Elm & Carlton Streets
Buffalo, New York 14263

Institutional Review Board

Approval Notice

This institution has an approved assurance of compliance on file with HHS which covers this activity FWA 00006731 Federal Wide Assurance identification number

May 6, 2017

Patrick Boland, MD

Patrick.Boland@RoswellPark.org

Dear Dr. Boland:

On 5/5/2017, the IRB reviewed the following submission:

Type of Submission:	Modification and Continuing Review
Type of Review:	☐ Full Board ☒ Expedited ☐ Exempt ☐ Non-Human Research
Title of Study:	Pralatrexate in Combination with Oxaliplatin in Advanced Esophagogastric Cancer: A Phase II Trial with Predictive Molecular Correlates
Investigator:	Patrick Boland
IRB ID:	MODCR00000245 / I 169210
Funding:	Name: NATIONAL COMPREHENSIVE CANCER NETWORK
Grant ID:	None
IND, IDE, or HDE:	None
Documents Reviewed:	• I 169210 IB V9 10113.pdf, Category: Drug Attachment; • I 169210 Oxaliplatin_ Drug information - UpToDate.pdf, Category: Drug Attachment; • I169219 PT. DIRAY.pdf, Category: Recruitment Materials; • I 169210 PROT AMD 08 CLN 090115.pdf, Category: IRB Protocol; • 169210 IND MEMO.pdf, Category: Drug Attachment; • I 169219 Consent V10 (data migration only)

The IRB approved the study from 5/5/2017to 5/17/2018 inclusive. Before 5/17/2018 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR.
If continuing review approval is not granted on or before 5/17/2018, approval of this study expires after that date.

Please be advised that only the IRB approved and stamped consent form can be used to enroll subjects.

The principal investigator is responsible for ensuring that the research complies with all applicable regulations. Any modifications in the research project are subject to approval by the Board prior to initiation by the investigator. The Board reserves the right to stop the research for violations of regulatory or IRB requirements.

A progress report must be submitted to the IRB at least one month prior to the expiration date noted above for continuing review as required by federal regulations and/or institutional requirements.

Please be advised that your research study may be audited periodically by the IRB for compliance.

This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This protocol fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device.

The study documents have been submitted to Clinical Research Services (CRS) Compliance Office for processing prior to release and protocol implementation. Please contact CRS Compliance for information regarding the protocol implementation release date.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103), including the reporting of Unanticipated Problems and any other Reportable New Information.

Sincerely,
Donald Handley MSc, MBA
Camille P Wicher, PhD, Esq., RN, MSN

Pralatrexate in combination with oxaliplatin in advanced esophago-gastric cancer: a phase II trial with predictive molecular correlates

RPCI # I 169210

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Amendment #8 (PI Change) 09.01.15

Date: 09.01.15

Network Investigator Signature Page

I 169210
Pralatrexate in combination with oxaliplatin in advanced esophago-gastric cancer: a phase II trial with predictive molecular correlates

PROTOCOL APPROVAL AND INVESTIGATOR AGREEMENT
I have read and familiarized myself with this protocol and I agree to conduct the study as described according to GCP and ICH guidelines.

Principal Investigator

_____ _____
Signed Date

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Printed

Address: _____

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_____

Phone: _____

Fax: _____

E-mail: _____

NOTE: Please see Appendix 2 for Network-specific instructions that will apply to your site.

Date: 09.01.15

STUDY SYNOPSIS:

Objectives:

Primary:

- To determine the overall response rate in patients with advanced esophago-gastric cancer to combination pralatrexate and oxaliplatin.

Secondary:

- To examine the toxicity and tolerability of this regimen
- To determine the time-to-progression and overall survival using this regimen
- To examine whether functionally relevant polymorphisms of genes of the folate metabolism pathway correlate with efficacy and toxicity of pralatrexate
- To examine whether response to pralatrexate can be predicted by microRNA expression profiling of the epithelial component of the tumor.

Design:

Phase II with a safety lead-in cohort of 6 patients to assess safety and tolerability of the combination. Total accrual will be 34 (single institution) and duration will be 4 years with early stoppage rules for futility.

Major Inclusion / Exclusion Criteria:

Inclusion

1. Histologically confirmed carcinoma of the esophagus, stomach or gastro-esophageal junction that is metastatic, or locally advanced and inoperable for cure. Histologic sub-types permitted include adenocarcinoma, squamous-cell carcinoma, or undifferentiated carcinoma. Small-cell carcinoma variant is not eligible.
2. No previous systemic therapy for metastatic or recurrent disease. Therapy (chemotherapy, radiotherapy, or both) administered in the neo-adjuvant, adjuvant, or definitive setting for previously localized disease is permitted, provided it was completed more than 6 months prior to enrollment. Palliative radiotherapy is permitted provided it is completed $\geq$ 3 weeks prior to study therapy initiation.
3. ECOG performance status 0-2.
4. Life expectancy $\geq$ 12 weeks.
5. Age $\geq$ 18 years.
6. Adequate hematologic parameters *(hemoglobin $\geq$ 9 g/dl, absolute neutrophil count $\geq$ 1500/mm^3, platelet count $\geq$ 100,000/mm^3)*.
7. Adequate biochemical parameters *(serum creatinine $\leq$ institutional upper limit normal (ULN), bilirubin $\leq$ 1.5 X ULN, transaminases $\leq$ 3 X ULN; for documented liver metastases, transaminases up to 5 X ULN is permitted)*.

8. No evidence of ≥ grade 2 peripheral neuropathy.
9. Patients with reproductive potential must be willing to use an adequate contraceptive method (e.g., abstinence, intrauterine device, oral contraceptives, barrier device with spermicide or surgical sterilization) during treatment and for three months after completing treatment. A negative pregnancy test is required for women of child-bearing potential. Nursing women are ineligible.
10. Written, informed consent.

Exclusion

1. Hypersensitivity to platinum compounds.
2. Uncontrolled inter-current illness including but not limited to active infection, symptomatic congestive heart failure, unstable angina, uncontrolled cardiac arrhythmia, or psychiatric illness that would limit compliance with study requirements.
3. Presence of brain metastases.
4. Patients with third-space (pleural, peritoneal) fluid not controllable with usual drainage methods are not eligible.
5. History of second primary malignancy within 3 years prior to enrollment, except for *in-situ* cervix carcinoma or non-melanoma skin cancer.
6. Undergone an allogeneic stem cell transplant.

Treatment Plan:

Following Vitamin B12 and folate supplementation, and appropriate anti-emetic therapy (to include a 5-HT_3 antagonist and a steroid), pralatrexate will be administered intravenously in a dose of 120 mg/m^2 over 3-5 minutes followed by oxaliplatin at 85 mg/m^2 intravenously over 2 hours. Cycles will be repeated every 2 weeks until disease progression, undue toxicity, or patient/physician discretion. For patients meeting response criteria to continue therapy, oxaliplatin will be discontinued after 12 cycles (maximum cumulative dose = 1020 mg/m^2) to reduce the risk of peripheral neuropathy.

The safety lead-in will start at cohort 1 with six patients. If this is tolerable, then all further accrual will be at this starting dose level. If not, successively decreasing cohorts will be investigated per the following table:

	Cohort 1	Cohort -1 (if needed)	Cohort -2 (if needed)
Oxaliplatin	85mg/m^2	85mg/m^2	65mg/m^2
Pralatrexate	120mg/m^2	100mg/m^2	80mg/m^2

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1. Hypothesis

The efficacy of current chemotherapy options in advanced esophago-gastric cancer has reached a plateau. Investigative strategies should focus on the development of new agents and on predictive markers to tailor the efficacy of drugs to specific patient or tumor characteristics. We hypothesize the following:

a. The combination of pralatrexate and oxaliplatin will be efficacious and tolerable in patients with advanced esophago-gastric cancer.
b. Polymorphisms in genes responsible for transport and metabolism of pralatrexate will correlate with response and outcome.
c. MicroRNA (miRNA) expression profiles generated using cell line data can predict response to pralatrexate using expression profiling of the epithelial component of the tumor isolated using laser-capture microdissection (LCM).

2. Background

Cancers of the stomach and esophagus are the second and sixth most common causes of cancer-related mortality world-wide.[1] With delayed symptoms, most cancers present at an advanced stage and the 5-year survival remains less than 20%. There has been a dramatic increase in the incidence of esophageal adenocarcinoma in the United States in the past two decades with a concurrent 7-fold increase in mortality.[2] Anatomically, most cancers of the upper gastrointestinal tract arise from the distal esophagus, gastro-esophageal junction (GEJ) or the proximal stomach, and are typically of the adenocarcinoma sub-type. There is no uniform global standard for the first-line therapy of advanced esophago-gastric cancer (EGC). Regimens include doublet or triplet-combination chemotherapy agents, usually platinum- or fluorouracil-based with response rates ranging from 15%-50%, achieving an overall survival of 9-11 months.[3-6]

Oxaliplatin, a novel antineoplastic platinum is a favorable alternative to cisplatin in terms of toxicity without compromise of efficacy.[7] It is less emetogenic, less nephrotoxic and less neurotoxic compared to cisplatin. It has been widely incorporated into combination treatment regimens in advanced EGC.[8-13] Our group has systematically demonstrated the feasibility and efficacy of oxaliplatin in combination with 5-fluorouracil (5FU), and with capecitabine in three successive trials in esophagus cancer (EC), all in combination with radiotherapy.[14-16] This regimen showed an impressive pathologic complete response rate of 33% for resectable EC in SWOG 0356, a multi-center phase II trial.[17] Major drawbacks of the oxaliplatin plus 5FU doublets are inconvenience of the 5FU infusion schedule and the need for central venous access and infusion pumps. Hence it is imperative to investigate newer agents in combination with platinums.

The anti-folate drugs methotrexate and pemetrexed have demonstrated clinical activity in EGC.[18-21] Pemetrexed has shown greater efficacy compared to 5FU against several gastric cancer cell lines.[22] This drug shows synergistic cytotoxicity when combined with

oxaliplatin in human HT29 colon cancer cells, including 5FU-resistant cells.[23] Recently, a phase II trial assessing pemetrexed (500mg/m^2) in combination with oxaliplatin (120mg/m^2) every 21 days in advanced gastric cancer demonstrated a response rate of 36%, with four complete responses and an impressive time-to-progression of 6.2 months.[24] With the more conventionally used biweekly regimen of oxaliplatin at 85mg/m^2, pemetrexed can be safely given every 14 days at a dose of 400-500mg/m^2 in patients without extensive prior therapy.[25] Similar combinations have been investigated in non-small cell lung cancer.[26] Taken together, these data suggest that the combination of oxaliplatin with the multi-targeted anti-folate pemetrexed is safe and efficacious in several tumor types, including gastric cancer.

Pre-clinical observations of increased cellular uptake and polyglutamylation of pralatrexate relative to other anti-folates methotrexate and pemetrexed make this an appropriate drug for study in clinical trials.[27-30] It has demonstrated synergy with platinum and taxane compounds.[29, 31] Phase I testing has evaluated weekly, biweekly and every 3-week dosing with stomatitis being the dose-limiting toxicity. The documented pre-clinical synergy with platinum makes it an ideal candidate for testing in a combination regimen. Of note, these trials did not utilize vitamin supplementation to mitigate toxicity. With this approach, higher doses of pralatrexate are currently being tested in clinical trials. In a phase I study, pralatrexate at 120mg/m^2 in combination with docetaxel at 35mg/m^2, both administered every other week was found to be safe and tolerable when vitamin supplementation was administered concurrently.[31]

Using this information, we propose to use pralatrexate at 120mg/m^2 every other week in combination with the standard dose of biweekly oxaliplatin (85mg/m^2) in this clinical trial for patients with advanced EGC. The lack of overlapping toxicities for these drugs makes this particularly attractive. As there is no phase I data for this specific combination, a safety lead-in cohort of 6 patients will be tested first.

