

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB/SWOG 80405

A PHASE III TRIAL OF IRINOTECAN/5-FU/LEUCOVORIN OR OXALIPLATIN/5-FU/LEUCOVORIN WITH BEVACIZUMAB, OR CETUXIMAB (C225), OR WITH THE COMBINATION OF BEVACIZUMAB AND CETUXIMAB FOR PATIENTS WITH UNTREATED METASTATIC ADENOCARCINOMA OF THE COLON OR RECTUM

Limited access companion study (for specified sites only): CALGB 70501

Investigational Agent: Cetuximab (C225) NSC #714692, CALGB-sponsored IND BB 11264, will be supplied by Lilly and distributed by Lilly

<input checked="" type="checkbox"/> Update:	<input type="checkbox"/> Status Change:
<input type="checkbox"/> Eligibility changes	<input type="checkbox"/> Activation
<input type="checkbox"/> Therapy / Dose Modifications / Study Calendar changes	<input type="checkbox"/> Closure
<input checked="" type="checkbox"/> Informed Consent changes	<input type="checkbox"/> Suspension / temporary closure
<input type="checkbox"/> Scientific / Statistical Considerations changes	<input type="checkbox"/> Reactivation
<input checked="" type="checkbox"/> Data Submission / Forms changes	
<input checked="" type="checkbox"/> Editorial / Administrative changes	
<input type="checkbox"/> Other :	

IRB approval (or disapproval) is required within 90 days. Expedited review is allowed. Please follow your local IRB guidelines

UPDATES TO THE PROTOCOL:

Cover Page (Page 1)

- Underneath the study title in the “Investigational Agent” line, references to BMS and Aptuit INC. (US) and Fisher-Acculogix (Canada) have been updated to Lilly, as Eli Lilly and Company will now handle supply and distribution of Cetuximab (C225). It now reads: “Investigational Agent: Cetuximab (C225) NSC #714692, CALGB-sponsored IND BB 11264, will be supplied by Lilly and distributed by Lilly.”
- Jennifer Warner has replaced Kathleen McEwen as the data coordinator. Contact information has been updated.

Cover Page (Page 2)

- The address to the Alliance Protocol Operations Program Office has been updated.
- As data management of CALGB/SWOG 80405 has moved from Duke University to Mayo, the contact information for the Alliance Statistics and Data Center at Duke University has been replaced with the contact information for the Alliance Statistics and Data Center at Mayo.

Section 5.5 Data Submission

All data should now be submitted to the Alliance Statistics and Data Center at Mayo Clinic. Therefore this section has updated instructions on how and where to submit case report forms for this study.

Section 11.9 Cetuximab (C-225)

Drug supply and distribution will now be handled by Eli Lilly and Company. Therefore, the Availability, Drug Ordering and Accountability, and Important Reorder Instructions subsections have been updated accordingly.

Appendix I Cancer Trials Support Unit (CTSU) Participation Procedures

- In the CTSU Address and Contact Information table, instructions on data submission procedures have been updated.
- In the “Drug Procurement” section, “Bristol-Myers Squibb” has been updated to “Eli Lilly and Company.”

UPDATES TO THE MODEL CONSENT:

References to “CALGB” and “Cancer and Leukemia Group B” outside of the study title throughout the document have been updated to “Alliance” and “Alliance for Clinical Trials in Oncology,” respectively.

Will my medical information be kept private?

- In the bullet point of the second paragraph of this section that names Bristol-Meyers Squibb, the word “former” has been inserted as follows: “Bristol-Meyers Squibb pharmaceutical company, the former makers of cetuximab.”
- Also, the following text has been added as the last bullet point: “Eli Lilly and Company, the makers of cetuximab.”

What are the costs of taking part in this study?

In the first sentence of the second paragraph of this section, “Bristo-Meyers Squibb” has been updated to “Eli Lilly and Company.” It now reads: “Eli Lilly and Company is supplying the cetuximab at no cost to you.”

Safeguards of Confidentiality in Studies Involving Genes (genetic studies)

- Throughout the first bullet point, references to the “The Cancer and Leukemia Group B Statistical Center” has been updated to the “Alliance for Clinical Trials in Oncology Statistics and Data Center at Mayo.”
- In the second bullet point, the reference to the “Cancer and Leukemia Group B Statistical Center at Duke University” has been updated to the “Alliance for Clinical Trials in Oncology Statistics and Data Center at Mayo.”

**Replacement protocol and informed consent documents have been issued.
This study remains closed to new patient accrual.**

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

CALGB/SWOG C80405

A PHASE III TRIAL OF IRINOTECAN/5-FU/LEUCOVORIN OR OXALIPLATIN/5-FU/LEUCOVORIN WITH BEVACIZUMAB, OR CETUXIMAB (C225), OR WITH THE COMBINATION OF BEVACIZUMAB AND CETUXIMAB FOR PATIENTS WITH UNTREATED METASTATIC ADENOCARCINOMA OF THE COLON OR RECTUM

Limited access companion study (for specified sites only): CALGB 70501

Investigational Agent: Cetuximab (C225) NSC #714692, CALGB-sponsored IND BB 11264, will be supplied by Lilly and distributed by Lilly

Alliance GI Committee and Study Chair

Alan Venook, M.D.
Univ. of California, San Francisco
1600 Divisadero Box 1705
San Francisco, CA 94115
Tel: 415-353-2745
Fax: 415-353-9959
venook@cc.ucsf.edu

Alliance Study Co-Chair

Bert H. O'Neil, MD
IU Simon Cancer Center
535 Barnhill Dr., Rm 130D
Indianapolis, IN 46077
Tel: 317-278-8845
Fax: 317-274-0396
bhoneil@iu.edu

SWOG Study Co-Chair

Heinz-Josef Lenz, M.D.
Univ. of Southern California
1520 San Pablo St. Suite 1000
Los Angeles, CA 90033
Tel: 323-865-3955
lenz_h@ccnt.hsc.usc.edu

SWOG Corr. Sci. Chair

Heinz Josef-Lenz, MD
Univ. of Southern California
Tel: 323-865-3955
lenz_h@ccnt.hsc.usc.edu

Alliance Corr. Sci. Co-Chair

Monica Bertagnolli, MD
Brigham and Women's Hosp.
Tel: 617-732-8910
mbertagnolli@partners.org

PET Co-Chair

Howard McLeod, PharmD
University of North Carolina
Tel: 919-966-0512
hmcLeod@unc.edu

QOL Co-Chair

Michelle Naughton, PhD
Wake Forest University
Tel: 336-716-2918
naughton@wfubmc.edu

Diet and Lifestyle Co-chair

Jeffrey Meyerhardt, MD
Dana Farber Cancer Inst.
Tel: 617-632-5136
jeffrey_meyerhardt@dfci.harvard.edu

Alliance Primary Statistician (GI)

Donna Niedzwiecki, PhD
Tel: 919-681-5030
Fax: 919-681-8028
donna.niedzwiecki@duke.edu

Alliance Primary Statistician (QOL)

Xiaofei Wang, PhD
Tel: 919-681-5406
Fax: 919-681-8028
xiaofei.wang@duke.edu

Alliance Primary Statistician (PET)

Kouros Owzar, PhD
Tel: 919-681-1829
Fax : 919-681-8028
kouros.owzar@duke.edu

SWOG Primary Statistician

Katherine A. Guthrie, PhD
Tel: 206-667-5595
Fax: 206-667-4408
kguthrie@fhcrc.org

Secondary Statistician

Xing (Cynthia) Ye, MS
Tel: 919-681-2044
Fax: 919-681-8028
xing.ye@duke.edu

Data Coordinator

Jennifer Warner
Tel: 507-538-1484
Fax: 507-284-1902
warner.jennifer4@mayo.edu

Protocol Coordinator

Vance N. Erese, MAS
Tel: 773-834-4518
Fax: 312-345-0117
verese@uchicago.edu

Participating Organizations

ALLIANCE / Alliance for Clinical Trials in Oncology; **SWOG** / SWOG; **NRG** / NRG Oncology
ECOG-ACRIN / ECOG-ACRIN Cancer Research Group; **NCIC-CTG** / NCIC Clinical Trials Research Group

<p>Alliance Protocol Operations Program Office 125 S. Wacker Dr., Suite 1600 Chicago, IL 60606-4422 Tel: 773-702-9171 Fax: 312-345-0117 www.allianceforclinicaltrialsinoncology.org</p> <p>Adverse Event Reporting https://eapps-ctep.nci.nih.gov/ctepaers</p> <p>Alliance Biorepository at Ohio State University Innovation Centre 2001 Polaris Parkway Columbus, OH 43240 Tel: 614-293-7073 Fax: 614-293-7967 path.calgb@osumc.edu</p>	<p>Alliance Statistics and Data Center Mayo Clinic 200 First St. SW Rochester MN 55905</p> <p>Alliance Patient Registration Tel: 507-284-4130 Fax: 507-284-0885</p> <p>CALGB 80405 Pharmacy Contact TBN</p> <p>CALGB 80405 Nursing Contact TBN</p>
--	--

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Institutions not aligned with the Alliance will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix.

- The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at <https://members.ctsu.org>
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to the CTSU logistical appendix for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to the CTSU logistical appendix for specific instructions and forms to be submitted.
- Data management will be performed by the Alliance. **Case report forms** (with the exception of patient enrollment forms), **clinical reports, and transmittals** must be sent to Alliance unless otherwise directed by the protocol. Do not send study data or case report forms to the CTSU Data Operations.
- **Data query and delinquency reports** will be sent directly to the enrolling site by Alliance. (generally via email but may be sent via fax or postal mail). Please send query responses and delinquent data to Alliance and do not copy the CTSU Data Operations. Query responses should be sent to Alliance via postal mail or fax (no transmittal form needs to accompany response). Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP AMS account contact information current. This will ensure timely communication between the clinical site and the Alliance Statistical Center.

Participating Organization Contacts:

ECOG-ACRIN

Howard Hochster, MD
Tel: 212-731-5100

howard.hochster@med.nyu.edu

ECOG-ACRIN

Coordinating Office

Ryan Palmer
617-632-3610

palmer.ryan@jimmy.harvard.edu

The pharmacogenomic component of this study is conducted as part of the NIH Pharmacogenomics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/pharmacogenomics/research_net.html for details)

A PHASE III TRIAL OF IRINOTECAN/5-FU/LEUCOVORIN OR OXALIPLATIN/5-FU/LEUCOVORIN WITH BEVACIZUMAB, OR CETUXIMAB (C225), OR WITH THE COMBINATION OF BEVACIZUMAB AND CETUXIMAB FOR PATIENTS WITH UNTREATED METASTATIC ADENOCARCINOMA OF THE COLON OR RECTUM

Patient Eligibility

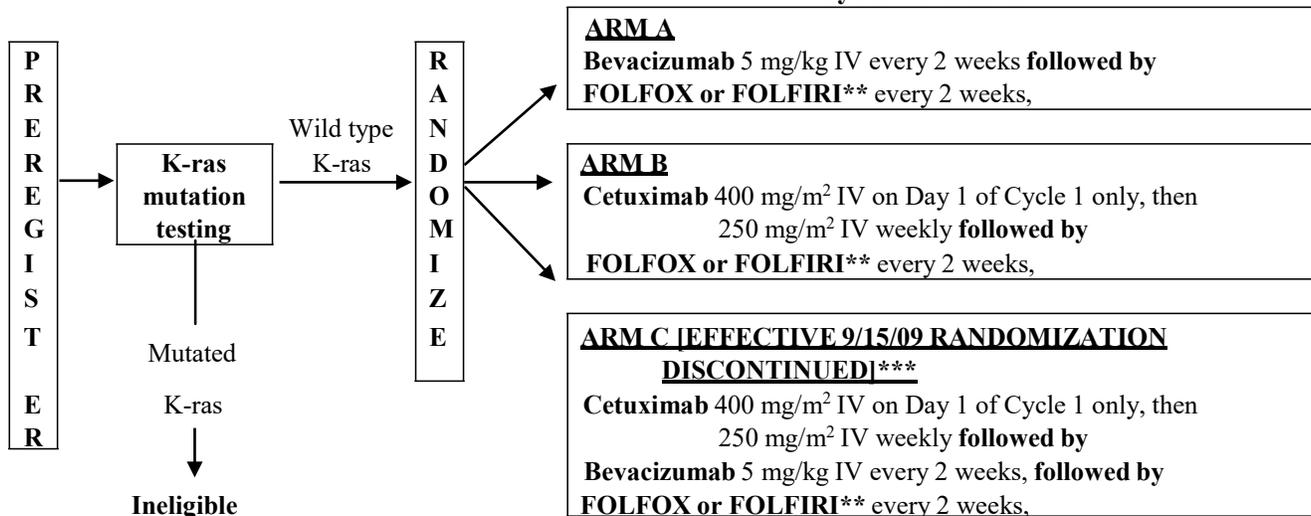
Histologically or cytologically documented locally advanced or metastatic adenocarcinoma of the colon or rectum (see §4.1)
 Only patients with a wildtype K-ras gene are eligible (see §4.2)
 No prior systemic treatment for advanced or metastatic colorectal cancer (see §4.3.1)
 - No prior radiotherapy to > 25% of bone marrow (see §4.3.2)
 - ≥ 4 weeks since major surgery (see §4.3.3)
 No previous or concurrent malignancy (see §4.4.1)
 For FOLFIRI patients: No evidence of Gilbert’s syndrome or of homozygosity for the UGT1A1*28 allele (see §4.4.2)
 No ≥ grade 2 sensory peripheral neuropathy for FOLFOX patients
 No known central nervous system metastases or carcinomatous meningitis
 No interstitial pneumonia or extensive and symptomatic interstitial fibrosis of the lung
 No pleural effusion or ascites that causes ≥ grade 2 dyspnea
 No predisposing colonic or small bowel disorders in which the symptoms are uncontrolled (see §4.4.7)
 No uncontrolled seizure disorder or active neurological disease
 No current congestive heart failure; hypertension must be well controlled (<160/90); and patients on full-dose anticoagulants must be on a stable dose of warfarin and have an in-range INR or be on a stable dose of LMW heparin (see §4.4.9 - 4.4.11)
 No significant history of bleeding events or GI perforation; no recent (within 6 months) arterial thrombotic events; and no serious or non-healing wound, ulcer or bone fracture (see §4.4.12 – 4.4.14)
 No known hypersensitivity to Chinese hamster ovary cell products or to recombinant human or murine antibodies (see §4.4.15)
 Not pregnant and not nursing (see §4.5)
 ECOG Performance Status: 0-1
 Age ≥ 18

Required Initial Laboratory Values	
Granulocytes	≥ 1500/μL
Hemoglobin	≥ 9 grams/dL
Platelet Count	≥ 100,000/μL
Creatinine	≤ 1.5 x ULN
Bilirubin	≤ 1.5 x mg/dL
Albumin	≥ 2.5 g/dL
Urinalysis	≤ 1+ protein*

* See [Section 4.7](#)

SCHEMA*

1 Cycle = 8 Weeks



Continue treatment until disease progression or unacceptable toxicity (see Sec. 7.1).

* See [Section 7.0](#) for complete treatment details.

** The decision to use either FOLFOX or FOLFIRI is at the patient/treating physician’s discretion, but must be declared prior to randomization and must not be changed during the course of the patient’s treatment.

*** **Prior to 9/15/09, patients were randomized to Arms A, B, and C; effective with the issuance of Update #6 to this protocol, patients are to be randomized to Arms A and B only.**

TABLE OF CONTENTS

1.0	INTRODUCTION.....	8
1.1	BACKGROUND INFORMATION ON 5-FU AND LEUCOVORIN	9
1.2	BACKGROUND INFORMATION ON IRINOTECAN.....	9
1.3	BACKGROUND INFORMATION ON OXALIPLATIN	9
1.4	BACKGROUND INFORMATION ON BEVACIZUMAB.....	10
1.5	BACKGROUND INFORMATION ON CETUXIMAB.....	10
1.6	STUDIES OF IRINOTECAN, 5-FU AND LV (FOLFIRI & IFL).....	10
1.7	STUDIES ON OXAL, 5-FU AND LV (FOLFOX).....	11
1.8	STUDIES WITH BEVACIZUMAB COMBINATIONS	12
1.9	STUDIES WITH CETUXIMAB COMBINATIONS.....	13
1.10	STUDIES OF FOLFOX/FOLFIRI + BEVACIZUMAB/CETUXIMAB COMBINATIONS.....	13
1.11	2009 CLINICAL TRIALS RESULTS: COMBINATION STUDIES	14
1.12	INCLUSION OF WOMEN AND MINORITIES.....	14
2.0	OBJECTIVES	14
2.1	PRIMARY OBJECTIVE.....	14
2.2	SECONDARY OBJECTIVES	14
3.0	ON-STUDY GUIDELINES.....	15
4.0	ELIGIBILITY CRITERIA	15
4.1	LOCALLY ADVANCED OR METASTATIC COLORECTAL CANCER.....	15
4.2	K-RAS STATUS.....	16
4.3	PRIOR TREATMENT	16
4.4	PATIENT HISTORY	17
4.5	NON-PREGNANT AND NOT NURSING.	18
4.6	AGE AND PERFORMANCE STATUS	18
4.7	REQUIRED INITIAL LABORATORY VALUES:	18
5.0	REGISTRATION/RANDOMIZATION, STRATIFICATION, AND DATA AND SAMPLE SUBMISSION	19
5.1	RANDOMIZATION REQUIREMENTS	19
5.2	CALGB PRE-REGISTRATION AND RANDOMIZATION PROCEDURES	19
5.3	REGISTRATION TO COMPANION STUDIES.....	21
5.4	STRATIFICATION FACTORS	22
5.5	DATA SUBMISSION	22
5.6	SPECIMEN SUBMISSION FOR K-RAS TESTING AND CORRELATIVE STUDIES	24
5.7	SUBMISSION OF QUALITY OF LIFE AND HEALTH SERVICES MEASURES.....	29
5.8	SUBMISSION OF DIET AND LIFESTYLE SURVEYS.....	30
6.0	REQUIRED DATA.....	31
7.0	TREATMENT PLAN.....	32
7.1	PATIENTS SHOULD RECEIVE A MINIMUM OF TWO CYCLES OF THERAPY.....	32
7.2	BEVACIZUMAB (ARMS A AND C)	33
7.3	CETUXIMAB (ARMS B AND C).....	33
7.4	CHEMOTHERAPY.....	33
7.5	CONCURRENT MEDICATIONS	34
8.0	DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY.....	34

8.1	DOSE LEVELS.....	34
8.2	HEMATOLOGIC TOXICITIES.....	35
8.3	GASTROINTESTINAL TOXICITIES:.....	36
8.4	PULMONARY TOXICITY	37
8.5	NEUROTOXICITY	37
8.6	EXTRAVASATION.....	38
8.7	HYPOMAGNESEMIA	38
8.8	BEVACIZUMAB DOSE MODIFICATIONS FOR HYPERTENSION	38
8.9	BEVACIZUMAB/PLACEBO DOSE MODIFICATIONS FOR REVERSIBLE POSTERIOR LEUKOENCEPHALOPATHY SYNDROME (RPLS)	38
8.10	DOSE MODIFICATIONS FOR CARDIOVASCULAR TOXICITIES:.....	39
8.11	BEVACIZUMAB DOSE MODIFICATIONS FOR HEMORRHAGE/BLEEDING.....	39
8.12	BEVACIZUMAB DOSE MODIFICATIONS FOR PROTEINURIA	40
8.13	OTHER BEVACIZUMAB TOXICITIES	40
8.14	CETUXIMAB DOSE MODIFICATIONS FOR CUTANEOUS TOXICITY.....	40
8.15	HYPERSENSITIVITY AND INFUSION REACTIONS	41
8.16	OTHER NON-HEMATOLOGIC TOXICITIES	42
8.17	DOSE MODIFICATION FOR OBESE PATIENTS	42
9.0	QUALITY OF LIFE AND HEALTH SERVICES COMPANION STUDIES	42
9.1	INTRODUCTION	42
9.2	OVERVIEW OF PRESCRIPTION DRUG COMPONENT	43
9.3	OVERVIEW OF PHARMACOECONOMIC COMPONENT	44
9.4	HRQL, PRESCRIPTION DRUG, AND PHARMACOECONOMIC HYPOTHESES	45
9.5	HRQL AND PRESCRIPTION DRUG COMPONENT ASSESSMENT INSTRUMENTS	46
9.6	DATA COLLECTION.....	47
10.0	DIET AND LIFESTYLE COMPONENT.....	48
10.1	INTRODUCTION	48
10.2	OBJECTIVES.....	48
10.3	BACKGROUND.....	48
10.4	HYPOTHESES.....	51
10.5	METHODS.....	52
11.0	DRUG FORMULATION, AVAILABILITY AND PREPARATION.....	54
11.1	QUALIFIED PERSONNEL	54
11.2	DRUG DISPOSITION	54
11.3	DRUG DOSING GUIDELINES	54
11.4	OXALIPLATIN [ELOXATIN].....	54
11.5	5-FLUOROURACIL (5-FU; FLUOROURACIL; ADRUCIL [®])	56
11.6	LEUCOVORIN CALCIUM (FOLINIC ACID) LEUCOVORIN CALCIUM (CALCIUM FOLINATE; CITROVORUM FACTOR; N 5-FORMYLTETRAHYDROFOLATE; 5-FORMYL-FH4; FOLINIC ACID).....	57
11.7	IRINOTECAN (CPT-11, CAMPTOSAR [®]).....	57
11.8	BEVACIZUMAB (RHUMAB VEGF, AVASTIN [®]) (NSC #704865)	58
11.9	CETUXIMAB (C-225).....	60
12.0	ANCILLARY THERAPY	63
12.1	SUPPORTIVE CARE	63
12.2	HORMONAL/OTHER CHEMOTHERAPEUTIC AGENTS.....	63
12.3	PALLIATIVE RADIATION THERAPY	63
12.4	CALGB 80405 POLICY CONCERNING THE USE OF GROWTH FACTORS	63

12.5	ANTIEMETICS	63
12.6	ANTICOAGULANTS	64
12.7	ACNE.....	64
13.0	CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE (SOLID TUMORS):..	64
13.1	TARGET LESIONS	64
13.2	NON-TARGET LESIONS	65
13.3	CYTOLOGY AND HISTOLOGY	65
13.4	EVALUATION OF BEST OVERALL RESPONSE	65
13.5	GUIDELINES FOR EVALUATION OF MEASURABLE DISEASE	66
13.6	CONFIRMATION MEASUREMENT/DURATION OF RESPONSE	66
14.0	REMOVAL OF PATIENTS FROM PROTOCOL THERAPY	67
14.1	CR, PR, OR SD	67
14.2	SURGICAL RESECTION	67
14.3	DISEASE PROGRESSION.....	67
14.4	EXTRAORDINARY MEDICAL CIRCUMSTANCES	67
15.0	STATISTICAL CONSIDERATIONS.....	67
15.1	STUDY DESIGN AND HYPOTHESES.....	67
15.2	PRIMARY HYPOTHESIS.....	68
15.3	SECONDARY HYPOTHESES.....	68
15.4	STATISTICAL TESTS OF PRIMARY HYPOTHESIS	68
15.5	STATISTICAL TESTS OF SECONDARY HYPOTHESES	70
15.6	TOXICITY MONITORING.....	71
15.7	INTERIM ANALYSIS	71
15.8	ACCRUAL, FOLLOW-UP, AND EARLY CLOSURE OF TREATMENT ARM(S).....	72
15.9	STATISTICAL CONSIDERATIONS FOR THE QUALITY OF LIFE AND PRESCRIPTION SUBSTUDY ..	72
15.10	STATISTICAL CONSIDERATIONS FOR THE DIET AND LIFESTYLE SUBSTUDY	76
16.0	ADVERSE EVENT REPORTING (AER).....	78
16.1	CALGB/SWOG 80405 REPORTING REQUIREMENTS FOR ALL ARMS OF THE STUDY:.....	78
16.2	ADDITIONAL INSTRUCTIONS OR EXCLUSIONS FROM CTEP-AERS EXPEDITED REPORTING REQUIREMENTS FOR PHASE 2 AND 3 TRIALS UTILIZING AN AGENT UNDER A CTEP-IND OR NON-CTEP IND:	79
16.3	COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LIST (CAEPR) FOR BEVACIZUMAB (RHUMAB VEGF, NSC 704865).....	80
16.4	COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LIST (CAEPR) FOR CETUXIMAB (NSC 714692).....	86
16.5	COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LIST (CAEPR) FOR OXALIPLATIN (NSC 266046).....	89
17.0	REFERENCES	96
	APPENDIX I	104
	INTRODUCTION.....	111
	APPENDIX II.....	112
	APPENDIX III	132
	APPENDIX IV	163
	APPENDIX V	171

APPENDIX VI 183
APPENDIX VII..... 197

1.0 INTRODUCTION

Colorectal cancer is the second-leading cause of cancer death in this country and has historically been refractory to most chemotherapeutics. Over the past five years, the introduction of irinotecan and oxaliplatin have enabled patients with advanced colorectal cancer to live longer than before, with a median survival for these patients of 16-18 months. These drugs, along with 5-fluorouracil (5-FU), are now commercially available and represent the conventional chemotherapeutic armamentarium for treating colorectal cancer.

5-FU is commonly administered with leucovorin (LV), which is a modulator of 5-FU activity. Irinotecan is active in advanced disease and was first approved by the FDA for patients with colorectal cancer refractory to 5-FU/LV. Approval was initially based on a response rate of about 15%, then confirmed with a randomized study showing improved survival for patients refractory to 5-FU who were treated with irinotecan compared to best supportive care [1]. Two combinations of irinotecan/5-fluorouracil/leucovorin have been compared to 5-FU/LV regimens, one with bolus 5FU/LV (IFL) [2] and another with continuous infusion 5FU (FOLFIRI) [3]. In both studies, the irinotecan containing combinations conferred a survival advantage, leading to the identification of these regimens as the standard front-line treatment for patients with metastatic colorectal cancer. The bolus 5FU/LV and irinotecan combination (IFL) has been favored in the U.S., although the IFL regimen appears to be more toxic than the FOLFIRI regimen, and the general consensus is that FOLFIRI merits further testing in the U.S. Oxaliplatin used in combination with infusional 5-FU has been approved by the FDA for patients with advanced colorectal cancer who have progressed following treatment with IFL; oxaliplatin as a single agent is much less active than in combination with 5-FU [4]. Oxaliplatin has been studied most extensively with 5FU regimens (FOLFOX) [5]. FOLFOX regimens (numbered 1-7) all incorporate similar treatment backbones: oxaliplatin infusion over 120 minutes followed by a variety of bolus and infusional 5-FU regimens. Oxaliplatin with 5-FU/LV (FOLFOX) is approved as first and second-line therapy in the U.S., and comparative studies suggest that FOLFOX is superior to the IFL combination in untreated patients [6]. In Europe, FOLFOX and FOLFIRI have been compared to each other, used sequentially [7], in the management of patients with advanced colorectal cancer, and there does not appear to be a significant difference in outcome based on the sequence of administration, although the toxicity profiles are dissimilar. The treatments have not been compared head-to-head in the U.S.

Bevacizumab (Avastin) is a recombinant humanized version of a murine anti-human VEGF monoclonal antibody. Bevacizumab has been clinically tested as a single agent and with cytotoxic chemotherapy in treatment of a number of metastatic solid tumor types. It has been approved for use in untreated patients with advanced colorectal cancer in combination with fluorouracil-based chemotherapy. Several trials have established the efficacy of bevacizumab in combination with chemotherapy, with the most dramatic result coming in first-line combination with IFL compared to IFL alone [8]. In that study, a survival advantage of more than four months was seen in the bevacizumab-treated patients. Bevacizumab also appears to improve survival when combined with FOLFOX following progression on IFL (ASCO, 2005). Bevacizumab does not appear to alter the toxicity profile of chemotherapy, although hypertension and proteinuria are commonly seen. Approximately 3-4% of patients receiving bevacizumab suffer arterial thrombotic events.

Cetuximab (C225) is a chimerized monoclonal antibody to the epidermal growth factor- receptor (EGFR). Approximately 70% of colorectal cancers express EGFR. As a single agent, cetuximab produces tumor regression in about 10% of EGFR (+), previously treated colorectal cancer patients [9]. Pre-clinical evidence suggests that inhibitors of EGFR can interact with chemotherapy, either increasing cytotoxicity or rendering resistant cancer cells sensitive to the same therapy [10]. Clinical trials have shown that the combination of cetuximab with irinotecan leads to tumor regression in about 20% of EGFR (+) patients who have demonstrated resistance to irinotecan chemotherapy [9]. The addition of cetuximab to irinotecan as a single agent did not cause any difference in irinotecan

toxicity although the use of cetuximab was accompanied by an acneiform skin rash in about 30% of patients.

The combination of cetuximab and bevacizumab has been tested in a pilot trial [11]. The study explored the safety and efficacy of the antibody combination with or without irinotecan in patients with advanced colorectal cancer who had previously progressed on irinotecan. None of these patients had received prior bevacizumab or cetuximab. The combination treatment was found to be well tolerated; with no evidence of toxicity greater than expected from either treatment individually. As to efficacy, response rates in the dual biologic/irinotecan arm were 38%; and in the dual biologic arm, were 23%. Time to tumor progression also appeared to be better than historical controls. The prospect of the combination of standard front-line chemotherapy with two antibodies targeting different pathways is very scientifically appealing. As noted above, oxaliplatin or irinotecan with fluorouracil and either bevacizumab or cetuximab have been tested in various combinations. Irinotecan plus cetuximab has been shown superior to irinotecan alone in second or third-line treatment [12]; FOLFOX plus bevacizumab is superior to FOLFOX alone in patients following progression on IFL.

This study will allow the treating physician to choose FOLFOX or FOLFIRI as the chemotherapy for patients. The control arm is the combination of FOLFIRI or FOLFOX with bevacizumab. This study will explore the possibility that the addition of cetuximab to either of the chemotherapy combinations or to the chemotherapy and bevacizumab combinations will lead to a superior outcome compared to the chemotherapy with bevacizumab. Correlative studies will attempt to identify predictors of response to these therapies and will also look to confirm the reported 70% incidence of EGFR positivity in patients with advanced colorectal cancer.

1.1 Background information on 5-FU and leucovorin

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.

5-FU and LV have formed the backbone of colorectal cancer treatment for more than the past decade, although the optimal treatment schedule remains open to discussion. 5-FU has a short half-life; so 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 to 48 hours.

1.2 Background information on irinotecan

Irinotecan is converted by carboxylesterase to its more active metabolite, SN-38. It works principally by binding and stabilizing topoisomerase I as it uncoils DNA during cell division; this complex of inhibitor-enzyme-DNA halts advancing replication forks, resulting in double-stranded DNA breaks and consequent apoptosis.

The mean-terminal half-life of SN-38 is longer than that for irinotecan, 11.5 hours compared to 6.8 hours. The time to peak concentration is highly variable, occurring 30 to 90 minutes after the end of infusion [13]. SN-38 is detoxified by glucuronide conjugation, which is a critical step in protecting against irinotecan-induced gastrointestinal toxicity. There is strong evidence that patients with defects in glucuronidation, such as those with Gilbert's Syndrome, may suffer severe irinotecan toxicity [14] and that this may more specifically be related to certain hepatic enzyme polymorphisms, such as UGT1A1*28 [15].

1.3 Background information on oxaliplatin

Oxaliplatin is a novel antineoplastic platinum derivative with a 1,2-diaminocyclohexane carrier ligand. Although the precise mechanism of action is unknown, oxaliplatin is thought to exert its

cytotoxic effects through the formation of DNA adducts. 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.

1.4 Background information on bevacizumab

There is compelling evidence linking tumor growth with angiogenesis. More specifically, microvessel counts in colorectal cancer specimens are correlated with size, metastases, and survival. Vascular endothelial growth factor (VEGF; also known as vascular permeability factor) is the most potent of the identified angiogenic factors, and has been identified as a crucial regulator of pathologic angiogenesis. VEGF induces endothelial cell mitogenesis/migration and production of proteinases, leading to remodeling of the extracellular matrix, increased vascular permeability, and enhanced survival of newly formed blood vessels. Increased expression of VEGF has been measured in many, if not most, human tumors examined to date, including cancers of the lung, breast, thyroid, gastrointestinal tract, kidney, bladder, ovary, and cervix, as well as angiosarcomas and glioblastomas. Intense expression of VEGF mRNA has been detected in human liver metastases from colorectal cancer.

Inhibition of VEGF using an anti-VEGF monoclonal antibody blocks the growth of a number of human cancer cell lines in nude mice, including the colorectal cancer types LS174T, HM-7, and LSLiM6. Anti-VEGF antibody and chemotherapy in combination resulted in an increased antitumor effect compared with antibody or chemotherapy treatment alone, in nude mice injected with human cancer xenografts.

Bevacizumab is a recombinant humanized version of a murine anti-human VEGF monoclonal antibody. It has been clinically tested as a single agent and with cytotoxic chemotherapy in treatment of a number of metastatic solid tumor types.

1.5 Background Information on cetuximab

Cetuximab is a recombinant DNA-derived, chimerized monoclonal antibody that was engineered by attaching the murine Fv regions of M225 (a murine antibody developed to block the ligand binding site of EGFR) to a human immunoglobulin G constant region segment. The resulting functional antibody is comprised of two heavy chains (gamma) and two light chains (kappa) with a molecular weight of 152 kD and has binding specificity identical to M225. Cetuximab binds to the extracellular region of the human EGFR and functions as a competitive antagonist to inhibit ligand binding to the EGFR. The binding affinity of cetuximab to the EGFR is greater than the natural ligand EGF and TGF- α and higher than the original M225 antibody; its increased affinity is due to a lower dissociation constant. By competing for ligand binding, cetuximab inhibits ligand-induced tyrosine kinase-dependent phosphorylation and downstream signaling of the receptor. Also, cetuximab binding induces internalization and degradation of the EGFR, leading to a reduction in cell surface receptor available for ligand stimulation and activation of the EGFR signaling pathway.

1.6 Studies of irinotecan, 5-FU and LV (FOLFIRI & IFL)

Irinotecan is active in advanced colorectal cancer and was first approved by the FDA for patients with colorectal cancer refractory to 5-FU/LV. Approval was initially based on a response rate of about 15%. A randomized study showing improved survival for patients refractory to 5-FU treated with irinotecan compared to best supportive care was later conducted; this study also confirmed a maintenance of quality of life in the irinotecan treated patients [1]. The principal dose-limiting toxicity of irinotecan is diarrhea, which can be ameliorated with the aggressive use of loperamide. Leukopenia is also seen with irinotecan.

5-FU is commonly administered with leucovorin (LV), which is a modulator of 5-FU activity. Many studies of various schedules of this combination have been published. No single study has demonstrated a clear response or toxicity difference between bolus or infusional schedules, although a meta-analysis would appear to favor the use of infusional compared to bolus schedules [16]. The principal toxicities of 5-FU depend upon the schedule of administration, with diarrhea, stomatitis and leukopenia being significant in the bolus schedules and hand-foot syndrome and diarrhea being prominent in the infusional schedules.

Because of their differing mechanisms of action but somewhat overlapping toxicities, substantial effort went into combining these therapies. Two regimens of irinotecan/5-fluorouracil/leucovorin were developed in phase I studies, one with bolus 5FU/LV [17] and another with continuous infusion 5FU (FOLFIRI) [3].

The bolus IFL regimen was developed in a phase I study by adding various doses of 5-FU and LV to a fixed dose of irinotecan. By this method, the IFL regimen of irinotecan 125 mg/m² with bolus 5-FU 500 mg/m² and LV 20 mg/m² given four consecutive weeks followed by a two-week rest was chosen to go forward into further studies. IFL was then compared to the standard 5-day bolus 5-FU/LV combination known as the Mayo Clinic regimen [18] in patients with untreated, advanced colorectal cancer [2]. The results of this 683 patient three-arm randomized trial (a third arm consisted of single agent irinotecan) showed a median survival of 14.8 vs. 12.6 months ($p = 0.04$) in favor of IFL compared to the 5-FU/LV treatment arm; progression-free survival and response rate also favored the IFL group. Diarrhea was more common in the IFL arm but there was no apparent difference among the arms in grade 4 toxicity.

Two FOLFIRI regimens were being studied at the same time in Europe. The regimens were identified in phase I testing: irinotecan 80 mg/m² with 5-FU 2300 mg/m² by 24-hour infusion plus LV 500 mg/m² weekly; or irinotecan 180 mg/m² on day 1 with 5-FU 400 mg/m² bolus and 600 mg/m² by 22-hour infusion plus LV 200 mg/m² on days 1 and 2 every other week. Each of these combinations was then compared in a randomized trial to their 5-FU/LV schedule counterpart for patients with untreated advanced colorectal cancer [3]. As in the trial cited above, the 387 patient trial also showed a response and survival advantage for the irinotecan-containing combinations; median survival was 17.4 versus 14.1 months ($p = 0.031$.) The irinotecan group had more diarrhea and neutropenia but despite this toxicity, the accompanying quality of life analysis suggested an improvement for the patients in the irinotecan group.

The results of both studies, demonstrating that the irinotecan/5-FU/LV combinations conferred a survival advantage, lead to the approval by the FDA of these irinotecan containing regimens as the front-line treatment options and the standards against which other therapies would be compared for patients with metastatic colorectal cancer. IFL and FOLFIRI remain standard treatments, although increasing clinical experience and a subsequent interim analysis of intergroup study N9741 [19] led to suggestions that IFL may be more toxic than initially appreciated. A rigorous analysis of IFL-related treatment deaths suggests

the bolus regimen is manageable [20], although a general sense favoring FOLFIRI is emerging in the cancer community.

The FOLFIRI regimen used in this study is a variation on the Douillard regimen, involving only one bolus 5-FU and LV treatment and a 46-48 hour 5-FU infusion at 2400 mg/m². This is the FOLFIRI combination studied in a recent comparative trial in Europe assessing FOLFOX and FOLFIRI [21]. It is being proposed as the treatment in this study because of the relative ease of administering just one bolus, mandating one fewer visit to the physician.

1.7 Studies on OXAL, 5-FU and LV (FOLFOX)

OXAL has been studied extensively in pre-clinical models and appears to interact with many different chemotherapeutics [22]. Oxaliplatin has been commercially available for many years

in Europe. It has been apparent since its introduction that, as a single agent, oxaliplatin has only minimal clinical activity for patients with advanced colorectal cancer, with a response rate of about 10%. In combination with 5-FU/LV, however, significant anti-tumor activity in previously untreated patients with colorectal cancer has been demonstrated [23]. The main toxicities of oxaliplatin consist of an acute neuropathy manifesting as a cold-induced sensory neuropathy as well as a chronic peripheral sensory neuropathy that appears to be dose-related.

The anti-tumor activity interaction between oxaliplatin and 5-FU/LV remains unexplained, but the clinical observation of such an interaction has been confirmed in the U.S. study which led to the FDA approval of oxaliplatin in combination with 5FU/LV as second-line therapy (unpublished data, Sanofi). In this study of patients who had progressed on IFL, no patients treated with continuous infusion 5-FU had objective response, 1% of patients treated with oxaliplatin alone had objective tumor response while the response rate for patients treated with the FOLFOX combination was 9%.

There are numerous published regimens that combine varying doses and schedules of oxaliplatin, 5-FU and LV. The most popular regimens were designed by Aimery de Gramont, a French oncologist. These combinations have been called FOLFOX 1-7. Each consists of OXAL infusion over 120 minutes, followed by LV and 5-FU as a bolus, then continuous infusion of 5-FU over 24 to 48 hours. The investigators suggest that this regimen offers the pharmacokinetic advantages of infusional 5-FU with the potential differential anti-tumor mechanisms of both bolus and infusional 5-FU. There is no clear evidence that any one FOLFOX regimen is superior to another.

The role of FOLFOX as front-line therapy for patients with advanced or metastatic colorectal cancer is supported by the findings of an intergroup study, N9741. Published in 2004, this study clearly demonstrated superiority of FOLFOX4 over IFL in terms of disease free survival and overall survival in untreated patients with colorectal cancer [6].

As studied recently, FOLFOX6 consists of the following chemotherapy regimen: Oxaliplatin 100 mg/m² over 120 minutes, then LV 400 mg/m² over 2 hour. (In published reports, the dose is reported as 200 mg/m² L-LV, which is equivalent to 400 mg/m² of racemic LV.) The LV is followed by 5-FU 400 mg/m² bolus then 5-FU 2400 mg/m² by 46-48 h infusion, repeated every 2 weeks [21]. For this trial, we will modify the oxaliplatin dose to 85 mg/m² every other week (based on personal communication with Sanofi.)

The modified FOLFIRI and FOLFOX described above have been compared to each other in at least two randomized studies. Tournigand [7] published the results of a randomized study which mandated cross-over from one arm to the other at the time of disease progression. While the study was under-powered to identify a small difference (only 200 + patients in total), the findings suggest that the initial chemotherapy choice is not important in determining outcome, with both arms having median survival of approximately 21 months. Toxicity differences are significant however; peripheral neuropathy is a cumulative toxicity in patients receiving more than 8-10 cycles of oxaliplatin-based therapy while diarrhea and neutropenia are more common in patients receiving irinotecan-based therapy.

CALGB 80203 also compared modified FOLFOX and FOLFIRI, with an added variable of cetuximab. That study was closed to accrual after 234 patients enrolled and the results are not yet available.

1.8 Studies with bevacizumab combinations

Bevacizumab has been studied in combination with fluorouracil and either irinotecan or oxaliplatin regimens. As noted above, bevacizumab has demonstrated added efficacy in both front-line and second-line settings compared to chemotherapy alone, and therefore serves as the standard arm for this trial.

1.9 Studies with cetuximab combinations

Cetuximab has been studied as a single agent for patients with advanced cancer, including colorectal cancer. The objective response rate is about 10%. Its main toxicity is an acneiform rash that occurs in about 89% of patients and is presumably related to the suppression of EGF in the skin. Allergic reactions occur rarely. Cetuximab has been combined with irinotecan for patients who have already progressed (with colorectal cancer) despite treatment with irinotecan and this combination produced about a 20% objective response rate; patients who did not develop an acneiform rash were very unlikely to have tumor regression [24].

Combinations of FOLFIRI or FOLFOX and cetuximab have been studied, most recently in CALGB 80203 described above. While results are not yet available, a Data and Safety Monitoring Board found no substantive toxicity differences between the two arms or compared to toxicity profiles expected for the chemotherapy alone.

1.10 Studies of FOLFOX/FOLFIRI + Bevacizumab/Cetuximab combinations

The combination of cetuximab and bevacizumab has been tested in a pilot trial [11]. The study explored the safety and efficacy of the double biologic combination with or without irinotecan in patients with advanced colorectal cancer who had previously progressed on irinotecan and more than 80% of whom had previously received oxaliplatin. None of these patients had previously received bevacizumab or cetuximab. The combination treatment was found to be well tolerated with no apparent toxicity beyond that expected from the use of either treatment individually. As to efficacy, the response rate in the dual biologic/irinotecan arm was 38% and was 23% in the dual biologic arm. Time to tumor progression was 8.5 months in the bevacizumab/cetuximab/irinotecan arm and was 6.9 months in the bevacizumab/cetuximab group, each of which is better than historical controls. As yet, no data with the biologic combinations and either FOLFOX or FOLFIRI has been reported. These experimental arms will be closely monitored by the Data and Safety Monitoring Board for any evidence of added toxicity or less activity.

[Added with Update #5:] Evolving data on cetuximab now suggests that there is a biomarker that predicts who will not benefit from the treatment. The K-ras gene is mutated in approximately 35% of colon cancers and this mutation appears to prevent response benefit from the use of anti-EGFR antibodies. This has been demonstrated for single agent(s) panitumumab and cetuximab as well as in combination with chemotherapy (Lievre et al, JCO, 2008). It has recently been demonstrated to impact first as well as subsequent lines of therapy.

In addition, a randomized phase III study of capecitabine, oxaliplatin and bevacizumab with or without cetuximab (CAIRO-2) conducted by the Dutch Colorectal Cancer Group has been presented as an abstract and showed a decrease in progression-free survival as assessed by the investigator, but no difference in overall survival for the dual biologic arm compared to the bevacizumab arm. However, the subset of K-ras wild type patients did not demonstrate that finding. This study will now evaluate whether the benefit of dual biologic therapy in combination with FOLFOX or FOLFIRI is of potential benefit for patients with K-ras wild-type tumors with careful monitoring.

Therefore, with Update #5 to this protocol, evidence of wildtype K-ras gene status will be an eligibility criterion for enrollment on this study. Patients will be tested for K-ras status at pre-registration, and those with evidence of K-ras mutation will not be eligible to continue to randomization.

Finally, in order to investigate whether there exists a correlation between serum and/or plasma K-ras mutation status and K-ras mutation status based on tumor tissue evaluation, as well as whether a correlation exists with progression-free survival, patients will be asked to provide an additional tube of serum and of plasma at pre-registration and before eligibility to this study has

been determined (see also, [Section 5.6](#) of the protocol and Section 3.5 in [Appendix V](#)). The serum and plasma from patients who are not deemed eligible for the study will be used only to assess the correlation between serum and/or plasma K-ras mutation status and K-ras mutation status based on tumor tissue evaluation.

1.11 2009 clinical trials results: Combination studies

In 2009, the results of two other studies investigating the effects of combining an EGFR antibody with bevacizumab became available. One study, CAIRO-2, compared a standard arm of capecitabine/oxaliplatin and bevacizumab with and without cetuximab [136]. Another study, PACCE, offered either FOLFOX or FOLFIRI as a chemotherapy backbone with bevacizumab, and randomized patients to receive or not receive panitumumab [137].

Both of these studies found that the addition of the EGFR antibody to the control arm failed to improve outcomes for patients. In fact, the PACCE trial was halted early because of inferiority in the double biologic arm with FOLFOX chemotherapy. When retrospectively analyzed by K-ras status, patients in CAIRO-2 had inferior results if mutant for K-ras and receiving the double biologic combination. Overall, K-ras wild type patients did no better with the combination biologics than with bevacizumab and experienced more toxicity.

Although the details of these studies raise uncertainty as to the applicability of the data to this study, the weight of the evidence suggests that the double biologic arm is unlikely to yield overall survival benefit. Because of this, and the necessity to compare first-line chemotherapy and bevacizumab with chemotherapy and cetuximab in K-ras wild type patients, the double biologic arm has been removed with the issuance of Update #6 to this protocol.

1.12 Inclusion of Women and Minorities

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 overall survival and the logistic regression model for EGFR status.

2.0 OBJECTIVES

2.1 Primary Objective

To determine if the addition of cetuximab to FOLFIRI or FOLFOX chemotherapy prolongs survival compared to FOLFIRI or FOLFOX with bevacizumab in patients with untreated, advanced or metastatic colorectal cancer who have K-ras wild type tumors.

2.2 Secondary Objectives

- 2.2.1** To evaluate response, progression-free survival (PFS), time to treatment failure (TTF), and duration of response (DR) among patients with unresectable advanced metastatic colon cancer treated with bevacizumab or cetuximab in addition to chemotherapy with FOLFIRI or FOLFOX.
- 2.2.2** To evaluate toxicity and, in particular, 60-day mortality among patients with unresectable advanced metastatic colon cancer treated with bevacizumab or cetuximab in addition to chemotherapy with FOLFIRI or FOLFOX.
- 2.2.3** To describe patients with unresectable locally advanced or metastatic colorectal cancer rendered “resectable” with chemotherapy.

Quality of Life

- 2.2.4** To determine if there are significant differences in health-related quality of life among study patients by treatment arm (i.e. FOLFOX or FOLFIRI plus bevacizumab; FOLFOX or FOLFIRI plus cetuximab; FOLFOX or FOLFIRI plus bevacizumab and cetuximab).

Prescription Drug and Pharmacoeconomic Components

- 2.2.5** To determine the degree to which patients enrolled in the clinical trial have access to prescription drug coverage to pay for the cost of supportive medications during chemotherapy and to identify the extent to which paying for costs of prescription medications is a source of hardship and worry for clinical trial participants.
- 2.2.6** To compare the effects of the different combinations of chemotherapy and biologic agents on resource utilization, cost, and utilities, and if applicable, to make estimates of marginal cost-utility.

Diet and Lifestyle Component

- 2.2.6** To prospectively assess the influence of diet, obesity, physical activity, and other lifestyle habits on treatment-related toxicity, progression-free survival and overall survival in patients with stage IV colorectal cancer.

[See Appendices II-IV for Correlative Sciences Objectives.]

3.0 ON-STUDY GUIDELINES

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. Although they will not be considered as formal eligibility (exclusion) criteria, as part of this decision making process, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness that would prevent the patient from giving informed consent.
- Patient is not deemed a candidate for FOLFIRI or FOLFOX based on overall condition and comorbidities.
- A medical condition such as active/uncontrolled infection or cardiac disease that would make this protocol unreasonably hazardous for the patient in the opinion of the treating physician.
- Life expectancy \leq 12 weeks.
- Unwillingness to use adequate contraception for the duration of treatment and for up to 8 weeks after the completion of protocol therapy.

4.0 ELIGIBILITY CRITERIA

All questions regarding eligibility criteria should be directed first to the Alliance Co-Chair (Dr. Bert O'Neil) then to the Alliance or SWOG Study Chair. Please note that the Study Chairs cannot grant waivers to eligibility requirements.

4.1 Locally Advanced or Metastatic Colorectal Cancer

- 4.1.1 Eligible patients must have histologically or cytologically documented adenocarcinoma of the colon or rectum. Patients must have either locally advanced (unresectable) or metastatic disease.** 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.

4.1.2 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 greater than five 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.

4.1.3 At the time of randomization, the intent of this treatment must be indicated: palliative or neoadjuvant chemotherapy with the potential for resection of all sites of metastatic disease.

4.2 K-ras Status

Only patients with a wildtype K-ras gene as determined by the laboratory at the SWOG Solid Tumor Repository or by a local CLIA-certified laboratory are eligible. Patients with a mutation in the K-ras gene are ineligible. All patients must have available for analysis of K-ras status at least one H and E slide and one paraffin block of the previously resected primary colorectal tumor and/or a tumor deposit. For patients registered and randomized based on local CLIA-certified laboratory results, SWOG analysis will be confirmatory only.

See [Section 5.2](#) for pre-registration instructions and [Section 5.6.2](#) for collection and shipment procedures.

4.3 Prior Treatment

4.3.1 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 prior adjuvant chemotherapy that included fluorouracil alone or in combination with fluorouracil and oxaliplatin or irinotecan (no more than 6 months); or radiation with radiosensitizing chemotherapy.
- The last course of adjuvant chemotherapy must have concluded > 12 months prior to colorectal cancer recurrence.
- Patients may have received neoadjuvant chemo-radiation with capecitabine or 5-fluorouracil.
- Patients may not have received itraconazole or ketoconazole less than 4 weeks prior to randomization.
- No prior exposure to any tyrosine kinase inhibitors or other agents (including protein products, monoclonal antibodies, antisense, etc.) that target VEGF or EGF receptors is allowed.
- No prior treatment with bevacizumab or cetuximab.

4.3.2 Patients may not have had prior radiotherapy to greater than 25% of bone marrow. (Standard adjuvant rectal cancer chemoradiation will not exclude patient from protocol entry.) Radiation must have concluded ≥ 4 weeks prior to randomization.

- 4.3.3 Patients should have completed any major surgery \geq 4 weeks from randomization.** Patients must have completed any minor surgery \geq 2 weeks prior to randomization. Patients must have fully recovered from the procedure. (Insertion of a vascular access device is not considered major or minor surgery.)

4.4 Patient History

- 4.4.1 No previous or concurrent malignancy is allowed except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer for which the patient has been disease-free for five years.**
- 4.4.2 For patients who are to receive FOLFIRI: No evidence of Gilbert's Syndrome or of homozygosity for the UGT1A1*28 allele.**
- Patients with Gilbert's Syndrome may have a greater risk of irinotecan toxicity due to the abnormal glucuronidation of SN-38. Evidence of Gilbert's Syndrome would include a prior finding of an isolated elevation of indirect bilirubin.
 - UGT1A1 genotyping is not required on this study. However, patients known to be homozygous for the UGT1A1*28 allele are not to receive FOLFIRI for this study.
Patients with Gilbert's Syndrome or who are found to be homozygous for the UGT1A1 allele who will receive FOLFOX are eligible.
- 4.4.3 No sensory peripheral neuropathy of \geq grade 2 at baseline for patients who are to receive FOLFOX.**
- 4.4.4 No known central nervous system metastases or carcinomatous meningitis.**
- 4.4.5 No interstitial pneumonia or extensive and symptomatic interstitial fibrosis of the lung.**
- 4.4.6 No pleural effusion or ascites that causes \geq grade 2 dyspnea.**
- 4.4.7 No predisposing colonic or small bowel disorders in which the symptoms are uncontrolled** as indicated by 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.
- 4.4.8 Patients must not have an uncontrolled seizure disorder, or active neurological disease.**
- 4.4.9 No current congestive heart failure** (New York Heart Association Class II, III or IV).
- 4.4.10 Patients with history of hypertension must be well controlled** ($<$ 160/90) on a regimen of anti-hypertensive therapy.
- 4.4.11 Patents on full-dose anticoagulation (e.g., warfarin) are eligible provided that both of the following criteria are met:**
- The patient has an in-range INR (usually between 2 and 3) on a stable dose of oral anticoagulant or be on a stable dose of low molecular weight heparin.
 - The patient has no active bleeding or pathological condition that carries a high risk of bleeding (e.g., 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.

4.4.12 No significant history of bleeding events or GI perforation.

- Patients with a history of significant bleeding episodes (e.g., hemoptysis, upper or lower GI bleeding) within 6 months of randomization are not eligible unless the source of bleeding has been resected.
- Patients with a history of GI perforation within 12 months of randomization are not eligible.

4.4.13 No 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 (i.e., claudication on less than one block) or any other arterial thrombotic event are also ineligible.

4.4.14 No serious or non-healing wound, ulcer or bone fracture.

4.4.15 Patients with known hypersensitivity to Chinese hamster ovary cell products or to recombinant human or murine antibodies are not eligible.

4.5 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 72 hours prior to randomization. This is because DNA alkylating agents are known to be teratogenic, and the effects of irinotecan, oxaliplatin, 5-FU, bevacizumab, and cetuximab on a developing fetus at the recommended therapeutic doses are unknown.

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 \geq 12 consecutive months; or women on hormone replacement therapy (HRT) with documented serum follicle stimulating hormone (FSH) level $>$ 35mIU/mL]. Even women who are using oral, implanted or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

4.6 Age and Performance Status

4.6.1 ECOG performance status of 0-1.

4.6.2 Age \geq 18.

4.7 Required Initial Laboratory Values:

Granulocytes	\geq 1500/ μ L
Hemoglobin	\geq 9.0 grams/dL (patient may be transfused to meet this criterion)
Platelet count	\geq 100,000/ μ L
Creatinine	\leq 1.5 x upper limits of normal
Bilirubin	\leq 1.5 mg/dL
Albumin	\geq 2.5 g/dL
Urinalysis	\leq 1+ protein*

- * Patients discovered to have $\geq 2+$ proteinuria at baseline must undergo a 24-hour urine collection that must demonstrate < 1 g of protein/24 hr or have a UPC < 1.0 to allow participation in the study.

5.0 REGISTRATION/RANDOMIZATION, STRATIFICATION, AND DATA AND SAMPLE SUBMISSION

5.1 Randomization Requirements

Informed Consent: the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form are required.

Protected Health Information: In order to be able to send questionnaires to those patients who choose to participate in the QOL/Health Services substudy, it will be necessary to collect those participants' names, addresses, and telephone numbers. This information will be sent to the Wake Forest University School of Medicine and will be destroyed upon completion of the study.

5.2 CALGB pre-registration and randomization procedures

5.2.1 CALGB patient pre-registration procedures

Pre-registration for this study will be accomplished using the CALGB Web-based Patient Registration system. Pre-registration will be accepted only through CALGB Main Member Institutions, selected affiliate institutions and CCOPs using the Web-based Patient Registration system. Pre-registration must occur before K-ras mutation tissue sample shipment (see [Section 5.6.2](#)).

Complete the Pre-registration Worksheet. Access the Web-based Patient Registration system via the Patient Pre-Registration tab on the CALGB Member Web site at www.calgb.org. If the registering clinical research associate (CRA) requires assistance, s/he may consult the on-line help file at the bottom of the screen or call the IS Help Desk at 1-888-44CALGB. If further assistance is required, the registering CRA may call the CALGB Registrar (919)-668-9396, Monday-Friday, 9 AM – 5 PM, Eastern Time. Enter the following information:

Study #
 Name of Group (CALGB)
 Name of institution where patient is being treated
 Name of treating physician
 Name of person in contact with the patient record (responsible contact)
 Protocol IRB approval date
 Date of signed consent
 Date of HIPAA authorization signed by the patient
 Patient initials (last initial, first initial, middle initial)
 Patient's Social Security #, date of birth, hospital ID #, and survival status
 Patient's gender
 Patient's race
 Patient's ethnicity
 Patient's postal code
 Disease (if available)
 Companion studies (if applicable)

When the patient is pre-registered, a CALGB patient identification number will be generated. Please write the number in your records. The pre-registration CALGB patient

ID should be entered into the SWOG SpecTrak system for pre-study sample submission and will be used to register/randomize the patient at a later date (see [Section 5.2.2](#)).

If a patient answers “yes” to either “I agree that my blood may be used for the research study described above” (Question #1) or “My tissue and blood may be kept for future unknown use in research to learn about, prevent, treat, or cure cancer,” (Question #4) in the Model Consent, s/he has consented to participate in the correlative science studies described in Appendices II, III and V. The patient should be pre-registered to CALGB 150506 at the same time that s/he is pre-registered to the treatment trial (80405). If the patient says “yes” to Question #1 in the Model Consent, two additional tubes of blood should be drawn (one for plasma and one for serum) per [Section 5.6](#).

A tissue block must be submitted to the SWOG Solid Tumor Repository for mandatory K-ras testing for all patients, including those who have been previously tested by a local CLIA-certified laboratory. Patients with a wildtype K-ras gene as determined by a local CLIA-certified laboratory may be registered and randomized to this trial immediately following the submission of the lab report with wildtype K-ras results and C-2035 to the CALGB Statistical Center. The required tumor block and H&E slide must be submitted to the SWOG Tumor Repository within 30 days after randomization. For all other patients, once K-ras mutation status has been determined by the laboratory at the SWOG Solid Tumor Repository, the K-ras mutation result will be communicated by secure email from the SWOG Specimen Repository to the institution. If the patient’s K-ras status is wildtype, CALGB institutions may then register/randomize the patient.

5.2.2 CALGB patient registration/randomization

This study uses the CALGB Web-based Patient Registration system. Randomization will be accepted only through CALGB Main Member Institutions, selected affiliate institutions and CCOPs using the Web-based Patient Registration system.

Confirm eligibility criteria ([Section 4.0](#)). Complete the Registration Worksheet. Access the on-line Patient Registration system via the patient registration icon on the CALGB Information Systems (IS) Application main menu. If the registering CRA requires assistance, he/she may consult the on-line help file located under the help menu of the CALGB IS Application. If further assistance is required, the registering CRA may call the CALGB Registrar (919-668-9396, Monday-Friday, 9 AM – 5 PM, Eastern Time). Enter the following information:

Study

Name of group (CALGB)

Name of institution where patient is being treated

Name of treating physician

Name of responsible contact (treating physician or responsible CRA)

CALGB patient ID #, if applicable

Patient’s initials

Patient’s Social Security # date of birth and or hospital ID #

Patient’s gender

Patient’s race and ethnicity

Patient’s height in centimeters, weight in kilograms, and ECOG performance status

Type of insurance (method of payment)

Disease type and stage, if applicable

Patient’s postal code (if applicable)

Treatment start date

Date of signed consent

Date of signed HIPAA authorization
 Companion studies (see [Section 5.3](#))
 Eligibility criteria met (no, yes)

Registration to optional companion substudies will be done at the same time as registration to the treatment study. Registration to both treatment and companion studies will not be completed if eligibility requirements are not met for all selected trials (treatment and companions).

After registration is complete, the patient may be randomized. The patient is randomized according to the stratification factors, which must be entered to obtain a treatment assignment. Once the randomization is complete, note the patient's treatment assignment in your records. Treatment is to begin within 14 days of randomization.

The Main Member Institution and registering institution will receive a Confirmation of Registration and a Confirmation of Randomization. Please check both confirmations for errors. Submit corrections in writing to the data coordinator at the CALGB Statistical Center, Data Operations, 2424 Erwin Rd, Ste 802 Hock Plaza, Durham, NC 27705, or fax to 919-668-9397.

5.3 Registration to companion studies

5.3.1 Substudies

There are four substudies within CALGB 80405. These embedded companion studies must be offered to all patients enrolled on CALGB 80405 (although patients may opt not to participate). The substudies included within CALGB 80405 are:

- Diet and Lifestyle substudy ([Section 10.0](#))
- Quality of Life and Health Services substudies: CALGB 70502 ([Section 9.0](#))**
- Predictors of Toxicity and Outcome in Patients Receiving Chemotherapy for Metastatic Colorectal Cancer, CALGB 150506 (Appendices [II](#), [III](#), and [V](#))
- Pharmacogenomic substudy, CALGB 60501 ([Appendix IV](#))

** *Effective August 31, 2007, patients enrolling to 70502 will complete only the health services assessments, Forms C-187 and C-1156. Please see [Section 5.5](#) for submission instructions.*

If a patient answers “yes” to “I choose to take part in the Diet and Lifestyle study and agree to complete the diet and lifestyle questionnaire,” (Question #2) in the Model Consent, s/he has consented to participate in the Diet and Lifestyle studies described in [Section 10.0](#). There is no separate registration for this substudy.

If a patient answers “yes” to “I choose to take part in the medical costs study and agree to complete the medical costs questionnaire,” (Question #3) in the Model Consent, s/he has consented to participate in the substudy described in [Section 9.0](#). **In order to be eligible to participate in the health services substudy, patients must be able to speak English or Spanish.** Patients should be registered to CALGB 70502 at the same time that s/he is registered to the treatment trial (80405).

