.3 below for instructions). Samples should be processed and frozen within 3 hours of collection. Label all samples with participant's initials, participant's study ID, DFCI protocol number, date collected, and type of specimen. Shipment of buffy coat samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers to control any spill or leakage. The outer container must be puncture-resistant (e.g., cardboard mail tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers. Samples should be shipped on Monday-Thursday on dry ice by overnight mail to:

Christine Ganser
Gastrointestinal Cancer Center
Dana-Farber Cancer Institute
450 Brookline Avenue, DA1B-16
Boston, MA 02215
Ph: 617-632-3773

8.2.1 Vitamin D Pathway Genotyping Analyses

Buffy coat for germline DNA extraction will be obtained at baseline to allow for genotyping of SNPs in several vitamin D pathway genes (see Section 2.4). Genotyping analyses will be performed in the Dana-Farber/Harvard Cancer Center (DF/HCC) High Throughput Polymorphism Core laboratory led by Dr. Immaculata De Vivo.

8.3 Submission of Plasma Specimens

Collection of plasma samples for assay of 25(OH)D levels is required of all participants enrolled on the study. Collection of plasma samples for assay of inflammatory and IGF pathway biomarkers and future research is optional. Blood samples will be collected and spun in a centrifuge at 1500 x g for 20-25 minutes. After centrifugation, 200 microliters of plasma will be aliquoted into a 1.8 mL cryogenic vial and stored at -80ºC for 25(OH)D assays. Two separate aliquots of 1 mL each will be placed into 1.8 mL cryovials and stored at -80ºC for inflammatory and IGF pathway biomarker assays, respectively. Remaining plasma should be aliquoted equally into three 1.8 mL cryovials and stored at -80ºC for future research purposes. Plasma is to be kept frozen until shipped on dry ice to:

Christine Ganser
Gastrointestinal Cancer Center
Dana-Farber Cancer Institute
450 Brookline Avenue, DA1B-16
Boston, MA 02215
Ph: 617-632-3773

Samples should be processed and frozen within 3 hours of collection. Label all samples with participant's initials, participant's study ID, DFCI protocol number, date collected, and type of specimen. Shipment of serum samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers to control any spill or leakage. The outer container must be puncture-resistant (e.g., cardboard mail tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers. Samples should be shipped on Monday-Thursday by overnight mail to ensure receipt.

8.3.1 Plasma 25(OH)D Assays

In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion. Plasma research samples will be sent by overnight delivery to Heartland Assays, Inc. (Ames, IA), and 25(OH)D concentrations measured by radioimmunoassay (RIA).[163] Masked quality control samples will be interspersed among the case samples, and all laboratory personnel will be blinded to participant outcome. The mean coefficient of variation of the assay is 8%.

8.3.2 Inflammatory Mediators

For participants who have consented, samples will be analyzed for plasma levels of CRP, IL-6, and TNFα-R2 (a stable, reliable marker of TNF-α system activation over time)[164] in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA) in a blinded fashion. CRP will be measured via a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring), IL-6 via a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay), and TNFα-R2 by an enzyme-linked immunosorbent assay (ELISA) kit utilizing immobilized monoclonal antibody to human TNFα-R2 (Genzyme). The coefficients of variation (CVs) are 3.8% for CRP, 5.9% for IL-6, and 6.2% for TNF-αR2.

8.3.3 IGF-Pathway Factors

For participants who have consented, samples will be analyzed for IGF-1, IGF-2, IGFBP-1, IGFBP-3, and C-peptide (a stable marker of serum insulin exposure) in the laboratory of Dr. Michael N. Pollak (Jewish General Hospital and McGill University). Plasma levels of C-peptide will be assayed by RIA (Linco Research), an assay with little or no cross-reactivity with proinsulin.[165] IGFBP-1 ELISAs will be performed using reagents from Diagnostic Systems Laboratory (Webster, TX). We have previously demonstrated excellent mean intra-assay coefficients of variation for these analytes from large numbers of blinded quality control samples. For example, the mean intra-assay CVs for C-peptide and IGFBP-1 were each <13%.[156]

9. STUDY CALENDAR

Screening evaluations are to be conducted within 14 days prior to start of protocol therapy. Scans must be done ≤4 weeks prior to the start of therapy.

All laboratory and other assessments must be performed prior to administration of any study medication, however +/- 7 days will be allowed to change patient schedule without PI or IRB approval.

	Pre-Study	Cycle 1, Day 1[a]	Cycle 2, Day 1	Cycle3, Day1	Cycle4, Day1	Cycle5, Day1	Cycles 6-8, Day1	Cycle9, Day1	All subsequent cycles, Day 1	Off Treatment
FOLFOX-bevacizumab		X[c]	X	X	X	X	X	X	X	
Vitamin D3[d]		X	X	X	X	X	X	X	X	
Informed consent	X									
Medical history	X									
Concurrent meds	X	X----------X								X
Complete physical exam, including ht, wt, VS	X									X
Limited physical exam, including wt and VS		X	X	X	X	X	X	X	X	
Performance Status	X									
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X
Serum chemistry [e]	X	X	X	X	X	X	X	X	X	X
PT, INR, PTT[f]	X									
Urinalysis				X		X		X	Repeated every 4 cycles	X
Urine Protein/creatinine ratio (UPCR)	X									
CEA	X	Repeated every 4 cycles (approximately every 8 weeks)								X
Review drug diaries and vitamin D3 pill counts			X----------X							X
Archival tumor tissue	X[g]									
Blood samples for future biomarker research	X					X		X		X
Blood sample for genotyping analyses and future research	X									
Adverse event evaluation		X----------X								X
Radiologic imaging/tumor measurements	X	Repeated after every 4 cycles . Documentation (radiologic) must be provided for participants removed from study for progressive disease.[h]								X
B-HCG	X[i]									
AE follow-up										X[j]
Survival follow-up										X[k]

[a] Labs do not have to be repeated on Cycle 1, Day 1 if they were done within 7 days.
[b] Off-treatment evaluation should occur within 30 days (+/-3 days) after the last dose of study treatment. The visit at which a response assessment shows disease progression may be used as the off-treatment visit.
[c] Bevacizumab may be held on Cycle 1 Day 1 at the treating investigator's discretion.
[d] Vitamin D3 is administered orally once daily throughout every cycle starting on cycle 1 day 1.
[e] Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, phosphorous, total bilirubin, alkaline phosphatase, total protein, albumin, SGOT [AST], SGPT [ALT]
[f] PT, INR, and PTT only required for patients on anticoagulation
[g] Paraffin-embedded tumor specimen or unstained paraffin slides must contain colorectal cancer; if slides are submitted, there must be at least 15 unstained slides. An additional tissue block or 15 additional slides will be collected from patients who undergo surgical resection and consent to the optional tissue sub-study. Submit all available archived specimens, if feasible. Submit the associated operative and pathology reports. Refer to Section 8.1 for instructions.
[h] Radiologic studies should be performed after every 4 cycles (i.e. prior to Day 1 of Cycle 5, Cycle 9, Cycle 13, etc.)
[i] Serum pregnancy test (women of childbearing potential)
[j] Follow for AE monitoring for a total of 30 days (+/- 3 days) from the last dose of study treatment. Participants with an AE that is ongoing at the off-treatment visit should be contacted at least monthly until the event has resolved or until the subject begins treatment with a new anti-cancer therapy.
[k] Follow for survival information every 3 months (±1 month) until 36 months from the date that the last participant was randomized or death, whichever occurs earlier.

10. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable and/or non-measurable disease will be assessed by RECIST criteria. For the purposes of this study, participants should be reevaluated after every 4 cycles.

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be re-evaluated for response after every 4 cycles. Response and progression will be evaluated in this study using the new revised international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[166] Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

10.1.1 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

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

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). A lesion in a previously irradiated area is not eligible for measurable disease unless there is objective evidence of progression of the lesion prior to study enrollment. Lesions in previously irradiated areas must be clearly identified as such.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to < 15mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas participant to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as

well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

FDG PET and PET/CT. The acquisition of FDG PET and FDG PET/CT scans should follow the NCI Guidelines for using FDG PET as an indicator of therapeutic response.[167] Participants should avoid strenuous exercise and be on a low carbohydrate diet for 24 hours prior to the scan. Participants should fast for 4 hours or longer prior to the FDG injection and should have a serum glucose of

less than 200 mg/dL at the time of FDG injection. A 10-20 mCi dose of FDG should be injected for typical adult patients. For longitudinal studies with multiple scans, particular attention should be paid to ensure consistent patient preparation and acquisition parameters between the follow-up scan and the baseline scan. When designing a study where PET scans are going to be utilized as one of the modalities to evaluate efficacy, it is important to consult with physicians in nuclear medicine in designing the appropriate criteria to be utilized.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

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

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

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5 mm

absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease 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.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

> **Note**: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.2 Evaluation of Non-Target Lesions

> Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
>
> **Note:** If tumor markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.
>
> Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.
>
> Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions.

Overall level of substantial worsening that merits discontinuation of therapy. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.3 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/Not evaluated	No	PR

SD	Non-CR/Non-PD/Not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	NonCR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

10.1.5 Duration of Response

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

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

10.1.6 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression or clinical disease progression as assessed by the treating investigator. Death will be regarded as a progression event.

10.1.7 Response Review

Central review of restaging scans will be performed by the DF/HCC Tumor Imaging Metrics Core.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).

- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators or designee will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Expedited Adverse Event Reporting

11.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

11.3.2 For multi-site trials where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, grade 4 toxicities and grade 5 (death) regardless of study phase or attribution.

11.3.3 The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the Overall Principal Investigator.

11.3.4 Expedited Reporting Guidelines for the DF/HCC IRB

Investigative sites within DF/HCC and DF/PCC will notify the Overall PI of any SAE according to timelines indicated in the DF/HCC Reportable AEs table

below. The DF/HCC or DF/PCC site will report Serious Adverse Events (SAEs) directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy and provide a copy of any DFCI Adverse Event Reporting Form to the Overall PI by fax or email.

Kimmie Ng, MD, MPH
Email: Kimmie_Ng@dfci.harvard.edu
Fax: 617-582-7988

Other investigative sites will report Serious Adverse Events (SAEs) to the Overall PI according to the DF/HCC Reportable AEs table below. These events will be reported to the Overall PI on the DFCI Adverse Event Reporting Form. SAEs will be reported to the respective site's IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI by fax or email to:

Kimmie Ng, MD, MPH
Email: Kimmie_Ng@dfci.harvard.edu
Fax: 617-582-7988

DF/HCC Reportable AEs Table

Attribution	DF/HCC Reportable AEs			
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected or Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	3 business days	3 business days
Possible Probable Definite	Not required	3 business days	3 business days	3 business days
Note: If the participant is in long term follow up, report the death at the time of continuing review.				