3. Research Design

This phase II study will consist of combination pralatrexate and oxaliplatin every 2 weeks in the first-line treatment of patients with advanced EGC following an initial safety-lead in cohort. Response will be assessed every 8 weeks and treatment will continue until disease progression. Oxaliplatin will be discontinued after 12 cycles of treatment to reduce the risk of neuropathy with cumulative dosing in patients with responding or stable disease. The primary clinical end-point is response rate with secondary end-points of describing toxicity and calculating time-to-progression and overall survival.

Blood (5ml) will be collected on all patients and sent to the Adjei laboratory for single nucleotide polymorphism (SNP) analysis. In addition, pre-treatment primary tumor biopsies will be undertaken by upper endoscopy for LCM for miRNA expression profiling. This will be sent to the Yendamuri laboratory. *See correlative section for details.*

4. Objectives:

4.1. Primary

To determine the overall response rate in patients with advanced EGC to combination pralatrexate and oxaliplatin.

4.2. Secondary

4.2.1. To examine the toxicity and tolerability of this regimen

4.2.2. To determine the time-to-progression and overall survival using this regimen

4.2.3. To examine whether functionally relevant polymorphisms of genes of the folate metabolism pathway correlate with efficacy and toxicity of pralatrexate

4.2.4 To examine whether response to pralatrexate can be predicted by microRNA expression profiling of the epithelial component of the tumor.

5. Inclusion of Women and Minorities

Women and minorities will be eligible for this study without any alteration in the eligibility criteria. National demographics for esophagus cancer indicate a relative rarity of this disease in women. Hence only 10-15% of patients enrolled on esophagus cancer trials are women. African-Americans comprise 5-10% of patients on esophagus cancer trials with most of these patients developing squamous-cell cancer. Our own experience suggests a similar demographic distribution and the current clinical trial would be expected to mirror a similar patient population.

6. Study Drugs

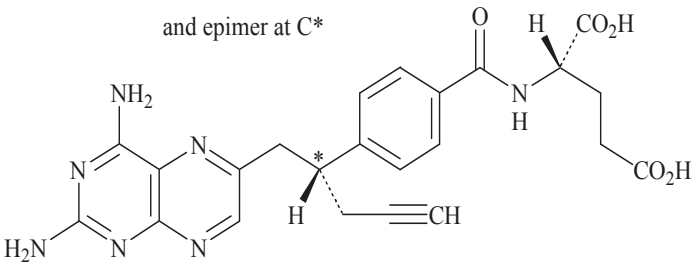
6.1 PRALATREXATE

PHARMACOLOGY

Pralatrexate is a synthetic 10-deazaaminopterin antifolate. The 10-deazaaminopterins are a class of folate analogues that demonstrate greater antitumor effects than methotrexate against human tumor cell lines and in human tumor xenograft models in mice.[27, 28, 32] Pralatrexate is a folate analogue that inhibits folate metabolism by binding to and inhibiting the enzyme dihydrofolate reductase (DHFR). The improved antitumor effects of pralatrexate are likely due to the more effective internalization via reduced folate transport, and the subsequent accumulation in tumor cells through the formation of polyglutamylated metabolites.[28, 33]

PHYSICAL, CHEMICAL, PHARMACEUTICAL PROPERTIES

Pralatrexate has the chemical name (2*S*)-2-[[4-[(1*RS*)-1-[(2, 4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid. The structural formula is as follows:



Pralatrexate is a 1:1 racemic mixture of *S*- and *R*- diastereomers at the C10 position (indicated with *)

The molecular formula is $C_{23}H_{23}N_7O_5$ and the molecular weight is 477.48 g/mol. Pralatrexate is an off-white to yellow solid. It is soluble in aqueous solutions at pH 6.5 or higher. Pralatrexate is practically insoluble in unbuffered water, chloroform, and ethanol. The pKa values are 3.25, 4.76, and 6.17.

Pralatrexate is supplied as a preservative free, sterile, isotonic, non-pyrogenic clear yellow aqueous parenteral solution contained in a single-use 2 mL-size clear glass vial for intravenous administration. Each 1 mL of solution contains 20 mg of pralatrexate, 0.6% sodium chloride to achieve an isotonic (280-300 mOsm) solution, and sufficient sodium hydroxide, and hydrochloric acid if needed, to adjust and maintain the pH at 7.5-8.5. Pralatrexate is supplied as either 20 mg (1 mL) or 40 mg (2 mL) single-use vials at a concentration of 20 mg/mL.

MECHANISM OF ACTION

Pralatrexate is a folate analog metabolic inhibitor that competitively inhibits DHFR. In *in vitro* studies, pralatrexate showed improved cytotoxic activity against a panel of cancer cell lines compared with methotrexate. The improved cytotoxic activity is likely due to pralatrexate being a more efficient permeant for reduced folate transport and it being more efficiently polyglutamylated by the enzyme folylpolyglutamyl synthetase (FPGS).[28, 33] Similar to what has been reported for methotrexate, cellular uptake of pralatrexate is thought to occur via the reduced folate carrier-1 (RFC-1) protein. RFC-1 is a membrane protein involved in cellular uptake of reduced folates. This carrier protein has evolved to efficiently transport reduced natural folates into highly proliferative cells in order to meet the demands for purine and pyrimidine nucleotides during cell replication. Inside the cell, pralatrexate is polyglutamylated by FPGS, an enzyme involved in polyglutamylation of reduced natural folates. Therefore, pralatrexate may act as a competitive inhibitor for polyglutamylation of natural folates. Further, it is believed

that the addition of glutamate residues to pralatrexate leads to increased intracellular half-life, thus allowing for prolonged drug action in malignant cells.

Folic acid (folate) is a B vitamin (B_9) and serves as a carrier of 1-carbon groups in many metabolic reactions that are critical to the life cycle of cells. Following absorption into the cell via RFC-1, dietary folate undergoes reduction to dihydrofolate. Dihydrofolate is then converted to tetrahydrofolate (THF) by the enzyme DHFR. THF is required for the synthesis and catabolism of several amino acids, formation of creatine and choline, synthesis of purines, methylation of ribonucleic acids (RNAs), and synthesis of thymidine. Pralatrexate is a competitive inhibitor of DHFR, and thus treatment with pralatrexate will lead to depletion of thymidine and other biological molecules the synthesis of which depends on one carbon transfer. Depletion of thymidine will result in inhibition of DNA replication and thus interference with cell proliferation. For detailed information about the general pharmacology, pharmacokinetics (PK), nonclinical toxicology and clinical efficacy studies of pralatrexate, refer to the current pralatrexate Investigator's Brochure (IB) accompanying this protocol.

PRECLINICAL INFORMATION

Data from in vitro and in vivo models of human NHL established the superior therapeutic activity of pralatrexate compared to methotrexate. In vitro experiments demonstrated that pralatrexate concentrations that result in 50% growth inhibition (IC_{50}) were consistently 10-fold lower than that typically seen for methotrexate in a library of lymphoma cell lines, and xenograft studies of lymphoma lines in mice consistently showed pralatrexate to be superior to methotrexate.[34]

Studies exploring the integration of pralatrexate with gemcitabine revealed that these 2 agents appear to be synergistic in models of lymphoma. One of the important observations from these studies was the importance of schedule dependency.[35] These observations were supported by results from in vitro cytotoxicity experiments, apoptosis assays, as well as in vivo xenograft experiments. The sequence of pralatrexate followed by gemcitabine was 5 times more effective at inducing apoptosis compared with the simultaneous exposure of pralatrexate plus gemcitabine, and in vivo, complete regressions were only observed in animals receiving pralatrexate followed by gemcitabine.

In cytotoxicity assays using a number of human NSCLC and breast cancer cell lines, pralatrexate was found to be superior to both methotrexate (13- to 40-fold lower IC_{50}) and another methotrexate analog, edatrexate (2- to 4-fold lower IC_{50}).[36]

Table 1: In Vitro Comparison of Methotrexate Analogs

	Pralatrexate	Edatrexate	Methotrexate
DHFR Inhibition, K_i (pM)	13.4	5.8	4.9
FPGS Activity, V_{max}/Km	23.2	10.3	2.2
In vitro Activity			
IC_{50} Concentration (μM):			
MDA-468 (breast)	0.11	0.39	4.5
SK-BR III (breast)	0.28	0.99	4.2
ZR-75-1 (breast)	0.26	0.86	3.5
SK-LC8 (NSCLC)	0.42	1.24	10.3
SK-LC16 (NSCLC)	0.11	0.26	2.1

K_i = inhibition constant
V_{max} = maximum velocity of a reaction catalyzed by a fixed enzyme concentration
Km = concentration of substrate which gives ½ V_{max}
IC_{50} = concentration of compound that inhibits cell growth by 50%

Screening of pralatrexate in the National Cancer Institute (NCI) 60-Cell Panel showed potent cytotoxic activity across a broad spectrum of tumor types. (Allos data on file)

CLINICAL STUDIES: HEMATOLOGIC MALIGNANCIES

Study PDX 02-078

In a "phase 2/1/2" study of pralatrexate in 57 patients with relapsed or refractory lymphomas, the initial phase 2 dose of 135 mg/m^2 every 2 weeks was associated with unacceptable mucositis, leading to phase 1/2 evaluation of 30 mg/m2 once weekly for 6 weeks in 7-week cycles. This schedule modification resulted in a 50% reduction in the major hematologic toxicities and abrogation of the grade 3 to 4 stomatitis. Overall response rate among assessable patients with PTCL was 54% (14/26), including 31% (8/26) with complete remission or complete remission unconfirmed, and 23% (6/26) with partial remission.[37, 38]

Study PDX-008

The pivotal clinical trial of pralatrexate for the indication of PTCL was a single-arm phase 2 clinical study (PROPEL) in which 115 patients with relapsed or refractory PTCL were enrolled. Patients received Pralatrexate 30 mg/m^2 once weekly for 6 weeks in 7-week cycles, and were treated until disease progression or unacceptable toxicity.[39, 40] Of the 111 patients treated with pralatrexate, 109 were histologically confirmed and

evaluable for efficacy. Efficacy was evaluated with IWC by independent central review at the end of cycle 1 and then every other cycle thereafter. The primary efficacy endpoint was overall response rate and the key secondary efficacy endpoint was duration of response until disease progression or death.

Seventy-five patients (68%) were male 34 (32%) were female. Most patients (72%) were White and other racial origins included: Black (12%), Hispanic (8%), Asian (6%), other and unknown (<1% each). Patients had an Eastern Cooperative Oncology Group (ECOG) performance status at study entry of 0 (39%), 1 (45%), or 2 (17%). The median time from initial diagnosis to study entry was 15.6 months (range 0.8 – 322.3). The median number of prior systemic therapies was 3 (range 1-12). Approximately one-fourth of patients (24%, n = 26) did not have evidence of response to any previous therapy. Approximately two-thirds of patients (63%, n = 69) did not have evidence of response to their most recent prior therapy before entering the study.

The overall response rate per IWC by independent central review was 29% (n = 32). Overall response rate by investigator assessment was 39% (n = 43). Of the responders, 63% responded by the first assessment at the end of Cycle 1. The median time to first response was 45 days (range, 37–349 days) and the median time to best response was 96 days (range, 37–483 days). The Kaplan-Meier estimates of the median duration of response and overall survival were 10.1 months (range, 1-673 days) and 14.5 months (95% CI: 11.2, not evaluable), respectively.

The mean duration of treatment was 112 days (range, 1–558 days). Most patients (69%, n = 77) remained at the target dose (30 mg/m^2 for 6 of 7 weeks per cycle) for the duration of treatment and 85% of scheduled doses were administered. The most frequent adverse events in PROPEL, regardless of causality, were mucositis (71% All Grade, 18% Grade 3, 4% Grade 4), thrombocytopenia (41% All Grade, 14% Grade 3, 19% Grade 4), nausea (41% All Grade, 4% Grade 3, 0 Grade 4), fatigue (36% All Grade, 5% Grade 3, 2% Grade 4), anemia (34% All Grade, 16% Grade 3, 2% Grade 4), constipation (34% All Grade, 0 Grade 3, 0 Grade 4), pyrexia (34% All Grade, 1% Grade 3, 1% Grade 4), and edema (31% All Grade, 1% Grade 3, 0 Grade 4).