If a patient answered “yes” to “I agree that my blood may be used for the research study described above.” (Question #1) OR “My tissue and blood may be kept for future unknown use in research to learn about, prevent, treat, or cure cancer,” (Question #4) in the Model Consent, the patient should have been pre-registered to CALGB 150506 at the same time that s/he was pre-registered to the treatment trial (80405) and samples submitted per

[Section 5.6](#). At this time, patients who were pre-registered to 150506 should be registered to the companion study.

If a patient answers “yes” to “I agree that my blood may be used for the genetic research studies described above,” (Question #7) in the Model Consent, s/he has consented to participate in the pharmacogenomic studies described in Appendix IV. The patient should be registered to CALGB 60501. Although it is preferable that patients are registered to 60501 at the same time that they are registered to 80405, registration to 60501 may occur up to 60 days following registration to the treatment trial. Samples should be submitted per [Section 5.6.6](#).

5.3.2 Limited Access Companion Study

CALGB 70501, “Collection of patient-reported symptoms and performance status via the internet”, is a separate limited access companion protocol available to a number of selected institutions. All patients enrolled or enrolling to CALGB 80405 at these specific institutions should be approached and invited to participate in CALGB 70501. Registration should occur simultaneously with the CALGB randomization to CALGB 80405; however, registrations to CALGB 70501 may take place later. Please note that CALGB 70501 participants must be registered prior to receiving therapy on scheduled clinic visit/cycle #2 of this study.

5.4 Stratification Factors

Patients will be stratified by:

1. Chemotherapy (see [Section 7.4](#)):
 - a) FOLFOX, b) FOLFIRI
2. Prior adjuvant chemotherapy:
 - a) no, b) yes
3. Prior pelvic radiation:
 - a) no, b) yes

5.5 Data Submission

Forms should be submitted to the Alliance Statistics and Data Center at Mayo Clinic Rochester, Data Operations in compliance with the Data Submission schedule below. This study will use NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 for routine toxicity reporting on study forms. There are two options for submitting forms that use the Teleform barcode and cornerstones:

- All data forms available online must be submitted electronically using the “Print and/or Submit to CALGB” button located at the bottom of the last page of each form. Forms submitted electronically should not be submitted by fax or mail.
- Supporting documentation (e.g. scan reports) and amended forms must be faxed at 507-284-1902 or mailed to the **Alliance Data Center**, Attention: CALGB 80405 Data Manager, RO FF-3-24-CC/NW Clinic, 200 First Street Southwest, Rochester, MN 55905 USA.

Data Submission: Submit forms to the Alliance Statistics and Data Center at Mayo Clinic Rochester at the following intervals:

Form	Submission Schedule
Baseline	
C-2035 Report# CALGB 80405 Institutional K-ras Results Form Lab Report with wildtype K-ras results	Submit prior to randomization if WT K-ras status has been determined by a local CLIA-certified lab.
C-1397 C-1700 Report# Report# Report# CALGB 80405 On-study Form Solid Tumor Evaluation Form CT, MRI, X-ray reports (chest, abdomen, pelvis) Pathology report Operative report from resection of primary tumor	Within one MONTH of randomization
Dietary and Lifestyle Questionnaire	Submit within the first month of therapy to DFCI (see Section 5.8)
C-187 C-1156 Background Information Form Prescription Drug Survey-Baseline	For patients who enroll to CALGB 70502 <u>after</u> 7/31/2007, complete at randomization.
C-1442 Pharmacogenomic Submission and Quality Assurance Form	See Section 5.6.6
Treatment	
C-1398 C-1399 C-1401 C-1412 C-1700 Report# CALGB 80405 Treatment and Response Form CALGB 80405 Adverse Event Form* CALGB 80405 Cetuximab Skin Adverse Event Form (only for patients receiving C225) CALGB 80405 Supplemental Adverse Event Form CALGB Solid Tumor Evaluation Form CT, MRI, X-ray reports (chest, abdomen, pelvis)	Submit every cycle during protocol therapy. Not required for post-surgical treatment.
Follow-up (after end of protocol treatment)	
C-1400 C-1700 Report# CALGB 80405 Follow-up and Response Form CALGB Solid Tumor Evaluation Form CT, MRI, X-ray reports (chest, abdomen, pelvis)	Submit every 2 months after the end of protocol therapy for 5 years or until tumor resection or disease progression, whichever comes first. Then submit only the C-1400 every 6 months for 5 years or until death, whichever comes first.
Other**	
C-1413 Report# 80405 Surgical Resection Form Operative and pathology reports	Submit if patient has resection after beginning protocol treatment.
C-1402 CALGB 80405 Emergency Room Visit/Hospitalization Form	At time of emergency room visit or hospitalization

* C-1399 should be submitted until protocol treatment related AEs are resolved. Sensory neuropathies must be graded using the Sanofi-defined grading system per [Section 8.5](#).

** The C-300 Off-Treatment Form, C-113 Notification of Death Form, and C-1001 New Malignancy Form are not submitted for this study.

Submit copies of all required reports to confirm eligibility and restaging results.

Common Toxicity Criteria: This study will utilize the NCI Common Terminology Criteria for Adverse Events version 3.0 for routine reporting on study forms.

5.6 Specimen submission for K-ras testing and correlative studies

The Southwest Oncology Group (SWOG) is the coordinating group for the correlative science substudies to this trial. All samples, with the exception of the whole blood for the pharmacogenomic studies, should be sent to the SWOG Solid Tumor Specimen Repository per the instructions below.

Submission of a tumor block for determination/confirmation of K-ras status is required for all patients. If a patient declines to participate in the other correlative studies, the SWOG bank will return the tumor block to the submitting institution.

All participating institutions must ask patients for their consent to participate in the other correlative studies planned for CALGB/SWOG 80405, although patient participation is optional. Rationale and methods for the scientific components of these studies are described in Appendices III-V. For patients who consent to participate, samples will be collected at the following time points for these substudies:

Type of specimen	Pre-registration	Pre-treatment (eligible patients only)	After any cetuximab infusion reaction*	At first restaging (8 weeks)	At the end of protocol therapy (e.g., disease progression, etc.)
Tumor Block¹ and 1 H & E Slide	<i>Required for all patients To SWOG</i>				
Whole (venous) Blood (EDTA/purple top)		1 x 5 mL ³ <i>To ABOSU</i>	1 x 5 mL ² <i>To SWOG</i>		
	Number and volume of tubes to draw				
Citrated Plasma² (blue top)	1 x 5 mL ^{**^} <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>
EDTA Plasma² (purple top)		3 x 5 mL <i>To SWOG</i>		3 x 5 mL <i>To SWOG</i>	3 x 5 mL <i>To SWOG</i>
Serum² (red top or SST)	1 x 5 mL ^{**^} <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>	1 x 5 mL <i>To SWOG</i>
[Total blood volume]	[10 mL]	(30 mL)	[15 mL]	[25 mL]	[25 mL]

1 Tissue submission at pre-registration is required for determination of K-ras status and eligibility (see Sec. 5.6.2). For patients who consent to participate in the optional correlative studies, tissue will be retained to be used for the correlative studies described in Appendices II and V (150506).

2 To be used for correlative studies described in Appendices III and V (150506).

3 To be used for the pharmacogenomic assays described in Appendix IV (60501).

* All patients who experience a cetuximab infusion reaction in any cycle should have samples (whole blood, plasma, and serum) drawn at the time of each reaction. These samples should be drawn as close to the time of the reaction as possible. Infusion reactions should be reported in the C-1412, CALGB Supplemental Adverse Event Form.

** For patients who answer “yes” to consent #1.

^ For patients determined to be K-ras WT by a local CLIA-certified laboratory and who consent to CALGB 150506, these specimens can be collected and shipped at the same time as the pre-treatment specimens, prior to the initiation of protocol treatment.

5.6.1 SWOG general specimen submission instructions

These instructions apply to all samples submitted with the exception of the baseline whole blood sample submission (for pharmacogenomic studies) described in [Section 5.6.6](#).

- a. All submitted specimens must be labeled with the protocol number (80405), CALGB patient number, SWOG patient ID, patient's initials and date and type of specimen collection (e.g., serum).
- b. The Federal guidelines for packaging are as follows:
 1. The specimen must be wrapped in an absorbable material.
 2. The specimen must be placed in an AIRTIGHT container (like a resealable bag).
 3. Pack the resealable bag and specimen in a Styrofoam shipping container.
 4. Pack the Styrofoam shipping container in a cardboard box.
 5. The cardboard box must be marked as "BIOHAZARD".
- c. Specimen tracking system

Tissue blocks, slides, plasma, and serum for patients registered on this study must be entered and tracked using the online SWOG Specimen Tracking System. All institutions may access this system at <https://gill.crab.org/SpecTrack/Logon.aspx>. Users at non-SWOG institutions will log on to the system using their CTSU user ID and password. If you do not have a CTSU User ID and password, you may obtain one via the CTSU members' website (<http://members.ctsu.org>). SWOG members will log on to the system using their SWOG Roster ID number and password.

ALL SPECIMENS MUST BE LOGGED VIA THIS SYSTEM (with the exception of the baseline whole blood sample submission). For procedural help with logging and shipping specimens, please refer to both the general instructions document on the Specimen Tracking home page, **and the Special Instructions document for C80405**. For further assistance, contact the SWOG Data Operations Center at 206/652-2267 to be routed to a SWOG Data Coordinator. To report technical problems with the SWOG Specimen Tracking System, such as database errors or connectivity issues, please email technicalquestion@crab.org.

A copy of the Shipment Packing List produced by the Specimen Tracking system must be printed and placed in the pocket of the specimen bag if it has one, or in a separate resealable bag.

- d. Shipping

In the online Specimen Tracking system, laboratory ID numbers are used to identify the laboratories to which specimens are shipped. The laboratory ID number for this study is #78 (Solid Tumor Specimen Repository). The shipping address can be found through the Specimen Tracking system. **Please direct any questions regarding submissions to Miguel Martinez at (303) 724-3086 or miguel.martinez@ucdenver.edu.**

Materials are to be shipped by overnight delivery Monday through Thursday (no holidays). Note that pre-registration materials (see [Section 5.6.2](#)) may be shipped Monday through Friday.

- e. The SWOG Solid Tumor Specimen Repository will forward the materials to the investigators responsible for the correlative testing. Specimens not consumed by testing specified in this protocol will be retained in the SWOG Solid Tumor Specimen Repository. With additional consent, specimens from eligible patients may be used for future unspecified research.

5.6.2 Submission of paraffin embedded specimens – REQUIRED PRIOR TO RANDOMIZATION

a. **The submission of tissue (previously resected primary colorectal tumor and/or a metastatic tumor deposit) is mandatory for all patients. This will be used for the pre-registration K-ras testing that determines/confirms eligibility (see [Section 4.2](#)).** With additional consent, tissue from eligible patients will also be used for gene expression analyses (see Appendices III and V for a description of laboratory methods).

b. The following materials **MUST** be submitted following pre-registration, prior to randomization:

- An FFPE tumor block
- One H&E stained slide
- Pathology report

Please contact Miguel Martinez at (303) 724-3086 or miguel.martinez@ucdenver.edu if your institution is unable to send tumor blocks.

c. Specimens must be logged into the SWOG Specimen Tracking System as described in [Section 5.6.1.c](#). Please note the following points when logging the pre-registration specimens:

- On the first page of the Specimen Tracking system, there is a link to special instructions for **C80405**. Please refer to this document for assistance.
- When logging the pre-registration specimens, the user will be required to enter the CALGB patient ID number. This CALGB patient ID number is obtained during the pre-registration process from either CALGB (for CALGB sites) or from CTSU (for non-CALGB sites).
- The user will be prompted to provide e-mail addresses for two contact people who will receive notification of the K-ras testing results.
- The user will be prompted to provide the FedEx tracking number.
- After logging the specimens, the system will generate a SWOG patient ID number. Please retain this SWOG patient ID number in addition to the CALGB patient ID number.

d. Specimens may be shipped Monday through Friday for receipt Tuesday through Saturday.

e. **The University of Colorado will notify the site of the K-ras testing result by secure e-mail within 3 business days after receipt of the specimens.** This notification will be sent to the contact people identified in the Specimen Tracking System.

Following that notification, and provided they meet all other eligibility criteria, patients with wild-type K-ras may be registered to the study.

f. Blocks will be carefully banked and processed for this study according to routine SWOG paraffin tissue bank policy procedures detailed below. The policy includes the following four components:

- Safeguards to address medical/legal concerns of submitting institutions' pathologists;
- Quality control of storage and sectioning of blocks;
- Quality assurance of stored/sectioned material;
- Scientific review process for utilization of specimens.

We respect and are sensitive to the medical/legal concerns of submitting institutions' pathologists and have developed SWOG pathology policy that incorporates the following criteria:

- All precautions are taken to prevent exhausting the tissue block.
- A minimum of three H & E sections (obtained at different thicknesses throughout the block) remain on file at the SWOG Solid Tumor Specimen Repository.
- A minimum of two unstained sections (4 micron thickness) remain on file at SWOG.
- Unused portions of blocks are stored at the SWOG Solid Tumor Specimen Repository, unless hospital policy prohibits such storage.
- Unused portions of blocks, H & E slides, and unstained slides are available to the submitting institution by overnight carrier for any emergent medical or legal need.

The treating institutional pathologist should screen and select the appropriate blocks for submission.

5.6.3 Plasma Submission

For patients who consent to participate, plasma samples will be used for the studies described in Appendices III and V.

- For those patients who consent to question #1, one 5 mL citrate tube should be drawn at pre-registration. For patients determined to be K-ras WT by a local CLIA-certified laboratory, these specimens can be collected and shipped at the same time as the pre-treatment specimens, prior to the initiation of protocol treatment.
- For those patients who consent to question #4, one 5 mL citrate tube and three 5 mL EDTA tubes should be drawn prior to the initiation of protocol therapy, at first restaging (8 weeks), and at the discontinuation of protocol therapy.

Centrifuge blood for 10 to 15 minutes at 1300 x g (or in accordance with collection tube manufacturer's instructions). Aliquot plasma into 1.8 mL cryovials at 0.5 mL per vial. Samples should be processed and frozen within 3 hours of collection and should be labeled per the instructions in [Section 5.6.1](#). Store samples at -80°C (-20°C acceptable for up to 72 hours).

All specimens will be shipped frozen (on dry ice, if possible) to the SWOG Solid Tumor Specimen Repository. Batching of samples is permitted.

5.6.4 Serum Submission

For patients who consent to participate, serum samples will be used for the studies described in Appendices III and V.

- For those patients who consent to question #1, one 5 mL red top or SST tube should be drawn at pre-registration. For patients determined to be K-ras WT by a local CLIA-certified laboratory, these specimens can be collected and shipped at the same time as the pre-treatment specimens, prior to the initiation of protocol treatment.
- For those patients who consent to question #4, one 5 mL red top or SST tube should be drawn prior to the initiation of protocol therapy, at first restaging (8 weeks), and at the discontinuation of protocol therapy.

Allow blood to clot in tube for 30 minutes at room temperature. Centrifuge clotted blood for 10 to 15 minutes at 1300 x g (or in accordance with manufacturer's instructions). Aliquot serum into two 1.8 mL cryovials. Samples should be processed and frozen within

3 hours of collection and should be labeled per the instructions in [Section 5.6.1](#). Store samples at -80°C (-20°C acceptable for up to 72 hours).

All specimens will be shipped frozen (on dry ice, if possible) to the SWOG Solid Tumor Specimen Repository. Batching of samples is permitted.

5.6.5 Sample Submission for the Infusion Reaction Study

For patients who have consented to participate in the correlative science sub-study and who experience a cetuximab infusion reaction, the following samples will be obtained within 30 to 60 minutes of each reaction:

- **Plasma:** One 5 mL citrate tube should be collected. Centrifuge blood for 10 to 15 minutes at 1300 x g (or in accordance with collection tube manufacturer's instructions). Aliquot plasma into two 1.8 mL cryovials. Samples should be processed and frozen within 3 hours of collection and should be labeled per the instructions in [Section 5.6.1](#). Store samples at -80°C (-20°C acceptable for up to 72 hours).
- **Serum:** One 5 mL red top or SST tube should be collected. Allow blood to clot in tube for 30 minutes at room temperature. Centrifuge clotted blood for 10 to 15 minutes at 1300 x g (or in accordance with manufacturer's instructions). Aliquot serum into two 1.8 mL cryovials. Samples should be processed and frozen within 3 hours of collection and should be labeled per the instructions in [Section 5.6.1](#). Store samples at -80°C (-20°C acceptable for up to 72 hours).
- **Whole Blood:** Five mL of venous blood should be collected in a lavender top (EDTA coagulant) tube. The tube should be inverted several times to mix the EDTA, frozen until shipped on dry ice by overnight mail. Label the tube per the instructions in [Section 5.6.1](#). The sample should be shipped the same day that the blood is drawn.

All specimens (plasma, serum, and whole blood) for the infusion reaction study are to be shipped frozen (on dry ice, if possible) to the SWOG Solid Tumor Specimen Repository. Batching of samples is permitted.

5.6.6 Whole blood submission for pharmacogenomic studies

A whole blood sample will be obtained prior to treatment for the pharmacogenomic studies described in Appendix IV. This sample will be taken at the time the other registration blood work is collected. 4.5 mL of venous blood should be collected in a lavender top (EDTA coagulant) vacutainer. Label with the patient's initials, CALGB/CTSU patient ID number (no name should accompany patient samples), study number (CALGB/SWOG 80405), and date of collection. Ship on cold pack by overnight mail to:

Alliance Biorepository
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Phone: 614-293-7073 FAX: 614-293-7967

The CALGB C-1442, Pharmacogenomic Submission and Quality Assurance Form, must be submitted with the sample and a copy submitted to the Alliance Statistics and Data Center.

Shipment on Monday through Friday by overnight service to assure receipt is encouraged. If shipping on Friday, FedEx or UPS must be used and the air bill must be marked "For Saturday delivery." Do not ship specimens on Saturdays.

5.7 Submission of quality of life and health services measures

*The following table only applies to patients enrolled to CALGB 70502 **prior** to August 31, 2007. For patients enrolled **after** August 31, 2007, only Forms C-187 and C-1156 are requested. Please see the Baseline section of the Data Submission Table ([Section 5.5](#)) for submission instructions.*

For patients who consent to participate, quality of life and health services data will be collected at the following timepoints:

Form		Baseline*	Week 6†	Month 3†	Month 6†	Month 9†
	[Assessment No.]	[#0]	[#1]	[#2]	[#3]	[#4]
C-419	QOL Assessment Summary Form	X	X	X	X	X
C-187	Background Information Form	X				
C-259	EORTC QLQ-C30	X	X	X	X	X
C-616	Changes in Function		X	X	X	X
C-1157	Dermatology QOL	X	X	X	X	X
C-903	EQ-5D	X	X	X	X	X
C-1156	Prescription Drug Survey-Baseline	X				
C-1414	Prescription Drug Survey-Follow-up			X		X

* To be submitted by the institutional CRA; see [Section 5.7.1](#).

† To be submitted by the Research Interviewer; See [Section 5.7.2](#).

5.7.1 Baseline Data Collection

The baseline assessment of the patients' Quality of Life (QOL) and Prescription Drug Survey will occur at the time the patients are recruited to the study, but prior to randomization. The institutional clinical research associate (CRA) will ask the patient to complete the initial QOL questionnaires and Prescription Drug Survey when informed consent is obtained. The estimated time to complete these baseline forms is 45-50 minutes.

The baseline assessments must be completed prior to randomization and submitted to the Alliance Statistics and Data Center with copies sent to:

Michelle Naughton, Ph.D.
Associate Professor
Department of Public Health Sciences
Wake Forest University Health Sciences
2000 W. First Street, Rm. 224
Winston-Salem, NC 27104
Tel: 336-716-2918 Fax: 336-716-7554

5.7.2 Follow-up Assessments

The follow-up evaluations will be conducted through telephone interviews conducted by staff at the Wake Forest University Health Sciences. The follow-up evaluations will take place at 6 weeks, and 3, 6 and 9 months following baseline. See [Section 9.5](#) for more information regarding these interviews.

5.8 Submission of Diet and Lifestyle Surveys

At registration, patients may elect to enroll in the diet and lifestyle questionnaire companion study ([Section 10.0](#)). Surveys will be sent directly to individual treatment centers within 1 week of registration. Patients should complete the survey within the first month of enrollment. It is recommended that the surveys be completed during a physician visit, likely during the infusion of chemotherapy. Questions regarding the survey should be directed to Nathalie Fadel, 617-632-3687. Completed surveys should be mailed directly to the following address:

Drs. Meyerhardt/Fuchs
c/o Nathalie Fadel
CALGB Diet and Lifestyle Survey Coordination Office
Dana Farber Cancer Institute
44 Binney Street
Boston, MA 02115

If the patient has refused participation in the diet and lifestyle study, the questionnaire should be returned to Drs. Meyerhardt and Fuchs with the notation that the “patient refused” participation.

6.0 REQUIRED DATA

Guidelines for Pre-Study Testing

To be completed within 16 DAYS before randomization:

- All blood work, history and physical, and pregnancy test.

To be completed within 28 DAYS before randomization:

- Any X-ray, scan of any type, which is utilized for (staging) tumor measurement.

To be completed within 42 DAYS before randomization:

- Any X-ray, scan of any type of uninvolved organs which is not utilized for tumor measurement.

	Prior to Randomization*	Every two weeks*	Day 1 of each cycle*	Post Treatment Follow up**
Tests & Observations				
History and Progress Notes	X		X	X
Physical Examination	X		X	X
Pulse, Blood Pressure	X	X	X	
Height	X			
Weight/BSA	X		X	
Performance Status	X	X	X	
Drug Toxicity Assessment		X	X	
Laboratory Studies				
CBC, Differential, Platelets	X	X#£	X	
Serum creatinine, BUN	X	X#£	X	
Electrolytes (Na, K, Cl, bicarb)	X	X#£	X	
AST, ALT, Alk. Phos, Bili	X	A	X	
Albumin, LDH	X			
Magnesium	X	E	E	
PT/INR	F	F	F	
Urinalysis	X	B	B	
Pregnancy test (UCG)	C		C	
Staging				
Chest x-ray, PA & Lateral***	X		D	X
CT Scan or MRI of abd/pelvis	X		D	X
Evaluation for resectability	X		X	
Companion Studies‡				
Whole blood, serum & plasma	<i>Baseline blood collection is to be completed prior to protocol treatment.</i>			
Tissue block	<i>Tumor samples/slides are to be submitted prior to registration for K-ras testing.</i>			
QOL Assessments	<i>Baseline QOL assessment is to be completed prior to randomization.</i>			
Dietary Questionnaire	<i>To be completed within the first month of therapy.</i>			
‡	For those patients who consent to participate; see Sections 5.6 , 5.7 and 5.8 for all collection timepoints.			

* Randomization tests, observations and laboratory studies completed within 7 days prior to the first day of treatment need not be repeated. Labs (except urinalysis) and physical exam may be obtained up to 24 hours prior to treatment for all other cycles.

** Every 2 months until 5 years after the end of protocol therapy or until disease progression or resection, whichever comes first.

*** If CT or MRI scan of the chest is performed, chest X-ray is not required.

On Arms B and C only, weekly laboratory studies are required for the first four weeks of treatment, then every 2 weeks.

£ PRN during a cycle for those patients who have discontinued FOLFOX or FOLFIRI due to toxicity per Sec. 8.0.

A On Arms B and C only, LFT's are to be done weekly for the first four weeks and then on Day 1 of each cycle.

B All patients receiving bevacizumab will have a urinalysis performed within 48 hours prior to every other bevacizumab dose (q 4 weeks); if urine protein is $\geq 2+$, 24-hour urine collection will be required (see [Section 8.12](#)). Urinalysis is not required after baseline for patients not receiving bevacizumab.

C For women of child-bearing potential (see Sec 4.19). Pregnancy tests must performed within 72 hours prior to registration and within 72 hours prior to the initiation of protocol treatment.

D After pre-study staging, within 7 days prior to Day 1 of each cycle. Cycle 1, Day 1, scans do not need to be repeated.

E Only for those patients receiving cetuximab.

F PT/INR monitoring is required only for those patients receiving coumadin or warfarin. For these patients, PT/INR should be monitored at baseline and weekly during treatment.

7.0 TREATMENT PLAN

Protocol treatment is to begin within 14 days of randomization. One cycle will be defined as 8 weeks of treatment. Questions regarding treatment should be directed to the Alliance Co-Chair (Dr. Bert O'Neil) first, then to the Alliance or SWOG Study Chair.

Prior to 9/15/09, patients were randomized to Arms A, B, and C; effective with the issuance of Update #6 to this protocol, patients are to be randomized to Arms A and B only.

ARM A:

Order of infusion:	Week							
	1	2	3	4	5	6	7	8
Bevacizumab	X		X		X		X	
FOLFOX/FOLFIRI	X		X		X		X	

ARM B:

Order of infusion:	Week							
	1	2	3	4	5	6	7	8
Cetuximab	X	X	X	X	X	X	X	X
FOLFOX/FOLFIRI	X		X		X		X	

ARM C: EFFECTIVE 9/15/09, RANDOMIZATION TO THIS ARM OF THE STUDY WAS DISCONTINUED

Order of infusion:	Week							
	1	2	3	4	5	6	7	8
Cetuximab	X	X	X	X	X	X	X	X
Bevacizumab	X		X		X		X	
FOLFOX/FOLFIRI	X		X		X		X	

7.1 Patients should receive a minimum of two cycles of therapy.

Treatment will be continued until disease progression, unacceptable toxicity, or surgery with curative intent as planned.

Order of Administration: Regimens will be given in the order listed in the tables above. Cetuximab should always be the first agent delivered (in Arms B and C*). FOLFOX and FOLFIRI regimens be delivered in the order listed in [Section 7.4](#), with 5-FU always being the last agent delivered.

For patients for whom elective surgery is contemplated, bevacizumab is to be discontinued for at least 8 weeks prior to surgery. Bevacizumab may be resumed after at least 4 weeks following surgery. These patients should also discontinue aspirin at least 1 week prior to surgery.

Patients who undergo complete resection of metastatic disease will discontinue protocol therapy and may receive further treatment at the treating physician's discretion.

For patients for whom non-elective surgery is required, hold bevacizumab as long as possible prior to surgery and for at least 6 weeks following surgery. Other protocol treatment may be given while bevacizumab is held at the discretion of the treating physician.

7.2 Bevacizumab (Arms A and C)

5 mg/kg IV every 2 weeks administered preceding FOLFOX or FOLFIRI (and following cetuximab on Arm C).

The initial dose is to be given over 90 minutes, second dose over 60 minutes, and all subsequent doses over 30 minutes if prior infusions are tolerated without infusion-associated adverse events.

7.3 Cetuximab (Arms B and C)

400 mg/m² IV over 2 hours on the first day of treatment, then 250 mg/m² IV over 1 hour weekly thereafter. Cetuximab will precede FOLFOX or FOLFIRI on Arm B and will precede bevacizumab on Arm C.

All patients must be premedicated with **diphenhydramine hydrochloride** 50 mg (or a similar agent) IV prior to the first dose of cetuximab in an effort to prevent an infusion or hypersensitivity reaction. Premedication is recommended prior to subsequent doses, but at the investigator's discretion the dose of diphenhydramine (or a similar agent) may be reduced. Pretreatment with acetaminophen may also be used.

7.4 Chemotherapy

Chemotherapy will consist of either FOLFOX or FOLFIRI at the doses described below. The decision to use either regimen is at the treating physician's discretion (while complying with eligibility criteria 4.5 and 4.6), but must be declared prior to randomization and must not be changed during the course of the patient's treatment. A change in the choice of chemotherapy will be considered a major protocol violation. FOLFOX or FOLFIRI will follow bevacizumab and/or cetuximab.

7.4.1 FOLFOX, every 2 weeks.

- **Oxaliplatin** 85 mg/m² IV infused over two hours, **followed by**
- **Leucovorin** 400 mg/m² IV over 2 hours. Alternatively, leucovorin may be administered (via separate infusion lines) concurrently with oxaliplatin, **followed by**
- **5-FU** 400 mg/m² IV bolus, then 2400 mg/m² continuous IV infusion over 46-48 hours.

Patients receiving oxaliplatin on this study 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 patient is at risk for these cold-induced sensory neuropathies is not well documented. Patients should exercise caution regarding cold exposure during the treatment period. Peripheral sensory neuropathies can occur at any time after receiving oxaliplatin therapy.

7.4.2 FOLFIRI, every 2 weeks

- **Irinotecan** 180 mg/m² IV infused over 90 minutes, **followed by**
- **Leucovorin** 400 mg/m² IV over 2 hours. Alternatively, leucovorin may be administered (via separate infusion lines) concurrently with irinotecan, **followed by**
- **5-FU** 400 mg/m² IV bolus following leucovorin, then 2400 mg/m² continuous IV infusion over 46-48 hours.

For the first two cycles of irinotecan, patients should remain in the treatment area for a minimum of one hour following completion of the irinotecan infusion in the event that acute abdominal cramping or diarrhea develops.

7.5 Concurrent Medications

7.5.1 Loperamide: For symptoms of diarrhea and/or abdominal cramping that occur at any time during a treatment cycle with irinotecan, patients 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 to 2 more bowel movements than usual in 1 day or (3) an increase in stool volume or liquidity. Loperamide should be taken 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. Patients may take loperamide 4 mg every 4 hours during the night. The maximum daily dose of loperamide is 16 mg/day. Patients should be provided with loperamide at the initial treatment visit 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. Patients should be instructed to increase fluid intake to help maintain fluid and electrolyte balance during episodes of diarrhea.

7.5.2 Antibiotics: Oral fluoroquinolone treatment should be initiated for any of the following:

- Diarrhea persisting for more than 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 patients who are hospitalized with prolonged diarrhea (even in the absence of neutropenia).

8.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

A new cycle of treatment (i.e., Week 1 of the next cycle) may not begin until the ANC is $\geq 1500/\text{mm}^3$, the platelet count is $\geq 100,000/\text{mm}^3$, and any treatment-related GI toxicity has resolved to \leq grade 1. If the initiation of a new cycle, or therapy during a cycle is delayed for ≥ 4 weeks, treatment with FOLFOX/FOLFIRI should be discontinued.

Dose modifications should be based on laboratory values obtained as described in the Required Data section (6.0). Interval counts (i.e., cbc's done on non-FOLFOX/FOLFIRI days) are not determinants of dose reductions unless associated with other issues such as febrile neutropenia.

8.1 Dose Levels

The table below indicates potential dose levels for each of the agents for which dose modifications will be allowed. Dose adjustments of each agent may be made independently based on the specific types of toxicities observed. Only those agents specified in the sections below should be dose reduced.

Patients who require multiple dose reductions during a cycle for grade 2 toxicity may, at the physician's discretion, begin the following cycle at one dose level higher than the final dose level during that cycle. If dose reduction beyond -3 for any agent is required, that agent should be discontinued. Dose reductions beyond -2 are not allowed for cetuximab.

If FOLFOX or FOLFIRI is discontinued due to toxicity, patients may continue on protocol therapy with bevacizumab and/or cetuximab only.

Agent*	Initial Dose	Level –1	Level –2	Level –3
Oxaliplatin	85 mg/m ²	65 mg/m ²	50 mg/m ²	40 mg/m ²
Irinotecan	180 mg/m ²	150 mg/m ²	120 mg/m ²	100 mg/m ²
5-FU Bolus	400 mg/m ²	320 mg/m ²	270 mg/m ²	230 mg/m ²
5-FU Infusion	2400 mg/m ² per 46-48 hrs	1920 mg/m ² per 46-48 hrs	1600 mg/m ² per 46-48 hrs	1360 mg/m ² per 46-48 hrs
Cetuximab**	250 mg/m ²	200 mg/m ²	150 mg/m ²	NA

* All doses are IV.

** **Cetuximab** initial dose is always 400 mg/m² IV. Dose levels in the table apply to cetuximab doses following the initial dose.

Leucovorin dose is always 400 mg/m², IV given prior to the infusion of 5-FU. If any infusion of 5-FU is to be skipped, leucovorin must also be skipped.

Bevacizumab dose is always 5 mg/kg IV. Bevacizumab may be skipped or discontinued as described below, but the dose is not reduced.

8.2 Hematologic toxicities

The following dose modifications are based on toxicity experienced during a cycle (i.e., after Day 1 of any cycle):

8.2.1 Grade 2 neutropenia or thrombocytopenia occurring during a cycle: Reduce 5-FU and oxaliplatin or irinotecan one dose level for the remainder of the cycle.

For the next cycle, resume 5-FU and oxaliplatin or irinotecan at the previous dose levels, provided ANC \geq 1500 and platelets \geq 100,000.

8.2.2 Grade 3 or 4 neutropenia or thrombocytopenia: Skip 5-FU and oxaliplatin or irinotecan. If, during the current cycle, counts recover to ANC \geq 1500 and platelets \geq 75,000, 5-FU and oxaliplatin or irinotecan may be resumed at one lower dose level for the remainder of the cycle. Skipped doses are not to be made up.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the reduced dose levels from the previous cycle.

8.2.3 Febrile neutropenia (defined as ANC < 1000 and T \geq 38.5°C): Skip 5-FU and either oxaliplatin or irinotecan. If fever and neutropenia resolve during the current cycle, 5-FU and either oxaliplatin or irinotecan may be resumed at one lower dose level for the remainder of the cycle, provided ANC \geq 1500 and platelets \geq 75,000. Skipped doses are not to be made up.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the reduced dose levels from the previous cycle.

8.2.4 No bevacizumab or cetuximab dose modifications will be made for hematologic toxicity. Continue bevacizumab and/or cetuximab when 5-FU and oxaliplatin or irinotecan are skipped for hematologic toxicities.

8.3 Gastrointestinal toxicities:

8.3.1 Diarrhea: Lacrimation, rhinorrhea, miosis, diaphoresis, hot flashes, flushing, abdominal cramping, diarrhea, or other symptoms of early cholinergic syndrome may occur during or shortly after receiving irinotecan. Atropine, 0.25-1.0 mg IV or SC may be used to treat these symptoms. In patients with troublesome or recurrent symptoms, prophylactic administration of atropine shortly before irinotecan therapy may be considered. Additional antidiarrheal measures may be used at the discretion of the treating physician. Combination anticholinergic medications containing barbiturates or other agents (e.g., Donnatal[®]) should not be used because these may affect irinotecan metabolism. Anticholinergics should be used with caution in patients with potential contraindications (e.g., obstructive uropathy, glaucoma, tachycardia, etc.).

Late diarrhea (e.g., developing more than 24 hours after irinotecan) should be managed with loperamide as described in [Section 7.5.1](#).

The following dose modifications are based on toxicity experienced during a cycle (i.e., after Day 1 of any cycle):

- **For grade 2 diarrhea,** reduce 5-FU and irinotecan one dose level for the remainder of the cycle. Oxaliplatin is not reduced.

For the next cycle, resume 5-FU and irinotecan at the previous dose levels, provided diarrhea has fully resolved.

- **For grade 3 or 4 diarrhea,** skip 5-FU and oxaliplatin or irinotecan. If diarrhea resolves to \leq grade 2 during the current cycle, 5-FU and oxaliplatin or irinotecan may be resumed at one lower dose level for the remainder of the cycle. Skipped doses are not made up.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the reduced dose levels from the previous cycle.

8.3.2 Mucositis: The following dose modifications are based the grade of mucositis seen on the day of treatment for any day after Day 1 in any cycle.

- **For grade 2 mucositis,** reduce 5-FU and oxaliplatin or irinotecan one dose level for the remainder of the cycle.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the previous dose levels, provided mucositis has fully resolved.

- **For grade 3 mucositis,** skip 5-FU and oxaliplatin or irinotecan. If mucositis resolves to \leq grade 2 during the current cycle, 5-FU and oxaliplatin or irinotecan will be resumed at one lower dose level for the remainder of the cycle. Skipped doses will not be made up.

For the next cycle, continue 5-FU at the reduced dose level from the previous cycle and resume oxaliplatin or irinotecan at the previous dose levels.

- **For grade 4 mucositis,** skip all protocol therapy. If mucositis resolves to \leq grade 2 during the current cycle, 5-FU and oxaliplatin or irinotecan may be resumed at one lower dose level for the remainder of the cycle. Bevacizumab and/or cetuximab will be resumed at the prior dose. Skipped doses will not be made up.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the reduced dose levels from the previous cycle.

8.3.3 Nausea/vomiting: The following dose modifications are based on the grade of nausea and vomiting occurring during a cycle (i.e., after Day 1 in any cycle).

- **For grade 3 nausea or vomiting**, reduce oxaliplatin or irinotecan one dose level for the remainder of the cycle.

For the next cycle, continue oxaliplatin or irinotecan at the reduced dose level from the previous cycle.

- **For grade 4 nausea or vomiting**, reduce 5-FU and oxaliplatin or irinotecan one dose level for the remainder of the cycle.

For the next cycle, continue 5-FU and oxaliplatin or irinotecan at the reduced dose levels from the previous cycle.

These dose reductions for vomiting and/or nausea should be made only if they persist/occur despite two treatments with adequate (combination) antiemetic therapy. The use of aprepitant is prohibited for those patients receiving FOLFIRI.

- 8.3.4** No bevacizumab or cetuximab dose modifications will be made for diarrhea, mucositis, nausea, or vomiting. Continue bevacizumab and cetuximab when 5-FU and oxaliplatin or irinotecan are skipped for these GI toxicities.

8.4 Pulmonary Toxicity

For grade 2 or worsening pulmonary symptoms unrelated to underlying cancer, cetuximab therapy should be stopped and symptoms investigated. Cetuximab therapy may resume when symptoms resolve to \leq grade 1.

For \geq grade 3 cough, dyspnea, hypoxia, pneumonitis, or pulmonary infiltrates, skip oxaliplatin and bevacizumab (and cetuximab) until interstitial lung disease is ruled out. Continue 5-FU. Discontinue all protocol therapy if interstitial lung disease is confirmed.

8.5 Neurotoxicity

Table 1
Toxicity Scale for the Sensory Neuropathies Associated with Oxaliplatin

	Symptoms
Grade 1	Paresthesias/dysesthesias* of short duration that resolve and do not interfere with function.
Grade 2	Paresthesias/dysesthesias* interfering with function, but not in activities of daily living (ADL)
Grade 3	Paresthesias/dysesthesias* with pain or with functional impairment that also interfere with ADL.
Grade 4	Persistent paresthesias/dysesthesias* that are disabling or life threatening.
	* May be cold-induced

- 8.5.1 For grade 2 neurotoxicity persisting between treatments:** Reduce oxaliplatin by one dose level for the remainder of the cycle and for subsequent cycles.

- 8.5.2** For grade 3 neurotoxicity resolving to \leq grade 2 between treatments: Reduce oxaliplatin by one dose level for the remainder of the cycle and for subsequent cycles.

- 8.5.3** For recurrent grade 3 neurotoxicity resolving to \leq grade 2 between treatments: Reduce oxaliplatin by one additional dose level for the remainder of the cycle and for subsequent cycles. Oxaliplatin will not be reduced beyond level -3. If further dose reduction is required for neurotoxicity, oxaliplatin will be discontinued. Patients should continue to receive other protocol therapy.
- 8.5.4 For grade 3 neurotoxicity persisting between treatments:** Discontinue oxaliplatin. Patients should continue to receive other protocol therapy.
- 8.5.5 For grade 4 neurotoxicity:** Discontinue oxaliplatin. Patients should continue to receive other protocol therapy.
- 8.5.6 For pharyngo-laryngeal dysesthesia:** Increase the duration of oxaliplatin infusion to 6 hours for all subsequent treatments.

Patients 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. Patients should continue to receive other protocol therapy and the oxaliplatin may be reintroduced subsequently.

8.6 Extravasation

Extravasation of oxaliplatin has been associated with necrosis; if extravasation is suspected, the infusion should be stopped and the drug administered at another site. Extravasation may be treated according to institutional guidelines.

8.7 Hypomagnesemia

Hypomagnesemia has been seen with cetuximab. Should hypomagnesemia occur, magnesium supplementation should be provided. No dose adjustment is required; however, continue careful monitoring.

8.8 Bevacizumab dose modifications for hypertension

- **For hypertension controlled with medication (to $< 160/90$):** Continue bevacizumab.
- **For persistent or symptomatic hypertension:** Skip bevacizumab. If bevacizumab treatment is delayed for more than 4 weeks due to uncontrolled hypertension, discontinue bevacizumab.
- **Grade 4 hypertension:** Discontinue bevacizumab.

Patients who skip or discontinue bevacizumab due to hypertension may continue other protocol therapy.

8.9 Bevacizumab/placebo dose modifications for 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 as described in [Section 11.8](#). 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.

8.10 Dose modifications for cardiovascular toxicities:

Patients should be carefully monitored for evidence of thromboembolic disease during treatment.

8.10.1 For grade 3 venous thrombosis or asymptomatic pulmonary embolism: Skip bevacizumab. If the planned duration of full-dose anticoagulation is < 2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is > 2 weeks, bevacizumab may be resumed during the period of full-dose anticoagulation if all of the following criteria are met:

- The patient must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin or be on stable dose of low molecular weight heparin prior to restarting bevacizumab treatment;
- The patient must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels);
- The patient must not have had hemorrhagic events while on study.

8.10.2 For grade 4 or for recurrent/worsening venous thromboembolic events after resumption of bevacizumab: Discontinue all protocol therapy.

8.10.3 For symptomatic pulmonary embolism, patients will discontinue all protocol therapy.

8.10.4 Arterial thrombotic events

NOTE: Per [Section 16.2](#), all arterial thrombotic events, regardless of grade, attribution, or treatment arm, are to be reported within 10 calendar days using CTEP-AERS expedited reporting.

- **For grade 2 arterial thrombotic events not present at baseline or worsened since the initiation of protocol therapy,** discontinue bevacizumab. Patients may continue other protocol therapy.
- **For grade 3 cerebrovascular ischemia, and/or peripheral or visceral arterial ischemia,** discontinue bevacizumab. Patients may continue other protocol therapy.
- **For grade 3 cardiac ischemia/infarction,** discontinue all protocol therapy.
- **For any grade 4 arterial thrombotic event,** including cerebrovascular ischemia, cardiac ischemia/infarction, peripheral or visceral arterial ischemia, discontinue all protocol therapy.

8.10.5 Left Ventricular dysfunction

- **For grade 3 LV dysfunction,** discontinue cetuximab and/or bevacizumab. Patients may continue other protocol therapy.
- **For grade 4 LV dysfunction,** discontinue all protocol therapy.

8.11 Bevacizumab dose modifications for hemorrhage/bleeding

- **For grade 3 hemorrhage/bleeding,** Discontinue bevacizumab and skip other protocol therapy; once hemorrhage or bleeding resolves, other protocol therapy may be continued at the treating physician's discretion.
- **For grade 4 hemorrhage/bleeding,** Discontinue all protocol therapy.

8.12 Bevacizumab dose modifications for proteinuria

- For proteinuria of $\geq 2+$: Confirm total urine protein with a 24-hour urine collection or urine protein to creatinine (UPC) ratio. For 2+ proteinuria, the scheduled dose of bevacizumab may be given while awaiting the results of the 24-hour collection or UPC ratio. For $> 2+$ proteinuria, skip bevacizumab while awaiting results of the 24-hour urine collection or UPC ratio. Other protocol therapy may be continued. (See Appendix VI for instructions on calculating UPC ratio.)
- If proteinuria is ≥ 2 g/24 hours or UPC ratio ≥ 2.0 , skip bevacizumab until urine protein recovers to < 2 g/24 hours or UPC < 2.0 , continue other protocol treatment. If bevacizumab is delayed more than 8 weeks due to proteinuria, discontinue bevacizumab.
- **If nephrotic syndrome (grade 4 proteinuria) occurs, discontinue bevacizumab.**

8.13 Other bevacizumab toxicities

- **For wound dehiscence requiring medical or surgical intervention:** Discontinue bevacizumab.
- **For any grade GI perforation, GI leak, or intraabdominal fistula:** Discontinue bevacizumab.

8.14 Cetuximab Dose Modifications for Cutaneous Toxicity

There will be no dose level reductions below a weekly dose of 150 mg/m² for cetuximab. Cetuximab dose reductions are permanent, that is, there will not be any re-escalation of dose.

Discontinue cetuximab if doses are skipped for more than 4 consecutive weeks.

Continue 5-FU, oxaliplatin or irinotecan, and bevacizumab if cetuximab is skipped or discontinued for cutaneous toxicity.

Table 2**Cetuximab Dose Modification/Rash Management Guidelines**

Grade 3 Rash	Cetuximab	Outcome	Cetuximab Dose Modification
1 st occurrence	Skip infusion 1 to 2 wks	If improvement: If no improvement:	Continue at 250 mg/m ² Discontinue cetuximab
2 nd occurrence	Skip infusion 1 to 2 wks	If improvement: If no improvement:	Reduce to 200 mg/m ² Discontinue cetuximab
3 rd occurrence	Skip infusion 1 to 2 wks	If improvement: If no improvement:	Reduce to 150 mg/m ² Discontinue cetuximab
4 th occurrence	Discontinue cetuximab		
Grade 4 Rash	Discontinue cetuximab		

For grade 3 nail disorders, cetuximab may be skipped for up to 3 weeks and if no improvement, reduce dose to 200 mg/m² for 1st occurrence, 150 mg/m² for 2nd occurrence. For 3rd occurrence discontinue cetuximab if there is no improvement after 3 weeks.

8.15 Hypersensitivity and infusion reactions

Note that the NCI CTCAE defines these reactions differently: “Cytokine release syndromes/acute infusion reactions are different from allergic/hypersensitivity reactions, although some of the manifestations are common to both AEs. An acute infusion reaction may occur with an agent that causes cytokine release (e.g., monoclonal antibodies or other biological agents). Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.” See the “Syndromes” section of the CTCAE version 4.0 for a complete list of signs and symptoms of “Cytokine release syndrome/acute infusion reaction;” and see the “Allergy/Immunology” section for a description of hypersensitivity.

Whole blood, plasma, and serum samples are to be collected from all patients who have consented to the correlative science substudy and who experience either a hypersensitivity or infusion reaction to cetuximab (see [Section 5.6.5](#)).

8.15.1 Dose modifications for hypersensitivity reactions (for all agents)

- **For grade 1 hypersensitivity reactions** (transient rash, drug fever < 38°C): Decrease the infusion rate by 50% until symptoms resolve, then resume at the initial planned rate.
- **For grade 2 hypersensitivity reactions** (urticaria, drug fever ≥ 38°C and/or asymptomatic bronchospasm): 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.
- **For grade 3 or grade 4 hypersensitivity reactions:** Stop the infusion. Discontinue all protocol treatment and notify the study chair.

8.15.2 Oxaliplatin-induced pharyngolaryngeal dysesthesias

Should a patient develop oxaliplatin-induced pharyngolaryngeal dysesthesia, her/his oxygen saturation should be evaluated via a pulse oximeter; if normal, an anxiolytic agent may be given and the patient observed in the clinic until the episode has resolved. Following this toxicity, patients may continue protocol therapy if it is felt appropriate. A table comparing pharyngo-laryngodysesthesia to platinum hypersensitivity reactions is presented in Table 3 below.

**Table 3
Comparison of the Symptoms and Treatment of Pharyngo-Laryngodysesthesias and Platinum Hypersensitivity Reactions**

Clinical Symptoms	Pharyngo-Laryngeal Dysesthesias	Platinum Hypersensitivity
Dyspnea	present	present
Bronchospasm	absent	present
Laryngospasm	absent	present
Anxiety	present	present
O ₂ saturation	normal	decreased
Difficulty swallowing	present (loss of sensation)	absent
Pruritis	absent	present
Urticaria/rash	absent	present
cold-induced symptoms	yes	no
BP	normal or increased	normal or decreased

Treatment	anxiolytics, observation in a controlled clinical setting until symptoms abate or at the physician's discretion	oxygen, steroids, epinephrine, bronchodilators; fluids and vasopressors, if appropriate
------------------	---	---

8.15.3 Bevacizumab dose modifications for infusion reactions

The initial bevacizumab dose should be administered over a minimum of 90 minutes. If no adverse reactions occur, the second dose should be administered over a minimum of 60 minutes. Again, if no adverse reactions occur, the third and subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, subsequent infusions should be administered over the shortest period that is well-tolerated. Patients may receive premedication with diphenhydramine 25 to 50 mg intravenously or orally 30 minutes prior to bevacizumab if they have previously experienced mild infusion reactions. Acetaminophen premedication may also be used.

8.15.4 Cetuximab dose modifications for infusion reactions

- **For grade 1 or 2 cetuximab infusion reactions:** Stop the infusion until symptoms resolve, then restart cetuximab at a 50% lower rate of infusion. All subsequent doses should be administered at the lower infusion rate.
- For \geq grade 3 cetuximab infusion reactions: Discontinue cetuximab. Other protocol therapy may be continued.

8.16 Other non-hematologic toxicities

For all other \geq grade 3 non-hematologic toxicities not described above, hold all protocol treatment and monitor toxicity at least weekly. If toxicity resolves to \leq grade 1 within 4 weeks, treatment may be resumed, with 5-FU and irinotecan or oxaliplatin at one lower dose level.

8.17 Dose Modification for Obese Patients

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 the patient's actual weight without any modification**. 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 administering chemotherapy dose based on actual body weight should not enroll obese patients on CALGB protocols.

9.0 QUALITY OF LIFE AND HEALTH SERVICES COMPANION STUDIES

Effective August 31, 2007, patients were no longer enrolled to the quality of life portion of CALGB 70502. The health services portion of CALGB 70502 remains open to accrual for patients registered after August, 31, 2007. Please see [Section 5.5](#) for data submission instructions.

9.1 Introduction

Health-related quality of life (HRQL) is a multidimensional concept that refers to an individual's overall well-being [25]. Recent years have witnessed a growing recognition among cancer researchers of the importance of systematically measuring health-related quality of life and psychosocial factors, both to characterize the health status and well-being of these patient populations, as well as to assess treatment efficacy.

The traditional endpoints in cancer clinical trials have been disease-free survival, tumor response, and overall survival. With advances in cancer treatments, and the availability of

different treatment options for the same disease, health-related quality of life (HRQL) has become an important additional endpoint in evaluating the efficacy of various interventions. The assessment of HRQL in clinical research provides the investigator with important information on how treatment and disease impact on the daily lives of their patients and can provide a more informed decision-making process for patients and their physicians with regard to various treatment options. HRQL is particularly important in trials where treatments are expected to produce similar survival rates but may differ in toxicity [26]

In the past decade, significant progress has been made in the reliable and valid assessment of health related quality of life (HRQL) and other psychosocial variables [26-30]. Although there has been some debate regarding the definition of HRQL, an international consensus conference composed of a panel HRQL experts reached agreement on the fundamental dimensions essential to any HRQL assessment [31]. These dimensions include physical functioning, psychological functioning, social functioning and role activities, and the individuals' overall life satisfaction and perception of their health status. Other dimensions of HRQL may be appropriate to assess in any given trial, depending on the goals of the study, the research questions to be addressed, the anticipated effects of treatment and therapy, and other considerations, such as staff and participant burden. Other typically measured dimensions of HRQL include sleep disturbance, pain, symptoms, neurocognitive functioning, and intimacy and sexual functioning.

The rationale for the inclusion of HRQL in this protocol is based on two premises. First, this study explores three treatment regimens that may yield different results in terms of survival and toxicity profiles. While these toxicities may not interfere with drug dosing, these toxicities may impact on quality of life. In this instance, a choice between clinically equivalent treatments might be based on whether one form of treatment has a preferred quality of life profile. Secondly, this investigation is also assessing the value of an EGF inhibitor. The classic toxicity of this group of agents is an acneiform skin rash, which is generally dismissed as a mere nuisance by oncology professionals, but may be considered very bothersome by patients, who may already have undergone changes in their physical appearance due to their cancer and/or therapy. This study will assess the social impact of severe acne on this population of patients, which has not heretofore been collected in a standardized manner in a randomized clinical trial.

The conceptual framework used to select HRQL measures for this study is a functional approach (i.e., how treatment and or the illness itself affects an individual's ability to function in chief life domains). Measures were selected to assess the primary dimensions of physical functioning, social/role functioning, emotional status, and overall life quality in order to provide a more comprehensive "picture" of the patients' overall health status. Symptom-specific measures were also included in this assessment to address chief treatment complaints of patients (i.e., acne; neuropathy), in addition to more traditional cancer-related physical symptoms commonly expressed by patients.

9.2 Overview of Prescription Drug Component

Access to prescription drugs is a pressing health and public policy issue. Much attention has focused on the Medicare population, where the near poor and chronically ill are particularly vulnerable to underinsurance and high out of pocket drug costs [32] But across the age spectrum, growing numbers of Americans face dwindling drug coverage and higher out-of-pocket costs for their medications [33, 34].

Drug plans vary in generosity. Research has shown that those with less generous plans fill fewer prescriptions [35] and incur higher out-of-pocket drug costs [36, 37]. They are also less apt to receive appropriate medications [38] Declining generosity of coverage has been linked to adverse health outcomes among the chronically ill and poor [39, 40]

There has been no work focusing on financial access to drugs among cancer patients. This is a remarkable omission because cancer is an area in which aging and chronic illness intersect, where Medicare is often the primary insurer, and where drugs are essential forms of primary treatment and palliation/support. Moreover, as cancer treatment has moved beyond the hospital—where Medicare has historically provided coverage—the traditional benefit has proved ill suited to supporting high quality and appropriate care. For example, the development of effective oral chemotherapeutic agents challenges a system in which coverage is determined by mode of administration. Similarly, the advent of parenteral supportive agents that can be self-administered (e.g., Epoetin, Enoxaparin, G-CSF) raises questions as to whether it is sensible to cover such treatments only when the injection is performed in the health care setting rather than when it is dispensed for self-administration. Finally, the greatest challenge comes in rationalizing access to supportive agents like high cost/high quality antiemetics and analgesics: to the extent that maintaining quality of life is a primary goal of cancer treatment, what is the impact of limited access to these modalities based on prescription coverage? There is no information regarding the degree to which the lack of prescription drug coverage and/or the high costs of prescription medications create hardship for cancer patients.

If lack of prescription drug coverage means that patients forego supportive medications necessary to make cytotoxic chemotherapy more tolerable, barriers to these medications could influence treatment toxicity and quality of life. Given the very limited data on access to prescription drugs for cancer patients, we propose a study to describe the extent to which this economic constraint is a source of hardship and worry for patients receiving chemotherapy on CALGB clinical trials.

Descriptive information will therefore be collected from patients regarding whether their insurance coverage is adequate to pay for medications and other incidentals related to their cancer therapy, any out-of-pocket expenses they may incur, the degree of anxiety they have regarding paying for medications, and methods they use to cope with the cost of prescription drugs. Since the information to be collected is descriptive and will be preliminary in nature, no formal hypotheses have been generated for these data. The baseline survey to be completed by all participants will permit a description of the extent to which clinical trial participants have access to prescription drug coverage. Follow-up interviews will be conducted at Month 3 and Month 9 on the subsample of participants enrolled in the quality of life companion. These interviews will evaluate the extent to which paying for prescription drugs during clinical trial participation is a hardship for patients with colorectal cancer.

9.3 Overview of Pharmacoeconomic Component

This phase III trial is designed to compare the survival time and health-related quality of life (HRQL) of patients with previously untreated metastatic colorectal cancer randomized to receive cetuximab, bevacizumab, or a combination of these two biologic agents in addition to standard chemotherapy consisting of either FOLFIRI or FOLFOX (at the discretion of the treating physician). Both cetuximab and bevacizumab are costly, and so any differences in outcome would have to be weighed against differences in cost from a policy point of view. An economic analysis will be able to relate these outcomes to one another and produce an estimate of cost-utility. Conversely, if no difference is detected in either survival or quality of life, economic results could prove to be a critical factor in determining the preferred regimen.

Resource Utilization: To permit maximum generalizability, data collection will focus on resource utilization rather than costs. Information about treatment delivery, complication management, and post-trial treatments will be captured for all patients through medical record review as part of the clinical trial. Because all

agents are commercially available, those not received on trial could be given after progression on trial. Moreover, first line systemic therapy could lead to subsequent potentially curative metastatectomy in certain situations depending on the response to treatment. As a result, knowledge of post-trial therapy is crucial for interpretation of both clinical and economic outcomes and will be captured as part of the study’s routine data collection. Consequently, additional data will not be collected specifically for the pharmacoeconomic substudy. Data will be obtained from the following sources:

Resource Utilization	Form
Dose administered, number of cycles	Treatment Form
Clinic assessments, ER visits, hospitalizations and length of stay, and ICU days due to adverse events. Non-investigational therapies, including blood products, rehydration, anticoagulants, cytokines, and surgical procedures.	Adverse Event Forms, Supplemental Adverse Event Form, Hospitalization Form
Post-trial therapies and their duration (including metastatectomy)	Follow-Up Form Surgical Resection Form
Survival	Follow-up Form
Utilities	EQ-5D (from the QoL Assessment)

Direct non-medical resources such as transportation, and indirect costs such as lost wages, will not be measured because of anticipated small differences between treatment arms and the substantial effort required to collect such data. Estimates of these costs will be drawn from the literature, however, and used in sensitivity analyses.

Cost estimates Cost estimates will be obtained by applying standard Medicare reimbursement rates to each item of resource utilization. Economic results will be presented in a disaggregated fashion, i.e.,

$$\text{cost} = \text{resource utilization} \times \text{unit cost},$$

where the units of resource use (e.g., number of hospital days) for each resource will be multiplied by the appropriate cost multiplier and summed for each arm of the study. This allows the potential for re-analysis using any cost inputs.

Utilities Utilities will be captured prospectively on all patients participating in the HRQL evaluation with the EQ-5D [41].

Survival: Survival is the primary endpoint of the clinical trial. Any survival differences found in the trial will be measured as the area between the survival curves for the purposes of the economic evaluation.

9.4 HRQL, Prescription Drug, and Pharmacoeconomic Hypotheses

Instrument selection, power calculations and plans for data analysis were chosen to faddress the following primary hypotheses.

- 9.4.1** At 3 and 9 months, there will be no significant differences in health-related quality of life among study patients by treatment arm (i.e. FOLFOX or FOLFIRI plus bevacizumab; FOLFOX or FOLFIRI plus cetuximab; FOLFOX or FOLFIRI plus bevacizumab and cetuximab). The patients in the three treatment groups, however, are anticipated to have difference types of toxicity profiles, which will lead to differential impact on their health-related quality of life.

- 9.4.2** At 3 months, there will be a significant difference in health-related quality of life due to acne for those patients receiving cetuximab (i.e., patients in arms B and C) versus those who do not receive cetuximab (Arm A). Patients receiving cetuximab are anticipated to have lower satisfaction with their appearance, reduced social functioning, and lower overall quality of life due to acne symptoms.
- 9.4.3** Descriptive analyses are proposed for the prescription drug component since there is very limited prior research in this area. These analyses will examine:
- a) The extent to which clinical trial participants have access to prescription drug plans which cover the costs of supportive medications and medications for other medical conditions. Access will be related to patient demographics, education and employment status.
 - b) The extent to which clinical trial participants utilize strategies during study enrollment to cope with high costs of medications such as not filling prescriptions, skimping on medications, or ordering from foreign pharmacies.
- 9.4.4** Higher cost for treatment is associated with longer survival or quality-adjusted survival will be explored using multivariate regression modeling.

9.5 HRQL and prescription drug component assessment instruments

All consenting English- and Spanish-speaking patients will be evaluated prospectively to assess the impact of therapy on their health-related quality of life and prescription drug coverage. The effects of treatment will be evaluated through repeated administration of the following validated instruments. All patients will be evaluated, prospectively to assess the impact of therapy on their health-related quality of life and prescription drug coverage. The effects of treatment will be evaluated through repeated administration of validated instruments. The following measures and subscales will compose the health outcomes assessment.

Background Information Form (C-187): The background information form from CALGB will be used to record the patient's educational attainment, marital status, employment status, and persons with whom they live.

European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) (C-259): This instrument will be used to assess the quality of life of the cancer patients [42]. The EORTC QLQ-C30 is a well-validated scale that assesses the overall quality of life of cancer patients. It is composed of six functional scales (physical, role, emotional, cognitive, social, and global health status/quality of life) and nine symptom scales/items (fatigue, nausea and vomiting, pain, dyspnea, sleep disturbance, appetite loss, constipation, diarrhea, financial impact). This instrument will be used to assess the primary dimensions of quality of life (i.e., physical, social, emotional functioning), as well as measure patients' cancer-related symptoms.

Changes in Function (C-616): The Subjective Significance Questionnaire developed by Osoba and colleagues [43] will be used to determine the significance to patients of changes in health-related quality of life, as measured by the EORTC-QLQ C30. This 4-item questionnaire asks the patients to rate changes in their health-related quality of life (e.g., moderately worse; a little better) in terms of their physical, emotional, social, and overall quality of life. This questionnaire will be used to calibrate changes in the EORTC scores in terms of small, moderate, or large changes in the quality of life of the patients.

Dermatology-Specific Quality of Life (C-1157): The Dermatology-Specific Quality of Life (DSQL) instrument will be used to assess the impact of patients' acne on daily life [44]. The DSQL is composed of five subscales (symptoms; daily activities; social functioning;

work/school performance; and personal perceptions) and eight global items assessing the interference of acne in daily life, the severity of the patients' skin condition, and the patients' satisfaction with specific areas of their life. Both subscale specific and an overall quality of life score can be obtained from this instrument. For these analyses, the total score will be used to determine the impact of acne and skin conditions on patients' health-related quality of life.

EuroQol/EQ-5D (C-903): The EQ-5D questionnaire, developed by the EuroQol group, will be used as a utility assessment of the patients' self-reported health status [41]. Respondents are asked to describe their own health status using five questions covering the domains of mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each of these dimensions records three levels of severity, which are indicated by numbers. No problems are coded '1', some or moderate problems '2' and extreme problems '3'. A health state can therefore be described with a five-digit number, for instance 12113. This means 'no problems' on the dimension of mobility, 'some problems' on the dimension of self-care, 'no problems' with respect to usual activities and pain/discomfort and 'severe problems' on the dimension of anxiety/depression. The classification system defines 243 health states. In addition, the states of unconsciousness and death are included.

