PHASE II STUDY OF NEOADJUVANT GEMCITABINE, CISPLATIN AND BEVACIZUMAB IN STAGE IIIA (N2) NON-SQUAMOUS CELL NON-SMALL CELL LUNG CANCER

Abbreviated Title: Neoadjuvant therapy in NSCLC

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Précis

- Stage IIIA-N2 is considered one of the most therapeutically challenging and controversial subsets of lung cancer. This heterogenous group of patients, have tumors which range from minimal N2 (found incidentally during or after surgery) to multi-station bulky N2 disease. The extent of mediastinal involvement has an inverse correlation with survival.
- The 5-year survival ranges from 5-8% in patients with bulky N2 disease, to nearly 35% in patients with single station, microscopic N2 involvement[1].
- Neo-adjuvant chemotherapy and chemo-radiotherapy have been shown to be superior to surgery alone.
- Platinum-based induction chemotherapy in early and locally advanced NSCLC results in a radiological down-staging in at least 50% of patients, and a pathological complete response rate of approximately 5%[2,3,4].
- Concurrent chemo-radiotherapy as an induction regimen increases the radiological and pathological down-staging rate, but at the cost of increasing the morbidity and mortality of a surgical intervention.
- Expectations have now turned towards a possible incremental effect of adding a targeted biological agent to a standard induction treatment.

Primary Objectives

- To determine the safety of neo-adjuvant Gemcitabine/Cisplatin and Bevacizumab in stage IIIA-N2 NSCLC
- To determine the pathological complete response rate
- To determine the resectability rate
- To determine the extent of surgery

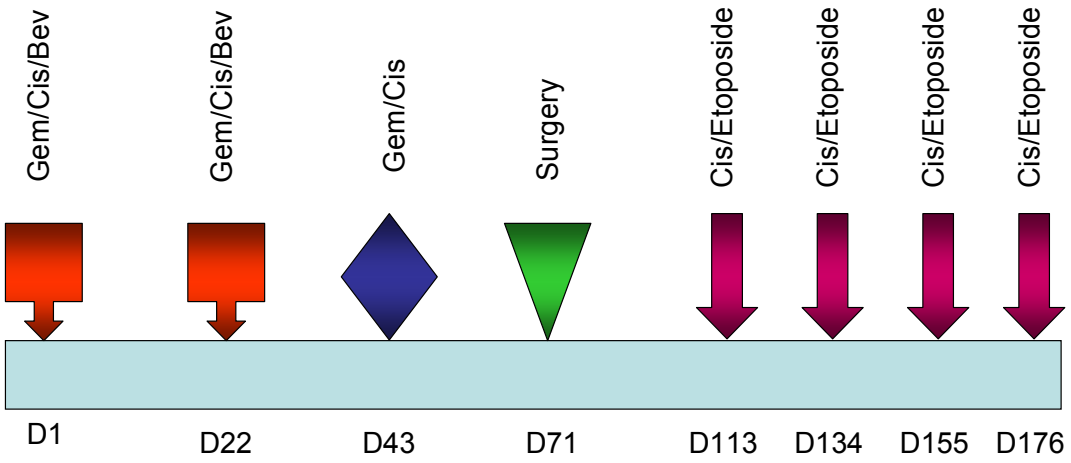
Eligibility

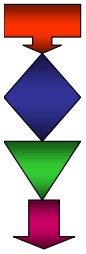
- Histologically confirmed stage IIIA-N2 NSCLC (non-squamous)
- No previous chemotherapy, radiotherapy, surgery or biological therapy for lung cancer
- Adequate organ and bone marrow function

Design

- Multi-center, International (USA/Croatia), open labeled phase II trial
- Following a Simon two-stage optimal design

Schema





Gemcitabine 1,250mg/m^2 on days 1 & 8, Cisplatin 80mg/m^2 on day 1 and Bevacizumab 7.5mg/Kg on day 1 every 21 days for 2 cycles only

Gemcitabine 1,250mg/m2 on days 1 & 8, Cisplatin 80mg/m2 on day 1 for one 21 day cycle

Surgical resection at least 4 weeks post C3 Gemcitabine/Cisplatin and 7 weeks post last Bevacizumab

Cisplatin 80mg/m^2 on day 1 & Etoposide 100mg/m^2 per day x 3 days on days 1-3, every 21 days x4 cycles

Table of Contents

1. Objectives

1.1 Primary protocol objectives

- Safety
- Pathological complete response rate
- Resectability rate
- Extent of surgery (Lobectomy Vs Pneumonectomy)

1.2 Secondary protocol objectives

- To evaluate progression free survival, median survival (MS) and overall survival (OS)
- Exploratory surrogate markers: (To be prioritized as follows)
    - Blood - Angiogenesis studies–CECs/CEPs, Myeloid subset analysis
    - Tissue
    1. Methylation microarray analysis
    2. KRAS mutation analysis
    3. Comparative genomic hybridization
    4. ERCC1 and RRM1 polymorphism and expression analysis

2. Background

2.1 Non-small cell lung cancer

Lung cancer is the leading cause of cancer related death in Europe and in the USA. Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancers and approximately 15% of patients with NSCLC are diagnosed with stage IIIA-N2[1]. This subset is perhaps the most controversial and therapeutically challenging of all lung cancer stages. Stage IIIA-N2 represents a heterogenous group of patients, ranging from minimal N2 (found incidentally during or after surgery) to multi-station bulky N2 disease. The extent of mediastinal involvement has an inverse correlation with survival. The 5-year survival ranges from 5-8% in patients with bulky N2 disease, to nearly 35% in patients with single station, microscopic N2 invovlement[1].

2.2 Systemic and Local Therapy

Platinum-based induction chemotherapy in early and locally advanced NSCLC results in radiological down-staging in at least 50% of patients, and a pathological complete response rate in only a small minority[5,6,7]. The simultaneous administration of chemotherapy and radiotherapy increases the radiological and pathological down-staging rate, but increases the morbidity and mortality of a surgical intervention, suggesting the gain in pathological response is lost by the operative mortality. In a meta-analysis, the combination of platinum-based chemotherapy and thoracic radiotherapy yielded a 5-year survival rate of 15% in locally advanced NSCLC[5], and this combination has been considered a standard treatment for patients with stage IIIA-N2 tumors with unresectable disease. Local

and distant failure rates remain high in patients treated with definitive chemo-radiation. Some authors continue to advocate in favor of the trimodality approach with surgical resection post chemo-radiation in selected patients with IIIA-N2 disease[6,7]. Trials of neo-adjuvant therapy followed by surgery have used both chemotherapy and chemo-radiotherapy as induction regimens[2,8].
Many institutions routinely use induction chemotherapy followed by surgery as treatment for stage IIIA-N2 disease. This schedule is based on small randomized studies showing that surgery alone is inferior to peri-operative chemotherapy and surgery[9,10]. The European Organization for Research and Treatment of Cancer (EORTC) performed a large multicenter randomized trial (08941) to compare surgery with radiotherapy in patients with stage IIIA-N2 NSCLC who showed response to induction chemotherapy[2]. Of the planned patients included in the study, 87% received the planned three cycles of induction chemotherapy, with an overall response rate of 61%. A 5% pathologic complete response rate was seen in those patients randomized to the surgical arm. Down-staging to NO or N1 disease was obtained in 41% of patients. There was no significant difference in median survival (17.5 months in the radiotherapy arm vs 16.4 months in the surgery arm), 5-year overall survival (14 vs 15.7%) or progression free survival. In a post hoc unplanned subgroup analysis on the surgical arm, 5-year survival was longer if a radical resection was performed, nodal down-staging was present or if a lobectomy was performed. Overall the author's conclusions were that if patients have a radiologic response to induction chemotherapy, surgery did not improve survival compared with radiotherapy.

In the US Intergroup trial 0139, patients with T1-3 pN2M0 NSCLC were randomized between induction chemo-radiotherapy followed by either surgery or consolidation radiotherapy to 61Gy[8]. Patients received two cycles of cisplatin and etoposide, concurrent with thoracic radiotherapy of 45Gy. Non-progressing patients then proceeded to either surgery or radiation and both arms received consolidation chemotherapy with two cycles of cisplatin/etoposide. Pathologic complete response was achieved in 15% of the patients. Progression-free survival was significantly better in the surgery group but overall survival did not differ, mainly due to postoperative mortality. Long term follow up confirms the significant improvement in progression-free survival, but not in overall survival, when surgery follows induction chemo-radiation. Three factors found in multivariate analysis to be associated with improved outcome were lobectomy, pathological down-staging and completeness of resection. Completeness of resection can only be defined post hoc, lymph node status is revised as part of pathological staging and no surgeon can be certain pre-operatively that a pneumonectomy will not be necessary for an individual patient with stage IIIA-N2 disease. This has been shown by the high rates of pneumonectomy (28-44%) in selected surgical series[11]. This figure is similar to the rate of 46% observed in EORTC 08941[2]. There are currently no comparative data showing that induction chemo-radiotherapy followed by surgery results in a better outcome than induction chemotherapy and surgery. Furthermore studies have shown that most

of the third-generation cytotoxic drugs have similar activity in locally advanced disease.

An improvement in our understanding of the molecular anomalies that lead to the development of lung cancer has lead to a renewed emphasis in developing targeted therapy that can be combined with standard cytotoxic chemotherapy in order to improve response rates and survival of patients with advanced lung cancer. Expectations have turned towards possible incremental benefits of adding the targeted biological agent, bevacizumab, to a standard induction regimen.

Bevacizumab is a novel targeted agent which has achieved proof of efficacy in combination with platinum-based doublet chemotherapy in NSCLC. A large phase III trial (Eastern Cooperative Oncology Group (ECOG) study 4599) evaluated bevacizumab plus chemotherapy in 878 treatment-naive patients with advanced nonsquamous NSCLC[12]. Patients were randomized to receive carboplatin (AUC of 6) / paclitaxel (200 mg/m^2) +/- bevacizumab (15 mg/kg) every 3 weeks for six cycles; bevacizumab monotherapy (15 mg/kg every 3 weeks) was then continued until progressive disease or intolerable toxicity, or up to 1 year. Patients receiving bevacizumab had significantly improved median overall survival (12.5 versus 10.2 months; P = 0.007), progression-free survival (6.4 versus 4.5 months; P < 0.0001), and response rates (27.2% versus 10.0%; P < 0.0001) compared with chemotherapy alone.

Based on E4599 results, the bevacizumab/carboplatin/paclitaxel regimen used in the trial has been adopted as the new ECOG standard of care for the first-line treatment of advanced NSCLC. The National Comprehensive Cancer Network has also recently incorporated bevacizumab (in combination with chemotherapy) into its treatment guidelines for NSCLC. In October 2006, the Food and Drug Administration (FDA) granted approval for the use of bevacizumab in combination with carboplatin/paclitaxel as first-line treatment for patients with advanced non-squamous NSCLC.

A large randomized phase III trial of cisplatin/gemcitabine +/- bevacizumab as first-line therapy in advanced non-squamous NSCLC (AVAiL) was performed in Europe[13]. A total of 1,044 patients were randomized to three arms to receive cisplatin/gemcitabine with or without one of two doses of bevacizumab (either low dose 7.5 mg/kg or high dose 15 mg/kg every 3 weeks). Preliminary analysis revealed that the 347 patients in the placebo arm had a median progression-free survival (PFS) time of 6.1 months, compared with 6.7 months for the 345 patients on low-dose bevacizumab, and 6.5 months for the 351 patients in the high-dose arm. A statistically significant reduction in the risk of relapse was observed in patients treated with bevacizumab and chemotherapy, HR: 0.75 (CI: 0.62, 0.91) P< 0.002625 in the low-dose arm and a HR: 0.82 (CI: 0.68, 0.98) P < 0.0301 in the high-dose arm. A statistically significant difference was found in the response rate (RR) observed in patients treated with bevacizumab and

chemotherapy, RR were 20%, 34% and 30% for placebo, low-dose bevacizumab, and high-dose arm, respectively. No improvement in overall survival has been reported.

Based on E4599 and AVAiL results, bevacizumab, was approved in August 2007, for the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC), in combination with platinum-based chemotherapy by the European Medicines Agency's (EMEA).

The aim of this study is to evaluate neo-adjuvant bevacizumab in combination with cisplatin and gemcitabine in stage IIIA N2 NSCLC prior to surgical resection.

2.3 VEGF Inhibition

The pioneering work of Folkman and others have shown that tumors require angiogenesis in order to grow beyond 1-2 mm^3 in size[14]. When a tumor acquires the ability to establish its own vasculature, its behavior becomes more aggressive[15]. The inhibition of new blood vessel growth has therefore become an important cancer control target. Vascular Endothelial Growth Factor (VEGF) is a potent endothelial cell mitogen, which is normally seen in certain physiologic situations (fetal development, menstruation, wound healing). High serum levels of circulating VEGF have been seen with a number of different tumor types.

Bevacizumab is a recombinant humanized monoclonal IgG_1 antibody that binds to and inhibits the biologic activity of human VEGF. Bevacizumab binds VEGF and prevents the interaction of VEGF to its receptors on the surface of endothelial cells. Two large randomized phase III clinical trials have shown a benefit in progression free survival, response rates and in one study overall survival when bevacizumab is added to a doublet of platinum based chemotherapy in the treatment of advanced non-small cell lung cancer[12,13].

There are limited data regarding the safety of peri-operative inhibition of VEGF. Roman, et al treated rats subjected to a lobectomy with SU5416, a VEGF flk-1/KDR receptor tyrosine kinase inhibitor at doses that had achieved a clear decrease in vascular flow assays in a previous murine model[16]. They observed no adverse effects on the animals' recovery and on their post operative wound healing. Additional experiments in different murine models suggest that there are likely enough differences between normal physiologic angiogenesis and neoplasm induced neo-angiogenesis to explain the fact that angiogenesis inhibition compromises tumor neo-vascularization sparing normal vascularization[17].

There are no studies to date investigating the safety and efficacy of bevacizumab added to gemcitabine and cisplatin in the neo-adjuvant setting in patients with resectable stage III NSCLC. The integration of novel effective cytotoxic biologic therapies with advanced surgical procedures has however transformed the

treatment of patients with colorectal cancer metastatic to the liver. In nonrandomized trials, the pre-operative administration of regimens containing chemotherapy in combination with bevacizumab appears to be both effective and safe.

The First BEAT study evaluated the efficacy and safety of bevacizumab with standard first-line fluoropyrimidine-based chemotherapy in patients with metatatic colorectal cancer[18]. The authors report potential curative surgery was perfomed in 12% of patients. A low rate of surgery related grade 3 AEs or SAEs were reported (wound healing complications 1.3%; bleeding 0.4%).

A second trial evaluated bevacizumab, capecitabine and oxalipaltin as neo-adjuvant therapy for patients with potentially curable metastatic colorectal cancer. The authors conclude that bevacizumab can be safely administered until 5 weeks before liver resection in patients with metastatic CRC without increasing the rate of surgical or wound healing complications or severity of bleeding[19].

2.4 Correlative studies background

2.4.1 Angiogenesis studies

Anti-angiogenic treatments for cancer, could be improved, if reliable surrogate markers of drug biological activity were available to help select and stratify the patients who are most likely to benefit from treatment. Many preclinical angiogenesis assays rely on the growth-factor-induced generation and quantification of new vessels at sites such as the cornea, skin or dorsal sac. Such surrogates are not adaptable to patients. To date the evaluation of microvessel density (MVD) in tumor biopsy samples has been used as a means of predicting and/or assessing the efficacy of anti-angiogenic therapies[20]. Another approach is the measurement of circulating levels of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), hepatocyte growth factor (HGF) and interleukin 8 (IL8), among others. Unfortunately no growth factor to date has been validated for predicting a response to anti-angiogenic therapies. Another possible approach to investigate anti-angiogenic activity is by utilizing functional imaging via dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to measure tumor blood flow and vascular permeability[21]. The effectiveness of this approach remains to be validated and its use requires expensive instrumentation that is limited to a small number of institutions.

The possibility that the measurement of circulating endothelial cells (CEC's) and circulating endothelial progenitors (CEP's) might be exploited as surrogate markers of angiogenesis and anti-angiogenic drug activity has been studied at both the preclinical and clinical levels and will be evaluated in this trial.

Recently, much research has focused on a population of myeloid cells, identified by the expression of the cell surface markers CD11b, which include a variety of cell types such as neutrophils, immature dendritic cells, monocytes and early myeloid progenitors. Interest in these cells which includes a class of immune suppressive cells termed myeloid-derived suppressor cells (MDSC), relates to their ability to promote tumor progression. Tie2-expressing monocytes have also been reported to promote tumor growth through secretion of proangiogenic factors[22] Recruitment of these cells may represent a cellular mechanism underlying the inherent refractoriness that tumors can develop in response to anti-VEGF therapy[23]. This study will analyze CEC's/CEP's, MDSC, Tie2+ monocytes and VEGFR1+ hemangiocytes before treatment and on specified dates after administration of Gemcitabine/Cisplatin and Bevacizumab.

2.4.2 Comparative Genomic Hybridization

Chromosomal aberrations are thought to be critical events in human tumorigenesis, and several genomic regions frequently harboring DNA gains (3q, 5p, 7q, 8q, 11q and 16p) and losses (3p, 4q, 5q, 6q, 8p, 9p and 13q, 17q) have been identified in NSCLC patients. Using array based comparative genomic hybridization (aCGH) and gene expression microarrays, DNA copy number changes and gene expression can be measured throughout the whole genome of tumor cells. We will combine the data from these analyses to obtain an integrated genome wide view of gene dosage aberrations and their effect on gene expression, which might help in identifying genes important in NSCLC.

2.4.3 Methylation microarray analysis

Epigenetic gene silencing is a molecular mechanism of silencing a gene by methylating its promoter region. Epigenetic silencing is involved in the initiation and progression of several types of cancer, including lung cancer[24,25]. The detection of epigenetic alterations with the use of methylation-specific polymerase-chain-reaction (PCR) assays may allow for the molecular staging of cancer. We will utilize methylation-specific PCR assays to define patterns of DNA methlyation that may delineate the behaviour of the primary tumor.

2.4.4 ERCC1 and RRM1 IHC/SNP analysis

Many published candidate gene association studies have assessed cancer risk by examining a single SNP per gene or a single locus at a time analysis approach. The ERCC1 gene encodes a subunit of the nucleotide excision repair complex required for the incision step of nucleotide excision repair (NER)[26]. Since the ERCC1 protein is essential for NER and influences genomic instability, polymorphisms in ERCC1 may play a role in carcinogenesis. A synonymous mutation at 500 (C>T) in the messenger RNA, both coding for asparagines (Asn, or N) at 118, was found to be correlated to important clinical endpoints of

platinum-based chemotherapy[47,48]. In general, the variant allele T was thought to result in decreased codon usage, which in turn compromises ERCC1 protein expression. Patients with T allele were found to respond to platinum-based chemotherapy better than those possessing C allele. In addition, ERCC1 expression level in the tumors was suggested to have prognostic value for platinum-based chemotherapy[49-52]. Patients with low ERCC1 expression showed survival benefit from platinum-containing regimens. This study will examine whether the genotype of the germ line mutation ERCC1 N118N (500 C>T), and/or ERCC1 expression level in tumors could be used to predict a survival benefit of chemotherapy.

RRM1, encodes the regulatory subunit of ribonucleotide reductase. It is located on chromosome segment 11p15.5, a region with frequent loss of heterozygosity in non small cell lung cancer[27,28]. Low levels of expression of the gene are associated with poor survival among patients with non small cell lung cancer[29]. An increase in the expression of the RRM1 protein increases the expression of the phosphatase and tensin homologue (PTEN), an inhibitor of cell proliferation; decreases the phosphorylation of focal adhesion kinase; and decreases cell migration and invasiveness[30]. RRM1 is also the predominant cellular determinant of the efficacy of the nucleoside analogue gemcitabine (2',2'-difluorodeoxycytidine)[31,32]. In addition to the gene expression, two genetic polymorphisms (-37 and -524) in the promoter region of RRM1 gene showed prognostic value for gemcitabine in patients with NSCLC[55]. The response rate was significantly higher in the patients carrying RR37AC-RR524CT genotype compared with the patients containing other genotypes (P = 0.039).

Blood samples will be collected from each patient enrolled in this study to extract the genomic DNA for genotyping of ERCC1 polymorphism. In addition, tumor tissues, stromal cells surrounding the tumors and a small amount of normal tissue from each patient will be collected to be used in the immuno-staining study.

2.5 Rationale

The combination of anti-VEGF therapies with platinum based doublets has been shown to improve overall survival in metastatic NSCLC. Neo-adjuvant chemotherapy utilizing the combination of Cisplatin and Gemcitabine has been shown to lead to a down-staging of stage IIIA N2 NSCLC and a pathological complete response rate in a small number of tumors. The addition of bevacizumab to this platinum based doublet in the neo-adjuvant setting has not previously been evaluated in a clinical study. It is hoped that this combination of cytotoxic chemotherapy with bevacizumab will be safe and effective in improving pathological complete response rates leading to improved resectability, progression free and overall survival.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1. Histologically or cytologically documented non squamous cell non-small cell lung cancer and confirmed by the pathological laboratories at participating centers.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques or as >10 mm with spiral CT scan. See section 9.2 for the evaluation of measurable disease.

3.1.3 Stage IIIA (N2) disease. All patients will require a baseline mediastinoscopy to ensure histological proof of N2 disease. (See appendix I for staging of non-small cell lung cancer).

3.1.4 No prior treatment for lung cancer including chemotherapy, radiotherapy, surgery or biological therapy.

3.1.5 Age ≥18 years (males or non-pregnant females).

3.1.6 Life expectancy of greater than 3 months

3.1.7 ECOG performance status 0-1 (Karnofsky >60%, see Appendix II)

3.1.8 Adequate pulmonary and cardiovascular function to tolerate planned surgical resection:

Pulmonary Function criteria:

- paO2 >65 mmHg, paCO2 <45 mmHg on room air ABG
- Anticipated post-op FEV1 > or equal to 40% predicted
- Anticipated post-op DLCO > or equal to 40% predicted
- If anticipated post-op FEV1 or DLCO < 40% predicted, must have VO2 >15ml/kg on oxygen consumption study

Cardiac criteria:

- LVEF > 40%
- No pulmonary hypertension or RV dysfunction
- No unstable angina

3.1.9 Serum Creatinine ≤1.5mg/dl

3.1.10 Hemoglobin (baseline) ≥ 10.0g/dl

3.1.11 Absolute neutrophil count ≥ 1,500/m^3 and platelets ≥ 100,000/m^3

3.1.12 AST/SGOT and ALT/SGPT ≤ 2.5 x ULN, total bilirubin ≤ 1.5 x ULN (In patients with evidence of Gilberts disease, elevated bilirubin should not be related to tumor or other liver diseases and should be less than or equal 2 x upper limit of normal)

3.1.13 The ability to understand and the willingness to sign a written informed consent document and the ability to comply with the requirements of the protocol.