There was an additional analysis of treatment responses among patients in the PROPEL trial who had refractory PTCL.[40] Refractory disease was defined as 1) no evidence of response to their most recent therapy or 2) no evidence of response to any prior therapies. Of the 111 patients evaluated for safety, 109 were evaluable for efficacy. Sixty-nine of these patients (63%) had no response to their most recent therapy and 26/109 (24%) had no response to any prior therapy. Prior therapies consisted mostly of CHOP-based chemotherapy (70%), platinum-containing combination chemotherapy (40%), and non-platinum containing combination chemotherapy (38%). Of the 69 patients with no response to their most recent therapy, the median number of prior systemic therapies was 3 (range 1-11). There were 18 patients with prior autologous stem cell transplants.

The ORR in the 69 patients with no response to their most recent therapy was 25% (17/69) based on central review and 36% (25/69) based on investigator review. According to central review, the response duration ranged from 41-673 days. In the 26 patients with no response to any prior therapy, 19% (5/26) had a response to pralatrexate according to central review and 27% (7/26) had a response based on investigator review (duration of response based on central review, 54-306 days). Of the 69 patients with no response to most recent therapy, 46 (67%) received full-dose therapy and the safety and tolerability in this subset was similar to the overall study population.

The safety and management of pralatrexate treatment in relapsed or refractory PTCL patients included in the PDX-008 was evaluated over 3 parameters: to assess safety according to duration of pralatrexate treatment, to evaluate both early- and late- onset pralatrexate toxicities, and to assess the impact of pralatrexate dose modification on toxicities.[41] Patients received pralatrexate 30 mg/m^2 administered as an intravenous push over 3-5 minutes once weekly for 6 weeks in 7-week cycles. All patients received vitamin B_{12} (1 mg) intramuscular injection every 8-10 weeks and oral folic acid (1.0-1.25 mg) daily. Patients with methylmalonic acid (MMA) levels ≥ 200 nmol/L or homocysteine (Hcy) ≥ 10 µmol/L at screening received supplementation > 10 days prior to the first pralatrexate dose. Pralatrexate doses were omitted in patients with platelets <50,000/µL, absolute neutrophil count (ANC) 500-1,000/µL with fever, ANC <500 µL, Grade 2-4 mucositis and any other Grade 3 treatment-related event. The dose was reduced to 20 mg/m^2 in patients with platelets < 25,000/µL on 2 occurrences, recurrence of ANC 500-1,000µL with fever, recurrence of ANC <500µL, recurrence of Grade 2 mucositis, Grade 3-4 mucositis, or any Grade 4 treatment-related event. Of the 111 patients evaluated for safety, 109 were evaluable for efficacy. Patients received pralatrexate for a median of 70 days (range 1-696); 19 of these patients received therapy for > 180 days; and 12 received therapy for ≥ 300 days. Two or more cycles were delivered in 64 patients, and ≥ 3 cycles were delivered in 43 patients. The median cumulative dose was 207.9 mg/m^2 (range 26.7-2108.8). There were 76 patients (68%) who needed no dose modification. Of the remaining 35 patients (32%), dose reduction occurred in cycle 1 for 20 patients, 8 in cycle 2, and 7 in cycle 3 or later. The most common reason for dose reduction was mucosal inflammation. Prior to dose modification, the frequency of Grade 2-4 mucosal inflammation was 28/35 compared with 15/35 after dose modification.

The most common adverse events in the overall population were mucosal inflammation, nausea, thrombocytopenia, and fatigue. There was no evidence of cumulative dose-toxicity effects.

A additional analysis evaluated the correlation between baseline methylomalonic acid (MMA) status and mucositis severity in the PROPEL study, and the implications for vitamin prophylaxis.[42] In addition to MMA, homocysteine (HCY) and red blood cell (RBC) folate levels were evaluated to assess the association between these baseline parameters and mucositis and thrombocytopenia. Mucositis and thrombocytopenia were the most common adverse events reported in the PROPEL study. Of the 111 patients evaluated for safety, 109 were evaluable for efficacy. The following percentages of patients had baseline levels available: MMA 89%, HCY 91%, RBC folate 75%.

There was a statistically significant linear relationship between mucositis grade and baseline MMA level. (slope estimate = 43.3 nmol/L, P=0.039). In the subset of patients enrolled in PROPEL for whom baseline MMA levels were obtained, those with higher levels of baseline MMA compared to the normal range experienced increased severity of mucosal inflammation despite concurrent vitamin supplementation. Although not significant, there was a trend toward a relationship between increasing severity of thrombocytopenia and increasing MMA levels (slope estimate= 17.6 nmol/L, P=0.267). There were no significant relationships between baseline HCY and RBC folate levels and mucosal inflammation or thrombocytopenia.

Patients with higher baseline levels of MMA experienced increased severity of mucositis. Based on the results of this study, vitamin supplementation with folic acid and vitamin B_{12} is appropriate in patients with relapsed or refractory PTCL who are treated with pralatrexate.

Study PDX-010

An open-label phase 1 trial was conducted to examine the safety and efficacy of pralatrexate in relapsed or refractory cutaneous T-cell lymphoma (CTCL). Results from 31 enrolled patients were presented at the American Society of Hematology 2009 Annual Meeting.[43] Eligible patients were required to have mycosis fungoides stage 1B or higher (MF), Sezary syndrome (SS), or primary cutaneous anaplastic large cell lymphoma (ALCL), and progression or relapse of disease after at least 1 systemic therapy and a ECOG Performance Status of ≤ 2. The 31 evaluable patients (22 with MF, 7 with SS, 1 with primary cutaneous ALCL, 1 with CTCL, NOS) were enrolled into 6 cohorts: pralatrexate 30 mg/m^2 for 3 of 4 weeks (n = 2), 20 mg/m^2 for 3 of 4 weeks (n = 3), 20 mg/m^2 for 2 of 3 weeks (n = 7), 15 mg/m^2 for 3 of 4 weeks (n = 6), 15 mg/m^2 for 2 of 3 weeks (n = 3), 10 mg/m^2 for 3 of 4 weeks (n = 10). All patients received vitamin B_{12} and folic acid supplementation. These patients were heavily pretreated with a median of 6 prior regimens (range, 1–25), and a median of 4 prior systemic regimens (range, 1–10).

Of the 31 evaluable patients, 12 achieved a response (39%), including complete response in 2 patients (1 each with MF/SS and primary cutaneous ALCL) and partial response in 10 patients (all with MF/SS). The ORR at pralatrexate dose intensity ≥ 15 mg/m^3 q3/4 week was 61% (11/18). A pralatrexate dose of ≥ 15 mg/m^3 seemed to be the threshold for substantial activity in CTCL and the optimal regimen was pralatrexate ≥ 15 mg/m^3 weekly 3 of 4 week cycle. Dose-limiting toxicities (DLTs) included Grade 2 anorexia (n=1), Grade 3 weakness (n=1), Grade 2 stomatitis (n= 3), Grade 2-3 stomatitis (n=1), Grade 3 LFT abnormalities (n=1), Grade 2 fatigue (n=1), Grade 2 dehydration (n=1), Grade 3 thrombocytopenia/neutropenia (n=1), Grade 3 skin lesion (n=1), and Grade 3 zoster (n=1). The most common treatment-related adverse events included stomatitis (Grade 1-3= 58%, Grade 3= 13%), nausea (Grade 1-3= 52%, Grade 3 = 0%), and fatigue (Grade 1-3= 48%, Grade 3 = 0%).

Pralatrexate shows activity in patients with relapsed /refractory CTCL (median of 4 prior systemic therapies) at a lower dose-intensity than in studies for PTCL. An expansion cohort is enrolling additional patients at pralatrexate 15 mg/m^2 weekly 3 of 4 week cycle.

Study PDX-009

It was demonstrated in non-Hodgkin's lymphoma (NHL) cell lines in vitro and xenografts in vivo that pralatrexate may synergize with gemcitabine in a schedule-dependent manner.[35] pralatrexate in combination with gemcitabine is being evaluated in an ongoing clinical study (Study PDX-009) in patients with certain relapsed or refractory lymphoproliferative malignancies, including Hodgkin's lymphoma and certain types of NHL (www.clinicaltrials.gov/show/NCT00481871). (Data on file, Allos Therapeutics) The phase 1 component of the study has been completed.[44] Various doses and schedules were evaluated in the following treatment groups:

- **Treatment Group A (n=7)**: pralatrexate (15 mg/m^2) followed the next day by gemcitabine (400 mg/m^2-starting dose), administered once weekly. One cycle of pralatrexate and gemcitabine was 4 weeks in duration consisting of 3 weeks of treatment followed by 1 week of rest. Accrual was closed in this treatment group due to dose limiting toxicities (DLT) of Grade 3-4 neutropenia and thrombocytopenia.
- **Treatment Group B (n=10)**: pralatrexate (10 mg/m^2) followed the next day by gemcitabine (300 mg/m^2- starting dose), administered once every 2 weeks. One cycle of pralatrexate and gemcitabine is 4 weeks in duration consisting of 2 doses each of pralatrexate and gemcitabine.
- **Treatment Group C (n=17)**: pralatrexate (10 mg/m^2) followed 1 hour later by gemcitabine (300 mg/m^2- starting dose), administered once every 2 weeks. One cycle of pralatrexate and gemcitabine is 4 weeks in duration consisting of 2 doses each of pralatrexate and gemcitabine.

Patients were enrolled into each treatment group until the maximum tolerated dose (MTD) was determined. The MTD was defined as the highest dose level at which ≤ 33% of patients experience a DLT. Potential DLTs in the phase 1 portion of the study included: Grade 4 neutropenia for ≥ 7 days, Grade 4 thrombocytopenia or any thrombocytopenia with clinically significant bleeding, ≥ Grade 3 febrile neutropenia, ≥ Grade 3 treatment-related nonhematological toxicity excluding nausea/vomiting in the absence of appropriate antiemetic therapy. Safety information was obtained for all patients, and patients who received ≥ 1 cycle of chemotherapy were eligible for the efficacy evaluation. Of the 35 patients treated in the phase 1 component, 33 were evaluable for response. The median number of prior regimens among the 35 patients was 3 (range 1-11). Lymphoma subtypes included: B-cell (n=15 [DLBCL= 11, follicular-4]), PTCL (n=12), Hodgkin's lymphoma (n=7), composite B- and T-cell lymphoma (n=1).

The MTD in Groups B and C were pralatrexate/gemcitabine 10/400 mg/m^2, when given on sequential days, and pralatrexate/gemcitabine 15/600 mg/m^2, when given on the same day. Grade 3-4 treatment-related adverse events (with either study drug) occurring in > 2 patients included anemia (29% Grade 3, 0% Grade 4), neutropenia (31% Grade 3, 11% Grade 4), thrombocytopenia (11% Grade 3 , 26% Grade 4), leukopenia (3% Grade 3, 9% Grade 4), and abnormal liver function test (9% Grade 3 , 0% Grade 4). In Group B, 33% of patients had DLTs (Grade 4 thrombocytopenia or any thrombocytopenia with clinically significant bleeding 17%, ≥ Grade 3 febrile neutropenia 17%, ≥ Grade 3 treatment-related nonhematological toxicity 33%). In Group C, 21% of patients had DLTs (Grade 4 thrombocytopenia or any thrombocytopenia with clinically significant bleeding 7%, ≥ Grade 3 treatment-related nonhematological toxicity 14%).
Response to therapy was evaluated using the International Workshop Criteria a minimum of every 3 cycles. Of the 33 evaluable patients, 8 (24%) had a partial response (Hodgkin's lymphoma 4/7, PTCL 2/11, B-cell lymphoma 2*/15 [*1 patient with mixed B- and T-cell histology]). The authors concluded that the combination of pralatrexate and gemcitabine can be administered on a q2w schedule with acceptable toxicity. Based on the results of the study, the phase 2 component is actively accruing using the MTD in Groups B (pralatrexate [10 mg/m^2] followed the next day by gemcitabine [300 mg/m^2- starting dose], administered once every 2 weeks, one cycle is 4 weeks duration) and C (pralatrexate [10 mg/m^2] followed 1 hour later by gemcitabine [300 mg/m^2- starting dose], administered once every 2 weeks, one cycle is 4 weeks duration) of the phase 1 component.