The EQ-5D also includes a 20 cm visual analogue scale (VAS) on which patients rate their current health state on a 0-100 scale. A similar VAS is used when valuing hypothetically.

The EQ-5D provides a simple descriptive profile and a single index value for health status from which QALY (Quality Adjusted Life Years) can be calculated. The EQ-5D has been specially designed to complement other quality of life measures such as the SF-36, and the EORTC-QLQ C30. This instrument will be used to assist in the planned health economic's companion to this trial to assess economic endpoints regarding the study treatments and the condition.

Access to Prescription Drugs Survey (C-1156 and C-1414): This form asks patients about their prescription drug coverage plans and the extent to which paying for the costs of outpatient prescription drugs represents a hardship. A slightly modified and shortened version of the baseline survey focussed on strategies used to cope with prescription drugs is to be administered by the Telephone Interviewer at follow-up. The form asks patients to rate the degree of hardship they have regarding paying for medications, and methods they use (such as leaving prescriptions unfilled, taking others' medications) to cope with the cost of prescription drugs.

QOL Assessment Summary Form (C-419): In order to track data collection, and reasons for missing data, a QOL Assessment Summary Form will be completed on each patient after each scheduled interview. This form will code whether the interview was conducted, and if not, the reason why. It also provides a place to document any problems the interviewer encountered in completing the interview with the patient.

9.6 Data Collection

9.6.1 Baseline Data Collection

The baseline assessment of the patients' HRQL and prescription drug coverage will occur at the time the patients are recruited to the study, but prior to randomization. The institutional data manager will ask the patient to complete the initial HRQL and prescription drug surveys when informed consent is obtained. Completion of these forms while the patient is in the clinic will ensure that the patients complete the questionnaires prior to randomization; will enable the patients to ask questions regarding the forms so that follow-up telephone assessments will be facilitated; and will minimize problems with missing data on the baseline forms. The estimated time to complete these baseline forms is 45-50 minutes.

The baseline assessments must be completed prior to randomization and should be submitted to the Alliance Data and Statistics Center with copies sent to Michelle Naughton (see [Section 5.7.1](#)).

9.6.2. Follow-up Assessments

The follow-up evaluations will be conducted through telephone interviews conducted by staff at the Wake Forest University Health Sciences. [Section 5.7](#) provides a summary of the number of follow-up assessments and questionnaires to be administered to each study participant.

The administration of these instruments will take approximately 30 minutes at the 6 week and 6 month assessment points, and approximately 30-40 minutes at months 3 and 9.

10.0 DIET AND LIFESTYLE COMPONENT

10.1 Introduction

We propose to assess the influence of diet and other exogenous factors on progression-free survival, overall survival and treatment-related toxicity among over 2,000 patients with stage IV colorectal cancer. Patients enrolled on CALGB 80405 will be asked to complete a food-frequency questionnaire within the first month of treatment. The questionnaire proposed has been extensively validated among large populations and provides comprehensive data on 131 food items and over 100 micronutrients [45]. The instrument will also ascertain leisure-time physical activity, cigarette smoking, height and weight, aspirin and non-steroidal anti-inflammatory drug use, vitamin/supplement use, and alternative medicine use. This questionnaire can be self-administered; the expectation is that patients would complete and return the questionnaire at their outpatient visit.

10.2 Objectives

Epidemiologic and scientific research indicate that diet and other lifestyle factors have a significant influence on the risk of developing colon cancer. Consumption of red meat [46, 47], alcohol [48-50], calcium [51, 52], fiber [53], aspirin [54, 55], and folic acid [46, 49, 53], obesity [56-63], physical activity [60, 61, 63], and cigarette smoking [58, 64-66] are among factors that have been suggested to influence the risk of developing colorectal cancer.

Little is known about the influence of diet and other factors on the outcome for individuals with established colorectal cancer. Patients often seek to understand what, if any, diet and lifestyle changes will improved the efficacy of their therapy for metastatic colorectal cancer as well as the potential toxicities associated with chemotherapy. We propose the following specific aims:

- 10.2.1 To prospectively assess the influence of diet, body mass index, physical activity and other lifestyle habits on the progression-free survival and overall survival among patients with metastatic colorectal cancer.
- 10.2.2 To assess the influence of diet, obesity, physical activity, and other lifestyle habits on the risk of toxicity associated with chemotherapy.
- 10.2.3 In addition, exploratory analyses will investigate the interaction of diet and molecular markers within tumors on the prognosis of patients with stage IV colorectal cancer.

10.3 Background

After the diagnosis of cancer, many patients seek information about diet, physical activity, vitamin and mineral supplements use, and complementary therapies [67]. The psychology literature strongly supports the notion that when patients can exert or believe they can exert

control over a tragic event or disease, they can adjust to it more successfully [68, 69]. Taylor and colleagues reported that 56% of women being treated for breast cancer felt they have some or a lot of control over their care; one-half of those patients believed that changes in their attitude and lifestyle (including dietary changes) gave them control [68].

10.3.1 Diet, Lifestyle and Cancer-Survival

Limited data are available on the impact of diet and lifestyle on outcomes of patients with cancer. In women with breast cancer, there appears to be a tendency towards worse prognosis disease (nodal status, hormone receptor status, stage of disease) with increasing body mass index [70, 71], increased fat intake [72, 73], and lower carbohydrate and fiber intake [72]. Weight gain after diagnosis of early breast cancer has been associated with worse disease-free survival and recurrence rates [74-76].

In a small, nonrandomized study on the influence of selected vegetables on the survival of 19 patients with stage III-IV non-small cell lung cancer also treated with conventional therapies, patients treated with selected vegetables had longer median survival than those on conventional therapies only [77]. The influence of diet and lifestyle on prognostic factors in colorectal cancer is largely unknown. In a study using patients with colon cancer identified through the Utah Cancer Registry as a part of two population-based case-control studies, Slattery et al observed an improved survival with increasing consumption of calories, fat, and protein [78]. By contrast, the highest level of fiber intake was associated with a decreased survival when compared with the lowest level of intake. This study was limited by the small number of patients with advanced disease (only 59), heterogeneous patient population, and inability to adjust for treatment and other residual confounding.

Diet and lifestyle may influence cancer survival through several possible mechanisms. Nutrients can interact with chemotherapeutic agents, thereby influencing their efficacy. Recent studies suggest that vitamin E may enhance the cytotoxicity of 5-fluorouracil on colon cancer cells [79]. This effect was mediated by induction of p21^{WAF1/CIP1}, an inhibitor of the cell cycle. Other micronutrients have been proposed to inhibit cancer cell growth. Mathijssen et al demonstrated that patients taking St. John's Wort concurrent with chemotherapy have decreased levels of SN-38 (the active metabolite of irinotecan), with associated less myelosuppression; however, such an effect may potentially reduce efficacy of irinotecan [80]. In the recent intergroup trial of first-line therapy regimens for metastatic colorectal cancer, over 50% of patients needed to discontinue oxaliplatin, 5-FU, leucovorin therapy due to treatment-related toxicity and not disease progression [19]; inability to maximize benefit of a chemotherapy regimen can influence a potential survival benefit.

Laboratory studies have shown that smoking and alcohol intake alter the epidermal growth factor receptor (EGFR) [81-85]. Using whole saliva and buccal biopsies, Wang et al. demonstrated that salivary EGF concentrations were 32% lower in smokers with high cotinine levels and that EGFR autophosphorylation was altered in smokers [84]. Similarly, alcohol inhibited epidermal growth factor-stimulated autophosphorylation of EGFR in adults rats [85]. The efficacy of EGFR inhibition in patients by smoking status and frequency and alcohol intake is unknown in patients with metastatic cancer.

10.3.2 Diet, Lifestyle, and Chemotherapy Toxicity

Dietary constituents may influence chemotherapy toxicity by several means, including the induction or inhibition of enzymes responsible for the biotransformation and metabolism of chemotherapeutic agents. Earlier studies suggested that a high-protein-low-carbohydrate diet was associated with reduced toxicity from 5-FU [86].

Irinotecan is metabolized by a series of enzymes that are potentially influenced by exogenous factors. The principal toxicities of the drug are diarrhea and neutropenia, although individual patient experience will vary. In fact, the interpatient coefficient of variability of metabolism for irinotecan is among the highest for chemotherapeutic agents. Such variability may reflect both genetic as well as environmental factors.

Irinotecan can be converted to aminopentanecarboxylic acid (APC) by cytochrome P450 3A4 or it can be bioactivated by carboxylesterases to SN-38 [87]. SN-38 is eliminated mainly through conjugation by hepatic uridine glucuronosyltransferase (UGT). Deconjugation of SN-38 by enteric beta-glucuronidase may contribute to enterohepatic recirculation of SN-38 and delayed intestinal toxicity. Little is known regarding the influence of exogenous factors on carboxylesterase activity. Phenobarbital does appear to increase carboxylesterase activity in humans.

Several factors have been shown to influence P450 enzyme activity. Diets high in protein, garlic, vitamin C, red meat, and ethanol appear to increase P450 activity whereas diets high in carbohydrate and flavonoids reduce activity [88]. In addition, cigarette smoking appears to induce P450 activity possibly mediated by heterocyclic amines.

Activity of hepatic uridine glucuronosyltransferase (UGT) can be markedly influenced by exogenous factors. Phenobarbital, phenytoin, and carbamazepine all induce UGT activity whereas valproate reduces activity. Studies in animals and humans suggest that cruciferous vegetable, ascorbic acid, carotenoid, protein, and ethanol intake increase UGT activity [89-94]. In addition, smoking and obesity have been reported to increase UGT activity [92].

Takasuna et al observed that prophylactic use of Kampo medicines, inhibitors of beta-glucuronidase, reduced the incidence of irinotecan toxicity [95]. Other dietary constituents appear to affect beta-glucuronidase activity. Increased consumption of vegetables and fiber decrease beta-glucuronidase activity whereas higher fat intake increases beta-glucuronidase activity [96-99]. Such dietary patterns could influence the rate of irinotecan toxicity.

Oxaliplatin undergoes nonenzymatic conversion in physiological solutions to active derivatives via displacement of the labile oxalate ligand. Several transient reaction species are formed, including monaquo and diaquo DACH platinum, which covalently bind with macromolecules, resulting in inter- and intra-strand platinum-DNA crosslinks. Similar to other platinum derivatives (cisplatin and carboplatin), oxaliplatin can cause significant neurotoxicity, possibly by accumulation of platinum within the peripheral nervous system [100]. Recently, Cascinu et al reported on the protective effects of the antioxidant glutathione on the development of oxaliplatin-induced neuropathy [101]. The effects of diet and supplementation may also affect the incidence and severity of peripheral neuropathy. Other toxicities associated with platinum drugs may be altered with diet. In mouse models, diets high in zinc increase the therapeutic index of carboplatin, with reduced hematological toxicity [102]. Further, guinea pigs fed low-protein diets had significantly higher hearing loss with cisplatin [103]. In a small randomized trial, Pace and colleagues demonstrated that supplementation with vitamin E significantly reduced incidence of neurotoxicity among patients treated with cisplatin [104].

10.3.3 Diet and Lifestyle Survey in Colon Cancer Patients

As part of the recently completed CALGB randomized trial of adjuvant chemotherapy for stage III colon cancer, our group collected semi-quantitative food frequency and lifestyle questionnaires on nearly 1,000 patients. Response rate to an initial survey administered at the start of chemotherapy was 93%. Initial analyses of these results suggest potential important results from these type of studies. Preliminary analyses show that consistent

aspirin users have a decreased risk of colon cancer recurrences (Fuchs, ASCO 2005). Further, patients who were more physically active 1 year after surgery had a decreased rate of cancer recurrence and improved overall survival (Meyerhardt, ASCO 2005).

10.4 Hypotheses

As part of our analysis to prospectively assess the influence of diet, body mass index, physical activity and other lifestyle factors on progression-free survival and overall survival among patients with metastatic colorectal cancer, we will address the following hypotheses:

10.4.1 Regular physical activity and avoidance of obesity will improve progression-free survival and overall survival. Sedentary lifestyle and obesity are each associated with an increased risk of developing colon cancer. Among several studies, the most physical active participants experienced a 50 percent reduction in risk (relative risk = 0.50) [60, 63]. Both factors appear to act late in the pathway of colon carcinogenesis. Moreover, recent observations indicate that each factor may be mediated by enhancing levels of insulin-like growth factors, known trophic hormones for colon carcinogenesis [105, 106]. No study has assessed the influence of sedentary lifestyle and obesity among patients with metastatic colorectal cancer.

10.4.2 Increased red meat and animal fat consumption will decrease progression-free and overall survival. Increased red meat intake is associated with an increased risk of developing colon cancer. Among participants in the Nurses' Health and Health Professionals Follow-up Study, the highest consumers of red meat experienced a 2.75 to 3.5 fold increase in risk in developing colon cancer [46, 47] No study has assessed the influence of red meat among patients with metastatic colon cancer.

Of note, recent *in vitro* data suggests that unsaturated fatty acids and oleic acid can activate the epidermal growth factor receptor signaling pathway [107]. The interaction between fat intake, fat composition and response to EGFR inhibitors is unknown.

10.4.3 Increased vitamin E intake (coded in quintiles; with and without supplements) will improve progression-free and overall survival. Recent studies suggest that vitamin E may enhance the cytotoxicity of 5-fluorouracil on colon cancer cells. Chinery et al. observed that the antioxidants pyrrolidinedithiocarbamate and vitamin E induced apoptosis in colorectal cancer cells [79]. This effect was mediated by induction of p21WAF1/CIP1, a powerful inhibitor of the cell cycle, through a mechanism involving C/EBPbeta (a member of the CCAAT/enhancer binding protein family of transcription factors), independent of p53. Antioxidants significantly enhanced colorectal cancer tumor growth inhibition by 5-fluorouracil *in vitro* and *in vivo*. To date, no prospective studies have assessed this relation in the metastatic setting.

10.4.4 Increased folate intake will improve progression-free and overall survival. Studies suggest that increased intake of fruits and vegetables reduces the risk of developing colon cancer, possibly mediated through the folic acid content in fruits and vegetables [108]. Recent studies show that increased folic acid intake reduces the risk of developing colon cancer by more than 40% [48, 49, 109, 110]. No study has assessed the influence of folate intake among patients with established colon cancer.

10.4.5 Smoking and alcohol intake will alter the efficacy of cetuximab therapy. Laboratory studies have shown that smoking and alcohol intake alter the epidermal growth factor receptor (EGFR) [81-85]. Using whole saliva and buccal biopsies, Wang et al demonstrated that salivary EGF concentrations were 32% lower in smokers with high cotinine levels and that EGFR autophosphorylation was altered in smokers [84]. Similarly, alcohol inhibited

epidermal growth factor-stimulated autophosphorylation of EGFR in adults rats [85]. The efficacy of EGFR inhibition in patients by smoking status and frequency and alcohol intake is unknown in patients with metastatic cancer.

10.4.6 Increased glycemic index will decrease progression-free survival and overall survival.

Carbohydrates with differing physical forms, particle sizes, chemical structures, and fiber contents induce different plasma glucose and insulin responses [111]. The physiological response to carbohydrates can be quantified by glycemic index [111-113]. The glycemic index is a qualitative assessment of foods based on the incremental glucose response and insulin demand they produce for a given amount of carbohydrates. Foods with low glucose indexes have reduced serum insulin and glucose responses, compared to foods with high glucose indexes [114-117]. Using data from a population-based case-control study with 1,993 cases of colon cancer, Slattery et al. demonstrated that in patients under age 67, highest levels of sucrose intake compared to lowest levels had an odds ratio for developing colon cancer of 1.59 (95% confidence interval 1.07-2.37) [118]. In all subjects studied, increased sucrose-to-dietary fiber ratio led to increased risk of colon cancer development. Similarly, Franceschi et al conducted a case-control study on colorectal cancer in Italy [119]. They showed an odds ratio for colorectal cancer development in the highest compared to lowest glycemic index quintile of 1.7 (95% confidence interval 1.4-2.0) and, for glycemic load, an odds ratio of 1.8 (95% confidence interval 1.5-2.2) [119]. The effects of carbohydrate intake on patients with colorectal cancer is unknown.

In further exploratory analyses, we will also consider looking at the relationship between calcium, vitamin D, fiber, aspirin and NSAID use, and alternative therapies on progression-free and overall survival. This latter goal is further supported by the recent finding by Mathijssen et al on the decreased SN-38 (the active metabolite of irinotecan) in patients taking St. John's Wort concurrent with chemotherapy [80]. Additionally, we will study the effects of diet and lifestyle factors on treatment-related toxicities.

10.5 Methods

This is a companion substudy to CALGB 80405, in which 2,289 patients with stage IV colorectal cancer will be treated with either FOLFIRI (5-FU, leucovorin, irinotecan) or FOLFOX (5-FU, leucovorin, oxaliplatin) and randomized to either cetuximab, bevacizumab or the combination of cetuximab and bevacizumab. Patients must have histologically-confirmed adenocarcinoma of the colon or rectum, had no prior chemotherapy for metastatic disease, have adequate bone marrow, liver and renal function, and meet other eligibility criteria listed in the protocol. In companion correlative studies, the relationship of specific germline polymorphisms and other putative prognostic markers will be correlated with overall survival and treatment-related toxicities.

In this companion study, we would propose that patients participating in the treatment trial be asked to complete a 131-item validated, food-frequency questionnaire within the first one months of treatment. The questionnaire proposed was designed by Dr. Walter Willett and colleagues for the Nurses' Health Study, has been extensively validated among both health professional and lay populations, and provides comprehensive data on over 100 micro-nutrients, with and without supplement use. This questionnaire can be self-administered. Patients would complete the questionnaire during their 1st or 2nd infusion of chemotherapy. Within the questionnaire, we will also include a series of questions about leisure-time physical activity, smoking habits, alcohol intake, and other habits that have also been validated in large populations. Height and weight will also be obtained as part of the adjuvant therapy. A similar study was initiated in the preceding intergroup trial (CALGB 80203) and 98% of patients completed the questionnaire.

Validation of the Semi-quantitative Food Frequency Questionnaire: The current version of the questionnaire consists of 131 food items plus vitamin and mineral supplement use that collectively account for over 90% of the intake of the nutrients assessed [45, 120-123]. For each food, a commonly used unit or portion size (e.g., one egg or slice of bread) is specified, and participants are asked how often, on average over the past year, they consumed that amount of each food. There are nine possible responses, which range from never to six or more times per day. We will compute nutrient intakes by multiplying the frequency of consumption of each food by the nutrient content of the specified portions, using composition values from Department of Agriculture sources supplemented with other data, including the components of specific vitamins and breakfast cereals. All nutrients will be adjusted for total energy intake by the residuals method [124].

In 1980, the food frequency questionnaire was administered twice to 173 individuals at an interval of approximately one year, and four one-week diet records for each subject were collected during that period. Diet records probably are the best measures of current, short-term food intake. Since the seven-day record provides information for a relatively short period of time, four one-week diet records in different seasons were collected. The mean calorie adjusted intakes from the four one-week diet records and those from the questionnaire were well-correlated [45, 122, 123]. In the 1986 diet validation study, the correlation between folate calculated from the semi-quantitative food frequency questionnaire (SFFQ) and red cell folate level was 0.55 [49]. Nutrients calculated from the expanded SFFQ were correlated with other corresponding biochemical indicators: plasma beta-carotene ($r = 0.30-0.42$) [125, 126], plasma vitamin E ($r = 0.41-0.53$) [125, 126], adipose linoleic acid ($r = 0.35-0.37$) [127, 128], adipose trans fatty acid ($r = 0.51$) [127, 128], and adipose N-3 fatty acids ($r = 0.48-0.49$) [127, 128]. To evaluate further the capability of the revised 131-item questionnaire to discriminate among subjects, Willett and colleagues asked 127 individuals to complete two weeks of diet records and the semiquantitative food frequency questionnaire in 1986. The mean calorie adjusted intakes from the diet records and those from the questionnaire were well-correlated [122].

These data indicate that the proposed self-administered dietary questionnaires provides highly informative and biologically relevant measurement of a wide variety of nutrients, thus allowing one to address the dietary hypotheses outlined in the specific aims.

In terms of other measures from the survey, Wolf et al reported on a detailed validation study of the physical activity questionnaire among a sample of 325 participants in the parallel Nurses' Health Study II (NHS II) (241 random cohort sample and 84 random sample of African American participants) [129]. Participants completed four 1-week activity recalls and four 7-day activity diaries over one year and then repeated the NHS II activity questionnaire. For the total activity score, the correlations of the last activity questionnaire with the diaries was 0.64 for the total cohort sample and 0.59 for the African American sample. Within the Health Professionals Follow-up Study, a parallel study of men, validity of the physical activity questionnaire was assessed among 238 randomly selected participants by comparisons with four 1-week activity diaries, four 1-week activity recalls, and resting and post exercise pulse rates [130]. Correlations with the activity diaries were 0.41 for inactivity (sitting) and 0.58 for vigorous physical activity. Vigorous activity assessed by the questionnaire was correlated with resting pulse ($r = -0.45$) and post-exercise pulse ($r = -0.41$).

Methods of Analysis for the Diet and Lifestyles Substudy: We plan to assess the influence of various dietary constituents and lifestyle habits on time to progression and overall survival in patients with metastatic colorectal cancer. Among the factors we propose to assess include: intake of red meat, fat, vitamin E, folate, alcohol, carbohydrates, calcium, fiber, vitamin D, and ASA/NSAIDs. We will also look at the influence of body mass index, physical activity, and cigarette smoking on the outcome of these patients. We will also assess the influence of diet and

other factors on toxicities associated with chemotherapy. Lastly, we will explore the interaction of diet and other factors with various molecular markers on the outcome of patients with stage IV colorectal cancer.

11.0 DRUG FORMULATION, AVAILABILITY AND PREPARATION

11.1 Qualified Personnel

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

11.2 Drug Disposition

Discard unused portions of injectable chemotherapeutic agents supplied as single-dose preparations within eight hours of vial entry to minimize the risk of bacterial contamination.

11.3 Drug Dosing Guidelines

The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose. It is not necessary to change the dose of chemotherapy or cetuximab administered unless the calculated dose changes by $\geq 10\%$.

11.4 Oxaliplatin [Eloxatin]

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 is commercially available. In the event that third party coverage is not available, the ELOXATIN Reimbursement hotline provides information regarding access to oxaliplatin via a patient assistance program. The phone number for the hotline is 1-877-435-6928 (hours for the hotline are Mon-Fri 9 AM to 8 PM ET)

When calling the hotline, notify the reimbursement specialist that the patient is enrolled on CALGB 80405. In addition, you will be asked to provide the following information:

- Physician's name, address, telephone and fax number, and tax ID number
- Patients name, address, telephone number and social security number
- Insured's name address, telephone number and social security number
- Name, address and telephone number of the patient's insurer
- Patient's insurance policy number and group coverage number
- Copy of claim (if applicable)
- Insurers explanation of denials of benefits (if applicable)

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.

Preparation

The calculated dose of oxaliplatin should be diluted for infusion with 250 mL to 500 mL 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.

Administration

Oxaliplatin will be administered by intravenous infusion over 120 minutes in patients receiving FOLFOX. Infusion time may be prolonged (up to 6 hours) in patients 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.

Toxicity

The most commonly observed oxaliplatin toxicities include neurotoxicity, GI toxicity, and myelosuppression. Three neurotoxicity syndromes have been seen: acute sensory neuropathy develops within hours to 2 days after oxaliplatin administration. Symptoms include, paresthesias, dysesthesias, and hypoesthesia of the hands, feet and perioral region. Jaw spasm, abnormal tongue sensation, dyarthria, eye pain and a sensation of chest pressure have also been noted. Acute sensory neuropathy symptoms may be exacerbated by exposure to cold temperature or cold objects. Symptoms are reversible, usually resolving within 14 days and commonly recurring with further dosing. This syndrome has been observed in about 56% of patients receiving oxaliplatin with 5-FU and leucovorin.

Acute pharyngolaryngeal dysesthesia is reported to occur in 1-2% of patients. This syndrome is characterized by a subjective sensation of difficulty breathing or swallowing without laryngospasm or bronchospasm or objective evidence of hypoxia. Avoidance of cold drinks, food and air is suggested in order to minimize pharyngolaryngeal dysesthesia. Antianxiety agents (e.g. lorazepam) may be used to treat pharyngolaryngeal dysesthesias once oxygen saturation has been documented to be normal.

Peripheral neuropathy persisting > 14 days is characterized by paresthesias, dysesthesias, and hypoesthesia. Abnormalities in proprioception may also be seen. Symptoms of persistent neuropathy may improve upon discontinuation of oxaliplatin.

Various agents have been used in an attempt to minimize neurotoxicity of oxaliplatin (e.g. carbamazepine, Mg⁺, Ca⁺⁺). Calcium and magnesium infusions appear to be beneficial in preventing neurotoxicity. Contrary to preliminary findings described in 2007, calcium and magnesium do not appear to interfere with tumor response to FOLFOX. Calcium and magnesium infusions are generally given before and after oxaliplatin, and should not be prepared in the same infusion solution as FOLFOX components.

Gastrointestinal toxicities include nausea, vomiting (oxaliplatin is considered to be moderately emetogenic) and diarrhea.

Neutropenia is reported in 73% of patients receiving oxaliplatin with 5-FU and leucovorin (44% grade 3 or 4). Grade 3 or 4 thrombocytopenia is reported to occur in 4% of patients receiving the combination.

Allergic reactions, similar to those seen with other platinum compounds, have also been observed in patients treated with oxaliplatin. Reactions range from rash to anaphylaxis.

Rarely, oxaliplatin has been associated with pulmonary fibrosis, which may be fatal. Oxaliplatin should be discontinued in the presence of unexplained pulmonary symptoms (e.g. nonproductive cough, dysphagia) or pulmonary infiltrates until interstitial lung disease or pulmonary fibrosis have been ruled out.

Recent reports of oxaliplatin extravasation suggest that tissue necrosis may result and that oxaliplatin should be considered a vesicant. No standard treatment exists for oxaliplatin extravasation although heat and sodium thiosulfate have both been suggested.

Veno-occlusive disease (VOD) of the liver is a rare complication associated with oxaliplatin and 5-FU. Clinical manifestations of VOD include hepato-megaly, ascites, and jaundice. Histologically, VOD is characterized by diffuse damage in the centrilobular zone of the liver. Sequelae of VOD include hepatomegaly, splenomegaly, portal hypertension, and esophageal varices. A recent analysis of resected liver metastases in 153 patients indicated histological findings consistent with VOD in 6/27 patients who received 5-FU alone, 4/17 patients who received 5-FU and irinotecan, 20/27 patients who received 5-FU and oxaliplatin, and 14/16 who received 5-FU, oxaliplatin and irinotecan. The remaining 66 patients had not received chemotherapy prior to resection. There were no such findings in these patients.

For more information on toxicities associated with oxaliplatin, please see the package insert.

11.5 5-Fluorouracil (5-FU; fluorouracil; Adrucil[®])

Please refer to the package insert for complete product information.

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.

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 antiemetics.

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.

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 to 48 hours.

Toxicity

Nausea, diarrhea, vomiting (mild); stomatitis: 5–8 days after treatment initiation; myelosuppression: granulocytopenia (9–14 days); thrombocytopenia (7–14 days); Alopecia; loss of nails; hyperpigmentation; photosensitivity; Maculopapular rash; palmar–plantar erythrodysesthesias: (42–82% receiving continuous infusion); CNS effects: cerebral ataxia (rare); Cardiotoxicity: MI, angina; asymptomatic S–T changes 68%; ocular effects: excessive lacrimation and less commonly, tear duct stenosis.

Drug Interactions

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. Patients receiving both drugs should be followed with weekly INRs.

11.6 Leucovorin Calcium (Folinic Acid) Leucovorin Calcium (calcium folinate; citrovorum factor; N 5-formyltetrahydrofolate; 5-formyl-FH4; folinic acid).

Please refer to the package insert for complete product information.

Availability

Leucovorin calcium is commercially available in: 50 mg, 100 mg, 350 mg vials for reconstitution.

Storage and Stability

Intact vials should be stored at room temperature and protected from light. Solutions reconstituted with BWI are stable for at least 7 days at room temperature.

Preparation

Leucovorin may be reconstituted with Bacteriostatic Water for Injection (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.

Administration

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

Toxicity

The only adverse reactions associated with leucovorin are allergic reactions. These are extremely uncommon.

11.7 Irinotecan (CPT-11, CAMPTOSAR®)*Availability*

Irinotecan is commercially available as a 20 mg/mL solution for injection in 2 mL and 5 mL vials.

Storage and Stability

Intact vials should be stored at controlled room temperature 59° to 86° F (15° to 30° C) and when protected from light. Solutions diluted in D5W are reported to be stable for 48 hours under refrigeration and protected from light. Irinotecan solutions should not be frozen as the drug may precipitate.

Preparation

Irinotecan is diluted in 5% dextrose (D5W) 500 mL to a final concentration of 0.12 – 1.1 mg/mL.

Administration

In this study irinotecan will be administered as an IV infusion over 90 minutes in patients receiving FOLFIRI.

Toxicities

Virtually all phase I and II studies of irinotecan have reported neutropenia and/or late diarrhea (diarrhea occurring more than 24 hours after irinotecan administration) as the dose-limiting toxicities (depending upon the schedule). Other commonly observed adverse events include nausea and vomiting, anorexia, abdominal cramping, alopecia, asthenia, lymphocytopenia, and anemia. Dehydration has occurred as a consequence of diarrhea, particularly when associated with severe vomiting. Patients may have an acute syndrome of lacrimation, diaphoresis, abdominal cramping, and diarrhea (early diarrhea) during or shortly after irinotecan administration; this syndrome is thought to be cholinergically mediated and may be treated and

subsequently prevented with atropine. Sporadic cases of pulmonary toxicity, manifested as shortness of breath, nonproductive cough, and transient infiltrates on chest X-ray have been reported. Infrequent occurrences of mucositis or colitis (sometimes with gastrointestinal bleeding) have been observed. Occasionally, abnormalities of serum creatinine, hepatic enzymes, or thrombocytopenia have been observed.

Further information regarding irinotecan may be obtained from the package insert.

11.8 Bevacizumab (rhuMAb VEGF, Avastin[®]) (NSC #704865)

Bevacizumab 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 vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.

Availability

Bevacizumab is available commercially in vials of 100 mg at a concentration of 25 mg/mL.

Storage and Stability

Intact vials of bevacizumab should be stored in a refrigerator (2° to 8° 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 eight hours after initial entry. Solutions diluted for infusion may be stored in a refrigerator for up to 8 hours.

Preparation

The calculated dose of bevacizumab should be diluted in 100 mL of 0.9% Sodium Chloride for Injection.

Administration

Bevacizumab is administered as an intravenous infusion. 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.

Toxicities

According to the package information for bevacizumab, the most serious adverse events associated with bevacizumab to date are gastrointestinal perforations/wound healing complications, hemorrhage, arterial thromboembolic events, hypertensive crises, nephrotic syndrome, and congestive heart failure.

Hypertension is among the most common adverse events associated with bevacizumab to date. Both new hypertension and worsening of existing hypertension have been reported. Hypertension may require treatment; ACE inhibitors, calcium channel blockers, beta blockers, and diuretics have all been reported to be effective in this setting. Although most hypertension can be controlled by medication, hypertensive crises have been reported in several studies, and the end organ consequences included CNS bleeding and ischemia, and congestive heart failure

Proteinuria, ranging from asymptomatic abnormal urinalysis to nephrotic syndrome, has been described in 10% or more of patients receiving bevacizumab. Proteinuria is managed with dose modifications as described in [Section 8.12](#).

Bleeding, including fatal CNS hemorrhage, has been reported. Bleeding at tumor sites or at sites of other pre-existing abnormalities (e.g., diverticulosis, hemorrhoids) has also been described. In a phase III study, fatal hemoptysis occurred in 2 of 55 patients with non-small cell lung cancer, both of whom had a history of hemoptysis. The rate of fatal hemoptysis in non-squamous NSCLC is estimated at 1-2%. Epistaxis is usually short-lived and resolves without treatment, although some episodes may require medical intervention. DIC has been described in a few patients receiving bevacizumab in combination with oxaliplatin, fluorouracil and leucovorin.

Thrombosis/embolism Both arterial and venous thromboses (including pulmonary embolism, mesenteric vein thrombosis, ischemic bowel, cerebral vascular accident), and myocardial infarction have been described in patients receiving bevacizumab. Fatal pulmonary embolus has also been described.

With regard to arterial thromboses (which include myocardial infarction, transient ischemic attack, cerebrovascular accident/stroke, and angina/unstable angina), recent studies indicate that the risk with bevacizumab and chemotherapy is 2-3 times (up to 5%) that of chemotherapy alone. Furthermore, certain baseline characteristics, specifically age > 65 years and prior thromboembolic event, conferred additional risk.

Hepatic Dysfunction: Reversible and marked elevations of liver function tests (total bilirubin and/or transaminase and AP) have been rarely reported when bevacizumab is used in combination with chemotherapy or concurrently with other drugs that are potentially hepatotoxic. The mechanism of such hepatic toxicities is unclear. It is possible that on rare occasions, bevacizumab may potentiate the liver side effect of a concurrent medication, although it is unclear at this time whether bevacizumab alone can cause LFT derangement.

Bowel perforation and bowel anastomotic dehiscence have been reported in clinical trials using bevacizumab alone or in combination with chemotherapy. Although these events were also related to co-existing factors such as tumor involvement, chemotherapy, recent invasive procedures or bowel inflammation, they have occurred at an increased rate in patients receiving bevacizumab. A fatal bowel perforation has been described. **GI perforation should be included in the differential diagnosis of patients receiving bevacizumab therapy presenting with abdominal pain or rectal/abdominal abscess.** Partial delay in wound healing has been demonstrated in animal models treated with anti-VEGF antibodies and it is possible that bevacizumab may delay or compromise wound healing in patients.

Reversible posterior leukoencephalopathy syndrome (RPLS) or similar leukoencephalopathy syndrome: RPLS or clinical syndromes related to vasogenic edema of the white matter have been recently reported in association with bevacizumab therapy. These syndromes have been seen in < 1% of patients to date. Clinical presentations are variable and may include altered mental status, seizure and cortical visual deficit. HTN is a common risk factor and was present in most (though not all) patients on bevacizumab who developed RPLS. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypointensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure or other CNS findings. RPLS is potentially reversible, but timely correction of the underlying causes, including control of BP and interruption of the offending drug, is important in order to prevent progression to irreversible tissue damage.

Infusion reactions, including fever, chills, rigors, rash, urticaria, dyspnea, and hypersensitivity reactions have been reported in approximately 3% of patients.

Neutropenia: When combined with chemotherapy, bevacizumab is reported to increase the risk of neutropenia over that of chemotherapy alone. Grade 3-4 neutropenia, febrile neutropenia, or

increased rate of infection were increased in studies in which bevacizumab with chemotherapy (IFL, paclitaxel and carboplatin) was compared to chemotherapy alone.

Other toxicities: Other reported or potential toxicities associated with bevacizumab include:

- Constitutional—Headache, infection without neutropenia, asthenia Cardiovascular—
- Hypotension, pericardial effusion, congestive heart failure
- Skin—Rash, urticaria
- Gastrointestinal—Nausea, vomiting, stomatitis/pharyngitis, colitis, intestinal obstruction
- Pulmonary—Pulmonary infiltration, dyspnea
- Musculoskeletal—Arthralgia, chest pain

Note that additional toxicities may be associated with combination chemotherapy. **For a comprehensive list of adverse events and potential risks (CAEPR), see [Section 16.3](#). See the most current version of the package insert for additional information.**

11.9 Cetuximab (C-225)

Please refer to the package insert and the Physician’s Desk Reference (PDR) for additional information. To obtain a copy of the Investigator’s Brochure contact the Alliance Central Office at (773) 702-9171.

Availability

Cetuximab will be supplied free-of-charge by Eli Lilly and Company (Lilly) in single-use, ready-to-use 50-mL vials at a concentration of 2 mg/mL.

Drug Ordering and Accountability

As of November 2015, Lilly will be distributing cetuximab in the US for this trial. Following submission of the required regulatory documentation to the RSS, a supply of cetuximab may be ordered. Depending on the patient’s BSA, 7-9 vials for an initial dose, and 4-6 vials for weekly maintenance doses will be needed. A suggested initial shipment is 20 vials. Allow 5-7 business days for shipment of drug from receipt of the request. Complete the Drug Request Form located on the Alliance (US) or CTSU (US) websites. The form should be submitted to the respective distributor via email.

All products will be shipped via Federal Express in a temperature-controlled container. Shipments will be made on Monday through Thursday for delivery Tuesday through Friday. There will be no weekend, holiday or Monday delivery of drugs. It is possible that sites may have more than one cetuximab clinical study ongoing at the same time. It is imperative that only product designated for CALGB 80405 (CA225245) be used for this study. The cetuximab supplies are study-specific but not patient-specific. Cetuximab ordered for CALGB 80405 may be used for multiple patients on this study.

Inside each shipping container, a disposable electronic unit (TagAlert[®]) to ensure the product has remained at the appropriate temperature during shipping may be included. This unit may be attached to an information card. The LCD display will show “OK” (indicating no alarm has been triggered) or a black bar and the number(s) 1-4 (indicating an alarm/alerts have been triggered). Display results should be recorded on the packing list. For questions regarding drug requisitioning or shipment contact the respective distributor via the contact information on the order form.

For temperature excursions or other product concerns, please review the slides on the Alliance website detailing product complaints, and complete the product complaint form, also found on the Alliance website. Email the form to the address found on the form, within 24 hours of discovery of the product concern.

Important Reorder Instructions

Reorders should be emailed directly to the distributor for shipment within 5 to 7 business days. When assessing need for resupply, institutions should keep in mind the number of vials used per treatment dose (~7-9 for initial dose, ~4-6 for weekly maintenance doses, dependent on patient's BSA), and the estimated delivery time.

Receipt of Drug Shipment

Study drug shipments may include a TagAlert[®] unit and attached information card (see above for description) and a clinical supply packing list (CSPL). If included, the pharmacist/study personnel responsible for the clinical study product will need to indicate the condition of the shipment, record the TagAlert[®] results, and sign the CSPL in the designated areas.

Drug Destruction and Return

All empty and partial vials will be destroyed at the site according to institution's policy for drug destruction. At the completion of the study, all unused study drugs will be destroyed at the site according to the institution's policy for drug destruction. Please maintain appropriate records of the disposal, including dates and quantities.

Storage and Stability

Intact vials of cetuximab must be stored under refrigeration at 2° to 8° C (36° to 46° F). Cetuximab vials can reportedly withstand "temporary excursion" to room temperature without adverse effect. Storage at room temperature, however, is not recommended. **DO NOT FREEZE.** Preparations of cetuximab in infusion containers are chemically and physically stable for up to 12 hours at 2° to 8° C (36° to 46° F) and up to 8 hours at controlled room temperature (20° to 25° C; 68° to 77° F).

Preparation

The calculated amount of cetuximab should be withdrawn from the appropriate number of vials and injected into an empty sterile container (glass, PVC or non-PVC plastic) for IV infusion. Cetuximab should not be mixed with other drugs or diluted with infusion solutions. **Do not shake.**

Administration

Cetuximab will be administered weekly in Arms B and C. The initial dose of 400 mg/m² is to be administered by IV infusion over 120 minutes. In the absence of infusion reactions, subsequent doses of 250 mg/m² are to be administered over 60 minutes weekly.

Cetuximab should be administered through a 0.22 micrometer in-line filter. The solution should be clear and colorless and may contain a small amount of easily visible white amorphous cetuximab particulates. **DO NOT SHAKE OR DILUTE.** Cetuximab can be administered via infusion pump or syringe pump but must not be administered as an IV push or bolus. Cetuximab should be piggybacked to the patient's infusion line. Prime the infusion line with cetuximab before starting the infusion. Maximum infusion rate should not exceed 5 mL/min (300 mL/hour). Use 0.9% saline solution to flush the line at the end of the infusion. Following the cetuximab infusion, a one hour observation period is recommended.

In an effort to prevent a hypersensitivity reaction, all patients should be premedicated with diphenhydramine hydrochloride 50 mg (or an equivalent antihistamine) by IV given 30-60 minutes prior to the first dose of cetuximab. Premedication may be administered prior to subsequent doses, but at the Investigator's discretion, the dose of diphenhydramine (or a similar agent) may be reduced.

Patients should be closely monitored for treatment-related adverse events, especially hypersensitivity reactions, during the infusion and the post-infusion observation hour. For the

duration that patients are on protocol therapy, adverse event monitoring will be done continuously. Patients will be evaluated for adverse events at each visit and are to be instructed to call their physician to report any clinically significant adverse events between visits.

Toxicities

Acne-like Rash: The most common adverse event associated with cetuximab administration is acne-like rash. Acne-like rash usually occurs on the face, upper chest, and back, but occasionally extends to the extremities and is characterized by multiple follicular or pustular appearing lesions characterized histologically as a lymphocytic perifolliculitis or suppurative superficial folliculitis. The onset of rash is generally within the first 2 weeks of therapy.

A number of therapeutic interventions have been attempted, including oral and topical antibiotics, topical steroids, and rarely, oral steroids. The value of these measures is unknown since definitive clinical trials have not been performed. The etiology of the acne-like skin rash is believed to be the result of cetuximab binding to EGFR in the epidermis.

Nail Disorder: Nail disorders were reported in 6.4% of patients receiving cetuximab as a single agent. The nail disorder is characterized as paronychia inflammation with associated swelling of the lateral nail folds of the toes and fingers. The most commonly affected digits are the great toes and thumbs. According to investigators, the nail disorder persists for up to 3 months after discontinuation of cetuximab. Soaks in aluminum acetate (Burow's) solution BID-QID will prevent secondary infection. Symptom relief may be achieved with standard bandages or with the application of liquid bandages (cyanoacrylate preparations). Preliminary analysis in patients treated at usual doses (400 mg/m² initial dose, followed by 250 mg/m² weekly), revealed that the incidence of nail disorders is greater in patients who received more than 6 infusions (approximately 10%) compared to patients treated with 6 or less infusion of cetuximab (approximately 3%).

Allergic Reactions: The majority of the allergic/hypersensitivity reactions described have been grade 1 to 2 toxicities with less occurrences of grade 3-4. All reactions responded promptly to appropriate medical intervention.

Infusion Reaction: Infusion reactions are distinct from allergic or hypersensitivity reactions, although some of the manifestation are overlapping. Infusion reactions generally develop during or shortly after the infusion. Mild infusion reactions (chills, fever, dyspnea) have been reported in 23% of patients receiving cetuximab as a single agent. Severe infusion reactions (airway obstruction [bronchospasm, stridor, hoarseness], urticaria, hypotension) were reported in 2% of patients receiving single agent cetuximab. Infusion reactions occur most often with the first dose.

Pulmonary Toxicity: Interstitial lung disease has been reported in less than 1% of patients who have received cetuximab for advanced colorectal cancer, and in one patient with head and neck cancer.

Cardiac Toxicity: Recent reports described the occurrence of cardiac dysfunction, chest pain, and/or cardiac ischemia/infarction. These events occurred in patients who were receiving cetuximab in addition to 5-FU-containing therapy. Cardiac ischemia/infarction and acute cardiomyopathy are known side effects of 5-fluorouracil-based chemotherapy. It is presently unclear if the addition of cetuximab may increase the risk of 5-FU-related cardiac events.

Other Toxicities: Other reported or potential toxicities associated with cetuximab include:

Skin—Pruritis (10%), alopecia

Gastrointestinal—Diarrhea (28%, 2% grade 3/4), nausea/vomiting (29%),
stomatitis/mucositis (11%), anorexia, constipation

Metabolites—Hypomagnesemia (rarely grade 4 requiring aggressive IV repletion)

Pulmonary—Interstitial pneumonitis was reported in 3 of 633 (<0.5%) patients; one patient died as a consequence.

EENT—Conjunctivitis (7%) Constitutional—

Fatigue/malaise, asthenia, infection

Musculoskeletal—Back pain Hematologic—

Anemia, leukopenia CNS—Headache (25%, 3% grade 3/4)

12.0 ANCILLARY THERAPY

12.1 Supportive Care

Patients should receive *full supportive care*, including transfusions of blood and blood products, epoetin, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded.

12.2 Hormonal/Other Chemotherapeutic Agents

Treatment with *hormones or other chemotherapeutic agents* may not be administered except for steroids given for adrenal failure; hormones administered for non-disease-related conditions (e.g., insulin for diabetes); and intermittent use of dexamethasone as an antiemetic.

12.3 Palliative radiation therapy

Palliative radiation therapy may not be administered except for whole-brain irradiation given for documented CNS disease. Do not hold protocol therapy during CNS irradiation. Irradiate a symptomatic lesion, or one that may produce disability (e.g., unstable femur) prior to study initiation, provided other measurable disease is present.

12.4 CALGB 80405 Policy Concerning the Use of Growth Factors

The use of epoetin (EPO) is **permitted** at the discretion of the treating physician. Filgrastim (G-CSF), pegfilgrastim, and sargramostim (GM-CSF) treatment for patients is discouraged. They may not be used:

- To avoid dose reductions or delays,
- Prophylactically because of concern about myelosuppression from prior chemotherapies,
- For the treatment of febrile neutropenia

The use of CSF's should not be routinely instituted as an adjunct to appropriate antibiotic therapy. However, the use of CSF's may be indicated in patients who have prognostic factors that are predictive of clinical deterioration such as pneumonia, hypotension, multi-organ dysfunction (sepsis syndrome) or fungal infection, as per the ASCO guidelines. Investigators should therefore use their own discretion in using the CSF's in this setting. The use of CSF must be documented and reported on the CALGB C-260 Remarks Addenda.

If a CSF is used, it must be obtained from commercial sources.

12.5 Antiemetics

Patients should receive antiemetics before irinotecan or oxaliplatin according to the treating physician and institutional guidelines.

The use of aprepitant is prohibited for those patients receiving FOLFIRI.

It has been suggested that prochlorperazine increases the risk of irinotecan toxicity and it should be avoided on the day of infusion of irinotecan. Patients should also avoid other phenothiazines while receiving irinotecan.

12.6 Anticoagulants

Prophylactic warfarin administration (1 mg/day) is allowed to prevent Portacath or central venous access thrombosis. If warfarin (or similar agents) are used, it is recommended that INR be monitored carefully (at least weekly). Subcutaneous heparin is permitted.

Prophylactic therapy for venous thromboembolic disease such as mini-dose heparin or low molecular weight heparin is encouraged for patients during periods of risk for thrombotic events (such as patients with limited activity due to hospitalization).

Patients with a history of coronary artery disease should be maintained on low-dose aspirin prophylaxis if medically indicated.

12.7 Acne

While there is no clear evidence of benefit, over-the-counter or prescription acne medications designed to treat the acne-like rash may be used for patients who experience the acne-like rash associated with EGFR inhibitors. The investigator could also consider concomitant treatment with topical and/or oral antibiotics; topical corticosteroids are not recommended.

13.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE (SOLID TUMORS):

For the purposes of this study, patients should be reevaluated every 8 weeks. At each interval evaluation, a determination of the possibility of a curative surgical intervention – R0 resection of all metastatic disease – should be considered. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

13.1 Target Lesions

All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

13.1.1 Complete Response: Disappearance of all target lesions.

13.1.2 Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.

13.1.3 Progression (PD): At least a 20% increase in the sum of the LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

13.1.4 Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started. Patients having a documented response with no reconfirmation of the response will be listed with stable disease.

13.2 Non-target Lesions

All other lesions (or sites of disease) not included in the “target disease” definition should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as “present” or “absent.”

13.2.1 Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

13.2.2 Non-complete response (non-CR)/Non-progression (non-PD): Persistence of one or more non-target lesion

13.2.3 Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

13.3 Cytology and Histology

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

These techniques can be used to differentiate between PR and CR in rare cases (for example, 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.

13.4 Evaluation of Best Overall Response

The best overall 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). In general, the patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria (see [Section 13.6.1](#)).

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “symptomatic deterioration” on the CALGB Treatment and Response Form (C-1398) under “other.” Every effort should be made to document the objective progression even after discontinuation of treatment.

- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

13.5 Guidelines for Evaluation of Measurable Disease

- 13.5.1 Clinical Lesions** will only be considered measurable when they are superficial (e.g., skin nodules, 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.
- 13.5.2 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.
- 13.5.3 Conventional CT and MRI** 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 the chest, abdomen, and pelvis.
- 13.5.4 Ultrasound (US)** should not be used to measure tumor lesions that are clinically not easily accessible when the primary endpoint of the study is objective response evaluation. It is a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- 13.5.5 Endoscopy and Laporoscopy** 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 can be useful to confirm complete pathological response when biopsies are obtained.
- 13.5.6 Tumor Markers** alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

13.6 Confirmation Measurement/Duration of Response

13.6.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks.

13.6.2 Duration of Overall Response

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

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

13.6.3 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.

14.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY**14.1 CR, PR, or SD**

Continue treatment until the appearance of disease progression.

14.2 Surgical Resection

Patients may be removed from protocol therapy to undergo surgical resection of metastatic disease.

14.3 Disease Progression

Patients should receive a minimum of two cycles of therapy. Patients should discontinue protocol therapy if they experience rapid disease progression. Document details, including tumor measurements.

14.4 Extraordinary Medical Circumstances

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair(s).
- Document the reason(s) for discontinuation of therapy on the CALGB C-260 Remarks Addenda form.
- Follow the patient as appropriate per study.

15.0 STATISTICAL CONSIDERATIONS**15.1 Study Design and Hypotheses**

With the implementation of Update #5 to this study, only patients with K-ras wild type (WT) tumors are eligible for treatment on this protocol.

The choice of chemotherapy between FOLFOX and FOLFIRI is at the treating physician's discretion, but must be declared prior to randomization and must not be changed during the course of the patient's treatment. Patients are then randomized with equal probability to treatment with the biologic bevacizumab or with cetuximab.

Random treatment assignments for K-ras WT patients will be generated according to randomly permuted blocks within strata. Patients will be stratified at the time of randomization by: 1) physician-selected chemotherapy (FOLFOX; FOLFIRI); 2) prior adjuvant therapy (no; yes); and

3) prior pelvic RT (no; yes). At the time of analysis, patients will also be stratified by: 4) time of enrollment (pre- K-ras WT amendment; post- K-ras WT amendment).

The primary goal of this trial is to assess the role of biologics in the treatment of patients with metastatic colorectal cancer having K-ras WT tumors. Based on the current literature no differences in outcome are expected between chemotherapy regimens. Stratification by chemotherapy with oxaliplatin or irinotecan in this trial is employed to remove potential bias associated with physician selection of these regimens. The primary hypothesis will be tested collapsing over treatment with oxaliplatin or irinotecan, assuming no chemotherapy by biologic treatment interactions.

15.2 Primary Hypothesis

Overall Survival (OS) will be compared between treatment with chemotherapy (oxaliplatin- and irinotecan-containing regimens) + bevacizumab and treatment with chemotherapy (oxaliplatin- and irinotecan-containing regimens) + cetuximab.

OS will be measured from study entry until death from any cause. Primary analyses will be performed among patients as randomized regardless of treatment received (intent-to-treat). The test will be conducted controlling for Type I error at $\alpha=0.05$. If the ineligibility rate is high (> 10%) the analysis may be conducted in the subset of eligible patients.

15.3 Secondary Hypotheses

Progression-free survival (PFS), time to treatment failure (TTF) and duration of tumor response (DR) will also be studied as secondary endpoints. PFS will be measured from study entry until first documented progression or death from any cause. TTF will be measured from study entry until documented progression of disease, death from any cause, or removal from protocol therapy. DR will be measured from documented CR or PR until documented relapse, progression, or death from any cause. Response to treatment (CR/PR) will be estimated and compared between treatment arms. Sixty-day mortality, defined as any death within 60 days of beginning protocol therapy, will also be compared between regimens. All secondary analyses will be performed among patients as randomized regardless of treatment received (intent-to-treat). If the ineligibility rate is high (> 10%) these analyses may also be conducted in the subset of eligible patients.

Update #8 allowed K-ras status for eligibility to be determined locally by a CLIA-certified laboratory with subsequent confirmation by the SWOG Solid Tumor Repository. It is anticipated that a majority of the patients yet to be enrolled (300-500 patients) will be randomized based on results from local laboratories. Agreement between results from local laboratories and results from central review will be estimated when 100, 250, and 350 patients, registered post Update #8, have K-ras status determined locally and centrally. Agreement will be estimated using the kappa statistic.

15.4 Statistical Tests of Primary Hypothesis

Administrative Summary

This trial was activated on September 15, 2005. On February 15, 2008, the protocol was amended to allow the interim analyses to begin sooner than originally specified (Update #4). The amendment allowed for immediate reporting of interim results on the primary hypotheses for OS and secondary hypotheses for PFS amended from the time when 20% of expected deaths had occurred. This change was prompted by the interim results reported on panitumumab in combination with bevacizumab (PACCE Trial) showing significantly inferior PFS and OS among patients receiving the combination and subsequent concerns regarding the impact of cetuximab in combination with bevacizumab on OS and PFS in C80405.

In this amendment, truncation of the Lan-DeMets interim stopping boundaries was also adjusted to correct the resulting inflation in the significance level at the final analysis (from 0.044 to 0.025). In addition, the futility stopping rule was modified from 2-sided to 1-sided; language was included specifying that the confidence interval estimates will be adjusted at each interim. No other design changes were made.

Two planned interim analyses were conducted in October 2007 for the November 2007 DSMB meeting and in May 2008 for the June 2008 DSMB meeting. In October 2007, 5.7% of the expected number of survival events ($n = 1,478$) and 14.0% of the expected number of progression-free survival events ($n = 1,620$) were observed. Similarly, in May 2008, 13.7% of the expected number of survival events and 27.0% of the expected number of progression-free survival events were observed. A Lan-DeMets spending function was used in both analyses. The table below provides the number and percent of patients for each level of the stratification factors at the time of the interim report in May 2008.

Patient frequencies for stratification factors by treatment arm at the time of the interim analysis in May, 2008

Treatment Arm	Bevacizumab N (%)	Cetuximab N (%)	Combination N (%)	Total N (%)
Chemotherapy				
FOLFOX	372 (80%)	375 (80%)	373 (81%)	1120 (81%)
FOLFIRI	91 (20%)	93 (20%)	87 (19%)	271 (19%)
Prior Adjuvant Chemotherapy				
No	397 (86%)	403 (86%)	398 (87%)	1198 (86%)
Yes	66 (14%)	65 (14%)	62 (13%)	193 (14%)
Prior Pelvic Radiation				
No	425 (92%)	427 (91%)	425 (92%)	1277 (92%)
Yes	38 (8%)	41 (9%)	35 (8%)	114 (8%)

Based on the results from external trials in colorectal cancer reporting on the relationship between K-ras mutational status and cetuximab therapy (CRYSTAL; CAIRO2), CALGB 80405 was temporarily suspended to accrual on June 6, 2008. At this time, 1,420 patients had been enrolled. The trial was amended to limit eligibility to patients with K-ras wild type (WT) tumors (Update #5). The two primary hypotheses did not change.

The trial was re-activated after Update #5 on November 7, 2008, Eighty-seven (87) patients with known K-ras WT tumors have been enrolled since Update #5. Due to sluggish accrual since re-activation (<20 patients per month, average) and lack of community interest in treating patients with the combination of biologics, the combination treatment arm (Arm C) has been closed to accrual with the issuance of Update #6.

Sample Size Estimation

Eight hundred forty-six events (846) are needed to detect an OS hazard ratio of 1.25 with 90% power (2-sided $\alpha=0.05$). Patients who enrolled prior to Update #5 with tumor samples identified as K-ras WT will be included in the analysis of OS. Presently, 860 of 1,420 patients enrolled prior to Update #5 are documented as having tumor samples available for K-ras testing. The current estimate of the prevalence of K-ras WT in this sample is 0.576 (based on 441 patient

samples analyzed); approximately two-thirds of these patients were randomized to arms A and B. Thus, approximately 326 patients with K-ras WT tumors who were enrolled over 32 months prior to Update #5 are expected to be included in the primary analysis. Among these 326 patients, 282 events are expected over the study period. (This computation assumes a follow-up period of approximately 5.0 years.) If 814 patients are enrolled prospectively over approximately 19 months with 3 years of follow-up, 567 OS events are expected. Thus, a total of 849 events will be observed, resulting in 90% power to test the null hypothesis. Since 58 patients with K-ras WT tumors have been randomized to arms A and B post Update #5, an additional 756 patients will be randomized.

At the time of analysis, patients will be stratified according to type of chemotherapy (oxaliplatin-containing; irinotecan-containing), prior adjuvant therapy (no; yes) and prior pelvic RT (no; yes); time of enrollment (pre- K-ras WT amendment; post- K-ras WT amendment).

15.5 Statistical Tests of Secondary Hypotheses

Proportions of patients responding to treatment (CR+PR) will be compared between treatment regimens (chemotherapy plus bevacizumab and chemotherapy plus cetuximab). Assuming 571 patients per treatment group, the power is approximately 0.92 to detect a difference of 0.10 in magnitude between response rates (0.4 versus 0.5, 2-sided $\alpha=0.05$).

Fisher's exact test will be used to compare the proportions of patients dying within 60 days of beginning treatment between the chemotherapy plus bevacizumab and chemotherapy + cetuximab regimens. The estimated power to detect the difference between 2% and 5% is 0.80 ($n = 571$ per treatment arm; 2-sided $\alpha = 0.05$).

PFS will also be studied as a secondary endpoint. The PFS hypothesis will be tested in the subgroup of patients with K-ras WT tumors at the end of the accrual period. The number of PFS events expected in this subgroup is 526. With 526 observed events, a hazard ratio of 1.30 for PFS can be detected with approximately 85% power (2-sided log rank test, $\alpha=0.05$).

For power computations, the following assumptions were made with respect to median PFS: median PFS among patients with K-ras WT tumors treated with chemotherapy + bevacizumab of 13.5 months; median PFS among patients with K-ras WT tumors treated with chemotherapy + cetuximab of 17.5 months. No additional follow-up was assumed.

Secondary analyses of OS, PFS, and response will also be conducted among all patients enrolled on the trial including the more than 500 patients randomized to the combination treatment arm and those with K-ras mutations. Pre-Update #5 data on approximately 370 patients with K-ras mutated tumors and 490 with K-ras WT tumors will be available. The K-ras mutational status by treatment interaction will be tested using the Cox regression model (See Appendix V).

The kappa statistic and its 90% lower confidence bound (LCB) will be used to estimate agreement between K-ras results from local laboratories (WT; mut) and results obtained by central review (WT; mut). The following table denotes the precision of the 90% LCB with 150, 250, and 350 patients studied. Agreement will be considered acceptable if the 90% LCB > 0.75 . However, agreement is expected to be high and the primary analysis will be conducted based on the K-ras results as determined by the central review.

Precision at 90% confidence based on the kappa statistic by sample size [138]:

n	Precision (standard error x 1.282)
150	0.1046
250	0.0769
350	0.0687

15.6 Toxicity Monitoring

The incidence of arterial thrombotic events (ATEs) on each treatment arm will be monitored closely throughout the trial. Evidence of an underlying ATE rate greater than 5% will be considered unacceptable. At each interim analysis the null hypothesis that the arterial thrombotic event (ATE) rate on each treatment is less than or equal to 5% will be tested against the alternative hypothesis that it is greater than 5% ($H_0: p \leq 0.05$ versus $H_A: p > 0.05$). With 571 patients, 90% power is achieved to test this null hypothesis versus the alternative hypothesis that the ATE rate is 8.0% (1-sided $\alpha = 0.05$). Formal interim analysis will begin when approximately 20% of the data is available ($n = 135$ patients per treatment arm) and occur every six months, thereafter, coinciding with the meetings of the Alliance DSMB. The Lan-DeMets analogue of the Pocock boundaries will be used at each interim test. If evidence of an unacceptable ATE rate is observed enrollment to a treatment arm may be suspended as necessary until the toxicity issue is addressed.

Results will be reported to the Alliance DSMB at scheduled meetings and as necessary between scheduled meetings.

15.7 Interim Analysis

Assuming no chemotherapy by biologic interaction, a test of the difference hypothesis for treatment with chemotherapy (oxaliplatin- and irinotecan-containing) plus bevacizumab versus treatment with chemotherapy (oxaliplatin- and irinotecan-containing) plus cetuximab will be conducted at each interim.

Interim analyses will be adjusted to reflect the revised expected number of events for OS ($n=849$) and PFS ($n=526$). Also, the nominal significance levels at the final analyses will be adjusted for the percent alpha spent for the interim analyses conducted prior to Update #5 limiting eligibility to patients with K-ras WT tumors.

Formal interim analyses for the comparison of chemotherapy plus bevacizumab and chemotherapy plus cetuximab will resume among patients with K-ras WT tumors when 15% (127/849) of the expected number of OS events in these treatment arms have been observed. The Kaplan-Meier survival curves for OS and PFS will be provided to the DSMB at each interim analysis for all patients treated on the trial. For the formal interim analyses, the 2-sided Lan-DeMets analogue of the O'Brien-Fleming boundaries will be used to test the null hypothesis at each subsequent interim analysis according to the stratified logrank test [132]. Interim analyses will be conducted to coincide with meetings of the Alliance Data and Safety Monitoring Board every six months.

For the OS endpoint, a maximum of eight interim analyses are expected, two during the accrual period and six during follow-up. If at any interim analysis the log of the targeted hazard ratio of 1.25 lies above the upper limit of the adjusted 95.0% confidence interval for the observed log hazard ratio, consideration will be given to terminating enrollment to the trial. The confidence interval estimates will be adjusted according to the Lan-DeMets analogue of the O'Brien-Fleming boundaries [132].

Results for the secondary endpoint, PFS, will also be reported at each interim analysis of the primary endpoint, OS. Three interim analyses of PFS are expected, all during the accrual period. The 2-sided Lan-DeMets analogue of the O'Brien-Fleming boundaries will be used to test the cetuximab hypotheses for PFS at each interim analysis according to the stratified logrank test.

At each interim analysis, Fisher's exact test will be used to compare the proportions of patients dying within 60 days of beginning treatment between the bevacizumab and cetuximab regimens. No error adjustment will be made in these analyses.