3.1.14 Women of childbearing potential must have a negative pregnancy test and both men and women must be willing to

consent to using effective contraception while on treatment and for at least 3 months thereafter

3.2 Exclusion Criteria

3.2.1 Squamous cell cancer or mixed tumors with small cell elements
3.2.2 Tumor of any histology in close proximity to a major vessel or cavitation (Any tumor abutting an interlobar, main pulmonary artery, vena cava or major vein will be exluded).
3.2.3 History of hemoptysis (bright red blood of ½ teaspoon or more [≥ 2.5 mL]) unrelated to any diagnostic procedure. (Patients who have a history of hemoptysis that occurred >3 months prior to study entry and that is assessed not to be related to tumor may be eligible)
3.2.4 Patients with metastatic disease
3.2.5 History of uncontrolled or labile hypertension, defined as blood pressure > 150/100mmHg (NCI CTCAE v.3.0 grade ≥ 2), systolic blood pressure > 180 mm Hg if diastolic blood pressure < 90 mm Hg, or diastolic blood pressure > 90 mm Hg, on at least 2 repeated determinations on separate days within 3 months prior to study enrollment. Patients who have medication controlled hypertension are eligible for the study.
3.2.6 Any of the following within 6 months prior to study enrollment: myocardial infarction, severe/unstable angina pectoris or uncontrolled angina pectoris, coronary/peripheral artery bypass graft, NYHA class III or IV congestive heart failure, clinically significant peripheral vascular disease (Grade II or greater)
3.2.7 Psychiatric or neurologic illness that would limit compliance with study requirements.
3.2.8 Patients with serious illness or medical condition
3.2.9 Active infection within 14 days before beginning treatment.
3.2.10 Patients may not be receiving any other investigational agents.
3.2.11 History of a malignancy in the last five years other than in situ carcinoma of the cervix, or non-melanomatous skin cancers.
3.2.12 Patients must not be on therapeutic anticoagulation or chronic daily treatment with aspirin 325mg/day within 10 days prior to day 1 on study. Prophylactic anticoagulation during peri-operative period is acceptable. Full dose aspirin post surgical resection is acceptable. Low dose aspirin 81mg/day and anticoagulation for line protection are allowed in the peri-operative period and the adjuvant setting.
3.2.13 Women who are breast feeding.
3.2.14 History of stroke or transient ischemic attack within 6 months
3.2.15 History of pulmonary embolism, deep venous thrombosis or other thrombo-embolic event within 6 months prior to study

3.2.16 Patients with a history of severe hypersensitivity reaction to compounds of similar chemical or biologic composition to cisplatin, gemcitabine, bevacizumab, etoposide or other agents used in the study.
3.2.17 History of a major surgical procedure, open biopsy, or a significant traumatic injury within 35 days prior to commencing treatment, or the anticipation of the need for a major surgical procedure during the course of the study prior to the predetermined date of tumor excision. Fine needle aspirations, core biopsies or mediastinoscopies within 7 days prior to commencing treatment.
3.2.18 History of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess or tracheo-esophageal fistula
3.2.19 Non-healing wound or ulcer
3.2.20 Evidence of coagulopathic disorder or hemorrhagic diathesis. INR greater than 1.5.
3.2.21 Patients with existing ototoxicity
3.2.22 Pregnancy (positive pregnancy test)
3.2.23 Urine protein: creatinine ratio ≥ 1.0 at screening.
3.2.24 Patients known to be HIV-positive or have active hepatitis B/C (due to possible interaction between chemotherapy and HAART and antiviral medications used for treatment of active hepatitis B/C)
3.2.25 Serious illness that may preclude adherence to the protocol.

3.3 On-Study research eligibility evaluation

3.3.1 Complete history and physical examination - including height, weight, vital signs and ECOG performance score (see appendix II).

3.3.2 Imaging Studies (Baseline) - Every patient should have a baseline radiological evaluation with PET/CT scan, CT scan of chest and either MRI or contrast CT of brain. Imaging of other areas of known or suspected disease involvement should also be performed prior to receiving treatment. This must be completed within 28 days prior to enrollment.

3.3.3 A baseline CXR,EKG, and arterial blood gas should be obtained within 14 days prior to enrollment. Pulmonary function tests, Thallium stress test and Echo are also required as baseline investigations to determine suitability for surgery.

3.3.4 Laboratory Evaluation [baseline is to be obtained within one week prior to enrollment].
3.3.4.1 Hematological Profile: Complete blood count (CBC) with differential and platelet count, Prothrombin time/International normalized ratio, activated partial thromboplastin time.

3.3.4.2 Biochemical Profile: Sodium, potassium, blood urea nitrogen (BUN), creatinine, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, lactic acid dehydrogenase (LDH), chloride and bicarbonate.

3.3.4.3 Serum or urine beta-hCG for female patients of childbearing age within 7 days prior to therapy.

3.3.4.4 Urinalysis for urinary protein or urinary protein creatinine (UPC) ratio

3.3.5 Histologic confirmation: The initial pathological diagnosis will be performed by either the NCI laboratory of pathology or the pathology department in Zagreb Croatia. A block of primary tissue from the time of staging (mediastinoscopy + bronchoscopy) and the surgically resected specimen (post neo-adjuvant treatment) will be required from each patient and sent to the laboratory department in the NCI to confirm the diagnosis (N2 disease) and to perform correlative studies.

3.4 Patient Registration

For patients at the NCI, CCR authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://intranet.cancer.gov/ccr/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office (CRO), CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information.

All patients must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. A protocol registration form and cover memo will be supplied by the Coordinating Center, NCI CCR and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To register a subject, fax the completed registration checklist and cover memo to the CRO at 301-480-0757. Please indicate on the protocol registration form whether the patient is screening or is eligible to start treatment. The CRO will notify you either by e-mail or fax that the protocol registration form has been received. The CRO will assign a unique "900" patient/subject ID number for each subject that will be used to enter data into the C3D data base. Questions about eligibility should be directed to the Coordinating Center's

Research Nurse, Michell Manu at 301-402-4423 or manumichell@mail.nih.gov. Technical questions about the form should be directed to the Central Registration Office (301-402-1732).

Off-Study Procedure: Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (http://intranet.cancer.gov/ccr/welcome.htm) main page must be completed and faxed to 301-480-0757. The coordinating center will provide sites with off study forms.

4.0 Study Implementation

4.1 Treatment Plan

Cycle 1 and 2:
Cisplatin 80mg/m^2 day 1 every 21 days for 3 cycles + Gemcitabine 1250mg/m^2 dose for two doses on days 1 and day 8 every 21 days for 3 cycles + Bevacizumab 7.5 mg/kg on day 1 every 21 days for **first 2 cycles only**

↓

Cycle 3:
Cisplatin 80mg/m^2 on day 1 + Gemcitabine 1250mg/m^2·dose for two doses on days 1 and 8. **No bevacizumab on 3rd cycle**.

↓

Re-staging and Response Evaluation following third cycle of chemotherapy, no more than 14 days prior to surgical resection

↓

Surgery: 4-6 weeks post completion of last cycle of Cisplatin/Gemcitabine.

↓

Adjuvant chemotherapy 4-8 weeks post surgery. All patients will Receive adjuvant chemotherapy irrespective of pathological staging

Post surgery. Cisplatin 80mg/m^2 on day 1, every 3 weeks x 4 cycles + Etoposide 100mg/m^2 IV per day for consecutive 3 days, on days 1 to 3 every 3 weeks for 4 cycles.

4.2 Drug Administration

4.2.1 Gemcitabine administration

Gemcitabine will be diluted in 250 – 500 mL 0.9% Sodium Chloride Injection (0.9%NS) maintaining a concentration ≥0.1 mg/mL, and administered intravenously before cisplatin at a fixed dose rate of 10 mg/m^2·minute (600 mg/m^2·hour).

4.2.2 Cisplatin administration

4.2.2.1 Hydration
Euvolemic patients will receive hydration with 1000 mL 0.9%NS intravenously over 1 hour before receiving cisplatin.

Patients who have received adequate hydration should receive Mannitol 12.5 grams intravenously over 5 to 10 minutes just prior to starting cisplatin.

- Osmotically-induced diuresis requires maintenance of adequate fluid input during and after mannitol administration.

Post-cisplatin hydration with 1000 mL 0.9%NS should be administered intravenously over 1 to 2 hours after cisplatin administration is completed. In the adjuvant setting 1000 mL 0.9% NS over 1 to 2 hours should be administered after Etoposide administration is completed.

- Additional IV fluids may be administered *ad libitum*.
- Encourage ambulatory outpatients to continue liberally ingesting water, sports drinks and other non-alcoholic beverages for at least 24 hours after receiving cisplatin.

4.2.2.2 Administration
Cisplatin will be diluted in 100 mL 0.9%NS and administered intravenously after gemcitabine over 60 minutes.

4.2.3 Bevacizumab administration
Bevacizumab will be diluted in a total volume of 100 mL 0.9%NS, and administered intravenously on day 1 after gemcitabine and cisplatin, every 21 days **ONLY during the first two cycles.**

- The initial Bevacizumab dose must be administered over at least 90 (± 10) min.

- The second Bevacizumab dose may be given over 60 (±10) min if administration over 90 min was well tolerated.
- In the event of an infusion-related event, bevacizumab administration should be interrupted until acute symptoms resolve. The PI or a medically responsible AI will be notified of the event at the time of the occurrence. After resolution of adverse signs and symptoms, bevacizumab administration may resume at a rate which increases the total planned infusion time by 30 minutes. For example, for a 60-minute infusion, the rate should be decreased to deliver the volume over 90 minutes (from 1.7 mL/h to 1.1 mL/h).

4.2.3.1 On days when bevacizumab is administered, the following evaluations should be performed: (Bevacizumab dose modifications see section 5.5)

- review of systems pertinent to bleeding and thrombosis,
- spot urine protein/creatinine ratio, &
- blood pressure measurement.

4.2.3.2 Dose timing adjustments are listed in section 5.3.

4.2.3.3 If a patient's weight changes by ≥ 10% during the course of the study, bevacizumab dose will be recalculated.

4.2.4 Etoposide administration (adjuvant setting)
If the calculated dose of etoposide is 100-200mg then dilute with 500ml of either 5% dextrose or 0.9% normal saline. If the calculated dose is 201-400mg then dilute with 1000ml of either 5% dextrose or 0.9% normal saline. Administer etoposide IV over at least 60 minutes, daily for 3 consecutive days every 21 days.

4.3 Supportive Care
Prophylactic antiemetics, during and after, cisplatin administration will be as per standard practice in each participating center and the choice of agents administered is at the discretion of the principal investigator at each site.

4.3.1 Antiemetics for cisplatin: (alternative antiemetic schedules are acceptable, at the discretion of the treating physician at each participating site).

Acute and Delayed emesis prophylaxis will be delivered with cisplatin chemotherapy. Alternative drugs and dosing strategies are listed.
Antiemetic Primary Prophylaxis:

Day 1: a serotonin receptor (5-HT_3) subtype antagonist + a high potency glucocorticoid + a neurokinin receptor (NK_1) subtype antagonist before cisplatin administration.

- e.g., ondansetron 16 – 24 mg PO *or* 8 – 12 mg IV + dexamethasone 12 – 20 mg PO *or* 10 – 12 mg IV + aprepitant 125 mg PO *or* fosaprepitant 115 mg IV

Days 2 & 3: a high potency glucocorticoid + a NK_1 subtype antagonist.

- e.g., dexamethasone 8 mg PO twice daily *or* 8 mg IV once daily + aprepitant 80 mg/day PO

Other acceptable Anti-emetics (administered as per recommended guidelines)

- 5-HT_3 receptor antagonists: palonosetron, granisetron
- D_2 receptor antagonists: metoclopramide, domperidone, prochloroperazine, thiethylperazine, metopimazine
- Benzodiazepines: lorazepam, alprazolam

4.3.2 Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should only be given for a platelet count below 10,000/μl. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count ≥ 50,000/μl.

4.3.3 Symptomatic anemia should be treated with appropriate red blood cell support and transfusion is recommended if the hemoglobin falls below 8 g/dl.

4.3.4 Central venous access devices such as a temporary internal jugular line, PICC lines via the brachial vein, semi-permanent HICKMAN®, GROSHONG®, or medi-port implanted devices are acceptable for this study. All devices will have nursing supervision to include patient self-care and cleaning/flushing of the devices.

4.3.5 Nutritional assessment and psychological support: Neoplasms are commonly complicated by malnutrition. Patients with weight loss or evidence of wasting syndrome should have a nutritional consult. Patients who are having emotional difficulties dealing with their treatment, and disease, or those patients who request assistance, will be referred to a Social Worker for evaluation and support.

4.4 Surgical evaluation/management

Patients with biopsy-proven or suspected primary lung cancers who are deemed potentially eligible for study will undergo comprehensive evaluation by thoracic surgery staff to determine/confirm tumor histology, pathologic stage, tumor resectability and operability. Evaluation will consist of physical exam, blood work as listed in Section 3.3, chest X-RAY, CT scan of the chest, PET/CT scan, and

brain MRI scan or CT brain with contrast. Additional imaging studies (ie bone scan, plain films, CT or MR scans of extremities or spine) will be obtained if clinically indicated.

Patients deemed to have clinical stage III disease will undergo bronchoscopy and cervical mediastinoscopy (CME) to confirm N2 disease and rule out N3 nodal metastases. CME will be considered complete if upper and lower right paratracheal (stations 2 and 4), left paratracheal (stations 2 and 4) and station 7 are biopsied. Other techniques such as video-assisted thoracoscopic or endobronchial FNA techniques may be used to assess these stations if they cannot be reached by CME due to inflammation/adhesions, as well as stations 5 and 6 (which cannot be assessed by standard CME) if clinically indicated for left-sided lesions. Tissue from patients with stage IIIA (N2) disease will be obtained for aforementioned staging purposes as well as translational endpoints.. Patients who have no evidence of N2 metastases as well as those found to have N3 (stage IIIB) disease will not be eligible for this study.

In addition to the aforementioned pathologic staging studies, patients will undergo evaluation of cardiopulmonary reserve via pulmonary function testing (arterial blood gas, spirometry with DLCO, and oxygen consumption studies, if indicated) as well as cardiology evaluation (EKG and thallium stress test and ECHO). Additional studies such as cardiac CT/MR, and coronary angiography, will be performed, if indicated. Patients deemed to have acceptable cardiopulmonary reserve to tolerate resection as indicated in section 3.1.8 (inclusion criteria) will undergo induction Cisplatin/Gemcitabine and Bevacizumab therapy as per protocol. Two to three weeks following completion of induction therapy patients will undergo repeat evaluation by Medical Oncology/Thoracic Surgery personnel to determine clinical response to therapy, and confirm potential tumor resectability and operability. Repeat staging studies will consist of CXR, CT scan of the chest, PET/CT scan and brain MR or CT brain with contrast, as well as additional imaging studies if clinically indicated. Any possible N3 or new sites of potentially distant disease will be evaluated by endo-bronchial FNA, CME, VATS, or CT-guided FNA techniques to rule-out inoperable disease prior to surgery. In addition, patients will undergo repeat pulmonary function tests with arterial blood gas, as well as EKG and ECHO to rule out potentially significant deterioration of cardiopulmonary status that could affect operability or peri-operative risk. All staging studies and pre-surgical tests obtained at outside participating institutions will be centrally reviewed by NCI Thoracic Surgery personnel to maintain consistency of surgical management.

Patients meeting criteria for resection (clinically stable disease or tumor regression, no evidence of new sites of disease, and stable cardiopulmonary status) will undergo bronchoscopy and thoracotomy with lobectomy/ pneumonectomy and mediastinal lymph node dissection. All patients will have thoracic epidurals placed for post-operative analgesia, unless technically or medically contra-indicated. Bronchoplastic/ angioplastic techniques will be used if

possible to avoid pneumonectomy. All reasonable efforts will be made to assure microscopically-negative margins of resection by intra-operative frozen section analysis of bronchial as well as chest wall or parenchymal margins in the case of extended lobectomy. Mediastinal lymph node dissection will include stations 9, 8, 7, 10, 4/3, and 2 for right-sided neoplasm, and stations 9, 8, 7, 10, 6, and 5 for left-sided lesions. Post-operatively, patients will be managed according to standard of care surgical guidelines regarding pulmonary hygiene, pain control, chest tube removal, etc. Patients will have routine beta-blocker or calcium channel antagonist prophylaxis for SVT, lovenox or heparin prophylaxis for DVT, acid-reduction regimen consisting of H2 antagonist or proton-pump inhibitor, and any other medications specifically indicated for co-morbidities. One month following surgery, patients will return to the clinic for evaluation by Thoracic Surgery staff prior to commencing post-operative chemotherapy per protocol.

4.5 Tissue sample acquisition and processing

4.5.1 At the time of biopsy of primary lung lesions, mediastinoscopy, and definitive lung cancer resections, tissue will be obtained for staging purposes. Additional tumor biopsies/aspirates will be obtained for evaluation of molecular endpoints. Tissue aspirates/biopsies will be immediately snap-frozen in liquid nitrogen and thereafter stored at -80°C..Tissues obtained from resections performed at the NCI will also be harvested in order to establish cell lines, and isolate cancer stem cells.

All samples from the NCI will be codified and anonomized upon transport to the Thoracic Oncology Laboratory, (Building 10; Rm 3-5848), using the NCI Lab Matrix Database. Peripheral blood samples (10 ml) will be collected at baseline, immediately prior to surgery, 1month, and three months post-op, and at all subsequent scheduled follow-up appointments. Serum from these samples will be stored at -80^{0}C. Samples from Croatian patients will be coded (by using the Harris Orcan unique patient identifying number assigned at the time of NCI central registration) at the time of sampling, frozen, tracked and shipped via FedEx or World Courier to the Laboratory of Dr William Figg (See Appendix IX – NIH policy manual 1340-1, permits for import and export of biological materials). Application form 75-3 CDC for a permit to import or transport etiologic agents, hosts or vectors of human disease can be downloaded from http://dohs.ors.od.nih.gov/pdf/PHS%20Application%20to%20Import.pdf.
(See appendix X - SOP describing all the procedures necessary to prepare biological samples for transport to the Clinical Pharmacology Program, Medical Oncology Branch, National Cancer Institute).

Packaging and shipping will comply with IATAs dangerous goods regulations and with department of transport (DOT) regulations 49 CFR 171-178 which can be found at http://www.access.gpo.gov/nara/cfr/waisidx_07/49cfrv2_07.html. For information on packaging and shipping training, visit the DOHS website at

http://dohs.ors.od.nih.gov/Resources_main.htm. A NIH commercial invoice form 1884-1 is attached as appendix XII.

Upon reaching the NCI, samples will be sent to the Lab of Dr William Figg, whereby, they will be codified, anonomized and stored for future analysis (See Appendix VII). Data will be entered as outlined in Appendix XI (SOP describes how to enter new sample data into LabSamples for the Clinical Pharmacology Program, Medical Oncology Branch, CCR, NCI). Thereafter, samples will be sent to the Laboratories of Dr Schrump, Dr Giaccone, Dr Meltzer and Dr Trepel. Tissues will be distributed to appropriate labs for micro-array, immuno-histochemistry, and ELISA analysis of DNA methylation gene expression and gene expression profiling, analysis of k-ras mutation status and ERCC1/RRM1 expression, and circulating VEGF levels.

4.5.2 Sample processing for angiogenesis studies

Draw blood into 3 CPT citrate tubes (blue/black tops). Samples are required at baseline (cycle 1 day 1), cycle 1 day 8, cycle 1 day 22, day of surgery and approximately 72 hours post surgery. Spin samples as per manufacturer's instructions within 3 hours of collection. Collect the following:

(1) The top 1ml (plasma) per tube and store at -80°celcius or in liquid nitrogen.
(2) The cells right above the gel (the cloudy interface between the plasma and the gel). Count the number of cells via a hemocytometer or a coulter counter and mix with cryomedium of approximately 5 x 10^6 per vial.

Store the samples at -80°celcius in an isopropanol container overnight and transfer to liquid nitrogen the next day.

Specimens that were not collected at the National Institutes of Health will be shipped to Lab of Dr William Figg as outlined above and then sent to the lab of Dr Jane Trepel (Building 10, Room 12N218).

4.6 Protocol evaluation

Baseline non-radiological evaluations are to be conducted within 1 week prior to start of study therapy. PET/CT and CT chest, abdomen and pelvis and either brain MRI or CT brain with contrast must be carried out within 28 days prior to the start of therapy.

4.7 Concurrent therapies

The combination of cisplatin, gemcitabine and bevacizumab is an approved chemotherapeutic drug combination in stage IV NSCLC, however, because there is a potential for interaction with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Patients will be advised to discuss

taking any drugs, over-the-counter medications, or alternative therapies with a member of the research team.

4.8 Reassessment

Patients will be seen in clinic at least every 1 week while receiving neo-adjuvant therapy and 3 weekly while receiving adjuvant therapy. A history and physical with sphygmomanometry and a review of systems that documents coagulopathy-related events must be charted in the medical record for each visit. PET/CT and CT/MRI scans will be obtained at baseline and within 2 weeks before surgery to monitor disease response. Measurable disease will be monitored as described in section 9. Blood pressure monitoring will be based on published recommendations (NHLBI, JNC7). The PI will be notified of any abnormal measurement (any systolic BP over 140 or diastolic BP > 90). Treatment will be determined by the BP on the day of the clinic visit surrounding the treatment. Urinalysis every 3 weeks for proteinuria and calculation of a urine/protein creatinine ratio will be determined prior to each administration of bevacizumab (Bevacizumab dose modifications see section 5.5).