The phase 2 expansions at the MTD will evaluate both sequential dosing (pralatrexate 10 mg/m2 and gemcitabine 400 mg/m2) and same-day dosing (pralatrexate 15 mg/m2 and gemcitabine 600 mg/m2) in a q2 week schedule.

CLINICAL STUDIES: SOLID TUMORS (Non-Small Cell Lung Cancer)

Study 97-006
The first Phase 1 study was conducted exclusively in patients with NSCLC, who had been previously treated with a median of 2 prior chemotherapy regimens. Initially, pralatrexate was administered to 6 patients at a dose of 30 mg/m^2 IV weekly for 3 weeks out of 4 weeks. Due to the excessive incidence of mucositis/stomatitis/pharyngitis (mucositis) in these patients, which became the dose-limiting toxicity (DLT), the dosing schedule for the remaining patients was changed to a once every 2 week treatment on a 28-day cycle, starting at 15 mg/m2 q 2 weeks and escalated to a maximum dose of 170 mg/m2 q 2 weeks. Based on these Phase 1 data, the recommended dose for subsequent Phase 2 studies was 150 mg/m2 administered q 2 weeks.[45]

Study 99-053
A subsequent single-agent Phase 2 study, in patients with Stage IIIB or IV NSCLC revealed activity of pralatrexate consistent with the activity of other single agents in this setting. Of 38 evaluable patients, 4 patients (11%) experienced a confirmed partial response (PR) lasting 4, 9, 12, and 15 months respectively, while 12 patients (31%) exhibited stabilization of their disease (SD).[46]

Study PDX-012
An exploratory Phase 2 trial in NSCLC has recently completed enrollment. The goal of this study is evaluate the effectiveness and safety of pralatrexate monotherapy at a starting dose of 190 mg/m2 every 2 weeks compared to erlotinib when given to NSCLC patients who are current or former cigarette smokers and who have received at least 1 prior treatment with a platinum drug (cisplatin or carboplatin).

SAFETY OVERVIEW

The most common adverse events observed in patients with peripheral t-cell lymphoma (PTCL) treated with pralatrexate were mucositis, thrombocytopenia, nausea, and fatigue. Patients receiving pralatrexate should be monitored closely because AEs may occur at any time during therapy. For detailed information about the safety profile of pralatrexate, refer to the current pralatrexate IB.

Clinical Trials Experience

The safety of pralatrexate was evaluated in 111 PTCL patients in a single-arm clinical study (PDX-008) in which patients received 30 mg/m^2 once weekly for 6 weeks in 7-week cycles. The median duration of treatment was 70 days (range 1-540 days).

Most Frequent Adverse Events

Table 1 summarizes the most frequent adverse events in PDX-008, regardless of causality, using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI CTCAE, version 3.0).

Table 2: Adverse Reactions Occurring in PTCL Patients (Incidence ≥ 10% of patients)

	PDX-008 (n=111)					
	Total		Grade 3		Grade 4	
Preferred Term	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Any Adverse Event	111	(100)	48	(43)	34	(31)
Mucositis[a]	78	(70)	19	(17)	4	(4)
Thrombocytopenia[b]	45	(41)	15	(14)	21	(19)[b]
Nausea	44	(40)	4	(4)	0	(0)
Fatigue	40	(36)	5	(5)	2	(2)
Anemia	38	(34)	17	(15)	2	(2)
Constipation	37	(33)	0	(0)	0	(0)
Pyrexia	36	(32)	1	(1)	1	(1)
Edema	33	(30)	1	(1)	0	(0)
Cough	31	(28)	1	(1)	0	(0)
Epistaxis	29	(26)	0	(0)	0	(0)
Vomiting	28	(25)	2	(2)	0	(0)
Neutropenia	27	(24)	14	(13)	8	(7)
Diarrhea	23	(21)	2	(2)	0	(0)
Dyspnea	21	(19)	8	(7)	0	(0)
Anorexia	17	(15)	3	(3)	0	(0)
Hypokalemia	17	(15)	4	(4)	1	(1)
Rash	17	(15)	0	(0)	0	(0)
Pruritus	16	(14)	2	(2)	0	(0)
Pharyngolaryngeal pain	15	(14)	1	(1)	0	(0)
Liver function test abnormal[c]	14	(13)	6	(5)	0	(0)
Abdominal pain	13	(12)	4	(4)	0	(0)
Pain in extremity	13	(12)	0	(0)	0	(0)
Back pain	12	(11)	3	(3)	0	(0)
Leukopenia	12	(11)	3	(3)	4	(4)
Night sweats	12	(11)	0	(0)	0	(0)
Asthenia	11	(10)	1	(1)	0	(0)
Tachycardia	11	(10)	0	(0)	0	(0)
Upper respiratory tract infection	11	(10)	1	(1)	0	(0)

[a] Stomatitis or Mucosal Inflammation of the gastrointestinal and genitourinary tracts.

[b]Five patients with platelets < 10,000/μL

[c]Alanine Aminotransferase, Aspartate Aminotransferase, and Transaminases Increased

HANDLING AND STORAGE OF PRALATREXATE

Pralatrexate Injection Formulation

Pralatrexate Injection will be supplied by Allos and will have been tested and released according to established specifications. Pralatrexate Injection is formulated as a sterile solution for injection and will be supplied in single-use clear glass vials containing pralatrexate at a concentration of 20 mg/mL as a **preservative-free**, sterile, clear yellow solution individually packaged for intravenous use.

Pralatrexate Injection Storage and Handling

Pralatrexate Injection is a cytotoxic agent. The institutional, local, and all applicable policies and procedures must be followed for proper handling and disposal of chemotherapy drugs.

Pralatrexate Injection must be stored refrigerated at 2-8°C (36-46°F) and protected from light. Pralatrexate vials should be stored in original carton until use. Unopened vial(s) of Pralatrexate are stable if stored in the original carton at room temperature for 72 hours. Any vials left at room temperature for greater than 72 hours should be discarded.

Preparation and Administration Precautions

Pralatrexate is a cytotoxic anticancer agent. Caution should be exercised in handling, preparing, and administering of the solution. The use of gloves and other protective clothing is recommended. If Pralatrexate comes in contact with the skin, immediately and thoroughly wash with soap and water. If Pralatrexate comes in contact with mucous membranes, flush thoroughly with water.

PREPARATION FOR INTRAVENOUS PUSH ADMINISTRATION

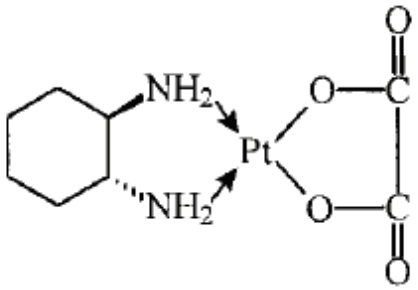
1. Pralatrexate vials should be refrigerated at 2-8°C (36-46°F) until use.
2. Pralatrexate vials should be stored in original carton to protect from light until use.
3. Pralatrexate is a clear, yellow solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use any vials exhibiting particulate matter or discoloration.
4. The calculated dose of Pralatrexate should be aseptically withdrawn into a syringe for immediate use.
5. Do not dilute Pralatrexate.
6. Administer Pralatrexate by IV push over 3 to 5 minutes via the side port of a free flowing 0.9% Sodium Chloride Injection, USP IV line.

7. Pralatrexate vials contain no preservatives and are intended for single use only. After withdrawal of dose, discard vial including any unused portion.
8. Unopened vial(s) of Pralatrexate are stable if stored in the original carton at room temperature for 72 hours. Any vials left at room temperature for greater than 72 hours should be discarded.

6.2 OXALIPLATIN

Chemical Name:	cis-[(1 R,2R)-1,2-cyclohexanediamine-N,N'] [oxalate(2-)-O,O']platinum.
International Non-Proprietary Name:	Eloxatin™
Empirical Formula:	$C_8H14N_2O_4Pt$
Relative Molecular Weight:	397.3
Physical Properties:	Slightly soluble in water Very slightly soluble in methanol Practically insoluble in ethanol and acetone

Structural Formula:



Oxaliplatin is a diaminocyclohexane (DACH) - platinum compound, active in several solid tumor types, including some cisplatin/carboplatin refractory diseases, and licensed in the U.S. for the treatment of colorectal cancer. The main mechanism of action of oxaliplatin, like cisplatin, is mediated through the formation of DNA-adducts, but DACH-platinum adducts are bulkier and more hydrophobic than cisplatin adducts. Oxaliplatin as a single agent has a broad spectrum of *in vitro* cytotoxic/antiproliferative activity against a variety of murine and human tumor cell lines. Oxaliplatin was also effective in cell lines with acquired cisplatin resistance.

Neurotoxicity is considered the dose-limiting toxicity of oxaliplatin. The most common acute side effect is a transient peripheral neurotoxicity characterized by paresthesias and dysesthesias in hands, feet and the peri-oral area, triggered and/or enhanced by contact with cold substances or ambient temperatures. Some patients report laryngo-pharyngeal dysesthesia when swallowing cold food or beverages. The intensity of symptoms is generally mild to moderate. These symptoms are often observed during an oxaliplatin infusion, may last for a few minutes to a few days, and are fully reversible. This acute toxicity, which may be observed at doses as low as (or, occasionally, lower than) 90 mg/m², increases in incidence at higher doses, to affect 75% of patients treated at 200 mg/m². Chronic neurotoxicity, by contrast, is a function of cumulative oxaliplatin dose,

with a median time of evolution to grade 3 of 23 weeks, but a high rate of reversibility once oxaliplatin therapy is discontinued. Women seem more likely than men to experience severe neurotoxicity. These symptoms disappear within 12 weeks of stopping treatment in 50% of patients and in the majority after 30 weeks.

At doses higher than 45 mg/m², oxaliplatin induces nausea and vomiting with rapid onset in the great majority of patients. This can last for 24 to 48 hours and is generally controlled by the standard anti-emetic measures used for all platinum derivatives. Diarrhea may occur in up to 25% of cycles; when administered with 5-FU/LV, grade 3/4 diarrhea has been reported in 12% of patients with previously untreated advanced colorectal carcinoma. Hematological toxicity is minor and sporadic. In monotherapy studies, 2% of patients experienced grade 3-4 anemia and neutropenia; grade 3/4 thrombocytopenia may also be seen in a small percentage of patients. No significant renal toxicity and <1% ototoxicity were reported with oxaliplatin. A single agent, pharmacokinetic study of oxaliplatin in patients with renal impairment was performed, and demonstrated that single agent oxaliplatin was well tolerated, even in patients with a creatinine clearances as low as 20 mg/mL/min. Allergic reactions to Oxaliplatin may occur in up to 11% of patients, but few are severe (<0.5% anaphylactic), while fever without infection may occur in up to a third of the patients.

Mechanism of Action

Like cisplatin, oxaliplatin reacts with DNA, forming mainly platinated intrastrand links with two adjacent guanines or a guanine adjacent to an adenine. However, DACH-platinum adducts formed by oxaliplatin are apparently more effective at inhibiting DNA synthesis and are more cytotoxic than cis-diammine-platinum adducts formed from cisplatin and carboplatin (21). The two mechanisms that reproducibly discriminate between cisplatin and oxaliplatin are defects in mismatch repair and enhanced replicative bypass (22). Several studies have demonstrated that the loss of the mismatch repair (MMR) enzyme complex is a contributor to intrinsic resistance to cisplatin, but not oxaliplatin (23). This resistance can be overcome by a platinum agent with a bulky carrier group, such as oxaliplatin, which can make bypass more difficult and can prevent the binding of the MMR complex.