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

11.3.5 Expedited Reporting Guidelines for the Food and Drug Administration (FDA)

Requirements of All Sites:
All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment and meet the criteria in section 11.1.2 must be reported to the DF/HCC Overall Principal Investigator on the Form FDA 3500A (Mandatory Reporting Form for investigational agents) within 3 calendar days of the event, or within 3 calendar days of learning of the event. Forms are available at http://www.fda.gov/medwatch/getforms.htm.

Forms will be faxed or emailed to:

Kimmie Ng, MD, MPH
Email: Kimmie_Ng@dfci.harvard.edu
Fax: 617-582-7988

Requirements of the Overall Principal Investigator:
The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

11.3.6 Expedited Reporting Guidelines for Genentech

Refer to Appendix C for reporting of SAEs to Genentech.

A copy of all submissions to Genentech must be sent to the DF/HCC Overall Principal Investigator:

Kimmie Ng, MD, MPH
Email: Kimmie_Ng@dfci.harvard.edu
Fax: 617-582-7988

11.4 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the NIH Office of Biotechnology Activities (OBA)

N/A

11.6 Reporting to the Institutional Biosafety Committee (IBC)

N/A

11.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

11.8 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect and manage data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration

Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this trial. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed with the Principal Investigator, statistician and study team members. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

  - Title 21 Part 56 – Institutional Review Boards
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
  - Title 21 Part 312 – Investigational New Drug Application
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-center Guidelines

The DF/HCC requirements for participating institutions, and the responsibilities of the DF/HCC Overall Principal Investigator (or Protocol Chair), Coordinating Center, and Participating Institutions are described in the Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (Appendix B). The DF/HCC Overall Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order FOLFOX-bevacizumab directly from the supplier and Vitamin D will be ordered through the lead site. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

13.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A

14. STATISTICAL CONSIDERATIONS

14.1 Study Design

This study is a multi-center, randomized, double-blind, phase II trial of vitamin D supplementation in combination with FOLFOX + bevacizumab in 140 previously untreated patients with metastatic colorectal cancer.

Participants will be accrued in one stage and randomized in a 1:1 ratio to two doses of oral vitamin D3 in combination with FOLFOX-bevacizumab: a standard dose of 400 IU daily in the control arm versus a higher dose of 8000 IU daily for two weeks (as a loading dose), followed by a maintenance dose of 4000 IU daily.

Participants will be assessed by CT scan approximately every 8 weeks and will remain on chemotherapy plus vitamin D until disease progression, intolerable toxicity, or participant withdrawal of consent. Plasma will be banked serially for 25(OH)D and other circulating markers, and germline DNA and archived tumor specimens collected. **In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. If there are concerns related to a participant's vitamin D status, the lead Principal Investigator should be contacted for further discussion.**

14.2 Analysis of Primary Endpoint

The primary endpoint of the study is PFS, which will be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan-Meier distribution will be determined, including the median PFS and the proportion of participants remaining progression-free at 9, 12, and 18 months. These statistics will be given as point estimates with 95% confidence intervals, and a log rank test used to compare the two arms. PFS will also be analyzed using a Cox proportional hazards model. Hazard ratios and 95% confidence intervals will be presented for the comparison of FOLFOX + bevacizumab in combination with standard-dose vitamin D (referent) versus higher-dose vitamin D. Models will be adjusted for relevant clinical and pathologic variables that are prognostic for survival. The primary analysis will be performed in the intent-

to-treat population, with a secondary efficacy analysis in evaluable participants, defined as those participants who received at least one dose of study treatment and underwent at least one tumor assessment. Participants who exhibit objective disease progression or die prior to the end of Cycle 1 will also be considered evaluable.

14.3 Sample Size/Accrual Rate

The expected PFS for metastatic colorectal cancer patients receiving first-line FOLFOX-bevacizumab is approximately 9 months.[74] The trial initially targeted 120 evaluable patients. Assuming that approximately 5 participants/month are accrued over 24 months and followed for at least 36 months, a one-sided log rank test with 60 participants in the control arm and 60 in the investigational arm achieves 80% power at a 20% significance level to detect a HR of 0.73. This sample size is intended to generate effect estimates that will support the design of a more definitive phase III study. The use of randomized phase II trials with sample sizes equal to or less than the current proposed study is now an accepted paradigm in the development of new anticancer therapies. For example, the strategy was successful in identifying bevacizumab as an effective treatment for metastatic colorectal cancer,[168] and pharmaceutical companies around the world are also utilizing this model (i.e., Amgen's "A Phase 1b/2 Study of AMG655 in Combination with Modified FOLFOX6 and Bevacizumab for the First-Line Treatment of Subjects with Metastatic Colorectal Cancer").

As of December 2015, 120 participants have been enrolled in this study. Eight of the 120 subjects are inevaluable and another 13 subjects came off study prior to disease progression to pursue liver resection. Consequently, we have now increased the total accrual to 140 subjects. We assume that approximately 130 subjects will be evaluable. Assuming that approximately 5 participants/month are accrued over 24 months and followed for at least 36 months, a one-sided log rank test with 65 participants in the control arm and 65 in the investigational arm achieves 80% power at a 20% significance level to detect an HR of 0.73. We expect to see a total of 118 events (progression or death) with this sample size. With multiple centers enrolling approximately 5 participants per month, the study should complete accrual of the additional 20 subjects over the next -5 months. Participants will be followed for survival until 36 months from the date that the last participant was randomized or death, whichever occurs earlier.

14.4 Analysis of Secondary Endpoints

14.4.1 Overall Survival

Overall survival will be will be analyzed using a Cox proportional hazards model. Hazard ratios and 95% confidence intervals will be presented for the comparison of FOLFOX + bevacizumab in combination with standard-dose vitamin D

(referent) versus higher-dose vitamin D. Models will be adjusted for relevant clinical and pathologic variables that are prognostic for survival. Overall survival will also be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan-Meier distribution will be determined, including the median survival and the proportion of participants remaining alive at 12, 18, and 24 months. These statistics will be given as point estimates with 95% confidence intervals, and a log rank test used to compare the two arms.

14.4.2 Objective Tumor Response Rate

At the time of the primary analysis, the proportion of participants with measurable disease at baseline achieving an objective response prior to any interventional therapy to size-reduce or remove any target lesions will be presented for each treatment arm with corresponding 95% confidence intervals calculated using the Clopper Pearson method. Any unscheduled tumor assessments will be included in the response classification. In addition, logistic regression adjusted for relevant prognostic clinical and pathologic variables will be used to express treatment differences in terms of an odds ratio and exact 95% confidence interval.

14.4.3 Toxicity

Each reported adverse event will be assigned a grade of severity in accordance with the NCI-CTCAE version 4.0 guidelines. All adverse events will be listed. Serious and non-serious adverse events occurring between informed consent and the first administration of investigational product will be listed for all participants who signed the informed consent. The participant incidence rate of treatment emergent adverse events will be summarized by treatment group and maximum severity (where appropriate) for all adverse events, serious adverse events, adverse events leading to discontinuation of protocol-specified treatment, adverse events leading to discontinuation from the study, and fatal adverse events. These summary tables will also be repeated for treatment-related adverse events.

14.4.4 Plasma 25(OH)D Endpoints

Descriptive statistics will be used to report the results of secondary endpoints related to plasma 25(OH)D levels. The mean and median baseline plasma 25(OH)D level will be reported for the overall population and by treatment arm, as well as the percentage of participants with vitamin D insufficiency (defined as plasma 25(OH)D <30 ng/mL) and vitamin D deficiency (defined as plasma 25(OH)D <20 ng/mL). The mean and median change in plasma 25(OH)D level from baseline to various time points (i.e., 8 weeks, 16 weeks, treatment discontinuation) will also be reported by treatment arm, as well as the time course of achieving levels ≥33 ng/mL in each arm. Lastly, we will also perform Cox proportional hazards regression, adjusting for other prognostic factors, to see if plasma 25(OH)D levels are associated with PFS and overall survival.

14.5 Analysis of Correlative Studies

14.5.1 Vitamin D Pathway Polymorphisms

We will compare the PFS of participants receiving higher-dose vitamin D versus standard dose, stratified by genotype status. Tests for statistical interaction will be performed by entering into the Cox model the cross-product term of treatment group with genotype status. Because this correlative study is exploratory in nature, only large differences are detectable with adequate power given the sample size of the main study. Assuming that 50% of participants will have documented disease progression at 9 months, a 50% prevalence of the variant genotype, and one-sided α=0.20, we will have 80% power to detect a HR of 0.64 for progression among participants with the variant genotype receiving higher-dose vitamin D compared to standard dose. In further exploratory analyses, we will also evaluate the association between genotype status and baseline 25(OH)D level and change in 25(OH)D levels induced by vitamin D supplementation.

14.5.2 VDR and 1-α-Hydroxylase Expression, and *KRAS* Mutation Status

We will compare the PFS of participants receiving higher-dose vitamin D versus standard dose, stratified by VDR expression or *KRAS* mutation status. Tests for statistical interaction will be performed by entering into the Cox model the cross-product term of treatment group with expression or mutation status. Because this correlative study is exploratory, only large differences are detectable with adequate power given the sample size of the main study. Assuming that 50% of participants will progress at 9 months, a 38% prevalence of VDR overexpression, and one-sided α=0.20, we will have 80% power to detect a HR of 0.60 for progression among participants with VDR overexpression receiving higher-dose vitamin D compared to standard dose. Assuming a 60% prevalence of *KRAS* wild-type tumors and one-sided α=0.20, we will have 80% power to detect a HR of 0.67 for progression among participants with wild-type *KRAS* receiving higher-dose vitamin D compared to standard dose.

14.5.3 Inflammatory and IGF Pathway Plasma Biomarkers

We will compare the PFS of participants receiving higher-dose vitamin D versus standard dose, stratified by high versus low levels of each plasma marker at baseline using the median value as the cutpoint. Tests for statistical interaction will be performed by entering into the Cox model the cross-product term of treatment group with the plasma marker as a continuous variable. Because this correlative study is exploratory in nature, only large differences are detectable with adequate power given the sample size of the main study. Assuming that 50% of participants will have documented disease progression at 9 months and one-sided α=0.20, we will have 80% power to detect a HR of 0.64 for progression among participants with either high or low levels of the plasma factor who are receiving higher-dose vitamin D compared to standard dose. In further exploratory analyses, we will evaluate the correlation between levels of the plasma inflammatory or IGF factor

with plasma 25(OH)D levels, as well as correlations between changes in each marker with changes in plasma 25(OH)D.

14.6 Reporting and Exclusions

14.6.1 Evaluation of toxicity. All participants who receive at least one dose of vitamin D will be evaluable for toxicity from the time of their first treatment.