4.9 Duration of therapy

Patients will receive three 3-week cycles of neo-adjuvant chemotherapy leading to 63 days/9 week duration of therapy prior to surgery. There will then be a 4 to 6 week period prior to surgery (7 to 9 weeks after completion of bevacizumab). Post-operatively (4-8 weeks) patients who are medically fit to receive adjuvant chemotherapy will receive up to 4 cycles of adjuvant chemotherapy (see section 4.0).

4.10 Off study criteria

1. Progression of disease during treatment on study protocol.
   No less than 4 weeks/28 days post completion of last cycle of Cisplatin/Gemcitabine and 7 weeks/49 days post completion of second infusion of Bevacizumab. No more than 12 weeks/85 days from the start of therapy. Decision will be made at the time of restaging (restaging prior to surgery). However, if clinically indicated, a decision may be made following the first or second cycle, after obtaining the appropriate staging studies.

2. Grade 3/4 adverse event lasting greater than 2 weeks or when therapy is judged detrimental to the patient's health.

3. Grade 4 hypersensitivity reaction to drug administration (anaphylaxis).

4. Patient non-compliance or voluntary withdrawal.

5. If a patient is deemed medically unfit to continue on study, at the discretion of the Principal Investigator.

Unacceptable toxicities that have not resolved at time of “off treatment” or “off study” must be followed until stabilization or resolution.

4.11 Post study evaluation (Follow-up)

Patients will be evaluated one month post adjuvant chemotherapy and then every 3 months for the first 2 years, every 6 months for 3 years and then yearly for 3 years. Follow up will include clinical evaluation, a chest x-ray and additional investigations when clinically indicated.

5.0 Dosing delays and dose modification

5.1 Gemcitabine/Cisplatin/Etoposide - Hematologic toxicities

5.1.1 Neutropenia/Thrombocytopenia

Patients will have a complete blood count with differential checked prior to each dose of chemotherapy, on day 1 and on day 8 of neo-adjuvant therapy. Patients not eligible for treatment due to low neutrophil count or platelet count must return in one week for reassessment.

- During cycle 1 (neoadjuvant setting), patients should not receive prophylactic filgrastim or pegfilgrastim to support their white blood cell count.
- Any grade 4 neutropenia, or grade 3-4 neutropenia associated with fever (one reading of temperature ≥ 38.5° C or two readings of >38° C one hour apart), in addition to commencing recommended institutional antibiotic therapy may be treated with filgrastim or pegfilgrastim at the discretion of the treating physician. If filgrastim or pegfilgrastim is used to support the neutrophil count, no dose reduction for neutropenia is required unless growth factor support is insufficient to prevent subsequent grade 3/4 neutropenia.
- Patients who experience grade 4 neutropenia on protocol (with or without fever), or require dose delay due to neutropenia, will receive prophylactic pegfilgrastim or filgrastim during cycle 2 and 3 of chemotherapy.
- See Table 1 for dose adjustment criteria.

Table 1: Day 1 Cisplatin/Gemcitabine/Bevacizumab

Absolute neutrophil count		Platelets	Treatment
≥ 1000/μl	and	≥100,000/μl	Treat
<1000/μl	or	<100,000/μl	Hold therapy until ANC ≥1000/μl and platelets ≥100,000/μl

Table 1: Day 8 Gemcitabine only

Absolute neutrophil count		Platelets	Treatment
>1000/μl	And	100,000/μl	Treat with full dose
750-1000/μl	And	75,000 - 100,000/μl	25% dose reduction of Gemcitabine
500-750/μl	And	50,000 - 75,000/μl	50% dose reduction of Gemcitabine
<500/μl	or	<50,000/μl	Hold Gemcitabine

5.2 Gemcitabine/Cisplatin/Etoposide – Non-Hematologic toxicities
All non hematological toxicities should be resolved to grade 1 or less at the start of each cycle with the exception of alopecia.

5.3 Dose modifications for subsequent cycles of Cisplatin/Gemcitabine

- Non hematological grade 3 or 4 toxicities during the previous cycle will require a 25% dose reduction in the dose of both Cisplatin and Gemcitabine with the exception of:

    a. ototoxicity (grade 3/4) which will require substitution of Carboplatin for Cisplatin
    b. nephrotoxicity (grade 3/4) which will require substitution of Carboplatin for Cisplatin
    c. neurotoxicity (recurring or worsening grade 2) which will require substitution of Carboplatin for Cisplatin

- Grade 3 or 4 toxicity of nausea and vomiting from a previous cycle will not require a dose reduction unless symptoms are refractory to antiemetic medications as outlined in section 4.3.1.

5.4 In the adjuvant setting the same dose reductions and modifications apply for Etoposide and Cisplatin as outlined above.

5.5 Bevacizumab dose modifications

1. Hypertension: Hypertension is one of the major toxicities that may be experienced with bevacizumab. All patients will have blood pressure measured and recorded weekly during the first 9 weeks of the study. Patients who have an asymptomatic increase > 20 mmHg (diastolic) or > 150/100mmHg recurrent or

persistent more than 24h: Antihypertensive therapy (or adding another category of antihypertensive drug if the patient already being treated for HTN) monotherapy will be initiated. Bevacizumab should be held for systolic BP (SBP) > 150 mmHg or diastolic BP (DBP) > 100 mmHg. If symptomatic, bevacizumab will be held until symptoms resolve. Bevacizumab should be discontinued for grade 4 hypertension. The presence of hypertension and proteinuria would favor the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, more so because angiotensin inhibitors restore nephrin expression.

2. Hemorrhage/Bleeding: Bevacizumab administration is contingent upon assessment of any persistent bleeding. Active bleeding such as new petechiae, epistaxis, intermittent minimal hemoptysis or nasal secretions, or other grade 1 or greater bleeding events (excluding dipstick positive urine without red blood cells by microscopic exam) will require additional workup and subsequent risk assessment. Grade 2/3/4 hemorrhage: Bevacizumab will be stopped and not re-introduced.

3.Proteinuria: A spot UPC ratio should be performed prior to each bevacizumab dose. Repeat testing should be performed no sooner than 4 days. If bevacizumab is delayed for more than 2 weeks due to proteinuria, discontinue bevacizumab.

Table 2: Bevacizumab dose modification (See appendix III)

UPC RATIO	**BEVACIZUMAB DOSE MODIFICATION**
<2	Continue Bevacizumab
$\geq 2 - 3.5$	Hold Bevacizumab and recheck UPC in 1 week. UPC must resolve <1.0 in order to resume Bevacizumab
> 3.5	Hold Bevacizumab and recheck UPC in 1 week. UPC must resolve <1.0 in order to resume Bevacizumab.

4. Surgical or periodontal procedures: If there is a need for a major surgical or serious periodontal procedure, bevacizumab should be held for 4 weeks prior to the procedure and must not be resumed until 4 weeks after the surgical procedure. Longer delays may be necessary if clinically indicated in order to insure that adequate healing has taken place prior to bevacizumab resumption. Minor oral or periodontal procedures or surgical procedures may be done with no delay at the discretion of the PI.

5. Thrombosis

Arterial Thromobsis: Patients will be taken off study in the event of arterial thrombosis. Arterial thrombosis includes CNS ischemia, cardiac ischemia, and any visceral or peripheral artery thrombosis.

Venous Thrombosis: For venous thrombosis requiring systemic anticoagulation, patients will not receive further bevacizumab.

6. Coagulopathy: Patients with grade 3-4 coagulopathy will be taken off study.

7. Thrombocytopenia (platelets < 50,000/μl - grade 3 or greater): Bevacizumab should be held until thrombocytopenia resolves to grade 1 or better (platelet count ≥ 75,000/μl). If treatment is delayed for more than 2 weeks, discontinue therapy. Thrombocytopenia due to Cisplatin and Gemcitabine will require dose reductions of these agents. (see cisplatin and gemcitabine dose modifications, section 5.1.1).

8. Infusion Reactions: Infusion reactions with the first dose of Bevacizumab are uncommon (<3%) and severe reactions can occur in 0.2% of patients. Infusion reactions reported in clinical trials include hypertension, hypertensive crises associated with neurologic signs and symptoms, wheezing, oxygen desaturation, NCI-CTC Grade 3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis. Adequate information on re-challenge is not available. Patients who develop grade 2 allergic reactions with urticaria and dyspnea or any grade 3/4 allergic/infusional reactions should have bevacizumab discontinued and not be re-challenged. Grade 1 Infusion reactions with transient flushing or rash canbe rechallenged 24 hours later after premedication with dexamethasone 20 mg IV 30 minutes before the infusion. The infusion rate should be increased to 3 hours. If tolerated, the subsequent dose can be given with 50% of the steroid dose and a decreased duration of infusion from 3 hours → 90 minutes.

9. Reversible posterior leukoencephalopathy syndrome (RPLS): Bevacizumab should be held in patients with signs and symptoms suggestive of reversible posterior leukoencephalopathy syndrome (RPLS), pending work-up and management, including control of blood pressure and MRI brain to rule out brain metastases. Bevacizumab should be discontinued upon diagnosis of RPLS.

10. Bevacizumab should be withheld in the event of Grade 3 LFT elevations (Liver transaminases or alkaline phosphatase greater than 5.0 x upper limit of normal, total bilirubin greater than 3.0 x upper limit of normal), and should not resume until the abnormalities have recovered to grade 1 or lower. If LFT elevations recur with retreatment, bevacizumab should be permanently discontinued.

6.0 Pharmaceutical Information

6.1 Gemcitabine (see product information appendix VIII)

6.2 Cisplatin (see product information appendix VIII)

6.3 Bevacizumab (see product information appendix VIII)

6.4 Etoposide (see product information appendix VIII)

7.0 Correlative Studies

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations), the principal investigator must amend the protocol and obtain informed consent from all research subjects.

Samples to be obtained for correlative studies (Section 4.5.1 for sample management):

1. Block of formalin-fixed, paraffin-embedded primary tumor for
--Immunohistochemistry for ERCC1 and RRM1
--DNA extraction for CGH and methylation assays
--KRAS mutation analysis

2. Peripheral blood drawn into 3 CPT citrate tubes for CEC, CEP, MDSC, $Tie2^+$ monocytes, $VEGFR1^+$ hemangiocytes (See section 4.5.2)

7.1 Angiogenesis studies

7.1.1 Method for identification of CEC and CEP

Multiparameter flow cytometry is currently the best way to identify CEC and CEP cells[33]. For this analysis, peripheral blood is drawn into 3 CPT citrate tubes (approximately 8 cc per tube) (See section 4.5.2 for sample processing).

Mononuclear cells are isolated by centrifugation, washed with PBS, and FcR solution (Miltenyi) is added to block non-specific binding. For identification of CECs and CEPs, cells are stained with FITC- conjugated anti-CD31 (BD), PerCP-conjugated anti-CD45 (BD), APC-conjugated anti-CD133 (Miltenyi) and PE-conjugated CD146 (Chemicon) for 30 min on ice. For isotype controls, cells are incubated with FITC-conjugated IgG1, PerCP-conjugated IgG1, APC-conjugated IgG1 and PE-conjugated IgG1. After washing with 0.1% BSA in PBS, the cells are incubated with Hoechst 33258 dye as an indicator of cell viability

and analyzed by multiparametric flow cytometry on an LSR II flow cytometer (BD Biosciences), using LSR II-equipped digital data acquisition and FlowJo cytometric data analysis software. The CEC and CEP cell concentrations are calculated as a percentage of the total number of mononuclear cells or as the number of cells/microliter of whole blood after an evaluation of a minimum of 10^5 cellular events, and preferable 10^6 cellular events. CEC cells are defined by the co-expression, or absence of expression on a single cell of the following parameters: CD45-, CD31+, CD133- and CD146+, Hoechst 33258- (viable CEC) or CD45-, CD31+, CD133- and CD146+, Hoechst 33258+ (nonviable CEC). CEP cells are defined by the co-expression, or absence of expression on a single cell of the following parameters: CD45- or dim, CD31+, CD133+ and CD146-, Hoechst 33258- (viable CEP) or CD45- or dim, CD31+, CD133+ and CD146-, Hoechst 33258+ (nonviable CEP). CEC and CEP markers may be modified or expanded in response to new studies in this rapidly developing field. Additional parameters may be added specific to the targeted agent, i.e. flow cytometric analysis of p53 levels or the level of p53 target gene products in CEP and other progenitor cells.

The assays will be performed by Dr. Min-Jung Lee and Dr. Yeong Sang Kim in Jane Trepel's laboratory in the Medical Oncology Branch, Building 10, Room 12N218. This group has particular expertise in multiparametric flow cytometry pharmacodynamic assays[34,35], including development of flow cytometric assays that were used for several published clinical trials[36,37], and a patent in the National Phase of Foreign Filing on the use of multiparameter flow cytometry for pharmacodynamic analysis[38].

The outcome measures will be the number of CEC and CEP per 10^6 mononuclear cells or per microliter of peripheral blood, analyzed in samples taken before and after treatment. These numbers will then be examined for correlations with various parameters to assess their potential utility as surrogate biomarkers for drug activity, for establishing the optimal biologic dose, for patient stratification, and monitoring of therapy-related side effects.

7.1.2 Myeloid Subset Analysis

Peripheral blood will be analyzed for 3 myeloid subsets: MDSC, (myeloid-derived suppressor cells), Tie2+ proangiogenic monocytes, and VEGFR1+ hemangiocytes, using multiparameter flow cytometry.

The markers to be used and the references for each subtype are included in table 3.

Note that 3 CPT citrate tubes collected should be sufficient for the 3 myeloid subpopulations as well as CEC and CEP cells. All analyses will be performed in accord with the number of cells available. Markers may be modified as more detailed studies become available.

Table 3: Angiogenesis studies

Sample	Target population	Marker	Ref.
PBMC 3 CPT citrate tubes (Becton Dickinson)	1. CEC/CEP		
	2. MDSC (Myeloid-derived suppressor cells)	lineage(CD3,14,19,56)- HLA-DR - CD33+ CD11b+	39,40,41,42
	3. Tie2+ monocyte	Tie2+ CD14 low CD16 +	39,40,43,44,45
	4. VEGFR1+ hemangiocyte	VEGFR1+ CD11b+	39,40,46

7.2 KRAS mutation analysis:
Tissue (whether fresh or archival) for KRAS mutation status will be analyzed at the pathology department in the National Cancer Institute.

7.3 Comparative Genomic Hybridization:
As exploratory analyses, in collaboration with Dr. Paul Meltzer, we will utilize patient's tumor tissue to determine if there is any correlation between chromosomal gains or losses in comparative genomic hybridization in non small cell lung carcinomas and clinical outcome.

7.3.1 Collection of Specimen(s):
Archival material: A block of archival tumor material will be requested from each patient. In addition, a recent resection sample or blocks of tissue from the original resection will be required (see section 4.5.1).

7.3.2 Handling of Specimens(s):
Comparative Genomic Hybridization (CGH) is a whole genome scanning method which can measure changes in copy number across the whole genome. This technique has the potential to uncover regions of the genome which undergo recurrent alteration during tumor progression. Recent advances allow this technique to be performed at gene level resolution using microarrays. Similarly, this technique can now be used to study the methylation status of thousands of sites in the genome. CGH and methylation assays will be performed on DNA extracted from formalin fixed paraffin embedded tumor tissue (FFPE) using previously collected excess material which is not necessary for diagnostic purposes. Procedures will be carried out in the Clinical Molecular Profiling Core

lab. One 5 micron section will be cut from the FFPE block and stained using hematoxylin and eosin to guide the collection of tumor tissue. If a suitable stained section is already available, that will be used instead. Using the stained section to identify the location of tumor within the block, a core of tissue will be collected using a 1mm punch. The block will then be stabilized by the insertion of a paraffin plug. If the FFPE material is not of sufficient size for tissue collection by the punch method, 5 unstained slides will be cut from the FFPE block and the tumor tissue scraped from the slides. DNA will be then be extracted from the FFPE tissue using the ATL tissue lysis buffer (Qiagen) and the DNeasy kit (Qiagen). 900ng DNA will be labeled for hybridization to CGH and 500ng utilized for methylation microarrays using standard methods (Barrett et al.; Bibikova et al.). If insufficient DNA is unavailable, 10-20ng of DNA will be amplified using the WGA2 kit (Sigma) and 2.5 micrograms of the amplification product will be used for CGH.

7.3.3 Site(s) Performing Correlative Study:
CGH will be performed in collaboration with Dr. Paul Meltzer, Clinical Molecular Profiling Core lab, Bldg. 37 room 6138, tel. (301) 496-5266.

7.4 Methylation microarray analysis

DNA and RNA will be isolated from tumor aspirates. Bisulfite modified DNA will be amplified using WGA procedures perfected in the Thoracic Oncology Laboratory. RNA will be amplified using established techniques. Genomic DNA methylation and gene expression signatures will be assessed by Illumina micro-arrays, as well as customized arrays containing a variety of cancer-testis gene sequences, developed in the Thoracic Oncology Lab, Surgery Branch, NCI. Software assisted higher order analysis will be performed to examine if DNA methylation and /or gene expression signatures appear to be associated with response to therapy. These assays, will be performed by Thoracic Oncology lab personnel in collaboration with Dr. Paul Meltzer's group at the NIH, are considered hypothesis generating; data from these preliminary studies may be further evaluated in a more formal manner in subsequent clinical trials.

7.5 ERCC1 and RRM1 polymorphism and expression measurement:

ERCC1 is the DNA repair gene involved in Nucleotide Excision Repair (NER) pathway that is mainly responsible for removing the DNA damage caused by cisplatin. A synonymous mutation at 500 (C>T) in the messenger RNA, both coding for asparagines (Asn, or N) at 118, was found to be correlated to important clinical endpoints of platinum-based chemotherapy[47,48]. In general, the variant allele T was thought to result in decreased codon usage, which in turn compromises ERCC1 protein expression. Patients with T allele were found to respond to platinum-based chemotherapy better than those possessing C allele. In addition, ERCC1 expression level in the tumors was suggested to have prognostic value for platinum-based chemotherapy[49-52]. Patients with low ERCC1

expression showed survival benefit from platinum-containing regimens. This study will examine whether the genotype of the germ line mutation ERCC1 N118N (500 C>T), and/or ERCC1 expression level in tumors could be used to predict a survival benefit of chemotherapy. Ribonucleotide reductase M1, also known as RRM1, encodes the regulatory subunit of this enzyme and is a molecular target of gemcitabine. Gemcitabine-treated patients with high tumoral RRM1 expression generally signal poor prognoses[53,54]. In addition to the gene expression, two genetic polymorphisms (-37 and -524) in the promoter region of RRM1 gene showed prognostic value for gemcitabine in patients with NSCLC[55]. The response rate was significantly higher in the patients carrying RR37AC-RR524CT genotype compared with the patients containing other genotypes (P = 0.039).

7.5.1 Collecting of Specimens(s):
Blood samples will be collected from each patient enrolled in this study to extract the genomic DNA for genotyping of ERCC1 polymorphism. In addition, tumor tissues, stromal cells surrounding the tumors and a small amount of normal tissue from each patient will be collected to be used in the immuno-staining study.

7.5.2 Handling of Specimens(s):
Immuno-staining of ERCC1 will be performed using paraffin-embedded tumor samples from previously collected material. Specimens will be exposed to 10 mM citrate buffer (pH 6.0) and heated for 30 minutes in a water bath. Sections of tumors will be incubated for 60 minutes with a monoclonal antibody specific against the full-length human ERCC1 protein (mouse, clone 8F1, Neomarkers). Binding of the antibody will be determined using NovaRED as the substrate and Mayers hematoxylin as the counterstain[56]. The normal tissue sections and the stromal cells surrounding the tumor will be used as external and internal positive controls. Total RNA will be extracted using PicoPure RNA isolation kit (KIT0204; Arcturus, Mountain View, CA) for the measurement of RRM1 expression. Complementary DNA will be synthesized using Superscript II and oligo-dT from Invitrogen (Carlsbad, CA). Real-time quantitative RT-PCR gene analysis will be performed in triplicate per sample and gene in 96-well plates using ABI prism 7700. Genotyping experiments will be performed using genomic DNA isolated from serum using the QIAmp DNA blood mini-kit (Qiagen, Inc, Valencia, CA). Polymerase chain reaction (PCR) will be performed using the Platinum Taq PCR Kit from Invitrogen (Carlsbad, CA) with gene-specific primers. Direct nucleotide sequencing PCR will be conducted using the Big Dye Terminator Cycle Sequencing Ready Reaction kit V3.1 (Applied Biosystems, Foster City, CA) and an ABI Prism 3130 Genetic Analyzer according to the manufacturers instructions.

7.5.3 Site(s) Performing Correlative Study:
These studies will be performed in collaboration with Dr. William Figg at the Medical Oncology Branch, CCR, NCI, Bldg 10 room 5A07, tel. (301) 451-5859.

8.0 Study calendar

Parameter	Prestudy[1]	Before Each Neoadjuvant Cycle[2]	Prior to Surgery	Before Each Adjuvant Cycle	Follow - Up[5]
History	X	X	X	X	X
Physical examination	X	X	X	X	X
Height (Prestudy only) and Weight	X	X	X	X	X
Measurement of tumor[4]	X		X		X
Performance status	X	X	X	X	X
CBC, differential, platelet count, Coagulation studies	X	X	X	X	X
Serum Chemistries (must include, bilirubin, Alk Phos, ALT and AST, Albumin, LDH)	X	X	X	X	X
Glucose and Electrolytes (Na, K, C, C1, CO_2, BUN, glucose, calcium, phosphorous, magnesium, bicarbonate), urinalysis and UP/C, 24 hr urine collection.[7]	X	X	X		X
Beta HCG[2,3]	X				
Tumor Imaging[4]	X		X		
Brain CT or MRI[6]	X		X		
CXR/EKG/PFT/ABG and Echo[1]	X		X		
Thallium Stress Test	X				

[1] Within 14 days before initiating chemotherapy and within 14 days prior to surgery
[2] Within 7 days prior to receiving chemotherapy
[3] For women of childbearing potential.
[4] This consists of a CT scan thorax through to the adrenals, and a PET/CT scan, within 28 days of initiation of therapy, and at the completion of neo-adjuvant chemotherapy within 14 days prior to surgery. Restaging CT scans at the end of adjuvant chemotherapy and every 3 months for 2 years, every 6 months for 3 years, then yearly for 3 years.