Oxaliplatin in Colorectal Cancer

The efficacy of oxaliplatin monotherapy in patients with advanced colorectal cancer was evaluated in five phase II trials, two with 63 previously untreated patients and three with 139 patients with metastatic disease previously treated with and mostly refractory to 5-FU. The objective response rate achieved with oxaliplatin as first-line therapy averaged 18% while that of oxaliplatin as second-line therapy averaged 10%. These overall response rates compare favorably with those of cisplatin (3%) and carboplatin (2.4%) resulting from single-agent therapy in patients with metastatic colorectal cancer. In addition, these results were achieved with only moderate toxicity. The efficacy of oxaliplatin combined with 5-FU/LV has been evaluated in 614 previously untreated patients with metastatic colorectal cancer: 136 patients in two-phase II trials and 478 patients in three phase III trials. Response rates varied from 29% to 67%. Median progression-free survival and overall survival ranged from 7.7 to 11 months and 12+ to

19 months, respectively. Major toxicities were diarrhea, stomatitis, peripheral sensory neuropathy, nausea and vomiting using oxaliplatin/5-FU/LV combination therapy.[47]

Intergroup N9741 was a large multicenter randomized phase III study conducted in the U.S., which investigated three possible combinations- oxaliplatin with 5-FU and leucovorin, irinotecan with 5-FU and leucovorin and irinotecan with oxaliplatin. This study reported a significant improvement in toxicity profile as well as a survival improvement with the first arm.[48, 49] In summary, clinical trials of oxaliplatin combined with 5-FU/LV in both chemotherapy-treated and -untreated patients with colorectal cancer have shown the effectiveness and tolerability of the oxaliplatin/5-FU/LV combination. Oxaliplatin is approved in the United States for the treatment of advanced colorectal cancer and for the adjuvant treatment of stage III colon cancer following complete resection of the primary tumor.

Oxaliplatin in cancer of the esophagus and stomach:

Oxaliplatin combinations are active in the treatment of several gastrointestinal cancers apart from colorectal cancer, including esophageal, gastric, biliary and pancreatic tumors. A variety of regimens have been studied in advanced EGC in phase II trials (in combination with 5-FU, capecitabine, irinotecan, docetaxel), with response rates ranging from 40-60%.[8-13, 50] An overview of the treatment options in these diseases is provided in reference #4. The efficacy of oxaliplatin in advanced EGC has been confirmed in two phase III trials.[7, 51] In the REAL-2 trial, therapy-naïve patients were randomized to one of four combination treatment arms, aiming to examine the efficacy of oxaliplatin (as compared to cisplatin) and capecitabine (as compared to 5-FU). There was no significant difference in response rates or progression-free survival between the arms. Survival in the two oxaliplatin-containing arms was comparable to the cisplatin-containing arms, with less neutropenia, alopecia, thromboembolism and renal insufficiency, but greater neuropathy and diarrhea. Overall survival for the epirubicin, oxaliplatin and capecitabine (EOX) arm was 11.2 months (significantly longer compared to the standard ECF arm, 9.9 months) on secondary analysis.[7] Similar results of were found in the AIO study comparing oxaliplatin with cisplatin, when combined with leucovorin-modulated 5-FU.[51]

Oxaliplatin is listed in the NCCN Drugs and Biologic Compendium™ for the treatment of advanced esophageal or gastric cancer and in the pre-operative or definitive treatment of localized disease (with or without radiotherapy).

HANDLING AND STORAGE OF OXALIPLATIN

Oxaliplatin will be obtained from commercial supply for this clinical trial.

Concentrate for solution for infusion:

Oxaliplatin is supplied in clear, glass, single-use vials containing 50 mg or 100 mg oxaliplatin as a sterile, preservative-free, aqueous solution at a concentration of 5 mg/ml. Tartaric acid, NF, Water for Injection, USP, and Sodium Hydroxide, NF are used as

inactive ingredients and/or in combination as a buffering system

NDC 61703-363-18: 50 mg / 10 ml single-use vial individually packaged in a carton.

NDC 61703-363-22: 100 mg / 20 ml single-use vial individually packaged in a carton.

Preparation of Infusion Solution:

Concentrate for solution for infusion

i. Do not freeze and protect from light the concentrated solution.
ii. A final dilution must never be performed with a sodium chloride solution or other chloride-containing solutions.
iii. The solution must be further diluted in an infusion solution of 250-500 mL of 5% Dextrose Injection, USP.
iv. After dilution with 250-500 mL of 5% Dextrose Injection, USP, the shelf life is 6 hours at room temperature [20-25°C (68-77°F)] or up to 24 hours under refrigeration [2-8°C (36-46°F)].
v. After final dilution, protection from light is not required.

Incompatibilities:

Oxaliplatin is incompatible in solution with alkaline medications or media (such as basic solutions of 5-FU) and must not be mixed with these or administered simultaneously through the same infusion line. The infusion line should be flushed with D5W prior to administration of any concomitant medication. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration and discarded if present.

Needles or intravenous administration sets containing aluminum parts that may come in contact with oxaliplatin should not be used for the preparation or mixing of the drug. Aluminum has been reported to cause degradation of platinum compounds.

Handling and Disposal:

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of infusion solutions prepared from oxaliplatin. The use of gloves is recommended. If a solution of oxaliplatin contacts the skin, wash the skin immediately and thoroughly with soap and water. If oxaliplatin contacts the mucous membranes, flush thoroughly with water.

Institutional procedures for the handling and disposal of anticancer drugs should be considered.

Storage:

Concentrate for solution for infusion:

Store under normal lighting conditions at 20˚C-25°C (68°F-77°F); excursions permitted to 15-30°C (59-86°F). Do not freeze.

7. Study Population

I. Stage: Advanced esophago-gastric cancer (metastatic or unresectable)

II. Major Inclusion / Exclusion Criteria

Inclusion

1. Histologically confirmed carcinoma of the esophagus, stomach or gastro-esophageal junction that is metastatic, or locally advanced and inoperable for cure. Histologic sub-types permitted include adenocarcinoma, squamous-cell carcinoma, or undifferentiated carcinoma. Small-cell carcinoma variant is not eligible.
2. No previous systemic therapy for metastatic or recurrent disease. Therapy (chemotherapy, radiotherapy, or both) administered in the neo-adjuvant, adjuvant, or definitive setting for previously localized disease is permitted, provided it was completed more than 6 months prior to enrollment. Palliative radiotherapy is permitted provided it is completed ≥ 3 weeks prior to study therapy initiation.
3. ECOG performance status 0-2.
4. Life expectancy ≥ 12 weeks.
5. Age ≥ 18 years.
6. Adequate hematologic parameters *(hemoglobin ≥ 9 g/dl, absolute neutrophil count ≥ 1500/mm^3, platelet count ≥ 100,000/mm^3)*.
7. Adequate biochemical parameters *(serum creatinine ≤ institutional upper limit normal (ULN), bilirubin ≤ 1.5 X ULN, transaminases ≤ 3 X ULN; for documented liver metastases, transaminases up to 5 X ULN is permitted)*.
8. No evidence of ≥ grade 2 peripheral neuropathy.
9. Patients with reproductive potential must be willing to use an adequate contraceptive method (e.g., abstinence, intrauterine device, oral contraceptives, barrier device with spermicide or surgical sterilization) during treatment and for three months after completing treatment. A negative pregnancy test is required for women of child-bearing potential. Nursing women are ineligible.
10. Written, informed consent.

Exclusion

1. Hypersensitivity to platinum compounds.
2. Uncontrolled inter-current illness including but not limited to active infection, symptomatic congestive heart failure, unstable angina, uncontrolled cardiac

arrhythmia, or psychiatric illness that would limit compliance with study requirements.
3. Presence of brain metastases.
4. Patients with third-space (pleural, peritoneal) fluid not controllable with usual drainage methods are not eligible.
5. History of second primary malignancy within 3 years prior to enrollment, except for *in-situ* cervix carcinoma or non-melanoma skin cancer.
6. Undergone an allogeneic stem cell transplant

8. Treatment Plan

8.1 Premedication

Folic acid (1-1.25 mg/d) will be given orally daily starting at least 10 days prior to the first dose of pralatrexate and must be continued during the full course of treatment with pralatrexate, and for 30 days after the last dose of pralatrexate. Please see appendix 1: Diary for folic acid medication

Vitamin B_{12} (1000 mcg) will be administered intra-muscularly no more than 10 weeks prior to the first dose of pralatrexate and repeated every 8-10 weeks thereafter while on treatment with pralatrexate. Subsequent B_{12} injections may be given the same day as pralatrexate.

8.2 Chemotherapy

Following appropriate anti-emetic therapy (to include a 5-HT_3 antagonist and a steroid), pralatrexate will be administered intravenously in a dose of 120 mg/m^2 over 3-5 minutes followed by oxaliplatin at 85 mg/m^2 intravenously over 2 hours. Cycles will be repeated every 2 weeks until disease progression, undue toxicity, or patient/physician discretion. For patients meeting response criteria to continue therapy, oxaliplatin will be discontinued after 12 cycles (maximum cumulative dose = 1020 mg/m^2) to reduce the risk of peripheral neuropathy.

The following table outlines the cohorts that may be examined based on tolerance of the combination. Details are outlined in the safety lead-in section 11 of the study.

	Cohort 1	Cohort -1 (if needed)	Cohort -2 (if needed)*
Oxaliplatin	85mg/m^2	85mg/m^2	65mg/m^2
Pralatrexate	120mg/m^2	100mg/m^2	80mg/m^2

*Examination of cohort -2 will only be explored at the discretion of the investigator following discussions with the biostatistician, NCCN and Allos Therapeutics, Inc.

General Guidelines for Chemotherapy Use:

- The dose of chemotherapy may be rounded off (up or down) to the nearest whole number or for purposes of minimizing wastage from a drug vial so long as the dose is within 5% of the actual calculated dose.
- It is not necessary to change the dose of chemotherapy administered in successive cycles unless the calculated dose changes by ≥ 10%.
- There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore all calculations will be based solely on the patients' actual body weight for the purpose of determining the body surface area. This will help minimize calculation errors and possible introduction of variation in dose administration. Failure to adhere to this will be considered a major protocol violation.
- Patients should be counseled to avoid ice-chips, cold drinks, and exposure to cold water or air as the neurotoxicity seen with oxaliplatin is often exacerbated by cold exposure. The duration of this cold-related neuropathy is not well defined and patients should exercise caution regarding cold exposure during the treatment cycle.

8.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse events(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

8.4 Duration of Follow-Up

Patients who have completed protocol therapy will be followed for toxicity assessment and resolution for up to 30 days after the last dose of study drug(s). This may be extended appropriately in case of non-resolution of toxicity. Subsequent follow-up will be per standard of care in order to record survival data. This will be until a maximum of 5 years or death, whichever occurs first.

9. Supportive Care Guidelines

9.1. Patients should receive full supportive care, including transfusion of blood and blood products, antibiotics and anti-emetics where deemed medically appropriate. The use of aprepitant is permitted if clinically indicated.

9.2. Growth Factor Use

The use of erythropoietin (EPO) and darbepoetin is permitted at the discretion of the treating physician.

The use of filgastrim (G-CSF), pegfilgastrim and sargramostin (GM-CSF) is discouraged. They may not be used:

- To avoid dose delays or reductions
- Prophylactically for concern of myelosuppression
- For the routine treatment of febrile neutropenia (except in the following circumstances)

However the use of CSFs is permitted per ASCO guidelines, e.g., patients with indicators of poor prognosis such as pneumonia, hypotension, multi-organ dysfunction and invasive fungal infection. In these cases, treating physician discretion will prevail. The reason and duration for CSF use should be recorded.