14.6.2 Evaluation of response. All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

15. PUBLICATION PLAN

The results of this study will be published as an original article in an appropriate peer-reviewed medical journal. The primary responsibility for publication of study results will be held by the Principal Investigator, Dr. Kimmie Ng, at the lead institution, Dana-Farber Cancer Institute.

16. REFERENCES

1. Meggouh F, Lointier P, Saez S. Sex steroid and 1,25-dihydroxyvitamin D3 receptors in human colorectal adenocarcinoma and normal mucosa. Cancer Res 1991;51(4):1227-33.
2. Vandewalle B, Adenis A, Hornez L, Revillion F, Lefebvre J. 1,25-dihydroxyvitamin D3 receptors in normal and malignant human colorectal tissues. Cancer Lett 1994;86(1):67-73.
3. Zehnder D, Bland R, Williams MC, et al. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 2001;86(2):888-94.
4. Vandewalle B, Wattez N, Lefebvre J. Effects of vitamin D3 derivatives on growth, differentiation and apoptosis in tumoral colonic HT 29 cells: possible implication of intracellular calcium. Cancer Lett 1995;97(1):99-106.
5. Diaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. Cancer Res 2000;60(8):2304-12.
6. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D3 analog induces a G1-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. Endocrinology 2000;141(11):3931-9.
7. Iseki K, Tatsuta M, Uehara H, et al. Inhibition of angiogenesis as a mechanism for inhibition by 1alpha-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 of colon carcinogenesis induced by azoxymethane in Wistar rats. Int J Cancer 1999;81(5):730-3.

8. Fernandez-Garcia NI, Palmer HG, Garcia M, et al. 1alpha,25-Dihydroxyvitamin D3 regulates the expression of Id1 and Id2 genes and the angiogenic phenotype of human colon carcinoma cells. Oncogene 2005;24(43):6533-44.
9. Lamprecht SA, Lipkin M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. Ann N Y Acad Sci 2001;952:73-87.
10. Evans SR, Shchepotin EI, Young H, Rochon J, Uskokovic M, Shchepotin IB. 1,25-dihydroxyvitamin D3 synthetic analogs inhibit spontaneous metastases in a 1,2-dimethylhydrazine-induced colon carcinogenesis model. Int J Oncol 2000;16(6):1249-54.
11. Shabahang M, Buras RR, Davoodi F, et al. Growth inhibition of HT-29 human colon cancer cells by analogues of 1,25-dihydroxyvitamin D3. Cancer Res 1994;54(15):4057-64.
12. Eisman JA, Barkla DH, Tutton PJ. Suppression of in vivo growth of human cancer solid tumor xenografts by 1,25-dihydroxyvitamin D3. Cancer Res 1987;47(1):21-5.
13. Giuliano AR, Franceschi RT, Wood RJ. Characterization of the vitamin D receptor from the Caco-2 human colon carcinoma cell line: effect of cellular differentiation. Arch Biochem Biophys 1991;285(2):261-9.
14. Zhao X, Feldman D. Regulation of vitamin D receptor abundance and responsiveness during differentiation of HT-29 human colon cancer cells. Endocrinology 1993;132(4):1808-14.
15. Beaty MM, Lee EY, Glauert HP. Influence of dietary calcium and vitamin D on colon epithelial cell proliferation and 1,2-dimethylhydrazine-induced colon carcinogenesis in rats fed high fat diets. J Nutr 1993;123(1):144-52.
16. Huerta S, Irwin RW, Heber D, et al. 1alpha,25-(OH)(2)-D(3) and its synthetic analogue decrease tumor load in the Apc(min) Mouse. Cancer Res 2002;62(3):741-6.
17. Fraser DR. Regulation of the metabolism of vitamin D. Physiol Rev 1980;60(2):551-613.
18. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet 1989;2(8673):1176-8.
19. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. Am J Epidemiol 1995;142(6):608-11.
20. Tangrea J, Helzlsouer K, Pietinen P, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control 1997;8(4):615-25.
21. Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004;13(9):1502-8.
22. Wactawski-Wende J, Kotchen JM, Anderson GL, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006;354(7):684-96.
23. Wu K, Feskanich D, Fuchs CS, Willett WC, Hollis BW, Giovannucci EL. A nested case control study of plasma 25-hydroxyvitamin D concentrations and risk of colorectal cancer. J Natl Cancer Inst 2007;99(14):1120-9.
24. Gorham ED, Garland CF, Garland FC, et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. Am J Prev Med 2007;32(3):210-6.
25. Ding EL, Mehta S, Fawzi WW, Giovannucci EL. Interaction of estrogen therapy with calcium and vitamin D supplementation on colorectal cancer risk: reanalysis of Women's Health Initiative randomized trial. Int J Cancer 2008;122(8):1690-4.

26. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007;85(6):1586-91.
27. Freedman DM, Looker AC, Chang SC, Graubard BI. Prospective study of serum vitamin D and cancer mortality in the United States. J Natl Cancer Inst 2007;99(21):1594-602.
28. Robsahm TE, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004;15(2):149-58.
29. Moan J, Porojnicu AC, Robsahm TE, et al. Solar radiation, vitamin D and survival rate of colon cancer in Norway. J Photochem Photobiol B 2005;78(3):189-93.
30. Ng K, Meyerhardt JA, Wu K, et al. Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer. J Clin Oncol 2008;26(18):2984-91.
31. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006;98(7):451-9.
32. Mezawa H, Sugiura T, Watanabe M, et al. Serum vitamin D levels and survival of patients with colorectal cancer: post-hoc analysis of a prospective cohort study. BMC Cancer 2010;10:347.
33. Ng K, Sargent DJ, Goldberg RM, et al. Vitamin D status in patients with stage IV colorectal cancer: Findings from Intergroup Trial N9741. J Clin Oncol 2011:in press.
34. Hershberger PA, McGuire TF, Yu WD, et al. Cisplatin potentiates 1,25-dihydroxyvitamin D3-induced apoptosis in association with increased mitogen-activated protein kinase kinase kinase 1 (MEKK-1) expression. Mol Cancer Ther 2002;1(10):821-9.
35. Light BW, Yu WD, McElwain MC, Russell DM, Trump DL, Johnson CS. Potentiation of cisplatin antitumor activity using a vitamin D analogue in a murine squamous cell carcinoma model system. Cancer Res 1997;57(17):3759-64.
36. Moffatt KA, Johannes WU, Miller GJ. 1Alpha,25dihydroxyvitamin D3 and platinum drugs act synergistically to inhibit the growth of prostate cancer cell lines. Clin Cancer Res 1999;5(3):695-703.
37. Cho YL, Christensen C, Saunders DE, et al. Combined effects of 1,25-dihydroxyvitamin D3 and platinum drugs on the growth of MCF-7 cells. Cancer Res 1991;51(11):2848-53.
38. Kulkarni AD, van Ginkel PR, Darjatmoko SR, Lindstrom MJ, Albert DM. Use of combination therapy with cisplatin and calcitriol in the treatment of Y-79 human retinoblastoma xenograft model. Br J Ophthalmol 2009;93(8):1105-8.
39. Liu G, Hu X, Chakrabarty S. Vitamin D mediates its action in human colon carcinoma cells in a calcium-sensing receptor-dependent manner: downregulates malignant cell behavior and the expression of thymidylate synthase and survivin and promotes cellular sensitivity to 5-FU. Int J Cancer 2010;126(3):631-9.
40. Trump DL, Hershberger PA, Bernardi RJ, et al. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. J Steroid Biochem Mol Biol 2004;89-90(1-5):519-26.
41. Yu WD, Ma Y, Flynn G, et al. Calcitriol enhances gemcitabine anti-tumor activity in vitro and in vivo by promoting apoptosis in a human pancreatic carcinoma model system. Cell Cycle 2010;9(15):3022-9.

42. Belochitski O, Ariad S, Shany S, Fridman V, Gavrilov V. Efficient dual treatment of the hormone-refractory prostate cancer cell line DU145 with cetuximab and 1,25-dihydroxyvitamin D3. In Vivo 2007;21(2):371-6.
43. Bernardi RJ, Trump DL, Johnson C. ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), enhances the antiproliferative effects of calcitriol (1,25-dihydroxyvitamin D3) in squamous cell carcinoma: Effects on ERK and Akt. Proceedings of the American Association for Cancer Research 2002;43:783 (abstract 3883).
44. Gavrilov V, Steiner M, Shany S. The combined treatment of 1,25-dihydroxyvitamin D3 and a non-steroid anti-inflammatory drug is highly effective in suppressing prostate cancer cell line (LNCaP) growth. Anticancer Res 2005;25(5):3425-9.
45. Jamshidi F, Zhang J, Harrison JS, Wang X, Studzinski GP. Induction of differentiation of human leukemia cells by combinations of COX inhibitors and 1,25-dihydroxyvitamin D3 involves Raf1 but not Erk 1/2 signaling. Cell Cycle 2008;7(7):917-24.
46. Flaig TW, Barqawi A, Miller G, et al. A phase II trial of dexamethasone, vitamin D, and carboplatin in patients with hormone-refractory prostate cancer. Cancer 2006;107(2):266-74.
47. Chan JS, Beer TM, Quinn DI, et al. A phase II study of high-dose calcitriol combined with mitoxantrone and prednisone for androgen-independent prostate cancer. BJU Int 2008;102(11):1601-6.
48. Tiffany NM, Ryan CW, Garzotto M, Wersinger EM, Beer TM. High dose pulse calcitriol, docetaxel and estramustine for androgen independent prostate cancer: a phase I/II study. J Urol 2005;174(3):888-92.
49. Johnson CS, Egorin MJ, Zuhowski R. Effects of high-dose calcitriol (1,25-dihyroxyvitamin D3) on the pharmacokinetics of paclitaxel or carboplatin: Results of two phase I studies. J Clin Oncol 2000;19:abstract 210.
50. Beer TM, Eilers KM, Garzotto M, Egorin MJ, Lowe BA, Henner WD. Weekly high-dose calcitriol and docetaxel in metastatic androgen-independent prostate cancer. J Clin Oncol 2003;21(1):123-8.
51. Blanke CD, Beer TM, Todd K, Mori M, Stone M, Lopez C. Phase II study of calcitriol-enhanced docetaxel in patients with previously untreated metastatic or locally advanced pancreatic cancer. Invest New Drugs 2009;27(4):374-8.
52. Fakih MG, Trump DL, Muindi JR, et al. A phase I pharmacokinetic and pharmacodynamic study of intravenous calcitriol in combination with oral gefitinib in patients with advanced solid tumors. Clin Cancer Res 2007;13(4):1216-23.
53. Muindi JR, Johnson CS, Trump DL, Christy R, Engler KL, Fakih MG. A phase I and pharmacokinetics study of intravenous calcitriol in combination with oral dexamethasone and gefitinib in patients with advanced solid tumors. Cancer Chemother Pharmacol 2009;65(1):33-40.
54. Muindi JR, Peng Y, Potter DM, et al. Pharmacokinetics of high-dose oral calcitriol: results from a phase 1 trial of calcitriol and paclitaxel. Clin Pharmacol Ther 2002;72(6):648-59.
55. Srinivas S, Feldman D. A phase II trial of calcitriol and naproxen in recurrent prostate cancer. Anticancer Res 2009;29(9):3605-10.
56. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. Am J Clin Nutr 2001;73(2):288-94.