[5] Every 3 months for 2 years, every 6 months for 3 years, then yearly for 3 years.
[6] At investigator's discretion, depending on symptoms
[7] Please see Appendix III for determination of UP/C. UP/C should be performed prior to all 3 cycles of neo-adjuvant therapy. 24 Hr. urine collection should be performed for all patients with a UP/C ≥ 2

9. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response at the end of treatment prior to surgery.

9.1 Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable," in reference to measurability, will not be used because it does not provide additional meaning or accuracy.

9.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.2 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

9.1.3 Target lesions

All measurable lesions up to a maximum of five lesions per organ should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated 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 by which to characterize the objective tumor response.

9.1.4 Non-target lesions

All other lesions should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

9.2 Guidelines for Evaluation of Measurable Disease

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

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

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

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

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm.

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

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases.
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.

9.3 Response Criteria

9.3.1 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions

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.

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

9.3.2 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.

Incomplete Response/Stable Disease (SD):
Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Although a clear progression of “non-target” lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail and the progression status should be confirmed at a later time by the review panel (or study chair).

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

9.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1).

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	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 classified as having "symptomatic deterioration." 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 on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

9.4 Confirmatory Measurement/Duration of Response

9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed four 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 and at a minimum interval of eight weeks (see section 9.3.3).

9.4.2 Duration of overall response

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

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

9.4.3 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.

9.5 Time to Progression and Overall Survival

- Time to progression: The time between the first day of treatment to the day of disease progression as described in section 9.3.
- Overall survival: The time between the first day of treatment to the days of death.

9.6 Response Review

Patients with measurable disease will be assessed by standard criteria. The purpose of tumor measurements will be to assess benefit to the patients from treatment and to determine appropriateness for continuing on study. For the purposes of this study, patients should be re-evaluated after the third cycle of neo-adjuvant treatment and before surgery. The response will be evaluated by the radiology departments at the Clinical Center Bethesda and in Zagreb Croatia.

10.0 REGULATORY AND REPORTING REQUIREMENTS

Adverse Event Definitions

10.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

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10.1.2 Serious Adverse Event (SAE)

Serious adverse drug experience (or SAE): Any adverse drug experience occurring during any study phase (treatment or follow-up) and at any dose that results in any of the following outcomes:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Disability: A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience: Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

10.1.3 Unexpected adverse drug experience:

Any adverse drug experience, the specificity or severity of which is not consistent with the current **package insert**. ''Unexpected,'' as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

10.2 NCI-IRB Adverse Event Reporting

10.2.1 NCI-IRB Expedited Reporting of and Adverse Events and Deaths

The Protocol PI will report to the NCI-IRB:

- All serious adverse events (SAEs) that are **not** in the consent form, but are possibly, probably or definitely related to the research. A SAE is defined as an untoward medical occurrence that
  - resulted in a death;
  - was life-threatening;
  - required or prolonged hospitalization;
  - caused persistent or significant disability/incapacity;
  - resulted in congenital anomalies or birth defects; or
  - required intervention to prevent permanent impairment or death.
- All other deaths not included in the SAE category above, except deaths due to progressive disease.

- All deaths that occur within 30 days of the last dose of study drug or treatment, except deaths due to progressive disease.
- All grade 3 and 4 (CTCAE) events that are not in the consent and that are possibly, probably or definitely related to the research.

Reports must be received by the NCI-IRB within 7 working days via iRIS or for participating sites using form supplies in Appendix VI

10.2.2 NCI-IRB Requirements for PI Reporting of Expected (In Consent) and Unexpected (Not in Consent) Adverse Events at Continuing Review

The protocol PI will report to the NCI-IRB:

- All Grade 2 events that are not in the consent form, but are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.
- **NOTE**: Grade 1 events are not required to be reported.

The form for reporting to the FDA is the Voluntary MedWatch 3500 Form which can be found at: http://www.fda.gov/medwatch/how.html. Only serious, unexpected, and unrelated events will be reported to the FDA. Outside sites will fill out the Voluntary MedWatch 3500 form and fax or email to the Coordinating Center. Forms can be faxed to Arlene Berman 301-480-2590 or emailed to arleneb@mail.nih.gov

Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released without patient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act.

11.0 STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

The primary objective of this study is to determine in patients with stage IIIA N2 NSCLC if the combination of bevacizumab, gemcitabine, and cisplatin is able to result in a rate of pathologic complete responses (PCR) which is approximately double that of platinum and gemcitabine alone.

The study will be conducted as an optimal two-stage phase II trial (Simon R, Controlled Clinical Trials 10:1-10, 1989), in order to rule out an unacceptably low 5% PCR rate (p0=0.05; that is, 5% which was observed for patients receiving platinum and gemcitabine alone) in favor of a targeted, improved rate consistent with 15% (p1=0.15). With alpha=0.10 (probability of accepting a poor regimen=0.10) and beta = 0.10 (probability of rejecting a good regimen=0.10), the study will initially enroll 28 evaluable patients, and if 0-1 of the 28 are able to have a PCR then no further patients will be accrued. If 2 or more of the first 28 patients have a PCR, then accrual would continue until a total of 66 patients have been treated. A temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 to 5 patients with a PCR in the total of 66 patients, then this would be an uninterestingly low rate, while if there were 6 or more patients of the 66 who have a PCR, this would be sufficiently interesting to warrant further study of this regimen in later trials. Under the null hypothesis (5% PCR rate), the probability of early termination is 59%.

At present there are no published trials involving neoadjuvant bevacizumab in NSCLC. Unpublished studies have documented the potential for an increase in pulmonary hemorrhage or tracheosophageal fistula formation. These studies have either involved concurrent chemoradiation or used a higher dose of bevacizumab (15mg/Kg) or a greater number of induction bevacizumab treatments (3 cycles). In this study the dose of bevacizumab is 7.5mg/kg, patients will receive 2 doses of bevacizumab only and radiation will not be administered.

An interim evaluation for safety will be performed. The incidence of grade 3 toxicities will be compared to existing data involving neoadjuvant gemcitabine/cisplatin (see table 4, 5, 6, 7) .If more than one patient in the first 10 enrolled is noted to have a fistula formation, or to have Grade 3 hemorrhaging within the first 10 patients enrolled, then the trial will be modified to reduce the likelihood of this occurring further. After 28 patients have been enrolled, a further evaluation of toxicities will take place and incidences noted of grade 3 toxicities will be compared to those associated with patients receiving standard chemotherapy (without bevacizumab). The magnitude of the noted toxicities relative to those associated with standard therapy will be used to guide further

treatment modifications if necessary. For example, if the associated lower 80% confidence interval bound for any individual toxicity incidence in this trial exceeds the actual rate known for the same type of toxicity in conventional treatment of similar patients, then this will trigger an evaluation to determine if modifications to the regimen are warranted.

Table 4: Hematologic Toxicity (N=47) for Induction Gemcitabine and Cisplatin for patients with stage IIIA-N2 NSCLC[57]

	Grade 0		**Grade 1**		**Grade 2**		**Grade 3**		**Grade 4**	
	No. Pts	%	No. Pts	%	No. Pts	%	No. Pts	%	No. Pts	%
Leukocytes	7	14.9.	10	21.3	16	34.0	14	29.8	---	
Neutrophils	16	34.0	2	4.3	11	23.4	8	17.0	10	21.3
Platelets	2	4.3	4	8.5	13	27.7	11	23.4	17	36.2
Hemoglobin	---		13	27.7	27	57.5	6	12.8	1	2.2

Table 5: Nonhematologic Toxicities (N=47) for Induction Gemcitabine and Cisplatin for patients with stage IIIA-N2 NSCLC[57]

	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**% G3/4**
Nausea	10	13	17	7	---	14.8%
Vomiting	14	10	18	5	---	10.6%
Headache	36	4	1	0	0	0
Neuropathy	37	9	0	0	0	0
Other neurotoxicity	23	8	8	3	2	10.6%
Alopecia	19	8	14	5	---	10.6%
Lethargy	12	16	16	3	---	6.4%
Creatinine	35	9	1	---	1	2.1%
Febrile neutropenia	46	---	---	1	---	2.1%
Infection	37	---	3	1	---	2.1%
Diarrhea	38	6	2	1	---	2.1%
Hemorrhage	29	11	1	1	---	2.1%
Weight loss	38	4	1	1	---	2.1%
Dyspnea	36	1	---	1	---	2.1%

Table 6: Hematologic Toxicity (N=136) for Induction Gemcitabine/Cisplatin and Docetaxel for patients with stage IIIA-N2 and IIIB (T4N0-1) NSCLC[58]

	Grade 0		Grade 1		Grade 2		Grade 3		Grade 4	
	No. Pts	%	No. Pts	%	No. Pts	%	No. Pts	%	No. Pts	%
Leukocytes	17	12.5	21	15.4	38	27.9	50	36.8	10	7.4
Neutrophils	19	14.0	10	7.4	22	16.2	34	25.0	51	37.5
Platelets	5	3.7	61	44.9	35	25.7	30	22.1	5	3.7
Hemoglobin	12	8.8	71	52.2	41	30.1	9	6.6	3	2.2

Table 7: Nonhematologic Toxicity (N=136) for Induction Gemcitabine/Cisplatin and Docetaxel for patients with stage IIIA-N2 and IIIB (T4N0-1) NSCLC[58]

	Grade 1		Grade 2		Grade 3		Grade 4		% G3/4	
N/V	37	27.2%	39	28.7%	13	9.6%	0	0%	0	0%
Neuropathy	4	2.9%	1	0.7%	1	0.7%	0	0%	1	0.7%
Alopecia	25	18.4%	37	27.2%	0	0%	0	0%	0	0%
Creatinine	0	0%	1	0.7%	0	0%	0	0%	0	0%
Diarrhea	14	10.3%	11	8.1%	7	5.1%	3	2.2%	10	7.4%
Asthenia	34	25.0%	37	27.2%	10	7.4%	0	0%	10	7.4%

11.2 Early Stopping Rule for Pulmonary Embolism (PE)

Following review by the Safety Monitoring Committee on August 4, 2010, an early stopping rule for pulmonary embolism (PE) will be implemented as follows: The study will continue to accrue patients until a total of 10 have been treated and evaluated for development of a PE. The expected incidence of PE is 7%. At present, 3 out of 7 patients have developed a PE. If there are 3 patients with PE after 10 have been treated, the lower one-sided 97.5% CI bound (corresponding to a two-sided 95% CI) is 6.7%. The lower one-sided 95% CI bound is 8.7% with 3/10. If the rate becomes 4/10, the lower one-sided 97.5% CI bound would be 12.2% and the lower one-sided 95% CI bound would be 15.0%. Thus, if 4 patients in the first 10 have a PE, this is likely to be in excess of what would be expected and accrual will stop pending re-evaluation of the treatments provided on the trial. Should accrual continue past 10 patients, the rate will be re-examined again after each additional 5 patients have been enrolled and if any of the subsequent evaluations result in a lower one-sided 95% bound for PE incidence exceeding 10%, the trial would be stopped at that time. If accrual stops and treatment is modified as a result, the stopping rules will start over beginning with the patients treated under the modified regimen. At this time there will be no prophylactic treatment for Pulmonary Embolisms but treatment will be determined on an individual basis.

11.3 Sample Size/Accrual Rate

It is anticipated that up to 3 patients per month may be enrolled onto this trial, and thus up to two years may be required to enroll 66 evaluable patients. To allow for a small number of patients who may be in-evaluable, the accrual ceiling will be set at 70.

11.4 Stratification Factors - N/A

11.5 Analysis of Secondary Endpoints

For all evaluable patients, Kaplan-Meier analyses of time to progression and overall survival will also be performed. Appropriate confidence intervals at selected time points will also be provided. Since these will be considered secondary endpoints, the results will be reported as part of the descriptive report of the study.

Toxicity will be tabulated by type and grade, for all types of toxicity identified as being at least possibly attributable to the agent.

All molecular and other secondary evaluations will be considered exploratory analyses. However, as an illustration for any given evaluation, if 10 paired results are available (before and after treatment); there is 80% power to declare an effect equal to one standard deviation of the change to be statistically significant at the 0.05 level with a two-tailed test. In general, it is anticipated that non-parametric analyses will generally be used for these evaluations because of the limited number of subjects. In view of the exploratory nature of these analyses, any p-values reported will not be adjusted for multiple comparisons, and results of any such analyses will be stated carefully as being hypothesis generating, requiring additional confirmation

11.6 Reporting and Exclusions

11.6.1 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Gemcitabine, Cisplatin, Bevacizumab and Etoposide.

11.6.2 Evaluation of response. All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not

assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 will be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible patients. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis will be clearly reported. The 95% confidence intervals will also be provided.

12.0 HUMAN SUBJECTS PROTECTIONS

12.1 Rationale for Subject Selection

This study will be open to all individual with stage IIIA N2 NSCLC regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, only pregnant women and children are excluded from this study. This study will be recruited through internal referral, our local physician referral base, and through physicians in Croatia. All individuals with newly diagnosed stage IIIA N2 NSCLC are eligible according to the eligibility criteria within section 3. This is a Phase II trial designed to evaluate the safety and efficacy of Gemcitabine, Cisplatin and Bevacizumab in stage IIIA N2 NSCLC, study the side effect profile of the combination of drugs, characterize the pharmacokinetics, and assess several biological endpoints. Patients should realize that we are hopeful that they may gain benefit from this study, but there is no objective evidence to support our optimism at this time. Patients must not have received prior treatment for their lung cancer. Subjects from both genders and all racial /ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section 3. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one

hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

12.2 Justification for Exclusions

Due to lack of knowledge of the effects of Gemcitabine, Cisplatin and Bevacizumab on the fetus or on infants, as well as the possibility of teratogenic effects, pregnant and nursing women will be excluded from this trial. Patients with unstable or serious medical conditions (ongoing or active infection, symptomatic congestive heart failure (AHA Class II or worse), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements) are excluded due to the possibility that the combination of Gemcitabine, Cisplatin and Bevacizumab may worsen their condition and the likelihood that the underlying condition may obscure the attribution of adverse events with respect to this chemotherapeutic regimen.

12.3 Participation of Children

Patients under the age of 18 will be excluded from study because of the relative infrequence of NSCLC in this group.

12.4 Evaluation of Benefits and Risks/Discomforts

The potential benefit to a patient who enters study is a reduction in the bulk of his/her tumor, which may or may not have a favorable impact on extent of surgical resection and/or survival. Potential risks include the possible occurrence of any of a range of side effects that are listed in the pharmaceutical section and the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described earlier.

12.4.1 Risks/Benefits Analysis

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland and the University hospital for lung diseases, Jordanovac, Zagreb, Croatia. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5 Consent and Assent Process and Documentation

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient at a subsequent visit. The original signed consent goes to Medical Records; copy placed in research record

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

12.6 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. Every effort will be made to recruit women and minorities in this study.

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Multi-Institutional guidelines

13.1.1 IRB Approvals: The PI will provide the NCI IRB and Central Registration Office with a copy of the participating institution's approved yearly continuing review. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB.

13.1.2 Amendments and Consents: The CCR PI will provide the NCI IRB with copies of all amendments, consents and approvals from each participating institution.

13.1.3 Data and Specimen Collection Procedures: The PI will provide specific guidelines for quality assurance, data collection and format, and data receipt by the coordinating institution (recommend at least quarterly). It is recommended that data collection forms/system be consistent for all institutions. All adverse events from participating institutions must be submitted to the NCI IRB according to current NCI IRB policy.

13.1.4 NCI Guidance for Reporting Serious Adverse Events for Multi-Center Trials: The reporting requirements for adverse events in multi-center trials when the NCI PI is responsible for the research and the coordination of the other research sites is the same as with any NCI

intramural research protocol. Serious adverse event reports should be submitted from the participating centers along with a PI assessment of the event in the same time frame as adverse event reports that occur at the NCI. The review of the event should be submitted when available. If the NCI is a participating site, rather than the responsible site, then the serious adverse event report should be submitted per protocol

13.1.5 Data Center Audits: The PI will provide guidelines for audits of participating institutions. Selected patient charts should be audited as well as the participating institutions Standard Operating Procedures (SOP) at the time of the visit. Data from participating institutions should be available when the protocol is audited at the NCI.

Drug Distribution: Lead Associate Investigators will order drug as per local guidelines.

Collection and Toxicity Reporting: Required data include, concurrent medications that are not disease related do not need to be entered into the NCI C3D database with the exception of steroids. Participating institutions must submit ADRs as specified under section 10.0. All data collection forms and the NCI IRB copy of the adverse event report must be submitted to the following address: Arlene Berman, RN, Building 10, Room 12N226, 10 Center Drive, Bethesda, MD 20892. FAX# 301-480-2590.

Data and Center Audits: Participating centers will be audited at least every 1 year. Selected patient charts will be audited as well as the participating institutions Standard Operating Procedures (SOP) at the time of the visit. Data from participating institutions should be available when the protocol is audited at the NCI.

13.2 Data Safety and Monitoring Plan:

This is an investigator initiated trial. The NCI PI will be responsible for patients enrolled at all institutions. Serious adverse event reporting is outlined in section 10.0. The participating sites will fax or email copies of SAEs to the Coordinating Center. These will be reviewed by the Research Team. Toxicity reports will be run quarterly to include data from all participating sites. These reports will be reviewed by the Principal Investigator and communicated to the Lead Associate Investigator. A site visit to Croatia will be made prior to opening the study to ensure understanding of protocol data collection requirements. Audits will be conducted yearly to insure data integrity and to provide quality control. The site will perform ongoing quality assurance checks in the C3D data base to look for data discrepancies. Issues will be brought to the attention of the PI and the site PI.

13.3 Data Collection:

The investigators will be responsible for the collection, maintenance, security and quality control of all study data. Meetings chaired by the principal investigator and/or lead associate investigator will be held on a monthly basis to review the study data for quality, completeness, and interim analysis. Data will be captured on the NCI C3D database.

- All toxicities related to hemorrhage will be collected regardless of grade and captured in C3D
- All toxicities related to wound healing will be collected regardless of grade and captured in C3D
- All toxicities related to hypertension will be collected regardless of grade and captured in C3D
- All toxicities related to proteinuria will be collected regardless of grade and captured in C3D
- All grade 3, 4 events related to the research (possibly, probably and definitely) will be captured in the C3D data base
- We will capture all grade 5 events

Data from Croatia will be directly entered in to the C3D data base by staff in Croatia. *All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.* Source documents will be maintained in the patient medical record.

13.4 Toxicity Criteria:

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. Adverse events occurring during the study will be graded according to the NCI Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) found at: http://ctep.cancer.gov/reporting/ctc_v30.html.

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Appendix I

Proposed Definitions for T, N, and M Descriptors

T (Primary Tumor)	
TX	Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor ≤3 cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e., not in the main bronchus)[a]
T1a	Tumor ≤2 cm in greatest dimension
T1b	Tumor >2 cm but ≤3 cm in greatest dimension
T2	Tumor >3 cm but ≤7 cm or tumor with any of the following features (T2 tumors with these features are classified T2a if ≤5 cm) Involves main bronchus, ≥2 cm distal to the carina Invades visceral pleura Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	Tumor >3 cm but ≤5 cm in greatest dimension
T2b	Tumor >5 cm but ≤7 cm in greatest dimension
T3	Tumor >7 cm or one that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium; or tumor in the main bronchus <2 cm distal to the carina[a] but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe
T4	Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, carina; separate tumor nodule(s) in a different ipsilateral lobe
N (Regional Lymph Nodes)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
M (Distant Metastasis)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Descriptors, Proposed T and M Categories, and Proposed Stage Groupings

Sixth Edition T/M Descriptor	Proposed T/M	N0	N1	N2	N3
T1 (≤2 cm)	T1a	IA	IIA	IIIA	IIIB
T1 (>2–3 cm)	T1b	IA	IIA	IIIA	IIIB
T2 (≤5 cm)	T2a	IB	**IIA**	IIIA	IIIB
T2 (>5–7 cm)	T2b	**IIA**	IIB	IIIA	IIIB
T2 (>7 cm)	T3	**IIB**	**IIIA**	IIIA	IIIB
T3 invasion		IIB	IIIA	IIIA	IIIB
T4 (same lobe nodules)		**IIB**	**IIIA**	**IIIA**	IIIB
T4 (extension)	T4	**IIIA**	**IIIA**	IIIB	IIIB
M1 (ipsilateral lung)		**IIIA**	**IIIA**	**IIIB**	**IIIB**
T4 (pleural effusion)	M1a	**IV**	**IV**	**IV**	**IV**
M1 (contralateral lung)		IV	IV	IV	IV
M1 (distant)	M1b	IV	IV	IV	IV

Cells in bold indicate a change from the sixth edition for a particular TNM category.

Revision of the TNM Stage Groupings in the forthcoming (Seventh) Edition of the TNM Classification of Malignant Tumors. Adapted from Goldstraw et al: J.Thorac.Oncol., Volume 2(8).August 2007.706-714

APPENDIX II

Performance Status Criteria

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

Appendix III
Bevacizumab Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension	
No dose modifications for grade 1/2 events	
Grade 3	If not controlled with medication, discontinue the subject from the study.
Grade 4	Discontinue the subject from the study.
Hemorrhage	
No dose modifications for grade 1/2 events	
Grade 3	Subjects who experience pulmonary hemorrhage will be removed from the study. Subjects who are also receiving full-dose anticoagulation will be discontinued from the study. All other subjects will have study treatment held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat Grade 3 hemorrhagic event will be discontinued from treatment.
Grade 4	Discontinue the subject from the study.
Venous Thrombosis	
No dose modifications for grade 1/2 events	
Grade 3/ Asymptomatic Grade 4	Subjects with lung cancer placed on anticoagulant therapy for a thrombotic event should be discontinued from study.
Symptomatic Grade 4	Discontinue the subject from the study.
Arterial Thromboembolic event	
(Angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)	
Any grade	Discontinue the subject from the study.
Proteinuria	
Subjects who develop more than 2g/24 hr proteinuria, or a urine protein/creatinine > 2.0 will have their dose held until the Up/C < 1.0 or the proteinuria is < 1g/24hr	
Grade 3 (>3.5 g/ 24 hr)	Hold study drug treatment until ≤ Grade 2
Grade 4 (nephrotic syndrome)	Discontinue the subject from the study
GI Perforation requiring medical or surgical therapy	Discontinue the subject from the study.
Wound dehiscence requiring medical or surgical therapy	Discontinue the subject from the study.