10. Required Data and Study Calendar

Guidelines for pre-study testing

- To be completed within 14 days of registration
  - Required laboratory data, history and physical
- To be completed within 28 days of registration
  - Any imaging used for staging purposes

	Prior to Registration*	Day 1 of every cycle*	Follow-up**
Tests and Observations			
History / Examination	X	X	X
Weight / BSA	X	X	
Performance Status	X	X	
Toxicity Assessment		X	X
Neurologic Examination	X	X	X
Laboratory Studies			
CBC / Differential[1]	X	X	
Comprehensive Metabolic Panel[1]	X	X	
LDH	X	X	
PT/INR	X		
Pregnancy Test[2]	X		
Staging / Imaging			
Contrast CT (CAP; Neck if indicated) or MRI[3]	X	X[3]	
PET (if indicated)[3]	X		
Correlative Studies			
EGD with biopsy[4]	X		
Blood for pharmacogenomics (5ml)[5]	X		

NOTE: If treatment day (day 0 of a given cycle) falls on a designated holiday, treatment can be administered on days -1 to +2, provided treatment parameters have been met..

* Tests, observations, and laboratory studies completed within 7 days prior to the first day of treatment need not be repeated; one cycle is defined as 2 weeks

** Upon completion of protocol related treatment until 30 days after the last dose of study drug(s); this can be repeated at weekly or biweekly intervals till resolution of drug-related toxicity. At least one visit must occur on or around the 30 day mark (or 7 days beyond)

1. Can be repeated as needed during a treatment cycle if indicated (e.g. toxicity purposes)
2. For women of child-bearing potential (urine or serum)
3. Preferred imaging is a contrast-enhanced CT scan (chest, abdomen and pelvis; neck, if indicated). In case of contrast allergy despite the use of recommended prophylaxis, a contrast- MRI or an FDG-PET may be used for staging and follow-up provided target lesions can be accurately measured. Imaging to be obtained every 8 weeks (+/- 7 days) while on treatment, even if dose delays or interruptions in therapy have occurred.
4. For patients whose primary tumor tissue block (formalin-fixed paraffin embedded) is not readily available. This can be performed at any time prior to start of therapy, but only after informed consent for the protocol has been obtained. Non-availability of primary site tumor tissue will not preclude enrollment on this study towards the primary end-point of efficacy. These patients will be excluded from the micro-RNA correlative study analysis (section 4.2.4)
5. Can be done at any time prior to or during protocol therapy – treatment does not affect genetic polymorphisms

11. Safety Lead-In Cohort

This initial portion of the study will be limited to the first 6 patients at cohort 1 for purpose of toxicity evaluation of the combination of pralatrexate and oxaliplatin. This dose level will be considered tolerable following completion of two cycles (28 days) of treatment in 5 out of 6 patients in the absence of dose limiting toxicity (DLT). If ≥ 2 patients experience DLT at this dose level, then 6 additional patients will be enrolled at

cohort -1. Cohort -1 will be considered tolerable if ≤ 1 patient experiences a DLT. If so, the remainder patients will be accrued at this level. If this criterion is not met (i.e. ≥ 2 DLTs observed), then further examination of a lower dose (cohort -2) will be explored at the discretion of the investigator following discussions with the biostatistician, NCCN and Allos Therapeutics, Inc.

Dose-limiting toxicity in this safety lead-in cohort will be defined as any of the following conditions related to study treatment occurring within the first 28 days of treatment (2 cycles):

1. Grade 4 neutropenia lasting > 7 days
2. Febrile neutropenia, defined as grade 3 or 4 neutropenia associated with a fever of ≥ 100.4 F (= 38.0 C)
3. Grade 4 thrombocytopenia
4. Any ≥ grade 3 non-hematologic toxicity with the following exceptions:
   a. Grade 3 diarrhea, nausea or vomiting in the absence of optimal anti-diarrheal or anti-emetic treatment, provided toxicity resolves within 7 days and no dose reduction is required.
   b. Grade 3 hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, or hypophosphatemia which responds to medical intervention, provided toxicity resolves within 7 days and no dose reductions are required.
5. Treatment delay of > 14 days for toxicity

Hypersensitivity reactions to oxaliplatin will not constitute a DLT – if this adversity necessitates discontinuation of the drug within the first 28 days of therapy for this cohort, the patient will be replaced. Patients who experience a DLT will be dose reduced to the next lower dose cohort for continuation on the study. If an observed toxicity is clearly known to be within the expected toxicity profile of one of the study drugs, the attribution of this towards a DLT would be considered appropriate only if the severity of observed toxicity is greater than what would be typically observed with that drug alone.

Accrual will be halted after the first 6 patients are enrolled to permit assessment of toxicity of the combination. At least 28 days must have elapsed following the initiation of therapy for the sixth patient to enable complete evaluation. Patients enrolled in the safety lead-in part will count towards full accrual, including primary and secondary objectives. If a patient in this cohort does not complete 28 days of therapy, he/she will be replaced provided the discontinuation was not secondary to toxicity. The data on the first six evaluable patients and interpretation thereof will be communicated to the biostatistician, RPCI Phase I committee and the IRB before continuation of enrollment commences.

12. Dose Delays and Dose Modifications

A new cycle of treatment may not begin until the ANC is $\geq$ 1500/mm^3 and the platelet count is $\geq$ 100,000/mm^3, and any treatment-related non-hematologic toxicity (except sensory neuropathy – see below) has resolved to $\leq$ grade 1. In this case, weekly evaluations (clinical and laboratory) will be required to assess for resumption of therapy. Prior to any pralatrexate dose, mucositis must be $\leq$ grade 1. Assessment and grading of toxicity will be per NCI CTCAE Version 4.0.

Dose modifications and/or delays will be based on laboratory parameters obtained on day 1 of a given cycle, or based on nadir counts, if obtained (or worst non-hematologic toxicity) during that cycle. Blood counts obtained within 1 day prior may also be used to determine continuation/modification of dosing. As an example – a patient enrolled who lives some distance away from RPCI may have counts done closer to home on the day prior to determine eligibility for treatment on a given day.

In the event of adverse events, dose modifications should apply as per this section with the following guidelines:

- For toxicities, which are considered by the investigator unlikely to develop into serious, or life–threatening events (e.g. alopecia, altered taste etc.), treatment will be continued at the same dose without reduction or interruption. In addition, no dose reductions or interruptions will be required for anemia as it can be satisfactorily managed by transfusions.

- For any event, which is apparent at baseline, the dose modifications will apply according to the corresponding shift in toxicity grade, if the investigator feels it is appropriate. (e.g. if a patient has grade 1 asthenia at baseline which increases to grade 2 during treatment, this will be considered as a shift of 1 grade and treated as a grade 1 toxicity for dose modification purposes).

- If in the opinion of the investigator, a noted toxicity is considered solely due to one drug, then the dose of the other drug need not be modified.

If the dose is modified to manage an adverse event, it is recommended that the patient be seen by the investigator prior to re-starting study drug. Once a dose has been reduced it will not be increased at a later time. If toxicity or other reason requires a dosing delay/interruption of both drugs of more than four weeks, the patient will be withdrawn from protocol therapy and the reason recorded.

The following dose modification table will be followed (for patients with starting dose at cohort 1):

	Starting Dose	First Dose Reduction	Second Dose Reduction
Oxaliplatin	85mg/m^2	65mg/m^2	50mg/m^2
Pralatrexate	120mg/m^2	100mg/m^2	80mg/m^2

The following dose modification table will be followed (for patients with starting dose at cohort -1):

	Starting Dose	First Dose Reduction	Second Dose Reduction
Oxaliplatin	85mg/m^2	65mg/m^2	50mg/m^2
Pralatrexate	100mg/m^2	80mg/m^2	60mg/m^2

The patient will be taken off protocol therapy if dose reduction beyond dose level -2 is required.

12.1 Dose modification for hematologic toxicity:

12.1.1 Grade 2 neutropenia or thrombocytopenia: No change in dose for subsequent cycles. If count recovery takes > 2 weeks, reduce both drug doses by one dose level.

12.1.2 Grade 3 neutropenia (uncomplicated): No change in dose for subsequent cycles. If count recovery takes > 2 weeks, reduce both drug doses by one dose level.

12.1.3 Grade 4 neutropenia, febrile neutropenia (defined as ANC ≤ 1000 and fever ≥ 38.0 C) or grade 3/4 thrombocytopenia: Decrease by one dose level (both drugs)

12.1.4 Anemia, regardless of grade, will not warrant dose modification unless deemed necessary by the investigator.

12.2 Dose modification for mucositis:

Mucositis Grade on Day of Treatment	Action[1]	Dose upon recovery to ≤ Grade 1
Grade 2	Omit dose	Continue prior dose
Grade 2 recurrence	Omit dose	↓ both drugs by one dose level
Grade 3	Omit dose	↓ both drugs by one dose level
Grade 4	Omit dose	↓ both drugs by two dose levels*

*If this occurs at dose level -1, discontinue all protocol therapy
[1] For both drugs

Management of mucositis: A suggested regimen is to start oral leucovorin 25mg q6 hours X 3-5 days for ≥ grade 2 mucositis. This should be discontinued at least 48 hours prior to the next planned dose of pralatrexate.[53]

12.3 Dose modification for diarrhea

Diarrhea Grade on Day of Treatment	Action[1]	Dose upon recovery to ≤ Grade 1
Grade 2	Omit dose	Continue prior dose
Grade 2 recurrence	Omit dose	↓ both drugs by one dose level
Grade 3	Omit dose	↓ both drugs by one dose level
Grade 4	Omit dose	↓ both drugs by two dose levels*

*If this occurs at dose level -1, discontinue all protocol therapy
[1] For both drugs

12.4 Dose modification for nausea and emesis

For grade 3 or 4 nausea and emesis that persist despite the use of aprepitant and steroids, reduce the dose of both drugs by one dose level.

12.5 Dose modification for hepatic toxicity

For ≥ Grade 3 elevation in AST (SGOT), ALT (SGPT), alkaline phosphatase or bilirubin, the dose of oxaliplatin and pralatrexate will be reduced by one level during the next and subsequent cycles of therapy.

12.6 Dose modifications for other non-hematologic toxicity

Toxicity Grade on Day of Treatment	Action[1]	Dose upon recovery to ≤ Grade 1
Grade 2	Omit dose*	Same
Grade 3	Omit dose	↓ both drugs by one dose level
Grade 3 re-occurrence at reduced dose	Omit dose	↓ both drugs by one dose level
Grade 4	Omit dose	↓ both drugs by two dose levels**

* If clinically significant; in the event of recurrent grade 2 toxicity resulting in treatment delays, a reduction of both drugs by one dose level may be unndertaken

**If this occurs at dose level -1, discontinue all protocol therapy

[1] For both drugs

12.7 Toxicities unique to oxaliplatin

12.7.1 Neurotoxicity

Evaluation of neurologic toxicity should occur prior to each oxaliplatin dose. The patient should be questioned for neurologic symptoms and an appropriate neurologic examination performed. See Table below for an appropriate neurologic toxicity grading scale.

Oxaliplatin Dose Modifications for Neurologic Toxicity			
Toxicity	**Duration of Toxicity**		**Persistent[a] between Cycles**
	1 - 7 Days	**> 7 Days**	
Paresthesias/dysesthesias[b] of short duration that resolve and do not interfere with function (Grade 1)	no change	no change	no change
Paresthesias/dysesthesias[b] interfering with function, but not activities of daily living (ADL) (Grade 2)	no change	no change	↓ one dose level
Paresthesias/dysesthesias[b] with pain or with functional impairment that also interfere with ADL (Grade 3)	1st time: ↓ one dose level 2nd time: ↓one dose level	1st time: ↓one dose level 2nd time: ↓ one dose level	Stop (continue pralatrexate only)
Persistent paresthesias/dysesthesias that are disabling or life-threatening (Grade 4)	Stop (continue pralatrexate only)	Stop (continue pralatrexate only)	Stop (continue pralatrexate only)
Pharyngo-laryngeal dysesthesias	SEE BELOW		

[a]Not resolved by the beginning of the next cycle.

[b]May be cold-induced.