57. Vashi PG, Trukova K, Lammersfeld CA, Braun DP, Gupta D. Impact of oral vitamin D supplementation on serum 25-hydroxyvitamin D levels in oncology. Nutr J 2010;9:60.
58. Davie MW, Lawson DE, Emberson C, Barnes JL, Roberts GE, Barnes ND. Vitamin D from skin: contribution to vitamin D status compared with oral vitamin D in normal and anticonvulsant-treated subjects. Clin Sci (Lond) 1982;63(5):461-72.
59. Stamp TC, Haddad JG, Twigg CA. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet 1977;1(8026):1341-3.
60. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 2003;77(1):204-10.
61. Amir E, Simmons CE, Freedman OC, et al. A phase 2 trial exploring the effects of high-dose (10,000 IU/day) vitamin D(3) in breast cancer patients with bone metastases. Cancer 2010;116(2):284-91.
62. Khan QJ, Reddy PS, Kimler BF, et al. Effect of vitamin D supplementation on serum 25-hydroxy vitamin D levels, joint pain, and fatigue in women starting adjuvant letrozole treatment for breast cancer. Breast Cancer Res Treat 2010;119(1):111-8.
63. Ross AC, Manson JE, Abrams SA, et al. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab 2010.
64. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60(5):277-300.
65. Kindler HL, Shulman KL. Metastatic colorectal cancer. Curr Treat Options Oncol 2001;2(6):459-71.
66. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005;352(5):476-87.
67. Cancer AJCo. AJCC Cancer Staging Handbook, 7th edition. New York, NY: Springer-Verlag; 2010.
68. Advanced Colorectal Cancer Meta-Analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. . J Clin Oncol 1992;10(6):896-903.
69. Maindrault-Goebel F, Louvet C, Andre T, et al. Oxaliplatin added to the simplified bimonthly leucovorin and 5-fluorouracil regimen as second-line therapy for metastatic colorectal cancer (FOLFOX6). GERCOR. Eur J Cancer 1999;35(9):1338-42.
70. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000;18(16):2938-47.
71. Goldberg RM, Sargent DJ, Morton RF, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2004;22(1):23-30.
72. Tournigand C, Andre T, Achille E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 2004;22(2):229-37.
73. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350(23):2335-42.

74. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 2008;26(12):2013-9.
75. Cranney A, Weiler HA, O'Donnell S, Puil L. Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. Am J Clin Nutr 2008;88(2):513S-9S.
76. Ng K, Meyerhardt JA, Chan JA, et al. Multivitamin use is not associated with cancer recurrence or survival in patients with stage III colon cancer: findings from CALGB 89803. J Clin Oncol 2010;28(28):4354-63.
77. Jacobs ET, Martinez ME, Alberts DS. Research and public health implications of the intricate relationship between calcium and vitamin D in the prevention of colorectal neoplasia. J Natl Cancer Inst 2003;95(23):1736-7.
78. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376(9736):180-8.
79. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;19(13):2739-45.
80. Ramagopalan SV, Heger A, Berlanga AJ, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 2010;20(10):1352-60.
81. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367(6460):284-7.
82. Durrin LK, Haile RW, Ingles SA, Coetzee GA. Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. Biochim Biophys Acta 1999;1453(3):311-20.
83. Arai H, Miyamoto K, Taketani Y, et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 1997;12(6):915-21.
84. Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 2000;14(3):401-20.
85. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res 1999;14(5):740-6.
86. Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res 1996;11(12):1850-5.
87. Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. Proc Natl Acad Sci U S A 1992;89(15):6665-9.
88. Haussler MR, Whitfield GK, Haussler CA, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 1998;13(3):325-49.
89. Slattery ML, Neuhausen SL, Hoffman M, et al. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. Int J Cancer 2004;111(5):750-6.

90. Wong HL, Seow A, Arakawa K, Lee HP, Yu MC, Ingles SA. Vitamin D receptor start codon polymorphism and colorectal cancer risk: effect modification by dietary calcium and fat in Singapore Chinese. Carcinogenesis 2003;24(6):1091-5.
91. Theodoratou E, Farrington SM, Tenesa A, et al. Modification of the inverse association between dietary vitamin D intake and colorectal cancer risk by a FokI variant supports a chemoprotective action of Vitamin D intake mediated through VDR binding. Int J Cancer 2008;123(9):2170-9.
92. Slattery ML, Wolff RK, Curtin K, et al. Colon tumor mutations and epigenetic changes associated with genetic polymorphism: insight into disease pathways. Mutat Res 2009;660(1-2):12-21.
93. Parisi E, Rene JM, Cardus A, et al. Vitamin D receptor levels in colorectal cancer. Possible role of BsmI polymorphism. J Steroid Biochem Mol Biol 2008;111(1-2):87-90.
94. Ochs-Balcom HM, Cicek MS, Thompson CL, et al. Association of vitamin D receptor gene variants, adiposity and colon cancer. Carcinogenesis 2008;29(9):1788-93.
95. Li C, Li Y, Gao LB, et al. Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population. Dig Dis Sci 2009;54(3):634-9.
96. Flugge J, Laschinski G, Roots I, Krusekopf S. Vitamin D receptor poly(A) microsatellite and colorectal cancer risk in Caucasians. Int J Colorectal Dis 2009;24(2):171-5.
97. Yaylim-Eraltan I, Arzu Ergen H, Arikan S, et al. Investigation of the VDR gene polymorphisms association with susceptibility to colorectal cancer. Cell Biochem Funct 2007;25(6):731-7.
98. Slattery ML, Herrick J, Wolff RK, Caan BJ, Potter JD, Sweeney C. CDX2 VDR polymorphism and colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007;16(12):2752-5.
99. Kadiyska T, Yakulov T, Kaneva R, et al. Vitamin D and estrogen receptor gene polymorphisms and the risk of colorectal cancer in Bulgaria. Int J Colorectal Dis 2007;22(4):395-400.
100. Flugge J, Krusekopf S, Goldammer M, et al. Vitamin D receptor haplotypes protect against development of colorectal cancer. Eur J Clin Pharmacol 2007;63(11):997-1005.
101. Sweeney C, Curtin K, Murtaugh MA, Caan BJ, Potter JD, Slattery ML. Haplotype analysis of common vitamin D receptor variants and colon and rectal cancers. Cancer Epidemiol Biomarkers Prev 2006;15(4):744-9.
102. Park K, Woo M, Nam J, Kim JC. Start codon polymorphisms in the vitamin D receptor and colorectal cancer risk. Cancer Lett 2006;237(2):199-206.
103. Murtaugh MA, Sweeney C, Ma KN, et al. Vitamin D receptor gene polymorphisms, dietary promotion of insulin resistance, and colon and rectal cancer. Nutr Cancer 2006;55(1):35-43.
104. Nykjaer A, Dragun D, Walther D, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. Cell 1999;96(4):507-15.
105. Malecki MT, Klupa T, Wanic K, Cyganek K, Frey J, Sieradzki J. Vitamin D binding protein gene and genetic susceptibility to type 2 diabetes mellitus in a Polish population. Diabetes Res Clin Pract 2002;57(2):99-104.
106. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009;89(2):634-40.

107. Prosser DE, Jones G. Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci 2004;29(12):664-73.
108. Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. Annu Rev Nutr 2002;22:139-66.
109. Ogunkolade BW, Boucher BJ, Fairclough PD, et al. Expression of 25-hydroxyvitamin D-1-alpha-hydroxylase mRNA in individuals with colorectal cancer. Lancet 2002;359(9320):1831-2.
110. Bises G, Kallay E, Weiland T, et al. 25-hydroxyvitamin D3-1alpha-hydroxylase expression in normal and malignant human colon. J Histochem Cytochem 2004;52(7):985-9.
111. Bareis P, Bises G, Bischof MG, Cross HS, Peterlik M. 25-hydroxy-vitamin d metabolism in human colon cancer cells during tumor progression. Biochem Biophys Res Commun 2001;285(4):1012-7.
112. Lopez ER, Zwermann O, Segni M, et al. A promoter polymorphism of the CYP27B1 gene is associated with Addison's disease, Hashimoto's thyroiditis, Graves' disease and type 1 diabetes mellitus in Germans. Eur J Endocrinol 2004;151(2):193-7.
113. Lopez ER, Regulla K, Pani MA, Krause M, Usadel KH, Badenhoop K. CYP27B1 polymorphisms variants are associated with type 1 diabetes mellitus in Germans. J Steroid Biochem Mol Biol 2004;89-90(1-5):155-7.
114. Matusiak D, Benya RV. CYP27A1 and CYP24 expression as a function of malignant transformation in the colon. J Histochem Cytochem 2007;55(12):1257-64.
115. Ebert R, Schutze N, Adamski J, Jakob F. Vitamin D signaling is modulated on multiple levels in health and disease. Mol Cell Endocrinol 2006;248(1-2):149-59.
116. Bettoun DJ, Burris TP, Houck KA, et al. Retinoid X receptor is a nonsilent major contributor to vitamin D receptor-mediated transcriptional activation. Mol Endocrinol 2003;17(11):2320-8.
117. Echchgadda I, Song CS, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase is a target for transcriptional induction by the vitamin D receptor. Mol Pharmacol 2004;65(3):720-9.
118. Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ, Evans SR. 1,25-Dihydroxyvitamin D3 receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. Cancer Res 1993;53(16):3712-8.
119. Evans SR, Schwartz AM, Shchepotin EI, Uskokovic M, Shchepotin IB. Growth inhibitory effects of 1,25-dihydroxyvitamin D3 and its synthetic analogue, 1alpha,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalcifero l (Ro 25-6760), on a human colon cancer xenograft. Clin Cancer Res 1998;4(11):2869-76.
120. Matusiak D, Murillo G, Carroll RE, Mehta RG, Benya RV. Expression of vitamin D receptor and 25-hydroxyvitamin D3-1{alpha}-hydroxylase in normal and malignant human colon. Cancer Epidemiol Biomarkers Prev 2005;14(10):2370-6.
121. Kure S, Nosho K, Baba Y, et al. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev 2009;18(10):2765-72.
122. Zheng W, Wong KE, Zhang Z, et al. Inactivation of the vitamin D receptor in APC(min/+) mice reveals a critical role for the vitamin D receptor in intestinal tumor growth. Int J Cancer 2011.

123. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 1998;90(9):675-84.
124. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 2001;85(5):692-6.
125. Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000;9(11):1193-7.
126. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66(8):3992-5.
127. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Br J Cancer 2007;96(8):1166-9.
128. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25(22):3230-7.
129. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 2007;67(6):2643-8.
130. Solomon C, Sebag M, White JH, Rhim J, Kremer R. Disruption of vitamin D receptor-retinoid X receptor heterodimer formation following ras transformation of human keratinocytes. J Biol Chem 1998;273(28):17573-8.
131. Solomon C, Kremer R, White JH, Rhim JS. Vitamin D resistance in RAS-transformed keratinocytes: mechanism and reversal strategies. Radiat Res 2001;155(1 Pt 2):156-62.
132. Qi X, Tang J, Pramanik R, et al. p38 MAPK activation selectively induces cell death in K-ras-mutated human colon cancer cells through regulation of vitamin D receptor. J Biol Chem 2004;279(21):22138-44.
133. Stedman L, Nickel KP, Castillo SS, Andrade J, Burgess JR, Teegarden D. 1,25-dihydroxyvitamin D inhibits vitamin E succinate-induced apoptosis in C3H10T1/2 cells but not Harvey ras-transfected cells. Nutr Cancer 2003;45(1):93-100.
134. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends Mol Med 2002;8(4):174-9.
135. Shoji T, Shinohara K, Kimoto E, et al. Lower risk for cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a haemodialysis population. Nephrol Dial Transplant 2004;19(1):179-84.
136. Teng M, Wolf M, Ofsthun MN, et al. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. J Am Soc Nephrol 2005;16(4):1115-25.
137. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond) 2008;5:10.
138. Mark BL, Carson JA. Vitamin D and autoimmune disease--implications for practice from the multiple sclerosis literature. J Am Diet Assoc 2006;106(3):418-24.
139. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006;56(2):69-83.

140. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol Endocrinol 2003;17(12):2386-92.
141. Froicu M, Zhu Y, Cantorna MT. Vitamin D receptor is required to control gastrointestinal immunity in IL-10 knockout mice. Immunology 2006;117(3):310-8.
142. Fichera A, Little N, Dougherty U, et al. A vitamin D analogue inhibits colonic carcinogenesis in the AOM/DSS model. J Surg Res 2007;142(2):239-45.
143. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 2000;130(11):2648-52.
144. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. Eur J Immunol 2005;35(1):217-24.
145. Lim WC, Hanauer SB, Li YC. Mechanisms of disease: vitamin D and inflammatory bowel disease. Nat Clin Pract Gastroenterol Hepatol 2005;2(7):308-15.
146. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Invest 2005;35(5):290-304.
147. Gilman J, Shanahan F, Cashman KD. Altered levels of biochemical indices of bone turnover and bone-related vitamins in patients with Crohn's disease and ulcerative colitis. Aliment Pharmacol Ther 2006;23(7):1007-16.
148. Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. Gut 2000;47(2):211-4.
149. Dresner-Pollak R, Ackerman Z, Eliakim R, Karban A, Chowers Y, Fidder HH. The BsmI vitamin D receptor gene polymorphism is associated with ulcerative colitis in Jewish Ashkenazi patients. Genet Test 2004;8(4):417-20.
150. Timms PM, Mannan N, Hitman GA, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? Qjm 2002;95(12):787-96.
151. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006;83(4):754-9.
152. Aaronson SA. Growth factors and cancer. Science 1991;254(5035):1146-53.
153. Rechler MM. Growth inhibition by insulin-like growth factor (IGF) binding protein-3--what's IGF got to do with it? Endocrinology 1997;138(7):2645-7.
154. Giovannucci E, Pollak MN, Platz EA, et al. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 2000;9(4):345-9.
155. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999;91(7):620-5.
156. Wolpin BM, Meyerhardt JA, Chan AT, et al. Insulin, the insulin-like growth factor axis, and mortality in patients with nonmetastatic colorectal cancer. J Clin Oncol 2009;27(2):176-85.
157. Fuchs CS, Goldberg RM, Sargent DJ, et al. Plasma insulin-like growth factors, insulin-like binding protein-3, and outcome in metastatic colorectal cancer: results from intergroup trial N9741. Clin Cancer Res 2008;14(24):8263-9.

158. Gomez JM, Maravall FJ, Gomez N, Navarro MA, Casamitjana R, Soler J. Relationship between 25-(OH) D3, the IGF-I system, leptin, anthropometric and body composition variables in a healthy, randomly selected population. Horm Metab Res 2004;36(1):48-53.
159. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007;7(9):684-700.
160. Huynh H, Pollak M, Zhang JC. Regulation of insulin-like growth factor (IGF) II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25(OH)2D3 and its analog EB1089. Int J Oncol 1998;13(1):137-43.
161. Ogino S, Kawasaki T, Brahmandam M, et al. Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn 2005;7(3):413-21.
162. Kraemer HC. Ramifications of a population model for kappa as a coefficient of reliability. Psychometrika 1979;44:461-72.
163. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol 1997;282:174-86.
164. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Haematol 1995;54(1):1-8.
165. Bonser AM, Garcia-Webb P. C-peptide measurement: methods and clinical utility. Crit Rev Clin Lab Sci 1984;19(4):297-352.
166. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45(2):228-47.
167. Shankar LK, Hoffman JM, Bacharach S, et al. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med 2006;47(6):1059-66.
168. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003;21(1):60-5.

17.

APPENDICES

Appendix A: Performance Status Criteria

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

DFCI IRB Protocol #: 11-436

APPENDIX B

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

Table of Contents

1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for a DF/HCC Multi-Center research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center (DF/HCC) Multi-center protocol will comply with Federal regulations and Health Insurance Portability and Accountability Act (HIPAA) requirements in accordance with the CTEP Multi-center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Components

The Multi-Center Data and Safety Monitoring Plan includes the following components:

DF/HCC Multi-center Protocol: One or more outside institutions collaborating with Dana-Farber/Harvard Cancer Center on a research protocol where DF/HCC is the Lead Institution. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: DFCI will be the Lead Institution and will be responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, FDA, OBA etc.). The Lead Institution is the home of the Overall PI.

DF/HCC Contract Principal Investigator: Investigator located at the Lead Institution who will be charged with the responsibility of the administration of the DF/HCC Project. This most often will be the Protocol Chair, but occasionally this may be the overall grant or contract holder, as applicable.

Protocol Chair: The Protocol Chair is the Principal Investigator for the DF/HCC protocol submitted as the Lead Institution. For applicable protocols, the Protocol Chair will be the single liaison with any regulatory agencies (i.e. CTEP Protocol and Information Office (PIO), FDA, OBA etc.).

Participating Institution: A Participating Institution is an institution that desires to collaborate with DF/HCC and commits to accruing participants to a DF/HCC protocol. The Participating Institution acknowledges the Protocol Chair as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-center Protocol. The Coordinating Center will provide the administrative support to the Protocol Chair in order that he/she may fulfill the responsibilities outlined in the DSMP and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In addition to the Lead Institution, the Quality Assurance Office for Clinical

Trials (QACT) provides support services to assist the Protocol Chair.

Clinical Trials Office: The clinical trials offices of the DF/HCC consortium members support investigators and their study teams with the coordination, submission and ongoing conduct of research protocols involving human subjects. Specifically, these offices support four core service areas including; pre-review of PI initiated protocols; assistance in the preparation and management of Investigational New Drug (IND) applications and subsequent required reporting to the FDA; regulatory consultation and guidance in the interpretation of local, federal, and ICH guidelines and policies; and the orientation and ongoing training support of clinical research personnel.

DF/HCC Quality Assurance Office for Clinical Trials: The DF/HCC QACT is a unit that has been developed to computerize, manage, and QC & QA data and DF/HCC trials. The DF/HCC QACT is located administratively in the office of the Senior Vice President for Clinical Research, at Dana-Farber Cancer Institute. The QACT uses DF/HCC computerized institutional databases for participant registrations and for the management of trial data as well as a set of quality assurance programs designed to audit DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

In accordance with the CTEP Multi-center Guidelines, the Protocol Chair, Coordinating Center (Lead Institution or designee), and the Participating Institutions will all agree to the general responsibilities as follows (specific procedures for these general responsibilities are detailed in the DSMP):

2.1 Protocol Chair (DF/HCC Principal Investigator)

The Protocol Chair, **Kimmie Ng, MD, MPH,** will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an inclusion to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants.
- For international trials, assure that the protocol is provided to Participating Institutions in the primary language spoken at the site.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI IRB, DF/HCC and other applicable (i.e. CTEP, FDA, OBA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.

- Act as the single liaison with CTEP/PIO Office (CTEP trials) or FDA (investigator-held IND trials) or OBA (gene therapy trials), as applicable.

2.2 Coordinating Center (Lead Institution)

The Coordinating Center is the DF/HCC Lead Institution's study team or designee (i.e Medical Monitor, Clinical Research Organization). The DF/HCC Lead Institution, **Dana-Farber Cancer Institute**, will ensure that all Participating Institutions within the Multi-Center Protocol demonstrate their intent and capability of complying with Federal Regulations and HIPAA requirements. To assist the Protocol Chair in meeting his/her responsibilities as required by the DSMP, the DF/HCC Lead Institution's study team or designee will assume the following general responsibilities:

- Assist in protocol review.
- Maintain copies of FWA and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain FDA or OBA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the Protocol Chair.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to Protocol Chair for timely review.
- Distribute Serious Adverse Event safety reports (both IND Safety reports and protocol specific SAEs).
- Monitor at Participating Institutions either by on-site inspection of selected participant records and/or with source documents and research records submitted to the Lead Institution.

In addition to the Lead Institution, the DF/HCC Quality Assurance Office for Clinical Trials provides the following support services to assist the Protocol Chair:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide Central Participant Registration.
- Verify that eligibility has been confirmed by the investigator and that appropriate consent has been obtained.
- Provide auditing services (funding and QACT approval required).

2.3 Participating Institution

Each Participating Institution will provide to the Coordinating Center a list of the key personnel assigned to the role for oversight of data management at their site. All sites must have office space, office equipment, and internet access that meet HIPAA standards.