Appendix IV

MANAGEMENT OF ACUTE HYPERSENSITIVITY

Severity of Symptoms	Treatment Guidelines
Mild symptoms: localized cutaneous reactions such as mild pruritus, flushing, rash.	• Consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient • Then, complete chemotherapy infusion at the initial planned rate
Moderate symptoms: any symptom that is not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP 80 mm Hg	• Interrupt chemotherapy infusion • Give diphenhydramine 50 mg IV with or without dexamethasone 10mg IV; monitor patient until resolution of symptoms • Resume chemotherapy infusion after recovery of symptoms; depending on the physician's assessment of the patient, chemotherapy infusion should be resumed at a slower rate, and then increased incrementally to the initial planned rate, (*eg. Infuse at an 8 hour rate for 5 minutes, then at a 4 hour rate for 5 minutes, then a 2 hour rate for 5 minutes, then finally, resume at the 1 hour infusion rate)* • Depending on the intensity of the reaction observed, additional oral or IV premedication with an antihistamine should also be given for the **next cycle** of treatment, and the rate of infusion should be decreased initially and then increased back to the recommended 1 hour infusion, (*eg, infuse at 8 hour rate for 5 minutes, then at a 4 hour rate for 5 minutes, then at a 2 hour rate for 5 minutes, ad finally, administer at the 1 hour infusion rate).*
Severe symptoms: any reaction such as bronchospasm, generalized urticaria, systolic BP, 80 mm Hg, angioedema	• Immediately discontinue chemotherapy infusion • Give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV and/or epinephrine as needed; monitor patient until resolution of symptoms • The same treatment guidelines outlined under moderate symptoms (i.e. the third and fourth bullets) should be followed.
Anaphylaxis(NCI grade 4 reaction)	NO FURTHER STUDY DRUG THERAPY

Appendix V

NIH Definitions:

Adverse events

Any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen.

Serious adverse events

Any untoward medical occurrences that:

(1) result in death,
(2) are life threatening,
(3) require (or prolong) hospitalization,
(4) cause persistent or significant disability/incapacity,
(5) result in congenital anomalies or birth defects, or
(6) are other conditions which in the judgment of the investigators represent significant hazards.

Expected Adverse Events

For approved and marketed drugs or devices, those adverse events described in the approved Package Insert (Label). For investigational new drugs or devices, those adverse events described in the FDA Investigator's Brochure. In clinical research studies, information on expected adverse events are also summarized in the protocol and in the consent form.

Unexpected Adverse Events

Those adverse events not described in the Package Insert, Investigator's Brochure, in published medical literature, in the protocol, or in the informed consent document.

Intensity or Severity of Adverse Events

Assignment of the grade of adverse events or side-effects of interventions based on intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of objective clinical signs or laboratory parameters (schemes for assessing and monitoring adverse events can be drawn from existing models or those customized for use in particular protocols must be justified by the PI and approved by the IRB).

Relatedness of Adverse Event to an Intervention

The best estimate of the PI at the time of reporting of the causal relationship between an experimental intervention and an adverse event; the degree of certainty about causality is graded as follows:

Unrelated:

Adverse event is clearly due to extraneous causes (e.g., underlying disease, environment)

Unlikely (must have 2):

Adverse event:

(1) does not have temporal relationship to intervention,
(2) could readily have been produced by the subject's clinical state,
(3) could have been due to environmental or other interventions,
(4) does not follow known pattern of response to intervention,
(5) does not reappear or worsen with reintroduction of intervention

Possible (must have 2):

Adverse event:

(1) has a reasonable temporal relationship to intervention,
(2) could not readily have been produced by the subject's clinical state,
(3) could not readily have been due to environmental or other interventions,
(4) follows a known pattern of response to intervention

Probable (must have 3):

Adverse event:

(1) has a reasonable temporal relationship to intervention,
(2) could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions,
(3) follows a known pattern of response to intervention,
(4) disappears or decreases with reduction in dose or cessation of intervention

Definite (must have all 4):

Adverse event:

(1) has a reasonable temporal relationship to intervention,
(2) could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions,
(3) follows a known pattern of response to intervention,
(4) disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure

Appendix VI

PROTOCOL SUBMISSION FORM **Expedited Adverse Event Report**	PROTOCOL NO.	PRINCIPAL INVESTIGATOR (NIH Employee Name, Inst/Br, Telephone and e-mail):

PROTOCOL TITLE:

ABBREVIATED TITLE:

Reference Number:

Date Principal Investigator Notified of Event:

Description of Participant:

Sex: ☐ Male ☐ Female Age: Diagnosis:

Is this an Initial or a Follow-Up Report? ☐ Initial ☐ Follow up

For Follow-Up - Initial AE reference #, if applicable:

If this is a follow up report, describe what was updated from the initial report:

Category of Adverse Event:

☐ Serious Adverse Event (related to the research but not in consent)
☐ Death from any cause (not classified as a SAE)
☐ Grade 3 or 4 adverse event (related to the research, not as SAE, and not in consent)

Outcome Category for Serious Adverse Event, if SAE:

☐ Death
☐ Life Threatening
☐ Hospitalization-Initial or Prolonged
☐ Disability / Incapacity
☐ Congenital Anomaly / Birth Defect
☐ Required intervention to prevent permanent impairment

Have similar adverse events occurred on this protocol? ☐ Yes ☐ No

Description of similar events:

What steps do you plan to take as a result of the adverse event reported?

☐ No action required
☐ Amend protocol
☐ Amend consent document
☐ Inform current subjects
☐ Terminate of suspend protocol
☐ Other

Specify Other Steps Taken:

Brief description of the nature of the adverse event:

Adverse Event Information:

CTC Term	Date of Event	Location of Event	CTC Version	CTC Grade	Attribution to Research	Attribution to IND Agent	Listed in Consent

LEGEND:

Location of Event	CTC Version	CTC Grade	Attribution to Research	Attribution to IND Agent	Listed in Consent
NIH	2	2	Unrelated	Unrelated	Yes
Elsewhere	3	3	Unlikely	Unlikely	No
		4	Possibly	Possibly	
		5	Probably	Probably	
			Definitely	Definitely	
			Related to Progressive Disease	Related to Progressive Disease	
				Related to non-treatment study	

SIGNATURE _____
Principal Investigator - signature and date

APPROVALS _____
Clinical Director - signature and date

_____
Chair, IRB Review - signature and date

Appendix VII

General Considerations for Sample Handling for Research Samples at the NIH

Shipment

Samples from Croatian patients will be batch shipped via FedEx or World Courier Priority Overnight on dry ice to the Clinical Pharmacology Program (CPP) (Dr William Figg) at the following address:

Dr. William D. Figg
National Cancer Institute
9000 Rockville Pike
Bldg 10 Rm 5A01
Bethesda, MD 20892
USA
Tel: 301-402-3622

Samples will be batch shipped to the National Cancer Institute every **TWO (2)** months. Samples must be packaged in accordance with IATA regulations. The following documentation must be included with the shipment: a Simple Letter of Agreement (SLA) and a shipping manifest. The shipping manifest should include all relevant sample information: Harris Orcan patient registration number, sample ID, sample material type, sample collection date/time, date/time placed in freezer, protocol timepoint (Cycle 1 Day 1), and any processing or collection issues. The manifest should also include contact information for the person(s) shipping the samples, the protocol title/number, and the shipment date. Patient names and medical record numbers will not be included on the manifest.

On the day of shipment, send an email notification to Dr. Figg at wdfigg@helix.nih.gov with the number of samples being shipped and the FedEx/World Courier tracking number.

Storage/Tracking

Research samples will be obtained from patients, as described in the protocol. Patient samples will be coded in the laboratory of Dr. William Figg and stored either at 80°C or in a Liquid Nitrogen freezer in the case of samples viably frozen. Records of sample acquisition will be held in the laboratory in Labrador (formerly known as LabSamples) a computerized database that is accessible by password. A limited number of laboratory staff will have access to identifying patient information. Assays as noted above will be carried out on patient samples.

Protocol Completion/Sample Destruction
Material remaining after completion of correlative studies will be returned to the laboratory of Dr. William Figg. These samples will be managed in accordance with the laboratory's policies and procedures. The PI will report any loss or destruction of samples to the IRB. Any new use of the samples, specimens, or data will require prospective IRB review and approval.

Labrador
All samples will be barcoded, with data entered and stored in the Labrador system (formerly known as LabSamples) utilized by the CPP. This is a secure program, with access to the system limited to defined CPP personnel, who are issued individual user accounts. The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.). For samples shipped from Croatia, any associated sample information sent with the shipment, such as the shipping manifest, will be scanned, uploaded, and attached to the sample(s) in Labrador.

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20, -80°C, or in liquid nitrogen according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. **Samples will be stored until requested** by Drs Giaccone, Schrump, Trepel and/or Meltzer. Samples will be brought to the requestor's lab on dry ice in an appropriate container by a member of Dr. Figg's laboratory with a copy of the shipping manifest. All requests are monitored and tracked in the Labrador system. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed

(or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of Labrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Appendix VIII

1. Gemcitabine HCl

Formulation
Gemzar® is commercially available as a sterile, white, lyophilized powder in single-use vials.

Ingredient	Vial Sizes	
Gemcitabine HCl, equivalent to gemcitabine free base (+ 0–3% excess)	200 mg	1000 mg
Mannitol, USP	200 mg*	1000 mg*
Sodium acetate, ACS and USP	12.5 mg*	62.5 mg*

* ± 10%

Hydrochloric acid and sodium hydroxide may have been added to adjust product pH.

Source
Gemcitabine HCl will be purchased from commercial sources by the NIH Clinical Center Pharmacy Department.

Preparation:
Vials should be reconstituted with 5 mL (200-mg vials) or 25 mL (1000-mg vials) 0.9% Sodium Chloride Injection, USP (0.9% NS) to produce a solution with a concentration = 38 mg/mL. The lyophilized powder adds a small amount to the total volume after reconstitution (200 mg gemcitabine/5.26 mL; 1000 mg/26.3 mL).
Reconstituted gemcitabine is suitable for direct administration or may be further diluted with 0.9%NS to concentrations as low as 0.1 mg/mL.

Gemcitabine doses will be diluted in 250-500mL of 0.9% NS.

Gemcitabine is compatible with either glass or PVC containers and PVC administration sets

Stability
Intact vials stored are labeled with the manufacturer's expiration date.

After reconstitution to a concentration of 38 mg/mL with either sterile Water for Injection, USP, or 0.9%NS in the original vials, gemcitabine was chemically and physically stable at 23°C (73.4°F) for at least 35 days. Crystalization occasionally occurred in vials stored under refrigeration for > 7 days. The crystals did not redissolve when the solutions were warmed to room temperature and HPLC analysis revealed gemcitabine losses of 20 – 35% in samples which contained crystals. Exposure or protection from fluorescent light did not affect gemcitabine stability.

Storage
Store intact vials at controlled room temperature (20° – 25°C [68° – 77°F]).
Gemcitabine solutions were found to be stable without loss of potency after reconstitution to 38 mg/mL, and after dilution with 5% Dextrose Injection, USP, to concentrations of 10 mg/mL or 0.1 mg/mL and storage in PVC containers for 7 days at 32°C in the dark. Reconstituted gemcitabine solutions should not be refrigerated, as crystallization may occur.

Dosage and Administration

Fixed Dose-Rate Administration.
Gemcitabine will be administered intravenously at a fixed dose-rate (FDR) of 10 mg/m^2 per min (600 mg/m^2·hour).

- Experimental data have demonstrated that small increases in intracellular concentrations of gemcitabine's active triphosphate metabolite profoundly affect its intracellular area under the curve, and that a fixed gemcitabine administration rate of 10 mg/m^2 per min maximize the rate at which the metabolite accumulates in peripheral blood mononuclear cells.

- This observation is the basis for recent clinical trials with FDR gemcitabine administration which attempt to correlate increases in intracellular gemcitabine triphosphate concentrations with improved objective responses to treatment and survival.

Toxicities

Hematological Adverse Effects

Myelosuppression is the primary dose-limiting effect of gemcitabine, and is characterized by thrombocytopenia with relative sparing of leukocytes without cumulative toxicity. Gemcitabine may also impair erythropoiesis, producing increased serum iron and ferritin, and a decrease in peripheral blood reticulocyte counts.

Grades 3 and 4 anemia have been reported in 5% and up to 0.8% of patients, respectively, but has rarely been cause for discontinuing gemcitabine. Anemia has tended to be more severe than leukopenia in some studies.

Hemolytic uremic syndrome (HUS) has been reported in 0.015 – 0.25% of patients during and immediately following gemcitabine treatment. Renal failure may be irreversible. Thrombotic microangiopathy secondary to HUS has been described in three case reports of long- term therapy with gemcitabine. A diagnosis of HUS should be considered if a patient develops anemia with evidence of microangiopathic hemolysis, increased serum bilirubin or LDH, reticulocytosis, severe thrombocytopenia, and/or evidence of renal failure (increased serum creatinine or BUN). Gemcitabine therapy should be discontinued immediately. Renal failure may not be reversible even with discontinuation of therapy and dialysis may be required.

Respiratory Adverse Effects

Dyspnea was reported in up to 10 – 23% of patients in clinical trials with gemcitabine; however, many patients with primary or metastatic lung cancer may have pre-existing pulmonary dysfunction prior to starting therapy. Bronchospasm has also been observed during gemcitabine therapy.

Parenchymal toxicity, including interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, and acute respiratory distress syndrome (ARDS), has been reported rarely following one or more doses of gemcitabine. Some patients experienced the onset of pulmonary symptoms up to two weeks after a dose of gemcitabine. Respiratory failure and death occurred very rarely in some patients despite discontinuation of therapy.

A patient developed acute respiratory distress after gemcitabine 900 mg/m^2 over 90 minutes weekly for 6 weeks, every 8 weeks. Lung biopsy confirmed chemical interstitial injury which was believed to be related to gemcitabine treatment. ARDS has also been reported with gemcitabine 1200 – 1250 mg/m^2 on days 1, 8, and 15 every four weeks. Initial signs and symptoms including dyspnea, cough, and bilateral pulmonary infiltrates consistent with edema appeared after two to four cycles. Among three patients who developed ARDS symptoms, one who showed temporary improvement with diuretics received three additional gemcitabine cycles before dying of rapidly progressing ARDS. A second patient died within three days after initial presentation. The survivor improved with steroids and received no further treatment with gemcitabine. Gemcitabine should be discontinued in the presence of unexplained, non-cardiogenic, acute or subacute pulmonary edema. Other cases have been reported, with at least one fatality.

Renal Adverse Effects

Proteinuria and hematuria are common and have been described in up to 45% of patients in clinical trials.

Azotemia and acute renal failure have been reported in association with gemcitabine treatment.

A retrospective systematic review of case reports and clinical trials through December 2005, revealed data on 56 patients who developed gemcitabine-associated thrombotic microangiopathy (TMA), within a range of 0.5 – 19 months (mean, 7.56 mo). Twenty-four patients had received prior chemotherapy, and an estimated cumulative dose of gemcitabine in the range, 2000 – 70,000 mg (median, 22,480 mg). Symptoms included worsening anemia, thrombocytopenia, increased serum creatinine and LDH, hypertension, dyspnea, peripheral edema, proteinuria, hematuria, neurological signs, and fragmented red blood cells. Development of TMA secondary to hemolytic uremic syndrome (HUS) has been described in case reports of long-term therapy with gemcitabine.

Deaths have been reported as a result of HUS or HUS-related complications in association with gemcitabine therapy. The classic triad of acute uremia, microangiopathic hemolytic anemia, and thrombocytopenia was noted in all patients with HUS associated with gemcitabine. Hypertension was the other most common finding [see additional information about HUS in the *Hematological Adverse Effects* section (above)].

Dermatological Adverse Effects

Generalized, erythematous, pruritic, maculopapular skin rashes (primarily affecting the neck and extremities) have been described in trials of gemcitabine (5 – 32% of patients). Rashes are reversible with local

treatment and do not often require dosage reduction or drug discontinuation. Antihistamines and topical steroids may also provide symptomatic improvement without having to alter or interrupt treatment. Gemcitabine may cause skin irritation and eye irritation after topical exposure, but it is not a vesicant drug. Up to 15% of patients treated with gemcitabine may experience hair loss. Alopecia and rashes seem to occur relatively frequently during gemcitabine therapy. Cellulitis, pruritus, radiation recall dermatitis, erythema, skin ulcerations, and pseudolymphoma have been reported. Skin desquamation and anal pruritis have been reported rarely. More severe skin reactions have also been reported occasionally in patients treated with gemcitabine, including bullous skin ulceration and severe erythema resembling scalded-skin syndrome. Radiation recall dermatitis has been reported in patients who received gemcitabine.

Metabolic Adverse Effects
Transient febrile episodes have been observed within 6 – 12 hours after a first dose in approximately 5 – 60% of patients and are commonly associated with other flu-like symptoms. Fevers generally are responsive to acetaminophen and nonsteroidal anti-inflammatory drugs.

Flu-like symptoms (with or without fever), including headache, malaise, myalgias, and rigors, are the most frequent adverse effects of gemcitabine, occurring in 20 – 100% of patients. Flu symptoms have been severe enough to be dose-limiting.

Fatigue or lethargy, occurring alone or in conjunction with other flu-like symptoms, occur commonly.

Hepatic Adverse Effects
Transient grade II or III liver transaminase elevations were observed in 7% of patients with non-small-cell lung cancer treated with gemcitabine. Gemcitabine has been associated with transiently increased serum transaminases in approximately 70% of patients. There is no evidence that incidence of hepatic toxicity correlates with duration of exposure or total cumulative gemcitabine doses.

Neurologic Adverse Effects
Paresthesia has been reported in 10% of patients during clinical trials with gemcitabine. Mild-to-moderate somnolence has been reported in approximately 9.1 – 11% of patients who received gemcitabine. In clinical trials, pain was reported by 48% of patients. In the treatment of pancreatic cancer, pain was reported by 6.8% and asthenia was observed in 6% of patients who received gemcitabine 1000 mg/m^2·week.

Cardiovascular Adverse Effects
Systemic capillary leak syndrome has been suggested to be the pathogenic mechanism for the pulmonary toxicity of gemcitabine.
Peripheral edema, usually mild, has been observed in 4% up to 20.3% of patients treated with gemcitabine, which was not associated with cardiac, hepatic, or renal failure.
Isolated cases of severe hypertension, and rarely, atrial fibrillation, dysrhythmias (predominantly supraventricular), myocardial infarction, and congestive heart failure have been reported in association with gemcitabine either alone or in combination with other cytotoxic agents.

Gastrointestinal Adverse Effects
Gemcitabine as a single agent is associated with low emetic risk, but may produce mild nausea and vomiting. Diarrhea and constipation have each occurred in patients during gemcitabine treatment. Stomatitis has been reported in 11% of patients who received gemcitabine, but G3 & G4 toxicity occurs infrequently.

Reproductive Toxicity
Gemcitabine is classified as a HAZARDOUS DRUG at the NIH Clinical Center.

Drug Interactions
Gemcitabine significantly increased systemic exposure to **fluorouracil** in 20 patients with advanced gastroenteric carcinoma. Patients who received the combination (with leucovorin) had a greater fluorouracil AUC and plasma half-life, and decreased mean total clearance and volume of distribution compared to controls. The fluorouracil AUC in patients who received gemcitabine and fluorouracil was 555 ±209 µg/mL·min and 244 ±89 in patients who did not receive gemcitabine.

Preclinical and clinical studies have shown that Gemzar has **radiosensitizing** activity. Toxicity associated with multimodality therapy is dependent on many different factors, including gemcitabine dose and

frequency of administration, dose of radiation, radiotherapy planning technique, the target tissue, and target volume.
Significant toxicity was observed when gemcitabine 1000 mg/m^2 was administered for up to 6 consecutive weeks in combination with thoracic radiation in patients with non-small cell lung cancer. Severe and potentially life-threatening mucositis, particularly esophagitis and pneumonitis, were observed particularly in patients who received large radiation volumes (median, 4795 cm^3).

One case has been reported in which a 63-year-old male stabilized on **warfarin** experienced increased international normalized ratio (INR) values while receiving gemcitabine. Repeated reductions in his warfarin dose were needed to maintain the INR in the target range. The patient experienced no emesis or weight loss as a result of gemcitabine therapy, and no antiemetics were given. After gemcitabine treatments were discontinued, the warfarin dose required an upward titration to its previous level.
Although a pharmacokinetic interaction is unlikely, it is possible that gemcitabine decreased the metabolic function of the cytochrome P450 enzyme system and consequently, warfarin metabolism. Hepatic toxicity associated with gemcitabine may also decrease clotting factors synthesis, resulting in a reduced warfarin requirement.

2. Cisplatin

Formulation & Source
Cisplatin is commercially available in amber, multiple-dose vials containing 50, 100, or 200 mg cisplatin in solution. Each milliliter of solution contains 1 mg of cisplatin and 9 mg sodium chloride in water for injection. HCl and sodium hydroxide may be added to adjust product pH.
Cisplatin Injection, USP, will be purchased from commercial sources by the NIH Clinical Center Pharmacy Department.

Preparation
Each dose of cisplatin will be diluted in 100 mL 0.9% Sodium Chloride Injection, USP. Protect diluted solutions from exposure to light.

Stability
Vials bear the manufacturer's expiration dating. After reconstitution to a concentration of 1 mg/mL, cisplatin is stable at room temperature (80°F [27°C]) for 20 hours. Further dilution to 0.05 or 0.5 mg/mL with 0.9%NS yields a solution that is stable for at least 24 hours at room temperature.

Storage
Intact vials and reconstituted solution should be maintained at room temperature.
Store intact vials at 15° – 25°C (59° – 77°F). Do not refrigerate cisplatin in vials or after dilution.
Cisplatin that remains in an amber vial after initial entry is stable for 28 days if protected from light or for 7 days under fluorescent room light.
Large-volume solutions do not need to be protected from light if used within 6 hours after preparation. For longer time periods, light protection is recommended. Light protection is required for intensive lighting conditions; e.g., direct sunlight exposure.
Although cisplatin slowly degrades to trichloroaminoplatinate (TCAP) on exposure to ambient lighting conditions, solution pH is the predominant factor affecting cisplatin stability. Solution pH > 4.3 (especially > 6.3) was associated with loss of cisplatin and a more rapid formation of TCAP.