15.8 Accrual, follow-up, and early closure of treatment arm(s)

Average monthly accrual to C80405 during 2007 was 51 patients, 68% of expected. With the issuance of Update #6 to remove the combination treatment arm, we expect approximately 75 patients to be pre-registered and 42 patients with K-ras WT tumors to be randomized to treatment arms A and B per month. The revised accrual goal for the two-arm study should be met in less than 2 years from reactivation of Update #6. Patients will be followed for a maximum of 5 years from the end of protocol treatment for progression endpoints.

To obtain 814 patients with K-ras WT tumors, approximately 1,430 patients will need to be screened for K-ras mutational status (assuming 57% of patients have K-ras WT tumors). A total of 1,420 patients were randomized prior to Update #5. We anticipate approximately 1,430 patients will be pre-registered to the two-arm trial and a total of 2,234 patients randomized from the time of first study activation in September 2005.

15.9 Statistical Considerations for the Quality of Life and Prescription Substudy

All patients enrolled on this study will be offered the chance to participate in the quality of life/health services companion substudy until 154 patients from each treatment arm agree to participate. The data that will be collected from a total of 462 patients across the three treatment arms, i.e. arm A: FOLFOX or FOLFIRI + BV; arm B: FOLFOX or FOLFIRI + C-225; arm C: FOLFOX or FOLFIRI + BV + C-225.

This companion study has three objectives:

- 1) To determine if there are significant differences in health-related quality of life among study patients by treatment arm at 3 and 9 months.
- 2) To determine if there is a significant difference in health-related quality of life due to acne for those patients receiving C-225 (arm B and arm C) versus those who do not receive C-225 (arm A).
- 3) To determine the degree to which patients enrolled in the clinical trial lack prescription drug coverage and perceive the costs of prescription drug medications as a source of hardship and worry.
- 4) To compare the effects of the different combinations of chemotherapy and biologic agents on resource utilization, cost, and utilities, and if applicable, to make estimates of marginal cost-utility.

The sample size is chosen to have adequate power to test the QOL objectives (Obj 1 and 2). No power calculations will be generated for the third objective given its descriptive nature.

Data for this companion will be collected prospectively and longitudinally. The baseline assessment will be completed in the clinic, and submitted directly to the QOL co-chair. Subsequent assessments will be collected by staff at the Wake Forest University School of Medicine via telephone interviews.

15.9.1 Sample size justification

Both short and long-term QOL are relevant to this study. This companion study is designed to detect the following differences that are of primary interest:

- At 3 months, differences between arms with and without C-225 in the overall QOL subscale from the EORTC QLQ-C30
- At 3 months, differences between arms with and without C-225 in the total score of the Dermatology-Specific Quality of Life (DSQL) Questionnaire.

- At 9 months, differences between three treatment arms in the overall QOL subscale from the EORTC QLQ-C30

The assumptions of the following power calculations are as follows:

- To account for there being 3 primary tests on QOL endpoints, power calculations will be generated with an adjustment for a multiplicity of tests. Specifically, we will assume a type I error of 0.0165 for each test.
- A median survival of 22 months is assumed for all treatment arms. Therefore, the proportion of patients who are alive at week 6, month 3, month 6, and month 9 are approximately 0.96, 0.91, 0.83, and 0.75, respectively.
- At each assessment, an additional 8-10% of patients will not be able to respond to QOL questionnaires due to failing health.
- 80% power and two-tailed test

EORTC Overall QOL Subscale at Month 3: Assuming a difference of 5 units between the two arms with the smallest QOL difference, and a difference of 12 units between the two arms with the largest QOL difference, with 126 patients in each treatment arm completing the assessment at month 3, the study has approximately 86% power to detect an overall difference among the three arms, and 92% power to detect the largest difference between two arms using a two-tailed test conducted at the 0.0165 level of significance. A difference of 12 units is equivalent to an effect size of 0.343, which is slightly smaller than the difference observed when comparing the QOL of patients with and without weight loss, patients with PS = 1 and PS = 0 [133].

Acne Endpoint at Month 3: Approximately 82% of the 462 patients who participate in the QOL companion will provide a month 3 assessment. Therefore, we expect that approximately 252 patients treated with cetuximab and 126 patients treated without cetuximab will be assessed at month 3. For a two-sided test conducted at the 0.0165 level of significance, there is 80% power to detect an effect size of 0.356—a “small” to “medium” effect size. Anderson et al. (1998), who developed the DSQL, report that a medium effect size is comparable to a 25% reduction in the dermatologists’ overall rating.

EORTC Overall QOL Subscale at Month 9: Assuming a difference of 5 units between the two arms with the smallest QOL difference, and a difference of 13 units between the two arms with the largest QOL difference, with 104 patients in each treatment arm completing the assessment at month 9, the study has approximately 85% power to detect an overall difference among the three arms, and 91% power to detect the largest difference between two arms using a two-tailed test conducted at the 0.0165 level of significance. The standard deviation associated with the global QOL subscale is reported by King (1996) to be approximately 25 units. Assuming that the standard deviation for the global QOL subscale is 25, the standard deviation for the difference between treatment groups is 35 units. A difference of 13 units is equivalent to an effect size of 0.367, which is comparable to the difference observed when comparing the QOL of patients with and without weight loss, patients with PS = 1 and PS=0 [133].

15.9.2 Analytic Methods for the QOL Endpoints

Analysis of Covariance (ANCOVA) will be used to test QOL differences between arms for each assessment point with adjustment for the baseline assessment and other patient characteristics. Results from Wilcoxon rank sum test will be used if normality assumption is violated. Repeated measures analysis, such as generalized estimation equation (GEE) will be used for a joint modeling of QOL differences over assessments before 6 months if missing data is less than 30%. It is likely that a percentage of QOL data collected at follow-

up assessments will be missing due to death, disease progression, or severe illness. If QOL assessments are missing more than 30%, a pattern-mixture approach [134] to modeling will be adapted in which a separate regression model is fit for each drop-out pattern. These longitudinal analyses will be conducted for each of the QLQ-C30 subscales, and the Dermatology-Specific Quality of Life questionnaire. If there are minimal amounts of data missing due to drop-out, an unstratified repeated measures analysis will be conducted. An unstratified repeated measures analysis will also be reported if response patterns are consistent across strata (i.e. drop-out patterns).

The Subjective Significance Questionnaire (SSQ) will be used to interpret the scores of the EORTC QLQ-C30 and to determine changes in scores that are significant to patients. The SSQ will be used to determine which patients report they have experienced improvement since their last assessment. The change in scores on the noted subscales will be examined to determine what magnitude corresponds to the patients' ratings of improvement.

15.9.3 Analytic Methods for the Prescription Drug component

The analysis of the prescription drug data will be descriptive since there is very limited preliminary data regarding this topic. Responses to survey items will be categorized according to information obtained from the background form on patient age, gender, race, education and employment status. Specifically, the following descriptive statistics will be generated:

From the baseline survey:

- The percentage of trial participants who lack prescription drug coverage and how perceived difficulty and anxiety vary based on coverage status.
- The strategies trial participants have used to cope with high drug costs prior to study enrollment.

From the follow-up interviews at months 3 and 9:

- The percentage of trial participants who report that since study enrollment paying for prescription medicines has been "somewhat" or "very difficult."
- The percentage of trial participants who discuss their concerns about the costs of their prescription drugs with their physicians.
- How frequently patients use various strategies for coping with the cost of prescription medicines during trial participation.

Logistic regression will be used to assess the effect of, insurance status, age, and estimated number of prescription drug medicines on each outcome. Fisher's exact test will be used to assess whether a relationship exists between a patient having prescription drug coverage and having a discussion with the physician about the costs of prescription drugs.

15.9.4 Analytic methods for the pharmacoeconomic component

The pharmacoeconomic analysis will consist of a post-hoc decision analytic model using prospectively collected data. The base-case perspective will be that of the health care payer.

The lifetime costs of each of the 6 potential initial treatment pathways (3 randomized and 3 due to the FOLFIRI versus FOLFOX option), including subsequent treatments after coming off trial, will be estimated and related to quality-adjusted overall survival. If one strategy is found to improve overall survival and/or quality of life, an incremental cost-utility ratio will be calculated comparing it to the least costly, next-most efficacious strategy. If, however, there is no interaction between initial chemotherapy regimen and

biologic agent in clinical outcomes, then the one that consumes the fewest resources will be preferred.

Cost analysis: Frequency distributions and measures of central tendency (e.g. means and medians) will be determined for each resource category for each arm of the study (e.g. length of hospital stay). Confidence intervals for the difference in costs and resource utilization between the strategies for each resource category will also be calculated. Univariate comparisons between the groups will be made primarily using nonparametric tests, such as Wilcoxon rank-sum test, and estimation of cost differences will be made using ANOVA if normality is not violated for cost data or generalized linear model (GLM) with a log link if cost data is right skewed.

Outcomes: Quality-Adjusted Life Years (QALYs) will be calculated by integrating survival and utility data over time. Each utility assessment will be considered the midpoint of a period of time with that utility. Missing data will be interpolated using mean values. Quality-adjusted survival will be calculated for each patient by summing the products of the utility x survival time for each interval.

Cost-utility analysis: If a cost-utility approach is appropriate, the cost-utility point estimate will be the ratio of the incremental costs to the incremental quality-adjusted life years (QALYs) afforded by each regimen, using the following equation:

$$\text{Cost-Utility} = \frac{\text{Cost}_A - \text{Cost}_B}{\text{QALY}_A - \text{QALY}_B}$$

and its 95% confidence interval will be given using bootstrapping technique.

The primary time horizon will be from the start of the trial to death. Analyses will also be done with time horizons of 5 years, the median follow-up duration, and the minimum follow-up duration. Both costs and benefits will be discounted at a 3% annual rate [135].

Sensitivity analyses: One and multi-way sensitivity analyses will be carried out around major cost drivers by varying the costs over their observed ranges and conducting threshold analyses where appropriate. Survival and utility estimates will be varied across their 95% confidence intervals. The effects of varying the discount rates from 0% to 6% will also be explored.

The hypothesis that higher cost for treatment is associated with longer survival or quality-adjusted survival will be explored using multivariate regression modeling.

Power considerations: The sample size for the trial is being determined by the overall survival endpoint. With 2289 patients randomized to one of the three treatment arms (763 per arm) and assuming a t-test comparison of untransformed cost data between arms is appropriate and that the mean cost in the least resource intense arm of \$60,000, the differences in cost that can be detected with 80% power at the 0.05 (two-tailed) level of significance across possible standard deviations are approximately:

Standard Deviation	Mean difference in cost detectable
\$5,000	\$718
\$10,000	\$1,435
\$15,000	\$2,153

15.10 Statistical Considerations for the Diet and Lifestyle Substudy

Time to progression will be measured from trial entry until documented progression of disease or death from any cause. Overall survival will be measured from trial entry until death from any cause.

Treatment-related toxicity will be measured primarily using two endpoints: 1) the proportion of patients experiencing grade 3 or greater diarrhea; 2) the proportion of patients experiencing grade 3 or greater neutropenia.

The median survival in this patient population treated with combination chemotherapy with bevacizumab is 22 months. Two thousand two hundred eighty-nine (2,289) patients will be randomized on this trial over 2.5 years and followed an additional 2 years. Analysis will occur approximately 5.0 years after study activation.

Patients will be asked to complete the self-administered questionnaire within 1 month of study entry. Responses to the questionnaire will be either dichotomous or assumed to have an underlying continuous distribution summarized using quintiles.

15.10.1 Power Estimation

Survival Endpoints (Overall Survival): For underlying continuous variables (total red meat, total fat, folate intake, glycemic index, and physical activity), power is estimated for testing the null hypothesis of independence versus specified alternatives in the 2-year overall survival (OS) over quintiles. For example, increased glycemic index is hypothesized to decrease OS. Under the alternative hypothesis approximately 50% of patients enrolled on the treatment trial are expected to survive two years. Based on the questionnaire response rates observed in CALGB 89803 we estimate that a minimum of 90% of patients will complete the questionnaire (n = 2060). The alternative hypothesis for glycemic index (hypothesis 6) presented in Table 4 is detectable with a power of 0.90.

Quintile	1st	2nd	3rd	4th	5th	Total
Proportion surviving 2-years	0.112	0.110	0.095	0.092	0.091	0.50
Proportion not surviving 2-years	0.088	0.09	0.0105	0.108	0.109	0.50
Total	0.20	0.200	0.20	0.200	0.20	1.000

Table 4. Alternative hypothesis 1 for glycemic index comparing OS at 2 years across quintiles based on glycemic index. The odds ratio comparing the lowest to the highest quintiles is 1.52 (associated χ -square non-centrality parameter, 14.8; n = 2060). Estimated power is 0.90 to test this hypothesis at a significance level of 0.01.

15.10.2 Survival Endpoints (Time to progression)

Patients who are obese or who consume > 10 g/day of alcohol are expected to have worse 1-year time to progression (TTP) than patients who are of normal weight or do not consume alcohol. Based on data from CALGB 89803 50% of patients are expected to be normal weight, 20% overweight and 30% obese. Similarly, 62% of patients are expected to have no alcohol intake, 24% are expected to have low to moderate alcohol intake and 14% are expected to consume 10 g/day or more of alcohol. Overall patients a 1-year TTP of 56% is anticipated.

Alcohol Consumption	No alcohol	Low to moderate alcohol	> 10 g/day alcohol	Total
Proportion progression-free at 1-year	0.37	0.12	0.07	0.56
Proportion progressed at 1-year	0.25	0.12	0.07	0.44
Total	0.62	0.24	0.14	1.0

Table 5. Alternative hypothesis comparing 1-year TTP over three alcohol-consumption categories. Estimated power is approximately 0.91 to test this hypothesis at a significance level of 0.01 (associated χ -square non-centrality parameter, 15.4; n = 2,060).

15.10.3 Toxicity Endpoints:

Associations between obesity and toxicity will be investigated over all biologic treatment arms. Two toxicity endpoints will be examined. Unacceptable toxicity will be defined as grade 3 or greater neutropenia and grade 3 or greater diarrhea. The null hypothesis of independence will be tested. Based on previously reported data, we expect approximately 19-47% of patients receiving FOLFOX [5] and 18% of patients receiving FOLFIRI as initial chemotherapy for metastatic disease, respectively, to experience grade 3 or greater neutropenia. Assuming 90% of patients will receive the FOLFOX regimen, grade 3 or greater neutropenia is expected in 33% of patients. Also, based on data from CALGB 89803 50% of patients are expected to be normal weight, 20% overweight and 30% obese. We estimate that obesity and toxicity data will be available on 90% of patients randomized (n = 2,060).

The alternative toxicity hypothesis illustrated in Table 6 is detectable with a power of 0.92.

Weight Status	Normal	Overweight	Obese	Total
Grade 3 or greater neutropenia	0.14	0.08	0.11	0.33
Less than Grade 3 neutropenia	0.36	0.12	0.19	0.67
Total	0.50	0.20	0.30	1.0

Table 6. Alternative hypothesis for comparing the occurrence of grade 3 or greater neutropenia over weight categories. Estimated power is approximately 0.92 to test this hypothesis at a significance level of 0.01 (associated χ -square non-centrality parameter, 15.9; n = 2,060).

The Cox model will be used to explore the simultaneous effect of diet and lifestyle variables on time to progression and overall survival. Logistic regression will be used to explore the relationship between the study variables and toxicity outcomes overall and within treatment arms. Exploratory analyses will also consider the relationships between other dietary factors such as calcium, vitamin D, fiber, aspirin and NSAID use, and alternative therapies on time to progression and overall survival. Models incorporating both dietary and molecular markers will be studied.

16.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Investigators are required to notify the Study Chair and their Institutional Review Board if a patient has a reportable serious adverse event. 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 beginning October 1, 2010. 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 Web site (<http://ctep.cancer.gov>). All reactions determined to be “reportable” in an expedited manner must be reported using the NCI CTEP Adverse Event Reporting System (CTEP-AERS).

See also, the Additional Instructions below regarding the reporting requirements for cardiac events.

16.1 CALGB/SWOG 80405 Reporting Requirements for all arms of the study:

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND or non-CTEP IND: CTEP-AERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days¹ of the Last Dose of the Investigational Agent.

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5 ²	Grades 4 & 5 ²
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	without Hospitalization	Expected with Hospitalization	without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hrs; 5 Calendar Days	10 Calendar Days

¹ Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND or non-CTEP IND require reporting as follows:

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events
- CTEP-AERS 10 calendar day report:
 - Grade 3 unexpected events with hospitalization or prolongation of hospitalization
 - Grade 5 expected events

² Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

March 2005

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
 - ★ “24 hours; 5 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
 - ★ “10 calendar days” - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND or non-CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

16.2 Additional Instructions or Exclusions from CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent under a CTEP-IND or non-CTEP IND:

- All adverse events reported via CTEP-AERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- Alliance holds the IND for cetuximab for this study. The reporting requirements for investigational agents under a CTEP IND should also be followed for all agents (any arm) in this trial.
- **All grade 3 cardiac events on any arm including expected events without hospitalization, regardless of attribution, require expedited reporting via CTEP-AERS within 10 calendar days.**
In addition, all arterial thrombotic events regardless of grade, attribution, or treatment arm, are to be reported within 10 calendar days using CTEP-AERS expedited reporting.
- Grade 3/4 hematosuppression and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results. All other grade 3 or 4 adverse events that precipitate hospitalization or prolong an existing hospitalization must be reported via CTEP-AERS.
- For the purposes of expedited adverse event reporting, the CAEPR (which includes expected adverse events) for bevacizumab may be found in [Section 16.3](#), below. Expected adverse events for the other agents used in this trial include those listed in [Section 11.0](#) and in the package inserts. **Note:** The ASAEL column of the CAEPR has been replaced with the specific protocol exceptions to expedited reporting (SPEER) list. This list now includes “expected” severity grades in addition to event terms.
- The reporting of adverse events described in the table above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see [Section 5.5](#) for required CALGB forms).
- Reporting of cases of secondary AML/MDS is to be done using the NCI/CTEP Secondary AML/MDS Report Form. New primary malignancies should be reported using study form C-1400.

16.3 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Bevacizumab (rhuMab VEGF, NSC 704865)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3540 patients.* Below is the CAEPR for bevacizumab (rhuMab VEGF).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, August 1, 2013¹

Adverse Events with Possible Relationship to Bevacizumab (rhuMab VEGF) (CTCAE 4.0 Term) [n= 3540]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		<i>Anemia (Gr 3)</i>
		Blood and lymphatic system disorders - Other (renal thrombotic microangiopathy)	
	Febrile neutropenia		<i>Febrile neutropenia (Gr 3)</i>
CARDIAC DISORDERS			
		Acute coronary syndrome ²	
	Cardiac disorders - Other (supraventricular arrhythmias) ³		<i>Cardiac disorders - Other (supraventricular arrhythmias)³ (Gr 3)</i>
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction ²	
		Ventricular arrhythmia	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		<i>Abdominal pain (Gr 3)</i>
	Colitis		<i>Colitis (Gr 3)</i>
	Constipation		<i>Constipation (Gr 3)</i>
	Diarrhea		<i>Diarrhea (Gr 3)</i>

	Dyspepsia		<i>Dyspepsia (Gr 2)</i>
		Gastrointestinal fistula ⁴	
	Gastrointestinal hemorrhage ⁵		<i>Gastrointestinal hemorrhage⁵ (Gr 2)</i>
	Gastrointestinal obstruction ⁶		
		Gastrointestinal perforation ⁷	
		Gastrointestinal ulcer ⁸	
	Ileus		
	Mucositis oral		<i>Mucositis oral (Gr 3)</i>
	Nausea		<i>Nausea (Gr 3)</i>
	Vomiting		<i>Vomiting (Gr 3)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		<i>Fatigue (Gr 3)</i>
	Infusion related reaction		<i>Infusion related reaction (Gr 2)</i>
	Non-cardiac chest pain		<i>Non-cardiac chest pain (Gr 3)</i>
	Pain		<i>Pain (Gr 3)</i>
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		<i>Allergic reaction (Gr 2)</i>
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection ⁹		<i>Infection⁹ (Gr 3)</i>
		Infections and infestations - Other (necrotizing fasciitis)	
	Infections and infestations - Other (peri-rectal abscess)		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Injury, poisoning and procedural complications - Other (anastomotic leak) ¹⁰	
	Wound complication		<i>Wound complication (Gr 2)</i>
	Wound dehiscence		<i>Wound dehiscence (Gr 2)</i>
INVESTIGATIONS			
	Alanine aminotransferase increased		<i>Alanine aminotransferase increased (Gr 3)</i>
	Alkaline phosphatase increased		<i>Alkaline phosphatase increased (Gr 3)</i>
	Aspartate aminotransferase increased		<i>Aspartate aminotransferase increased (Gr 3)</i>
	Blood bilirubin increased		<i>Blood bilirubin increased (Gr 2)</i>

	Cardiac troponin I increased		
Neutrophil count decreased			<i>Neutrophil count decreased (Gr 3)</i>
	Platelet count decreased		<i>Platelet count decreased (Gr 4)</i>
	Weight loss		<i>Weight loss (Gr 3)</i>
	White blood cell decreased		<i>White blood cell decreased (Gr 3)</i>
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		<i>Anorexia (Gr 3)</i>
	Dehydration		<i>Dehydration (Gr 3)</i>
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		<i>Arthralgia (Gr 3)</i>
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia) ¹¹		
	Myalgia		<i>Myalgia (Gr 3)</i>
	Osteonecrosis of jaw ¹²		
NERVOUS SYSTEM DISORDERS			
	Dizziness		<i>Dizziness (Gr 2)</i>
	Headache		<i>Headache (Gr 3)</i>
		Intracranial hemorrhage	
		Ischemia cerebrovascular ²	
	Peripheral sensory neuropathy ¹³		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		<i>Hematuria (Gr 3)</i>
	Proteinuria		<i>Proteinuria (Gr 2)</i>
		Renal and urinary disorders - Other (Nephrotic Syndrome)	
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Reproductive system and breast disorders - Other (ovarian failure) ¹⁴			

		Vaginal fistula	
	Vaginal hemorrhage		<i>Vaginal hemorrhage (Gr 3)</i>
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		<i>Allergic rhinitis (Gr 3)</i>
		Bronchopleural fistula	
		Bronchopulmonary hemorrhage	
	Cough		<i>Cough (Gr 3)</i>
	Dyspnea		<i>Dyspnea (Gr 2)</i>
	Epistaxis		<i>Epistaxis (Gr 3)</i>
	Hoarseness		<i>Hoarseness (Gr 3)</i>
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		<i>Pruritus (Gr 2)</i>
	Rash maculo-papular		<i>Rash maculo-papular (Gr 2)</i>
	Urticaria		<i>Urticaria (Gr 2)</i>
VASCULAR DISORDERS			
Hypertension			<i>Hypertension (Gr 3)</i>
	Thromboembolic event		<i>Thromboembolic event (Gr 3)</i>
		Vascular disorders - Other (arterial thromboembolic event) ^{2,15}	

¹This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

²The risks of arterial thrombosis such as cardiac or CNS ischemia are increased in elderly patients and in patients with a history of diabetes.

³Supraventricular arrhythmias may include supraventricular tachycardia, atrial fibrillation and atrial flutter.

⁴Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

⁵Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

⁶Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

⁷Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, Small intestinal perforation, and other sites under the GASTROINTESTINAL DISORDERS SOC.

⁸Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

⁹Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

¹⁰Anastomotic leak may include Gastric anastomotic leak; Gastrointestinal anastomotic leak; Large intestinal anastomotic leak; Rectal anastomotic leak; Small intestinal anastomotic leak; Urostomy leak; Vaginal anastomotic leak

¹¹Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

¹²Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

¹³Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

¹⁴*Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥ 30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level < 30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.*

¹⁵Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack and stroke.

Also reported on bevacizumab (rhUMAb VEGF) trials but with the relationship to bevacizumab (rhUMAb VEGF) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Bone marrow hypocellular; Disseminated intravascular coagulation; Hemolysis

CARDIAC DISORDERS - Atrioventricular block complete; Atrioventricular block first degree; Cardiac arrest; Myocarditis; Pericardial effusion; Restrictive cardiomyopathy; Right ventricular dysfunction

EAR AND LABYRINTH DISORDERS - Ear and labyrinth disorders - Other (tympanic membrane perforation); Hearing impaired; Tinnitus; Vertigo

ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism

EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (blindness); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (corneal epithelial defect); Eye disorders - Other (floaters); Eye disorders - Other (ischemic CRVO); Eye disorders - Other (macular pucker); Eye disorders - Other (transient increased IOP $>$ or $= 30$ mm Hg); Eye disorders - Other

(vitreous hemorrhage); Eye pain; Keratitis; Optic nerve disorder; Photophobia; Retinal detachment; Retinal tear; Retinopathy; Watery eyes

GASTROINTESTINAL DISORDERS - Ascites; Chelitis; Colonic stenosis; Dry mouth; Dysphagia; Enterocolitis; Esophageal pain; Esophageal stenosis; Flatulence; Gastrointestinal disorders - Other (peritonitis); Oral pain; Pancreatitis; Proctitis; Rectal mucositis; Rectal stenosis; Typhlitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Edema limbs; Edema trunk; Facial pain; Fever; Flu like symptoms; Gait disturbance; Injection site reaction; Localized edema; Multi-organ failure; Sudden death NOS

HEPATOBIILIARY DISORDERS - Cholecystitis; Gallbladder necrosis; Gallbladder obstruction; Hepatic failure; Hepatic necrosis

INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Burn; Dermatitis radiation; Fracture

INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood antidiuretic hormone abnormal; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Electrocardiogram QT corrected interval prolonged; Forced expiratory volume decreased; GGT increased; INR increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain

METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hyponatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Fibrosis deep connective tissue; Generalized muscle weakness; Head soft tissue necrosis; Joint effusion; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Neck pain; Pain in extremity; Pelvic soft tissue necrosis; Soft tissue necrosis lower limb

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Arachnoiditis; Ataxia; Central nervous system necrosis; Cerebrospinal fluid leakage; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Encephalopathy; Extrapyrimal disorder; Facial nerve disorder; Hydrocephalus; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (increased intracranial pressure); Paresthesia; Peripheral motor neuropathy; Pyramidal tract syndrome; Seizure; Somnolence; Tremor; Vasovagal reaction

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Psychosis

RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Renal and urinary disorders - Other (dysuria); Renal and urinary disorders - Other (ureterolithiasis); Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Erectile dysfunction; Irregular menstruation; Pelvic pain; Vaginal discharge

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Hypoxia; Nasal congestion; Pulmonary fibrosis; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (dry nares); Respiratory, thoracic and mediastinal disorders - Other (pulmonary infarction)

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Nail loss; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (diabetic foot ulcer); Skin and subcutaneous tissue disorders - Other (skin breakdown/ decubitus ulcer); Skin hyperpigmentation; Skin induration; Skin ulceration; Stevens-Johnson syndrome

VASCULAR DISORDERS - Flushing; Hot flashes; Hypotension; Lymphocele; Phlebitis; Vasculitis

Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

16.4 Comprehensive Adverse Events and Potential Risks List (CAEPR) for Cetuximab (NSC 714692)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with **bold** and *italicized* text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification. *Frequency is provided based on 2282 patients.* Below is the CAEPR for Cetuximab.

Version 2.1, March 31, 2010¹

Adverse Events with Possible Relationship to Cetuximab (CTCAE 4.0 Term) [n= 2282]			EXPECTED AEs FOR CTEP-AERS REPORTING Agent Specific Adverse Event List (ASAEL)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	<i>Expected</i>
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		
EAR AND LABYRINTH DISORDERS			
	External ear inflammation		
	Tinnitus		
EYE DISORDERS			
	Conjunctivitis		<i>Conjunctivitis</i>
	Dry eye		<i>Dry eye</i>
	Uveitis		<i>Uveitis</i>
	Watering eyes		<i>Watering eyes</i>
GASTROINTESTINAL DISORDERS			
	Abdominal pain		<i>Abdominal pain</i>
	Cheilitis		<i>Cheilitis</i>
	Constipation		<i>Constipation</i>
Diarrhea			<i>Diarrhea</i>
	Dry mouth		<i>Dry mouth</i>
	Dyspepsia		<i>Dyspepsia</i>
	Mucositis oral		<i>Mucositis oral</i>
Nausea			<i>Nausea</i>

	Vomiting		<i>Vomiting</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		<i>Chills</i>
	Edema limbs		
Fatigue			<i>Fatigue</i>
Fever			<i>Fever</i>
	Flu like symptoms		<i>Flu like symptoms</i>
	Infusion related reaction		<i>Infusion related reaction</i>
	Non-cardiac chest pain		
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		<i>Allergic reaction</i>
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection ²		
		Infections and infestations – Other (aseptic meningitis)	
INVESTIGATIONS			
	Neutrophil count decreased		
	Weight loss		<i>Weight loss</i>
	White blood cell decreased		<i>White blood cell decreased</i>
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		<i>Anorexia</i>
	Dehydration		<i>Dehydration</i>
	Hypocalcemia		
	Hypomagnesemia		<i>Hypomagnesemia</i>
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		<i>Arthralgia</i>
	Back pain		<i>Back pain</i>
	Myalgia		<i>Myalgia</i>
NERVOUS SYSTEM DISORDERS			
Headache			<i>Headache</i>
	Syncope		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		<i>Allergic rhinitis</i>
	Bronchospasm		
	Cough		<i>Cough</i>
	Dyspnea		<i>Dyspnea</i>
	Hoarseness		<i>Hoarseness</i>
		Pneumonitis	
		Respiratory, thoracic, and mediastinal disorders Other (non-cardiogenic pulmonary edema)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		<i>Alopecia</i>

Dry skin			Dry skin
	Nail loss		Nail loss
		Palmar-plantar erythrodysesthesia syndrome	
	Photosensitivity		Photosensitivity
	Pruritus		Pruritus
	Purpura		
Rash acneiform			Rash acneiform
Rash maculo-papular			Rash maculo-papular
	Skin ulceration		
	Urticaria		Urticaria
VASCULAR DISORDERS			
	Hypotension		Hypotension
	Thromboembolic event		Thromboembolic event

¹This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

²Infection could include all 75 sites of infections under the INFECTIONS AND INFESTATIONS SOC.
Also reported on cetuximab trials but with the relationship to cetuximab still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Disseminated intravascular coagulation; Hemolysis

CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Chest pain - cardiac; Left ventricular systolic dysfunction; Myocardial infarction; Paroxysmal atrial tachycardia; Pericardial effusion; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia

EAR AND LABYRINTH DISORDERS - Hearing impaired

EYE DISORDERS - Blurred vision; Extraocular muscle paresis; Eyelid function disorder; Keratitis; Photophobia; Vitreous hemorrhage

GASTROINTESTINAL DISORDERS - Colitis; Dysphagia; Esophagitis; Gastritis; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal hemorrhage (including Colonic or Gastric hemorrhage or hemorrhage in other sites under the GASTROINTESTINAL DISORDERS SOC); Gastrointestinal perforation (Colonic perforation, Duodenal perforation, or perforation in other sites under the GASTROINTESTINAL DISORDERS SOC); Gastrointestinal ulcer (ulcer includes Duodenal ulcer, Rectal ulcer, or ulcer in other sites under the GASTROINTESTINAL DISORDERS SOC); Ileus; Pancreatitis; Rectal fistula

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Sudden death NOS

HEPATOBIILIARY DISORDERS - Cholecystitis; Hepatic failure

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Wound dehiscence

INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Creatinine increased; Platelet count decreased; Serum amylase increased

METABOLISM AND NUTRITION DISORDERS - Hyperkalemia; Hyperuricemia; Hypokalemia; Hyponatremia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (myasthenia); Musculoskeletal and connective tissue disorder - Other (Sudeck's Atrophy)

NERVOUS SYSTEM DISORDERS - Ataxia; Dizziness; Dysgeusia; Extrapyrmidal disorder; Intracranial hemorrhage; Nervous system disorders - Other (cholinergic syndrome); Neuralgia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Tremor

PSYCHIATRIC DISORDERS - Agitation; Depression

RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (acute renal failure)

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Reproductive system and breast disorders - Other (balanitis); Vaginal inflammation

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Bronchopulmonary hemorrhage; Epistaxis; Pleural effusion; Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans-organized pneumonia [BOOP])

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hirsutism; Skin hypopigmentation; Skin and subcutaneous tissue disorders - Other (skin fissures)

VASCULAR DISORDERS - Flushing; Hypertension; Lymphedema; Vasculitis

Note: Cetuximab in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

16.5 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Oxaliplatin (NSC 266046)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with **bold** and *italicized* text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1141 patients.* Below is the CAEPR for oxaliplatin.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Adverse Events with Possible Relationship to Oxaliplatin (CTCAE 4.0 Term) [n= 1141]			Specific Protocol Exceptions to Expedited Reporting (SPEER) (formerly known as ASael)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			<i>Anemia (Gr 3)</i>
	Disseminated intravascular coagulation		<i>Disseminated intravascular coagulation (Gr 2)</i>
	Febrile neutropenia		<i>Febrile neutropenia (Gr 3)</i>
	Hemolysis		<i>Hemolysis (Gr 2)</i>
		Thrombotic thrombocytopenic purpura	
CARDIAC DISORDERS			
	Atrial fibrillation		<i>Atrial fibrillation (Gr 2)</i>
	Atrial flutter		<i>Atrial flutter (Gr 2)</i>
	Paroxysmal atrial tachycardia		<i>Paroxysmal atrial tachycardia (Gr 2)</i>
	Sinus bradycardia		<i>Sinus bradycardia (Gr 2)</i>
	Sinus tachycardia		<i>Sinus tachycardia (Gr 2)</i>
	Supraventricular tachycardia		<i>Supraventricular tachycardia (Gr 2)</i>
	Ventricular arrhythmia		<i>Ventricular arrhythmia (Gr 2)</i>
	Ventricular fibrillation		<i>Ventricular fibrillation (Gr 2)</i>
	Ventricular tachycardia		<i>Ventricular tachycardia (Gr 2)</i>
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		<i>Hearing impaired (Gr 2)</i>
	Middle ear inflammation		<i>Middle ear inflammation (Gr 2)</i>
EYE DISORDERS			
	Conjunctivitis		<i>Conjunctivitis (Gr 2)</i>
	Dry eye		<i>Dry eye (Gr 2)</i>
	Eye disorders - Other (amaurosis fugax)		<i>Eye disorders - Other (amaurosis fugax) (Gr 2)</i>
	Eye disorders - Other (cold-induced transient visual abnormalities)		<i>Eye disorders - Other (cold-induced transient visual abnormalities) (Gr 2)</i>
	Eyelid function disorder		<i>Eyelid function disorder (Gr 2)</i>
	Papilledema		<i>Papilledema (Gr 2)</i>
GASTROINTESTINAL DISORDERS			
	Abdominal pain		<i>Abdominal pain (Gr 3)</i>
	Ascites		<i>Ascites (Gr 2)</i>
	Colitis		<i>Colitis (Gr 3)</i>
	Constipation		<i>Constipation (Gr 3)</i>

Diarrhea			<i>Diarrhea (Gr 3)</i>
	Dry mouth		<i>Dry mouth (Gr 2)</i>
	Dyspepsia		<i>Dyspepsia (Gr 2)</i>
	Dysphagia		<i>Dysphagia (Gr 2)</i>
	Enterocolitis		<i>Enterocolitis (Gr 3)</i>
	Esophagitis		<i>Esophagitis (Gr 3)</i>
	Flatulence		<i>Flatulence (Gr 2)</i>
	Gastritis		<i>Gastritis (Gr 2)</i>
		Gastrointestinal disorders - Other (pneumatosis intestinalis)	
	Gastrointestinal hemorrhage ²		<i>Gastrointestinal hemorrhage² (Gr 3)</i>
	Gastrointestinal necrosis ³		<i>Gastrointestinal necrosis³ (Gr 2)</i>
	Gastrointestinal ulcer ⁴		<i>Gastrointestinal ulcer⁴(Gr 2)</i>
	Ileus		<i>Ileus (Gr 3)</i>
	Mucositis oral		<i>Mucositis oral (Gr 3)</i>
Nausea			<i>Nausea (Gr 3)</i>
	Pancreatitis		<i>Pancreatitis (Gr 2)</i>
	Small intestinal obstruction		<i>Small intestinal obstruction (Gr 3)</i>
Vomiting			<i>Vomiting (Gr 3)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		<i>Chills (Gr 2)</i>
	Edema face		<i>Edema face (Gr 2)</i>
	Edema limbs		<i>Edema limbs (Gr 2)</i>
Fatigue			<i>Fatigue (Gr 3)</i>
	Fever		<i>Fever (Gr 2)</i>
	Gait disturbance		<i>Gait disturbance (Gr 2)</i>
	General disorders and administration site conditions - Other (Hepato-renal syndrome)		<i>General disorders and administration site conditions - Other (Hepato-renal syndrome) (Gr 2)</i>
	Injection site reaction		<i>Injection site reaction (Gr 2)</i>
	Non-cardiac chest pain		<i>Non-cardiac chest pain (Gr 2)</i>
HEPATOBIILIARY DISORDERS			
		Cholecystitis	
	Hepatic failure		<i>Hepatic failure (Gr 2)</i>
	Hepatobiliary disorders - Other (hepatic enlargement)		<i>Hepatobiliary disorders - Other (hepatic enlargement) (Gr 2)</i>
	Hepatobiliary disorders - Other (veno-occlusive liver disease)		<i>Hepatobiliary disorders - Other (veno-occlusive liver disease) (Gr 2)</i>
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		<i>Allergic reaction (Gr 2)</i>

INFECTIONS AND INFESTATIONS		
	Infection ⁵	<i>Infection⁵ (Gr 3)</i>
INVESTIGATIONS		
	Activated partial thromboplastin time prolonged	<i>Activated partial thromboplastin time prolonged (Gr 2)</i>
Alanine aminotransferase increased		<i>Alanine aminotransferase increased (Gr 3)</i>
	Alkaline phosphatase increased	<i>Alkaline phosphatase increased (Gr 2)</i>
Aspartate aminotransferase increased		<i>Aspartate aminotransferase increased (Gr 3)</i>
	Blood bilirubin increased	<i>Blood bilirubin increased (Gr 3)</i>
	Creatinine increased	<i>Creatinine increased (Gr 3)</i>
	GGT increased	<i>GGT increased (Gr 2)</i>
	INR increased	<i>INR increased (Gr 2)</i>
	Lymphocyte count decreased	<i>Lymphocyte count decreased (Gr 3)</i>
	Neutrophil count decreased	<i>Neutrophil count decreased (Gr 4)</i>
Platelet count decreased		<i>Platelet count decreased (Gr 4)</i>
	Weight gain	<i>Weight gain (Gr 2)</i>
	Weight loss	<i>Weight loss (Gr 2)</i>
	White blood cell decreased	<i>White blood cell decreased (Gr 4)</i>
METABOLISM AND NUTRITION DISORDERS		
	Acidosis	<i>Acidosis (Gr 4)</i>
	Anorexia	<i>Anorexia (Gr 3)</i>
	Dehydration	<i>Dehydration (Gr 3)</i>
	Hyperglycemia	<i>Hyperglycemia (Gr 2)</i>
	Hyperuricemia	<i>Hyperuricemia (Gr 2)</i>
	Hypoalbuminemia	<i>Hypoalbuminemia (Gr 3)</i>
	Hypocalcemia	<i>Hypocalcemia (Gr 3)</i>
	Hypoglycemia	<i>Hypoglycemia (Gr 2)</i>
	Hypokalemia	<i>Hypokalemia (Gr 4)</i>
	Hypomagnesemia	<i>Hypomagnesemia (Gr 4)</i>
	Hyponatremia	<i>Hyponatremia (Gr 3)</i>
	Hypophosphatemia	<i>Hypophosphatemia (Gr 3)</i>
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia	<i>Arthralgia (Gr 2)</i>
	Back pain	<i>Back pain (Gr 2)</i>
	Bone pain	<i>Bone pain (Gr 2)</i>
	Myalgia	<i>Myalgia (Gr 2)</i>
	Trismus	<i>Trismus (Gr 2)</i>
NERVOUS SYSTEM DISORDERS		

	Ataxia		<i>Ataxia (Gr 3)</i>
	Depressed level of consciousness		<i>Depressed level of consciousness (Gr 2)</i>
	Dizziness		<i>Dizziness (Gr 3)</i>
	Dysgeusia		<i>Dysgeusia (Gr 2)</i>
	Dysphasia		<i>Dysphasia (Gr 2)</i>
	Extrapyramidal disorder		<i>Extrapyramidal disorder (Gr 2)</i>
	Headache		<i>Headache (Gr 2)</i>
	Intracranial hemorrhage		<i>Intracranial hemorrhage (Gr 2)</i>
	Ischemia cerebrovascular		<i>Ischemia cerebrovascular (Gr 2)</i>
	Nerve disorder ⁶		<i>Nerve disorder⁶ (Gr 2)</i>
	Nervous system disorders - Other (multiple cranial nerve palsies)		<i>Nervous system disorders - Other (multiple cranial nerve palsies) (Gr 2)</i>
	Peripheral motor neuropathy		<i>Peripheral motor neuropathy (Gr 3)</i>
Peripheral sensory neuropathy			<i>Peripheral sensory neuropathy (Gr 3)</i>
	Seizure		<i>Seizure (Gr 2)</i>
PSYCHIATRIC DISORDERS			
	Anxiety		<i>Anxiety (Gr 2)</i>
	Confusion		<i>Confusion (Gr 3)</i>
	Depression		<i>Depression (Gr 2)</i>
	Insomnia		<i>Insomnia (Gr 2)</i>
RENAL AND URINARY DISORDERS			
		Acute kidney injury	<i>Acute kidney injury (Gr 3)</i>
	Hematuria		<i>Hematuria (Gr 2)</i>
	Renal hemorrhage		<i>Renal hemorrhage (Gr 2)</i>
	Urinary frequency		<i>Urinary frequency (Gr 2)</i>
	Urinary retention		<i>Urinary retention (Gr 2)</i>
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
	Hematosalpinx		<i>Hematosalpinx (Gr 2)</i>
	Ovarian hemorrhage		<i>Ovarian hemorrhage (Gr 2)</i>
	Prostatic hemorrhage		<i>Prostatic hemorrhage (Gr 2)</i>
	Spermatic cord hemorrhage		<i>Spermatic cord hemorrhage (Gr 2)</i>
	Testicular hemorrhage		<i>Testicular hemorrhage (Gr 2)</i>
	Uterine hemorrhage		<i>Uterine hemorrhage (Gr 2)</i>
	Vaginal hemorrhage		<i>Vaginal hemorrhage (Gr 2)</i>
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome	<i>Adult respiratory distress syndrome (Gr 2)</i>
	Allergic rhinitis		<i>Allergic rhinitis (Gr 2)</i>
	Bronchopulmonary hemorrhage		<i>Bronchopulmonary hemorrhage (Gr 2)</i>
	Bronchospasm		<i>Bronchospasm (Gr 2)</i>

	Cough		<i>Cough (Gr 2)</i>
	Dyspnea		<i>Dyspnea (Gr 4)</i>
	Hiccups		<i>Hiccups (Gr 2)</i>
	Pneumonitis		<i>Pneumonitis (Gr 3)</i>
	Pulmonary fibrosis		<i>Pulmonary fibrosis (Gr 4)</i>
	Sinus disorder		<i>Sinus disorder (Gr 2)</i>
	Voice alteration		<i>Voice alteration (Gr 2)</i>
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		<i>Alopecia (Gr 2)</i>
	Dry skin		<i>Dry skin (Gr 2)</i>
	Hyperhidrosis		<i>Hyperhidrosis (Gr 2)</i>
		Palmar-plantar erythrodysesthesia syndrome	<i>Palmar-plantar erythrodysesthesia syndrome (Gr 2)</i>
	Pruritus		<i>Pruritus (Gr 2)</i>
	Rash maculo-papular		<i>Rash maculo-papular (Gr 2)</i>
	Urticaria		<i>Urticaria (Gr 2)</i>
VASCULAR DISORDERS			
	Flushing		<i>Flushing (Gr 2)</i>
	Hot flashes		<i>Hot flashes (Gr 2)</i>
	Hypertension		<i>Hypertension (Gr 2)</i>
	Hypotension		<i>Hypotension (Gr 3)</i>
	Phlebitis		<i>Phlebitis (Gr 2)</i>
	Thromboembolic event		<i>Thromboembolic event (Gr 4)</i>
	Vascular disorders - Other (hemorrhage with thrombocytopenia)		<i>Vascular disorders - Other (hemorrhage with thrombocytopenia) (Gr 2)</i>

¹This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

²Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

³Gastrointestinal necrosis includes Anal necrosis, Esophageal necrosis, Gastric necrosis, Pancreatic necrosis, Peritoneal necrosis, and Rectal necrosis under the GASTROINTESTINAL DISORDERS SOC.

⁴Gastrointestinal ulcer includes Anal ulcer, Colonic ulcer, Duodenal ulcer, Esophageal ulcer, Gastric ulcer, Ileal ulcer, Jejunal ulcer, Rectal ulcer, and Small intestine ulcer under the GASTROINTESTINAL DISORDERS SOC.

⁵Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

⁶Nerve disorder includes Abducens nerve disorder, Accessory nerve disorder, Acoustic nerve disorder NOS, Facial nerve disorder, Glossopharyngeal nerve disorder, Hypoglossal nerve disorder, IVth nerve disorder, Oculomotor nerve disorder, Olfactory nerve disorder, Trigeminal nerve disorder, and Vagus nerve disorder under the NERVOUS SYSTEM DISORDERS SOC.

⁷Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

Also reported on oxaliplatin trials but with the relationship to oxaliplatin still undetermined:

CARDIAC DISORDERS - Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Pericardial effusion

EYE DISORDERS - Eye pain

GASTROINTESTINAL DISORDERS – Gastrointestinal perforation⁷

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Injury to superior vena cava; Vascular access complication

INVESTIGATIONS - Cardiac troponin I increased; Lipase increased; Serum amylase increased

METABOLISM AND NUTRITION DISORDERS - Hypercalcemia; Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness

NERVOUS SYSTEM DISORDERS - Syncope

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Hypoxia

VASCULAR DISORDERS - Visceral arterial ischemia

Note: Oxaliplatin in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

17.0 REFERENCES

1. Cunningham, D., et al., Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet*, 1998. **352**(9138): p. 1413-8.
2. Saltz, L.B., et al., Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med*, 2000. **343**(13): p. 905-14.
3. Douillard, J.Y., et al., Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet*, 2000. **355**(9209): p. 1041-7.
4. Rothenberg, M.L., et al., Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. *J Clin Oncol*, 2003. **21**(11): p. 2059-69.
5. de Gramont, A., et al., Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol*, 2000. **18**(16): p. 2938-47.
6. Goldberg, R.M., 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): p. 23-30.
7. Tournigand, C., et al., FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol*, 2004. **22**(2): p. 229-37.
8. Hurwitz, H., et al., Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*, 2004. **350**(23): p. 2335-42.
9. Saltz, L.B., N.J. Meropol, and e. al. Single agent IMC225 (Erbix) has activity in CPT-11 refractory colorectal cancer that expresses epidemral growth factor receptor (EGFR). in ASCO. 2002. Orlando, FL.
10. Mendelsohn, J., Targeting the epidermal growth factor receptor for cancer therapy. *J Clin Oncol*, 2002. **20**(18 Suppl): p. 1S-13S.
11. Saltz, L.B. and e. al. in ASCO. 2005.
12. Cunningham, D., et al., Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*, 2004. **351**(4): p. 337-45.
13. Xie, R., et al., Clinical pharmacokinetics of irinotecan and its metabolites: a population analysis. *J Clin Oncol*, 2002. **20**(15): p. 3293-301.
14. Wasserman, E., et al., Severe CPT-11 toxicity in patients with Gilbert's syndrome: two case reports. *Ann Oncol*, 1997. **8**(10): p. 1049-51.
15. Iyer, L., et al., UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J*, 2002. **2**(1): p. 43-7.
16. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. Meta-analysis Group In Cancer. *J Clin Oncol*, 1998. **16**(1): p. 301-8.
17. Saltz, L.B., et al., Phase I clinical and pharmacokinetic study of irinotecan, fluorouracil, and leucovorin in patients with advanced solid tumors. *J Clin Oncol*, 1996. **14**(11): p. 2959-67.

18. Poon, M.A., et al., Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. *J Clin Oncol*, 1991. **9**(11): p. 1967-72.
19. Goldberg, R., et al. N9741: Oxaliplatin (oxal) or CPT-11 + 5-fluorouracil (5FU)/leucovorin (LV) or oxal + CPT-11 in advanced colorectal cancer (CRC): Initial toxicity and response data from a GI intergroup study. in ASCO. 2002. Orlando, FL.
20. Rothenberg, M.L., et al., Mortality associated with irinotecan plus bolus fluorouracil/leucovorin: summary findings of an independent panel. *J Clin Oncol*, 2001. **19**(18): p. 3801-7.
21. Tournigand. FOLFIRI followed by FOLFOX versus FOLFOX followed by FOLFIRI in metastatic colorectal cancer: final results of a phase III study. in ASCO. 2001.
22. Raymond, E., et al., Oxaliplatin: a review of preclinical and clinical studies. *Ann Oncol*, 1998. **9**(10): p. 1053-71.
23. Comba, A., C. Blajman, and e. al, A randomised phase II study of oxaliplatin alone versus oxaliplatin combined with 5-fluorouracil and folinic acid (Mayo Clinic regimen) in previously untreated metastatic colorectal cancer patients. *Eur J Cancer*, 2001. **37**: p. 1006-1013.
24. Saltz, L.B., M. Rubin, and e. al. Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer that expresses epidermal growth factor receptor (EGFR). in ASCO. 2001.
25. Naughton, M.J. and S.A. Shumaker, The case for domains of function in quality of life assessment. *Qual Life Res*, 2003. **12 Suppl 1**: p. 73-80.
26. Naughton, M.J. and S.A. Shumaker, Assessment of Health-related Quality of Life, in *Fundamentals of Clinical Trials*, L.M. Friedman, C.D. furberg, and D.L. DeMets, Editors. 1996, Mosby Press: St. Louis.
27. Spilker, B.e., *Quality of Life Assessment in Clinical Trials*. 1990, New York: Raven Press.
28. Spilker, B.e., *Quality of Life and Pharmacoeconomics in Clinical Trials*. 1996, Philadelphia: Lippincott-Raven Publishers.
29. Stewart, A.L. and J.E.e. Ware, *Measuring Function and Wellbeing: The Medical Outcomes Study Approach*. 1992, Durham, NC: Duke University Press.
30. Wilken, D., L. Hallam, and M. Doggett, *Measures of Need and Outcome for Primary Health Care*. 1992, New York: Oxford Medical.
31. Berzon, R., R.D. Hays, and S.A. Shumaker, International use, application and performance of health-related quality of life instruments. *Qual Life Res*, 1993. **2**(6): p. 367-8.
32. Soumerai, S.B. and D. Ross-Degnan, Inadequate prescription-drug coverage for Medicare enrollees--a call to action. *N Engl J Med*, 1999. **340**(9): p. 722-8.
33. Gabel, J., et al., Job-based health benefits in 2002: some important trends. *Health Aff (Millwood)*, 2002. **21**(5): p. 143-51.
34. McCormack, L.A., et al., Retiree health insurance: recent trends and tomorrow's prospects. *Health Care Financ Rev*, 2002. **23**(3): p. 17-34.

35. Artz, M.B., R.S. Hadsall, and S.W. Schondelmeyer, Impact of generosity level of outpatient prescription drug coverage on prescription drug events and expenditure among older persons. *Am J Public Health*, 2002. **92**(8): p. 1257-63.
36. Adams, A.S., S.B. Soumerai, and D. Ross-Degnan, The case for a medicare drug coverage benefit: a critical review of the empirical evidence. *Annu Rev Public Health*, 2001. **22**: p. 49-61.
37. Joyce, G.F., et al., Employer drug benefit plans and spending on prescription drugs. *Jama*, 2002. **288**(14): p. 1733-9.
38. Blustein, J., Medicare and drug coverage: a women's health issue. *Womens Health Issues*, 2000. **10**(2): p. 47-53.
39. Blustein, J., Drug coverage and drug purchases by Medicare beneficiaries with hypertension. *Health Aff (Millwood)*, 2000. **19**(2): p. 219-30.
40. Tamblyn, R., et al., Adverse events associated with prescription drug cost-sharing among poor and elderly persons. *Jama*, 2001. **285**(4): p. 421-9.
41. EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group. *Health Policy*, 1990. **16**(3): p. 199-208.
42. Aaronson, N.K., et al., The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst*, 1993. **85**(5): p. 365-76.
43. Osoba, D., et al., Interpreting the significance of changes in health-related quality-of-life scores. *J Clin Oncol*, 1998. **16**(1): p. 139-44.
44. Anderson, R. and R. Rajagopalan, Responsiveness of the Dermatology-specific Quality of Life (DSQL) instrument to treatment for acne vulgaris in a placebo-controlled clinical trial. *Qual Life Res*, 1998. **7**(8): p. 723-34.
45. Willett, W.C., et al., Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*, 1985. **122**(1): p. 51-65.
46. Giovannucci, E., et al., Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Res*, 1994. **54**(9): p. 2390-7.
47. Willett, W.C., et al., Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *N Engl J Med*, 1990. **323**(24): p. 1664-72.
48. Giovannucci, E., et al., Alcohol, low-methionine--low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst*, 1995. **87**(4): p. 265-73.
49. Giovannucci, E., et al., Folate, methionine, and alcohol intake and risk of colorectal adenoma. *J Natl Cancer Inst*, 1993. **85**(11): p. 875-84.
50. Wu, A.H., et al., Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. *Br J Cancer*, 1987. **55**(6): p. 687-94.
51. Baron, J.A., et al., Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med*, 1999. **340**(2): p. 101-7.
52. Kampman, E., et al., Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control*, 2000. **11**(5): p. 459-66.

53. Howe, G.R., et al., Dietary intake of fiber and decreased risk of cancers of the colon and rectum: evidence from the combined analysis of 13 case-control studies. *J Natl Cancer Inst*, 1992. **84**(24): p. 1887-96.
54. Arber, N. and R.N. DuBois, Nonsteroidal anti-inflammatory drugs and prevention of colorectal cancer. *Curr Gastroenterol Rep*, 1999. **1**(5): p. 441-8.
55. Thun, M.J., Aspirin and gastrointestinal cancer. *Adv Exp Med Biol*, 1997. **400A**: p. 395-402.
56. Caan, B.J., et al., Body size and the risk of colon cancer in a large case-control study. *Int J Obes Relat Metab Disord*, 1998. **22**(2): p. 178-84.
57. Chute, C.G., et al., A prospective study of body mass, height, and smoking on the risk of colorectal cancer in women. *Cancer Causes Control*, 1991. **2**(2): p. 117-24.
58. Chyou, P.H., A.M. Nomura, and G.N. Stemmermann, A prospective study of colon and rectal cancer among Hawaii Japanese men. *Ann Epidemiol*, 1996. **6**(4): p. 276-82.
59. Ford, E.S., Body mass index and colon cancer in a national sample of adult US men and women. *Am J Epidemiol*, 1999. **150**(4): p. 390-8.
60. Giovannucci, E., et al., Physical activity, obesity, and risk for colon cancer and adenoma in men. *Ann Intern Med*, 1995. **122**(5): p. 327-34.
61. Le Marchand, L., et al., Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. *Cancer Res*, 1997. **57**(21): p. 4787-94.
62. Lee, I.M. and R.S. Paffenbarger, Jr., Quetelet's index and risk of colon cancer in college alumni. *J Natl Cancer Inst*, 1992. **84**(17): p. 1326-31.
63. Martinez, M.E., et al., Leisure-time physical activity, body size, and colon cancer in women. Nurses' Health Study Research Group. *J Natl Cancer Inst*, 1997. **89**(13): p. 948-55.
64. Giovannucci, E., et al., A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. *J Natl Cancer Inst*, 1994. **86**(3): p. 192-9.
65. Giovannucci, E. and M.E. Martinez, Tobacco, colorectal cancer, and adenomas: a review of the evidence. *J Natl Cancer Inst*, 1996. **88**(23): p. 1717-30.
66. Giovannucci, E., et al., A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. men. *J Natl Cancer Inst*, 1994. **86**(3): p. 183-91.
67. Brown, J., et al., Nutrition during and after cancer treatment: a guide for informed choices by cancer survivors. *CA Cancer J Clin*, 2001. **51**(3): p. 153-87; quiz 189-92.
68. Taylor, S.E., R.R. Lichtman, and J.V. Wood, Attributions, beliefs about control, and adjustment to breast cancer. *J Pers Soc Psychol*, 1984. **46**(3): p. 489-502.
69. Thompson, S.C., Will it hurt less if i can control it? A complex answer to a simple question. *Psychol Bull*, 1981. **90**(1): p. 89-101.
70. Cui, Y., et al., Body mass and stage of breast cancer at diagnosis. *Int J Cancer*, 2002. **98**(2): p. 279-83.
71. Daling, J.R., et al., Relation of body mass index to tumor markers and survival among young women with invasive ductal breast carcinoma. *Cancer*, 2001. **92**(4): p. 720-9.
72. Holm, L.E., et al., Dietary habits and prognostic factors in breast cancer. *J Natl Cancer Inst*, 1989. **81**(16): p. 1218-23.

73. Verreault, R., et al., Dietary fat in relation to prognostic indicators in breast cancer. *J Natl Cancer Inst*, 1988. **80**(11): p. 819-25.
74. Camoriano, J.K., et al., Weight change in women treated with adjuvant therapy or observed following mastectomy for node-positive breast cancer. *J Clin Oncol*, 1990. **8**(8): p. 1327-34.
75. Chlebowski, R.T., et al., Long-term survival following relapse after 5-FU but not CMF adjuvant breast cancer therapy. *Breast Cancer Res Treat*, 1986. **7**(1): p. 23-30.
76. Levine, E.G., J.M. Raczynski, and J.T. Carpenter, Weight gain with breast cancer adjuvant treatment. *Cancer*, 1991. **67**(7): p. 1954-9.
77. Sun, A.S., et al., Phase I/II study of stage III and IV non-small cell lung cancer patients taking a specific dietary supplement. *Nutr Cancer*, 1999. **34**(1): p. 62-9.
78. Slattery, M.L., et al., Diet and survival of patients with colon cancer in Utah: is there an association? *Int J Epidemiol*, 1989. **18**(4): p. 792-7.
79. Chinery, R., et al., Antioxidants enhance the cytotoxicity of chemotherapeutic agents in colorectal cancer: a p53-independent induction of p21WAF1/CIP1 via C/EBPbeta. *Nat Med*, 1997. **3**(11): p. 1233-41.
80. Mathijssen, R.H., et al., Effects of St. John's wort on irinotecan metabolism. *J Natl Cancer Inst*, 2002. **94**(16): p. 1247-9.
81. Chang, K.M., et al., Epidermal growth factor in gingival crevicular fluid and its binding capacity in inflamed and non-inflamed human gingiva. *Arch Oral Biol*, 1996. **41**(7): p. 719-24.
82. van Oijen, M.G., et al., Increased expression of epidermal growth factor receptor in normal epithelium adjacent to head and neck carcinomas independent of tobacco and alcohol abuse. *Oral Dis*, 1998. **4**(1): p. 4-8.
83. Wang, S.L., et al., Smoking-related alterations in epidermal growth factor and insulin receptors in human placenta. *Mol Pharmacol*, 1988. **34**(3): p. 265-71.
84. Wang, S.L., et al., Effect of cigarette smoking on salivary epidermal growth factor (EGF) and EGF receptor in human buccal mucosa. *Toxicology*, 1992. **75**(2): p. 145-57.
85. Wang, S.L., et al., Chronic ethanol feeding alters the structure and function of the epidermal growth factor receptor in rat stomach. *Alcohol*, 1996. **13**(5): p. 461-6.
86. Flanigen-Roat, D.T., R.J. Milholland, and M.M. Ip, Effect of a high-protein-low-carbohydrate diet on toxicity and antitumor activity of 5-fluorouracil in mice. *J Natl Cancer Inst*, 1985. **74**(3): p. 705-10.
87. Kuhn, J.G., Pharmacology of irinotecan. *Oncology (Huntingt)*, 1998. **12**(8 Suppl 6): p. 39-42.
88. Guengerich, F.P., Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. *Am J Clin Nutr*, 1995. **61**(3 Suppl): p. 651S-658S.
89. Anderson, G.D., A mechanistic approach to antiepileptic drug interactions. *Ann Pharmacother*, 1998. **32**(5): p. 554-63.
90. Astorg, P., et al., Effects of beta-carotene and canthaxanthin on liver xenobiotic-metabolizing enzymes in the rat. *Food Chem Toxicol*, 1994. **32**(8): p. 735-42.
91. Burchell, B. and M.W. Coughtrie, Genetic and environmental factors associated with variation of human xenobiotic glucuronidation and sulfation. *Environ Health Perspect*, 1997. **105 Suppl 4**: p. 739-47.