The general responsibilities for each Participating Institution are as follows:

- Commit to accrual to the Lead Institution's (DF/HCC) protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder.
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event reports to local IRB and directly to the Coordinating Center.
- Submit deviations and violations to local IRB and the Coordinating Center.
- Secure investigational agents per federal guidelines and protocol requirements.
- For protocols using investigational agents, the Participating Institution will order their own investigational agents regardless of the supplier (i.e. NCI, pharmaceutical company)

3.0 PROTOCOL DEVELOPMENT

3.1 Activation of a Protocol

The Protocol Chair is responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting SAEs, violations and deviations per DFCI IRB guidelines and if applicable FDA or OBA Guidelines. Further, the Protocol Chair will be the single liaison with the FDA or OBA, as applicable.

To meet these requirements, the Protocol Chair will be responsible for the following minimum standards:

- Inclusion of the DF/HCC Multi-Center Data and Safety Monitoring Plan in the protocol as an appendix.
- Identify, qualify and initiate Participating Institutions and obtain accrual commitments.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Ensure that there is only one version of the Protocol and that all Participating Institutions use the correct version.
- Oversee the development of data collection forms (case report forms) that are of common format for use at all the Participating Institutions.

3.2 Coordinating Center Support Function

The DF/HCC Lead Institution's study staff or designee will provide administrative and clerical support to the Protocol Chair for the development and distribution of the protocol.

The tasks to be performed by the DF/HCC Lead Institution's study staff or designee include:

- Maintain Regulatory documents for all Participating Institutions.
- Review of the protocol and consent to check for logistics, spelling, and consistency. Provide the Protocol Chair a list of queries related to any inconsistencies.
- Provide necessary administrative sections, including paragraphs related to registration logistics, data management schedules, and multi-center guidelines.
- Maintenance of contact list of all Participating Institutions in the DF/HCC Multi-center Protocol and the distribution of updates to the sites as needed.
- Derivation of the study calendar, if applicable.
- Assistance in preparation and maintenance of case report forms.
- Conduct regular communications with all Participating Institutions (conference call, emails, etc)
- Maintain documentation of all communications.

4.0 PROTOCOL MANAGEMENT

The Coordinating Center is responsible for assuring that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the Coordinating Center must maintain copies of all IRB approvals, for each Participating Institution.

4.1 Protocol Distribution

The Coordinating Center will distribute the final approved protocol and any subsequent amended protocols to all Participating Institutions.

4.2 Protocol Revisions and Closures

The Participating Institutions will receive phone, fax, mail or e-mail notification of protocol revisions from the Lead Institution or designee. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

Non life-threatening revisions: Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Lead Institution or designee. Non-life-threatening protocol revisions should be IRB approved and implemented within 90 days from receipt of the notification.

Revisions for life-threatening Causes: Participating Institutions will receive telephone notification from the Lead Institution or designee concerning protocol revisions required to protect lives with follow-up by fax, mail or e-mail. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval

Protocol Closures and Temporary Holds: Participating Institutions will receive fax, e-mail, or phone notification of protocol closures and temporary holds from the Lead Institution or designee. Closures and holds will be effective immediately. In addition, the Lead Institution or designee will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

4.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for participating institutions. The Participating Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC guidance document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Site.

Participating sites are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Lead Site for their revision prior to submission to the participating site's IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. **It is DF/HCC policy that only attending physicians can obtain informed consent and re-consent to drug and/or device trials.**

4.4 IRB Documentation

The following must be on file with the DF/HCC Lead Institution or designee and must be submitted and approved by the DFCI IRB prior to participant registration:

- Approval Letter of the institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution’s IRB
- IRB approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the DF/HCC Lead Institution their IRB approval for Amendments to a protocol.

4.5 IRB Re-Approval

Annual IRB re-approval from the Participating Institution is required in order to continue research and register participants onto a protocol. There is no grace period for continuing approvals.

Protocol registrations will not be completed if a re-approval letter is not received by the DF/HCC Lead Institution from the Participating Institutions on or before the anniversary of the previous approval date.

4.6 Participant Confidentiality and Authorization Statement

The HIPPA of 1996 contains, as one of its six major components, the requirement to create privacy standards for health care information that is used or disclosed in the course of treatment, payment or health care operations. The original Privacy Rule, as it has come to be known, was published in December 2000. The Final Rule was published on August 14, 2002, which modified the privacy rule in significant ways vis-à-vis research.

In order for covered entities to use or disclose protected health information during the course of a DF/HCC Multi-Center Protocol, the study participant must sign an Authorization. This Authorization may or may not be separate from the Informed Consent. The DF/HCC Multi-Center Protocol, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide an Informed Consent template, which covered entities (DF/HCC Multi-Center Protocol Participating Institutions) must use.

The DF/HCC Multi-Center Protocol will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per National Cancer Institute requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

4.7 Participant Registration and Randomization

Refer to protocol section 4.2 for registration and randomization procedures.

4.8 DF/HCC Multi-center Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and written on all data and QACT correspondence for the participant.

4.9 DF/HCC Multi-center Protocol Registration Policy

4.9.1 Initiation of Therapy: Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's Registration Confirmation memo from the DF/HCC QACT. Therapy must be initiated per protocol guidelines. The Protocol Chair and DFCI IRB must be notified of any exceptions to this policy**.**

4.9.2 Eligibility Exceptions: The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval.

4.9.3 Verification of Registration, Dose Levels, and Arm Designation: A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one working day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

4.9.4 Confidentiality: All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Lead Institution or designee must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number and protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

4.10 Schedule of Data Submission

The DF/HCC QACT develops a set of either paper or electronic case report forms, (CRF/eCRFs) for use with the DF/HCC Multi-Center Protocol. QACT provides a web based training for eCRF users. These forms are designed to collect data for each study. *Note: It is necessary to send only ONE copy of all paper Case Report Forms, if applicable.*

4.10.1 Eligibility Checklist

Purpose - Outlines protocol-specific eligibility criteria and includes the following:

Participant Demographics (address, zip code, sex, race, ethnicity, initials, date of birth)

1) Parameters for eligibility
2) Parameters for exclusion
3) Parameters for stratifications

If a time frame is not specified in the protocol, tests must be completed as follows:

- Lab tests required for eligibility must be completed within 14 days prior to study enrollment by the QACT.
- For protocols requiring measurable disease, lab baseline measurements must be completed within 14 days prior to study enrollment by the QACT. Examples: flow cytometry, HLA typing, fluid cytology, tumor markers and hormones (CEA, CA-27-29, CA-125).
- Non-lab tests required for eligibility must be performed within 30 days prior to study entry. Example: radiological scans
- For bone marrow transplant (BMT) protocols and non-protocol treatment plans, eligibility tests must be completed within 42 days prior to enrollment

by the QACT. The extended period of time is allowed to facilitate insurance approval while ensuring participant safety.

4.10.2 On-study Form(s)

Purpose - documents the following items:

- Demographic data
- Prior therapy
- Past medical and surgical history
- Description of participant's physical status at protocol registration
- Disease site specific data

4.10.3 Baseline Assessment Form(s)

Purpose – Documents objective and subjective disease status as defined by the protocol**.** Records all pertinent radiographic and laboratory measurements of disease utilized in determining response evaluations.

4.10.4 Treatment Form(s)

Purpose - Records the following information related to the time the participant receives protocol treatment:

- Participant, Protocol information
- Protocol treatment and supportive therapy per treatment cycle
- Protocol specific laboratory values per treatment cycle
- All medications other than protocol chemotherapy agents used to treat concomitant diagnoses, if applicable

4.10.5 Adverse Event Report Form(s)

Purpose – Documents adverse events that occur while the participant is receiving treatment and for up to 30 days after the last dose of treatment. All adverse events are to be graded by number using the toxicity grading scale required by the protocol. *This form is not for IRB submission, but for recording the AE in the research database.*

4.10.6 Response Assessment Form(s)

Purpose – Documents objective and subjective response as defined by the protocol. Records all pertinent radiographic and laboratory measurements of disease utilized in determining response evaluations.

4.10.7 Off Treatment and Off Study Form(s)

Purpose - The Off Treatment and Off Study Forms are submitted when the participant is removed from the study or has completed all protocol treatment.

Note: If the participant dies while on protocol**,** the Off Study Form is the last form submitted.

4.10.8 Follow up / Survival Form

Purpose - Summarizes participant status at a given point in time after being removed from treatment.

4.11 Data Form Review

When data forms arrive at the DF/HCC QACT, they are reviewed for:

Completeness:
Is all the information provided as required per protocol?

Adverse Events (Toxicities):
Did the participant experience adverse events (toxicities or side effects) associated with the treatment? Was the treatment delayed due to the adverse event? What was the most severe degree of toxicity experienced by the participant?

Notations concerning adverse events will address relationship to protocol treatment for each adverse event grade. All adverse events encountered during the study will be evaluated according to the NCI Common Toxicity Criteria assigned to the protocol and all adverse events must be noted on the participant's Adverse Event (Toxicity) Forms.

Response:
Did the participant achieve a response? What level of response did they achieve? On what date did the participant achieve the response and how was the response determined?

Response criteria are defined in the protocol. A tumor assessment must be performed prior to the start of treatment and while the participant is on treatment as specified by the protocol.

Objective responses must have documentation such as physical measurements, x-rays, scans, or laboratory tests.

A subjective response is one that is perceived by the participant, such as reduction in pain, or improved appetite.

4.12 Missing and Deficient Memorandum

Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following policies and procedures:

<u>Incomplete or Questionable Data</u>

If study forms are received with missing or questionable data, the submitting institution will receive a written query from the DF/HCC QACT Data Analyst. Responses to the query should be completed and returned within 14 days. Responses may be returned on the written query or on an amended case report form. In both instances the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the DF/HCC QACT noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed monthly.

5.0 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol. Please refer to section 7.

5.1 CTEP Trial Routine Drug Requisitions

N/A

5.2 Medical Emergency Drug Ordering

N/A

6.0 SAFETY ASSESSMENTS AND TOXICITY MONITORING

All participants receiving investigational agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported to the investigator by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB).

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

6.1 Serious Adverse Events

A serious adverse event (SAE) is any adverse drug experience at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical

judgment, they may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions in a participant who has never had seizure activity in the past that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

6.2 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Serious Adverse Events (SAEs) will be followed as is delineated in the protocol Section 11.3.

The Lead Institution will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating all SAEs to all sites conducting the trial.

Participating Institutions must report the AEs to the Protocol Chair and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

6.3 Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The Protocol Chair will review all IND Safety Reports and is ultimately responsible for forwarding the IND Safety Reports to the Participating Institutions. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

7.0 PROTOCOL VIOLATIONS AND DEVIATIONS

Neither the FDA nor the ICH guidelines define the terms “protocol violation” or “protocol deviation.” All DF/HCC Protocol Chairs must adhere to those policies set by the DFCI IRB, the definitions for protocol violation and deviation as described by the DFCI IRB will be applied for reporting purposes for all Institutions Participating in the DF/HCC Multi-center Protocol.