Dosage and Administration
Cisplatin will be diluted in 100 mL 0.9%NS and administered intravenously after gemcitabine over 60 minutes.

Adverse Effects
The following 'Black Box' warnings appear in FDA approved product labeling for Cisplatin Injection, June 2004. Bedford Laboratories™, Bedford, OH:

> Cisplatin injection should be administered under the supervision of a qualified physician experienced in the use of cancer chemotherapeutic agents. Appropriate management of therapy and complications is possible only when adequate diagnostic and treatment facilities are readily available.
>
> Cumulative renal toxicity associated with cisplatin is severe. Other major dose-related toxicities are myelosuppression, nausea, and vomiting.

Ototoxicity, which may be more pronounced in children, and is manifested by tinnitus, and/or loss of high frequency hearing and occasionally deafness, is significant.

Anaphylactic-like reactions to cisplatin have been reported. Facial edema, bronchoconstriction, tachycardia, and hypotension may occur within minutes of cisplatin administration. Epinephrine, corticosteroids, and antihistamines have been effectively employed to alleviate symptoms.

Exercise caution to prevent inadvertent cisplatin overdose. Doses greater than 100 mg/m^2/cycle once every 3 to 4 weeks are rarely used. Care must be taken to avoid inadvertent cisplatin overdose due to confusion with carboplatin or prescribing practices that fail to differentiate daily doses from total dose per cycle.

Renal Adverse Effects
Renal toxicities are dose limiting and may be irreversible with high doses of cisplatin and repeated courses. The severity of toxicity correlates directly with the amount of cisplatin administered in individual and cumulative doses. Manifestations include increased BUN and serum creatinine and uric acid, and decreased creatinine clearance. Renal toxicity may be mitigated by delaying cisplatin administration in patients who are clinically dehydrated, by giving intravenous hydration during and after cisplatin administration with or without the use of diuretics (to maintain urine output of at least 100 mL/h), diluting cisplatin for administration in vehicle solutions which contain chloride concentrations > 0.225%, avoiding rapid administration (avoid rates > 1 mg cisplatin/kg·hour), and avoiding administration of other nephrotoxic drugs concomitantly.

Electrolyte disturbances are a consequence of renal tubular damage, and may include hypomagnesemia, hypocalcemia, hyponatremia, hypokalemia, and hypophosphatemia. Hypomagnesemia is a persistent and possibly permanent adverse effect of cisplatin.

Decreased serum sodium concentrations has been observed within 24 – 72 hours following cisplatin infusion. Serum sodium deficits may resolve, but chronic salt wasting and hyponatremia may persist after discontinuing cisplatin.
Cisplatin-induced hyponatremia was associated with decreased mental awareness, episodic seizures, and decerebrate posturing.

Hemolytic uremic syndrome has rarely been associated with cisplatin use.

Neurologic Adverse Effects
Peripheral neuropathy is a dose-limiting toxicity. The features of neuropathy are uniform and consistent with damage primarily to large sensory fibers. Numbness and tingling are often early symptoms; impaired reflexes, vibratory sensation, and proprioception are associated with progressive toxicity, and sensory ataxia generally occurs late. Lhermitte sign is often present. Pain and temperature sensation is usually preserved. The severity of neuropathy varies with the dose intensity and the total cumulative dose administered. The onset of neuropathy may begin or progress after cisplatin has been discontinued. Neuropathy is often reversible at least in part, but recovery often follows a protracted course. Persons with neuropathic symptoms due to diabetes, hereditary neuropathies, or prior exposure to neurotoxic chemotherapy may be at increased risk of neurotoxicity.

Other neurotoxic effects reported include encephalopathy with or without seizures; reversible posterior leukoencephalopathy syndrome; and visual disturbances, including: optic neuritis, papilledema, retrobulbar neuritis, cortical blindness, blurred vision and altered color perception.

Ototoxicity manifested by tinnitus or hearing loss occurs in up to 31% of patients who receive cisplatin 50 mg/m^2. Severity correlates with the magnitude of individual doses and total cumulative dose administered, and toxicity appears to be irreversible. Risk factors for hearing loss include age > 40 years and preexisting hearing loss. Vestibular toxicity has also been observed following cisplatin therapy.

Hypersensitivity Reactions
Hypersensitivity reactions including anaphylactoid reactions may occur with cisplatin. Cross-sensitivity among alternate platinating agents (cisplatin, carboplatin, oxaliplatin) may occur.

Gastrointestinal Adverse Effects
Acute emetic risk is high according to current antiemetic guidelines, and vomiting occurs in > 99% of patients who do not receive primary antiemetic prophylaxis. Patients who receive cisplatin dosages ≥ 50 mg/m^2 also are at risk for delayed symptoms; i.e., nausea and emesis which occur > 24 h after cisplatin administration.

Hematologic Adverse Effects
Transient and moderate myelosuppression, including leukopenia, thrombocytopenia, and anemia in 25 – 30% of patients. Leukocyte and platelet nadirs occur between 18 – 23 days after treatment and generally recover by day 39. Leukopenia occurs most frequently (27% of patients treated) followed by thrombocytopenia (16%), and anemia (11%). Leukocyte counts < 2000/mm^3 and platelet counts < 50,000 platelets/mm^3 are rare with intermittent doses of 50 – 60 mg/m^2.

Thromboembolic events associated with cisplatin treatment have included deep venous thrombosis, pulmonary embolism, arterial occlusions, and cerebrovascular accident. Prophylactic anticoagulation should be considered for patients with risk factors for thromboembolic disease.

Dermatologic Adverse Effects
Moderately severe cellulitis and fibrosis following cisplatin extravasation has been observed in one patient.

Hepatic Adverse Effects
ALT/SGPT and AST/SGOT may be transiently increased after cisplatin administration.

Cardiovascular Adverse Effects
Ciosplatin administration has been associated with arrythmias, bradycardia, and has been etiologically implicated in cases of myocardial infarction, and angina pectoris with proven ischemia.

Reproductive Adverse Effects
Sperm concentrations, rates of azoospermia, and FSH and LH concentrations were found significantly altered in male patients who received cumulative cisplatin dosages > 400 mg/m^2 for testicular germ cell tumors in comparison with patients who did not receive chemotherapy.

Carcinogenicity, Mutagenicity, Genotoxicity
Cisplatin is classified as a HAZARDOUS DRUG at the NIH Clinical Center.

The incidence of secondary leukemias was 1.5% in a Pediatric Oncology Group study of 198 children (age < 3 y) with malignant brain tumors who received cyclophosphamide plus vincristine alternating with cisplatin and etoposide for two years. The latency period between chemotherapy initiation and secondary malignancy ranged from 2.8 – 7.7 years.
Of 28,971 patients with ovarian cancer, 96 developed a secondary leukemia while being treated with platinum-based chemotherapy. The relative risk of developing a secondary leukemia for treatment with cisplatin was 3.3.
A case-control study based on international population-based cancer registry data of 18,567 males with testicular cancer diagnosed between 1970 and 1993 determined that the adjusted relative risk of secondary leukemia with a cumulative cisplatin dose of 650 mg was 3.2 (95% CI, 1.5 – 8.4). Investigators equated this relative risk with an excess of 16 leukemia cases per 10,000 patients over 15 years. The relative risk of leukemia was found correlated directly with cumulative cisplatin doses.
Concomitant ionizing radiation therapy or melphalan administration with cisplatin further increase the relative risk of developing a secondary leukemia.

Cisplatin is teratogenic and embryotoxic in animal models. In humans, normal pregnancies have been reported following cisplatin use alone and in combination with other chemotherapeutic agents.

Cisplatin exhibits mutagenic activity in vitro in the Ames microbial mutagenicity assay and embryotoxicity in animal models.

Although the quantity of drug appearing in breast milk is likely to be small, cisplatin is inherently cytotoxic, and the risks to a breastfeeding infant are unknown.

Breast (milk-to-plasma ratios = 0.1 and 1.1) have been reported. A conflicting report detected no cisplatin in the breast milk of a patient who received cisplatin 100 mg/m^2 IV over 26 hours plus doxorubicin 70 mg/m^2 IV over 15 min. Although doxorubicin was detected in milk samples, no platinum was found in six samples

collected between 0.25 – 71.25 hours after starting cisplatin administration. Although most of the platinum in breast milk may be bound to plasma proteins, no data are available on infant exposure to cisplatin through breast milk, and breastfeeding during cisplatin therapy is not recommended.

Drug Interactions
Hypersensitivity reactions have been reported in patients who received combination regimens containing sequential high dose **aldesleukin** and cisplatin. Erythema, pruritus, and hypotension has occurred within hours after chemotherapy administration.

Aluminum reacts with carboplatin causing precipitation and loss of drug potency. DO NOT PREPARE OR ADMINISTER cisplatin with needles, dispensing pins, administration sets that contain aluminum parts which may come into contact with the drug.

Concomitant use of cisplatin and **aminoglycosides (amikacin, gentamicin, streptomycin, tobramycin)** has the potential to increase the risk of nephrotoxicity.

Concomitant use of cisplatin was implicated in decreasing **carbamazepine** and **valproic acid** plasma concentrations to subtherapeutic ranges which resulted in seizure activity. The mechanistic basis for the putative interaction is not know.

In a study of patients with metastatic or advanced tumors, **docetaxel** in combination with cisplatin induced more severe neuropathic effects than either drug as a single agent in similar doses.

In a retrospective study in a series of 23 patients, Cagnoni, et al. showed the mean area under the curve for both cyclophosphamide (76,600 vs 90,600 mg/mL per min, P = 0.001) and cisplatin (525 vs 648 mg/mL per min, P = 0.01) were significantly less in patients who received **ondansetron** in a combination antiemetic regimen than in 129 historical control patients who received an antiemetic regimen in which prochlorperazine was used instead of ondansetron.

Combined therapy with cisplatin and **phenytoin** or **fosphenytoin** may cause decreased phenytoin concentrations and increased seizure activity and require compensatory increases in phenytoin dosages to maintain therapeutic plasma levels.

Cisplatin may affect **melphalan** kinetics by inducing renal dysfunction and subsequently altering melphalan clearance.

Cisplatin is physically incompatible in admixtures with **mesna**.

In a phase I trial, sequential infusions of escalating doses of **paclitaxel** (110 – 200 mg/m^2) and cisplatin (50 or 75 mg/m^2) caused more profound myelosuppression when paclitaxel was given after cisplatin than the alternate sequence of administration. Paclitaxel clearance was decreased approximately 33% when paclitaxel was administered after cisplatin.

In a randomized trial of cisplatin (37.5 mg/m^2 or 75 mg/m^2 IV, day 1) and altretamine (200 mg/m^2 PO, days 8–21) with and without concurrent **pyridoxine** (300 mg/day PO. days 1–21) in patients with ovarian cancer, pyridoxine was implicated in multivariate analysis among factors that adversely affected response duration in previously untreated patients. Consequently, pyridoxine should not be administered concurrently with altretamine and cisplatin.

3. Bevacizumab

Formulation

The commercial product (Avastin®) is packaged in single-use clear glass vials containing either 100 mg or 400 mg bevacizumab solution in a uniform concentration of 25 mg/mL. The product is formulated in α,α-trehalose dihydrate, sodium phosphate (monobasic, monohydrate), sodium phosphate (dibasic, anhydrous), polysorbate 20, and Water for Injection, USP.

Source

Bevacizumab will be provided by Genentech and Roche. The supply of bevacizumab being administered to patients enrolled on this study is intended for use only in clinical trials. It is expected to be very similar in safety and activity to the commercially marketed drug (Avastin®), but it is possible that some differences may exist.

Preparation

Withdraw from a vial an amount of bevacizumab sufficient to prepare a patient's dose and add it to a volume of 0.9%NS sufficient to produce a total volume of (Q.S.) 100 mL in a PVC, polyolefin, or glass container. Gently invert the container to mix diluted bevacizumab solution. Discard unused bevacizumab that remains in a vial.
DO NOT MIX bevacizumab with dextrose solutions.

Storage and Stability

Store intact vials under refrigeration at 2° – 8°C (35.6° – 46.4°F) and protect them from light. DO NOT FREEZE solutions containing bevacizumab.
DO NOT SHAKE intact vials or diluted solutions

After dilution in 0.9%NS, bevacizumab was stable for up to 24 hours under the following conditions:

Bevacizumab Concentrations (mg/mL)	Storage Temperatures	Container Composition
0.9 2.25 3 6.6 7.5 16.5	30°C (86°F)	PVC, polyolefin, glass
1 12.5 25 (undiluted drug)	2° – 8°C 30°C	Polyolefin

Dosage and Administration

DO NOT ADMINISTER bevacizumab by intravenous push or bolus injection
Administer bevacizumab by intravenous infusion after chemotherapy. The INITIAL DOSE is given over 90 minutes. If the first infusion is well tolerated, the administration duration may be decreased to 60 minutes during a second infusion. If a 60-minute infusion was well tolerated, subsequent treatments may be administered over 30 minutes

DO NOT ADMINISTER, mix, or flush bevacizumab with dextrose solutions

Adverse Effects

The following 'Black Box' warnings appear in FDA approved product labeling for Avastin® (Bevacizumab) For Intravenous Use, March 2008. Genentech, Inc. South San Francisco, CA:

Gastrointestinal Perforations
Avastin administration can result in the development of gastrointestinal perforation, in some instances resulting in fatality. Gastrointestinal perforation, sometimes associated with intra-abdominal abscess, occurred throughout treatment with Avastin (i.e., was not correlated to duration of exposure). The incidence of gastrointestinal perforation (gastrointestinal perforation, fistula formation, and/or intra-abdominal abscess) in patients with colorectal cancer and in patients with non-small cell lung cancer (NSCLC) receiving Avastin was 2.4% and 0.9%, respectively. The typical presentation was reported as abdominal pain associated with symptoms such as constipation and vomiting. Gastrointestinal perforation should be included in the differential diagnosis of patients presenting with abdominal pain on Avastin. Avastin therapy should be permanently discontinued in patients with gastrointestinal perforation.

Wound Healing Complications
Avastin administration can result in the development of wound dehiscence, in some instances resulting in fatality. Avastin therapy should be permanently discontinued in patients with wound dehiscence requiring medical intervention. The appropriate interval between termination of Avastin and subsequent elective surgery required to avoid the risks of impaired wound healing/wound dehiscence has not been determined.

Hemorrhage
Fatal pulmonary hemorrhage can occur in patients with NSCLC treated with chemotherapy and Avastin. The incidence of severe or fatal hemoptysis was 31% in patients with squamous histology and 2.3% in patients with NSCLC excluding predominant squamous histology. Patients with recent hemoptysis (≥1/2 tsp of red blood) should not receive Avastin.

Cardiovascular Adverse Effects
Genentech, Inc. informed healthcare professionals of reports of several cases of microangiopathic hemolytic anemia (MAHA) in patients with solid tumors who received bevacizumab in combination with sunitinib malate. Twenty-five patients were enrolled in a phase I dose-escalation study which included three treatment strata using a fixed dose of bevacizumab 10 mg/kg, IV every 2 weeks and escalating doses of sunitinib 25, 37.5, and 50 mg/day orally for four consecutive weeks followed by two weeks without sunitinib, every 6 weeks. Five of 12 patients at the highest sunitinib dose level exhibited laboratory findings consistent with MAHA. Two of these cases were considered severe with evidence of thrombocytopenia, anemia, reticulocytosis, reductions in serum haptoglobin, schistocytes on peripheral smear, modest increases in serum creatinine levels, and severe hypertension, reversible posterior leukoencephalopathy syndrome, and proteinuria. The findings in these two cases were reversible within three weeks after discontinuing both drugs without additional interventions.
<http://www.fda.gov/medwatch/safety/2008/MAHA_DHCP.pdf>

Arterial thromboembolic events (ATE) (e.g., cerebral infarction, transient ischemic attacks, myocardial infarction, angina) have been reported more often in patients receiving bevacizumab plus chemotherapy compared to patients receiving chemotherapy alone. Resuming bevacizumab therapy following resolution of an ATE has not been studied.

Bevacizumab increases the risk of hypertension, proteinuria, hypercoagulopathy, and arterial thrombosis by decreasing endothelial nitric oxide synthase activity, stimulating PAI-1 expression, and altering renal podocyte function and renal vasculature. Angiotensin converting enzyme inhibitors, in addition to their primary antihypertensive activity, may also prevent arterial thrombosis by decreasing serum PAI-1 levels and bevacizumab-induced proteinuria. Therefore, ACE inhibitors may be the preferred antihypertensive agent in the management of bevacizumab- induced hypertension.

In a single, open-label, randomized, multicenter study, congestive heart failure (G3 & G4) occurred in 2.2% of patients with metastatic breast cancer who received bevacizumab plus paclitaxel compared with 0.3% of patients who received paclitaxel alone. The incidence of CHF was found to be increased in patients who received anthracyclines prior to bevacizumab or concurrently.
Modest increases in diastolic and systolic blood pressures (BP) and clinical hypertension have are reported frequently during bevacizumab therapy (23 – 34% of patients). In most cases, increased BP is mild or moderate and controlled with antihypertensives. Severe hypertension has been reported more often in bevacizumab-treated patients compared to controls, with 8% to 18% of patients in clinical trials experiencing G3 or G4 hypertension. Up to 1.7% of patients required hospitalization or discontinuation of bevacizumab as a result of developing or worsening hypertension. Additionally, hypertension may persist after discontinuing bevacizumab.

Bevacizumab-induced hypertension may result in complications including hypertensive encephalopathy, which may sometimes be fatal.

Left ventricular dysfunction G2 – G4 was reported in 1.7% of patients who received bevacizumab in clinical studies. The safety of continuing or resuming bevacizumab therapy in patients with cardiac dysfunction has not been studied.

Dermatologic Adverse Effects

Alopecia.

Impaired wound healing (see the *Black Box* warning, above). The ideal interval between the termination of bevacizumab and elective surgery in order to avoid impaired wound healing or wound dehiscence has not been determined. In clinical studies, wound dehiscence was reported in 0.8% of patients who received chemotherapy plus bevacizumab, and has resulted in fatalities. The longest interval between a dose of bevacizumab and wound dehiscence was 56 days which occurred in a patient who received bolus irinotecan, fluorouracil, leucovorin, and bevacizumab. Do not initiate bevacizumab for at least 28 days following major surgery, and ensure a surgical incision is entirely healed before commencing or resuming bevacizumab.

Skin rashes (type unspecified) have been described in patients following bevacizumab infusion.

Metabolic Adverse Effects

In a randomized, open-label, clinical trial, G3 – G5 dehydration occurred in 10% of patients with metastatic colorectal carcinoma who received fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) plus bevacizumab, in 6% of patients who received bevacizumab monotherapy, and in 5% of patients who received FOLFOX4 only.

In a multicenter, randomized, open-label stud, hyponatremia G3 – G5 occurred in 4% of patients with previously untreated locally advanced, metastatic or recurrent non-small cell lung cancer who received paclitaxel, carboplatin, and bevacizumab compared to 1% of patients who received paclitaxel and carboplatin only.

Gastrointestinal Adverse Effects

Abdominal pain G1 – G4 was reported in 50 – 61% of patients with metastatic colorectal carcinoma. In a randomized, open-label, clinical trial, abdominal pain ≥G3 occurred in 8% of patients with metastatic colorectal carcinoma who received fluorouracil, leucovorin, oxaliplatin (FOLFOX4) and bevacizumab and in 8% of patients receiving bevacizumab monotherapy compared with 5% of patients who received FOLFOX4 only.

Constipation, any grade was reported in 29 – 40% of patients with metastatic colorectal carcinoma. Constipation G3 & G4 was reported in 4% and 2% of patients who received bolus-IFL (irinotecan, fluorouracil, leucovorin) plus bevacizumab and IFL plus placebo arms, respectively.

Diarrhea G≥3 has been reported in 2% of patients in association with bevacizumab monotherapy and up to 34% of patients with metastatic colorectal carcinoma who received bevacizumab in combination with chemotherapy.

Gastrointestinal hemorrhage in up to 24% of patients with metastatic colorectal carcinoma who received bevacizumab in association with chemotherapy.

Gastrointestinal perforation, sometimes associated with intra-abdominal abscess, has been reported in patients receiving bevacizumab (see the *Black Box* warning, above). GI perforation did not correlate with duration of exposure and occurred at various points during therapy. In pooled data, the incidence of GI perforation was up to 3.7%. In clinical trials, GI perforation with or without intraabdominal abscess or fistula formation was reported in 2.4% of patients with colorectal cancer and 0.9% of patients with non-small cell lung cancer treated with bevacizumab plus chemotherapy compared with 0.3% and 0% of patients treated with chemotherapy alone. GI perforation was typically associated with abdominal pain, constipation, and emesis.

In postmarketing analysis, cases of gastrointestinal perforation (approximately 30% being fatal) were reported in 1% of patients usually within 50 days after receiving bevacizumab (range, 1 wk to > 1 y).

Anorexia (G1 – G4), nausea (low emetic risk/ASCO criteria), stomatitis, altered taste.

Tracheoesophageal (TE) fistula occurred in a study in which patients with limited-stage small cell lung cancer (SCLC) received four cycles of concurrent irinotecan, carboplatin, radiation therapy, and bevacizumab followed by maintenance bevacizumab for up to 6 months.
Bevacizumab is not indicated for use in SCLC.
Two confirmed serious events of TE fistula (one fatal) were reported in the first 29 study participants. A third, fatal event (upper aerodigestive tract hemorrhage and death of unknown cause), was also reported, in which TE fistula was suspected but not confirmed. All three events occurred during the maintenance phase of the study in the context of persistent esophagitis. As of March 22, 2007, six cases of TE fistula have also been reported in other lung and esophageal cancer studies involving the use of bevacizumab and chemotherapy alone or with concurrent radiation treatment.
There is limited information in the published literature on the background rate of TE fistula in patients with limited-stage SCLC, but is estimated to be <1%, which was exceeded in the trial. Due to the small number of patients treated in the setting of limited-stage SCLC and the non-randomized nature of the trial, it was not possible to distinguish the toxicity observed in this trial from other risk factors for the development of TE. The study was closed to further accrual on March 12, 2007.
A description of cases of gastrointestinal tract fistula formation in patients with colorectal cancer and other types of cancer treated with Avastin® in clinical studies and post-marketing reports is included in the current US prescribing information.
[REF: Dear Healthcare Provider letter, entitled "IMPORTANT DRUG WARNING Regarding AVASTIN® (bevacizumab)" from Genentech, Inc., April 2007.
Linked to the US FDA MedWatch site at
www.fda.gov/medwatch/safety/2007/Avastin_DHCP_TEF_Final_April2007.pdf].