Laryngopharyngeal Dysesthesia:

An unusual laryngopharyngeal dysesthesia (LPD), a loss of *sensation* of breathing without any *objective* evidence of respiratory distress (laryngospasm, bronchospasm or hypoxia) has also been observed. This neurotoxicity may be induced or exacerbated upon exposure to cold and should be distinguished from a hypersensitivity reaction. If a patient develops LPD, the patient's oxygen saturation should be evaluated via a pulse oximeter and, if normal, reassurance, a benzodiazepine or other anxiolytic agent should be considered and the patient should be observed in the clinic until the episode has resolved. The oxaliplatin infusion may then be continued at a reduced rate, 33% of the original rate. Because this syndrome may be associated with the rapidity of oxaliplatin infusion, subsequent doses of oxaliplatin should be administered as 6-hour infusions. To minimize the risk of LPD, patients will be instructed to avoid ice and cold drinks the day of treatment.

Hypersensitivity reactions:

Oxaliplatin, as is the case with all platinum-containing compounds, is associated with a measurable (approximately 11%) incidence of hypersensitivity reactions, usually after multiple doses of treatment. This may present as bronchospasm, hypotension, and even hemolytic anemia. Pretreatment with glucocorticoids and antihistamines may be useful for some patients, but may not always prevent the development of anaphylactoid reactions, especially in patients with a prior history of hypersensitivity to this agent. For patients who have experienced a Grade 1 or 2 acute hypersensitivity reaction that is assessed as related to oxaliplatin administration, the following premedication is recommended prior to each subsequent dose of oxaliplatin (patients who have grade 3 or 4 acute hypersensitivity reactions should discontinue oxaliplatin therapy):

Dexamethasone 20 mg PO or IV, 12 and 6 hours prior to the oxaliplatin dose;
OR
Dexamethasone 20 mg PO or IV, as well as diphenhydramine 50 mg IV, and one of the following: cimetidine 300 mg IV, ranitidine 50 mg IV, or famotidine 20 mg IV 30-60 minutes prior to oxaliplatin administration. If these prophylactic measures fail to prevent oxaliplatin-related hypersensitivity, therapy with oxaliplatin should be discontinued. In this case, pralatrexate alone will be continued.

Pulmonary Fibrosis:

In the case of unexplained respiratory symptoms such as nonproductive cough, dyspnea or radiological pulmonary infiltrates, oxaliplatin should be discontinued until further investigation excludes interstitial pulmonary fibrosis. If interstitial pulmonary fibrosis is confirmed, oxaliplatin therapy should be terminated.

Hemolytic-Uremic Syndrome:

Upon monitoring weekly hematologic and renal parameters, if new onset of renal failure and/or hemolytic anemia is found, oxaliplatin will be held until the etiology of the renal failure and/or hemolytic anemia can be determined. Laboratory parameters that may be be used to determine whether hemolytic uremic syndrome (HUS) is present include: CBC, differential, platelets, PT, PTT, fibrinogen, FDP, ATIII, VWF, ANA, RhF, C3, C4, CH50, antiplatelet antibodies, platelet-associated IgG, and ciruculating immune complexes. If oxaliplatin is determined to be the cause of renal failure, hemolytic anemia or HUS, the drug will be permanently discontinued.

13. Endpoints

Primary: The primary end-point of this phase II trial is the overall response rate (ORR) to combination pralatrexate and oxaliplatin in advanced EGC. This will be determined using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 guidelines every 8 weeks while on treatment.[52] Objective responses will be confirmed 4 weeks after first documentation of response.
Secondary: The toxicity of this regimen will be recorded for all patients who received at least one dose of therapy. Other end-points will include calculation of time-to-progression and overall survival. Definitions of these variables are in the statistical section.
Correlative: The pharmacogenomic and tissue micro-RNA profiling end-points of this trial are outlined in the respective correlative science section.

14. Statistical Analysis

The study is a two-stage, single arm, unblinded Phase II trial allowing for early termination in case of futility. In the standard of care therapy, overall response rates (complete response + partial response) for advanced esophago-gastric cancer are around 0.20. An exact two-stage design proposed by Kepner and Chang tests the null hypothesis that the proposed treatment has an overall response rate that is at-most equal to the standard of care, against an alternative that it is greater.[54] The following study design has 80% power to detect a 0.40 overall response rate with the study treatment, while controlling to 5% the probability of erroneously finding a truly ineffective treatment as worthy of further research.

Seventeen eligible patients will be enrolled in the first stage of the trial. If a maximum of 3 of these patients achieve complete or partial response, the treatment will be declared ineffective, and the study terminated. If 4 or more patients achieve complete or partial response, an additional 16 patients will be enrolled. If 11 or more of the 34 total patients achieve complete or partial response, the treatment will be deemed effective and considered for further study.

Time to tumor progression (TTP) will be measured from the date of study enrollment to the first observation of progressive disease. Overall survival (OS) will be measured from the date of study enrollment to the time of death from any cause. These variables will be estimated using the Kaplan-Meier method. Statistics describing the time to event distributions will be obtained from Kaplan-Meier methods and Proportional Hazards models. Continuous variables will be summarized with commonly used statistics (mean, standard deviation, median, etc.), with sub-group associations tested using the Wilcoxon Rank Sum test. Categorical variables will be summarized in contingency tables, with associations of interest assessed using Fisher's Exact Test. Given the exploratory nature of this study, all p-values less than 0.05 will be deemed statistically significant. No adjustment will be made to the significance threshold to control for the effects of multiple testing on the overall Type I error rate.

The analyses for the correlative science studies are outlined in the correlative study section.

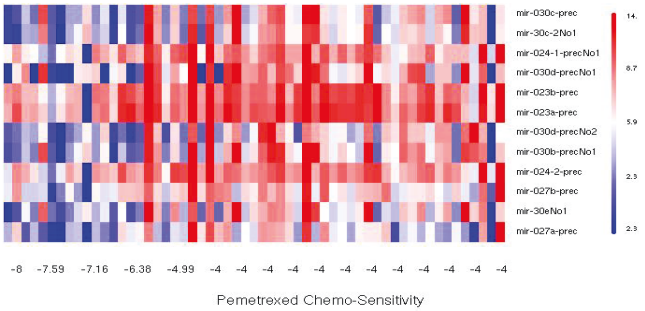
15. Correlative Studies

15.1 Background:

Pralatrexate like pemetrexed is a novel antifolate with activity in non small cell lung cancer (NSCLC), Hodgkin's disease, mesothelioma and bladder cancer. Pralatrexate is an analog of methotrexate and acts as a potent dihydrofolate reductase (DHFR) inhibitor. Studies indicate that pralatrexate selectively enters cells expressing reduced folate carrier (RFC-1) which is encoded by *SLC19A1*; and its increased retention in cells results from its high affinity for folylpolyglutamate synthase (FPGS). Like pemetrexed, the steady state accumulation of pralatrexate polyglutamates will depend primarily on activation/inactivation processes by FPGS and GGH respectively.[55-57] In previous studies with pemetrexed in NSCLC we identified correlations between haplotype-tagged single nucleotide polymorphisms (ht-SNPs) in *SLC19A1*, the gamma glutamyl hydrolase gene,*GGH*, and *FPGS* and efficacy as well as toxicity. In this proposal, we hypothesize that ***functionally relevant polymorphisms in these 3 genes as well as selected additional tagSNPs for genes from the folate pathway such as MTHFR and TYMS and DHFR either singly or in combination, play a role in the efficacy and/or toxicity of pralatrexate (Correlative Section Specific Aim 1).***

MicroRNAs (miRNAs) are small non-coding RNAs that were first described in the nematode *C. elegans* more than a decade ago[58], and are now known to regulate the expression of protein coding genes in various organisms. Over 800 miRNAs have been identified in humans. While changes in expression patterns of mRNA have been shown to be promising in chemosensitivity prediction in several human cancers, there are significant problems in their utilization in everyday clinical use. The requirement of fresh frozen RNA that is carefully preserved is one such significant limitation. MicroRNAs are stable to degradation over long periods of time.[59] This enables the use of RNA extracted from paraffin-embedded tissue, thereby enhancing and simplifying clinical use. The

number of miRNA known at this time is smaller, making miRNA profiles more robustly analyzable statistically than mRNA profiles. Therefore a microRNA signature is potentially more clinically feasible for everyday use. Limited data exists for the utilization of microRNA signatures for chemosensitivity prediction. As shown in the figure below, cell lines with increasing resistance (increasing IG50) show different microRNA profiles. This difference may be utilized in chemosensitivity prediction. ***In the Correlative Section Specific Aim 2, we hypothesize that response to pralatrexate can be predicted by microRNA expression profiling of the epithelial component of the tumor.***



15.2 Preliminary Data:

We have resequenced *FPGS* and obtained polymorphism (SNP) information.[60] We also utilized data from NCBI and HapMap databases to obtain polymorphism information on *GGH* and *SLC19A1*. We identified SNPs in these three candidate genes, *SLC19A1* and *GGH* and *FPGS*, and genotyped for the SNPs in two studies with pemetrexed–treated patients.[61-63] In these small studies,SNPs in *SLC19A1, FPGS* and *GGH* correlated with efficacy and toxicity of pemetrexed. In the pemetrexed-gemcitabine study, patients heterozygous for polymorphisms in *FPGS* and *GGH* had more grades 3/4 adverse events including SGPT (ALT) elevation, dyspnea and fatique, although not statistically significant. Similarly, polymorphisms in *GGH* associated with confirmed response.[62] As well, three SNPs in *SLC19A1* predicted for statistically significant differences in overall survival in the study: IVS4(2117)T>C; IVS5(9148) A>C and Exon6(2522) C>T, p=0.03. A similar analysis in our recently completed phase II study (50 patients) of pemetrexed in combination with bevacizumab in second-line NSCLC also identified SNPs in *FPGS, GGH* and *SLC19A1* which correlated with toxicity and efficacy.[63]

15.3 Study Design and Methods (Pharmacogenomics):

<u>Specific Aim 1</u>: Genotype DNA samples from patients enrolled in the clinical trial for tagged SNPs in candidate genes responsible for the *activation, inactivation and transport of pralatrexate*. The genes are *FPGS*, *GGH* and *SLC19A1*. We propose to evaluate the possible contribution of pharmacogenetics (PG) to variable molecular responses and toxicity of pralatrexate by correlating clinical activity and toxicity with intragene haplotypes in *FPGS*, *GGH* and *SLC19A1*. The tagged SNPs will be correlated

with toxicity (myelosuppression, mucositis, LFT elevation) and efficacy (objective responses, TTP) of pralatrexate.

15.3.1 FPGS, SLC19A1, GGH and other folate pathway polymorphisms: We have identified tagSNPs from our resequencing data and the HapMap mining studies for *FPGS*, *SLC19A1* and *GGH*.[60, 62, 63] Samples from this clinical study will be genotyped for all tagSNPs identified. Other polymorphisms that will be genotyped for include well-known SNPs in *MTHFR* and *TYMS*, namely C677T, A1298C in *MTHFR* and the 28bp-repeat polymorphism, and 1494del6 of the *TYMS,* as well as SNPs in *DHFR*. These SNPs have been associated to varying extents with methotrexate toxicity and efficacy.[64-68] A list of 49 tagSNPs to be genotyped is show below. To generate these tagSNPs, the SNP databases including HapMap, dbSNP and SeattleSNP were used. The SNP genotype datasets for the genes above were loaded into Haploview v 4.0[69] and polymorphisms with frequencies greater than 5% were selected for haplotype analysis at an r^2 threshold of 0.8. Haplotypes close to or above 2% were then organized into single blocks from which ht-SNPs were derived. In addition, the tagger software was used to generate additional tagSNPs.