92. Miners, J.O. and P.I. Mackenzie, Drug glucuronidation in humans. *Pharmacol Ther*, 1991. **51**(3): p. 347-69.
93. Neumann, C.M. and V.G. Zannoni, Ascorbic acid deficiency and hepatic UDP-glucuronyltransferase. *Drug Metab Dispos*, 1988. **16**(4): p. 551-6.
94. Ritter, J.K., et al., Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: evidence for both genetic and environmental influences. *Hepatology*, 1999. **30**(2): p. 476-84.
95. Takasuna, K., et al., Protective effects of kampo medicines and baicalin against intestinal toxicity of a new anticancer camptothecin derivative, irinotecan hydrochloride (CPT-11), in rats. *Jpn J Cancer Res*, 1995. **86**(10): p. 978-84.
96. Hambly, R.J., et al., Effects of high- and low-risk diets on gut microflora-associated biomarkers of colon cancer in human flora-associated rats. *Nutr Cancer*, 1997. **27**(3): p. 250-5.
97. Johansson, G.K., L. Ottova, and J.A. Gustafsson, Shift from a mixed diet to a lactovegetarian diet: influence on some cancer-associated intestinal bacterial enzyme activities. *Nutr Cancer*, 1990. **14**(3-4): p. 239-46.
98. Kwei, G.Y., et al., Enzyme activities associated with carcinogen metabolism in liver and nonhepatic tissues of rats maintained on high fat and food-restricted diets. *J Nutr*, 1991. **121**(1): p. 131-7.
99. Ling, W.H. and O. Hanninen, Shifting from a conventional diet to an uncooked vegan diet reversibly alters fecal hydrolytic activities in humans. *J Nutr*, 1992. **122**(4): p. 924-30.
100. Quasthoff, S. and H.P. Hartung, Chemotherapy-induced peripheral neuropathy. *J Neurol*, 2002. **249**(1): p. 9-17.
101. Cascinu, S., et al., Neuroprotective effect of reduced glutathione on oxaliplatin-based chemotherapy in advanced colorectal cancer: a randomized, double-blind, placebo-controlled trial. *J Clin Oncol*, 2002. **20**(16): p. 3478-83.
102. Doz, F., et al., Experimental basis for increasing the therapeutic index of cis-diamminedicarboxylatocyclobutaneplatinum(II) in brain tumor therapy by a high-zinc diet. *Cancer Chemother Pharmacol*, 1992. **29**(3): p. 219-26.
103. Lautermann, J., et al., Diet is a risk factor in cisplatin ototoxicity. *Hear Res*, 1995. **88**(1-2): p. 47-53.
104. Pace, A., et al., Neuroprotective effect of vitamin E supplementation in patients treated with cisplatin chemotherapy. *J Clin Oncol*, 2003. **21**(5): p. 927-31.
105. Giovannucci, E., 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): p. 345-9.
106. Ma, J., 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): p. 620-5.
107. Vacaresse, N., et al., Activation of epithelial growth factor receptor pathway by unsaturated fatty acids. *Circ Res*, 1999. **85**(10): p. 892-9.
108. Potter, J.D., Colorectal cancer: molecules and populations. *J Natl Cancer Inst*, 1999. **91**(11): p. 916-32.

109. Giovannucci, E., et al., Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med*, 1998. **129**(7): p. 517-24.
110. Glynn, S.A., et al., Colorectal cancer and folate status: a nested case-control study among male smokers. *Cancer Epidemiol Biomarkers Prev*, 1996. **5**(7): p. 487-94.
111. Jenkins, D.J., et al., Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr*, 1981. **34**(3): p. 362-6.
112. Foster-Powell, K. and J.B. Miller, International tables of glycemic index. *Am J Clin Nutr*, 1995. **62**(4): p. 871S-890S.
113. Wolever, T.M., et al., The glycemic index: methodology and clinical implications. *Am J Clin Nutr*, 1991. **54**(5): p. 846-54.
114. Frost, G., et al., Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycemic diet. *Metabolism*, 1998. **47**(10): p. 1245-51.
115. Miller, J.C., Importance of glycemic index in diabetes. *Am J Clin Nutr*, 1994. **59**(3 Suppl): p. 747S-752S.
116. Slabber, M., et al., Effects of a low-insulin-response, energy-restricted diet on weight loss and plasma insulin concentrations in hyperinsulinemic obese females. *Am J Clin Nutr*, 1994. **60**(1): p. 48-53.
117. Wolever, T.M. and D.J. Jenkins, The use of the glycemic index in predicting the blood glucose response to mixed meals. *Am J Clin Nutr*, 1986. **43**(1): p. 167-72.
118. Slattery, M.L., et al., Dietary sugar and colon cancer. *Cancer Epidemiol Biomarkers Prev*, 1997. **6**(9): p. 677-85.
119. Franceschi, S., et al., Dietary glycemic load and colorectal cancer risk. *Ann Oncol*, 2001. **12**(2): p. 173-8.
120. Colditz, G.A., et al., Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol*, 1986. **123**(5): p. 894-900.
121. Hu, F.B., et al., Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr*, 1999. **69**(2): p. 243-9.
122. Willett, W.C., et al., The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol*, 1988. **127**(1): p. 188-99.
123. Willett, W.C., et al., Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. *Am J Clin Nutr*, 1983. **38**(4): p. 631-9.
124. Willett, W. and M.J. Stampfer, Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*, 1986. **124**(1): p. 17-27.
125. Ascherio, A., et al., Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J Nutr*, 1992. **122**(9): p. 1792-801.
126. Stryker, W.S., et al., Contributions of specific foods to absolute intake and between-person variation of nutrient consumption. *J Am Diet Assoc*, 1991. **91**(2): p. 172-8.
127. Hunter, D.J., et al., Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. *Am J Epidemiol*, 1992. **135**(4): p. 418-27.

128. London, S.J., et al., Fatty acid composition of subcutaneous adipose tissue and diet in postmenopausal US women. *Am J Clin Nutr*, 1991. **54**(2): p. 340-5.
129. Wolf, A.M., et al., Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol*, 1994. **23**(5): p. 991-9.
130. Chasan-Taber, S., et al., Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology*, 1996. **7**(1): p. 81-6.
131. Freidlin, B., E.L. Korn, and S.L. George, Data monitoring committees and interim monitoring guidelines. *Control Clin Trials*, 1999. **20**(5): p. 395-407.
132. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. *Biometrika*, 1983 **70**(3):659-663.
133. King, M.T., The interpretation of scores from the EORTC quality of life questionnaire QLQ-C30. *Qual Life Res*, 1996. **5**(6): p. 555-67.
134. Hogan, J.W. and N.M. Laird, Mixture models for the joint distribution of repeated measures and event times. *Stat Med*, 1997. **16**(1-3): p. 239-57.
135. Gold M.R., J.E. Siegel, L.B. Russell, M.C.E. Weinstein, *Cost-effectiveness in health and medicine*. New York: Oxford University Press, 1996.
136. Tol J, Koopman M, Cats A, et al: Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med*, 2009. **360**: p. 563-572.
137. Hecht JR, Mitchell E, Chidiac T, et al: A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol*, 2009. **27**: p. 672-680.
138. Fleiss JL. *Statistical Methods for Rates and Proportions*, Second Edition. John Wiley & Sons. New York. 1981. pp 212-225.

APPENDIX I

CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION:

<p>To submit site registration documents:</p> <p>CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone: 1-888-823-5923 Fax: 215-569-0206</p>	<p>For patient enrollments:</p> <p>CTSU Patient Registration Phone: 1-888-462-3009 Fax: 1-888-691-8039 Hours: 9:00 AM – 5:30 PM Eastern Time, Monday – Friday (excluding holidays)</p> <p>[Registrations received after 5:00 PM ET will be handled the next business day. For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376 between 9:00 a.m. and 5:30 p.m.]</p>
--	--

Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:

Alliance Statistics and Data Center
Attention: CALGB 80405 Data Manager
RO FF-3-24-CC/NW Clinic
200 First Street Southwest
Rochester, MN 55905 USA
Tel: 507-284-5380
Fax: 507-284-1902

See [Section 5.5](#) Data Submission Section for details on forms submission.

Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility or treatment related questions: Contact the Alliance Co-Chair first, then the Alliance or SWOG Study Chair.

For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail:
CTSU General Information Line – 1-888-823-5923, or ctscontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.

The CTSU Public Web site is located at: www.ctsu.org
The CTSU Registered Member Web site is located at <http://members.ctsu.org>

REGISTRATION/RANDOMIZATION

Prior to the recruitment of a patient for this study, investigators must be registered members of the CTSU. Each investigator must have an NCI investigator number and must maintain an “active” investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU registered member Web site or by calling the PMB at 240-276-6575 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site at <http://members.ctsu.org>.

All forms and documents associated with this study can be downloaded from the C80405 Web page on the CTSU registered member Web site (<https://members.ctsu.org>). Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS.

Requirements for C80405 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

Prestudy requirements for patient enrollment on C80405:

- Patient must meet all inclusion criteria, and no exclusion criteria should apply
- Patient has signed and dated all applicable consents and authorization forms, and the patient decision whether to permit use of tissue for related studies and future studies has been documented.
- All baseline laboratory tests and prestudy evaluations performed within the time period specified in the protocol.

CTSU PROCEDURES FOR PATIENT ENROLLMENT

Pre-Registration Step:

Note: Pre-registration must occur at the time of tissue submission for K-ras mutation testing (see [Section 5.6](#)).

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 a.m. and 5:30 p.m. Eastern Time, Mon-Fri.. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g. within one hour, call the registrar cell phone at 1-301-704-2376.
2. Complete the following forms:
 - CTSU Patient Enrollment Transmittal Form
 - CALGB 80405 Pre-Registration Worksheet
3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 9:00 a.m. and 5:30 p.m., Mon-Fri, Eastern Time (excluding holidays); however, please be aware that registrations received after 5:00 p.m. will be processed the next day. This is limited to the operating hours of the CALGB Registration Office. The CTSU registrar will check the investigator and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and will follow-up with the site to resolve any discrepancies.
4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB **within the confines of CALGB's registration hours**. The CTSU registrar will access the CALGB's on-line registration system, to pre-register the patient and obtain assignment of a unique patient ID. The CTSU registrar will confirm registration by fax.

Registration/Randomization Step:

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 a.m. and 5:30 p.m. Eastern Time, Monday-Friday. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g. within one hour, call the registrar cell phone at 1-301-704-2376.
2. Complete the following forms:
 - CTSU Patient Enrollment Transmittal Form
 - CALGB C80405 Registration Worksheet

3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 9:00 a.m. and 5:50 p.m., Mon-Fri, Eastern Time (excluding holidays); however, please be aware that registrations received after 5:00 p.m. will be processed the next day. Registration is limited to the operating hours of the CALGB Registration Office (9 AM – 5 PM ET). The CTSU registrar will check the investigator and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and follow-up with the site to resolve any discrepancies.
4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, within the confines of CALGB's registration hours. The CTSU registrar will access the CALGB's on-line registration system, to obtain assignment of treatment arm and assignment of a unique patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.
 - Protocol treatment should begin within 14 days of randomization.
 - Registration to the companion studies for those patients who have agreed to participate will be performed at the same time as registration to the treatment study.

Procedures for late enrollment onto CALGB 60501 (pharmacogenomic companion):

- Submit CTSU Patient Enrollment transmittal form (with note indicating delayed registration to the pharmacogenomic companion study).
- Submit revised C80405 Registration Worksheet (indicating patient consent for CALGB 60501).

Note: Although it is preferable that patients are registered to 60501 at the same time they are registered to 80405, registration to 60501 may occur up to 60 days following registration to the treatment trial.

DATA SUBMISSION AND RECONCILIATION

1. All case report forms (CRFs) and transmittals associated with this study must be downloaded from the C80405 Web page located on the CTSU registered member Web site (<https://members.ctsu.org>). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.
2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the Alliance Statistics and Data Center, [see contact table and [Section 5.5](#) of protocol] unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.
3. The Alliance Statistics and Data Center will send (generally via email but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the Alliance Statistics and Data Center (via postal mail or fax) and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP AMS account contact information current**. This will ensure timely communication between the clinical site and the Alliance Statistics and Data Center.

SPECIAL MATERIALS OR SUBSTUDIES

There are four substudies embedded within C80405. All require patient consent.

- Diet and Lifestyle Substudy
- Quality of Life and Health Services: CALGB 70502 – requires separate registration
- Predictors of Toxicity and Outcome in Patients Receiving Chemotherapy for Metastatic Colorectal Cancer: CALGB 150506 – requires separate registration.
- Pharmacogenomic Substudy: CALGB 60501 – requires separate registration.

1. Specimen Submission for Correlative Studies (Protocol [Section 5.6](#))

- Collect, prepare, and submit specimens as outlined in the protocol.
- Do not send specimens, supporting clinical reports, or transmittals to the CTSU.
- All specimens (except whole blood for pharmacogenomic studies) submitted for this study must be entered and tracked using the SWOG on-line Specimen Tracking System, as specified in protocol [Section 5.6](#). You can also access the Tracking System from the CTSU Member Web Site. Go to the C80405 protocol page and click on the link provided under the Case Report Forms header.

2. Quality of Life (Protocol Sections [5.7](#) and [5.8](#))

- Send completed surveys as outlined in Sections [5.7](#) and [5.8](#) of the protocol.
- Do not send form/surveys to the CTSU.

SERIOUS ADVERSE EVENT (AE) REPORTING ([SECTION 16.0](#))

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.
2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Reporting System (CTEP-AERS) from either the Adverse Events tab of the CTSU member homepage (<https://members.ctsu.org>) or by selecting Adverse Event Reporting Forms from the document center drop down list on the protocol number Web page.
3. Do not send adverse event reports to the CTSU.
4. Secondary AML/MDS/ALL reporting: Report occurrence of secondary AML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of CTEP-AERS. Submit the completed form and supporting documentation as outlined in the protocol.

DRUG PROCUREMENT ([SECTION 11.0](#)):

Investigational agents: Cetuximab (Distributed by Eli Lilly and Company, see [Section 11.9](#) for details on drug ordering)

Commercial agents: 5-FU, Leucovorin, Oxaliplatin, Irinotecan, Bevacizumab

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in [Section 11.0](#) of the protocol.
2. You may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the C80405 Web page.

REGULATORY AND MONITORING

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314 and HHS 45 CFR 46] and National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

Responsibility for assignment of the audit will be determined by the site's primary affiliation with a Cooperative Group or CTSU. For Group-aligned sites, the audit of a patient registered through CTSU will become the responsibility of the Group receiving credit for the enrollment. For CTSU Independent Clinical Research Sites (CICRS), the CTSU will coordinate the entire audit process.

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial.

Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTSU Operations Manual located on the CTSU Member Web site.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

The HIPAA Privacy Rule establishes the conditions under which protected health information may be used or disclosed by covered entities for research purposes. Research is defined in the Privacy Rule referenced in HHS 45 CFR 164.501. Templated language addressing NCI-U.S. HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU website.

The HIPAA Privacy Rule does not affect participants from outside the United States. Authorization to release Protected Health Information is NOT required from patients enrolled in clinical trials at non-US sites.

Clinical Data Update System (CDS) Monitoring

This study will be monitored by the Clinical Data System (CDS-web). Cumulative CDS data will be submitted quarterly to CTEP by electronic means. The sponsoring Group fulfills this reporting obligation by electronically transmitting to CTEP the CDS data collected from the study-specific case report forms.

APPENDICES II, III, IV, V, and VI

**PREDICTORS OF TOXICITY AND OUTCOME IN PATIENTS RECEIVING CHEMOTHERAPY FOR
METASTATIC COLORECTAL CANCER**

COMPANION STUDIES TO CALGB 80405

Correlative Science Investigators:

Heinz-Josef Lenz, MD *University of Southern California*
Norris Comprehensive Cancer Center
1441 East Lake Avenue, Room 3456
Los Angeles, CA 90033
Tel: (323) 865-3955

Monica Bertagnolli, MD *Brigham and Women's Hospital*
Tel: 617-732-8910

Herb Hurwitz, MD *Duke University*
Tel: 919-681-5257

Dhavalkumar Patel, MD *University of North Carolina*
dhavalkumar_patel@med.unc.edu

Najjia Mahmoud, MD *University of Pennsylvania*
najjia.mahmoud@uphs.upenn.edu

Pharmacogenomic Investigators:

Howard McLeod, MD *Washington University School of Medicine*
660 E. Euclid Ave.
St. Louis, MO 63110
Tel: 314-747-2060
hmcLeod@im.wustl.edu

Apurva Desai, MD *University of Chicago*
Tel: 773-834-0737

Mark Ratain, MD *University of Chicago*
Tel: 919-773-4400

INTRODUCTION

Nine major areas of correlative studies are planned for this trial for which we collect:

Tumor tissues	1) Gene Expression Level	(Appendix II)
	2) Immunohistochemistry	(Appendix II)
Serum/Plasma	3) Proteinomic Studies	(Appendix III)
	4) Serum Biomarkers	(Appendix III)
Serum/Plasma/Whole blood	5) Infusion Reactions	(Appendix III)
Whole blood	6) Pharmacogenomic Studies	(Appendix IV)
Tumor tissue/Serum/Plasma	7) K-ras, EGFR Ligand, PTEN Studies	(Appendix V)
Tumor tissues	8) BRAF Mutation Detection	(Appendix VI)
	9) PTEN Gene Sequencing	(Appendix VI)

The administration of chemotherapy is limited by significant inter-individual variations in response and toxicity. Such variations are often due to genetic alterations (gene expression levels and/or genomic polymorphisms) in enzymes responsible for drug metabolism, DNA repair, angiogenesis or the EGFR pathway. Current concepts and clinical practice regarding prognosis and therapy for patients with colon cancer rest on the gross clinical/pathological staging. Identification of molecular determinants of drug efficacy and toxicity to chemotherapies which are combined with cetuximab or bevacizumab might become important in the design of individualized chemotherapy based on the individual's molecular tumor and genomic profiles. The goal of this correlative project is to identify germline variations and gene expression levels and arrays associated with clinical toxicity and outcome in patients treated with chemotherapy in combination with cetuximab or bevacizumab. We also included projects to identify serum biomarkers and proteomics profiles potentially predictive of clinical outcome and toxicity.

To identify relevant markers, this intergroup clinical trial will focus on two key pathways: 1) angiogenesis and 2) the EGFR pathway. It is expected that specific gene expression levels involved in angiogenesis (VEGF) and the EGFR pathway (EGFR) in the tumor will primarily predict time to progression and overall survival, whereas haplotypes of candidate gene with germline variations, which are present in all normal cells as well as the tumor, will be related to toxicity and possibly clinical outcome. We will also test whether proteinomic profiles and serum biomarkers from serum/plasma will be associated with clinical outcome and toxicity again with the primary focus on angiogenesis and the EGFR pathway.

Appendices II, III, and IV describe pathways as well as technologies that reflect the state of art at the time of protocol submission. However the knowledge base will increase significantly during the course of this protocol along with development of improved and novel technologies. Analyses of the samples collected within this protocol will incorporate new discoveries and new technologies (Appendices V and VI). Tumor tissues, blood and serum samples will be collected and stored at the SWOG GI Tumor bank under the direction of Dr. Fenoglio-Preisser. The priority of molecular analyses as well as the laboratories chosen for these analyses will be identified by the co-chairs (Drs. Lenz and Bertagnolli) of correlative science of this protocol who will closely work with Drs. Hurwitz, Fuchs, Meyerhardt, Fenoglio-Preisser, Ratain and McLeod to assure the highest quality of analyses.

APPENDIX II

PART A

GENE EXPRESSION LEVELS: PREDICTORS OF TOXICITY AND OUTCOME IN PATIENTS RECEIVING CHEMOTHERAPY FOR METASTATIC COLORECTAL CANCER ON 80405

1.0 Background

The primary endpoints of this gene expression study are the EGFR and VEGF pathway. The secondary endpoints focus on the drug metabolism of 5-FU, Irinotecan and DNA repair. Preliminary analyses on gene expression profiles using paraffin embedded tumor tissues will also be performed.

Primary Endpoints:

1. Angiogenesis Pathway
2. EGFR Pathway

Secondary Endpoints:

3. 5-FU Metabolism
4. Oxaliplatin/DNA Repair Pathway
5. Irinotecan Pathway
6. Molecular Mechanisms of Toxicity
7. Gene Expression Arrays

- 1.1 Molecular Determinants of Angiogenesis:** Our primary hypothesis is that gene expression levels of enzymes of VEGF and VEGFR are associated with clinical outcome and toxicity in patients treated with bevacizumab based chemotherapy. Our second hypothesis that gene expression levels of genes involved in the angiogenesis pathway such as TGFB, IL-8, COX-2 and TP are associated with clinical outcome and toxicity in patients treated with bevacizumab based chemotherapy.

The angiogenic factors such as VEGF, TGF- β , IL-8 and IL-10 have been suggested to play an important prognostic role in colorectal cancer and very recently thymidine phosphorylase (TP) has been shown to increase COX activity and COX-2 protein expression in several cell types (19). There are preliminary data suggesting that COX-2 overexpression increases adhesion to the extracellular matrix. One of the potential molecular effects of COX-2 inhibition is on the integrin family particular $\alpha_v\beta_1$ and β_3 based on the findings that COX-2 expression has been associated with increased attachment to laminin and matrigel. VEGF has been found to be closely associated with microvessel density formation in various cancers (20,21). A recent study showed that TP and VEGF expression in colon cancer appeared to be anti-coordinated; that is, in tumors with a high vessel density, TP expression was high when VEGF was low and vice versa (20). These data suggest that VEGF and COX-2 may play a significant role in tumor progression in patients with colorectal cancer. Vessel count and expression of VEGF have been shown to predict distant recurrence in patients with node-negative colon cancer (21). Thus, if VEGF is also associated with clinical outcome, the use of TP, COX-2 and VEGF expressions

simultaneously may identify patients who will more likely respond to therapy and ultimately may allow the development of more effective treatment strategies.

Our preliminary data suggest that the gene expression levels of VEGF in the normal and tumor tissue and germline polymorphism of VEGF and TGF- β may be associated tumor recurrence. In addition, the intratumoral mRNA levels of VEGF were significantly higher compared to the normal mucosa ($p < 0.001$) indicating that the tumor has a higher angiogenic potential. Our hypothesis is that high expression levels of genes involved in angiogenesis will be associated with clinical outcome and toxicity in patients treated with targeted chemotherapy. We will also test whether gene expression profiles will be associated with the pattern of recurrence and progression.

In the future, a combination of novel genes in metabolic and biological pathways might become important predictors of response, survival and toxicity. Angiogenesis is a good example because nucleotide excision is a symphony of enzymes working together to repair DNA defects. Enzymes such as VEGF, TP, COX-2, IL-8 and others all depend on each others' function; alterations of any of these genes can affect clinically relevant angiogenesis.

- 1.2 Epithelial Growth Factor Receptor (EGFR) Pathway:** Our primary hypothesis is that gene expression of enzymes involved in the EGFR pathway such as EGFR and EGF are associated with clinical outcome and toxicity to cetuximab based chemotherapy. Our second hypothesis is that gene expression in the EGFR pathway such as IL-8, VEGF and COX-2 and others are associated with clinical outcome and toxicity to cetuximab based chemotherapy.

EGF-R is a commonly expressed transmembrane glycoprotein of the tyrosine kinase growth factor family. EGFR are frequently overexpressed in many types of human cancers, including CRC (colon and rectal cancers) and their overexpression typically confers a more aggressive clinical behavior. The level of EGFR expression is primarily regulated by the abundance of its mRNA and the nature of the EGFR overexpression is believed to be due to an increase in the rate of EGFR transcription. Our data suggest that EGFR-mediated activation of the overall DNA-repair capacity might override the stimulation of tumor proliferation by the receptor in human colorectal cancers. This enhanced DNA-repair may result in an impaired efficacy of the administered oxaliplatin.

Our preliminary data in patients with metastatic colorectal cancer treated with cetuximab who had low gene expression levels of Cox-2, EGFR and IL-8 showed a significantly longer overall survival which was independent from skin toxicity. These data suggest that Cox-2 and IL-8 may be important factors in the EGFR signaling pathway. Cox-2, a rate-limiting enzyme involved in the conversion of arachidonic acid to prostaglandins, is well known to affect tumorigenesis in the colon by the regulation of apoptosis, angiogenesis and tumor cell invasiveness (22). Pai et al. showed recently that prostaglandin E₂, produced by the cyclooxygenase enzymes, transactivates EGFR in gastric epithelial and colon cancer cells, suggesting that Cox-2 induces the pathogenesis of cancer development by the activation of EGFR (23). Perrotte et al. showed that treatment with cetuximab in human transitional cell carcinoma growing orthotopically in nude mice inhibited mRNA and protein production of IL-8 accompanied by the involution of blood vessels (24). These data suggest that inhibition of angiogenesis by cetuximab may be by down-regulation of IL-8. Our findings implicate that Cox-2 may be an important regulator of EGFR, while IL-8 may play a part in cetuximab-related inhibition of angiogenesis.

The most common side-effect of cetuximab treatment is an acneiform follicular or perifollicular dermatitis that generally appears during the first 3 weeks of therapy

(25). In our study, 85% of patients presented with such acne-like rash. Interestingly, study patients with a grade 2-3 cutaneous reaction had significantly lower Cox-2 gene expression levels compared with patients that had grade 0-1 toxicity. Patients with lower EGFR mRNA

amount are more likely to have severe skin reactions compared with patients that have higher EGFR expression levels. These results imply that low levels of Cox-2 induce low levels of EGFR, leading to a down-regulated signaling pathway and therefore leading to the acne-like rash. However, it has been suggested that Cox-2, via the up-regulation of prostaglandins, is mainly involved in the pathogenesis of oral mucositis, a common side effect in 5FU and radiation treated patients (26).

We will test whether gene expression levels of genes involved in the EGFR pathway will predict with response, time to tumor progression and overall survival as well as toxicity. We will, however, review the literature, and refine our choices at the beginning of analyses of this project to incorporate relevant new findings as well as new technologies.

1.3 Mechanism of 5-FU chemosensitivity: Our hypothesis is that elevated intratumoral gene expressions levels of TS, DPD and TP and other genes in the 5-FU pathway will be associated with tumor response, survival and toxicity in patients with metastatic colorectal cancer treated with 5-FU based chemotherapy.

Thymidylate synthase catalyzes the intracellular conversion of deoxyuridylate to deoxythymidylate - the sole de novo source of thymidylate in the cell (1). The active metabolite of 5-FU, 5-fluorodeoxyuridylate (5-FdUMP), binds to TS and inhibits it by forming a stable ternary complex (2).

The rationale to incorporate these findings into clinical analyses and protocols was provided by earlier studies by Leichman et al. (3) and others (4-6). Leichman et al. were the first to demonstrate a significant inverse relationship between intratumoral TS gene expression and response to 5-FU based chemotherapy. In a retrospective analysis of 42 colorectal tumor samples pretreatment TS expression was determined using a RT-PCR based protocol. The median TS expression level among the 12 patients (29%) who responded to 5-FU/LV was 1.7×10^{-3} (as TS/ β -actin ratio). Non-responding tumors showed a median TS expression level of 5.6×10^{-3} (as TS/ β -actin ratio). Patients with low TS gene expression levels showed a superior median survival of 13.6 months, compared to 8.2 months in patients whose tumors had an increased TS-level (TS/ β -actin ratio of 3.5×10^{-3} was determined as cut-off) ($p = 0.02$) (3). Further clinical evaluation identified low TS expression as predictive for superior response to 5-FU/oxaliplatin (6), raltitrexed (4) or other fluoropyrimidine-based chemotherapy (5,7) in the treatment of colorectal cancer. Dihydropyrimidine dehydrogenase (DPD) represents the key enzyme of 5-FU metabolism. This rate-limiting enzyme of 5-FU catabolism inactivates > 80% of the drug in the liver (8). Retrospective analyses of gene expression revealed that low DPD levels may be important for superior response to fluoropyrimidine treatment (9). It has been suggested that low levels of DPD may increase bioavailability of the drug, thereby improving response. Our group, in collaboration with Dr. Diasio, has demonstrated that intratumoral gene expression of DPD can predict all patients who will respond to 5-FU chemotherapy (9). In vitro data suggested that oxaliplatin may down regulate TS and DPD and therefore increase the potential threshold known for TS and DPD expression levels (4,5). These data warrant further studies to establish what role the genes in the 5-FU pathway play in combination chemotherapies with Irinotecan or Oxaliplatin or with targeted agents.

Other enzymes in this pathway may be also critical for the efficacy of 5-FU based chemotherapy. Recently, Isshi et al., (10) demonstrated that high levels of orotate phosphorybosyl transferase (OPRT) may be associated with increased sensitivity to 5-FU based chemotherapy. OPRT catalyzes the reduction of FUDP to the actively TS inhibiting metabolite FdUMP indicating a role of chemosensitivity to 5-FU. There are other enzymes in this pathway such as dUTPase which will be evaluated.

- 1.3 DNA repair associated with oxaliplatin sensitivity and toxicity:** Our hypothesis is that intratumoral gene expressions levels of DNA repair enzymes will be associated with tumor response, overall survival and clinical toxicity in patients with metastatic colorectal cancer treated with oxaliplatin based chemotherapy.

Oxaliplatin cytotoxicity can be mediated by detoxification and DNA repair. Of the platinum detoxifying enzymes, glutathione-S-transferase Pi (GST P1) has been associated with anti-tumor effect. GST P1 will be assessed using a monoclonal antibody, with the hypothesis that overexpression of GST P1 will be associated with a poorer survival after oxaliplatin-containing therapy than patients with lower expression (11). The nucleotide excision repair enzymes XRCC1 and ERCC2 will also be evaluated using quantitative RT-PCR, with the hypothesis that high expression levels of XRCC1 or ERCC2 or other DNA repair enzymes will be associated with poorer survival after oxaliplatin-containing therapy (12).

Recently, our group developed an RT-PCR based method to precisely and reproducibly measure non-abundant mRNAs such as that of TS and ERCC1 in paraffin-embedded tissue specimens (13). This technology allows us to easily measure expression levels of genes of interest in microdissected tumor tissues and correlate gene expression levels with clinical outcome and toxicity in patients treated with chemotherapy. We tested the hypothesis that gene expression levels of enzymes involved in the 5-FU metabolism and DNA repair will predict tumor response and overall survival in patients with metastatic colorectal cancer treated with 5-FU/oxaliplatin chemotherapy. We measured TS and ERCC1 gene expression levels in microdissected paraffin embedded tumor tissue from 45 patients with metastatic colorectal cancer treated with 5-FU/oxaliplatin chemotherapy. Our data demonstrated that intratumoral gene expression levels of TS and ERCC1 are associated with overall survival. Our data also showed that TS expression was associated with response to 5-FU/oxaliplatin chemotherapy, however the low response rate in second line chemotherapy makes it difficult to detect associations of markers with response and toxicity. These data are particularly significant since in vitro studies showed oxaliplatin downregulating TS and DPD activities (14,15). Recently, in vitro data showed ERCC-1 expression protected against oxaliplatin damage (16). All these preliminary data indicate that genes involved in 5-FU metabolism as well as DNA repair may play a significant role in resistance to chemotherapy with 5-FU in combination with oxaliplatin.

- 1.4 Molecular Determinants of Resistance to irinotecan:** Our hypothesis is that intratumoral gene expressions levels of topoisomerase 1, carboxylesterase and COX-2 and others will be associated with tumor response, overall survival and clinical toxicity in patients with metastatic colorectal cancer treated with Irinotecan based chemotherapy.

Irinotecan must be activated to SN-38 by carboxylesterase 2 (17). SN-38 then inhibits topoisomerase 1, leading to cell death (18). The hypothesis is that patients with low carboxylesterase 2 will have a poorer survival after irinotecan-containing therapy than patients with high carboxylesterase 2. Also, patients with low tumor topoisomerase 1 will have a poorer survival after irinotecan-containing therapy than patients with high topoisomerase 1.

Irinotecan (CPT-11), a camptothecin derivate, acts as a topoisomerase I poison and has become a standard player in the chemotherapy of colorectal cancer, usually in combination with fluoropyrimidines or platinum compounds. Distinct differences of tolerability and response rates of irinotecan have been reported. Genetically defined variations of drug metabolism may be explain in part by inter-individual differences of drug effects. The metabolic pathways of irinotecan are highly complex involving numerous enzymes for activation, transport and deactivation.

Topoisomerase I inhibitors have cytotoxic mechanisms, which depend on DNA damage detection, DNA repair, cell cycle arrest, and cell death by apoptosis. Cancer cells often have

defects within these control systems, and these defects may confer selective sensitivity to appropriately designed drug therapy. Such correlations could guide the selection of drugs for therapy based on molecular diagnosis of individual tumors. Based on preclinical and clinical data, the potential molecular determinants of response to irinotecan are: 1) topoisomerase 1, 2) cell cycle kinetics/apoptosis, and 3) DNA repair.

In irinotecan resistant cells a marked decrease of Topoisomerase I protein was observed, indicating that the decreased protein content of Topoisomerase I may cause the decreased activity of Topoisomerase I and the decreased sensitivity to Topoisomerase I inhibitors. In vitro, Topoisomerase I gene copy number was highly correlated with topoisomerase I expression, and inversely correlated to sensitivity to a 1 hour exposure to SN38. This illustrates the significant impact of altered topoisomerase I gene copy number on intrinsic drug sensitivity and influences potential mechanisms for acquisition of drug resistance (18). Levels of topoisomerase I expression have been shown to vary widely between and within tumor types but the basis for this is poorly understood. Preliminary clinical data from patients with colorectal cancer suggest that topoisomerase I gene expression may be a predictor of clinical outcome (17,18). High topoisomerase I gene expression levels were associated with response to irinotecan, thus high expression levels of topoisomerase I may be predictive of responsiveness to irinotecan. Interestingly, the topoisomerase I gene expression levels were independent from the TS expression level indicating that response to irinotecan is possible despite increased TS expression levels which is associated with resistance to 5-FU. In 11 patients with banked tumor tissues who had been treated with irinotecan, three of four responders had TS levels above 4, the cut off for response to 5-FU/LV. The median gene expression level of topoisomerase 1 in responders was 8.8 and 1.8 in non-responders to irinotecan treatment indicating that topoisomerase 1 expression may predict response to irinotecan.

Additional genes have been and will be identified which have a putative role in regulating the activity and/or toxicity of 5FU, irinotecan, oxaliplatin, or cetuximab or bevacizumab. Additional genes of interest to this clinical study will also be identified in the future. Association of these candidate genes with toxicity and outcome will be conducted. In addition, analyses will be carried out in those patients with toxicities that cannot be explained by the genes described above. Those patients will be screened to identify expression in other candidate proteins that might be responsible for the observed toxicity or efficacy phenotype.

- 1.5 Mechanisms of Toxicity:** Our hypothesis is that gene expression levels of enzymes involved in the sodium channel pathway (SCN with neurotoxicity), DNA repair (XPD, ERCC-1 with neurotoxicity and GI toxicity), oxidative stress (GST-P1 with overall toxicity and neurotoxicity) and drug metabolism of 5-FU (TS,DPD, MTHFR, OPRT with GI toxicity and mucositis/stomatitis) and Irinotecan (CE, MDR1 with neutropenic fever and diarrhea) are associated with specific chemotherapy associated toxicities.

Mechanisms of Neurotoxicity: Neurotoxicity is the most frequent dose-limiting toxicity of oxaliplatin. Acute neurotoxicity is characterized by the rapid onset of cold-induced distal dysesthesia and/or paresthesia. Sensory symptoms may also be accompanied by cold-dependent muscular contractions of the extremities or the jaw. The symptoms, often occurring during or shortly after infusion, are usually transient and mild. A persistent sensory peripheral neuropathy may also develop with prolonged treatment, eventually causing superficial and deep sensory loss,

sensory ataxia, and functional impairment. Studies have shown patients with acute sensory symptoms to display little or no axonal degeneration, suggesting a specific effect of oxaliplatin on sensory neurons and/or motor neurons or muscle cells that is not observed with other platinum agents. The similarity of the acute symptoms induced by oxaliplatin with those caused by several drugs or toxins acting on neuronal or muscular ion channels suggests that these symptoms may

result from a specific interaction of oxaliplatin with ion channels located in the cellular membrane. There is no indication at the moment that a common cellular mechanism induces both the acute and the cumulative neurotoxicity of oxaliplatin (27,28). The medical interest of preventing acute neurotoxic side effects of oxaliplatin by infusing Ca(2+) and Mg(2+) was recently shown to decrease neurotoxicity (29).

The mechanism underlying this hyperexcitability has been investigated using rat sensory nerve preparations, dorsal root ganglia and hippocampal neurons (30). Oxaliplatin resulted in an increase of the amplitude and duration of compound action potentials. It lengthened the refractory period of peripheral nerves suggesting an interaction with voltage-gated Na(+) channels. Application of oxaliplatin to dorsal root ganglion neurons resulted in an increase of the Na(+) current, a block of the maximal amplitude and a shift of the voltage-response relationship towards more negative membrane potentials. We have some preliminary data suggesting that genes involved in DNA repair such as XPD or GSTP1 are associated with neurotoxicity and that the activity of the Sodium channels may be associated with risk of neurotoxicity.

Mechanisms of GI toxicity: It has been shown that the toxic response to the antifolate drug methotrexate (MTX), is substantially increased among patients who have reduced activity in a key enzyme in folate metabolism, 5,10-methylenetetrahydrofolate reductase (MTHFR) (31-33). These patients experienced a > 30% increase in severity of oral mucositis, a > 30% delay in platelet-recovery after hematopoietic stem-cell transplantation, and a greater frequency of other serious toxicities (31-33). Similarly, patients with low expression levels of TS were found to respond differently to treatment regimens by 5-FU or MTX (34,35): Recently, TS activity was shown to be associated with human homocysteine and folate levels suggesting a possible link to toxicity (36).

- 1.6 Gene expression arrays from paraffin embedded tissues:** Our hypothesis is that intratumoral gene expressions profiles generated from microdissected paraffin embedded tumor sections will be associated with tumor response, overall survival and clinical toxicity in patients with metastatic colorectal cancer treated with oxaliplatin based chemotherapy.

One of the limitations of measuring intratumoral gene expression is the availability of fresh frozen tissues in patients with GI cancer. In collaboration with Peter and Kathy Danenberg (ResponseGenetics), we developed a technology to isolate RNA from paraffin-embedded tissues, incorporating a laser captured microdissection technology to avoid any normal cell contamination (USC patent), to measure quantitatively intratumoral gene expression levels of genes of interest. They have successfully developed this technology for paraffin embedded tumor sections. Our hypothesis is that gene expression profiles will be associated with response, time to tumor progression, overall survival and toxicities in all arms of this clinical trial.

2.0 Objectives

- 2.1 To prospectively assess whether tumor expression of potential tissue-based markers of treatment response can independently predict response rate, time-to-tumor progression, and survival and toxicity in patients with metastatic colorectal cancer (CRC) treated with 1st line chemotherapy plus bevacizumab, cetuximab, or both. The markers chosen for this primary analysis consist of gene expression levels measured in microdissected formalin-fixed, paraffin-embedded tumor sections of the following mRNA levels: VEGF, VEGFR (bevacuzimab) and EGFR, EGF (cetuximab).

Secondary

- 2.2 To prospectively assess whether gene expression levels of genes involved in the 5-FU pathways (TS, DPD, TP, OPRT), DNA repair (ERCC-1,ERCC-2, XRCC1,GST-P1), irinotecan pathways (CE, topoisomerase 1, ERCC-1, MDR1) are independent predictors of response rate, time-to-tumor progression, and survival and toxicity in patients with metastatic CRC treated with 1st line chemotherapy plus bevacizumab, cetuximab, or both.

3.0 Methods

The technologies discussed in this section will take advantage of Laser captured microdissection of tumor sections from paraffin embedded tissues using Taqman based technologies. These technologies will be improved and novel technologies developed to evaluate mRNA levels of genes of interests in important pathways targeted with the proposed chemotherapies.

[See Section 8.0 of this appendix for Statistical Considerations for Gene Expression Analysis]

4.0 References

1. Kundu, N. G. and Heidelberger, C. Cyclopenta(f)isoquinoline derivatives designed to bind specifically to native deoxyribonucleic acid. 3. Interaction of 6-carbamylmethyl-8-methyl-7H-cyclopenta(f)isoquinolin-3(2H)-one with deoxyribonucleic acids and polydeoxyribonucleotides. *Biochem Biophys Res Commun*, 60: 561-568, 1974.
2. Danenberg, P. V. Thymidylate synthetase - a target enzyme in cancer chemotherapy. *Biochim Biophys Acta*, 473: 73-92, 1977.
3. Leichman, C. G., Lenz, H. J., Leichman, L., Danenberg, K., Baranda, J., Groshen, S., Boswell, W., Metzger, R., Tan, M., and Danenberg, P. V. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. *J Clin Oncol*, 15: 3223-3229, 1997.
4. Farrugia, D., Ford, H., Cunningham, D., Danenberg, K., Danenberg, P., Brabender, J., McVicar, A., Aherne, G., Hardcastle, A., McCarthy, K., and Jackman, A. Tymidylate Synthase Expression in Advanced Colorectal Cancer Predicts for Response to Raltitrexed. *Clin Cancer Res*, 9: 792-801, 2003.
5. Ichikawa, W., Uetake, H., Shirota, Y., Yamada, H., Nishi, N., Nihei, Z., Sugihara, K., and Hirayama, R. Combination of Dihydropyrimidin Dehydrogenase and Tymidylate Synthase Gene Expression in Primary Tumors as Predictive Parameters for the Efficacy of Fluoropyrimidine-based Chemotherapy for Metastatic Colorectal Cancer. *Clin Cancer Res*, 9: 786-791, 2003.
6. Shirota, Y., Stoehlmacher, J., Brabender, J., Xiong, Y. P., Uetake, H., Danenberg, K. D., Groshen, S., Tsao-Wei, D. D., Danenberg, P. V., and Lenz, H. J. ERCC1 and thymidylate

CALGB/SWOG C80405
Appendix II A

- synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol*, 19: 4298-4304, 2001.
7. Kubota, T., Watanabe, M., Otani, Y., Kitayima, M., and Fukushima, M. Different pathways of 5-fluorouracil metabolism after continuous venous or bolus injection in patients with colon carcinoma: possible predictive value of thymidylate synthase mRNA and ribonucleotide reductase for 5-fluorouracil. *Anticancer Res*, 22: 3537-3540, 2002.
 8. Heggie, G. D., Sommadossi, J. P., Cross, D. S., Huster, W. J., and Diasio, R. B. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res*, 47: 2203-2206, 1987.
 9. Salonga, D., Danenberg, K. D., Johnson, M., Metzger, R., Groshen, S., Tsao-Wei, D. D., Lenz, H. J., Leichman, C. G., Leichman, L., Diasio, R. B., and Danenberg, P. V. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res*, 6: 1322-1327, 2000.
 10. Isshi, K., Sakuyama, T., Gen, T., Nakamura, Y., Kuroda, T., Katayuma, T., and Maekawa, Y. Predicting 5-FU sensitivity using human colorectal cancer specimens: comparison of tumor dihydropyrimidine dehydrogenase and orotate phosphoribosyl transferase activities with in vivo chemosensitivity to 5-FU. *Int J Clin Oncol*, 7(6): 335-342, 2002.
 11. Sutoh I, Kohno H, Nakashima Y, Hishikawa Y, Tabara H, Tachibana M, Kubota H, Nagasue N. Concurrent expressions of metallothionein, glutathione S-transferase-pi, and P-glycoprotein in colorectal cancers. *Dis Colon Rectum*. 2000;43:221-32.
 12. Crnogorac-Jurcevic T, Efthimiou E, Nielsen T, Loader J, Terris B, Stamp G, Baron A, Scarpa A, Lemoine NR. Expression profiling of microdissected pancreatic adenocarcinomas. *Oncogene*. 2002;21:4587-94.
 13. Shirota Y, Stoehlmacher J, Brabender J, Xiong Y, Danenberg KD, Groshen S, Tsao-Wei DD, Danenberg PV, Lenz H. ERCC1 mRNA Levels and Thymidylate Synthase (TS) mRNA Levels Predict Survival Colorectal Cancer Patients Receiving Combination Oxaliplatin and Fluorouracil Chemotherapy. *J Clin Oncol*, 19:4298-4304, 2001.
 14. Magne N. Pivot X. Etienne-Grimaldi MC. Francois E. Renee N. Thyss A. Schneider M. Demard F. Milano G. Impact of platinum complexes on dihydropyrimidine dehydrogenase activity in 5-fluorouracil-treated patients. [Letter] *Annals of Oncology*. 14(8):1329-30, 2003 Aug.
 15. Yeh KH. Cheng AL. Wan JP. Lin CS. Liu CC. Down-regulation of thymidylate synthase expression and its steady-state mRNA by oxaliplatin in colon cancer cells. *Anti-Cancer Drugs*. 15(4):371-6, 2004 Apr.
 16. Youn CK. Kim MH. Cho HJ. Kim HB. Chang IY. Chung MH. You HJ. Oncogenic H-Ras up-regulates expression of ERCC1 to protect cells from platinum-based anticancer agents. [Journal Article] *Cancer Research*. 64(14):4849-57, 2004
 17. Xu G, Zhang W, Ma MK, McLeod HL. Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. *Clin Cancer Res*. 2002;8:2605-11.
 18. Boonsong A, Curran S, McKay JA, Cassidy J, Murray GI, McLeod HL. Topoisomerase I protein expression in primary colorectal cancer and lymph node metastases. *Hum Pathol*. 2002;33:1114-9.

CALGB/SWOG C80405
Appendix II A

19. Akarasereenont P, Bakhle Y, Thimermann C, Vane JR. Cytokine-mediated induction of cyclooxygenase-2 by activation of tyrosine kinase in bovine endothelial cells stimulated by bacterial LPS. *Br J Pharma* 115: 401-408, 1995.
20. Takahashi Y, Bucana CD, Liu W, Yoneda J, Cleary KR and Ellis LM. Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. *J Natl Cancer Inst* 88: 1146-1151, 1996.
21. Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR, Ellis LM. Vessel counts and expression of VEGF as prognostic factors in node-negative colon cancer. *Arch Surg* 132(5):541-6, 1997.
22. Kinoshita T, Takahashi Y, Sakashita T, et al: Growth stimulation and induction of epidermal growth factor receptor by overexpression of cyclooxygenases 1 and 2 in human colon carcinoma cells. *Biochim Biophys Acta* 1438:120-130, 1999
23. Pai R, Soreghan B, Szabo IL, et al: Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 8:289-293, 2002
24. Perrotte P, Matsumoto T, Inoue K, et al: Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* 5:257-265, 1999
25. Busam KJ, Capodieci P, Motzer R, et al: Cutaneous side-effects in cancer patients treated with the anti-epidermal growth factor receptor antibody C225. *Br J Dermatol* 144:1169-76, 2001
26. Sonis ST, O'Donnell KE, Popat R, et al. The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. *Oral Oncol* 40:170-176, 2004
27. Gamelin E. Gamelin L. Bossi L. Quasthoff S. Clinical aspects and molecular basis of oxaliplatin neurotoxicity: current management and development of preventive measures *Seminars in Oncology*. 29(5 Suppl 15):21-33, 2002 Oct.
28. Grothey A. Oxaliplatin-Safety: Neurotoxicity. *Seminars Oncology* 30(4) 2003, 5-13.
29. Gamelin E. et al *Clin Cancer Research*, 2004
30. Grolleau F. Gamelin L. Boisdron-Celle M. Lapied B. Pelhate M. Gamelin E. A possible explanation for a neurotoxic effect of the anticancer agent oxaliplatin on neuronal voltage-gated sodium channels. *Journal of Neurophysiology*. 85(5):2293-7, 2001
31. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, Morgan G. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults [see comments]. *Proc Natl Acad Sci USA*, 96: 12810-12815, 1999.
32. Wiemels J L, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF, United Kingdom Childhood Cancer Study. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. *Proc Natl Acad Sci USA*, 98: 4004-4009, 2001.
33. Gaughan DJ, Kluijtmans LA, Barbaux S, McMaster D, Young IS, Yarnell JW, Evans A, Whitehead AS. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. *Atherosclerosis*, 157: 451-456, 2001.

34. Skibola CF, Smith MT, Hubbard A, Shane B, Roberts AC, Law GR, Rollinson S, Roman E, Cartwright RA, Morgan GJ. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. *Blood*, 99: 3786-3791, 2002.
35. Krajcinovic M, Costea I, Chiasson S. Polymorphism of the thymidylate synthase gene and outcome of acute lymphoblastic leukaemia. *Lancet*, 359: 1033-1034, 2002.
36. Trinh BN, Ong CN, Coetzee GA, Yu MC, Laird PW. Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. *Human Genetics*. 111(3):299-302, 2002.

Part B

PROGNOSTIC AND PREDICTIVE IMPORTANCE OF SPECIFIC IMMUNOHISTOCHEMICAL (IHC) MARKERS FOR PATIENTS TREATED WITH 1ST LINE CHEMOTHERAPY PLUS BEVACIZUMAB, OR CETUXIMAB, OR BOTH IN METASTATIC COLORECTAL CANCER

5.0 Background

Colorectal cancer is a complex, heterogeneous disease. Despite extensive characterization of the nature of the adenoma-to-carcinoma transition during CRC development, few molecular events have been definitively linked to tumor behavior. An important challenge in this work is the fact that many important tumor-associated markers can only be identified through the acquisition, processing, and quality control of frozen tissues. These studies, therefore, are difficult or impossible to study in large multi-institutional settings. In this portion of the correlative science protocol, we will use a standard, widely available resource, namely that of formalin-fixed, paraffin embedded tissue, to identify markers of treatment response. The post-resection processing of this type of tissue limits the nature of our studies, and as a result, we will organize our studies according to primary and secondary aims.

First, we will undertake as a primary analysis a study of markers of CRC clinical behavior for which we have confidence in our ability to obtain definitive results. The markers chosen have all been tested in large, multi-institutional settings, and produce results that are consistent and reproducible. In addition, substantial data supports a role for each of these markers in CRC treatment outcome. These markers include expression of vascular endothelial growth factor (VEGF), a marker directly related to the efficacy of bevacizumab. Two IHC markers, MLH1 and MSH2 expression, will determine whether or not the tumors under treatment exhibit microsatellite instability, a pathway of tumor progression that is present in approximately 15-20% of CRCs and, when present, imparts specific biological behavior. In addition, IHC detection of MGMT, a protein necessary for proper repair of methylG:T DNA mismatches, will be performed. This assay will help identify a subset of CRCs that exhibit a worse prognosis due to widespread methylation-induced silencing of tumor suppressor genes, yet may also be likely to show better response to 5-FU based chemotherapy.

The secondary aims of our examination of paraffin-embedded tissues analyses include two goals. First, we will perform exploratory studies of several additional IHC markers. Analyses of these markers are secondary because, although they should be associated with bevacizumab and/or cetuximab response based upon the mechanisms of activity of these agents, they are not well-studied in large human treatment trials. These markers include tumor and/or stromal expression of cyclooxygenase-2 (COX-2), EGFR, AKT-p-Ser473, and p44/42 MAP kinase. Because of a lack of preliminary data concerning these markers, they will be performed first upon a randomly selected subset of specimens from CALGB protocol 80405, and proceed to full analysis only if they exhibit promise in these preliminary analyses. In addition to these IHC studies, we will ensure maximal use of the tissue resources provided by this protocol by construction TMAs to include tumor and non-tumor tissue cores from each patient treated on this protocol. Although no specific analyses of these TMAs will be described here, the intent is to use this resource in the future for relevant FISH and/or aCGH analyses.

5.1 Tumor Angiogenesis and Vascular Endothelial Growth Factor Expression: New blood vessel formation, termed angiogenesis, is considered a fundamental requirement for tumors to grow beyond a few millimeters in greatest dimension, and to set up new colonies at metastatic sites (1). VEGF is the most potent known endothelial mitogen and is widely expressed in solid tumors (2, 3). In colorectal cancer, expression of VEGF and its receptors are up-regulated in comparison to normal bowel and inhibition of VEGF activity is a promising addition to chemotherapy for management of metastatic disease (4, 5). Recent retrospective studies have indicated that measures of angiogenesis may be clinically useful as prognostic indicators in colorectal cancer, and that VEGF expression is associated with a poorer treatment outcome (1, 6). Prospective studies to address this issue in stage II and III CRC are underway (CALGB protocols 9581 and 89803).

5.2 Prognostic significance of mismatch repair deficiency in CRC: A subset of CRCs arise in the setting of a loss of the ability to repair small segment DNA mismatches, a characteristic known as microsatellite instability (MSI). The subset of CRC characterized by high-level DNA microsatellite instability (MSI-H) differs from other CRC subsets in clinically significant ways. For example, MSI-H tumors are more likely to occur in females, show a strong predilection for the proximal colon, have a relatively good prognosis, are characterized at the pathologic level by poor differentiation, mucinous histology and lymphocytic infiltration, and probably arise in hyperplastic polyps rather than traditional adenomas (7). Clinical studies indicate that, in addition to conveying a better prognosis overall, the presence of MSI may predict a better outcome following chemotherapy (8).

MSI can be identified when IHC reveals the absence of staining for the two mismatch repair enzymes commonly lost, MLH1 and MSH2. This method detects MSI-H tumors with a sensitivity of ~79% and a specificity of ~89%, compared to 98% for the much more labor intensive and expensive method of microsatellite analysis by Bethesda criteria (9).

5.3 Methylation-associated silencing of tumor suppressor genes in CRC: DNA methylation is a chemical modification that specifically alters cytosine nucleotides in CpG sites. Some cancers show concordant methylation across genetic loci, a characteristic described as the CpG island methylator phenotype (CIMP) (10). Methylation of CpG islands within gene promoter sequences produces gene silencing. Human CRC studies show that genes encoding tumor suppressors, including TGF β R, TP53, MLH1, p16, APC, PTEN, and the estrogen receptor, are sometimes silenced by methylation. These data suggest that, in addition to the well recognized mechanisms of chromosomal instability (CIN) and MSI, DNA methylation provides an important mechanism for inactivating tumor suppressor genes.

Because the DNA repair gene hMLH1, can be silenced by promoter methylation, there is an important link between DNA methylation and MSI (11). Despite this, tumors exhibiting CIMP generally lack the MSI-H phenotype but often show MSI-L status. The mechanism of MSI-L status has not been fully explained. *O*-6-Methylguanine DNA Methyltransferase (*MGMT*) is a gene encoding a protein that repairs alkylating DNA damage by removing alkyl groups from O-6-guanine. Methylation-induced silencing of *MGMT* has been associated with MSI-L (12) and occurs in approximately 40% of CRC (13). CRC exhibiting CIMP appear to have a poor prognosis, however, there also is evidence that DNA methylation predicts chemoresponsiveness for 5-FU based adjuvant therapy (14, 15). These preliminary data suggest that 5-FU based chemotherapy is beneficial primarily in the ~40% of subjects with CRC in which *MGMT* is methylated.

- 5.4 COX-2 and CRC Prognosis:** COX-2 is an inducible enzyme involved in arachidonic acid metabolism that plays an important role in inflammation and tumorigenesis. COX-2 is highly expressed in both tumor cells and tumor-associated endothelial cells, and its prostaglandin products promote angiogenesis in human tumors (16, 17). These observations led to the hypothesis that COX-2 upregulation supports tumor angiogenesis. Prostaglandin products of COX-2 activity may also be involved in activation of EGFR (18). COX-2 expression by IHC has been associated with reduced survival in patients undergoing resection of colorectal cancer (19). Measurement of COX-2 expression by IHC has been challenging, however, due to problems with antibody specificity and consistency of staining.
- 5.5 Markers of Treatment Response for Anti-EGFR Therapies:** EGFR is a transmembrane receptor tyrosine kinase whose activity is stimulated by growth factors such as EGF and TGF- α (20). Increased expression of this cellular oncogene is observed in many different types of solid tumors, and is associated with reduced disease-free and overall survival in patients with head and neck, ovarian, bladder, and esophageal carcinomas (21). Studies of EGFR expression in CRC have suffered from lack of uniform patient populations and variable protein detection methods. By immunohistochemistry (IHC) techniques, EGFR overexpression is observed in approximately 60-80% of CRCs compared to normal intestinal mucosa (22, 23). The relationship between tumor EGFR expression and colon cancer survival is unclear, although a few studies suggest that EGFR overexpression is associated with poor tumor grade and decreased disease-free (24) and overall survival (25, 26). The relationship between EGFR expression and anti-tumor response to antibody blockade of EGFR is likewise not well understood (27).

An understanding of the relationship between receptor expression and activation of downstream signaling pathways is crucial to improving the results of these targeted therapies. Pre-clinical studies have identified the MAP kinase, PI₃K, and AKT pathways as key signaling mechanisms affected by EGFR activation (28). Recently, reagents have been developed for use in paraffin sections that can indicate activation of EGFR-associated mediators. These include antibodies recognizing EGFR autophosphorylation (EGFR-p-Y1068), activated AKT (AKT-p-Ser473), and activated MAP kinase (p44/42 MAP kinase). In the secondary aims of this study, we will investigate the use of these new markers for patients treated with and without antibody blockade of EGFR.

6.0 Objectives

- 6.1** To prospectively assess whether tumor expression of tissue-based markers can independently predict response rate, time-to-tumor progression, and survival in patients with metastatic colorectal cancer (CRC) treated with 1st line chemotherapy plus bevacizumab, cetuximab, or both. The markers chosen for this primary analysis consist of IHC detection in formalin-fixed, paraffin-embedded tumor sections of the following proteins: VEGF, MSH2, MLH1, and MGMT.

Secondary

- 6.2** To prospectively assess whether markers of epidermal growth factor receptor (EGFR) activity, including tumor IHC analyses of COX-2, AKT-p-Ser473, and p44/42 MAP kinase and EGFR expression, are independent predictors of response rate, time-to-tumor progression, and survival in patients with metastatic CRC treated with 1st line chemotherapy plus bevacizumab, cetuximab, or both.
- 6.3** To create tissue microarrays (TMAs) containing both tumor and non-tumor samples from each patient treated on CALGB protocol 80405. These TMAs will be used to develop new markers

of treatment response using fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (cCGH) techniques.

7.0 Research Approach and Methods:

Tumor samples will be obtained at the time of study entry, processed as paraffin blocks, and sent to the SWOG Solid Tumor Specimen Repository for storage, processing, and distribution. Immunohistochemistry will be performed on paraffin sections to determine tumor expression of VEGF, MSH1, MLH2, MGMT, COX-2, EGFR, EGFR-p-Y1068, AKT-p-Ser473, and p44/42 MAP kinase. Paraffin sections of tumor samples to be used for immunohistochemistry will be sectioned at the SWOG Solid Tumor Specimen Repository immediately before assay and shipped to the appropriate research laboratory. Tumor samples obtained for this study may come from a variety of sources, including primary tumor, metastatic lymph node deposits, and liver metastases. Every attempt will be made to obtain a sample of primary tumor from each study participant. When available, samples from metastatic locations will also be evaluated. Data analyses will take into consideration tumor location. IHC stains will be performed at Brigham and Women's Hospital, under the direction of Dr. Mark Redston, on deparaffinized, rehydrated 4-6 micron sections of formalin-fixed tissue. Sections from positive and negative control tumors (which have been analyzed previously for all markers and found to be strongly positive or completely negative) and controls in the absence of primary antibody will be utilized routinely.

- 7.1 Primary analyses:** IHC techniques to detect proteins listed as primary analyses are all well-established, and will be conducted according to standard techniques. Antibodies to be used will include: VEGF: polyclonal antisera VEGF 165 (Santa Cruz); MLH1: monoclonal anti-MLH1, G168-728 (PharMingen); MSH2: monoclonal anti-MSH2, FE11 (Oncogene Research Products); MGMT: monoclonal anti-MGMT, MT3.1, Neomarkers (Lab Vision Corp). Each primary marker analysis will be performed in a coded manner independently of the standard histopathologic assessment by two investigators. All slides will be scored and recorded without knowledge of the patient's outcome. The primary examiner will re-score 10% of the samples assayed in a blinded fashion to determine intra-observer variability. A second examiner will score 10% of the samples assayed to determine inter-observer variability.

Marker expression will be evaluated for intensity of staining (weak, moderate, or strong) and percent of cells positive, and will be recorded as continuous variables. The values will also be assigned to ranges (0, <10, 10-50, 50-75, or >75% percent positive). The data will be converted to high or low, positive or negative, defined according to published criteria (9, 11, 15).

- 7.2 Secondary studies:** IHC techniques for determination of COX-2 expression and EGFR activation are not well established. Our approach to optimizing these assays will be as follows:

We have established conditions for COX-2 immunostaining in adenomas and have also used this antibody in esophageal carcinoma studies (29). Five micron sections will be cut from formalin-fixed paraffin-embedded tissue blocks. Immunodetection of COX-2 is performed using the tyramide signal amplification (TSA) indirect amplification method. The sections are deparaffinized and rehydrated, and sequentially treated to block endogenous peroxidase (3% hydrogen peroxide in methanol) and biotin (avidin-biotin blocking kit, #SP-2001, Vector Corp.). The sections are then incubated with a monoclonal antibody for COX-2 (Caymen Chemical, Ann Arbor, MI) at 4°C overnight, followed by incubation with a labeled polymer, HRP (anti-mouse) RT (DAKO, Corp. Carpinteria, CA) for 1 hour. Tissue sections are then treated in streptavidin-peroxidase for 30 min at room temperature, incubated with biotinylated tyramide at a 1:50 dilution for 15 min at room temperature, and finally incubated in streptavidin-peroxidase for 30 minutes at a dilution of 1:250. After staining with DAB followed by a light hematoxylin counterstain, the sections are dehydrated and coverslipped. A colon carcinoma

case known to strongly express COX-2 is used as a positive control. Omission of the primary antibody is used as the negative control.

To detect evidence of EGFR activation, IHC will be done by a standard technique using the DAKO clone 2-18C9 EGFR antibody (Dako Corporation, Carpinteria, CA). Specificity procedures for this clone will include its recognition of EGFR in paraffin sections from cell blocks prepared from A431 cells, and demonstration of increased expression of EGFR-p-Y1068, AKT-p-Ser473, and p44/42 MAP kinase in A431 cells treated with EGF. Sections from positive and negative control tumors (which have been analyzed previously for either EGFR, EGFR-p-Y1068, AKT-p-Ser473, and p44/42 MAP kinase and found to be strongly positive or completely negative) and controls in the absence of primary antibody will be utilized routinely. A standard control for EGFR expression, normal epidermis, will also be used. Whenever possible, internal controls will be utilized, including staining of the perineuria within the bowel. The antibodies used will be: EGFR (Dako H11, clone 2-18C9), EGFR-p-Y1068 (Cell Signaling #2231), AKT-p-Ser473 (Cell Signaling #9271), and p44/42 MAP kinase (Cell Signaling #9102). The intensity of EGFR and EGFR-p-Y1068 in the cytoplasm of tumor cells will be graded according to the following four-tier system (6):

- 0: No reactivity
- 1+: Weak reactivity. Faint or light brown reactivity that is membranous, cytoplasmic, or both.
- 2+: Moderate activity. Shades of brown staining of intermediate darkness (intensity), with complete or incomplete membrane reactivity.
- 3+: Strong reactivity. Dark brown to black staining that is almost always in a complete membrane pattern.