Hematologic Adverse Effects
Severe and fatal hemorrhage occurred more frequently in bevacizumab-treated patients versus patients who received chemotherapy alone (see the *Black Box* warning, above). Minor bleeding or hemorrhage (e.g., G1 epistaxis) have been reported following the administration of bevacizumab.

Administration of bevacizumab, in combination with chemotherapy, has been associated with serious hemorrhage and thromboembolic events. Other hematologic toxicities include neutropenia, febrile neutropenia, leukopenia, and thrombocytopenia. Some data suggest that vascular endothelial growth factors (VEGFs) may play a role in maintaining endothelial integrity of the tumor microvasculature, and that receptor blockade by bevacizumab may initiate a coagulation cascade.

Microangiopathic hemolytic anemia (MAHA) was reported in patients with solid tumors who received bevacizumab in combination with **sunitinib malate**.
In a phase 1 dose-escalation study, patients received a fixed dose of bevacizumab 10 mg/kg, IV every 2 wks and escalating doses of sunitinib that included 25, 37.5, or 50 mg/day orally for 4 consecutive wks followed by 2 wks without sunitinib. Five of 12 patients at the highest sunitinib dose level exhibited laboratory findings consistent with MAHA. Two cases were considered severe with evidence of thrombocytopenia, anemia, reticulocytosis, reductions in serum haptoglobin, schistocytes on peripheral smear, modest increases in serum creatinine, severe hypertension, reversible posterior leukoencephalopathy syndrome (RPLS), and proteinuria.
The findings in these two cases were reversible within three weeks upon discontinuation of both drugs without additional intervention. The information above led to the closure of a Genentech-sponsored phase 2 trial of sunitinib at 50 mg ± bevacizumab with a similar dosing schedule in which two of seven patients enrolled were found to have MAHA.
Two additional Genentech-sponsored randomized, Phase 2 studies of Avastin in combination with sunitinib and chemotherapy in patients with solid tumors were also closed due to poor tolerability primarily due to myelosuppression, fatigue and gastrointestinal complications (e.g., diarrhea, anorexia, dehydration, stomatitis). No events of MAHA have been reported in these studies.
Bevacizumab has not received FDA approval for use in combination with sunitinib malate, and is not recommended.
[REF: Dear Healthcare Provider letter, entitled "IMPORTANT DRUG WARNING: Microangiopathic Hemolytic Anemia (MAHA) in Patients treated with Avastin® (bevacizumab) and sunitinib malate" from Genentech, Inc., July 2008. Linked to the US FDA MedWatch site at
www.fda.gov/medwatch/SAFETY/2008/MAHA_DHCP.pdf].

Neutropenia, including febrile neutropenia, has been reported more frequently in patients who received bevacizumab plus chemotherapy compared to patients who received chemotherapy alone.

Hypersensitivity (Infusion) Reactions
In clinical trials and postmarketing surveillance, infusion reactions, including hypertension, hypertensive crises, wheezing, oxygen desaturation, G3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis, have been observed during bevacizumab administration. First dose infusion reactions have been reported in <3% of patients in clinical studies; 0.2% of patients reported a severe reaction. Bevacizumab therapy should be interrupted and appropriate management instituted in patients who experience a severe reaction. There is no information available regarding rechallenge or identification of patients who can be safely treated following severe infusion reactions.

Arthralgia has been reported occasionally after infusion with bevacizumab.

Neurologic Adverse Effects
Reversible posterior leukoencephalopathy syndrome (RPLS) has been reported in clinical trials (incidence <0.1%) and during postmarketing surveillance. Clinical presentation included headache, lethargy, seizures, confusion, blindness, and other visual and neurologic disturbances. Onset of symptoms has ranged from 16 h to 1 y after initiation of bevacizumab. Although some patients have experienced ongoing neurologic sequelae, symptoms usually resolve or improve within days. The safety of resuming bevacizumab therapy following RPLS is not known.

In a randomized, open-label, clinical trial, sensory neuropathy (G3 – G5) occurred in 17% of patients with metastatic colorectal carcinoma who received fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) plus bevacizumab and in 1% of patients who received bevacizumab alone compared with 9% of patients who received FOLFOX4 only. In a single, open-label, randomized, multicenter study in patients with metastatic breast cancer, sensory neuropathy (G3 – G5) occurred in 24.2% of patients who received bevacizumab plus paclitaxel *vs.* 17.5% of patients receiving paclitaxel alone.

Mild asthenia has been reported in up to 70% of patients during bevacizumab therapy. In contrast, asthenia G3 and G4 occurred in 10% of patients with metastatic colorectal carcinoma who received bolus irinotecan, fluorouracil, and leucovorin (bolus-IFL) plus bevacizumab compared with 7% of patients who received IFL plus placebo.

Headache, usually mild in severity, has been reported in up to 50% of patients receiving bevacizumab. In one study of patients being treated with bevacizumab for breast cancer, headache, with nausea and vomiting, was dose-limiting with bevacizumab 20 mg/kg·dose every 2 weeks.
Dizziness (G1 – G4).

Ophthalmologic Adverse Effects
In a retrospective case series, retinal pigment epithelial tears (RPE) occurred in 2.2% of patients with neovascular age-related macular degeneration treated with intravitreally administered bevacizumab.

Genitourinary Adverse Effects
Bladder and vaginal fistula formation.

Nephrotic syndrome and proteinuria have been reported in clinical trials at a higher rate in patients who received bevacizumab compared with control patients. In addition, proteinuria occurred at a higher rate in clinical trials in patients receiving bevacizumab compared with control patients. During these trials, bevacizumab was typically interrupted when urine protein was ≥2 grams/24 h, and subsequently resumed when urine protein levels returned to <2 g/24 h. The safety of continued therapy in patients with moderate to severe proteinuria has not been studied and improvement and resolution should be appropriately monitored with 24-hr urine collections. Bevacizumab therapy should be discontinued in patients experiencing nephrotic syndrome.

Respiratory Adverse Effects
Fatal pulmonary hemorrhage and hemoptysis have been reported in patients with non-small cell lung cancer treated with bevacizumab and chemotherapy, with life-threatening or fatal hemoptysis occurring in 31% of patients with squamous histology and 2.3% of patients with non-squamous non-small cell lung cancer.

In a randomized, double-blind, clinical trial, dyspnea (G1 – G4) occurred in 26% of patients with metastatic colorectal carcinoma who received bolus irinotecan, fluorouracil, and leucovorin (bolus-IFL) plus bevacizumab and 25% of patients received fluorouracil, leucovorin, and bevacizumab compared with 15% of patients who received IFL plus placebo.

In a randomized, double-blind, clinical trial, epistaxis (G1 – G4) occurred in 35% of patients with metastatic colorectal carcinoma receiving bolus irinotecan, fluorouracil, and leucovorin (bolus-IFL) plus bevacizumab and 32% of patients received fluorouracil, leucovorin, and bevacizumab compared with 10% of patients who received IFL plus placebo.

Bronchopleural fistula formation, pulmonary hypertension, voice alteration.

In clinical studies or postmarketing experience, nasal septum perforation has been reported in patients receiving bevacizumab.

Reproductive Adverse Effects
There is insufficient clinical experience with bevacizumab to confirm its safety in pregnancy. The potential to disrupt angiogenesis during fetal development following the administration of bevcizumab is likely to result in adverse effects on pregnancy. Persons should be counseled after discontinuing bevacizumab use regarding prolonged exposure of the drug, given a half-life of approximately 20 days, and the possible effects on the fetus.
No human studies of pregnancy outcomes after exposure to bevacizumab have been published, and there are no reports of outcomes following inadvertent exposure during pregnancy. Studies conducted in rabbits given twice the recommended human dose based on a mg/kg basis resulted in teratogenic effects, including decreases in fetal and maternal weights, increased fetal resorptions and an increased incidence of specific gross and skeletal alterations.

It is not known whether bevacizumab is excreted into human breast milk and the potential for adverse effects in a nursing infant from exposure to the drug are unknown. It is not known if bevacizumab affects the quantity or composition of breast milk.

Other Adverse Effects
Fatigue, low-grade fever has been reported with variable frequency during bevacizumab therapy.

4. Etoposide

Formulation

Etoposide Injection, USP, is commercially available in sterile, multiple-dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each milliliter of drug product contains 20 mg etoposide, USP; 2 mg citric acid; 30 mg benzyl alcohol; 80 mg modified polysorbate 80/tween 80; 650 mg polyethylene glycol 300; and 30.5% (v/v) alcohol.

Etoposide Injection, USP, will be purchased from commercial sources by the NIHClinical Center Pharmacy Department.

Stability
Intact vials are stable for 24 months at room temperature (25°C, 77°F).

The stability of etoposide is concentration dependent. When diluted as described (above) in either glass or plastic containers and stored at room temperature under normal room fluorescent light, etoposide demonstrates stability as follows:

solution concentration	**Duration of stability**
0.2 mg/mL	96 hours
0.4 mg/mL	48 hours
0.6 mg/mL	12 hours
1.0 mg/mL	2 hours

Storage
Store intact vials and diluted solutions at controlled room temperatures, 15° – 30°C (59° – 86°F). DO NOT REFRIGERATE etoposide in vials or diluted solutions.

Preparation
Etoposide Injection will be diluted in a volume of 5% Dextrose Injection, or 0.9% Sodium Chloride Injection, USP, sufficient to produce a concentration between 0.2 – 0.4 mg/mL prior to administration:

calculated dose	**Vehicle solution volume**
100 – 200 mg	500 mL
201 – 400 mg	1000 mL

Dosage and Administration
Administer etoposide intravenously over at least 60 minutes, daily for three consecutive days, every 21 days. Longer infusion durations may be used if the volume of fluid to be administered is of concern, or if a patient experiences hypotension or evidence of hypersensitivity.

Adverse Effects
In general, toxicities are increased in patients with decreased serum albumin or hyperbilirubinemia due to decreased protein binding and an increased fraction of unbound etoposide, and in persons with renal impairment
in whom elimination of etoposide and its metabolites is decreased.

Hematological
Myelosuppression is dose-related and dose-limiting. Leukopenia is the most common adverse effect associated with oral or intravenous etoposide. Granulocyte nadirs characteristically occur 7 – 14 days after etoposide administration, platelet nadirs occur 9 – 16 days after administration, and bone marrow recovery is usually complete by 20 days after etoposide administration. Cumulative myelosuppression has not been reported.

Concurrent anemia is an independent risk factor for myelotoxicity, in part because it decreases the volume of distribution of epipodophyllotoxins and increases the circulating concentration of the unbound drug.
Fever and infection have also been reported in patients with neutropenia. Death has been reported in association with myelosuppression.

Acute leukemia with or without a preleukemic phase has been reported rarely in patients treated with etoposide in association with other antineoplastic agents. Secondary leukemia occurred from 9 – 68 months after diagnosis of the first cancer; treatment responses have been poor in most patients.

Acute leukemia with or without a preleukemic phase has been reported rarely in patients treated with etoposide in association with other antineoplastic agents for acute lymphocytic leukemia (ALL) or solid tumors. The incidence of acute myeloid leukemias (AML) in three retrospective case series ranged from 0.37 – 8.1%. Patients with childhood ALL who received treatment containing etoposide have been found to develop an acute nonlymphocytic leukemia characterized by a translocation in chromosome 11 (11q23). The site is the locus for the mixed-lineage leukemia gene which regulates pluripotent stem cell proliferation. The secondary leukemic cells were found morphologically similar to acute monocytic or monomyelocytic leukemia cells.

Etoposide-related leukemia is characterized by a short interval between the end of treatment and onset of leukemia (1 – 3 years) and the absence of a myelodysplastic period preceding leukemia, which distinguish it from a 4- to 5-year interval to secondary leukemias among patients who received treatment with alkylating agents. Ten of 205 patients who received treatment for ALL which included etoposide developed secondary AML. The risk of secondary AML at 4 years was 5.9 ±3.2% which suggests a strong link
between etoposide use and secondary AML since none of the patients had received alkylating agents or irradiation. Patients who receive etoposide on weekly or twice-weekly schedules, with cumulative doses > 2000 mg/m^2, may be at increased risk for developing a secondary leukemia.

Gastrointestinal
The risk of nausea and vomiting after parenteral etoposide administration is low (approximately 15% of patients who do not receive antiemetic prophylaxis), but symptoms occur more frequently (up to 90% of patients) and may be the dose-limiting toxicity with oral administration. In general, the severity of emetic symptoms is mild to moderate.

Mucositis is directly related to etoposide dosage and the duration of exposure, and is reported in 1 – 6% of patients treated with etoposide at FDA approved dosages and schedules.

Diarrhea has been reported in 1 – 13% of patients receiving etoposide.

Anorexia has been reported in approximately 10 – 13% of patients treated with either intravenous or oral etoposide

Hypotension
Transient hypotension following rapid intravenous administration has been reported in 1 – 2% of patients, and may be avoided by slow intravenous administration over at least 30 minutes. Hypotension has not been associated with cardiac toxicity or ECG changes. Delayed hypotension has not been noted If hypotension occurs, it usually responds to interrupting etoposide. administration and, if necessary, administration of fluids or other supportive therapy as appropriate. When resuming intravenous administration after a hypotensive event, a slower rate should be used.

Hypersensitivity
Anaphylactoid reactions characterized by chills, fever, tachycardia, hypotension, bronchospasm, flushing, exanthema, dyspnea, tightness, cyanosis, and hypertension have been reported in 0.7 – 2% of patients who receive etoposide intravenously and in < 1% of patients treated with etoposide capsules administered orally. Hypersensitivity reactions often respond promptly to the cessation of etoposide administration and administration of pressor agents, glucocorticoids, antihistamines, or volume expanders as appropriate; however, fatal reactions have occurred. Hypertension and flushing have also been reported. Blood pressure usually normalizes within a few hours after discontinuing intravenous etoposide administration. Anaphylactoid reactions have been observed during initial exposure to etoposide.

Although an immunological basis for hypersensitivity cannot be excluded, hypersensitivity reactions are reported to occur less frequently with orally administered etoposide and etoposide phosphate which, unlike formulations of etoposide for parenteral administration, do not contain polysorbate 80.
Face and tongue swelling, coughing, diaphoresis, cyanosis, throat tightness, laryngospasm, back pain, and loss of consciousness have sometimes occurred in association with other hypersensitivity reactions. An apparent hypersensitivity-associated apnea has been reported rarely.

Rash, urticaria, and pruritus have infrequently been reported at FDA approved dosages and schedules.

Skin and Integument
Reversible alopecia occurs commonly (8 – 93% of patients) and may progress to total baldness. Ultraviolet light-induced 'recall' dermatoses,
StevensJohnson syndrome, rashes, cutaneous eruptions, urticaria and pruritis, exfoliative dermatitis, erythrodysesthesia, and onycholysis have been associated with etoposide use.

Chemical phlebitis has been reported during etoposide use, and is thought to be due to solvent components in the drug formulation. Rarely, extravasation has been associated with necrosis and venous induration.

Pulmonary
Bronchospasm, pneumonitis, pulmonary toxicity, dyspnea and apnea have been

described with etoposide use.

Hepatotoxicity
Hepatotoxicity has been reported in up to 3% of patients who received etoposide, and is particularly evident after high-dose treatment. Hepatotoxicity may include hepatitis with transiently increased hepatic enzymes (alkaline phosphatase, total bilirubin, ALT), hepatocellular necrosis, hyperammonemia, hyperbilirubinemia, and ascites. Hepatocellular necrosis has been reported following high dose etoposide (600 – 2400 mg/m^2).

Reproduction, Fertility, and Gestation
Ovarian failure, amenorrhea, anovulatory cycles, and hypomenorrhea have been described in association with etoposide therapy. Etoposide is potentially gonadotoxic and should be used with caution in young females.
Etoposide is teratogenic and may increase the risk of congenital malformations if given during the first trimester of pregnancy.
Etoposide is categorized among Hazardous Drugs at the NIH Clinical Center.

Other Adverse Effects
The following adverse reactions have been infrequently reported: abdominal pain, aftertaste, constipation, dysphagia, asthenia, fatigue, malaise, somnolence, transient cortical blindness, optic neuritis, fever, and seizure (occasionally associated with allergic reactions).
Although rare, cerebral edema due to a capillary leak syndrome, angina pectoris, myocardial infarction, and congestive heart failure have been described with etoposide in clinical use.

Drug Interactions
Cytochrome P450 (CYP) CYP3A4 inhibition by **aprepitant** or **fosaprepitant** theoretically may result in increased concentrations of etoposide in plasma and potentially increased etoposide side effects.

Vaccination with **live virus vaccines** (mumps, poliovirus, rotavirus, rubella, rubeola, varicella, variola, yellow fever virus) and **live bacteria vaccines** (BCG, typhoid) in patients with immunity compromised by chemotherapeutic agents has resulted in severe and fatal infections. At least three months should elapse between the discontinuation of chemotherapy and vaccination with a live vaccine.

Coadministration of etoposide and **cyclosporine** may increase etoposide serum concentrations and risk of adverse reactions.

Glucosamine induced resistance to the topoisomerase II inhibitors etoposide and doxorubicin in *in vitro* experiments. It is currently not known whether oral glucosamine administration concurrent with etoposide may compromise the latter drug's antineoplastic effect. Therefore, the combination should be avoided.

Concomitant **grapefruit juice** ingestion decreased the bioavailability of etoposide by a mean 26% in a randomized crossover trial. The effect may be attributable to grapefruit juice induction of P-glycoprotein and enhanced extracellular transport of etoposide into the gut lumen. Concomitant use of etoposide and grapefruit juice and grapefruit products should be avoided.

Etoposide is a substrate for metabolism by CYP3A4 and extracellular transport by P-glycoprotein (MDR1, ABCB1). **St. John's Wort** induces CYP3A4 and P-glycoprotein and, consequently, may decrease the effectiveness of etoposide while potentially increasing the formation of leukemogenic etoposide metabolites via CYP3A4. Use of St. John's Wort should be avoided in patients who receive treatment with etoposide.

Combined use of **valspodar** with chemotherapeutic regimens containing etoposide has resulted in significant decreases in etoposide clearance (40 – 60%), associated with significant increases in the etoposide area under the plasma concentration-*vs*-time curve and elimination half-life and a reduced volume of distribution. Dose reductions of etoposide of up to 66% have been

required to minimize toxicity.

In patients who receive **warfarin**, concomitant etoposide may increase the International Normalized Ratio (INR) and bleeding. Monitor INR closely and for signs of bleeding when warfarin and etoposide are used concomitantly

Appendix IX

NIH POLICY MANUAL

1340-1 - Permits for Import or Export of Biological Materials
Issuing Office: OD/OM/ORS/DOHS (301) 496-2960
Release Date: 02/01/08

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1. **Explanation of Material Transmitted:** This manual chapter describes the NIH policy and procedures concerning the requirements for the importation or shipment of etiological agents, their vectors, animals, and plants and for the exportation of biological materials. This chapter is being revised to update organizational references and to add 2 new required sections: Records Retention and Management Controls.

2. **Filing Instructions:**

   Remove: NIH Manual 1340-1, dated 11/15/96
   Insert: NIH Manual 1340-1 dated 02/01/08

PLEASE NOTE: For information on:

- Content of this chapter, contact the issuing office listed above.
- On-line information, enter this URL: http://www1.od.nih.gov/oma/manualchapters/
- To sign up for e-mail notification of future changes, please go to the NIH Manual Chapters LISTSERV Web page.

A. Purpose:

This chapter describes the NIH policy and procedures concerning the requirements for permits for the importation or shipment of etiological agents, their vectors, animals and plants, and for the exportation of biological materials. This chapter is being revised to comply with the requirement that chapters be reviewed every 5 years. In addition, 2 new sections have been added (a) Records Retention and (b) Management Controls.

B. Background:

In recent years, the concern over the safe and secure transport of hazardous material has intensified in an effort to ensure personal and public safety during transport of etiological

agents, their vectors, animals and plants, and for the exportation of biological materials. In the United States, all hazardous material packages that are offered for domestic transport are subject to the DOT regulations. All hazardous material packages shipped internationally are subject to the requirements established by the United Nations International Civil Aviation Organization (ICAO) whose guidelines are adopted by the International Air Transport Association (IATA). Both regulatory bodies establish definitions and requirements for the classification, packaging, marking, labeling, and documentation of hazardous material packages. Personal, civil and criminal penalties have been established for willful violation of these regulations.

Failure to comply with import and export requirements may result in shipment release delays or shipment confiscation and destruction by the Quarantine Officer at the port of entry.

C. Policy:

NIH will conform to all applicable laws and regulations for the importation and shipment of etiological agents and vectors.

It is the policy of the NIH to ensure that all packages being offered for transport comply with all Federal and international regulations for ground and air transport in order to protect the safety of the laboratory staff, support staff, the environment, and the public.

No person at NIH shall make arrangements to receive or ship an etiological agent, vector, animal, or plant before ascertaining the necessity for a permit and obtaining a permit when required.

Etiological agents and vectors of human or animal disease cannot be transported in a privately owned vehicle (POV). To transport via land, a government vehicle may be used. All applicable packaging requirements of the U. S. Department of Transportation (DOT) regulations must be followed (49 CFR Parts 171- 178).

Any person at the NIH wanting to personally transport etiological agents and/or vectors of human or animal disease via air must have the material packaged by an appropriately trained and certified individual, following IATA packaging instructions. The material must be declared prior to departure.

No person at NIH shall transfer PHS-permitted materials to another laboratory or facility within NIH or to another Federal or private facility without prior authorization by the Division of Occupational Health and Safety, Quarantine Permit Service Office (QPSO).

NIH Policy Manual 1340-1 sets forth the responsibilities and specific requirements for acquiring appropriate import and export permits for shipping hazardous materials. All hazardous materials shipped from the NIH Bethesda campus must be shipped through the Office of Logistics and Acquisitions Operations, Freight Forwarding Team. Remote NIH

facilities must establish site specific requirements for the transport of hazardous material packages to facilitate compliance with this policy.