7.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is prospectively approved prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not prospectively approved by the IRB prior to its initiation or implementation.

7.2 Reporting Procedures

The Protocol Chair: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The Protocol Chair will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from DFCI IRB. The Participating institution must submit the deviation request to the Protocol Chair or designee, who will submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation should be submitted to the Participating Institution's own IRB, per its institutional policy.
A copy of the Participating Institution's IRB report and determination will be forwarded to the DF/HCC Lead Institution or designee by mail, facsimile, or via e-mail within 10 business days after the original submission.

All protocol violations must be sent to the DF/HCC Lead Institution Protocol Chair or designee in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the DF/HCC Lead Institution or designee will submit the report to the Protocol Chair for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

8.0 MONITORING: QUALITY CONTROL

The quality control process for a multi-center clinical trial requires ongoing monitoring to ensure all participating centers are compliant with the most current protocol, that the study is being conducted according to all pertinent regulatory requirements and to verify data accuracy. As the Coordinating Center, the DF/HCC Lead Institution, with the aid of a Clinical Trial Specialist, will provide quality control oversight for this DF/HCC Multi-center Protocol.

8.1 Site Qualification

The DF/HCC Lead Institution will send a Site Selection and Feasibility Questionnaire and protocol to the site lead investigator at each proposed Participating Institution. The questionnaire will ask the co-investigator to consider DF/HCC policies regarding informed consent, serious adverse event reporting and documentation, and pharmacy guidelines. The questionnaire will also require site consideration of IRB procedures, participant enrollment, appropriateness of his/her facility for carrying out the proposed protocol, plans for data submission and monitoring, and the identification of staff availability and credentials. Co-investigators will be offered the opportunity to discuss

the questionnaire and accompanying checklist with the DF/HCC Lead Institution as appropriate. Site lead investigators will be instructed to return the completed questionnaire to the DF/HCC Lead Institution along with a CV, which has been signed and dated.
The Overall Principal Investigator will review the Site Selection and Feasibility Questionnaire and determine whether or not the site is qualified to participate. Sites believed to be qualified to participate will be invited to submit the protocol to their institutional IRB for review.

8.2 Regulatory Documents

8.2.1 Required Documents

The Participating Institution will provide the DF/HCC Lead Institution with the required regulatory documents prior to enrolling any patients. Regulatory documents include, but are not limited to: CVs for all participating investigators and laboratory director, current medical licenses, financial disclosure forms, original 1572, laboratory CLIA certificates (or equivalent), laboratory normal ranges and/or any applicable SOPs. The Participating Institution will forward any updated/current documents to the DF/HCC Lead Institution as needed.

8.2.2 Informed Consent

The DF/HCC Lead Institution will provide the Participating Institution with a copy of the informed consent to be used as a template. The Participating Institute must send a copy of their informed consent to the DF/HCC Lead Institution for approval prior to submitting to their local IRB. Any changes made to the informed consent during the Participating Institution's review process and/or during the duration of the study, must also be approved by the DF/HCC Lead Institution prior to submission to the Participating Institution's IRB.

8.2.3 IRB Approval

Once a proposed Participating Institution receives institutional IRB approval, all approval documentation and IRB-approved related documents will be emailed to the DF/HCC Lead Institution and an amendment to add a site will be submitted to the DF/HCC IRB. The Participating Institution will not be able to enroll patients until the amendment has been activated at DF/HCC and the DF/HCC Lead Institution has confirmed the Participating Institution can begin enrollment.

8.2.4 Participating Site Regulatory Binder

All Participating Institutions will be instructed to maintain and update all essential regulatory documents according to the template provided by the DF/HCC Lead Institution. A designated member of the DF/HCC Lead Institution study team will be responsible for maintaining the Trial Master File which will include copies

of all regulatory documentation for the DF/HCC Lead Institution and each individual Participating Institution. This study team member will maintain a record of all approvals and licensure expirations and will request documents as needed from each Participating Institution.

8.3 Training, Delegation of Responsibility and Oversight

Once the Participating Institution receives the training materials, a teleconference/virtual training will take place. The DF/HCC Lead Institution will lead the call. A Site Initiation Checklist will be used to document who from the Participating Institution is in attendance and what material has been reviewed. Staff will review all aspects of participant eligibility, screening, consenting, enrollment, protocol procedures/testing, event reporting, deviation/violation reporting, study objectives, specimen collection, data collection, oversight and study meeting schedules.

This training must occur prior to the enrollment of any participants at the Participating Institution.

Participating Institutions will be instructed to complete a Delegation of Responsibility Log which will be reviewed/approved by the Overall Principal Investigator. A copy of this document will be retained in the Trial Master File. Participating Institutions will be clearly instructed to inform the DF/HCC Lead Institution immediately should there be a change to study personnel. An updated training and Delegation Log will need to be completed as soon as possible.

The training teleconference and all subsequent trainings and communications will be documented on the training log or in email correspondence, which will be retained in each Participating Institution's regulatory binder and in the Trial Master File retained at the DF/HCC Lead Institution.

Teleconferences will be held monthly. The purpose of these calls will be to review study accrual, safety updates, and information pertinent to promoting protocol compliance. Calls will be held more frequently, if needed. Agendas, minutes, and attendance will be documented in the regulatory binders.

The DF/HCC Lead Institution will also be available to all Participating Institutions in order to discuss questions and concerns on an as-needed basis.

8.4 Site Approval

All Participating Institutions are required to submit all institutional IRB correspondences and approvals to be retained in the Trial Master File at the DF/HCC Lead Institution. The DF/HCC Lead Institution will review all working study documents and ensure the most current IRB-approved protocol and study documents are being used by the Participating Institutions.

8.5 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the DF/HCC Lead Institution for remote monitoring by the Clinical Trial Specialist. Also, the Participating Institution will be participant to at least one on-site monitoring visit conducted by the Clinical Trial Specialist.

The DF/HCC Lead Institution will implement monitoring activities ongoing to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Additional monitoring practices may include but are not limited to: source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management.
Monitoring will occur before the clinical phase of the protocol begins and will continue during protocol performance through study completion.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Lead Institution or designee and if applicable QACT Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution's Principal Investigator and research team.

The DF/HCC Lead Institution will maintain regular and ongoing communication to Participating Institutions about study related information.

8.5.1 Consent

The Participating Institution must follow the DF/HCC Lead Institution's consenting policy, which requires that an attending physician consent the participant to the research study. This physician must also be listed on the 1572 and the DF/HCC Lead Institution must have all current regulatory documents on file, including signed CV, medical license and financial disclosure form.

8.5.2 Eligibility

In order to register to the trial, the Participating Institutions will submit the signed Informed Consent Form, Eligibility Checklist and supporting source documentation to the DF/HCC Lead Institution immediately for review.

The DF/HCC Lead Institution will review each consent form to ensure 1) it is the current version, 2) that the participant information is included on all pages and that all pages of the consent have been submitted, 3) that the participant has signed and dated the consent form, 4) that a physician has reviewed and signed

the consent form on the same day/time as the participant, and that 5) all appropriate items have been completed.

The DF/HCC Lead Institution will carefully review the Eligibility Checklist and supporting source documentation in order to confirm participant eligibility to participate in the trial. The DF/HCC Lead Institution will then fax the Informed Consent and Eligibility Checklist to the QACT. QACT will provide an additional review of eligibility prior to registering the participant to protocol. **A participant cannot initiate study treatment until the Participating Institution receives an email Confirmation of Registration from the DF/HCC Lead Institution.**

Once the first several participants (but no more than 5) have been enrolled at the Participating Institution, the Clinical Trial Specialist will review the participant study charts to ensure eligibility criteria have been met and appropriate documentation and data collection has taken place. Assuming no issues are identified, further eligibility documentation will be verified at pre-set monitoring visits of randomly assigned participant study charts.

8.5.3 Data Monitoring

Participating Institutions will be required to enter data into the electronic data capture system (eDC) in a timely manner. The Participating Institution will provide supporting source documentation to the DF/HCC Lead Institution as requested. The source documentation will include, but may not be limited to, source supporting the primary and/or secondary study endpoints. The data and all supporting documentation will be reviewed by the Clinical Trial Specialist. In addition to data queries generated in the eDC system, data queries will be submitted to Participating Institutions via email in order to clarify any discrepancies. All data and supporting documentation will be housed in the password-protected study file (in secure Partners Firewall protected server) and in participant study charts housed in locked areas at the DF/HCC Lead Institution and Participating Institution.

Once the first several participants (but no more than 5) have been enrolled at a Participating Institution, the Clinical Trial Specialist will review the participant study charts to ensure appropriate and accurate data entry and collection. Assuming no issues are identified, further data monitoring will be verified at pre-set monitoring visits of randomly assigned participant study charts.

8.5.3 Adverse Event Reporting

Each Participating Institution will be instructed to maintain an Adverse Event Tracking Log. Adverse events must be reported as instructed in the protocol with the Log serving as record for all events at each Participating Institution. The DF/HCC Lead Institution will be notified of all AE and SAE's as per protocol. Each Participating Institution will be asked to submit their site's Event Log once a month to the Coordinating Center. The Coordinating Center will maintain a

master event list to be shared with all collaborators during regularly scheduled teleconferences.

8.5.3 Drug Accountability

Each participant will be given a Drug Diary providing instructions for taking study drug and for tracking doses. Participants will be instructed to bring the signed Drug Diary, and any unused medication, with them to every visit. The Drug Diary and unused medication will be collected by the Participating Institution's research team. The Participating Institution will be responsible for documenting the number of returned study drug capsules and for returning any unused study drug to the Participating Institutional Pharmacy for destruction per institutional policy. Participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipments of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug.

8.5.4 Accrual of Eligible Participants

Annual accrual rates for eligible participants enrolled onto therapeutic clinical trials are calculated for each institution. Participating Institutions are expected to maintain the minimum annual average accrual as defined by the protocol grant or contract.

8.5.5 Quality Improvement Report:

N/A

9.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. The main focus in auditing is to measure if the standards and procedures set are being followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and the data were generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs) and the Code of Federal Regulations.

9.1 NCI Sponsored Trials

N/A

9.2 DF/HCC Sponsored Trials

Each site that enrolls three subjects who receive protocol treatment will be audited once, at a minimum, by the QACT. Approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited. Additional audits may be requested at the determination of the overall PI (DF/HCC Sponsor).

9.3 Participating Institution

It is the Participating Institution's responsibility to notify the DF/HCC Lead Institution of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve the DF/HCC Multi-Center Protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the DF/HCC Lead Institution or designee within 12 weeks after the audit date.