EGFR reactivity is generally noted to be heterogeneous, therefore scoring will reflect the staining level of the most reactive tumor regions, which are most often located in the deepest portions of the tumor. The staining levels of AKT-p-Ser473 and p44/42 MAP kinase in tumor cells will be graded according to the following four-tier system:

- 0: No reactivity
- 1+: Weak reactivity, with 0-10% of tumor cells showing positive nuclear staining.
- 2+: Moderate activity, with 10-50% of tumor cells showing positive nuclear staining.
- 3+: Strong reactivity, with >50% of tumor cells showing positive nuclear staining.

7.3 Creation of TMAs: Outcome-linked paraffin-embedded tissues from patients on randomized treatment trials constitute a valuable but consumable resource for studies of tumor biology and treatment response. In order to make maximal use of the tissue resources provided by CALGB/SWOG C80405, we will construct TMAs containing tumor and non-tumor tissue from all participants. TMAs will be constructed at the SWOG Solid Tumor Specimen Repository. Briefly, H&E stained slides will be reviewed, and areas marked from which the donor cores should be taken. In general, areas measuring at least 7 mm in diameter are preferred in order to facilitate the harvesting of at least 6 cores of tissue (3 in the primary TMA block and 3 in the duplicate). Slides and corresponding blocks will be given to the TMA Core for TMA construction. Arrays are constructed with 3 additional cores of normal corresponding tissue at one end for orientation.

8.0 Statistical Considerations

8.1 Studies of Gene Expression and Immunohistochemistry:

At the time of study enrollment, patients will be asked to provide a tumor sample (from a biopsy or from an archival specimen). Although the study will require institutions to seek consent from all patients, we anticipate that some patients will refuse, or that a proportion of tumor samples will be unavailable. For the estimates below, we assume 80% (approximately 1411) will provide a tumor sample that is suitable for analysis.

8.2 General Analysis Plans for Gene Expression and Immunohistochemistry

A key goal of the proposed correlative studies is to assess whether gene expression variables, gene polymorphisms, and immunohistochemistry are predictive of clinical outcome and toxicity to treatment.

The primary outcome measure for this trial is overall survival, with progression-free survival a secondary endpoint. Because both of these outcomes involve censored data, analyses will be done using the Cox Proportional Hazards Model. This analysis will allow the assessment of marker-treatment interactions and main effects of marker, while adjusting for the effect of known prognostic factors such as stage and performance status.

Toxicities are coded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. Because of the large number of individual toxicities, most of our analyses will focus on summaries based on: 1) maximum grade experienced by a patient; and 2) maximum grade within global categories (hematologic, gastrointestinal, etc) and 3) individual toxicity codes related to specific treatments (e.g. rash for cetuximab treated patients). Primary analysis of toxicity will be a comparison between patients experiencing Grades 0-2 versus Grades 3-5. These analyses will involve use of logistic regression. These models allow an assessment of the relationship of markers to toxicity, while adjusting for the chemotherapy treatment and other potential covariates of interest such as age and performance status, and assessment of potential interactions among variables. It is anticipated that the primary analyses will be done within individual biologic treatment arms, although treatment-marker interactions will also be assessed.

The measures of gene expression are quantitative measures, and as such it will be necessary to consider appropriate ways to handle these variables in the analytic models. Since the distributions of these data often are not symmetric (and prone to outliers), analysis of the raw values in our models may be inappropriate. Thus, ways of grouping the data will be assessed, perhaps dichotomously into high/low gene expression, for example. There is no single best approach to this. As a first step, individual gene expressions may be split at the median, or could be split into tertiles. This approach has the benefit of providing equal samples within each level, and is not dependent upon the outcome variable. An alternate adaptive approach will be to select the split that maximizes the logrank statistic comparison of survival between the two levels. (95) It should be emphasized that there is no single 'best' way to categorize gene expression for a single marker, and thus caution will be required when generalizing these results.

Immunohistochemistry staining is reported as weak, moderate or severe. These categories can be treated as ordered in our analyses, or can be combined into a high and low using exploratory techniques. Similarly, it is anticipated that the continuous measure of percent staining will be grouped as described above.

8.3 Analytic Issues

The primary goals are to determine which markers are 1) prognostic of patient outcome; 2) predictive of which patients respond better to specific agents; 3) predictive of toxicity to specific agents.

A marker is prognostic if it is correlated with outcome in a way that is independent of treatment. This corresponds to a main effect in the Cox Proportional Hazards Model. A marker is predictive if its relationship with outcome differs between treatment arms. This corresponds to an interaction between treatment and marker in the Cox model.

The comparison between the Bevacizumab only arms and the Cetuximab only arms provide the most direct assessment of interaction and main effects. If significant interactions exist between a marker and treatment, then assessment of prognosis must be done separately in the two treatment arms, and interpretation will be specific to the treatment. We will also have the ability to make comparisons between the single biologic arm and the combined biologic arm, although this comparison will be secondary.

8.4 Primary Hypotheses

The correlative aims of this protocol involve analysis of a number of variables, introducing concerns regarding multiple comparisons. We define a number of ‘primary’ hypotheses, and provide power calculations based on these analyses. It is assumed that analysis of any remaining markers will be exploratory. Thus, the primary hypotheses are:

- For measures of gene expression, the priority will be to assess the relationship between survival and VEGF and EGF.
- For Immunohistochemistry, the primary endpoints will be of VEGF and EGF intensity and percent.

8.5 Power estimates:

Below we give an indication of the power we have to detect these primary hypotheses. We recognize, however, that with many other markers of potential interest, care must be taken in the interpretation of our analyses, due to problems with multiple comparisons. Results based on analyses of these secondary markers, as well as other exploratory analyses will require validation in future studies.

Gene expression and immunohistochemistry will be assessed using tumor tissues. It is anticipated that we will collect specimens from approximately 1411 patients, 935 from patients on the two arms using single biologic agents.

The estimates below are examples of the ability we have to detect certain marker relationships. For these calculations, we focus on overall survival (OS) as the primary endpoint for clinical outcome, and summary measures for the toxicity endpoints.

Table 7 indicates the power available to detect interactions between a marker and treatment, assuming gene expression for a particular gene is coded as high/low. As an example, we have 93% power to detect an interaction hazard ratio of 2. There are numerous scenarios that yield such an hr. Table 7 gives the simplest scenario for such a hazard ratio, namely that the median survival is the same in all but one of the treatment groups. In this example, we show a marker highly predictive of patients who will improve on bevacizumab (median 44 months survival), with all other patients having similar survival experience (median 22 months). While differing patterns between treatment arms (all with the same HR) may have slightly different power, these calculations are reasonable guidelines.

These calculations are based on using an alpha level of .01 to accommodate the fact we have multiple primary hypotheses for gene expression and IHC. These calculations also assume a roughly equal split between patients either high or low for a marker. Since power calculations are not greatly affected by moderate divergence from the 50:50 split, these approximations are reasonable for a number of the markers. However, for other markers, the power will be reduced.

Bev, Low	Bev, High	C225, Low	C225, High	Interaction Hazard ratio	Power (based on $\alpha=.01$)
37.4	22	22	22	1.7	72%
49.6	22	22	22	1.8	82%
41.8	22	22	22	1.9	89%
44	22	22	22	2.0	93%

Table 7: Power to detect interaction between marker and treatment for different interaction hazard ratios. Numbers in the first 4 columns are median survival

Assuming there is no interaction detected, it is then reasonable to assess main effects across treatment arms (with suitable adjustment for treatment assignment in the Cox model). If a significant interaction exists, then an assessment of the prognostic significance would need to be done separately within treatment, and must be interpreted with caution, since prognosis is now treatment dependent. Table 8 provides power to detect main effects either using the combined patients (from single biologic arms) or within a treatment arm. Thus, even if we have to assess markers within treatment, we have sufficient power to detect hazard ratios on the order of 1.6 with 89% power.

Hazard Ratio	Power, N=467	Power, N=935
1.4	60%	92%
1.5	78%	98%
1.6	89%	99%
1.7	96%	99%

Table 8. Power to detect selected hazard ratios for overall survival based. Two-sided test, alpha = .01.

We will also assess the relationship of genomic polymorphisms and target enzymes to toxicity from chemotherapy. Logistic regression models will be used to evaluate the relationship between markers and toxicities. As with the survival

examples above, we will assess If marker-treatment interactions are noted. We also anticipate that analyses within treatment arms will be undertaken.

Within treatment arm, assuming a .01 type one error, we estimate >95% power to detect differences of 20% or more in the proportion of severe toxicities between high and low marker groups. If the rates are relatively rare, we have similar power to detect differences of 15% or more.

Some of this work will be exploratory in nature. Interpretation of results from these studies must be based not merely on p-values, but on an assessment of the strength of the measure effect, and the width of the confidence interval. Some of the observations from this study might require independent validation before being incorporated into future clinical studies.

9.0 References

1. Bergsland, E.K. 2004. Vascular endothelial growth factor as a therapeutic target in cancer. *Am J Health Syst Pharm.* 61:S4-11.
2. Arii, S., A. Mori, S. Uchida, K. Fujimoto, Y. Shimada, and M. Imamura. 1999. Implication of vascular endothelial growth factor in the development and metastasis of human cancers. *Hum Cell.* 12:25-30.

CALGB/SWOG C80405
Appendix II B

3. Rak, J., and J.L. Yu. 2004. Oncogenes and tumor angiogenesis: the question of vascular "supply" and vascular "demand". *Semin Cancer Biol.* 14:93-104.
4. Stoeltzing, O., W. Liu, N. Reinmuth, A. Parikh, S.A. Ahmad, Y.D. Jung, F. Fan, and L.M. Ellis. 2003. Angiogenesis and antiangiogenic therapy of colon cancer liver metastasis. *Ann Surg Oncol.* 10:722-733.
5. Hurwitz, H. 2004. Integrating the anti-VEGF-A humanized monoclonal antibody bevacizumab with chemotherapy in advanced colorectal cancer. *Clin Colorectal Cancer.* 4:S62-S68.
6. Ellis, L.M. 2004. Preclinical data targeting vascular endothelial growth factor in colorectal cancer. *Clin Colorectal Cancer.* 4:S55-S61.
7. Olschwang, S., R. Hamelin, P. Laurent-Puig, B. Thuille, Y. De Rycke, Y.J. Li, F. Muzeau, J. Girodet, R.J. Salmon, and G. Thomas. 1997. Alternative genetic pathways in colorectal carcinogenesis. *Proc Natl Acad Sci USA.* 94:12122-12127.
8. Ribic, C.M., D.J. Sargent, M.J. Moore, S.N. Thibodeau, A.J. French, R.M. Goldberg, S.R. Hamilton, P. Laurent-Puig, R. Gryfe, L.E. Shepherd, D. Tu, and M. Redston. 2003. Tumor microsatellite instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *New Engl J Med.* 349:247-257.
9. Shia, J., D.S. Klimstra, K. Nafa, K. Offit, J.G. Guillem, A.J. Markowitz, W.L. Gerald, and N.A. Ellis. 2005. Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms. *Am J Surg Pathol.* 29:96-104.
10. Toyota, M., N. Ahuja, M. Ohe-Toyota, J.G. Herman, S.B. Baylin, and J.-P.J. Issa. 1999. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA.* 96:8681-8686.
11. Kane, M.F., M. Loda, G.M. Gaida, J. Lipman, R. Mishra, H. Goldman, J.M. Jessup, and R. Kolodner. 1997. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.* 57:808-811.
12. Whitehall, V.L.J., M.D. Walsh, J. Young, B.A. Leggett, and J.R. Jass. 2001. Methylation of O-6-Methylguanine DNA Methyltransferase characterises a subset of colorectal cancer with low level DNA microsatellite instability. *Cancer Res.* 61:827-830.
13. Esteller, M., R.A. Risques, M. Toyota, G. Capella, V. Moreno, M.A. Peinado, S.B. Baylin, and J.G. Herman. 2001. Promoter hypermethylation of the DNA repair gene O6-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. *Cancer Res.* 61:4689-4692.
14. van Rijnsoever, M., H. Elsaleh, D. Joseph, K. McCaul, and B. Iacopetta. 2003. CpG island methylator phenotype is an independent predictor of survival benefit from 5-fluorouracil in stage III colorectal cancer. *Clin Cancer Res.* 9:2898-2903.
15. Nagasaka, T., G.B. Sharp, K. Notohara, T. Kambara, H. Sasamoto, H. Isozaki, D.G. MacPhee, J.R. Jass, N. Tanaka, and N. Matsubara. 2003. Hypremethylation of O-6-methylguanine-DNA methyltransferase may predict non-recurrence after chemotherapy in colorectal cancer cases. *Clin Cancer Res.* 9:5306-5312.
16. Hanahan, D., and J. Folkman. 1996. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell.* 86:353-364.
17. Masferrer, J.L., K.M. Leahy, A.T. Koki, and a. al. 2000. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* 60.

CALGB/SWOG C80405
Appendix II B

18. Dannenberg, A.J., S.M. Lippman, J.R. Mann, K. Subbaramaiah, and R.N. DuBois. 2005. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. *J Clin Oncol.* 23:254-266.
19. Soumaoro, L.T., H. Uetake, T. Higuchi, Y. Tagai, M. Enomoto, and K. Sugihara. 2004. Cyclooxygenase-2 expression: A significant prognostic indicator for patients with colorectal cancer. *Clin Cancer Res.* 10:8465-8471.
20. Gill, G.N., T. Kawamoto, C. Cochet, and e. al. 1984. Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. *J Biol Chem.* 259:7755-7760.
21. Nicholson, R.I., J.M.W. Gee, and M.E. Harper. 2001. EGFR and cancer prognosis. *Eur J Cancer.* 37:S19-S15.
22. Kay, E.W., H. Mulcahy, C.B. Walsh, M. Leader, and D. O'Donoghue. 1994. Cytoplasmic c-erb-B-2 protein expression correlates with survival in Dukes' B colorectal carcinoma. *Histopathology.* 25:455-461.
23. Yasui, W., H. Sumiyoshi, J. Hata, and e. al. 1988. Expression of epidermal growth factor receptor in human gastric and colon carcinomas. *Cancer Res.* 48:137-141.
24. De Jong, J.K., R. Stellema, A. Karrenbeld, and e. al. 1998. Clinical relevance of transforming growth factor alpha, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. *Hepatology.* 28:971-979.
25. Kapitanovic, S., S. Radosevic, M. Kapitanovic, and e. al. 1997. The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. *Gastroenterology.* 112:1103-1113.
26. Goldstein, N.S., and M. Armin. 2001. Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer stage IV colon adenocarcinoma. *Cancer.* 92:1331-1346.
27. Baslega, J. 2001. The EGFR as a target for anticancer therapy - focus on cetuximab. *Eur J Cancer.* 37:S16-S22.
28. Mendelsohn, J. 2002. Targeting the epidermal growth factor receptor for cancer therapy. *J Clin Oncol.* 20:1s-13s.
29. Kulke, M.H., R.D. Odze, J.D. Mueller, H.H. Wang, and M.M. Bertagnolli. 2004. Increased Expression and Prognostic Significance of Tissue VEGF and COX-2 in Patients Receiving Pre-operative Chemotherapy and Radiation for Esophageal Cancer. *J Thoracic and Cardiovascular Surgery.* 127:1579-1586.

APPENDIX III

Part A

PROGNOSTIC AND PREDICTIVE IMPORTANCE OF THE IGF AXIS WITH 1ST LINE CHEMOTHERAPY PLUS BEVACIZUMAB, CETUXIMAB, OR BOTH IN METASTATIC COLORECTAL CANCER

1.0 Background:

Insulin has been shown to be a colon tumor promoter in an animal model [1] and high insulin levels have been related prospectively to colon cancer risk [2]. Supraphysiologic levels of insulin are required to activate the insulin growth factor (IGF) receptor and stimulate cell division; thus the cancer-enhancing effect of insulin is likely to act through a mediator. Insulin can increase the bioactivity of insulin-like growth factor-1 (IGF-1) by inhibiting the synthesis of certain IGF-binding proteins (IGFBPs) [3]. Further, insulin enhances growth hormone-stimulated IGF-1 synthesis [4]. Insulin-like growth factor-1 (IGF-1) is an important mitogen required by some cell types to progress from the G₁ phase to the S phase of the cell cycle [5]. IGF-1 has been reported to have potent ability to protect a broad range of cells from a variety of pro-apoptotic challenges. These actions are mediated through the type 1 IGF-receptor (IGF-1R), a tyrosine kinase that resembles the insulin receptor [6]. The availability of free IGF for interaction with the IGF-1R is modulated by the IGF binding proteins (IGFBPs), especially IGFBP-3. IGFBPs can have opposing actions to IGF-1, in part by binding IGF-1 [3], but also by direct inhibitory effects on target cells [7]. More than 90% of circulating IGFs are complexed with IGFBP-3 [8]. There is evidence that determinants of tissue IGF bioactivity are regulated in parallel with circulating IGF level [9, 10].

In prospective studies, relatively high plasma IGF-1 and low IGFBP-3 levels were associated with greater risk of prostate cancer [11, 12], lung cancer [13], colorectal cancer [14, 15], and breast cancer among premenopausal women [16]. Among women participating in the Nurses' Health Study, high IGF-1 and low IGFBP-3 levels were associated with an increased risk of colorectal cancers and adenomas ≥ 1 cm in diameter or those with a villous component (tubulovillous, villous, in situ cancers) [15]. For these endpoints combined, the RR was 2.38 (95% CI, 1.12-5.05) for high versus low tertile of IGF-1, and 0.30 (95% CI, 0.14-0.65) for high versus low tertile of IGFBP-3. Small, tubular adenomas were unrelated to IGF-1 or IGFBP-3. These results suggest that the IGF axis may increase risk of adenoma progression and colorectal cancer. Similarly, in a study of U.S. male physicians, men in the top quintile of IGF-1 had a relative risk (RR) of colon cancer of 1.36 (95% CI, 0.72-2.55). After further adjustment for IGFBP-3, the RR was 2.51 (95% CI, 1.15-5.46). For IGFBP-3, the RR for top versus bottom quintile was 0.47 (95% CI, 0.23-0.95); when further adjusted for IGF-1, the RR was 0.28 (95% CI, 0.12-0.66). The associations were similar even after the first 6 years of follow-up were excluded, strong evidence against any effect of undiagnosed tumor on plasma IGF [14].

IGF-1 has also been shown to be important for the survival of transformed cells [17]. Normal colon epithelial and cancer cells express IGF-1 receptors, which stimulate mitogenesis when activated by IGF-1 in vitro [18-20]. Several studies have shown that the IGF system contributes to homeostasis and functional integrity of intestinal epithelium by regulating several basic cellular functions such as proliferation and differentiation [21]. In vitro studies demonstrate that IGF-1 renders colon cancer cells resistant to apoptosis [22]. Other studies indicate that IGF-1 protects both colon and breast cancer cell lines against death induced by cytotoxic chemotherapy [18, 23-26]. Recently, Vadgama examined the influence of serum IGF-1 on survival among 130 women with breast cancer [27]. IGF-1 was inversely associated with progression-free and overall survival. In IGF-1 deficient mice, Wu

et. al. observed decreased colonic tumor growth and metastases compared to control mice; and injections of IGF-1 in the deficient mice restored this growth and metastatic potential [28].

Levels of IGF-I are stable and vary widely between individuals (e.g. 95th intervals range from 80-290 in older men) [29]. Changes in IGF-1 levels occur over a longer term of days to weeks [30]. Also single measures of IGF-1 have shown reasonable correlations with numerous physiologic parameters supporting the usefulness of a single measure of IGF-1 [29, 30]. Six IGF binding proteins (BP) are thought to influence IGF-1 bioactivity by direct and/or indirect mechanism [8]. We propose to measure IGFBP-3, which binds 80-90% of circulating IGF-1. Further, C-peptide levels (a marker of insulin secretion) have been associated with increased risk of the development of colorectal cancer [31]. C-peptide levels can be collected in patients that have not fasted and can be corrected based on time of last meal.

Recently, we reported preliminary results of the association between treatment efficacy and survival among patients with untreated metastatic colorectal cancer participating in Intergroup 9741 [32]. We found that higher IGFBP-3 levels are associated with increased likelihood of tumor response to chemotherapy and longer time-to-progression (TTP) and overall survival (OS). In contrast, IGF-1 was not correlated with response rate but increased levels were correlated with prolonged TTP and OS. However, this study was limited by sample size and the lack of data on recent weight change and caloric intake of these patients. The current Intergroup study will also include a diet and lifestyle questionnaire, along with questions regarding weight loss prior to diagnosis. If levels of IGFs were confirmed to be predictive of survival, these results would have important implications not only on our ability to predict patient outcome, but also on our understanding of cancer biology and treatment.

2.0 Hypothesis:

Levels of IGF-1 and C-peptide are inversely associated with response rate, time-to-tumor progression, and overall survival, whereas levels of IGFBP-3 are positively associated with these outcomes.

3.0 Research Approach and Methods:

This proposal would represent a companion study to the proposed CALGB 80405. In this proposed study, patients with stage IV colorectal cancer will be treated with either infusional 5-FU, leucovorin, oxaliplatin (FOLFOX) or infusional 5-FU, leucovorin, irinotecan (FORFIRI) and randomized to additional cetuximab, bevacizumab or cetuximab + bevacizumab. Blood samples for analysis will be obtained at study entry and serum will be assessed prospectively for levels of IGF-1, IGFBP-3 and C-peptide. IGF-1, IGFBP-3, and C-peptide will be assayed in the laboratory of Dr. Michael Pollak, using ELISAs with reagents provided by Diagnostic Systems Laboratory (Webster, Texas). This methodology is more reproducible than and highly correlated ($r=0.98$) with a radioimmunoassay technique previously employed [9]. The IGFBP assays do not cross-react with other IGF binding proteins. Blinded quality control samples are included in each batch so that the mean intrapair coefficients can be assessed. The inter- and intra-assay coefficients are 8 and 6%. IGF-1 (interclass correlation 0.98) and IGFBP-3 (0.96) concentrations have been shown to be stable in chilled whole blood over 24 to 36 hours, the approximate time between blood draw and arrival by overnight courier, compared to immediate processing and freezing [11]. To assess consistency of IGF-1 over time, we collected two blood specimens (in EDTA) approximately 8 weeks apart from 16 subjects (6 men and 10 women), ages 20 to 40 years. IGF-1 samples were analyzed (blinded) by two methods (ELISA and RIA). The correlations for IGF-1 between the 2 methods were quite high ($r = 0.79$, time 1, and $r = 0.90$, time 2). Moreover, the correlation between times 1 and 2 were 0.66 for ELISA and 0.63 for RIA, demonstrating that IGF-I levels track over time. The correlations for these biomarkers reflect both biological variability over time and laboratory error, and are similar or higher in magnitude to other markers widely used to predict disease risk based on a single estimation, such as blood pressure or serum cholesterol.

4.0 Statistical Considerations:

The influence of IGF-1, IGFBP-3 and C-peptide will be assessed using the response rate, progression-free survival, and overall survival among patients receiving chemotherapy for stage IV colorectal cancer. We hypothesize that levels of IGF-1 and C-peptide are inversely associated with response rate, time-to-tumor progression, and overall survival, whereas levels of IGFBP-3 are positively associated with these outcomes. Serum levels of IGF-1, IGFBP-3 and C-peptide will be categorized into quintiles. Since levels of these proteins can be modified by burden of disease and cachexia, we will also stratify by performance status (ECOG 0/1 vs 2), caloric intake (less than median vs greater than median), weight change (less than median vs greater than median; to be ascertained from the diet / lifestyle questionnaire that is a part of this trial) and LDH (less than or equal to institutional laboratory normal value vs greater than normal). Further, we will adjust for these factors and other potential determinants of response and survival using Cox regression. Finally, the presence or absence of diabetes mellitus will be obtained as a question on the diet and lifestyle instrument.

Overall survival (OS) will be measured from trial entry until death from any cause. Progression-free survival (PFS) will be measured from trial entry until documented progression of disease or death from any cause. Treatment-related toxicity will be measured primarily using two endpoints: 1) the proportion of patients experiencing Grade 3 or greater diarrhea; 2) the proportion of patients experiencing Grade 3 or greater neutropenia. The median survival in this patient population treated with combination chemotherapy is estimated to be 20-24 months. Median PFS is approximately 12 months. Two thousand two hundred eighty-nine (2,289) patients will be randomized on this trial in 2.5 years and followed an additional 2 years. Analysis will occur approximately 5.0 years after study activation.

IGF-1, C-peptide and IGFBP-3 will be measured at study entry. Each is assumed to have an underlying continuous distribution that will be summarized using quintiles. We anticipate that data on approximately 1000 patients will be available for this analysis. Power estimates are provided for the OS and PFS endpoints. Power computations are estimated based on testing the null hypothesis of independence of 2-year OS over IGF quintiles . Higher IGF-1 and C-peptide levels are hypothesized to be associated with decreased OS. Approximately 50% of patients enrolled on the treatment trial are expected to survive two years. Estimated power is approximately 0.91 ($\alpha = 0.01$) and 0.97 ($\alpha = 0.01$) to detect the difference illustrated in Table 9.

Quintile	1st	2nd	3rd	4th	5th	Total
Proportion surviving 2-years	0.115	0.111	0.100	0.090	0.084	0.50
Proportion not surviving 2-years	0.085	0.089	0.100	0.110	0.116	0.50
Total	0.200	0.200	0.200	0.200	0.200	1.00

Table 9. Alternative hypothesis for independence comparing OS at 2 years across quintiles based on IGF levels. The odds ratio comparing OS for the lowest to highest quintiles is 1.87 (associated χ -square non-centrality parameter,13.8; n=1000). Estimated power to test this hypothesis is 0.87 and 0.96 at significance levels of 0.01 and 0.05, respectively.

An odds ratio comparing OS for the lowest to highest quintiles based on IGFBP-3 of 0.53 is detectable with the same approximate power.

At one year 50% percent of patients are expected to have progressed/ died. The alternative hypothesis given in Table 10 for the test of independence of progression or death at 1 year over quintiles based on IGF levels is detectable with power of approximately 0.67 ($\alpha = 0.01$) and 0.85 ($\alpha = 0.05$).

Quintile	1st	2nd	3rd	4th	5th	Total
----------	-----	-----	-----	-----	-----	-------

CALGB/SWOG C80405
Appendix III A

Proportion not progressed at 1 year	0.1150	0.1044	0.0994	0.0954	0.0858	0.50
Proportion progressed at 1 year	0.0850	0.0956	0.1006	0.1046	0.1142	0.50
Total	0.2000	0.2000	0.2000	0.2000	0.2000	1.00

Table 10. Alternative hypothesis for independence comparing PFS at 1 year across quintiles based on IGF levels. The odds ratio comparing PFS for the lowest to highest quintiles is 1.80 (associated χ -square non-centrality parameter, 9.1; n = 1000). Estimated power is 0.67 and 0.85 testing at significance levels of 0.01 and 0.05, respectively.

An odds ratio comparing PFS for the lowest to highest quintiles based on IGFBP-3 of 0.56 is detectable with the same approximate power.

In exploratory analyses, we will assess whether levels of IGF-1, IGFBP-3 and C-peptide correlate with other clinical predictors of survival, including tumor stage, grade of differentiation, and nodal status. We will also investigate whether levels of IGF-1, IGFBP-3, and C-peptide modify the influence of systemic chemotherapy on response rate, time-to-tumor progression, and survival.

PROGNOSTIC AND PREDICTIVE IMPORTANCE OF SPECIFIC SERUM AND PLASMA BIOMARKER PROFILES WITH 1ST LINE CHEMOTHERAPY PLUS BEVACIZUMAB, CETUXIMAB OR BOTH IN METASTATIC COLORECTAL CANCER.

5.0 Background:

The importance of developing blood-based biomarkers has been extensively reviewed [33]. Compared to repeated tumor biopsies, the advantages of plasma and serum markers include increased safety, convenience, cost, the potential to assess a potentially greater number of factors at one time, and the ability to monitor repeatedly. The development of blood based biomarkers is often limited by many methodological issues, including the use of modestly sized, single institution experiences, use of retrospective clinical data, and inclusion of variable cancers, treatment regimens, clinical outcomes, and laboratory methodologies. For these reasons, both the development and the validation of individual biomarkers and biomarker profiles are best done in the context of large high quality randomized clinical trials. Recent multiplex technologies may facilitate this approach by reducing both costs and sample quantities [34]. If successful, patient profiling may allow more targeted or personalized treatment regimens and may identify targetable mechanisms of primary and acquired resistance.

Luminex bead technology offers an attractive alternative approach for cytokine analysis compared to more conventional ELISA methods. This novel multiplexing technology allows for the simultaneous measurement of several analytes [34]. The method is based on the use of microparticles that have been pre-coated with specific antibodies. These particles are then mixed with sample and the captured analytes are detected using specific secondary antibodies. This allows for up to 100 different analytes to be measured simultaneously in a single microplate well. The advantages of this flow cytometry-based method compared to traditional ELISA assays are conservation of patient samples as well as significant savings in terms of cost and labor. Also, this system offers increased versatility, accuracy, and reproducibility, as well as having a wider dynamic range than conventional microplate-based assays. At this time, the availability of different bead sets are limited; however, this technology is evolving quickly. For this reason, where we can validate the use of luminex technology to replace standard ELISA methods, we will do so.

- 5.1 Angiogenesis Efficacy and Resistance Markers:** The importance of tumor angiogenesis for colorectal cancer has recently been validated by the clinical benefit of bevacizumab in colorectal cancer, in both first line [35] and second line settings [36]. However, no molecular target profile has yet been shown to predict for efficacy of bevacizumab or other anti-VEGF strategies. VEGF has been shown to be a negative prognostic factor in many studies [37]. The most definitive data to date come from the pivotal 1st line colorectal cancer study of IFL +/- bevacizumab. In this study, VEGF expression from paraffin embedded samples was analyzed *by in situ* hybridization and by immunohistochemistry [38, 39]. By both methods, VEGF levels were not predictive of response to bevacizumab [38]. While this lack of prediction may be partially explained by the fact that most samples were from the original primary tumor not metastases, subset analysis of metastatic tumor samples did not show a marked difference between metastatic and primary tumor samples. The explanation is therefore more likely related to the complexity of the VEGF axis signaling, which includes promiscuity in ligand and receptor binding [40, 41], and the complexity of angiogenic signaling networks [42], which include dozens of angiogenic factors.

In preclinical models, multiple potential mechanisms of primary and acquired resistance to anti-VEGF therapies have been identified. For example, inhibition of one component of the VEGF signaling pathway has been shown to upregulate other parts of this pathway [43, 44]. In addition, other angiogenic factors have been shown to synergize with the proliferative and anti-apoptotic effects of VEGFA and to modulate sensitivity to anti-VEGF treatments. These include VEGFB [40, 45], VEGFC [46, 47], VEGFD [48, 49], PlGF [50], bFGF [51, 52], PDGF [53], PDEGF [54], IGF [55], TGF β [56, 57], TSP1 [58], IL8 [59], HGF [60], IL6 [61], and angiopoetin [62], among others.

In clinical studies, increased expression of tumor-associated angiogenic factors, including VEGF, has also been associated with worse clinical outcome, although this is not always the case. In fact, many conclusions should be considered as preliminary since most of these studies are of very limited size. In colorectal cancer patients, increased tissue expression of VEGFB [63], VEGFC [64], VEGFD [65, 66], PlGF [40], bFGF [67], PDGF [53], PDEGF [68], IGF [55], TGF β [69], TSP1 [70], IL8 [71], HGF [72], IL6 [73] and angiopoetin [74, 75], have been reported to be prognostic factors. Increased plasma levels of the following angiogenic factors have also been shown to have negative prognostic importance for colorectal cancer: VEGFA [76] [77], VEGFB [78], VEGFC [79], VEGFD [80], bFGF [76, 77], PDGF [81], PDEGF [68], IGF [14], TGF β [77, 82], IL8 [83], HGF [77], IL6 [84], and angiopoetin [74, 75]. Again, it must be noted that many of these studies are preliminary in nature, and further work is needed to clarify the prognostic value of these indices.

Despite often robust and durable clinical responses, essentially all patients treated with bevacizumab have progressed, implying primary or acquired resistance, or both. Treatment related changes in VEGF ligands and receptors have been reported in response to anti-VEGF therapy in preclinical models [42, 85, 86]. Treatment related changes have also been noted for many additional angiogenic factors, including PDGF, bFGF, and IGF, all of which have been shown to potentially mediate sensitivity and resistance to anti-VEGF therapy [51, 52]. Despite the known importance of numerous angiogenic factors in both preclinical models and in patients, there has not yet been a broad and systematic evaluation of either baseline or treatment related changes of multiple angiogenic factors in response to bevacizumab. This information is important since it may identify patterns of regulation among the network of angiogenic factors, including in the setting of treatment response and resistance, which in turn may suggest strategies for future combination anti-angiogenic regimens.

5.2 Angiogenesis Markers for Toxicity and Morbidity: The inter-relationship between tumor angiogenesis and the coagulation system is illustrated by the many components of the coagulation system that play significant roles in tumor angiogenesis, and by the many angiogenic factors have been shown to increase thrombotic tendencies [87]. For example, VEGF increases vascular permeability, which provides access for plasma proteins to the thrombogenic tissue matrix [86]. Fibrinogen is deposited in the perivascular space, leading to the production of a hydrophilic fibrin gel, which serves to further increase extravasation and retention of vascular fluids [86]. In endothelial cells, VEGF also leads to upregulation of TF (tissue factor) [88], vWF (von willebrand factor) [89], UPA1 (urinary plasminogen activator-1) [90], and UPAR (urinary plasminogen receptor) [91]. Many endogenous anti-angiogenic factors are also cryptic components of coagulation and matrix proteins, including angiostatin (fragment of plasminogen) [87], endostatin (fragment of collagen 18) [87], tumstatin (a fragment of collagen IV alpha3 chain) [92]), and vasostatin (fragment of calreticulin) [93]. Since the pathophysiology of venous thromboembolism (VTE) involves many processes that are altered in tumor angiogenesis, treatments designed to reduce one pathology may favorably impact the other. Anti-coagulants used for treatment of deep venous thrombosis [94], and statins used for

treatment of hyperlipidemia [95] have both been reported to reduce the risk of developing cancer.

Vascular events are a major source of morbidity and mortality for patients with metastatic colorectal cancer. In the pivotal study of IFL +/- bevacizumab, venous thromboembolic events were seen in 19.4% in the IFL/placebo group and 16.2% in the IFL/bevacizumab group [35]. While bevacizumab does not appear to increase the risk of VTE in this patient population, the high background rate is itself significant. This risk is greater than the risk of recurrent DVT in non-cancer patients after primary treatment of an initial DVT, particularly when adjusted for the rate of VTE per year [96]. While a hypercoagulable state has been described for many cancers [97, 98], including colorectal cancer [99], the mechanisms behind this increased clotting risk have not been systematically studied, particularly with respect to the impact of standard chemotherapy and anti-angiogenic therapy. By decreasing tumor angiogenesis, anti-angiogenic therapies are expected to downregulate most markers of vascular activation. The effect of chemotherapy on systemic markers of vascular activation and inflammation has not been well studied. Chemotherapy may have direct vascular and systemic toxicities, which may increase systemic markers of vascular activation and inflammation. Conversely, by reducing tumor bulk, systemic chemotherapy may also reduce these markers. In addition, homocysteine levels may be affected directly by 5FU via altered methionine pools, and indirectly by other chemotherapeutics via alterations in glutathione pools. Identifying patients at highest and lowest risk of VTE may allow more selective approaches to reducing this risk, particularly as novel therapies targeting distinct components of the clotting cascade are developed [100, 101].

In a pooled analysis of 5 randomized studies, arterial vascular events (ATE) were also increased with the addition of bevacizumab to chemotherapy (1.9% vs 4.4%) [102]. The risks were increased for both cerebrovascular events (0.5% vs 1.9%) and cardiovascular events (1.0% vs 2.1%). The risk of arterial events with bevacizumab were higher in patient 65 years or older (2.9% vs 8.5%) than in patients under age 65 years (1.4% vs 2.1%). Whether prognostic markers for ATE risk used in non-cancer populations predict for ATE risk in colorectal cancer patients treated with bevacizumab is not yet known. The effect of bevacizumab and chemotherapy on these risks factors is also not known. Larger and more definitive studies are needed to define the importance of these biomarkers for predicting and managing both VTE and ATE risks.

5.3 EGFR/Her Axis Markers of Efficacy and Resistance: The biology of the Her/Erb B family of receptors and ligands has been extensively reviewed [103, 104], as has their prognostic importance in targeted cancer therapies [105, 106]. The ErbB family of receptors is a group of 4 homologous Type I tyrosine kinase receptors: the epidermal growth factor receptor (EGFR/ErbB1/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors all have an extracellular binding domain, transmembrane lipophilic segment, intracellular tyrosine kinase domain, and regulatory -COOH terminal segment. The only exception is that HER3 has no tyrosine kinase domain. Several different ligands bind to the ErbB receptors. These include: EGF (epidermal growth factor) [107], TGF-alpha [108], amphiregulin (AR), betacellulin (BTC) [109], heparin-binding EGF (HB-EGF) [110], epiregulin, and epigen. [111-113] Heregulins, or neuregulins, bind HER3 and/or HER 4 [114, 115]. The EGFR ligands are integral membrane proteins that are released from the cell surface by metalloproteases, specifically ADAM10 and ADAM17 [116].

After ligand binding, the receptors become activated by homodimerization or heterodimerization. HER2 is the preferred co-receptor of EGFR, HER3, and HER4 [117, 118]. After dimerization, the PK becomes activated and tyrosine autophosphorylation occurs, leading to activation of several pathways, including the Ras-Raf-MAPK pathway [119], the PI3k-Akt pathway [120], the Jak-Stat pathway, and the PKC pathway. The increased activation of these mitogenic pathways is thought to be the mechanism of the correlation of overexpression of EGFR with poor prognosis in several malignancies, and the possible role of EGFR in tumorigenesis [121]. In addition, EGFR activation has been associated with increased IL-8 levels [122] and increased metalloprotease levels, specifically MMP9 [123] and MMP3 [124].

Aside from a limited set of tumors with EGFR mutations, clinically defining the target or target profile for anti-EGFR therapy has not yet been successful. This likely reflects both the complexity of the Her axis outlined above as well as assay methodologies. Most of this work has been done using tissue analysis. Tissue based markers of EGFR over-expression have generally been reported to have negative prognostic impact [125]. While cetuximab is active and approved for EGFR expressing colorectal cancer, the importance of EGFR status for predicting response to cetuximab in CRC remains controversial. In mCRC, EGFR staining intensity did not predict for the magnitude of benefit [126]. In addition, cetuximab may have activity in EGFR negative tumors as well [127]. The findings may reflect the complexity of the Her axis, differences between primary and metastatic disease, especially in the setting of chemotherapy resistance, and limitations of current assay methods.

While evaluation of the metastatic tumor tissue represents the gold standard for examining the biology of the Her axis, evaluation of blood based biomarkers has the theoretical advantages of far greater convenience and safety, particularly when repeated measurements are desirable, such as in the evaluation of treatment related changes that may mediate cetuximab sensitivity and resistance. The correlation of tissue and plasma markers has also not been evaluated in large studies.

The prognostic and predictive importance of Her receptors and ligands in serum has not yet been well studied. EGFR is readily measurable in serum [128]. ELISA based studies have noted elevated serum EGFR levels in cancer patients (636 +/- 299 pmol/L) compared to healthy controls (336 +/- 228 pmol/L) [128]. Mean EGFR concentrations of 336 +/- 228 pmol/L and 636 +/- 299 pmol/L have been seen in healthy patients versus cancer patients, respectively [129]. Pilot studies in patients with refractory non-small cell lung cancer and colorectal cancer have shown that measurement of Her receptors and ligands (EGFR, Her2, TGF β and amphiregulin) is feasible, although these markers were neither prognostic nor predictive in these studies, which may relate to many factors including the limited activity of gefitinib in these diseases and settings and the very small number of patients studied. Larger and more definitive studies are needed to define the importance of these biomarkers to optimize use of cetuximab and possibly other anti-EGFR strategies in colorectal cancer.

5.4 EGFR/Her Axis Markers of Toxicity and Morbidity: Cetuximab (ErbixTM) use is associated with a significant incidence (4%) of anaphylactoid infusion reactions of an unclear etiology. Like those with other biologic agents, cetuximab-related infusion reactions occur within 30 to 60 minutes of the start of the first infusion and often do not occur in patients who are re-challenged, arguing against an IgE-mediated anaphylactic reaction.

Table 11

	All (n = 2127)		Cetuximab-related (n=2127)	
	All Grades	Grades 3-4	All Grades	Grades 3-4
Hypersensitivity reaction	6.4%	2.5%	5.3%	2.4%
Infusion reaction	18.4%	3.1%	15.6%	2.7%

The frequency of cetuximab hypersensitivity and infusion reactions are listed below (taken from cetuximab Investigator Brochure v.10.0). Hypersensitivity reaction is defined as “Allergic reaction” or “Anaphylactoid reaction”. Infusion reaction is defined as “Allergic reaction” or “Anaphylactoid reaction” at anytime during therapy and/or “Fever”, “Chills”, or “Dyspnea” which occurred within the 24 hours following the first dose. The frequency of any reaction to cetuximab (hypersensitivity or infusion reaction) is approximately 18.3% -21.5%, which represents a significant clinical problem.

Clinically, the term anaphylaxis is most often used to describe rapidly developing generalized reactions that include pruritus, urticaria, angioedema (especially laryngeal edema), hypotension, wheezing and bronchospasm, nausea, vomiting, abdominal pain, diarrhea, uterine contractions, and/or direct cardiac effects, including arrhythmias. These clinical manifestations can occur singly or in various combinations and usually occur within moments of exposure. However, signs and symptoms may begin 30 to 60 minutes after exposure, and in some cases onset may be delayed for longer than an hour. In general, these type of severe reactions are either anaphylactic (IgE mediated) or anaphylactoid. Anaphylaxis is defined as an immediate systemic reaction caused by rapid, IgE-mediated immune release of potent mediators from tissue mast cells and peripheral blood basophils. Anaphylactoid reactions are immediate systemic reactions that mimic anaphylaxis but are not caused by IgE-mediated immune responses. The known causes of these types of reactions are non-IgE mediated mast cell/basophil degranulation, complement activation by immune complexes, or cytokine release syndromes. There may be causes of which we are not aware.

The mechanism behind infusion reactions has not been well studied for most drugs, including monoclonal antibodies such as cetuximab. Treatment strategies to minimize the frequency and severity of these anaphylactoid reactions will depend upon characterizing the mechanism behind them. There have been no mechanistic studies on cetuximab-related infusion reactions to date. Thus, we wish to define first whether the reactions are anaphylactic (IgE-mediated) or anaphylactoid (non-IgE-mediated release of anaphylatoxins from mast cells and basophils). Second, we wish to better define the pathologic mechanisms by which biologic infusion-related reactions occur.

6.0 Hypotheses:

- 6.1 That the baseline profile of multiple angiogenic factors will be prognostic for patients with metastatic colorectal cancer treated with 1st line chemotherapy and will be predictive of clinical benefit for bevacizumab.
- 6.2 That treatment-related changes in the angiogenic profile will correlate with response and resistance to bevacizumab therapy.
- 6.3 That increased coagulation and inflammatory markers at baseline or induced by treatment will correlate with increased risk of venous and arterial thrombotic events in both the control and bevacizumab groups.
- 6.4 That the baseline profile of multiple factors related to activation of the EGFR/Her axis will be prognostic for patients with metastatic colorectal cancer treated with 1st line chemotherapy and will be predictive of clinical benefit for cetuximab.
- 6.5 That treatment-related changes in the EGFR/Her axis profile will correlate with response and resistance to cetuximab therapy.
- 6.6 That cetuximab associated anaphalactoid infusion reactions will correlate with indices of mast cell degranulation, compliment activation, and/or IgE mechanisms.

7.0 Research Approach and Methods:

- 7.1 **That the baseline profile of multiple angiogenic factors will be prognostic for patients with metastatic colorectal cancer treated with 1st line chemotherapy and will be predictive of clinical benefit for bevacizumab:** Most angiogenic factors have been reported to have negative prognostic impact, consistent with the many unfavorable biological characteristics associated with a highly angiogenic phenotype [87]. However, the relationship of complementary angiogenic pathways in colorectal cancer have not yet been well described, nor has the prognostic value of multiple anigogenic factors been evaluated concurrently. The prediction is that the number of pro-angiogenic factors elevated (above median), and the number of anti-angiogenic factors decreased (below median), will correlate with worse prognosis in patients treated without bevacizumab. In addition to prognostic importance, for patients treated with bevacizumab, the number of pro-angiogenic factors elevated (above median), and the number of anti-angiogenic factors downregulated (below median), will correlate with survival and with the magnitude and duration of response. More complex models, where the impact of each factor is weighted, are also possible and will also be explored. To determine whether tumor and plasma profiles of angiogenic factors are correlated, the levels of these factors measured in tissue and baseline plasma will be compared. Using ELISA and, in some cases, Luminex bead approaches, plasma levels of the following angiogenic factors will be evaluated at baseline, at 2 months of treatment (the time most patients will have a partial response) and at disesase progression: VEGFA, VEGFB, VEGFC, VEGFD, PIGF, bFGF, PDGF, PDEGF, IGF, TGF, TSP1, IL8, HGF, IL6, e-selectin, VCAM, MMP2, MMP9, soluble VEGFR1, soluble VEGFR2, and soluble VEGFR3.
- 7.2 **That treatment related changes in the angiogenic profile will correlate with response and resistance to bevacizumab therapy:** Specifically, we hypothesize that the greater the number of anigogenic factors that decrease, and the greater the number of anti-angiogenic factors that increase, the larger the magnitude and duration of response will be. Conversely, increases in the number of angiogenic

factors, or decreases in anti-angiogenic factors, will correlate with more rapid disease progression. More complex models, where the impact of each factor is weighted, are also possible and will also be explored. Using ELISA and Luminex bead approaches, the same markers tested at baseline will be evaluated at 2 months on therapy (the time most patients will first achieve a partial response) and at the time of disease progression.

- 7.3 That increased coagulation and inflammatory markers at baseline or induced by treatment will correlate with increased risk of venous and arterial thrombotic events in both the control and bevacizumab groups:** Specifically, we hypothesize that baseline markers of activated coagulation pathways and markers of vascular activation and/or inflammation at baseline will correlate with the risk of deep venous thrombosis or pulmonary embolus and myocardial infarction, angina, cerebrovascular accident, transient ischemic attack, or other vascular event. These factors have been associated with increased risk of vascular events in non-cancer populations. Thrombin-antithrombin complexes are a general marker for thrombin activation. In addition, treatment related increases in these markers at 2 months (i.e., baseline – cycle 2 values) will also predict for these vascular events. Lastly, these markers will also increase at disease progression, consistent with the prediction that tumor growth is associated with increased tumor angiogenesis. Using ELISA and Luminex bead approaches, baseline Factor VII, fibrinogen, D-dimer, von Willebrand factor activity, von Willebrand factor antigen, Factor VIII, C-reactive protein, thrombin-antithrombin complexes, homocysteine, IL6, VCAM, and e-selectin levels will be evaluated at baseline, at 2 months on therapy (the time most patients will first achieve a partial response), and at the time of disease progression.
- 7.4 That the baseline profile of multiple factors related to activation of the EGFR/Her axis will be prognostic for patients with metastatic colorectal cancer treated with 1st line chemotherapy and will be predictive of clinical benefit for cetuximab:** Markers of increased EGFR activation will predict for worse prognosis and for better treatment response. Specifically, elevated baseline levels of sEGFR and EGFR ligands (EGF, HB-EGF, amphiregulin, TGF α , and betacellulin) will predict for clinical benefit from Cetuximab, and elevated sHer2, which may be a marker of increased EGFR1-Her2 heterodimerization, will predict for resistance. Alternatively, higher serum EGFR levels may predict resistance to cetuximab, since serum EGFR may serve as a sink for cetuximab, reducing delivery to the tumor cell membrane where it is biologically active. The prediction is that the number of EGFR factors elevated (above median) will correlate with worse prognosis in patients treated without cetuximab. In addition to prognostic importance, for patients treated with cetuximab, the number of Her factors elevated (above median) will correlate with survival and with the magnitude and duration of response. More complex models, where the impact of each factor is weighted, are also possible and will also be explored. To determine whether tumor expression and serum levels of EGFR/Her axis factors are correlated, the level of these factors measured in tissue and baseline serum will be compared. Using ELISA and Luminex bead approaches, plasma levels of the following EGFR/Her axis factors will be evaluated at baseline: EGF, HB-EGF, amphiregulin, TGF α , betacellulin, soluble EGFR and soluble Her2, IGF1, and IGFBP3.
- 7.5 That treatment related changes in the EGFR/Her axis profile will correlate with response and resistance to cetuximab therapy:** Specifically, we hypothesize that the greater the number of Her axis factors that decrease, the larger the magnitude and duration of response will be. Conversely, increases in the number of Her axis factors will correlate with more rapid disease progression. More complex models, where the impact of each factor is weighted, are also possible and will also be explored. Using ELISA and Luminex bead approaches, the same markers tested at baseline will be evaluated at 2 months on therapy (the time most patients will first achieve a partial response) and at the time of disease progression.

- 7.6 That cetuximab-associated anaphylactoid infusion reactions are mediated by mast cell degranulation, compliment activation, and IgE mechanisms:** In order to determine the cause of cetuximab-related infusion reactions, we propose to take two approaches: 1) hypothesis-driven approaches and 2) hypothesis-generating approaches. To determine whether there has been either an anaphylactic or an anaphylactoid reaction, we will test for mast cell degranulation, by measuring serum tryptase levels by ELISA. To differentiate between an anaphylactic and an anaphylactoid reaction, we will determine if the reaction is IgE-mediated, by measuring product-specific IgE levels by ELISA using Cetuximab (IgG) adsorbed onto ELISA plates. To define if immune complex activation of complement is a component of the reaction, we will measure complement levels and complement split products C3a and C5a by ELISA. To define if a cytokine release syndrome is a component of the cetuximab-related reactions, we will measure 17 cytokines by a multiplexed-bead based assay.

Using a systems biology approach, we will define the changes that occur in individuals who develop reactions, focusing on soluble protein expression and leukocyte transcription. We will utilize both bead-based and chip-based approaches to define the soluble proteins in serum and plasma. We will define the transcriptional profile of leukocytes in individuals with a reaction compared to those who did not have a reaction. We will focus on oxidative stress, NFAT, and NFkB pathways. However, we do not know whether other, important pathways will be affected. Thus, we will be unbiased in our approach to analysis of the transcriptome.

Cetuximab infusion reaction biomarkers will be drawn 30-60 min after the end of cetuximab infusion in cycle one only in the first 250 patients randomized to receive cetuximab. As of September 9, 2008, samples from 250 patients have been collected. Patients experiencing an infusion reaction should have infusion reaction biomarkers drawn as close to the time of the reaction as possible. Patients who experience an infusion reaction(s) in subsequent cycles should have infusion reaction biomarkers drawn at the time of each reaction.

Using ELISA and radioimmunoassay approaches, baseline and post infusion serum levels of the following will be evaluated in patients with and without infusion reactions: serum tryptase, complement, complement split products C3a and C5a, and product-specific IgE.

8.0 Statistical Considerations:

- 8.1 Analysis of Angiogenesis and EGFR/Her axis biomarker profiles:** Angiogenesis and EGFR/Her axis biomarkers will be studied according to the statistical testing procedure outlined below.

Statistical Testing Procedure: The statistical analysis of all biomarkers will begin when the clinical data have been finalized, i.e., follow-up and data cleaning have been completed. Angiogenic biomarkers will be measured at three timepoints, baseline, two months after the initiation of treatment (response assessment) and at time of progression. Approximately 90% of samples are expected at baseline (n=2,060), 85% at two months (n = 1,946) and 67% at progression (n = 1,534). Differences between the continuous measurements at baseline and two months, between the measurements at baseline and progression and between the measurements at two months and baseline will be computed.

Pre-treatment markers and marker differences over time will be identified as prognostic for outcome (OS, PFS, DR, clinical response of CR/PR) according to the following procedure. Markers will first be screened for prognostic value in a randomly-selected subset of 200 patients from each treatment arm (chemotherapy + bevacizumab; chemotherapy + cetuximab, chemotherapy + bevacizumab + cetuximab). Statisticians will provide laboratory investigators with the lists of patients whose tumors have been randomly selected for study in the screening group. (If technical or practical considerations limit the numbers of biomarkers that can be

CALGB/SWOG C80405
Appendix III B

evaluated in one screening group, a second screening group of equal size will be randomly-selected.)

Approximately 480 pre-treatment samples in each treatment group will remain for additional testing. Biomarker measurements (ELISA) will be considered as continuous variables. The statistical distributions of each biomarker will be described. Data transformations will be used, if appropriate, to normalize distributions. If no significant differences are found using the continuous data, qualitative differences will be explored based on the biomarker distribution. Other statistical methods such as the maximum chi-square methodology may also be employed to determine subgroups resulting in maximum discrimination.

A Type I error rate of 0.20 will be used for the screening and will not be adjusted for multiple looks. Relationships between clinical outcome and biomarker status (biomarker by treatment interactions) will be descriptively compared among/between treatment groups. At screening preliminary data/results will be available for biomarkers in each treatment group. Relationships between outcome (OS) and biomarker status can be descriptively compared among/between treatment groups. Power computations within each treatment group are provided for baseline biomarkers and the primary endpoint of OS. At the end of follow-up the numbers of expected deaths among 200 patients studied on each treatment arm are approximately 134 for the chemotherapy + bevacizumab, and 116 for each of the chemotherapy + cetuximab and chemotherapy + bevacizumab + cetuximab treatment arms, respectively.

Power computations are based on the logrank test of OS categorizing each biomarker as “high” or “low” at the median. Table 12 provides approximate detectable differences and the required numbers of events (expected deaths among 200 patients randomly selected for study at the end of follow-up) associated with powers of 0.8, 0.7 and 0.6 for testing exploratory hypotheses on each treatment arm (2-sided $\alpha = 0.20$, equal allocation by biomarker status is assumed).

CALGB/SWOG C80405
Appendix III B

Detectable Hazard Ratio	Required Number of Events	Power
1.42	146	0.80
1.44	134	0.80
1.48	116	0.80
1.50	110	0.80
1.56	90	0.80
1.35	146	0.70
1.37	134	0.70
1.40	116	0.70
1.46	90	0.70
1.48	86	0.70
1.31	134	0.60
1.33	116	0.60
1.36	100	0.60
1.38	90	0.60
1.40	82	0.60

Table 12: Approximate hazard ratios detectable and required numbers of events associated with approximate powers of 80%, 70% and 60% (2-sided logrank test $\alpha = 0.20$, equal allocation by biomarker status is assumed). These power estimates are also approximately correct for biomarker distributions of 0.30 to 0.70 (“high” versus “low”).

For example, on the chemotherapy + bevacizumab treatment arm approximately 80% power is achieved to detect an OS hazard ratio (“high” versus “low” pre-treatment cut at the median value of the marker) of 1.44 with 134 events observed (2-sided logrank test $\alpha = 0.2$).

Markers not found to be significant in any treatment subgroup in the screening data set will be dropped from further investigation. Potentially predictive markers at the 0.2 level in at least one treatment subgroup will be studied further in an additional 480 patients. A maximum of 680 samples will be tested in each treatment arm. For validation analyses the overall Type I error rate of 0.10 will be adjusted for the numbers of potential predictors using a Bonferroni correction. At the end of follow-up the numbers of expected deaths among 480 patients studied on each treatment arm are approximately 323 for the chemotherapy + bevacizumab treatment arm and 278 for each of the chemotherapy + cetuximab and chemotherapy + bevacizumab treatment arms, respectively. Table 13 provides approximate detectable differences and the required numbers of events associated with powers of 0.8, 0.7 and 0.6 for testing 8 hypotheses on each of the treatment arms among 480 patients at the end of follow-up.

CALGB/SWOG C80405
Appendix III B

Detectable Hazard Ratio	Required Number of Events	Power
1.42	360	0.80
1.47	300	0.80
1.50	270	0.80
1.38	360	0.70
1.42	300	0.70
1.45	270	0.70
1.34	360	0.60
1.38	300	0.60
1.40	270	0.60

Table 13. Approximate hazard ratios detectable and required numbers of events associated with powers of 80%, 70% and 60% (2-sided logrank test $\alpha=0.10$ adjusted for testing of 8 biomarkers, equal allocation by biomarker status at the median value is assumed). These power estimates are also approximately correct for biomarker distributions of 0.30 to 0.70 (“high” versus “low”).

Reasonable differences in OS are detectable with adequate power. A subset of biomarkers significantly related to OS for each treatment group will be identified and the inter-relationships among these variables will be investigated. Multivariable analysis will be used to determine the simultaneous impact of combinations of biomarkers on outcome. The prognostic value of the number of biomarkers in this subset on which a patient is scored negatively (with respect to outcome) will also be explored both as a continuous and categorical variable.

Under the proportional hazards model, the 2 x 2 hypotheses of no interaction between biomarker level and treatment (at two levels) will be tested. Three-way interactions will not be considered. Table 14 provides power estimates for the test of no interaction between treatment regimens (Chemotherapy + bevacizumab; Chemotherapy + cetuximab) and biomarker levels (low; high) for cases where high levels of the biomarker are associated with comparable or superior survival on the chemotherapy + bevacizumab regimen and inferior survival on the chemotherapy + cetuximab regimen, relative to patients with low levels of the biomarker.

Median OS in months (Chemotherapy+ BV; “high” biomarker)	Median OS in months (Chemotherapy+ BV; “low” biomarker)	Median OS in months (Chemotherapy + C225; “high” biomarker)	Median OS in months (Chemotherapy+ C225; “low” biomarker)	Power
26.0	19.0	27.5	27.5	0.44
26.5	18.5	27.5	27.5	0.58
27.0	17.0	27.5	27.5	0.84
26.5	18.5	26.0	29.0	0.84

Table 14. Estimated power to detect the specified 2 x 2 biomarker by treatment interactions assuming equal sample sizes per group (n = 480; overall 2-sided $\alpha=0.1$ adjusting for 8 comparisons). Median OS in each treatment group was selected to illustrate detectable differences based on the assumptions of the primary hypothesis. Power computations are also based on categorizing the biomarker as “high” or “low” at the median.

Reasonable differences are detectable with adequate power. If an interaction is present survival comparisons by biomarker status will be made within treatment arm (n = 480 patients per treatment).

8.2 Analysis of Coagulation and Inflammatory Markers and Venous and Arterial Thromboembolic Events: Markers of coagulation and inflammation associated with Venous Thromboembolic Events (VTEs), and Arterial Thromboembolic Events (ATEs) will be assessed in all available patient samples (n = 2,060). VTEs and ATEs are expected in approximately 20% and 5% of patients, respectively.

Venous Thromboembolic Events (VTE): Assuming a VTE rate of 20% on all treatment arms we expect approximately 412 patients of 2,060 to experience a VTE. The proportions of patients experiencing VTEs will be compared between “high” and “low” levels of each biomarker. Biomarker measurements will be categorized as “high” and “low” according to each biomarker distribution at the median or another cut-point based on the biomarker distribution.

Proportion of patients with high levels of the marker experiencing VTEs	Proportion of patients with low levels of the marker experiencing VTEs	Power (Fisher’s Exact Test, 2-sided $\alpha=0.01/0.05$)
0.24	0.16	0.97/0.99
0.235	0.165	0.91/0.97
0.23	0.17	0.78/0.91

Table 15. Approximate power estimates to detect the specified differences comparing the proportion of patients experiencing VTEs of Grade 3 or greater between “high” and “low” levels of each biomarker (n = 2,060). Power computations assume the biomarker is categorized at the median.

Small differences are detectable with good power. Power estimates in Table 15 are approximate for distributions of “high” levels (versus “low”) of between 0.30 and 0.70 and are lower outside this range.

Arterial Thromboembolic Events (ATE) Assuming a ATE rate of 5% on all treatment arms we expect approximately 103 patients of 2,060 to experience a ATE. The proportions of patients experiencing ATEs will be compared between “high” and “low” levels of each biomarker. Biomarker measurements will be categorized as “high” and “low” according to each biomarker distribution at the median or another cut-point based on the biomarker distribution.

Proportion of patients with high levels of the marker experiencing ATEs	Proportion of patients with low levels of the marker experiencing ATEs	Power (Fisher’s Exact Test, 2-sided $\alpha=0.01/0.05$)
0.075	0.025	0.99/0.99
0.07	0.03	0.93/0.98
0.065	0.035	0.67/0.86

Table 16. Approximate power estimates to detect the specified differences comparing the proportion of patients experiencing ATEs of Grade 3 or greater between “high” and “low” levels of each biomarker (n=2,060). Power computations assume the biomarker is categorized at the median.

Small differences are detectable with good power. Power estimates in Table 16 are approximate for distributions of “high” levels (versus “low”) of between 0.30 and 0.70 and are lower outside this range. (Power estimates based on comparisons of Poisson parameters are comparable.)

8.3 Cetuximab-associated infusion reactions

Tryptase, IgE, complement (C3, C4, C3a, C5a) and cytokine levels will be measured pre and post infusion of Erbitux in patients who experience anaphylactoid reactions and in a control group of patients who do not experience anaphylactoid reactions. In each of the cetuximab treatment arms, 2%-4% of patients are expected to experience grade 3-4 reactions (15-30 patients), and 15%-19% of patients are expected to experience grade 1-2 reactions (114-145 patients). All patients who experience any cetuximab infusion or hypersensitivity reaction will be studied, although formal analysis will focus primarily on those with grade 3-4 reactions. Up to 250 patients who do not have reactions will be studied as potential controls. Distributions of the marker levels will be described and transformations will be used as appropriate. In one previous study the mean (sd) total tryptase was 4.9 ng/mL (2.3) in 56 normal control subjects. Table 17 contains power estimates to detect the specified differences in pre-infusion tryptase levels with at least 30 and 60 patients studied. Differences in the changes in levels (pre versus post) will also be compared. The hypotheses of increased tryptase and IgE levels among patients with infusion reactions will be tested (1-sided $\alpha = 0.025$). Comparisons of complement and cytokine levels will be considered exploratory.