D. References: Legislative Sources:

1. Department of Health and Human Services, Public Health Service quarantine regulations task the Centers for Disease Control and Prevention (CDC) with management of human etiological agent import/transfer program. The regulation states: "A person may not import into the United States, nor distribute after importation, any etiological agent or any arthropod or other animal host or vector of human disease, or any exotic living arthropod or other animal capable of being a host or vector of human disease unless accompanied by a permit issued by the Director." (42 CFR 71.54).

2. U.S. Department of Agriculture (USDA) regulations state: "No organisms or vectors shall be imported into the United States or transported from one State or Territory or the District of Columbia to another State or Territory or the District of Columbia without a permit issued by the Secretary and in compliance with the terms thereof...." (9 CFR 122.2).

Note: The USDA will not permit the importation of cell cultures, monoclonal antibodies, ascites fluid or bovine serum from countries where rinderpest and foot-and-mouth disease are present unless the imported materials are determined to be virus-free.

3. Similar USDA regulations are concerned with agents and vectors of plant disease (7 CFR 330). These regulations seldom affect the work of biomedical investigators but are applicable.

4. The United States Fish and Wildlife Service (USFWS), U.S. Department of Interior, is responsible for regulations involving the prevention and control of wildlife diseases and for the importation of wildlife and eggs thereof (50 CFR 23). In addition, the USFWS represents the United States to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). If biomedical investigators wish to use biological materials or tissues derived from fauna or flora listed in Appendices I, II or III of the convention, a CITES permit is required (50 CFR 23). The USFWS has granted the NIH a Designated Port Exemption permit to allow for the importation of materials transiting through Washington Dulles International Airport, however, the importation of certain animals and birds is prohibited.

5. The U. S. Department of Commerce (DOC), Bureau of Industry and Security, is responsible for implementing and enforcing the Export Administration Regulations (EAR), which regulate the export and re-export of most commercial items.

6. Biological materials and etiological agents are subject to packaging and shipping requirements of various Federal and International regulations. Proper packaging

is the primary consideration and of utmost importance in the safe transportation of hazardous materials. The DOT regulations (49 CFR 171 – 178) and IATA's Dangerous Goods Regulations dictate the proper packaging requirements necessary for most biological materials.

E. Delegation:

The Centers for Disease Control and Prevention (CDC) Etiological Agent Import Permit Program Atlanta, Georgia, has authorized NIH to issue permits for the importation of etiological agents and vectors of human disease into NIH laboratories. It is a condition of this authorization that NIH maintains a record of each permit issued and documents the transfer of all PHS-permitted material.

NIH has established the Quarantine Permit Service Office (QPSO), Division of Occupational Health and Safety (DOHS), Office of Research Services (ORS), Building 13, Room 3K04 for matters involving the import or export of biological materials. Permit information is available by calling (301) 496-2960 or by going online at http://dohs.ors.od.nih.gov/shipping_biological_material.htm.

F. Responsibilities:

1. The NIH Quarantine Permit Service Office is responsible for:

a. Providing information and guidance to NIH components on the requirements for import or export of biological materials.

b. Issuing PHS import/transfer permits or letters of non-infectious import for biological materials arriving to the NIH.

c. Executing export declarations for biological materials leaving the United States.

d. Maintaining records and the submission of reports to regulatory agencies.

Note: In some cases, more than one permit may be required for the import/transfer or export of a biological material. Although the QPSO does not issue USDA or USFWS permits, the office will provide assistance in obtaining these permits. Should QPSO determine a DOC validated license is required for the export or re-export of a biological material, the office will notify the investigator and apply directly to the DOC on his/her behalf.

The transfer of select agents and toxins is managed by the NIH Select Agent Program, following the regulations promulgated in 42 CFR 73, "Select Agents and Toxins". The QPSO requires that a request for permit to import an etiological agent requiring Biosafety Level 3 (BSL3) or Biosafety Level 4 (BSL4) containment be submitted with the written concurrence of the IC Scientific Director.

2. IC Scientific Directors are responsible for:

a. Providing written concurrence for the import of an etiological agent requiring Biosafety Level 3 (BSL3) or Biosafety Level 4 (BSL4).

b. Ensuring staff compliance with QPSO policy and regulations involving the import and export of etiological agents, disease vectors, animals and plants, and other biological materials.

G. Procedures:

1. Imports:

   a. To ascertain the need for a permit to import or transfer a human etiological agent, vector of human or animal disease, animal or plant, contact the QPSO at least six weeks before the date of shipment in order to allow adequate time for processing the permit request.

   b. A person wishing to import biological material, or transfer previously permitted material to another NIH/PHS laboratory, must obtain a PHS import permit by submitting the application, Form CDC 75-3, "Application for Permit to Import or Transport Agents or Vectors of Human Disease" to the QPSO via fax (301) 480-0671. This form is available on the DOHS website at http://dohs.ors.od.nih.gov/forms.htm. The QPSO will determine whether a PHS permit or letter of non-infectious import is required.

   c. A person wanting to apply for an import/export/re-export permit of wildlife samples and/or biomedical samples collected from an endangered species must complete USFWS Form 3-200-29, “Import, Export, Re-export of Wildlife Samples and/or Biomedical Samples”. This permit application is submitted directly to USFWS and is available online at: http://www.fws.gov/permits/ . Applicants should be prepared to wait 60-90 days for a determination and be aware that an application fee applies. Once approval is granted, a copy of the USFWS permit must be forwarded to the QPSO.

   d. A person wanting to apply for a USDA Animal and Plant Health Inspection Service (APHIS) permit must submit the permit application directly to the USDA using the ePermit system available at http://www.aphis.usda.gov/permits/. Both USDA and PHS permits may be required for the importation of some biological materials. An application fee applies. Once approval is granted, a copy of the USDA permit must be forwarded to the QPSO. Applicants must contact the QPSO prior to shipment if live animals are to be received at the Washington Dulles International Airport under the NIH designated port exemption.

   e. No person at the NIH shall distribute a permitted etiological agent or vector of

human disease unless the intended recipient provides a copy of the appropriate permit authorizing the receipt of the material.

2. Exports:

   a. In general, biological materials may be exported to most countries under the provisions of the Export Administration Regulations (EAR), DOC. A person wanting to export a biological material must submit Form NIH-2388, "Declaration for Exportation of Biologic Materials", to the QPSO. This form is available on the NIH Forms website at http://forms.nih.gov/adobe/procurement/NH2388.PDF and can be faxed to (301) 480-0671. Authorization is dependent upon the nature of the biological agent or material, recipient, proposed country of destination, and commercial value of the shipment.

   b. The recipient's country may impose import restrictions or require that the recipient obtain an import permit from the appropriate issuing agency in the recipient's country. If an import permit is required, the permit number or a copy of the import permit must be included with the "Declaration for Exportation of Biologic Materials", and provided to the QPSO.

3. Interstate Shipments:

   In general, indigenous etiological agents and vectors are not subject to control by Federal or other agencies for U.S. interstate shipments, however there are exceptions (e.g., establishment of a colony of *Aedes aegypti* mosquitoes, interstate transport of plant pests, blue tongue virus, etc.). Contact the QPSO for guidance before transporting etiological agents that may be subject to interstate shipment restrictions.

4. Packaging Requirements:

Biological materials, including diagnostic specimens, are subject to packaging requirements described in the DOT regulations (49 CFR 171 – 178) and the International Air Transport Association (IATA) Dangerous Goods Regulations. Shipments known or suspected to contain a pathogen or toxin are subject to additional packaging and shipping requirements described in these regulations.

Only a person that has been properly trained may package hazardous materials for shipping. All packaging must follow DOT and IATA regulations. For information on packaging and shipping training, visit the DOHS website at http://dohs.ors.od.nih.gov/Resources_main.htm.

H. Records Retention and Disposal:

For this chapter, records pertaining to NIH Permits For Import or Export of Biological Materials are retained and disposed of under the authority of NIH Manual 1743 "Keeping

and Destroying Records," Appendix 1, "NIH Records Control Schedule," Item 1300-B-3, safety management subject files.

NIH e-mail messages. NIH e-mail messages (messages, including attachments, that are created on NIH computer systems or transmitted over NIH networks) that are evidence of the activities of the agency or have informational value are considered Federal records. These records must be maintained in accordance with current NIH Records Management guidelines. Contact your IC Records Officer for additional information.

All e-mail messages are considered Government property, and, if requested for a legitimate Government purpose, must be provided to the requester. Employees' supervisors, NIH staff conducting official reviews or investigations, and the Office of the Inspector General may request access to, or copies of, e-mail messages. E-mail messages must also be provided to Congressional oversight committees, if requested, and are subject to Freedom of Information Act requests. Since most e-mail systems have back-up files that are retained for significant periods of time, e-mail messages and attachments are likely to be retrievable from a back-up file after they have been deleted from an individual's computer. The back-up files are subject to the same request as the original messages.

I. Management Controls:

The purpose of this Manual Chapter is to establish the NIH policy and procedures concerning the requirements for permits for the importation, transfer, export or shipment of etiological agents, their vectors, animals and plants.

1. **Office Responsible for Reviewing Management Controls Relative to this Chapter (Issuing Office):** Through this Manual Chapter, the Quarantine Permit Service Office (QPSO), Division of Occupational Health and Safety (DOHS), Office of Research Services (ORS) is responsible for the method used to ensure that management controls are implemented and working.

2. **Frequency of Review:** Annual review

3. **Method of Review:** The QPSO, DOHS will maintain oversight and ensure compliance with this policy.

   The QPSO shall maintain a registry of all NIH personnel who have completed the DOHS sponsored *Shipping Infectious Substances* training course. The QPSO shall also maintain files of all export declarations and import/transfer permits issued. In addition, the QPSO shall maintain a database of all shipping related incidents which are reported to the DOHS.

4. **Review Reports are sent to:**

The QPSO is required to submit an annual summary report to the CDC regarding the total number of imports/transfers of biological material.

The QPSO will provide an annual report at the end of the calendar year of the issued import/transfer permits, export declarations and shipping incidents investigated to the Director, DOHS, the Associate Director for Research Services and the Deputy Director for Management.

Appendix X

SOP No. 6 Version 3 Inception Date: 12/15/04

Title: Preparation and shipping of biohazard

Filename: NCI10B.10_G1:Group:Clinical Pharmacology:SOPs:SOP No_6 Sample HandlingV3.doc

This SOP describes all the procedures necessary to prepare biological samples for transport in the Clinical Pharmacology Program, Medical Oncology Branch, National Cancer Institute.

Version No.	Date Revised	Revision Summary
1.	12/15/04	Original
2.	3/26/07	Updated titles and names
3.	9/30/08	Updated to comply with new CFR-49 and IATA regulations

Inception by:

William D. Figg, Head, CPP, MOB, CCR, NCI
Signature: _____ Date: _____

Erin Gardner, CPP, SAIC-F
Signature: _____ Date: _____

Kathryn Compton, CPP, SAIC-F
Signature: _____ Date: _____

4 pages

Approval of this page indicates approval of all pages of this SOP.

Approval Signature: _____ Date: _____

Annual Review Signature and Date:

_____ _____
_____ _____
_____ _____

SOP No. 6 Version 3 Inception Date: 12/15/04

1.0 Scope and Application

This is a Standard Operating Procedure (SOP) outlining the procedures, which should be carried out in order to prepare biological samples for shipment or transport off campus.

It complies with federal regulations outlined in 42 CFR 72.2 and 49 CFR.

2.0 Responsibility

All laboratory employees who pack samples for shipment or transport off campus are responsible for ensuring that sample packaging complies with federal regulations. A one day Shipping Biological Materials certificate course is offered by the NIH and should be attended by all personnel who will regularly be packaging biological samples for shipment.

3.0 Definitions

3.1 Biohazard label – OSHA Biohazard Label Marking is required to be used on the outside of the package per 29 CFR part 1910.1030 for blood borne pathogens requirements.

3.2 Carbon Dioxide, Solid (Dry Ice) – produced by expanding liquid carbon dioxide to vapour and "snow" in presses that compact the product into blocks. Used primarily for cooling (~ -79°C) and can cause severe burns to skin upon direct contact. As it converts to gaseous carbon dioxide, it takes in heat from its surroundings. The resulting gas is heavier than air and can cause suffocation in confined areas as it displaces the air. Packages containing this must be designed and constructed so as to prevent build-up of pressure due to the release of carbon dioxide.

3.3 Category B – an infectious substance which does not meet the criteria for inclusion in Category A. These substances must be assigned to UN 3373.

3.4 Infectious Substances – substances which are known or reasonably expected to cintain pathogens.

3.5 Overpack – an enclosure used by a single shipper to cintain one or more packages and to form one handling uint for convenience of handeling and stowage. Dangerous goods packages contained in the overpack, must be properly packed, marked, labelled and in proper condition as required by these Regulations.

3.6 Pathogens – micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals.

3.7 Patient Specimens – those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood or its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

3.8 UN Number – the four-digit number assigned by the Unite Nations Committee of Experts on the Transport of Dangerous Goods to identify a substance or a particular group of substances. (The prefix "UN" must always be used on conjunction with these numbers.)

4.0 Procedure

4.1 Unless otherwise known, all samples must be treated as hazardous.

4.2 Federal requirements for biological samples as contained in 42 CFR 72.2 and 49 CFR must be followed.

4.2.1 Primary Containment – sample must be within a sealed container. In most cases, a sample vial will be the primary containment.

4.2.1.1 Category A specimens - primary container volume must not exceed 50 ml.

4.2.1.2 Category B specimens – primary container volume must not exceed 1 liter.

4.2.2 Secondary Containment – the primary container must be placed within a sealed secondary container. This can take the form of a sealed plastic bag or other plastic container suitable for containing the sample in the case of thawing and/or breakage of the primary container.

4.2.3 Absorbent Material – absorbent material must be enclosed between the primary and secondary containers, for any liquid samples (including those that are frozen). This material must be able to absorb the total volume held in the primary container.

4.2.4 Itemized List – an itemized list of the contents must be enclosed between the secondary container and the outer shipping container.

4.2.5. Outer shipping Container – this container will enclose the secondary container(s). The outer shipping container must contain no more than 4 liters.

4.2.6 If dry ice is to be used, it must be between the secondary container and the outer shipping container. The outer shipping container must be designed to permit the release of carbon dioxide gas and to prevent the buildup of pressure. A means of support must be used to maintain the secondary container in its original position to prevent movement as dry ice dissipates.

Area Count

4.2.7 The shipping container must have appropriate labels and markings for biohazard agents and dangerous goods.

4.2.7.1 Category A specimens – UN2814 marking and "Infectious substances, affecting humans" marking

4.2.7.2 Category B specimens – UN3373 marking and "Biological substance, Category B" marking

4.2.7.3 Dry Ice – "Dry Ice UN1845" marking and Class 9 label

4.3 Specific instructions for packaging of boxes containing cryovials

4.3.1 These will be packaged following regulations in Section 3.2.

4.3.2 Place each box within a resealable biohazard bag.

e.g. LabGuard Reclosable Biohazard Bags
Size: 20.3 x 25.4 (8 x 10)
VWR catalog # 56766-372

4.3.3 Insert an absorbent strip into each bag and seal.

e.g. Saf-T-Pak STP-152
12 in, 250 ml absorbent strip
www.saf-t-pak.com

4.3.4 Place all bags inside a box with a Styrofoam cooler and surround with sufficient dry ice to keep samples frozen.

4.3.5 Affix biohazard label to outside of box.

Appendix XI

SOP No. 4 Version 2 Inception Date: 11/23/04

Title: Entering Sample Data into the Patient Sample Data Management System

Filename: Group Drive: Clinical Pharmacology/SOPs/SOP No_4 Sample Data Entry v2.doc

This SOP describes how to enter new sample data into LabSamples for the Clinical Pharmacology Program, Medical Oncology Branch, CCR, NCI.

Version No.	Date Revised	Revision Summary
1.	11/23/04	Original Approved SOP
2.	3/26/07	Updated titles and names

Inception by:

W. D. Figg, Head, CPP, MOB, CCR, NCI
Signature: _____ Date: _____

Erin Gardner, CPP, SAIC-F
Signature: _____ Date: _____

Kathy Compton, CPP, SAIC-F
Signature: _____ Date: _____

3 pages

Approval of this page indicates approval of all pages of this SOP.

Approval Signature: _____ Date: _____

Annual Review Signature and Date:

_____ _____
_____ _____

SOP No. 4 Version 1 Inception Date: 10/21/04

1.0 Scope and Application

This SOP describes how to enter new sample data into LabSamples.

2.0 Responsibility

2.1 All laboratory employees are required to follow this SOP when using LabSamples.

3.0 Procedure

3.1 Log into LabSamples (Refer to SOP 3 Sample System Access – 3.0 Procedure to Log on to Sample System).

3.2 Select 'Enter New Sample' button from main menu.

3.3 After 'Create Sample Data' screen loads, type in the patient's last name in the 'Last Name' field or, if known, the Patient ID and hit return on the keyboard.

3.4 If there is more than one patient with that last name (e.g. Smith) then a list of all patients Dr. Figg's lab has access to will appear in a new window 'Patient Search Results'.

3.5 If the correct first name appears in the list, click on the name to highlight it and then choose the 'Select Record' button.

3.6 If unsure, double-check the record by selecting it and verifying the correct trial on the next screen (Patient Data Screen). If you are still unsure, do not enter the sample data into the system. Check with the research nurse and other blood processing personnel to find out the correct patient name.

3.7 Once the correct record is found and selected, the 'Create Sample Data' window will appear again with the selected Patient Information. Be sure that the correct protocol for the blood sample is shown in the 'Protocol' drop down menu. If the protocol is not listed, double check the patient data to ensure the name is correct.

3.8 Select the correct type of sample from the 'Sample Type' drop down menu (i.e. Plasma for green top tube, Serum for red top tubes, etc.).

3.9 The Integrity of the Sample should be set to 'Unknown at Present', if you are entering data before the sample has been processed. Otherwise, please choose the best descriptor, and make a note if necessary (see section 3.16).

3.10 The 'Sample Drawn' date/time should be entered as the day and time the research nurse gave as the sample draw time. They normally place this information on the tube or the bag the tube was in. If no time is given, the research nurse needs to be contacted to find the correct time. **Do not estimate the draw time.** (Note: most tube labels state the draw time as 8AM. This is generally incorrect and should not be used as the draw time.)

3.11 The 'Placed in Freezer' date/time should be set as the current date/time.

3.12 Unless otherwise noted, all samples should be listed as 'On Study' in the 'Relationship to Protocol' field.

3.13 In the 'Number of Aliquots' field, select the correct number of aliquots to be created – typically two.

3.14 While the LabSamples does not require 'Storage Information' to be given in order to save data, all blood samples must be placed in boxes before data is saved. Select the correct freezer box to place the sample in from the 'Box' drop down menu.

3.15 If the correct box is unknown, locate the current 'box list' next to the main blood processing computer to check and/or look for the most current boxes in the -80°C freezer 7.

3.16 Information about pharmacokinetic samples must be entered into the 'Sample Notes' screen (i.e. Pre/Post drug infusion hour information). This is located under a tab at the top left side of the window. A 'Subject' and 'Sample Note' must be entered. Then click on the 'Add to List' button and save.

3.17 Any other uncharacteristic information about that particular blood sample should be noted in the sample notes. (Such as any possible human error when entering into LabSamples or an unknown blood draw time.)

3.18 Select ‘Save’ from the buttons at the bottom of the window.

3.19 Select ‘Print Barcodes’.

3.20 Once barcodes are printed, cancel out of windows and enter the next sample. If entering samples for the same patient, select the ‘Copy Info’ button. This prevents superfluous data entry.

This process needs to be completed for each blood tube received

Appendix XII

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
Building :_____
Room :_____
(301) 496- _____

COMMERCIAL INVOICE

RECEIVER'S COMPLETE ADDRESS AND PHONE NUMBER

NUMBER OF PACKAGES AND DETAILED LISTING OF CONTENTS
(If biologics or chemicals, etc., note if infectious or harmful to humans, dry ice, milliliters, kilograms, etc. If not, state as such.)

COUNTRY OF ORIGIN: USA

COUNTRY OF ULTIMATE DESTINATION: _____

REASON FOR EXPORT: Medical Research Related Purposes

TOTAL COMMERCIAL VALUE: _____

I/we hereby certify that the information on this invoice is true and correct and that the contents of this shipment are as stated above. I/we do hereby authorize the selected carrier to execute any additional documents necessary for the export of goods described herein on my/our behalf.

Typed Name of Sender	Title of Sender

Signature	Date

NIH 1884-1 (Rev. 12/89)

Information for Completing "Commercial Invoice"
(Form NIH 1884-1)

When should I prepare a commercial invoice?

The best rule of thumb is to ***always*** prepare a commercial invoice for your international shipments (except for letters). Since the rules vary and are constantly changing from country to country, using the commercial invoice ensures that your shipment will be delivered directly to the receiver's address. Following this rule will help avoid delays in clearing your shipment through customs.

What happens to a package that does <u>not</u> have a commercial invoice?

When the shipment does not have the required commercial invoice, the package will be delivered to the nearest customs airport. The carrier's agent then notifies the receiver who must arrange for customs clearance and delivery. The receiver pays for customs taxes and delivery charges. If you wish, you may let the receiver know the flight information so that he or she can be at the airport when the shipment arrives, and to clear and pick up the package. (To obtain flight information, call the shipping office on 496-5921, after 3 p.m. on the day of shipment.)

What are some helpful hints in preparing a commercial invoice?

1. Please type the form. Fill in your complete address in the letterhead (building, room, and phone number).

2. Type the receiver's complete address and phone number. Do not use P.O. box addresses!

3. Be specific in describing the contents of your shipment. If you type "biologics" or "chemicals," explain what kind, and whether or not it is hazardous, infectious, or harmful to humans. Include the amount (i.e. 25 milliliters), and how it is packaged (in 30 lbs. of dry ice, wet ice, or ice packs, etc.). If a permit or shipper's declaration is included with your paperwork, state it in this section of the invoice.

4. If you are sending printed matter or something that is priceless (i.e. biologics, chemicals, samples), type "$5.00" for the Total Commercial Value. If, however, you are sending equipment, medical supplies, tapes, etc., estimate the current commercial resale value, and use this figure.

   Note: State whether you want "airport" or "door to door" service in Item 15 ("Additional Information") of Form NIH 1884, "Request for Shipment."

NIH 1884-1 (Rev. 12/89) Back