9.4 Coordinating Center (Lead Institution or designee)

The Protocol Chair will review all DF/HCC Multi-Center Protocol Final Audit reports and corrective action plans if applicable. The Lead Institution or designee must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the Protocol Chair to implement recommendations or require further follow-up. For unacceptable audits, the Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

9.5 Sub-Standard Performance

The Protocol Chair and DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the DF/HCC Multi-Center Protocol.

9.5.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal guidelines, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the Protocol Chair for revocation of participation.

Appendix C

SAFETY REPORTING OF ADVERSE EVENTS TO GENENTECH

ASSESSMENT OF SAFETY

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to Bevacizumab, all events of death, and any study specific issue of concern.

Adverse Events

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of Bevacizumab or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood

dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are collected and reported to the FDA, appropriate IRB(s), and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to Bevacizumab (see following guidance), and actions taken.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the protocol, Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

Specific Instructions for Recording Adverse Events

The Principal Investigator should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases).

b. Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

d. Hospitalizations for Medical or Surgical Procedures

Requirement or prolongation of inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before

the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 30 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the Bevacizumab should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior Bevacizumab exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for Bevacizumab. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange quarterly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

h. AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the product. The Avastin AESIs are:

- Hypertension ≥ grade 3
- Proteinuria ≥ grade 3
- GI perforation, abscesses and fistulae (any grade)
- Wound healing complications ≥ grade 3
- Haemorrhage ≥ grade 3 (any grade CNS bleeding; ≥ grade 2 haemoptysis)
- Arterial thromboembolic events (any grade)
- Venous thromboembolic events ≥ grade 3
- PRES (any grade)
- CHF ≥ grade 3
- Non-GI fistula or abscess ≥ grade 2

I. SAE Reporting

Sites must report all SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682
OR
(650) 225-5288

Copies of all Medwatch/case reports must be sent to the Sponsor-Investigator at:

Email: Christine_Ganser@dfci.harvard.edu

- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.
- Serious AE reports that are related to the Bevacizumab and AEs of Special Interest (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
- Serious AE reports that are unrelated to the Bevacizumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- Additional Reporting Requirements to Genentech include the following:
- Any reports of pregnancy following the start of administration with the Bevacizumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- All Non-serious Adverse Events originating from the Study will be forwarded in a quarterly report Genentech.

Note: Investigators should also report events to their IRB as required.

MEDWATCH 3500A REPORTING GUIDELINES

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

FOLLOW-UP INFORMATION

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

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm

RANDOMIZATION CODES FOR BLINDED CLINICAL TRIALS

The blind will be broken for ADR reports that are Serious and Unexpected, unless otherwise agreed with applicable regulatory authorities.

All written IND Safety Reports submitted to the FDA by the Investigator must also be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-5288

And to the Site IRB:

Reference protocol section 11.3.4.

For questions related to safety reporting to Genentech, please contact Genentech Drug Safety:

Tel: (888) 835-2555

Fax: (650) 225-4682 OR (650) 225-5288

IND ANNUAL REPORTS (FOR SPONSOR-INVESTIGATOR ONLY)

Copies to Genentech:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-5288

Study Close-Out (For Sponsor-Investigator Only)

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to

Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study:

Avastin (bevacizumab) Protocols
Email: avastin-gsur@gene.com
Fax : 650-745-0978

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 225-4682

Alternate Fax No: (650) 225-5288

Genentech Study Number	ML28899
Principal Investigator	Kimmie Ng, MD MPH
Site Name	
Site Responsible Investigator	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

Important Notification for Participants on 11-436

IMPORTANT NOTICE FOR PROVIDERS Patient Name : _____ Patient Number : _____ **This person is enrolled in a clinical trial called:** Randomized, Double-Blind, Phase II Trial of Vitamin D Supplementation in Participants with Previously Untreated Metastatic Colorectal Cancer. **Please contact study physician prior to checking vitamin D levels, which could compromise the blinding of the study** **Please contact study physician before prescribing additional supplements, such as vitamin D, calcium, or multivitamin, while the patient is on study** **For questions or medical emergency contact the Study Doctor:** Name:_____ Phone Number : _____ (OVER)	**FRONT**
Information for the study participant: You should always keep this card with you and present to all medical doctors you meet during the course of the study. **Information for the treating provider:** Please contact the study doctor mentioned on the front of this card before starting medical treatment or changing a drug regimen.	**BACK**

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**11-436**
Protocol Name:	**Randomized, Double Blind, Phase II Trial of Vitamin D Supplementation in Patients with Previously Untreated Metastatic Colorectal Cancer.**
DFCI Site PI:	Kimmie Ng, MD
DFCI Research Nurse:	Christopher Graham, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	Vitamin D receptor (VDR) is expressed in colon cancer cells. Preclinical data supports the addition of Vitamin D to 5-FU/platinum based therapy for CRC. This study is comparing two different doses of Vitamin D in combination with standard treatment (FOLFOX and Bevacizumab) for advanced stage colorectal cancer. Vitamin D binds to the receptor in the cancer cells; it may stop cancer cells from growing abnormally & may cause cell death. Vitamin D may be beneficial in the treatment of colorectal cancer. Please refer to the protocol Schema & Study Design Section 1.1. Cycle = 14 Days (Section 5.0)
Dose Calc	Vitamin D 3 is administered as flat doses. There is no calculation needed. Bevacizumab, Oxaliplatin, Leucovorin & 5-FU are calculated per institutional standard. (Section 6.0)
Study Drug &Administration	**Vitamin D 3:** *Administration instructions are found in Section 5.2.1* *All site study personnel (except pharmacist) and all study participants will be blinded to the treatment arm assignment.* *Arm A & Arm B: One Arm receives a lower dose and one Arm receives a higher dose of Vitamin D 3* • For Cycle 1 only, Participants will take one capsule from each of two bottles. (two capsules total) • Capsules are taken orally, at the same time daily, for the first 14 days, without regard for food. • For Cycle 2 and beyond, Participants will take ONE capsule once daily. • Do not repeat vomited doses unless the entire capsule can be seen intact in the vomit. • Missed (forgotten), doses can be made up if it is within 12 hours of the regularly scheduled dose. **FOLFOX-Bevacizumab** *administration instructions are found in Section 5.2.2 Table 2. & Section 7.0* • Administered intravenously, Day 1 (+/- 7 days) of every two-week cycle per institutional standard, to all participants in both arms. • Criteria to treat for a new cycle is found in Section 5.2.2 • Pre-medications prior to 5-FU & Oxaliplatin administration is found in Section 5.2.2 • Sequence of Administration of Drugs in FOLFOX-Bevacizumab is found in Section 5.2.2 Administration of Bevacizumab prior to FOLFOX is intended as a **guideline**. Participating sites may use their Institutional Standards of care.(Section 5.2.2) • The Cycle 1 Day 1 dose of Bevacizumab may be held at the investigator's discretion. (Section 5.0)
Dose Modifications and Toxicity	• A new cycle of Vitamin D3 may not be administered if the serum calcium is > institutional ULN (correct if albumin is abnormal). (Section 5.2.1) • Up to a 4-week delay is allowed For Vitamin D3 in the initiation of a new cycle of treatment for resolution of hypercalcemia to ≤ grade 1. (Section 5.2.1) • If Vitamin D3 is held for toxicities, FOLFOX-bevacizumab may continue. (Section 5.2.1) • If FOLFOX is permanently discontinued, Bevacizumab may continue on Day 1 of every 2-week cycle and Vitamin D3 should continue. (Section 5.2.2) • Dose Mods for Chemotherapeutic agents are found in Section 6.0 • Dose Mods for Vitamin D3 are found in Section 6.2
Concom Meds	*Concomitant Medication and Supportive Care Guidelines are found in Section 5.4* • Loperamide administration should begin for symptoms of diarrhea/abdominal cramping anytime during a treatment cycle. The Maximum dose is 16mg in a 24 hour period. • Participants should avoid the cold, (drinks, water, air) which often exacerbates the neurotoxicity often seen with Oxaliplatin. Growth Factors are permitted. Guidance on their use is found in Section 5.4.
Req Data	• Study Calendar with assessments is found in Section 9.0 • Correlative Studies Blood Draws: (Section 5.1.5) • In order to maintain blinding, plasma 25(OH)D levels should not be routinely checked at screening or during the study by the treating investigator. Vitamin D levels will be assayed only as part of the research blood samples collected during the study. (Section 8.)

Charting Tips	Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record • If there is a discrepancy in the infusion time, delay in administration, or the infusion takes longer than is permitted by the guidelines of the protocol, please document the reason for the discrepancy in the medical record. Please be sure to also DOCUMENT any required observation periods, any additional vital signs, routes of administration, or injection sites

11-436 Vitamin D diary **Cycle 1 (Loading dose)**

Name: _____ **MRN: _____**

Start taking Vitamin D capsules on day 1 of Cycle 1 either before or after your chemotherapy.

Take (two) capsules at the same time each day, one from each bottle.

Take with or without food. If you vomit or miss a dose, record on the diary. Do not take another capsule until the next day unless you see the entire capsule in vomitus or you can take a missed dose within 12 hours of your usual scheduled dose.

Please return pill container (including any extra capsules) and this diary to study team at next visit.

Cycle 1 Day of Cycle	Date	Check box when Vitamin D is taken (1 capsule from each bottle)	Comments: (if dose is missed, please note why)
1		☐	
2		☐	
3		☐	
4		☐	
5		☐	
6		☐	
7		☐	
8		☐	
9		☐	
10		☐	
11		☐	
12		☐	
13		☐	
14		☐	

Patient Signature: _____ Date: _____

To Be Completed By Study Team:

Date Dispensed: _____

# of Caps Returned: _____

Provider notified of any missed doses or discrepancy: _____

_____

Reviewer Signature: _____Date: _____

11-436 Vitamin D diary

Name: _____ MRN: _____

Take one capsule at the same time each day. Take with or without food. If you vomit or miss a dose, record on the diary. Do not take another capsule until the next day unless you see entire capsule in vomitus or you can take a missed dose within 12 hours of your usual scheduled dose.

Please return pill container (including any extra capsules) and this diary to study team at next visit.

Cycle:	Date	Check box when Vitamin D is taken	Comments: (if dose is missed, please note why)
1		☐	
2		☐	
3		☐	
4		☐	
5		☐	
6		☐	
7		☐	
8		☐	
9		☐	
10		☐	
11		☐	
12		☐	
13		☐	
14		☐	
Cycle:			
1		☐	
2		☐	
3		☐	
4		☐	
5		☐	
6		☐	
7		☐	
8		☐	
9		☐	
10		☐	
11		☐	
12		☐	
13		☐	
14		☐	

Patient Signature: _____ Date: _____

To Be Completed By Study Team:

Date Dispensed: _____

# of Caps Returned: _____

Provider notified of any missed doses or discrepancy: _____

_____

Reviewer Signature: _____Date: _____