Hypothesized Mean Tryptase Levels (SD) – No Infusion reactions	Hypothesized Mean Tryptase Levels (SD) – Infusion reactions	n per group	Power (1-sided $\alpha=0.025$)
5.0 (2.5)	10.0 (9.0)	15	0.50
5.0 (2.5)	15.0 (13.5)	15	0.75
10.0 (6.7)	20.0 (18.0)	15	0.48
10.0 (6.7)	30.0 (27.0)	15	0.74
5.0 (2.5)	10.0 (9.0)	30	0.81
5.0 (3.4)	10.0 (9.0)	30	0.79
5.0 (2.5)	15.0 (13.5)	30	0.97
10.0 (6.7)	20.0 (18.0)	30	0.79
10.0 (6.7)	30.0 (27.0)	30	0.97

Table 17. Power estimates to detect specified differences per treatment group (total samples obtained n = 680) in pre-infusion tryptase levels for approximate 2% and 4% incidence of infusion reactions (Welch approximation to t-test, 1-sided $\alpha = 0.025$).

Detection of a 3-fold difference in tryptase and IgE levels is of interest. Differences between 2- and 3-fold are detectable with adequate power.

PROTEOMIC PROFILING WITH 1ST LINE CHEMOTHERAPY PLUS BEVACIZUMAB, CETUXIMAB OR BOTH IN METASTATIC COLORECTAL CANCER.**9.0 Background:**

9.1 Approach with biomarkers: Biomarkers that predict outcome, therapeutic response, or therapeutic toxicity would have tremendous patient benefit. Research efforts to improve diagnosis capabilities and disease stratification with biomarkers have often focused on elucidating the spectrum of genetic changes associated with cancer using gene or oligonucleotide microarrays. These studies have led to the identification of formerly unrecognized cancer-specific gene products as well as novel tumor classifications based on patterns of gene expression. While the impact this technology has had in improving the stratification of cancer is invaluable, given the disparity between gene transcription and protein expression, and post-translational modifications, it is possible that relevant phenotypic characteristics of cancer may be overlooked by investigations of gene expression alone. Because of this, the value of studying disease-specific changes in protein expression directly is becoming increasingly more evident.

9.2 Proteomics: The ability of proteomic analyses to complement transcript-level based microarray studies has been well documented. Using 2D-GE to separate the proteins, numerous alterations in the expression levels of proteins specific to cancer have been discovered. It is postulated that the spectrum of proteins expressed by a tumor cell is a reflection of its phenotype, thus elucidation of comprehensive protein profiles will allow us to gain more insight into the biology of the disease and, hence, lead to advances in tumor classification, stratification, and prognosis.

While protein expression profiling traditionally begins with 2D-GE for protein separation prior to mass spectrometry identification of gel spots, certain characteristics of 2D-GE makes comparisons between groups of specimens difficult. Hence, this has stimulated interest in developing alternative methods. One recently described method, differential in-gel electrophoresis (DIGE), is a new approach that has been used to compare expression profiles between groups of specimens. Other investigators have begun to explore the use of alternative separation methods followed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry, or MALDI-TOF MS. Although MALDI-TOF MS is most often used to identify proteins first separated by 2D-GE, several recent studies have suggested that direct analysis of MALDI spectra (with or without a pre-separation step) can provide diagnostic information. MALDI-TOF MS platforms are especially advantageous because they are amenable to the analysis of samples containing small amounts of protein. Previous studies by Patz and colleagues and by others have generated complex protein spectra using as little as 0.5 µg total protein [131, 132]. Xu et al. have also reported the generation of excellent spectra from 10 laser captured mouse colon crypt cells [133].

MALDI-TOF MS has excellent resolution in the low mass ranges and is unaffected by pH extremes. Although the resolution of MALDI spectra may be optimal at m/z values of 30,000 or less, the instrumentation is able to detect proteins of greater than 200 kDa. While the ability to detect a particular protein spot on a 2D gel depends on the abundance of the protein, the same is not always true with MALDI-TOF MS. Other factors such as protein mass, the presence or absence of specific amino acid sequences, and specific physicochemical interactions between the protein and the MALDI-TOF MS matrix all contribute to the detection efficiency of a given protein in the MALDI-TOF-MS experiment. Most importantly and in contrast to array-based

methods, these types of discovery platforms make absolutely no assumptions about known or unknown proteins, which allows the process to be completely independent of preconceived expectations of protein expression.

- 9.3 Protein expression platforms:** The Patz laboratory has developed a MALDI platform that can be applied to any biologic specimen. In pilot studies, this approach was used to generate spectra from complex samples from as little as 0.5 µg total protein with excellent resolution in the low mass range. The platform includes separation of proteins, MALDI-TOF MS analysis of each fraction, computer-based merging of spectra from all fractions, statistical comparison of the resulting composite spectra to identify differentially expressed proteins, protein identification, and assay development for validation in large clinical trials. Preliminary results with DIGE technology for biomarker development have also been encouraging.
- 9.4 Summary:** The purpose of the current proposal is to identify protein biomarkers that predict benefit from 1st line chemotherapy for metastatic colorectal cancer. This type of translational project has overwhelming implications for clinical management, in addition to the fact that this approach is amenable to address a broad spectrum of diagnostic issues in oncology. This proposal will provide significant insight into the development of serum based biomarkers for therapeutic response. This high-throughput platform is a powerful translational technique capable of rapidly addressing many issues in oncology. We expect this should prove to be a rich source of disease-specific proteins that can lead to the discovery of novel protein biomarkers.
- 9.5 Preliminary Studies:** MALDI-TOF Proof of principle study: Several different strategies are being pursued in the discovery of tumor biomarkers, although the ultimate goal of this application is to develop a sensitive and specific non-invasive diagnostic test that can predict response to therapy in colon cancer. As proof of principle and confirmation of our expression-profiling platform, Patz et. al. explored the possibility of creating protein expression profiles from small amounts of lung cancer tissue and normal lung tissue. The rationale for this initial set of experiments was that these 2 tissues of distinctly disparate biology (lung cancer tissue vs. normal lung tissue), although of similar origin, must possess different protein profiles, and thus we wanted to verify that our platform could elucidate differences in expression. The details of this exploratory project have been reported [131]. Briefly, protein expression profiles in the form of MALDI-TOF spectra were generated on 10 different patient tumor samples and on an equivalent sized piece of matched normal lung tissue from each of the 10 patients. All tumor/normal specimens were obtained at the time of surgical resection. By using matched pairs (tumor and normal specimen from the same patient), we could eliminate protein expression patterns unique to an individual. We prepared soluble protein extracts from 10 mg sections of the frozen tissue and subjected each extract to MALDI-TOF MS. We then compared the lung tumor profiles to that of the matched normal lung specimens from the same patient using a computer assisted pattern recognition program, and initially identified two proteins (cyclophilin A and macrophage migratory inhibitory factor) that were overexpressed by the tumors and not by the matched normal lung tissue.

We then confirmed these data in an additional 24 lung tumor specimens with the normal matched lung lysate pairs and 6 lung specimen lysates from patients with non-malignant lung disease to address specificity of the markers and the possibility that peaks resulted from a generalized host response such as inflammation. Overall, both of the distinguishing ion signals were seen in 27/34 tumor lysates, and in only 1/40 non-malignant tissue lysates (83% sensitivity, 98% specificity). Ion signals at either m/z 12,338 or m/z 17,882 were found in 30/34 tumor lysates and 3/40 non-malignant lysates (89% sensitivity, 93% specificity).

- 9.6 Serum profiling:** In the work proposed here the goal is to analyze patient serum directly for potential protein biomarkers as opposed to the patient's actual tumor tissue itself. Serum

sampling has enormous practical advantages in terms of convenience, cost, and sample availability compared to fresh tumor biopsies, especially for use in clinical settings involving large numbers of patients with advanced disease. To demonstrate the feasibility of protein profiling from serum, we have previously compared the serum protein profiles of patients with newly diagnosed lung cancer to that from age and sex matched individuals without evidence of cancer. We reasoned that protein expression differences revealed by a comparison between the composite peak lists from lung cancer and normal serum should result in the identification of potential lung cancer biomarkers. These studies have identified several differentially expressed peaks, one of which was at m/z 11,700. Using multiple techniques, this protein was identified as serum amyloid A (SAA) [134]. We will use a similar strategy for the current proposal.

- 9.7 DIGE approach:** Although the value of 2D-GE as a proteomics tool is undeniable, the lack of absolute reproducibility with regard to the precise location of a given protein spot on different gels limits the utility of the technique when it comes to multi-gel comparisons. One technique that has effectively eliminated this shortcoming is DIGE. DIGE was originally designed to compare the 2D spot patterns of pairs of samples run on the same gel. Differentiation of spots was achieved by labeling the proteins in each sample with spectrally resolvable fluorescent dyes prior to mixing the samples together and running the gel. A fluorescent scanner was then used to scan the gel once for one color and then again for the other color. Since the spots being compared were on the same gel, the question of matching the gels spot-for-spot was eliminated. While this edition of DIGE proved effective at comparing pairs of samples, the problem of spot matching still existed for comparisons between groups of specimens.

A recent improvement in the DIGE protocol deals with the problem of gel-to-gel variation by including an internal standard, consisting of a mixture of all specimens that is run on each gel [135]. The mixture is labeled with a third fluorescent dye, allowing spot intensities to be normalized to this internal standard. The utility of this methodology is elegantly demonstrated in a recently published study of colon cancer [136].

10.0 Hypothesis and specific aims

The primary goal of this proposal is to discover novel serum biomarkers to predict clinical benefit from 1st line chemotherapy for metastatic colorectal cancer. We will use two innovative proteomics approaches, a MALDI-TOF based platform and DIGE technology, to generate protein expression profiles from the serum of patients with colon carcinoma that are predictive of survival benefit from (1) all chemotherapy regimens, (2) bevacizumab based chemotherapy, and (3) cetuximab based chemotherapy. These techniques will elucidate patterns of protein expression that will determine which patients will benefit most and least from therapy. A major strength of this proposal is its translational approach, in particular, the use of novel basic science techniques to address a clinically relevant issue in oncology.

We will address this hypothesis through the following Specific Aims:

- 10.1 Generate protein expression profiles using a MALDI-TOF based platform and DIGE technology from serum samples.
- 10.2 Analyze and compare protein expression profiles to elucidate proteins that differentiate patients with greater survival benefit from those with lesser survival benefit from treatment with bevacizumab based chemotherapy, cetuximab based chemotherapy, or bevacizumab plus cetuximab based chemotherapy.
- 10.3 Identify differentially expressed proteins from these profiles that differentiate patients with greater survival benefit from those with lesser survival benefit from treatment with bevacizumab based chemotherapy, cetuximab based chemotherapy or bevacizumab plus cetuximab based chemotherapy.
- 10.4 Develop quantitative assays for each of these proteins
- 10.5 Validate the protein markers identified on a larger study population.

11.0 Research Approach and Methods:

- 11.1 **Generate protein expression profiles using a MALDI-TOF based platform and DIGE:** The purpose of this application is to discover and validate biomarkers that predict efficacy to 1st line chemotherapy for patients with colorectal adenocarcinoma. We will use approaches similar to the ones we have used previously to elucidate novel markers using our novel proteomics platform. To identify proteins potentially associated with outcome a representative sample of 40 patients will be selected for study within each treatment group (total n=120). Patients will be randomly-selected within treatment. Potential proteins will be identified based on their associations with OS controlling for the number of comparisons according to the methodology proposed by Jung [Jung S, Owzar K, George SL. “A multiple testing procedure to associate gene expression levels with survival.” *Statistics In Medicine*. In Press.]. Simulated power to detect OS associations with 40 patients will be provided.

Serum samples from each patient will be fractionated using a Rotofor and run through the MALDI 20 times, addressing some of the concerns associated with noise and reproducibility of the MALDI platform. Our methods are explicitly designed to try to recover meaningful features in this kind of small sample size. The same 40 samples will also be analyzed using the DIGE protocol.

Identification of potential proteins will be made using the data from both assays. The advantage of this design is the use of an internal standard created by pooling equal parts of each sample and aliquoting the pool among the total number of gels. In our design a total of 20 gels will be run for each arm. On each gel, three samples will be loaded and run simultaneously including a pooled internal standard. Equal amounts of protein from each sample will first be labeled with one of three CyDye minimal dyes. The internal standard will consistently be labeled with CyDye-2. The other two CyDyes will be CyDye-3 and CyDye-5. Prior to labeling, the sample may be rid of contaminating lipids, or nuclear material using a 2D clean-up kit (Amersham, Piscataway, NJ) in order to improve the spot resolution on each of the gels.

Once labeled with the respective CyDye, the samples are combined and actively rehydrated on a 14 cm, pH 4-7 IPG strip. This strip is then loaded on a 12% gel bound to non-fluorescent glass and run at 200V for 14 hours. Once completed, the gels will be imaged using a Typhoon™ 9410 image system. The gel attached to one of the glass plates will be stained with a coomassie stain which has proven compatible with our sequencing and identification methods.

- 11.2 Analyze and compare protein expression profiles to elucidate proteins that differentiate patients with greater survival benefit from those with lesser survival benefit.** A critical component to the success of our proposed research will be the analytical techniques that we developed and employ to extract the most relevant diagnostic information possible from the MALDI-TOF MS data collected from serum. Our preliminary results have demonstrated clearly that strong diagnostic signals are present in these data and are detectable using sound analytical techniques, developed in collaboration with Dr. Simon Lin in Bioinformatics. We will use similar analysis schemes for this study.

The DIGE results will be analyzed using the DeCyder DIA (Differential In-gel Analysis) software. Each gel produces 3 images visible at the wavelength specific for the respective CyDye. Initially, spots from each of the 60 images (20 gels, 3 images per gel) will be detected, verified manually to create a spot map, and normalized to the corresponding internal standard. Normalized protein abundance values for each spot will be expressed as a volume ratio and later organized into an .XML file for each of the 20 gels. The files will then be loaded into the DeCyder BVA (Biological Variation Analysis) software module to conduct gel-to-gel matching and statistical analysis of the normalized protein abundance ratios generated from spots identified with the initial DIA analysis across the 20 gels.

- 11.3 Identify differentially expressed proteins from these profiles that differentiate patients with greater and lesser survival benefit.** Our overall strategy for the identification of the proteins responsible for these ion peaks consists of following each ion peak by MALDI-TOF MS through partial purification until the majority of contaminating proteins have been removed. We then subject the partially purified fractions to 2-D gel electrophoresis, in-gel tryptic digestion, MALDI peptide fingerprinting, and/or MS/MS peptide sequencing. Identified proteins, or protein fragments, are then correlated with the original ion peak initially by molecular mass. Further correlation is achieved using immunological techniques. We will use this same strategy that was developed to identify the lung tumor-associated proteins CyP-A and MIF from MALDI-TOF ion peak data of lung tumor lysates and in identifying SAA from the serum.

For DIGE the differentially expressed spots will be selected and then identified from the coomassie stained gel. A spot picker will locate and acquire the spot, which then will be subjected to tryptic digestion and MALDI peptide fingerprinting, and/or MS/MS peptide sequencing. Identified proteins will be verified using immunological techniques such as ELISA or immunoblots as explained previously. If the spot cannot be identified due to insufficient abundance, then a traditional 2D gel loaded with more of the sample in which the target spot exists will be used for spot picking.

- 11.4 Develop quantitative assays for each of these proteins:** In order to validate the proteins as biomarkers, it is necessary to quantitate the levels of the individual proteins in a larger set of patient serum specimens. Therefore we believe the production of a rapid assay system will be essential for a clinically useful diagnostic tool. Once the serum level of each protein has been ascertained, the correlation of individual proteins, or of groups of proteins, with response to therapy can be determined.

Protein quantitation is most often accomplished using immunological techniques, which offer the most favorable mix of specificity, protocol flexibility, and robustness. The most commonly used immunological techniques are immunoblots and enzyme-linked immunosorbent assays (ELISAs). While excellent for determining if a particular antigen molecule is present in a given specimen or extract, immunoblots have limited dynamic range and are not easily amenable to large sample numbers. ELISAs, on the other hand, have excellent dynamic range, are easily adaptable to large sample numbers, and are very robust. This strategy was successfully applied to the ion peak protein at m/z 11,700 identified as SAA, and the ELIAS results for 25 patients are reported in a recent publication [134]. Antibodies for ELISA development will be either purchased (if commercially available) or custom generated by Biosource International (Camarillo, CA).

- 11.5 Validate the protein markers on a larger study population.** Once the optimal assays for the identified proteins are developed, the relationships between protein expression and OS will be investigated in a larger patient sample. Cox regression will be used to test associations between protein levels and OS. Known prognostic factors and combinations of markers will also be considered in these models.

12.0 References:

1. Tran, T.T., A. Medline, and W.R. Bruce, *Insulin Promotino of Colon Tumors in Rats*. Cancer, Epidemiology, Biomarkers adn Prevention, 1996. **5**: p. 1013-1015.
2. Schoen, R.E., et al., *Increased blood glucose and insulin, body size, and incident colorectal cancer*. J Natl Cancer Inst, 1999. **91**(13): p. 1147-54.
3. Rechler, M.M., *Growth inhibition by insulin-like growth factor (IGF) binding protein-3--what's IGF got to do with it?* Endocrinology, 1997. **138**(7): p. 2645-7.
4. Kaaks, R. and A. Lukanova, *Energy balance and cancer: the role of insulin and insulin-like growth factor-I*. Proc Nutr Soc, 2001. **60**(1): p. 91-106.
5. Aaronson, S.A., *Growth factors and cancer*. Science, 1991. **254**(5035): p. 1146-53.
6. Grimberg, A. and P. Cohen, *Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis*. J Cell Physiol, 2000. **183**(1): p. 1-9.
7. Rajah, R., B. Valentinis, and P. Cohen, *Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism*. J Biol Chem, 1997. **272**(18): p. 12181-8.
8. Jones, J.I. and D.R. Clemmons, *Insulin-like growth factors and their binding proteins: biological actions*. Endocr Rev, 1995. **16**(1): p. 3-34.
9. Pollak, M., et al., *Effect of tamoxifen on serum insulinlike growth factor I levels in stage I breast cancer patients*. J Natl Cancer Inst, 1990. **82**(21): p. 1693-7.
10. Huynh, H., X. Yang, and M. Pollak, *Estradiol and antiestrogens regulate a growth inhibitory insulin-like growth factor binding protein 3 autocrine loop in human breast cancer cells*. J Biol Chem, 1996. **271**(2): p. 1016-21.
11. Chan, J.M., et al., *Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study*. Science, 1998. **279**(5350): p. 563-6.
12. Wolk, A., et al., *Insulin-like growth factor I and prostate cancer risk: a population-based, case-control study*. J Natl Cancer Inst, 1998. **90**(12): p. 911-5.
13. Yu, H., et al., *Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis*. J Natl Cancer Inst, 1999. **91**(2): p. 151-6.
14. Ma, J., 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): p. 620-5.
15. Giovannucci, E., et al., *A prospective study of plasma insulin-like growth factor-I and binding protein-3 and risk of colorectal neoplasia in women*. Cancer Epidemiol Biomarkers Prev, 2000. **9**(4): p. 345-9.
16. Hankinson, S.E., et al., *Circulating concentrations of insulin-like growth factor-I and risk of breast cancer*. Lancet, 1998. **351**(9113): p. 1393-6.
17. Baserga, R., *The insulin-like growth factor I receptor: a key to tumor growth?* Cancer Res, 1995. **55**(2): p. 249-52.
18. Guo, Y.S., et al., *Characterization of insulinlike growth factor I receptors in human colon cancer*. Gastroenterology, 1992. **102**(4 Pt 1): p. 1101-8.

CALGB/SWOG C80405
Appendix III C

19. Koenuma, M., T. Yamori, and T. Tsuruo, *Insulin and insulin-like growth factor 1 stimulate proliferation of metastatic variants of colon carcinoma*. *Jpn J Cancer Res*, 1989. **80**(1): p. 51-8.
20. Watkins, L.F., L.R. Lewis, and A.E. Levine, *Characterization of the synergistic effect of insulin and transferrin and the regulation of their receptors on a human colon carcinoma cell line*. *Int J Cancer*, 1990. **45**(2): p. 372-5.
21. Drucker, D.J., *Epithelial cell growth and differentiation. I. Intestinal growth factors*. *Am J Physiol*, 1997. **273**(1 Pt 1): p. G3-6.
22. Remacle-Bonnet, M.M., et al., *Insulin-like growth factor-I protects colon cancer cells from death factor-induced apoptosis by potentiating tumor necrosis factor alpha-induced mitogen-activated protein kinase and nuclear factor kappaB signaling pathways*. *Cancer Res*, 2000. **60**(7): p. 2007-17.
23. Culouscou, J.M., et al., *Simultaneous production of IGF-I and EGF competing growth factors by HT-29 human colon cancer line*. *Int J Cancer*, 1987. **40**(5): p. 646-52.
24. Geier, A., et al., *Insulin-like growth factor-I inhibits cell death induced by anticancer drugs in the MCF-7 cells: involvement of growth factors in drug resistance*. *Cancer Invest*, 1995. **13**(5): p. 480-6.
25. Gooch, J.L., C.L. Van Den Berg, and D. Yee, *Insulin-like growth factor (IGF)-I rescues breast cancer cells from chemotherapy-induced cell death--proliferative and anti-apoptotic effects*. *Breast Cancer Res Treat*, 1999. **56**(1): p. 1-10.
26. Ellis, M.J., et al., *Insulin-like growth factors in human breast cancer*. *Breast Cancer Res Treat*, 1998. **52**(1-3): p. 175-84.
27. Vadgama, J.V., et al., *Plasma insulin-like growth factor-I and serum IGF-binding protein 3 can be associated with the progression of breast cancer, and predict the risk of recurrence and the probability of survival in African-American and Hispanic women*. *Oncology*, 1999. **57**(4): p. 330-40.
28. Wu, Y., et al., *Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis*. *Cancer Res*, 2002. **62**(4): p. 1030-5.
29. Abbasi, A.A., et al., *Low circulating levels of insulin-like growth factors and testosterone in chronically institutionalized elderly men*. *J Am Geriatr Soc*, 1993. **41**(9): p. 975-82.
30. Burroughs, K.D., et al., *Insulin-like growth factor-I: a key regulator of human cancer risk?* *J Natl Cancer Inst*, 1999. **91**(7): p. 579-81.
31. Kaaks, R., et al., *Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women*. *J Natl Cancer Inst*, 2000. **92**(19): p. 1592-600.
32. Fuchs, C., et al. *Insulin-like growth factor-I (IGF-1), IGF binding protein-3 (IGFBP-3), and outcome in metastatic colorectal cancer (CRC): Results from Intergroup Trial N9741*. in *Proc Meet Am Soc Clin Oncol*. 2004.
33. Poon, R.T., S.T. Fan, and J. Wong, *Clinical implications of circulating angiogenic factors in cancer patients*. *J Clin Oncol*, 2001. **19**(4): p. 1207-25.
34. Keyes, K.A., et al., *Circulating angiogenic growth factor levels in mice bearing human tumors using Luminex Multiplex technology*. *Cancer Chemotherapy & Pharmacology*, 2003. **51**(4): p. 321-7.

CALGB/SWOG C80405
Appendix III C

35. Hurwitz, H., et al., *Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer*. N Engl J Med, 2004. **350**(23): p. 2335-42.
36. Giantonio, B., et al. *High-dose bevacizumab in combination with FOLFOX4 improves survival in patients with previously treated advanced colorectal cancer: Results from the Eastern Cooperative Oncology Group (ECOG) study E3200*. in *Proc Am Soc Clin Oncol*. 2005.
37. Kuroi, K. and M. Toi, *Circulating angiogenesis regulators in cancer patients*. Int J Biol Markers, 2001. **16**(1): p. 5-26.
38. Koeppen, H.W., et al. *Molecular tumor characteristics and response to bevacizumab plus irinotecan/5-fluorouracil/leucovorin in metastatic colorectal cancer*. in *NCI-EORTC-AACR*. 2004.
39. Jubb, A.M., et al., *Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer*. submitted.
40. Carmeliet, P., et al., *Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions*. Nature Medicine, 2001. **7**(5): p. 575-83.
41. Ferrara, N., H.P. Gerber, and J. LeCouter, *The biology of VEGF and its receptors*. Nat Med, 2003. **9**(6): p. 669-76.
42. Kerbel, R.S., et al., *Possible mechanisms of acquired resistance to anti-angiogenic drugs: implications for the use of combination therapy approaches*. Cancer Metastasis Rev, 2001. **20**(1-2): p. 79-86.
43. Kim, E.S., et al., *Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma*. Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(17): p. 11399-404.
44. Gerber, H.P. and N. Ferrara, *The role of VEGF in normal and neoplastic hematopoiesis*. Journal of Molecular Medicine., 2003. **81**(1): p. 20-31.
45. Yoon, Y.S. and D.W. Losordo, *All in the family: VEGF-B joins the ranks of proangiogenic cytokines*. Circ Res, 2003. **93**(2): p. 87-90.
46. He, Y., T. Karpanen, and K. Alitalo, *Role of lymphangiogenic factors in tumor metastasis*. Biochim Biophys Acta, 2004. **1654**(1): p. 3-12.
47. He, Y., et al., *Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling*. J Natl Cancer Inst, 2002. **94**(11): p. 819-25.
48. Karpanen, T., et al., *Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth*. Cancer Res, 2001. **61**(5): p. 1786-90.
49. Lohela, M., et al., *Lymphangiogenic growth factors, receptors and therapies*. Thromb Haemost, 2003. **90**(2): p. 167-84.
50. Carmeliet, P., *Fibroblast growth factor-1 stimulates branching and survival of myocardial arteries: a goal for therapeutic angiogenesis?[comment]*. Circulation Research, 2000. **87**(3): p. 176-8.
51. Ferrara, N., et al., *Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer*. Nat Rev Drug Discov, 2004. **3**(5): p. 391-400.

52. McCarty, M.F., et al., *Promises and pitfalls of anti-angiogenic therapy in clinical trials*. Trends in Molecular Medicine, 2003. **9**(2): p. 53-8.
53. Bergers, G., et al., *Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors.[see comment]*. Journal of Clinical Investigation, 2003. **111**(9): p. 1287-95.
54. Fujiwaki, R., et al., *Vascular endothelial growth factor expression in progression of cervical cancer: correlation with thymidine phosphorylase expression, angiogenesis, tumor cell proliferation, and apoptosis*. Anticancer Res, 2000. **20**(2B): p. 1317-22.
55. Warren, R.S., et al., *Induction of vascular endothelial growth factor by insulin-like growth factor 1 in colorectal carcinoma*. J Biol Chem, 1996. **271**(46): p. 29483-8.
56. Battegay, E.J., *Angiogenesis: mechanistic insights, neovascular diseases, and therapeutic prospects*. Journal of Molecular Medicine, 1995. **73**(7): p. 333-46.
57. Ohm, J.E. and D.P. Carbone, *VEGF as a mediator of tumor-associated immunodeficiency*. Immunologic Research., 2001. **23**(2-3): p. 263-72.
58. Iruela-Arispe, M.L., F. Vazquez, and M.A. Ortega, *Antiangiogenic domains shared by thrombospondins and metallopondins, a new family of angiogenic inhibitors*. Annals of the New York Academy of Sciences, 1999. **886**: p. 58-66.
59. Rosenkilde, M.M. and T.W. Schwartz, *The chemokine system -- a major regulator of angiogenesis in health and disease*. Apmis, 2004. **112**(7-8): p. 481-95.
60. Xin, X., et al., *Hepatocyte growth factor enhances vascular endothelial growth factor-induced angiogenesis in vitro and in vivo*. American Journal of Pathology, 2001. **158**(3): p. 1111-20.
61. Wei, L.H., et al., *Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway*. Oncogene., 2003. **22**(10): p. 1517-27.
62. Maisonpierre, P.C., et al., *Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis.[see comment]*. Science., 1997. **277**(5322): p. 55-60.
63. Kawakami, M., et al., *Expression analysis of vascular endothelial growth factors and their relationships to lymph node metastasis in human colorectal cancer*. J Exp Clin Cancer Res, 2003. **22**(2): p. 229-37.
64. Furudoi, A., et al., *Clinical significance of vascular endothelial growth factor C expression and angiogenesis at the deepest invasive site of advanced colorectal carcinoma*. Oncology, 2002. **62**(2): p. 157-66.
65. Achen, M.G., et al., *Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4)*. Proc Natl Acad Sci U S A, 1998. **95**(2): p. 548-53.
66. Achen, M.G., et al., *Localization of vascular endothelial growth factor-D in malignant melanoma suggests a role in tumour angiogenesis*. J Pathol, 2001. **193**(2): p. 147-54.
67. George, M.L., et al., *Plasma basic fibroblast growth factor levels in colorectal cancer: a clinically useful assay?* Clinical & Experimental Metastasis, 2002. **19**(8): p. 735-8.
68. Matsumura, M., et al., *Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression correlated with tumor angiogenesis and macrophage infiltration in colorectal cancer*. Cancer Lett, 1998. **128**(1): p. 55-63.
69. Xiong, B., et al., *TGF beta1 expression and angiogenesis in colorectal cancer tissue*. World J Gastroenterol, 2002. **8**(3): p. 496-8.

CALGB/SWOG C80405
Appendix III C

70. Kaio, E., et al., *Clinical significance of thrombospondin-1 expression in relation to vascular endothelial growth factor and interleukin-10 expression at the deepest invasive tumor site of advanced colorectal carcinoma*. Int J Oncol, 2003. **23**(4): p. 901-11.
71. Fox, S.H., et al., *Angiogenesis in normal tissue adjacent to colon cancer*. J Surg Oncol, 1998. **69**(4): p. 230-4.
72. Kataoka, H., et al., *Activation of hepatocyte growth factor/scatter factor in colorectal carcinoma*. Cancer Res, 2000. **60**(21): p. 6148-59.
73. Wu, F.P., et al., *The effects of surgery, with or without rhGM-CSF, on the angiogenic profile of patients treated for colorectal carcinoma*. Cytokine, 2004. **25**(2): p. 68-72.
74. Yoshida, Y., et al., *Expression of angiostatic factors in colorectal cancer*. Int J Oncol, 1999. **15**(6): p. 1221-5.
75. Ochiumi, T., et al., *Clinical significance of angiopoietin-2 expression at the deepest invasive tumor site of advanced colorectal carcinoma*. Int J Oncol, 2004. **24**(3): p. 539-47.
76. Dirix, L.Y., et al., *Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients*. Br J Cancer, 1997. **76**(2): p. 238-43.
77. Fuhrmann-Benzakein, E., et al., *Elevated levels of angiogenic cytokines in the plasma of cancer patients*. Int J Cancer, 2000. **85**(1): p. 40-5.
78. Salven, P., A. Orpana, and H. Joensuu, *Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor*. Clin Cancer Res, 1999. **5**(3): p. 487-91.
79. Duff, S.E., et al., *Immunodetection and molecular forms of plasma vascular endothelial growth factor-C*. Int J Oncol, 2003. **22**(2): p. 339-43.
80. Alitalo, K. and P. Carmeliet, *Molecular mechanisms of lymphangiogenesis in health and disease*. Cancer Cell, 2002. **1**(3): p. 219-27.
81. Augustin, H.G., *Translating angiogenesis research into the clinic: the challenges ahead*. Br J Radiol, 2003. **76 Spec No 1**: p. S3-10.
82. Huang, A., et al., *Increased serum transforming growth factor-beta1 in human colorectal cancer correlates with reduced circulating dendritic cells and increased colonic Langerhans cell infiltration*. Clin Exp Immunol, 2003. **134**(2): p. 270-8.
83. Haraguchi, M., et al., *Elevated IL-8 levels in the drainage vein of resectable Dukes' C colorectal cancer indicate high risk for developing hepatic metastasis*. Oncol Rep, 2002. **9**(1): p. 159-65.
84. Salgado, R., et al., *Arterio-venous gradients of IL-6, plasma and serum VEGF and D-dimers in human cancer*. Br J Cancer, 2002. **87**(12): p. 1437-44.
85. Rafii, S., et al., *Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy?* Nature Reviews Cancer, 2002. **2**(11): p. 826-35.
86. Dvorak, H.F., *Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy*. Journal of Clinical Oncology, 2002. **20**(21): p. 4368-80.
87. Folkman, J., *Fundamental concepts of the angiogenic process*. Curr Mol Med, 2003. **3**(7): p. 643-51.

CALGB/SWOG C80405
Appendix III C

88. Shen, B.Q., et al., *Vascular endothelial growth factor KDR receptor signaling potentiates tumor necrosis factor-induced tissue factor expression in endothelial cells*. J Biol Chem, 2001. **276**(7): p. 5281-6.
89. Zanetta, L., et al., *Expression of Von Willebrand factor, an endothelial cell marker, is up-regulated by angiogenesis factors: a potential method for objective assessment of tumor angiogenesis*. Int J Cancer, 2000. **85**(2): p. 281-8.
90. Mandriota, S.J. and M.S. Pepper, *Vascular endothelial growth factor-induced in vitro angiogenesis and plasminogen activator expression are dependent on endogenous basic fibroblast growth factor*. J Cell Sci, 1997. **110 (Pt 18)**: p. 2293-302.
91. Behzadian, M.A., et al., *VEGF-induced paracellular permeability in cultured endothelial cells involves urokinase and its receptor*. Faseb J, 2003. **17**(6): p. 752-4.
92. Sund, M., L. Xie, and R. Kalluri, *The contribution of vascular basement membranes and extracellular matrix to the mechanics of tumor angiogenesis*. Apmis, 2004. **112**(7-8): p. 450-62.
93. Pike, S.E., et al., *Vasostatin, a calreticulin fragment, inhibits angiogenesis and suppresses tumor growth*. J Exp Med, 1998. **188**(12): p. 2349-56.
94. Ridker, P.M., et al., *Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism*. N Engl J Med, 2003. **348**(15): p. 1425-34.
95. Poynter, J.N., et al. *HMG CoA reductase inhibitors and the risk of colorectal cancer*. in Proc Am Soc Clin Oncol. 2004.
96. Ridker, P.M., *Inherited risk factors for venous thromboembolism: implications for clinical practice*. Clin Cornerstone, 2002. **4**(6): p. 18-30.
97. Falanga, A., *Mechanisms of hypercoagulation in malignancy and during chemotherapy*. Haemostasis, 1998. **28 Suppl 3**: p. 50-60.
98. Mousa, S.A., *Low-molecular-weight heparins in thrombosis and cancer: emerging links*. Cardiovasc Drug Rev, 2004. **22**(2): p. 121-34.
99. Xu, G., Y.L. Zhang, and W. Huang, *Relationship between plasma D-dimer levels and clinicopathologic parameters in resectable colorectal cancer patients*. World J Gastroenterol, 2004. **10**(6): p. 922-3.
100. Chang, P., *New anticoagulants for venous thromboembolic disease*. IDrugs, 2004. **7**(1): p. 50-7.
101. Bauer, K.A., *Selective inhibition of coagulation factors: advances in antithrombotic therapy*. Semin Thromb Hemost, 2002. **28 Suppl 2**: p. 15-24.
102. Hurwitz, H., et al. *Wound healing/bleeding in metastatic colorectal cancer patients who undergo surgery during treatment with bevacizumab*. in Proc Am Soc Clin Oncol. 2004.
103. Yarden, Y., *Biology of HER2 and its importance in breast cancer*. Oncology, 2001. **61 Suppl 2**: p. 1-13.
104. Yarden, Y. and M.X. Sliwkowski, *Untangling the ErbB signalling network*. Nat Rev Mol Cell Biol, 2001. **2**(2): p. 127-37.
105. Yarden, Y., J. Baselga, and D. Miles, *Molecular approach to breast cancer treatment*. Semin Oncol, 2004. **31**(5 Suppl 10): p. 6-13.

CALGB/SWOG C80405
Appendix III C

106. Arteaga, C., *Targeting HER1/EGFR: a molecular approach to cancer therapy*. Semin Oncol, 2003. **30**(3 Suppl 7): p. 3-14.
107. Cohen, S., *The stimulation of epidermal proliferation by a specific protein (EGF)*. Dev Biol, 1965. **12**(3): p. 394-407.
108. Derynck, R., et al., *Human transforming growth factor-alpha: precursor structure and expression in E. coli*. Cell, 1984. **38**(1): p. 287-97.
109. Shing, Y., et al., *Betacellulin: a mitogen from pancreatic beta cell tumors*. Science, 1993. **259**(5101): p. 1604-7.
110. Higashiyama, S., et al., *A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF*. Science, 1991. **251**(4996): p. 936-9.
111. Strachan, L., et al., *Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily*. J Biol Chem, 2001. **276**(21): p. 18265-71.
112. Klapper, L.N., et al., *The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors*. Proc Natl Acad Sci U S A, 1999. **96**(9): p. 4995-5000.
113. Alroy, I. and Y. Yarden, *The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions*. FEBS Lett, 1997. **410**(1): p. 83-6.
114. Burden, S. and Y. Yarden, *Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis*. Neuron, 1997. **18**(6): p. 847-55.
115. Holmes, W.E., et al., *Identification of heregulin, a specific activator of p185erbB2*. Science, 1992. **256**(5060): p. 1205-10.
116. Sahin, U., et al., *Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands*. J Cell Biol, 2004. **164**(5): p. 769-79.
117. Lemmon, M.A. and J. Schlessinger, *Regulation of signal transduction and signal diversity by receptor oligomerization*. Trends Biochem Sci, 1994. **19**(11): p. 459-63.
118. Carraway, K.L., 3rd and L.C. Cantley, *A new acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signaling*. Cell, 1994. **78**(1): p. 5-8.
119. Lewis, T.S., P.S. Shapiro, and N.G. Ahn, *Signal transduction through MAP kinase cascades*. Adv Cancer Res, 1998. **74**: p. 49-139.
120. Vivanco, I. and C.L. Sawyers, *The phosphatidylinositol 3-Kinase AKT pathway in human cancer*. Nat Rev Cancer, 2002. **2**(7): p. 489-501.
121. Dannenberg, A.J., et al., *Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention*. J Clin Oncol, 2005. **23**(2): p. 254-66.
122. Zhao, D., et al., *Metalloproteinase-dependent transforming growth factor-alpha release mediates neurotensin-stimulated MAP kinase activation in human colonic epithelial cells*. J Biol Chem, 2004. **279**(42): p. 43547-54.
123. P, O.c., et al., *Epidermal growth factor-like ligands differentially up-regulate matrix metalloproteinase 9 in head and neck squamous carcinoma cells*. Cancer Res, 2000. **60**(4): p. 1121-8.
124. Ongusaha, P.P., et al., *HB-EGF is a potent inducer of tumor growth and angiogenesis*. Cancer Res, 2004. **64**(15): p. 5283-90.

CALGB/SWOG C80405
Appendix III C

125. Mansour, O.A., et al., *Tissue and serum c-erbB-2 and tissue EGFR in breast carcinoma: three years follow-up*. *Anticancer Res*, 1997. **17**(4B): p. 3101-6.
126. Cunningham, D., et al., *Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer*. *N Engl J Med*, 2004. **351**(4): p. 337-45.
127. Chung, K.Y., et al., *Cetuximab Shows Activity in Colorectal Cancer Patients With Tumors That Do Not Express the Epidermal Growth Factor Receptor by Immunohistochemistry*. *J Clin Oncol*, 2005.
128. Payne, R.C., et al., *Automated assay for HER-2/neu in serum*. *Clin Chem*, 2000. **46**(2): p. 175-82.
129. Partanen, R., et al., *The detection of increased amounts of the extracellular domain of the epidermal growth factor receptor in serum during carcinogenesis in asbestosis patients*. *J Occup Med*, 1994. **36**(12): p. 1324-8.
130. Peterson, B. and S.L. George, *Sample size requirements and length of study for testing interaction in a 2 x k factorial design when time-to-failure is the outcome [corrected]*. *Control Clin Trials*, 1993. **14**(6): p. 511-22.
131. Campa, M.J., et al., *Protein expression profiling identifies macrophage migration inhibitory factor and cyclophilin a as potential molecular targets in non-small cell lung cancer*. *Cancer Res*, 2003. **63**(7): p. 1652-6.
132. Schwartz, S.A., M.L. Reyzer, and R.M. Caprioli, *Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation*. *J Mass Spectrom*, 2003. **38**(7): p. 699-708.
133. Xu, B.J., et al., *Direct analysis of laser capture microdissected cells by MALDI mass spectrometry*. *J Am Soc Mass Spectrom*, 2002. **13**(11): p. 1292-7.
134. Khan, N., et al., *Clinical utility of serum amyloid A and macrophage migration inhibitory factor as serum biomarkers for the detection of nonsmall cell lung carcinoma*. *Cancer*, 2004. **101**(2): p. 379-84.
135. Knowles, M.R., et al., *Multiplex proteomic analysis by two-dimensional differential in-gel electrophoresis*. *Proteomics*, 2003. **3**(7): p. 1162-71.
136. Friedman, D.B., et al., *Proteome analysis of human colon cancer by two-dimensional difference gel electrophoresis and mass spectrometry*. *Proteomics*, 2004. **4**(3): p. 793-811.

APPENDIX IV

PHARMACOGENETIC PREDICTORS OF TOXICITY AND OUTCOME IN PATIENTS RECEIVING CHEMOTHERAPY FOR METASTATIC COLORECTAL CANCER ON 80405

1.0 Background

The recent advances in the human genome project have led to great opportunities to better understand the molecular basis for variability in drug toxicity and response. Building on the considerable knowledge of the biochemical pathways regulating drug activity, a candidate gene approach will be used in this study to identify predictors of toxicity and outcome after treatment with 5-fluorouracil (5FU), oxaliplatin, irinotecan, cetuximab, and/or bevacizumab (Goldberg and Gill, 2004; Iqbal and Lenz, 2004; Marsh and McLeod, 2004).

CALGB 80405 offers an excellent opportunity to evaluate the role of genetic variants in several relevant genes responsible for activation, degradation, transport, or mechanism of action of 5FU, oxaliplatin, irinotecan, cetuximab, or bevacizumab. In particular, the randomization schema allow for the assessment of genetic variants which are predictive for toxicity or efficacy from bevacizumab (Arms A & C versus Arm B) or cetuximab (Arms B & C versus Arm A). Of these, the most compelling data for an influence on genetic polymorphism on gene expression and drug effect is available for cetuximab.

The epidermal growth factor receptor (EGFR) is a regulator of tumor biology and an important new therapeutic target. A polymorphic CA dinucleotide repeat in intron 1 of the EGFR has been described in colorectal cancer patients (McKay et al, 2002; Liu et al, 2003; Zhang et al, 2005). An inverse relationship between CA repeat length and EGFR protein expression has been described in breast tumors (Buerger et al, 2000). To date, alleles of 14 to 22 CA repeats in length have been described in Caucasian subjects (Chi et al, 1992; Liu et al, 2003). The most common CA repeat in the European American population has 16 repeats (allele frequency 43%) (McKay et al, 2002; Liu et al, 2003). Recently, the haplotype of the EGFR promoter region has been elucidated, providing clear evidence of a putative role for genetic polymorphism in the regulation of EGFR transcription (Liu et al, 2005). Specifically, variants at -216 (G/T) and -191 (C/A) were associated with gene transcription, with the -216 variant having the greatest impact. The -216 T allele produced significantly higher in vitro expression, compared to the -216 G allele. This allele has a frequency of 34% in the European American population and is not in strong linkage with the intron 1 repeat (Liu et al, 2005). Of the EGFR haplotypes identified in the predominant US population groups, haplotype 2 would be anticipated to result in superior activity of cetuximab (due to the presence of both -216T and CA16). The frequency of this haplotype is 17%, which means that 30% of the US population would be expected to have one or two copies of this haplotype (Liu et al, 2005). These data provide the basis for the following primary hypothesis:

2.0 Objectives

- 2.1 EGFR haplotype 2 (~30% of patients) is a favorable predictive marker for progression-free survival (PFS) in patients receiving cetuximab (Arms B & C), but not bevacizumab (Arm A).

Secondary

- 2.2 We will also explore the relationship of polymorphisms in other genes that are hypothesized to affect the pharmacokinetics or pharmacodynamics of 5-FU, irinotecan, oxaliplatin, cetuximab, or bevacizumab. These will be exploratory analyses, which will generate hypotheses potentially testable on other sample sets. In addition, whole genome typing will be considered if financially feasible, in order to generate hypotheses about genes not currently suspected to relate to the pharmacokinetics or pharmacodynamics of the aforementioned drugs.

Supporting data for secondary objectives: We briefly describe some of the results to date in regard to the pharmacogenetics of the drugs being utilized in this protocol. The cellular target for 5-FU chemotherapy is thymidylate synthase (TS), which is encoded by the *TYMS* gene. TS expression appears to be regulated by a highly polymorphic tandem repeat in the TS promoter enhancer region (TSER) (Marsh et al, 1999; Kwakami et al, 1999). This polymorphism is of significance as greater *in vitro* transcription occurs with the triple repeat than observed for the double repeat (Marsh et al, 1999). There are some data to suggest that patients with *TSER**3/*3 do not derive significant clinical benefit from 5-FU (Pullarkat et al, 2001). Additional genetic variants in TS have been identified, including a 6 bp deletion in the 3' UTR (Ulrich et al, 2000). The association of these additional TS variants with 5-fluorouracil efficacy and toxicity will also be evaluated.

5FU activity is influenced by the cellular folate pools, which in turn are influenced by the enzyme methylenetetrahydrofolate reductase (MTHFR). A C to T missense mutation at nucleotide 677 of the MTHFR gene has been associated with altered tissue folate levels and toxicity from 5-fluorouracil-containing chemotherapy (Toffoli et al, 2000).

Irinotecan is a prodrug and must form SN-38 before it can inhibit topoisomerase I and have antitumor activity. Irinotecan and its active metabolite SN-38 are substrates for several cellular transporter proteins, including ABCB1 (MDRI) (Iyer et al, 2002a), ABCC2, and ABCG2. Single nucleotide polymorphisms in these genes have been identified. SN-38 undergoes glucuronide conjugation via UGT1A1. UGT1A1 has a variable number tandem repeat in its promoter, which has been associated with neutropenia (Iyer et al, 2002b; Innocenti et al, 2004).

The pharmacology of oxaliplatin is less well defined. However, all platinum agents appear to be influenced by intracellular levels of the glutathione S-transferase (GST) family (Nishimura et al, 1998). This multigene family is a key component of detoxifying pathways and is responsible for conjugation of reactive radicals. A G to A single nucleotide polymorphism in *GST P1* codon 105 (AA frequency 51%) has been associated with survival in patients receiving 5-FU and oxaliplatin (Stoehlmacher et al, 2002). In addition, genetic variation in XRCC1 (Stoehlmacher et al, 2001), and ERCC2 (Park et al, 2001) has been associated with response to oxaliplatin.

Given that bevacizumab directly neutralizes VEGF, it is plausible that *VEGF* genetic variants will influence the toxicity and efficacy of this agent. One such common variant (936C>T) in the 3'UTR of the *VEGF* gene has been associated with VEGF plasma levels, such that individuals with the C/C genotype had significantly higher VEGF levels than individuals with C/T or T/T genotypes (Krippel, et al, 2003). The majority of the European American population have a C/C genotype (71%).

Additional genetic variants have been identified in candidate genes which have a putative role in regulating the activity and/or toxicity of 5FU, irinotecan, oxaliplatin, cetuximab or bevacizumab. Additional genetic variants of interest to this clinical study will also be identified

in the future. Association of these candidate genes with toxicity and outcome will be conducted. In addition, analyses will be carried out in those patients with toxicities that cannot be explained by the polymorphisms described above. Those patients will be screened to identify other variants in candidate genes that might be responsible for the observed toxicity phenotype

3.0 Methods

Blood Samples (5 mL in EDTA tube) from patients will be processed for the isolation of DNA, using commercial DNA isolation kits, such as Puregene (Gentra Systems, Inc., Minneapolis, MN). Samples will be collected in EDTA (purple top Vacutainer, Becton Dickson) and stored at 2-8°C for ≤ 5 days to obtain optimum DNA isolation results. RBCs will be separated, and DNA isolated from WBCs, using an RNase to remove contaminating RNA. Genomic DNA will be isolated by precipitation with alcohol and dissolved in a tris-EDTA buffer (pH = 8.0). Initial DNA extraction will be performed by the Pathology Core Laboratory. DNA will be stored there and aliquoted as appropriate to the laboratories involved in the genotyping, as determined by the study team.

Genotype analysis will be performed on the above genetic variants, using pyrosequencing or related genome analysis tools.

4.0 Statistical considerations

4.1 Primary Hypothesis

The primary endpoint for this pharmacogenetic companion study is the interaction between cetuximab and the EGFR haplotype 2 in modeling progression-free survival PFS. The analysis will be carried out in the context of log-linear Cox model whose hazard function is canonically presented as $l_{ijk} = l[t; z] = l_0[t] \exp[Y[z]]$ where

$$Y_{ijk} = Y[Z] = b_1 Z_1 + b_2 Z_2 + b_3 Z_3 + b_{12} Z_1 Z_2 + b_{23} Z_2 Z_3 \quad (1)$$

for $i, j, k \in \{0, 1\}$, $Z = (Z_1, Z_2, Z_3)^T$, $Z_1 = 1$ if patient receives cetuximab (0 otherwise), $Z_2 = 1$ if patient receives bevacizumab (0 otherwise) and $Z_3 = 1$ if patient has the EGFR haplotype 2 (0 otherwise).

It should be noted that

- it is assumed that there is no interaction between bevacizumab and the EGFR haplotype in modeling PFS;
- it is assumed that there is no interaction between bevacizumab and cetuximab in modeling PFS;
- the model does not take into account the effect given of the 5-FU based chemotherapy, including potential interactions with the biologic agents present in all three arms of the study.

The hypotheses of interest can be canonically presented as testing $H_0: b_{23} = 0$ versus the two-sided hypothesis $H_0: b_{23} \neq 0$.

4.2 Sample size and haplotype prevalence rates

The total sample size expected for the clinical design of this study is $N=2289$. It is expected that 80 % of these patients will be available for this PKG companion. As such, it is expected to have about $n=1830$ ($n_1=n_2=n_3=610$) per arm. As the putative relative frequency for being

heterozygous or homozygous for EGFR haplotype 2 is 0.3, it is expected that within each arm the frequency of the dominant EGFR haplotype is 183 (versus 427).

4.3 Putative cell probabilities

We will assume that the time-to-event distribution within each of the six cells is exponential. Furthermore, we will assume that the putative median PFS for the bevacizumab arm is 12 months (corresponding to a hazard rate of $\lambda_1=0.0578$). The hazard rates of each of the six cells are listed in Table 18.

bevacizumab	cetuximab	EGFR	λ_{ijk}
1	0	0	λ_1
1	0	1	λ_1
0	1	0	$\lambda_1 D_1^{-1}$
0	1	1	$\lambda_1 D_2^{-1}$
1	1	0	$\lambda_1 D_1^{-1} D^{-1}$
1	1	1	$\lambda_1 D_2^{-1} D^{-1}$

Table 18: Listing of the putative cell hazard rates.

It is noted that in construction of table

- as no bevacizumab/EGFR/PFS interaction is assumed we have set $\lambda_{100}=\lambda_{101}=\lambda_1$
- as cetuximab, regardless of EGFR haplotype, is hypothesized to offer improvement of bevacizumab, then $D_1>1$ and $D_2>1$.
- D_1 and D_2 quantify the improvements (over getting 5FU alone) due to cetuximab. As a cetuximab/EGFR/PFS interaction is hypothesized then $D_1<D_2$
- D quantifies the improvement attributed to bevacizumab for patients already getting cetuximab. It is noted that D is assumed not to depend on the EGFR haplotype.

4.4 Power Calculations

We will assume a 2-year PFS probability of $\alpha = 0.47$ for the

marker (heterozygous or homozygous for EGFR haplotype 2) on the cetuximab arm. The protocol hypothesizes a median PFS of 16.5 years for the cetuximab arm. This corresponds to a 2-year PFS probability of 0.32 for patients without EGFR haplotype 2 EGFR haplotype on the cetuximab arm. According to the protocol $\alpha = 1$. The cell $\alpha = 0.05$ level these assumptions and hypotheses, are listed in Tables IV-2-6. At the two-sided of significance, the empirical power, based on 10000 simulations, for testing $H_0: b_{23} = 0$ against the local alternative $H_0: b_{23} = \log[1.22/1.84]$ is 0.93. If we assume 2-year PFS probability of 0.44, 0.45 and 0.46 for the dominant EGFR on the cetuximab arm, the empirical power is given to be 0.70, 0.80 and 0.88 respectively. The power calculations are summarized in the following tables:

α	D_1	D_2	α	b_{23}	Power
0.44	1.26	1.69	1.00	-0.293	0.70
0.45	1.24	1.74	1.00	-0.333	0.80
0.46	1.23	1.79	1.00	-0.372	0.88
0.47	1.22	1.84	1.00	-0.412	0.93

Table 19: Summary of power calculations

bevacizumab	cetuximab	EGFR	l_{ijk}	Median $_{ijk}$
1	0	0	0.058	12.00
1	0	1	0.058	12.00
0	1	0	0.046	15.11
0	1	1	0.034	20.26
1	1	0	0.046	15.11
1	1	1	0.034	20.26

Table 20: Assuming a baseline median PFS of 12 for the bev arm, a 24 -year PFS probability of 0.44 and $D_1 = 1.26, D_2 = 1.69$

bevacizumab	cetuximab	EGFR	l_{ijk}	Median $_{ijk}$
1	0	0	0.058	12.00
1	0	1	0.058	12.00
0	1	0	0.046	14.94
0	1	1	0.033	20.83
1	1	0	0.046	14.94
1	1	1	0.033	20.83

Table 21: Assuming a baseline median PFS of 12 for the bev arm, a 24 -year PFS probability of 0.45 and $D_1 = 1.245, D_2 = 1.74$

CALGB/SWOG C80405
Appendix IV

bevacizumab	cetuximab	EGFR	l_{ijk}	Median _{ijk}
1	0	0	0.058	12.00
1	0	1	0.058	12.00
0	1	0	0.047	14.76
0	1	1	0.032	21.42
1	1	0	0.047	14.76
1	1	1	0.032	21.42

Table 22: Assuming a baseline median PFS of 12 for the bev arm, a 24 -year PFS probability of 0.46 and $D_1=1.23$, $D_2= 1.79$

bevacizumab	cetuximab	EGFR	l_{ijk}	Median _{ijk}
1	0	0	0.058	12.00
1	0	1	0.058	12.00
0	1	0	0.048	14.59
0	1	1	0.031	22.03
1	1	0	0.048	14.59
1	1	1	0.031	22.03

Table 23: Assuming a baseline median PFS of 12 for the bev arm, a 24 -year PFS probability of 0.47 and $D_1=1.216$, $D_2= 1.84$

4.5 Secondary Objectives

The above analysis will also be applied to other implicated haplotypes and polymorphisms. Other clinical endpoints such as toxicity and survival will be considered as well. In the case of binary type endpoints, a logistic model with Z as its mean response will be employed.

5.0 References

- Buerger H, Gebhardt F, Schmidt H, Beckmann A, Hutmacher K, Simon R, Lelle R, Boecker W, Brandt B. Length and loss of heterozygosity of an intron 1 polymorphic sequence of egfr is related to cytogenetic alterations and epithelial growth factor receptor expression. *Cancer Res.* 2000;60:854-7
- Goldberg RM, Gill S. Recent phase III trials of fluorouracil, irinotecan, and oxaliplatin as chemotherapy for metastatic colorectal cancer. *Cancer Chemother Pharmacol.* 2004;54 Suppl 1:S57-64.
- Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramirez J, Rudin CM, Vokes EE, Ratain MJ. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol.* 2004;22:1382-8.
- Iqbal S, Lenz HJ. Angiogenesis inhibitors in the treatment of colorectal cancer. *Semin Oncol.* 2004;31(6 Supp 17):10-6.
- Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, Di Rienzo A, Ratain MJ. Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther.* 1999;65:576-82.
- Iyer L, Ramirez J, Shepard DR, Bingham CM, Hossfeld DK, Ratain MJ, Mayer U. Biliary transport of irinotecan and metabolites in normal and P-glycoprotein-deficient mice. *Cancer Chemother Pharmacol.* 2002a;49:336-41.
- Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL, Ratain MJ. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J.* 2002b;2:43-7.
- Johnston PG, Lenz H-J, Leichman CG, et al: Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Res* 55:1407-1412, 1995.
- Krippel P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Haas J, Samonigg H. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. *Int J Cancer.* 2003;106:468-71.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet.* 2001;27:383-91.
- Kwakami K, Omura K, Kanehira E, Watanabe Y: Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. *Anticancer Res* 19:3249-3252, 1999.
- Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Garcia Y, Li J, Leichman L. Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. *J Clin Oncol.* 1996;14:176-82.
- Liu W, Innocenti F, Chen P, Das S, Cook EH Jr, Ratain MJ. Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. *Clin Cancer Res.* 2003;9:1009-12.

CALGB/SWOG C80405
Appendix IV

- Liu W, Innocenti F, Wu MH, Desai AA, Dolan ME, Cook EH Jr, Ratain MJ. A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. *Cancer Res.* 2005;65:46-53.
- Marsh S, Collie-Duguid ESR, Li T, et al: Ethnic variation in the thymidylate synthase enhancer region polymorphism among Caucasian and Asian populations. *Genomics* 58:310-312, 1999.
- Marsh S, McKay JA, Cassidy J, McLeod HL. Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. *Int J Oncol.* 2001;19:383-6.
- Marsh S, McLeod HL. Pharmacogenetics of irinotecan toxicity. *Pharmacogenomics.* 2004;5:835-43.
- McKay JA, Murray LJ, Curran S, Ross VG, Clark C, Murray GI, Cassidy J, McLeod HL. Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumours and lymph node metastases. *Eur J Cancer.* 2002;38:2258-64.
- McLeod HL, CollieDuguid ESR, Vreken P, et al: Nomenclature for human DPYD alleles. *Pharmacogenetics* 8:455-459, 1998.
- Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol.* 1998;16:309-16.
- Nishimura T, Newkirk J, Sessions RB, et al: Association between expression of glutathione-associated enzymes and response to platinum-based chemotherapy in head and neck cancer. *Chem Biol Interactions* 112:187-198, 1998.
- Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ. A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. *Cancer Res.* 2001;61:8654-8.
- Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, Warren R, Tsao-Wei D, Groshen S, Lenz HJ. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J.* 2001;1:65-70.
- Stoehlmacher J, Ghaderi V, Iobal S, Groshen S, Tsao-Wei D, Park D, Lenz HJ. A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. *Anticancer Res.* 2001;21:3075-9.
- Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, Yu MC, Lenz HJ. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst.* 2002;94:936-42.
- Toffoli G, Veronesi A, Boiocchi M, Crivellari D. MTHFR gene polymorphism and severe toxicity during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluorouracil (CMF) *Ann Oncol.* 2000;11:373-4.
- Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. *Cancer Epidemiol Biomarkers Prev.* 2000;9:1381-5.
- Zhang W, Park DJ, Lu B, Yang DY, Gordon M, Groshen S, Yun J, Press OA, Vallbohmer D, Rhodes K, Lenz HJ. Epidermal Growth Factor Receptor Gene Polymorphisms Predict Pelvic Recurrence in Patients with Rectal Cancer Treated with Chemoradiation. *Clin Cancer Res.* 2005;11:600-605.

APPENDIX V

PREDICTIVE AND PROGNOSTIC VALUE OF K-RAS MUTATION STATUS, EGFR LIGAND GENE EXPRESSION LEVELS AND PTEN STATUS IN ADVANCED COLORECTAL CANCER

1.0 Background

1.1 Ras, Amphiregulin and Epiregulin as biomarkers in colorectal cancer (CRC)

The Ras family of proteins plays a critical role as molecular switches in pathways that control proliferation, cell adhesion, apoptosis, and cell migration. The Ras pathway mediates cellular response to growth signals via transmission of growth-promoting signals from cell surface receptors toward the nucleus where the signals affect the production and regulation of other key proteins.¹ When Ras genes become mutated, they become more active in signaling. The most common mutations are point mutations in codons 12, 13 and 61.² Human tumor studies identified activating mutations in the K-Ras gene in 20-50% of CRC.³⁻⁵ The majority (85%) of these mutations were restricted to codons 12 and 13.⁵

K-Ras mutations have been evaluated as prognostic markers in many solid tumors, including CRC. In addition, the prognostic significance of codon 12 mutations may differ in CRC.⁶ The codon 12 valine-for-glycine mutant (G12V) has been reported to be associated with higher stage and decreased survival compared with the codon 12 aspartate-for-glycine mutant (G12D). GTPase activity of G12V is one-quarter that of G12D and one-tenth of wild type Ras. In addition, the affinity of the GTP analogue GppNp to bind to G12D appears to be 8-fold weaker than its binding to G12V or wild type Ras, which may result in a stronger oncogenic signal.

Several investigators have reported that K-Ras mutation status can predict disease response in patients with metastatic CRC (mCRC) who receive anti-EGFR antibodies.⁷⁻¹⁰ The purpose of this sub-study is to provide these data for patients with metastatic CRC who are treated on this protocol.

1.2 Cetuximab

Lievre and colleagues assessed K-Ras mutation status in 30 patients with mCRC that received cetuximab.⁷ All patients had a primary tumor that expressed EGFR. Eleven of the 30 patients (37%) responded to cetuximab. The median duration of response was 33.9 months. Six patients had stable disease with 18.3-months median duration of stabilization. Tumors from 13 patients (43%) were found to have K-Ras mutations. In 11 patients who experienced a clinical response to cetuximab, no K-Ras mutation was found. The presence of a K-Ras mutation was significantly associated with a lack of response to treatment.

A prospective study to identify candidate predictive markers in patients with mCRC was conducted at sites in the U.S., Canada and Spain.⁸ In this trial, 110 patients were treated with cetuximab. Tumor samples from pretreatment biopsies and blood were analyzed to identify markers that correlate with clinical response. Gene expression profiles generated from 80 patients demonstrated that patients with tumors that express high levels of the EGFR ligands epiregulin and amphiregulin were more likely to have disease control with cetuximab (EREG, $P = 0.000015$; AREG, $P = 0.000025$). Additionally, patients whose tumors did not have K-Ras mutations had a significantly higher disease control rate than patients with K-Ras mutations ($P = 0.0003$). Furthermore, patients with tumors that had high expression of EREG or AREG also had significantly longer progression-free survival (PFS) than patients with low expression

(EREG: P = 0.0002, hazard ratio [HR] = 0.47, and median PFS, 103.5 v 57 days, respectively; AREG: P = 0.0001, HR = 0.44, and median PFS, 115.5 v 57 days, respectively).

Data from this study was derived from fresh biopsy material collected from the metastatic site. Recent development of sensitive and quantitative assays of gene expression using PCR technology and microarray technology also permit gene expression analysis on formalin fixed paraffin embedded tissue (FFPET). A recent study conducted by Bristol-Myers Squibb in collaboration with Genomic Health, Inc. evaluated the expression of 100 candidate genes and their association with response in over 250 patients treated with cetuximab monotherapy (unpublished data). This analysis revealed that the expression of the EGFR ligands epiregulin and amphiregulin, in addition to DUSP-6 and PHLDA-1 were highly associated with increased TTP and RR (P ≤ 0.00001). In addition, an abstract recently presented by Tejpar, et al (2007 AACR-NCI-EORTC meeting) reported the results of an analysis of gene expression and K-Ras in FFPET primary tumor from 95 patients treated with cetuximab in combination with irinotecan. In this analysis, estimated median OS was 16.7 wks (95% CI [12.2-21.2]) in K-Ras Mut vs. 45.9 wks (95% CI [38.4-53.3]; Log-rank; p = 0.0001) in WT pts. Estimated median OS was 43.0 wks (95% CI [37.2-48.8]) in high amphiregulin vs. 22.9 wks (95% CI [17.0-28.7]; p = 0.008) in low amphiregulin patients. Estimated median OS of K-Ras WT pts with high amphiregulin expression was 49.4 wks (95% CI [27.9-70.9]) vs. 30.6 wks (95% CI [13.9-47.3]; p = 0.01) in low expressors. In K-Ras Mut patients OS was similar in high and low amphiregulin expressors (15.86 wks (95% CI [13.7-18.1]) vs. 15.86 wks (95% CI [11.2-20.5]; p = 0.405). Data for epiregulin were similar.

The identification of these response predictors in both fresh frozen biopsy and FFPET suggests amphiregulin, epiregulin and K-Ras mutation status are essential mediators of tumor biology and may be valuable biomarkers in determining outcome for patients with mCRC treated with cetuximab and chemotherapy.

Cell proliferation in response to activation of EGFR is a result of signaling via the Ras/Raf/MEK/ERK or PI3K/PTEN/AKT pathways. Phosphatase and Tensin homolog (PTEN) gene is a tumor suppressor that encodes for a protein that regulates the cell cycle. PTEN protein is a phosphatase that inhibits the phospho-AKT pathway. PTEN causes cells to undergo programmed cell death. Loss of PTEN is common in cancers and results in uncontrolled cell proliferation.

Frattini and colleagues reported that loss of PTEN expression predicts a poor response to cetuximab.⁹ Eleven out of 27 (42%) tumors were negative for PTEN expression by immunohistochemistry (IHC) and none experienced a benefit from treatment with cetuximab (p < 0.001).

- 1.3 Panitumumab (Pmab)** is a fully humanized antibody against the EGFR that achieves response rates around 10% as a single agent in mCRC. Results of a Phase III study with panitumumab were recently reported at the 14th European Cancer Conference. In this study, activating K-Ras mutations were detected using real-time PCR on DNA from fixed tumor sections. Treatment with panitumumab significantly increased progression free survival compared to best supportive care in EGFR expressing mCRC that was refractory to oxaliplatin and irinotecan.¹⁰

K-Ras status was determined in 427 patients; 43% of pts had tumors bearing mutant K-Ras. Median time to PFS in Pmab pts was 12.3 wks and 7.4 wks in the WT and mutant K-Ras groups, respectively. Median time to PFS in pts who had best supportive care (BSC) alone was 7.3 wks in both K-Ras groups. For Pmab pts, 17% responded and 34% had stable disease in the WT K-Ras group compared with a 0% response rate and 12% with stable disease in the mutant K-Ras group. Of 168 BSC pts who crossed over to receive Pmab (77% of all BSC pts; median time of 7 wks), 20/91 (22%) in the WT K-Ras subset and 0/77 (0%) in the mutant K-Ras subset

responded. When treatment arms were combined, WT K-Ras status identified a group with longer OS compared with mutant K-Ras (HR = 0.67, 95% CI, 0.55–0.82).

Freeman, et al. combined samples from four monotherapy studies with panitumumab.¹¹ Twenty-one tumors out of 59 (36%) were found to have a K-Ras mutation. No responders had a tumor that harbored a K-Ras mutation. The Hazard ratio was 1.7 from a Cox PH model for K-Ras mutation as a predictor of PFS.

1.4 Detection of free-circulating tumor-associated DNA in serum and plasma samples

Neoplastic cells proliferate at abnormal rates, and also undergo apoptosis at a high frequency. As a result, small fragmented DNA is released from tumor cells into the circulation. Numerous studies have shown that this circulating DNA harbors tumor-associated mutations that can be used to define tumor characteristics. The detection of genetic alterations in the serum of patients with colorectal cancer could be a useful non-invasive marker for monitoring response to therapy. Several investigators have reported the ability to find genetic mutations, including K-ras, in the plasma and serum of patients with colorectal cancer.¹²⁻¹⁹

Kopreski, et al used polymerase chain reaction (PCR) to determine the presence of mutant K-ras DNA in the serum or plasma of 31 patients with metastatic or unresectable colorectal cancer.¹⁴ DNA was also isolated from 28 normal volunteers. The serum or plasma from 12 of 31 patients (39%) had mutant K-ras DNA detected by PCR and was confirmed by sequencing while none of the DNA from volunteers had K-ras mutations detected. The presence of K-ras mutations in serum or plasma was not dependent on gender, location of primary tumor or metastatic disease, or temporal relation to treatment with surgery or chemotherapy.

Other investigators evaluated the prognostic value of circulating K-ras mutations in patients receiving initial treatment for colorectal cancer.¹⁸ Serum from 86 patients was fractionated and mutations in codon 12 of the K-ras gene were examined in the serum and primary tumors. K-ras mutations were found in the tumor tissue of 28 patients (33%) and of these, 10 patients (36%) had circulating K-ras mutations in serum. A multivariate analysis of K-ras mutation status and Dukes' stage revealed that patients with Dukes' stage D and circulating K-ras mutations had worse survival. All 7 patients with Stage D disease and circulating K-ras mutations died of their disease within 24 months of initial treatment.

More recently, Ryan and colleagues prospectively evaluated the prognostic value of mutations in circulating K-ras2 in the serum of patients with colorectal cancers who were undergoing surgery.¹⁹ They analyzed mutations in codon 12 and 13 of K-ras. Serum was obtained from 78 patients before surgery (Group A) and from 94 patients before surgery and every three months for three years during follow-up (Group B). K-ras2 mutations were detected in 53% of the tumors and 41% of preoperative sera of patients in Group A. The identical mutation was found 31 of 41 (76%) of patients with K-ras2 mutations in both sera and tumor samples. In Group B, 64% of patients (60 of 94) had K-ras2 mutations in their primary tumor and 16 (27%) of these patients had serum that was persistently positive for K-ras2 serum mutations obtained after surgery. None of the patients who were negative for serum K-ras2 mutations became positive despite recurrence in 9 of 34 patients. The relative hazard of disease recurrence was 6.37 (2.26–18.0; $p = 0.000$) for postoperative serum mutant K-ras2 positive patients.

1.5 Study rationale

The purpose of this sub-study is to determine whether mutations in K-Ras correlate with tumor response to cetuximab and predict patient outcome to treatment. In addition, we will evaluate whether a relative treatment effect on PFS is related to the level of expression of the EGFR ligands amphiregulin and epiregulin and their prognostic implication. The effect of PTEN loss on these outcomes will also be evaluated. Cell-free serum and plasma will be evaluated for K-ras mutations by PCR and for patients with a mutation detected in serum or plasma, the ability to identify the same mutation in the corresponding tumor tissue mutation will be determined. These latter studies will provide data essential for development of a blood test for tumor K-ras mutational status, yielding a new diagnostic method that will greatly facilitate patient care. Results from this sub-study will improve patient selection for treatment with cetuximab and be critical in the design of future studies that include cetuximab and potentially other anti-EGFR targeted therapies.

2.0 Objectives

2.1 Primary objectives

- a) To determine whether the relative effect of treatment (cetuximab vs bevacizumab) on progression-free survival (PFS) depends on K-Ras mutation status.
- b) To determine whether the relative effect of treatment (cetuximab vs bevacizumab) on PFS depends on levels of expression of genes encoding amphiregulin and epiregulin.
- c) To determine whether the relative effect of treatment (cetuximab vs bevacizumab) on PFS depends on loss of PTEN function.

2.2 Secondary objectives

- a) To determine whether K-Ras mutation status in tumor is prognostic for PFS;
- b) To determine whether tumor expression levels of amphiregulin and epiregulin are prognostic for PFS;
- c) To determine whether tumor PTEN status is prognostic for PFS;
- d) To determine whether one or more of these tumor markers is predictive or prognostic for OS and response (among patients with measurable disease);
- e) To determine whether one or more of these tumor markers is predictive for response in patients treated on the bevacizumab-containing arms;
- f) To determine potential relationships among different tumor markers relative to outcome;
- g) To determine whether tumor gene expression levels and tumor K-Ras mutational status are associated with toxicity;
- h) To correlate serum and/or plasma K-ras mutation status with tumor K-ras mutation status; and
- i) To correlate serum and/or plasma K-ras mutation status with PFS.

3.0 Research approach and methods

3.1 Prospective K-Ras mutation testing for determination of eligibility

Sampling the tissue block: The H&E slide received along with the tissue block will be examined by a pathologist and an appropriate region of highly cellular viable tumor will be dotted with a cytology marking pen. The corresponding paraffin block will then be secured on a Beecher manual tissue microarrayer. The dotted area on the slide will be aligned with the paraffin block and sampled using a 0.6 mm coring needle. The resulting tissue core will be placed in a solvent resistant test tube for deparaffinization.

Deparaffinization: Deparaffinization and rehydration is a slightly more lengthy process for tissue cores than it is for cut slides, but is an important part of the testing procedure and a frequent source of error for those unused to dealing with paraffin-embedded material. Tissue cores prepared from the paraffin blocks are placed in 1.5 mL xylene for 2 hours with three changes of solvent. Tissue is then processed through a series of progressively more dilute ethanols to water over a period of 1 hour. At the end of this time, rehydrated xylene free/formalin free tissue fragments are pelleted by centrifugation and resuspended in 180 mL ATL buffer (Qiagen, QIAamp FFPE DNA Extraction Kit). Deparaffinization is accomplished either manually or robotically overnight.

Proteinase K digestion and DNA extraction: 40 mL proteinase K solution (>600mAU/mL) is added to the suspension which is then incubated for 2 hours at 56° C (until the sample is completely lysed) followed by incubation at 90° C for one more hour. At the end of this time, 200 mL AL and 200 mL ethanol are added to the solution. The lysate is loaded on to the Qiacube automated DNA extraction system and purified using the QIAamp FFPE DNA Extraction Kit protocol. The purified DNA is quantified using a Nanodrop 1000 spectrophotometer and ~20 ng of DNA is used as starting material for K-Ras analysis.

K-Ras detection using the DxS kit: The DxS K-Ras Mutation Detection Kit will detect seven KRAS mutations in codons 12 and 13 (Table 24).

Gly12Ala	(GGT>GCT)
Gly12Asp	(GGT>GAT)
Gly12Arg	(GGT>CGT)
Gly12Cys	(GGT>TGT)
Gly12Ser	(GGT>AGT)
Gly12Val	(GGT>GTT)
Gly13Asp	(GGC>GAC)

Table 24
KRAS Mutations Detected by the DxS Kit (Mutation Base Change)

Detection of the mutations is possible in a background of wild-type genomic DNA in a real time PCR assay based on DxS Scorpions technology. This method can detect approximately 1% of mutant in a background of wild-type genomic DNA.

Scorpions real time assays use the number of PCR cycles necessary to detect a signal above a background as a measure of the target molecules present at the beginning of the reaction. The threshold at which the signal is detected above background fluorescence is called the Cycle threshold (Ct). Sample Δ Ct values are calculated as the difference between the mutation assay

Ct and control assay Ct. Samples are classed as positive if they give a Δ Ct that is less than the 1% Δ Ct value for the assay.

A control assay is used to assess the total DNA in a sample. This Scorpions assay amplifies a region of exon 4 of the KRAS gene. The primers and probe have been designed to avoid any known KRAS polymorphisms.

The mutation assays contain one Scorpion plus one ARMS primer for discrimination between the wild-type DNA and the mutant DNA detected by that assay. All assays also contain a Scorpions assay for an exogenous control monitored by the JOE detector. This controls for the presence of inhibitors, which may lead to false negative results.

Assay performance characteristics: The mutation assay performance characteristics are given in the Table 25 below. The positive control for each assay is a mixed standard containing 7 synthetic constructs (one positive control for each mutation assay), in 2 ng/ μ L of genomic DNA. The mixed standard Δ Ct values, given in Table 25, should be used to ascertain that the assays are working correctly. Deviations of ± 2 from these values are expected due to differences in threshold settings between runs.

All assays have been shown to detect 1% mutant. The 1% Δ Ct has been determined over a range of DNA concentrations down to 2 ng per PCR.

Assays	Mixed Standard Δ Ct	1% Δ Ct
12ALA	0.5	6.5
12ASP	2.0	8.0
12ARG	2.0	8.0
12CYS	0.5	7.0
12SER	2.5	9.0
12VAL	1.5	6.5
13ASP	3.5	9.0

Table 25
Assay Performance Characteristics

If the sample Δ Ct is higher than the 1% value (given in Table 25) the sample is classed as negative or below the limits of the kit. If the sample value is lower than the 1% value, the sample is classed as positive. Samples that give a Δ Ct close to the 1% value should be repeated in triplicate and all replicates should be in the positive zone for the sample to be classed as mutation positive. Samples with a mutation Ct of greater than 38 should also be repeated in triplicate and all replicates should be in the positive zone for the sample to be classed as mutation positive.

Sensitivity and specificity of the DxS assay compared to sequencing: In a comparison of the DxS mutation test to DNA sequencing (N=137) there was 100% concordance between the presence of a mutation detected by DNA sequencing and detection of that mutation by the DxS test.

Sequencing	Arms	
	Positive	Negative
Positive	16	0
Negative	5	116

In 5 of the 137 samples a mutation was detected by the DxS test that was not detected by DNA sequencing. Given the nature of the samples and the known limitation of sequencing this was expected. The 5 samples which were positive by ARMS but negative by direct sequencing were subsequently analysed by PCR amplification of exon 1 of the RAS gene followed by cloning and sequencing of individual clones. All 5 samples were shown to be true positives by this method. For the samples tested the DxS assay was 100% sensitive whereas the corresponding sensitivity for DNA sequencing was 76%.

The DxS assay is rapid, mutation specific and highly sensitive. The test is thus applicable to clinical trials requiring high accuracy and rapid turnaround.

3.2 Retrospective K-Ras testing of tissue collected prior to Update #5

An H&E slide will be prepared from the submitted block and the same procedure will be followed as described above. If slides were submitted instead of a block, one will be used for H&E and the quality of the tumor sample will be assessed to ensure that the appropriate amount of neoplastic cells are present for K-Ras mutation testing. Approximately two 10 µm-thick paraffin-embedded tumor tissue sections will be needed for the K-Ras analysis.

3.3 Gene Expression Levels of Amphiregulin, Epiregulin and Related Genes

Three 10 µm-thick paraffin-embedded tumor tissue sections and an H&E slide will be required for gene expression. These will be sent to Dr. Lenz for analysis. Specimens will be inspected before RNA isolation and must contain at least 20 square millimeters of tumor cells for processing.

RNA extraction from the FFPE specimens will be performed using a proprietary procedure (US patent number 6,248,535) developed by the Lenz laboratory. Following RNA isolation, cDNA will be prepared from each sample as described previously.²²

Real time PCR for measuring mRNA levels: Quantitation of cDNA's and an internal reference gene (β -actin) will be done using a fluorescence based real-time detection method (ABI PRISM 7700 Sequence Detection System [TaqMan®], Perkin Elmer Applied Biosystems, Foster City, CA.) as previously described.^{22,23} In brief, this method uses a dual labeled fluorogenic oligonucleotide probe that anneals specifically within the forward and reverse primers. Laser stimulation within the capped wells containing the reaction mixture causes emission of a 3' quencher dye (TAMRA) until the probe is cleaved by the 5' to 3' nuclease activity of the DNA polymerase during PCR extension, causing release of a 5' reporter dye (FAM). Production of an amplicon thus causes emission of a fluorescent signal that is detected by the TaqMan®'s CCD (charge-coupled device) detection camera, and the amount of signal produced at a threshold cycle within the purely exponential phase of the PCR reaction reflects the starting copy number of the sequence of interest. Comparison of the starting copy number of the sequence of interest with the starting copy number of the reference gene provides a relative gene expression level. The PCR reaction mixture consisted of 600 nM of each primer, 200 nM probe, 5 U AmpliTaq Gold Polymerase, 200 µM each dATP, dCTP, dGTP, 400 µM dUTP, 5.5 mM MgCl₂, and 1 x Taqman Buffer A containing a reference dye, to a final volume of 25 µL (all reagents Perkin Elmer (PE) Applied Biosystems, Foster City, CA). Cycling conditions were 50° C for 10s, 95° C for 10s, followed by 42 cycles at 95° C for 15s and 60° C for 1 min. Taqman assays have been established for amphiregulin, VEGF, cyclin D, epiregulin, Il-8, CXCR2, EGFR.^{22,23}

Quality Control for mRNA levels: Briefly, ranging studies were performed by processing dilutions of identical specimens multiple times on different days. The range included Taqman cycle thresholds of 29 to 35. The CV, precision and accuracy were determined in this range by running identical experiments on multiple days. The variation in measurement of the relative gene expression (TS/Actin) was less than 15% on a single day when analyzing more than 50

data points over the usable range of 29 to 35 cycle thresholds. The coefficient of variation (CV) between identical samples run on different days was not more than 20%. A series of control RNAs were purchased from Stratagene. These include RNA isolated from diseased colon, lung and breast specimens. The relative gene expression of these standards was determined for a number of targets by averaging twenty measurements. These RNAs are used as PCR plate callibrators. PCR data will not be reported from an experiment when the value of these controls varies more than 15% from the known value. When possible, RNA specific primers are used for quantitation. However, this is not always possible. Therefore, a nonreverse transcribed sample of the isolated RNA (no-RT control) is analyzed in order to quantitate the level of contaminating DNA in the specimen.

Data from specimens containing more than 25% DNA will not be reported. Data from specimens measured at greater than 37 cTs (cycle thresholds) will likewise not be reported. All specimens are measured in triplicate by the delta cT method and an average value is reported for the ratio of the target gene to the housekeeping gene, β -Actin.

3.4 Immunohistochemistry for PTEN Expression:

The Bertagnoli lab will assess PTEN protein expression status by IHC using a single 5 μ m tissue section as described in the literature.^{24,25} Anti-PTEN Ab-3 will be applied at 1:50 dilution. PTEN negative tumors will be defined as tumors showing a striking reduction or absence of immunostaining in at least 50% of cells, as compared with the internal or additional tissue controls of normal intestinal mucosa.

The PTEN IHC analysis is part of a larger group of IHC studies already described in Appendix II, above (MAPK, Akt-p-Ser473, VEGF, MSH2, MLH1, MGMT, and Cox-2).

Additional methods to assess PTEN status may also be evaluated.

3.5 Analysis of Serum and Plasma K-Ras Mutations

Serum and plasma samples are to be collected, prepared and shipped to the SWOG Solid Tumor Specimen Repository as described in [Section 5.6](#). One of the two aliquots of serum and plasma submitted at baseline (pre-treatment) will be sent to the laboratory of David Sidransky at Johns Hopkins for the analysis of K-Ras mutations. Serum contains higher amounts of DNA than plasma, due to its release during cell disintegration during blood clotting. However, in patients with cancer, DNA can also be isolated from plasma, and in this case has a higher ratio of tumor DNA to non-tumor DNA.

To isolate DNA from the serum or plasma specimen, two volumes of 6 mol/L guanidine thiocyanate will be mixed with the serum or plasma by inverting the mixture eight times. One mL of resin from the Wizard DNA isolation reagent set (Promega) will be added, and the mixture incubated for 2 hours at room temperature. The resin-DNA mixture will be transferred to a mini-column (Promega) and washed, eluting the DNA with H₂O. Isolated DNA will be quantified by real-time PCR with human albumin primers.²⁷ Following DNA extraction, mutations in K-Ras colon 12 and 13 will be identified using the DxS kit in a manner similar to that employed for tumor DNA.¹⁴

4.0 Statistical Considerations

The primary endpoint is progression-free survival (PFS) measured from study entry until first documented progression (RECIST criteria) or death from any cause. Overall survival (OS) and tumor response, defined as first CR+PR according to the RECIST criteria, will be studied as secondary endpoints. Response will be studied among patients with measurable disease. Power computations to test the primary hypothesis for PTEN, amphiregulin and epiregulin are based on accrual to the chemotherapy + bevacizumab (Arm A total expected accrual n = 990) and chemotherapy + cetuximab (Arm B total expected accrual n = 990) treatment arms. However, tissue samples from patients treated with chemotherapy + bevacizumab + cetuximab (Arm C) will also be analyzed for biomarkers. The power calculations were performed under the assumption of no association between bevacizumab response and marker expression. For analysis of PTEN, amphiregulin and epiregulin, a maximum of 1,785 tumor samples will be analyzed, approximately 595 samples per treatment arm. Power computations for the analysis of K-Ras are given below.

The primary hypothesis for each marker is that marker status impacts PFS among patients treated with cetuximab and has no impact on PFS among patients who are not treated with cetuximab (the marker is predictive of outcome). Marker status is defined as negative versus positive by IHC for PTEN expression; low versus high by RT-PCR for amphiregulin and epiregulin; and mutation versus no mutation for K-Ras in the tumor specimen by DxS K-RAS test kit and in serum/plasma by PCR. The hypothesis of no “treatment by marker” interaction will be tested using a Cox regression model. Forty to 45% of patients are expected to have PTEN negative tumors; 40-50% of patients are expected to have K-Ras mutations; approximately 35% of patients are expected to have tumors exhibiting high expression of amphiregulin and epiregulin. Secondary analyses will be conducted with and without marker status among patients treated on the C80405 combination arm. Multivariable analyses will be conducted to simultaneously assess the relationships between marker status and outcome. Combinations of markers will also be considered. These analyses will be considered exploratory.

Table 26 provides approximate power estimates to test the hypotheses of no interaction by underlying hazard ratio assuming 60% of tissue samples are available for marker analysis. Comparisons are between the bevacizumab and the cetuximab-alone treatment arms (targeted number of patients enrolled n = 1,980; n = 1,188 patients with tissue samples on these treatment arms is assumed).

Detectable Hazard Ratio for Interaction	Power estimate, $\alpha = 0.0125$
1.50	0.52
1.60	0.67
1.65	0.74
1.70	0.79
1.75	0.84
1.80	0.88

Table 26: Power estimates to detect the specified treatment by marker interaction for 60% of samples obtained in the bevacizumab-alone and cetuximab-alone treatment arms of C80405 (n = 1,188). Approximately 632 PFS events are expected among patients with available specimens (assumes the overall PFS rate of 1581 events/2970 patients). Power estimates assume equal sample sizes within subgroups and are approximate for marker prevalence between 30 and 70%. The significance level is adjusted for the multiple tests of four markers (2-sided $\alpha=0.0125$).

Moderate to large differences are detectable with adequate power. If a significant interaction is detected for a given marker, relationships between marker status and PFS will be assessed within

CALGB/SWOG C80405
Appendix V

treatment arm. Approximately 90% power is achieved to detect a hazard ratio of 1.5 for PFS differences by marker within treatment arm if 316 PFS events are observed ($n = 595$; 2-sided $\alpha = 0.0125$).

If no significant treatment by marker interaction is detected for a given marker, we will examine associations between marker status and PFS using the combined data from all treatment arms, adjusting for treatment assignment.

K-Ras: The primary hypothesis for K-ras mutational status is that marker status impacts PFS among patients treated with cetuximab and has no impact on PFS among patients who are not treated with cetuximab (the marker is predictive of outcome). The hypothesis of no “treatment by marker” interaction will be tested using a Cox regression model among patients with available specimens enrolled prior to the restriction of eligibility to K-Ras WT. K-ras mutational status determined by the two assay methods (tissue-based; serum/plasma based) will be studied. As of May 15, 2008, 913 patients treated on CALGB 80405 (approximately 70%) have primary tumors or unstained slides banked in the SWOG Solid Tumor Specimen Repository; approximately 320 (35%) of these patients are expected to have K-ras mutations. Assuming the overall PFS rate of 0.53, approximately 484 PFS events are expected.

Detectable Hazard Ratio for Interaction	Power estimate, $\alpha = 0.0125$
1.70	0.66
1.75	0.71
1.80	0.76
1.85	0.81
1.90	0.84
2.00	0.90

Large differences are detectable with adequate power. If a significant interaction is detected for K-ras mutational status, relationships between marker status and PFS will be assessed within treatment arm. Greater than 90% power is achieved to detect a hazard ratio of 1.6 for PFS differences by marker within treatment arm if 242 PFS events are observed ($n = 455$; 2-sided $\alpha = 0.0125$).

If no significant treatment by marker interaction is detected for K-ras mutational status, we will examine associations between marker status and PFS using the combined data from all treatment arms, adjusting for treatment assignment.

Agreement between k-ras mutational status determined using tumor tissue and K-ras mutational status determined using serum and/or plasma will be estimated using the kappa statistic.²⁸ The study sample will include the 913 patients with available specimens enrolled prior to the amendment to restrict eligibility to patients with K-ras WT tumors. Assuming 35% of tumors will have a K-ras mutation by both methods and 70% (639) of the 913 patients with tissue samples have an available serum/plasma sample, the standard error of kappa is 0.10 (under the null hypothesis). If agreement is greater than 0.6 with 90% confidence, agreement between the assay methods will be validated prospectively in 1,400 patients registered and tested for K-ras mutational status post-amendment. With, 1,400 patients kappa can be estimated with standard error of 0.071.

5.0 References

1. Peyssonaux C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* 2001;93:53-62.
2. Barbacid M. ras genes. *Annu Rev Biochem*. 1987;56:779-827.
3. Bas J,L, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. *Nature* 1987;327:293-297.
4. Forrester K, Almoguera C, Han K, et al. Detection of high incidence of K-ras oncogenes during human colorectal cancer tumorigenesis. *Nature* 1987;327:298-303.
5. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal tumor development. *N Engl J Med* 1988;319:525-532.
6. Mulla A, Milner-White E, Going JJ, Birnie GD. Structural differences between valine-12 and aspartate-12 Ras proteins may modify carcinoma aggression. *J Pathol* 1999;187:433-438.
7. Lievre A, Bachet J-B, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66:3992-3995. ECCO abstract.
8. 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;25:3230-3236.
9. Frattini M, Saletti P, Romagnani R, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal patients. *Br J Cancer* 2007;97:1139-1145.
10. Amado RG, Wolf M, Peeters M, et al. Wild-Type KRAS Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer. *J Clin Oncol* Apr 1 2008;26:1626-1634.
11. Freeman D, Juan T, Meropol NJ et al. Association of somatic KRAS gene mutations and clinical outcome in patients (pts) with metastatic colorectal cancer (mCRC) receiving panitumumab monotherapy. *Eur J Canc Supplement* 2007;5:239.
12. Anker P, Lefort F, Vasioujkin V, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. *Gastroenterology* 1997;112:1114-1120.
13. Hibi K, Robinson R, Booker, et al. Molecular detection of genetic alterations in the serum of colorectal cancer patients. *Cancer Res* 1998;58:1405-1407.
14. Kopreski MS, Benko FA, Kwee C, et al. Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer. *Br J Cancer* 1997;76:1293-1299.
15. Lecomte T, Berger A, Zinzindohoue F, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. *Int J Cancer* 2002;100:542-548.
16. Frattini M, Gallino G, Signorini S, et al. Quantitative analysis of plasma DNA in colorectal cancer patients: a novel prognostic tool. *Ann NY Acad Sci* 2006;1075:185-190.
17. Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *PNAS* 2005;102:16368-16373.
18. Trevisiol C, DiFabio F, Nascimbeni R, et al. Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastasis. *Int J Biol Markers* 2006;21:223-228.
19. Ryan BM, Lefort F, McManus R, et al. A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: strong prognostic indicator in postoperative follow up. *Gut* 2003;52:101-108.

CALGB/SWOG C80405
Appendix V

20. Thelwell N, Millington S, Solina A et al. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res* 2000;28:3752-3761.
21. Whitcombe D, Theaker J, Guy S, et al. Detection of PCR products using self-probing amplicons and fluorescence. *Nat Biotech* 1999;17:804-807.
22. Heid CA, Stevens J, Livak KJ, et al: Real time quantitative PCR. *Genome Res* 1996;6: 986-994.
23. Gibson UE, Heid CA, and Williams PM: A novel method for real time quantitative RT-PCR. *Genome Res* 1996;6: 995-1001.
24. Frattini M, Signoroni S, Pilotti S, Bertario L, Benvenuti S, Zanon C, Bardelli A, Pierotti MA. Phosphatase protein homologue to tensin expression and phosphatidylinositol-3 phosphate kinase mutations in colorectal cancer. *Cancer Res* 2005;65:11227.
25. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 2005;65:2554–2559.
26. Botezatu I, Serdyuk O, Potapova G, et el. Genetic analysis of DNA excreted in urine: a new approach for detecting specific genomic DNA sequences from cells dying in an organism. *Clin Chem* 2000;46:1078-1084.
27. Wang M, Block, TM, Steel L, et al. Preferential isolation of fragmented DNA enhances the detection of circulating mutated k-ras DNA. *Clin Chem* 2004;50:211-213.
28. Fleiss JL. Statistical Methods for Rates and Proportions, Second Edition. John Wiley & Sons. New York. 1981. pp 212-236.

APPENDIX VI
PART A

**EVALUATING BRAF MUTATIONS AS PREDICTORS OF EFFICACY IN CETUXIMAB-TREATED
COLORECTAL CANCER PATIENTS**

A RETROSPECTIVE STUDY OF TISSUES FROM CALGB/SWOG

SPECIFIC AIMS

The goal of this proposal is to evaluate the utility of determining the presence of BRAF mutations for predicting benefit from cetuximab-containing chemotherapy for patients with advanced colorectal cancer (CRC). Specifically, we propose to identify BRAF mutations in tumor tissue and correlate with treatment outcome.

BACKGROUND

Approximately 15-20% of colorectal cancers have an activating mutation in BRAF, a serine-threonine kinase. This particular BRAF mutation (BRAF V600E) has also been tied to microsatellite instability (MSI) in colorectal cancer. Like PTEN, BRAF signaling is downstream of KRAS in normal cells. As a result, it is plausible that activating mutations in BRAF such as BRAF V600E will mimic the biological consequences of KRAS mutation. As a result, patients whose tumors harbor the BRAF V600E mutation should not respond to cetuximab.

PRELIMINARY DATA

Investigators evaluated the prognostic and predictive value of BRAF mutations in patients with KRAS wild-type colorectal cancer.¹ Outcome data and mutation status was evaluated in 113 patients with metastatic colorectal cancer who received cetuximab or panitumumab. Specifically, the BRAF V600E mutation was found in 11 of 79 patients with wild-type KRAS. None of these patients with BRAF V600E mutations responded to treatment and, among the responders, none carried the mutation ($P = 0.029$). Patients with BRAF V600 E mutations also had a shorter progression-free survival (PFS) and overall survival (OS). Evaluating BRAF V660E mutation in addition to KRAS mutation may aid in selecting therapy for patients with metastatic colorectal cancer.

Study Objectives

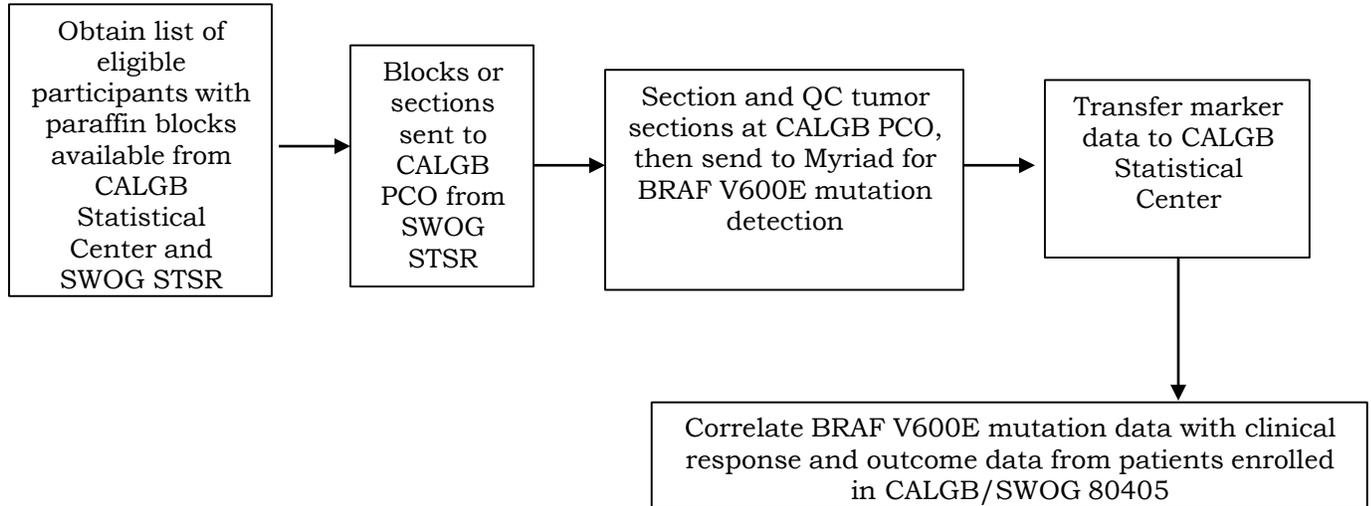
The objective of this study is to determine, among patients with advanced CRC, whether the effect of treatment (cetuximab vs bevacizumab) on progression-free survival (PFS) depends on tumor BRAF V600E mutational status. The relationships between tumor BRAF V600E mutational status, OS, and tumor response will also be studied as secondary endpoints.

Eligibility

The following are eligibility requirements for this study:

- Participation in CALGB/SWOG 80405
- Available specimens at the PCO for BRAF mutation detection
- Patient consent for use of samples

SCHEMA



SUBMISSION OF SAMPLES

Formalin-fixed and paraffin embedded (FFPE) baseline tumor samples are stored at SWOG's Solid Tumor Specimen Repository (STSR) for CALGB/SWOG 80405. The trial is currently designed to study 1,142 patients with KRAS WT tumors. Prior to the trial amendment excluding patient with KRAS mutant tumors, tumor blocks were also received from at least 214 patients with KRAS mutant tumors. At least three hundred twenty-eight patients enrolled prior to amendment #5 were identified as having KRAS WT tumors.

To address objective 1 above, we intend to analyze the entire cohort of patients enrolled on the trial who have KRAS WT tumors and were randomized to treatment with either bevacizumab or cetuximab alone. Patients randomized to the combination arm will not be included in the analysis. To address objective 2, patients with KRAS results (WT or mut) who consented to these analyses prior to amendment #5 will be included.

METHODS AND TECHNICAL FEASIBILITY:

Five 10 µm slides will be sent to Myriad for determination of BRAF V600E mutation. Additional assays such as PTEN sequencing will use this material as well. Any unused material will be returned to CALGB/SWOG.

Myriad has developed a highly sensitive PCR/sequencing based assay to detect V600E variants in the BRAF gene. The method employs a peptide nucleic acid (PNA) oligo that specifically hybridizes to wild-type BRAF sequence and subsequently inhibits the amplification of DNA molecules containing wild-type sequence. This technique (often called PNA clamping) has been used successfully to detect mutations in KRAS.² A PNA has been developed to detect BRAF V600E that will be used in this study. FFPE-derived DNA is amplified in presence and absence of PNA probe. PCR product is sequenced to identify the specific base change. This technique is robust to non-tumor contamination, and in fact, we have been able to detect one mutation in the presence of 10,000 wild-type DNA copies.

STATISTICAL CONSIDERATIONS:

The primary endpoint is progression-free survival (PFS) as measured by RECIST. The interactions between tumor BRAF V600E mutational status and treatment will be tested using a proportional hazards regression model. One thousand one hundred forty-two (n=1,142) patients with KRAS WT tumors will be enrolled on C80405 and treated with bevacizumab alone or cetuximab alone. Table 1 provides power computations for testing the interaction of BRAF mutation status (present; absent) and treatment (bevacizumab; cetuximab).³ The numbers of expected events among patients with KRAS WT tumors were computed for patients enrolled pre-amendment #5 and post-amendment #5. Pre-amendment #5, it is assumed that data from 328 patients with KRAS WT tumors enrolled over 32 months and followed 48 months will be available for analysis; post amendment #5, it is assumed that data from 472, 586, or 700 patients with KRAS WT tumors enrolled over 19 months and followed 36 months will be available for analysis.

Table 1. Power to detect an interaction hazard ratio of 0.59 for PFS by BRAF status (WT; mut) and treatment arm (bevacizumab; cetuximab) for the specified sample size and 70%, 80%, and 90% of 1,142 patients with KRAS WT tumors. The prevalence of BRAF mutations is assumed to be 0.15 and patients are assumed to be equally distributed by treatment (2-sided $\alpha=0.05, 0.1$). The estimated numbers of PFS events are 702, 797, and 893 for the samples sizes of 800, 914, and 1028, respectively. Hypothesized median PFS by BRAF status and treatment under the alternative hypothesis is provided in Table 2.

Sample Size	Power estimate, 2-sided $\alpha =0.05$	Power estimate, 2-sided $\alpha =0.10$
800	0.72	0.82
914	0.78	0.86
1028	0.82	0.89

Table 2. Median PFS in months under the alternative hypothesis by BRAF status and Treatment. No difference in PFS is assumed between BRAF mutant and WT for patients treated with bevacizumab.

Median PFS (mos)	Treatment	
BRAF	cetuximab	bevacizumab
mut	13.5	13.5
WT	22.0	13.0

CALGB/SWOG C80405
Appendix VI A

If a significant interaction is detected, relationships between BRAF mutational status and PFS will be assessed within treatment arm. Approximately 80% power is achieved to detect a hazard ratio of 1.55, 1.50, and 1.47 for PFS differences by BRAF mutational status within treatment arm, respectively, if 400, 457, and 514 patients are studied (2-sided $p=0.05$). These sample sizes correspond to 328, 372, and 417 PFS events observed, respectively.

If no significant treatment by BRAF interaction is detected, we will examine associations between BRAF mutational status and PFS using the combined data from both treatment arms, adjusting for treatment assignment. A PFS hazard ratio of 1.4 is detectable with approximately 88%, 91%, and 94% power in the combined treatment groups (for $n=800, 914, 1028$, respectively; 2-sided $p=0.05$).

Patient and tumor characteristics and outcomes will be compared between patients with and without tumor samples available for this analysis.

References

- 1) Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008, Dec 10;26(35):5705-12.
- 2) Tatsumi K, Mitani Y, Watanabe J, et al. Rapid screening assay for KRAS mutations by the modified smart amplification process. *J Mol Diagn*. 2008 Nov;10(6):520-6.
- 3) Peterson B, George SL. Sample size requirements and length of study for testing interaction in a 2 x k factorial design when time-to-failure is the outcome. *Controlled Clin Trials* 14:511-522, 1993

APPENDIX VI: PART B

**EVALUATING PTEN MUTATIONS AS PREDICTORS OF EFFICACY IN CETUXIMAB-TREATED
COLORECTAL CANCER PATIENTS**

A RETROSPECTIVE STUDY OF TISSUES FROM CALGB/SWOG

SPECIFIC AIMS

The goal of this proposal is to evaluate the utility of determining PTEN gene sequence changes for predicting benefit from cetuximab-containing chemotherapy for patients with advanced colorectal cancer (CRC). Variations from normal, i.e., PTEN gene mutations, will be correlated with advanced CRC treatment outcome (PFS and OS). In addition, the PTEN mutational status will be correlated with PTEN IHC results and also evaluated for its utility in predicting treatment outcome (either independently or as an adjunct to IHC).

BACKGROUND AND PRELIMINARY STUDIES: PTEN STATUS

Recent clinical trials have shown that activating mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS) protein, which partially transduces the activation signal from EGFR, abrogate the therapeutic effect of cetuximab.¹⁻⁴ Specifically, cetuximab-treated patients whose tumors carried KRAS mutations showed no response to therapy as assessed by either reduction in tumor volume or improvement in survival. Thus, selection of patients for cetuximab treatment by prescreening tumors for activating mutations in KRAS promises to significantly improve our ability to identify patients who may benefit from cetuximab. Despite this, patient selection is far from perfect. While the negative predictive value of KRAS mutations is nearly ideal, the positive predictive value is only fair, as almost 60% of the patients with wild type KRAS fail to respond to the therapy. This lack of response suggests that there are other important molecular determinants of cetuximab response. In addition to activating KRAS mutation, activation of PI3K/AKT, a signaling pathway downstream of EGFR, has been associated with a lack of clinical response to cetuximab. This suggests that simultaneous evaluation of key regulatory members of this pathway may provide an integrated assessment of the likelihood of drug response.

The phosphatase and tensin homologue deleted on chromosome 10 gene, PTEN encodes a dual specificity phosphatase that dephosphorylates tyrosine and serine/threonine residues.⁵ PTEN functions as a tumor suppressor by dephosphorylating phosphatidylinositol-3,4,5-phosphate (PIP3), thereby negatively regulating the PI3K/AKT signaling pathway downstream of KRAS activation.⁶ PTEN also regulates TP53 protein levels and activity via both phosphatase-dependant and independent mechanisms.⁷ In metastatic colorectal cancer, PTEN expression is frequently lost and like mutations in KRAS, activates EGFR-signaling pathways.⁸ In addition, PTEN activity has been associated with clinical response to targeted therapies directed against EGFR and/or EGFR-family members. Specifically, loss of PTEN expression has been correlated with lack of efficacy of Tarceva, Iressa (anti-EGFR receptor tyrosine kinase inhibitors) and Herceptin (a monoclonal antibody directed against HER2).⁹⁻¹¹ Additional data from human colorectal cancer studies indicated that molecular characteristics of tumors may provide more information than IHC for determination of PTEN activity. PTEN mutations can be detected by amplifying coding sequences of exons 5, 6, 7, 8 and 9. By this method, inactivating mutations in PTEN occur in 10-20% of CRCs.¹² A small study found that 10% of patients with metastatic CRC had PTEN mutations, and none of the patients with PTEN mutated tumors responded to treatment with cetuximab.¹³

CALGB/SWOG C80405
Appendix VI B

The relationship between PTEN expression by IHC and PTEN mutational status has not been determined in a large CRC cohort. The available data suggest that PTEN function correlates with cetuximab response in KRAS wildtype tumors, and that PTEN mutational analysis may add to the accuracy of PTEN IHC in predicting treatment response. CALGB/SWOG 80405 provides the ideal patient cohort to study this potential biomarker. The trial randomized 1400 patients with advanced CRC to treatment with either irinotecan/5-FU/leucovorin or oxaliplatin/5-FU/leucovorin with bevacizumab (Arm A), or cetuximab (Arm B), or with the combination of bevacizumab and cetuximab (Arm C) prior to an amendment (Update #5) limiting randomization to patients with KRAS WT tumors. A subsequent update to the trial (Update #6) curtailed randomization to the combination Arm C. The trial continues to enroll patients with a targeted sample size of 1,140 patients with KRAS WT tumors to be randomized on treatment Arms A and B, including patients identified with KRAS WT tumors randomized prior to Update #5.

Tumor specimens from CALGB/SWOG 80405 will be assessed for PTEN expression by IHC (Appendix V), as part of a previously approved correlative science substudy. We will extend this analysis by further evaluating how tumor PTEN mutational status impacts cetuximab treatment. The study of tumors from CALGB/SWOG 80405 will also allow us to evaluate the role of these variables as predictors of response to bevacizumab-based therapy. This is a highly relevant analysis, since potential diagnostics arising from this study might be used to help physicians discriminate between these treatment options.

STUDY OBJECTIVES

The objectives of this study are:

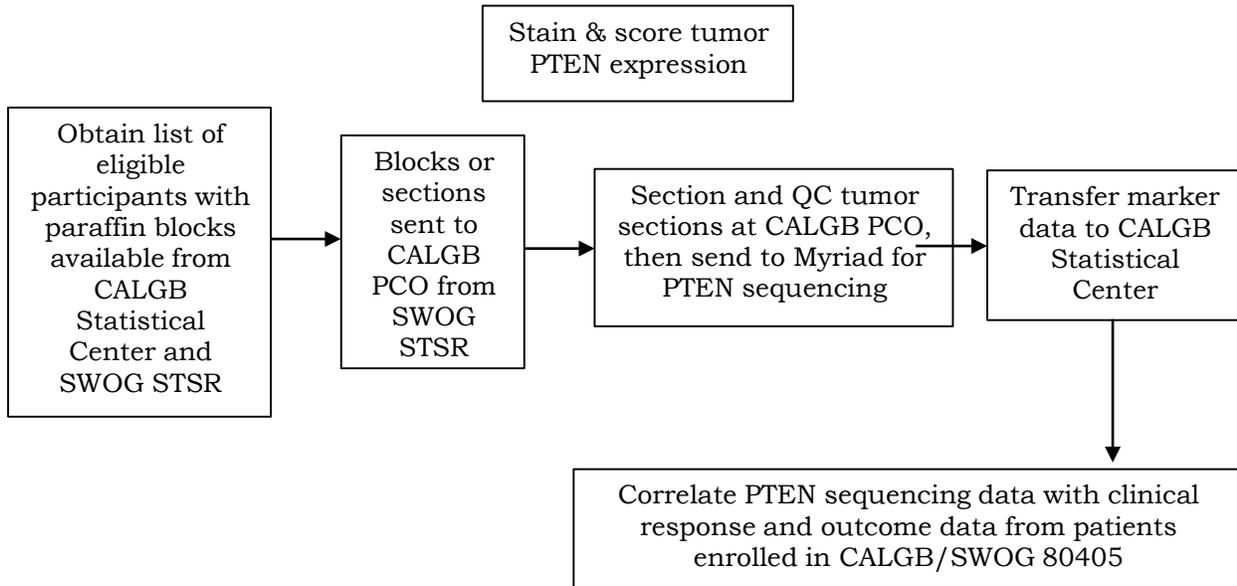
- 1.) To determine, among patients with advanced CRC, whether the effect of treatment (cetuximab vs bevacizumab) on progression-free survival (PFS) depends on PTEN mutational status. The relationships between PTEN mutational status, OS, and tumor response will also be studied as secondary endpoints.
- 2.) To determine the association between PTEN mutational status and PTEN expression measured by IHC.
- 3.) To determine the combined impact of tumor KRAS and tumor PTEN IHC and/or mutational status on outcome for patients with advanced CRC treated with a cetuximab-containing chemotherapy.

ELIGIBILITY

The following are eligibility requirements for this study.

- Participation in CALGB/SWOG 80405
- Specimens available at the PCO for *PTEN* gene sequencing
- Patient consent for use of samples.

SCHEMA



SUBMISSION OF SAMPLES

Formalin-fixed and paraffin embedded (FFPE) baseline tumor samples are stored at SWOG’s Solid Tumor Specimen Repository (STSR) for CALGB/SWOG 80405. The trial is currently designed to study 1,142 patients with KRAS WT tumors. Prior to the trial amendment excluding patient with KRAS mutant tumors, tumor blocks were also received from at least 214 patients with KRAS mutant tumors. At least 328 patients enrolled prior to amendment #5 were identified as having KRAS WT tumors.

To address objectives 1 and 2 above, we intend to analyze the entire cohort of patients enrolled on the trial who have KRAS WT tumors, were randomized to treatment with either bevacizumab or cetuximab alone, and consented to the use of their tissue for these analyses. Patients randomized to the combination arm will not be included in the analysis. To address objective 3, only patients with K-RAS (WT or mut) who consented to these analyses prior to amendment #5 will be included.

METHODS AND TECHNICAL FEASIBILITY

The analysis of PTEN mutational status will require DNA to be prepared from tumor sections containing at least 50-60% tumor. DNA will be isolated from five 10 µm sections and used for this as well as other marker analyses including BRAF.

For confirmation of novel PTEN mutations, genomic DNA has been isolated from whole blood samples and is available.

SEQUENCING METHODS

Myriad has a long history in generating sequence data for clinical applications, and that experience and expertise will be applied to this project. A CLIA certified laboratory will be used to generate PTEN sequence data from FFPE tumor samples. The DNA will have been previously prepared from

CALGB/SWOG C80405
Appendix VI B

tumor sections containing at least 50-60% tumor. However, the sequencing technology employed here will be robust to higher levels of contamination (see below). DNA will be isolated from Macro dissected FFPE slices using commercially available kits with some modifications to improve DNA quality. The sequencing assay will interrogate all nine exons of the PTEN coding region. The exons will be PCR amplified using in-house developed primer pairs and protocols. Some of the exons will be divided into more than one sequencing amplicon to ensure robust amplification from FFPE derived DNA. Each sequencing amplicon will be amplified with two independent pair of primers (Figure 1). This will eliminate failures due to rare polymorphisms or somatic mutations occurring under a primer, and enable immediate confirmation of any observed mutation.

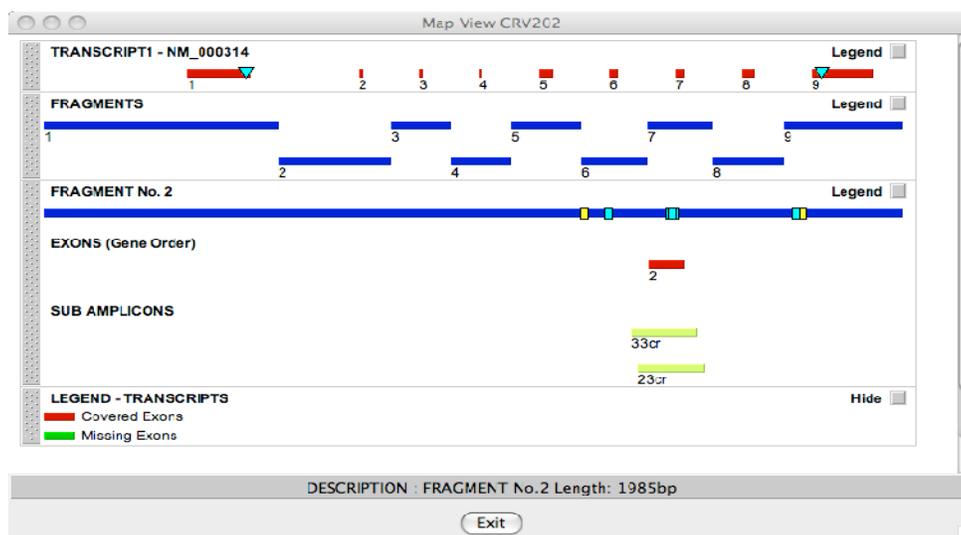


Figure 1. A screen shot of PTEN in Myriad’s mutation screening database. The exons of PTEN are shown (1-9) as well as the sequencing amplicons for exon 2 (33cr and 23cr).

PCR amplified products will be sequenced using Myriad’s BigDye primer chemistry on ABI 3730XL sequencer. The sequencing data will be analyzed with Myriad’s proprietary software that has been developed specifically for mutation screening FFPE tumor samples.

Mutation detection in tumor samples is challenging because of significant amount of non-tumor DNA presenting tumor samples. A computer program has been developed for mutation detection in tumor samples. The program compares a tumor sequencing chromatogram with a corresponding wild type chromatogram. This comparison is being used to (1) evaluate quality of the chromatogram and (2) detect mutations. Evaluating chromatogram quality is essential because it allows us to distinguish between numerous sequence artifacts and rare real mutations. The program has been tested extensively on multiple FFPE tumor samples. We found that the program detects reliably heterozygous mutations in up to 70% non-tumor background and homozygous mutations in up to 85% non-tumor background.

In this study, all nonsense mutations will be classified as deleterious. For novel missense mutations, we will analyze the germline status when possible.

We expect that multiple mutations will be detected within the same sample for only a small fraction of patients.

STATISTICAL CONSIDERATIONS

The primary endpoint is progression-free survival (PFS) measured from study entry until first documented progression (RECIST criteria) or death from any cause. Overall survival (OS), defined as death from any cause, and tumor response, defined as first CR+PR according to the RECIST criteria, will be studied as secondary endpoints. Response will be studied among patients with measurable disease. Patient and tumor characteristics and outcomes will be compared between patients with and without tumor samples available for analysis.

Power calculations are performed under the assumption of no association between response to bevacizumab and PTEN mutational status. Power computations for the analysis of PTEN and KRAS are given below.

PTEN mutational status as predictive of PFS

The primary hypothesis is that PTEN mutational status (at least one mutation versus no mutation) impacts PFS among patients treated with cetuximab and has no impact on PFS among patients who are not treated with cetuximab (PTEN mutational status is predictive of outcome). The hypothesis of no “treatment by PTEN” interaction will be tested using a Cox regression model. Based on results reported for immunohistochemistry, 20% of patients are assumed to have PTEN mutations.¹⁴⁻¹⁶ One thousand one hundred forty-two (n=1,142) patients with KRAS WT tumors will be enrolled on C80405 and treated with bevacizumab alone or cetuximab alone.

Power to detect an interaction hazard ratio of 0.59 for PFS by PTEN status (WT; mut) and treatment arm (bevacizumab; cetuximab) is provided in Table 1 for the specified sample sizes, 70%, 80%, and 90% of 1,142 patients with KRAS WT tumors.¹⁷ It is also assumed that 80% of patient samples will have sufficient tumor for analysis, PTEN prevalence is 0.20, and patients are equally-distributed across treatment. The numbers of expected events among patients with KRAS WT tumors were computed for patients enrolled pre-amendment #5 and post-amendment #5. Hypothesized median PFS by PTEN status and treatment under the alternative hypothesis is provided in Table 2.

Table 1. Power to detect an interaction hazard ratio of 0.59 for PFS by PTEN status (WT; mut) and treatment arm (bevacizumab; cetuximab) for the specified sample sizes, 70%, 80%, and 90% of 1,142 patients with KRAS WT tumors and 80% of samples with at least 50% tumor. The prevalence of PTEN mutations is assumed to be 0.20 and patients are assumed to be equally distributed by treatment (2-sided $\alpha=0.05, 0.1$). The estimated numbers of PFS events are 561, 637, and 713 for the samples sizes of 640, 732, and 822, respectively. Hypothesized median PFS by PTEN status and treatment under the alternative hypothesis is provided in Table 2.

Sample Size	Power estimate, 2-sided $\alpha=0.05$	Power estimate, 2-sided $\alpha=0.1$
640 (.7*.8*1142)	0.71	0.81
732 (.8*.8*1142)	0.77	0.85
822 (.9*.8*1142)	0.81	0.88

Table 2. Median PFS in months under the alternative hypothesis by PTEN status and Treatment. No difference in PFS is assumed between PTEN mutant and WT for patients treated with bevacizumab.

Median PFS	Treatment	
PTEN	cetuximab	bevacizumab

CALGB/SWOG C80405
Appendix VI B

mut	13	13.5
WT	22	13.5

If a significant interaction is detected, relationships between PTEN mutational status and PFS will be assessed within treatment arm. Approximately 80% power is achieved to detect a hazard ratio of 1.54, 1.50, and 1.47 for PFS differences by PTEN mutational status within treatment arm, respectively, if 320, 366, and 411 patients are studied (2-sided $\alpha=0.05$). These sample sizes correspond to 264, 300, and 336 PFS events observed, respectively.

If no significant treatment by PTEN by treatment interaction is detected, we will examine associations between PTEN mutational status and PFS using the combined data from both treatment arms, adjusting for treatment assignment. A PFS hazard ratio of 1.4 is detectable with approximately 89%, 92%, and 94% power in the combined treatment groups (for 561 of 640, 637 of 732, 713 of 822 PFS events, respectively; 2-sided $\alpha=0.05$).

The association between PTEN mutational status and PTEN expression measured by IHC will be estimated using Kendall's tau.¹⁸ We expect that paired assessments will be available for a minimum of 300 patients studied. Assuming 300 paired assessments, the standard deviation of this estimate is 0.038 (under the null hypothesis) and tau can be estimated to within +/- 0.074 with 95% confidence.

PTEN mutation and KRAS status

Data are available on a minimum of 542 patients with KRAS status (214 KRAS mutant tumors and 328 KRAS WT) treated with bevacizumab alone or cetuximab alone (pre-amendment #5). To maintain the population distribution of KRAS WT and mutation, the interaction between PTEN status, KRAS status, and treatment will be explored among these patients.

A combined biologic variable will be defined according to patient status on both PTEN (WT; mut) and KRAS (WT; mut), resulting in the following 4 levels (PTEN WT; KRAS WT), (PTEN WT; KRAS mut), (PTEN mut; KRAS WT), and (PTEN mut; KRAS mut). The 2 x 4 interaction between treatment (cetuximab; no cetuximab) and the combined biomarker variable will be investigated. Loupakis, et. al., retrospectively investigated the role of PTEN expression and KRAS on the activity of cetuximab plus irinotecan in 45 patients with metastatic colorectal cancer. The distribution of PTEN expression and KRAS was as follows: 0.16 PTEN-; KRAS mutant, 0.22 PTEN -; KRAS WT, 0.24 PTEN +; KRAS mutant, 0.38 PTEN +; KRAS WT.¹⁴ Table 3 illustrates an alternative hypothesis for this test that is comparable to the hypothesis in Table 2.

Table 3. Hypothesized median PFS in months for the test of the interaction between a combined biomarker variable based on PTEN and KRAS status and treatment. The marginal distribution of PTEN and KRAS status reported by Loupakis is assumed with equal probability of being treated with cetuximab or bevacizumab. Independence between treatment and PTEN, KRAS mutational status is also assumed.

CALGB/SWOG C80405
Appendix VI B

Treatment	Marker Status			
	PTEN mut; KRAS mut	PTEN mut; KRAS WT	PTEN WT; KRAS mut	PTEN WT; KRAS WT
Cetuximab	13.0	18.0	18.0	22.8
Bevacizumab	13.5	13.5	13.5	13.5

The estimated power to detect this interaction for sample sizes of 560 and 640 patients is 0.69 and 0.74, respectively, testing at 2-sided $\alpha=0.2$. This analysis will, thus, be considered exploratory.

Patient and tumor characteristics and outcomes will be compared between patients with and without tumor samples available for this analysis.

References

- 1) Lièvre A, Bachet JB, Boige V, *et al.* KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol.* 2008 Jan 20;26(3):374-9.
- 2) Lièvre A, Bachet JB, Le Corre D, *et al.* KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res.* 2006 Apr 15;66(8):3992-5.
- 3) 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.* 2008 Aug;5;99(3):551-2.
- 4) De Roock W, Piessevaux H, De Schutter J, *et al.* KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol.* 2008 Mar;19(3):508-15.
- 5) Li, DM, Sun H. TEP1, encoded by a candidate tumor suppressor locus is a novel protein tyrosine phosphatase regulated by transforming growth factor β . *Cancer Res.* 1997;57:2124-2129.
- 6) Steck PA, Pershouse MA, Jasser SA, *et al.* Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet.* 1997;15:356-262.
- 7) Li DM, Piluso LG, Cai X, *et al.* Mechanistic insights into maintenance of high p53 acetylation by PTEN. *Mol Cell.* 2006;23:575-587.
- 8) Nassif NT, Lobo GP, Wu X, *et al.* PTEN mutations are common in sporadic microsatellite stable colorectal cancer. *Oncogene.* 2004 Jan 15;23(2):617-28.
- 9) Mellinghoff IK, Wang MY, Vivanco I, *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med.* 2005 Nov 10;353(19):2012-24.
- 10) Nagata Y, Lan KH, Zhou X, *et al.* PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell.* 2004 Aug;6(2):117-27.
- 11) Berns K, Horlings HM, Hennessy BT, *et al.* A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell.* 2007 Oct;12(4):395-402.
- 12) Danielsen SA, Lind GE, Bjornslett M, *et al.* Novel mutations of the suppressor gene PTEN in colorectal carcinomas stratified by microsatellite instability- and TP53 mutation- status. *Human Mutation.* 2008;29:E252-E262.
- 13) Perrone F, Lampid A, Orsenigo M, *et al.* PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol.* 2009;20:84-90.
- 14) Loupakis F, Pollina L, Stasi I, *et. al.*: PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* 27(16):2622-2629, 2009

- 15) Laurent-Puig, Cayre A, Manceau G, et. al.: Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 27(35):5924-5930, 2009
- 16) Frattini, Saletti P, Romagnani E, et. al.: PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 97:1139-1145, 2007
- 17) Peterson B, George SL. Sample size requirements and length of study for testing interaction in a 2 x k factorial design when time-to-failure is the outcome. *Controlled Clin Trials* 14:511-522, 1993
- 18) Kendall, M. A New Measure of Rank Correlation. *Biometrika* 30 (1-2): 81–89, 1938

APPENDIX VII

UPC (Urine Protein to Creatinine) Ratio

The UPC (urine protein to creatinine) ratio directly correlates with the grams of protein found in a 24 hr urine. The UPC ratio can be used in the place of a 24-hour urine.

Procedure for Obtaining a Urine Protein/Creatinine Ratio:

1. Obtain at least 4 mL of a random urine sample in a sterile container (does not have to be a 24-hour urine sample).
2. Determine protein concentration (mg/dL).
3. Determine creatinine concentration (mg/dL).
4. Divide #2 by #3 above:

UPC Ratio =

$$\frac{\text{Protein Concentration (mg/dL)}}{\text{Creatinine Concentration (mg/dL)}}$$