Genes showing TagSNPs to be genotyped						
Gene	*FPGS*	*GGH*	*SLC19A1*	*MTHFR*	*TYMS*	*DHFR*
dbSNP id rs# / or SNP location	5FR -573	rs11545077	rs914232	rs2184226	rs2853533	rs2560424
	rs10760502	rs3780126	rs2838958	rs1537514	rs502396	rs1677693
	IVS1 (28)	rs11990678	rs2297291	rs4846049	rs2244500	rs11951910
	IVS9 (48)	rs3780130	rs2838956	rs2274976	rs2847153	rs11742668
		rs7010484	rs3788189	rs3818762	rs2847149	rs13161245
		rs11995525	rs1051298	rs1476413	rs15872	rs10072026
		rs12677953		rs1801131	rs699517	rs1650697
				rs12121543	rs2853542	
				rs6541003	rs34489327	
				rs1801133		
				rs17421511		
				rs11121832		
				rs7533315		
				rs9651118		
				rs17367504		
				rs13306561		

15.3.2 Sample Collection and genotyping: Five (5) ml of blood will be collected into an EDTA tube at baseline or at any time during the study (since therapy does not affect gene polymorphisms). Genomic DNA will be extracted and stored till analysis as previously described.[63] All genotyping are PCR-based and will be performed, using established methods at RPCI on the Sequenom MassARRAY platform as described in Adjei et al., 2009.[63] Network Sites please see Appendix 2 for shipping instructions.

For patients enrolled at network site(s), 5 ml of blood collected in an EDTA tube (non-glass) should be frozen at -80°C and shipped on dry ice to the following address:

Attn: Dr. Huayi Huang
Adjei laboratory,
GCDC Building, Room 127
Roswell Park Cancer Institute

Buffalo, NY 14263

Phone (716) 845-7656
e-mail: huayi.huang@roswellpark.org

The site(s) will be requested to contact the receiving laboratory personnel prior to shipping. It would be preferable to have samples batched for shipping.

15.3.3 Statistical Analysis: The primary hypothesis tested will be the association of dichotomous genomic events (i.e., homozygote/heterozygote, or presense/absense of polymorphisms, etc.) with adverse avents. If 5 or fewer subjects are observed for a particular genotype, e.g. homozygous, we will test for the rate of adverse events among heterogygotes, by a clopper pearson exact binomial test, against a 0.15 null rate of adverse events . If at least 6 subjects are observed as homozygous, tests of independence will be performed by Fishers Exact test. Type I error control will be performed, by bounding the probability of more than 5 false positives to 5%. Correlations of the haplotype-tagged SNPs with response, OS, and TTP will be *explored* by multivariate logistic and Cox proportional hazards models, to explore the impact of these germline SNPs on binary and time to event outcomes. These exploratory comparisons will be carried out on the entire cohort, as well as within different patient subgroups such as responders, and progression-free and stable patients if feasible.

15.3.4 Sample Size: A limitation of the present study is the small sample size (34 patients), which lacks the power to make robust associations between SNPs and treatment outcome (toxicity and efficacy). We see these correlative studies as hypothesis-generating. SNPs identified in this study as potentially predictive of efficacy or toxicity will be tested in future studies. If as many as 5 subjects are observed as homozygous, the design provides for 75% power to detect a true adverse event rate of 37%. If 6 subjects are observed as homozygous, there will be 75% power to detect an OR of 16.5, and for 10 observed homozygotes, an OR of 9.44.

15.4 Study Design and Methods (MicroRNA profiling)

Specific Aim 2: MicroRNA expression profiles generated using cell line data can predict response to pralatrexate chemotherapy using expression profiling of the epithelial component of the tumor isolated using laser-capture microdissection (LCM). For these experiments, it is crucial that we use only the epithelial component of tumors because the cell line signatures that we will attempt to validate are based on only the epithelial component. There is data that strongly suggests that the gene expression of whole tumors is different from that of the epithelial component isolated by LCM[70]. This is especially important when the gene expression measurements are those of miRNA because the changes seen in miRNA expression classifiers typically have small fold changes and using whole tumors may therefore mask relevant signals. Network Sites please see Appendix 2 for shipping instructions.

15.4.1 Experimental plan: Existing chemosensitivity data of the NCI-60 cell line panel to pemetrexed will be used to generate a miRNA signature. The feasibility of using this has been demonstrated using publicly available chemosensitivity data (http://dtp.nci.nih.gov/index.html) along with publicly accessible NCI-60 miRNA microarray data[71] in the figure above. Such an analysis will be performed using data from an updated miRNA microarray platform (Affymetrix Inc.) using RNA from the NCI-60 cell line panel. This database is currently being generated by the Yendamuri laboratory and is scheduled to be completed by April 2010. The use of a new platform is important as the publicly available data was generated using older platforms using a large amount of input total RNA. Therefore, the older platforms cannot be used to profile RNA obtained by LCM. We have tested the feasibility of generating profiles from 100 ng of total RNA input using the updated platform and have successfully profiled RNA from both the epithelial and stromal components of formalin fixed paraffin embedded (FFPE) lung cancer tissue. Therefore the methods required to perform the experiment have been standardized. Once the pemetrexed sensitivity signature is finalized based on the above data, the RNA obtained from the LCM-derived epithelial component of the primary esophago-gastric tumor tissue (FFPE) will be used to generate epithelium-specific miRNA profiles of the pre-treatment biopsy specimens using the Affymetrix microRNA microarray platform. The primary tumor tissue will be obtained by biopsy (those with primary in place) or from the original FFPE block (for those patients who underwent prior resection, or definitive therapy without local relapse). Non-availability of primary site tumor tissue will not preclude enrollment on this study towards the primary end-point of efficacy. These patients will be excluded from the micro-RNA correlative study analysis. The specimens will then be classified into "sensitive" and "specific" categories. This classification will then be compared to the clinical responsiveness of the tumor as judged by imaging data. We use pemetrexed sensitivity data in this proposal because pralatrexate chemosensitivty data of the NCI-60 cell line panel is not available and because of the close approximation of the mechanism of action of both drugs.

For network site(s) as well as tissue samples collected at outside institutions, the tissue sample (formalin-fixed paraffin block or 5-10 unstained formalin-fixed paraffin sections on appropriate slides along with one hematoxylin and eosin stained section) will be sent to the following address:

Yendamuri Laboratory
Attn: Eric Kannisto
Roswell Park Cancer Institute
MRC 207
Elm and Carlton Streets
Buffalo, NY 14263

Phone: (716) 845-8364
e-mail: eric.kannisto@roswellpark.org

The site(s) will be requested to contact the receiving laboratory personnel prior to shipping.

15.4.2 Statistical analysis: The investigators realize that the limited number of samples assayed in this experiments permits only exploratory analyses to be performed. Initial studies will be conducted to test and optimize individual miRNAs for maximum sensitivity and specificity to predict chemosensitivity, inferring differences in the distributions of biomarkers with stringent statistical multiple testing error control procedures. Affymetrix CEL files will be loaded into the affy bioconductor package, and preprocessed with a custom cdf library, using the RMA routine. We will perform a 2-sample two-ways test of mean differences in mir log expression by discrete treatment outcome (toxicity or efficacy), with a wilcoxon rank sum test. We will have 70% power to detect a 2 standard deviation difference in means, assuming 10 responders and 23 non-responders, bounding the probability of more than 10 false positives to 5%. We will visually explore the distributions of candidate mir signature profiles, by outcome, for artifacts, outliers, symmetry and spread. Data transformations,and discretizations will be evaluated for supervised and partially unsupervised prediction performance. We will investigate signature refinement with advanced penalized version of well known prediction methods, such as Bayesian Additive Regression Trees, Support Vector Machines, and Fisher's Discriminant with nested class Partitioning Around Mediods, considering univariate and multivariate variable selection methods. We will evaluate the prediction performance with ROC and Threshold/Recall Analysis conducted under k-fold monte carlo cross validation. Significant differences in sensitivity, specificity and area under the curve will be inferred with bootstrap techniques. Further exploration will include measuring the marginal predictive utility of the miRNA signatures, accounting for known and potential risk factors.

16. Adverse Event Reporting

IDENTIFICATION, RECORDING AND HANDLING ADVERSE EVENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. An adverse event is any adverse change (developing or worsening) from the patient's baseline (pre-treatment) condition, including intercurrent illness, which occurs during the course of a clinical study after treatment has started, whether considered related to treatment or not. All adverse events encountered during the clinical study will be reported on the case report form (CRF).The intensity of clinical adverse events will be graded according to the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, which have recommended grading.

Adverse events not listed on the CTC grading system will be graded according to the following scale:

Mild:	Discomfort noticed but no disruption of normal daily activity
Moderate:	Discomfort sufficient to reduce or affect normal daily activity
Severe:	Incapacitating with inability to work or perform normal daily activity
Life–threatening:	Self–explanatory

Adverse Events Monitoring

The collection of AE information should begin at the time the patient receives the initial **study drug** infusion and must continue throughout the study until 30 days after the last dose, unless specified otherwise in the protocol. This includes pre-existing conditions or symptoms that worsened during the study, or whose relationship to the investigational product changed; but does not include pre-planned hospitalizations or procedures for pre-existing conditions. In addition, the investigator should notify **National Comprehensive Cancer Network (NCCN)** of any SAE or outcome that may occur after this time period that he/she believes to be related to **pralatrexate and oxaliplatin**. All SAEs or AEs related to **study drugs** must be followed to resolution, stabilization or return to baseline, or deemed irreversible. An AE report must contain the following four basic elements: (1) an identifiable patient; (2) a suspect medicinal product; (3) an identifiable reporting source; and (4) an identifiable event or outcome. All identified AEs must be recorded and described on the appropriate page of the CRF. If known, the diagnosis of the underlying illness or disorder should be recorded in addition to the presenting symptoms. The following information should be captured for all AEs: the date of the onset and resolution; the severity of the event (see definitions in National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] Version 4.0); the investigator's opinion of the relationship of the event to the investigational product (certainly/definitely, probably, possibly, unlikely, or not related); the treatment required for the AE; information regarding resolution/outcome; and if the AE is serious, a clear identification of the seriousness outcome. In addition, all Roswell Park Cancer Institute initiated trials will be monitored periodically by the Compliance Monitor.

Definitions

An **Adverse Event** is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment.

Serious Adverse Events (SAE)

A serious adverse event as defined by ICH is any adverse experience that at any dose meets any of the following conditions:

- results in death
- is life-threatening (The patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may

not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

Hospitalizations that do not meet these criteria are:

- reasons described in the protocol, e.g., drug administration, protocol-required testing
- social reason in the absence of an AE
- surgery or procedure planned prior to entry into the trial

Nonserious Adverse Events
Any adverse event that is not an SAE is, by default, a non-serious AE.

An **Unexpected Adverse Event** is an event that is not listed in the current Clinical Investigator's Brochure (CIB) / Package Insert or an event that may be mentioned in the CIB / Package Insert, but differs from the event because of greater severity or specificity.

For SAEs occurring in the current study, the Expectedness of an Adverse Event will be determined by identifying the term in the table of Expected Adverse Events by Body System, provided in the latest version of the CIB. If the term is not contained in the list under the relevant body system, the term is to be considered UNEXPECTED.

For comparative drugs, expectedness is determined by using the pertinent reference text: the US Package Insert (if marketed drug) or Clinical Investigator Brochure (if Investigational New Drug).

Causality is a determination of whether there is a reasonable possibility that the drug may have caused or contributed to an adverse event. It includes assessing temporal relationships, dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) or a lack of one or more likely causes. The Investigator must determine if an adverse event is in some way related to the use of the study drug. This relationship should be described as follows:

Unlikely or Unrelated: The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug.

Possible: The event follows a reasonable temporal sequence from administration of the study drug or the event follows a known response pattern to the study drug *BUT* the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be

unlikely related to the use of the study drug or the event could be the effect of a concomitant medication.

Probable: The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition *or* the event cannot be the effect of a concomitant medication

Definite: The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug

Unknown: Based on the evidence available, causality cannot be ascribed

Adverse Events Emerging Subsequent to Study Cessation

For 30 days subsequent to study completion or withdrawal, new onset adverse events will be captured. Follow up of these events will follow the same procedure as described above for AEs observed during the study period.

Safety Reporting Requirements

Safety Reporting Requirements and Timelines

The Investigator will utilize the MedWatch Form for the reporting of adverse events and follow up information to those events.

All serious adverse events regardless of severity or relationship must be reported to Allos within 24 hours of the investigational staff's knowledge.

Spectrum Pharmaceuticals
157 Technology Drive
Irvine, CA 92618
Phone: (949) 788-6700
Fax: (949) 788-6706; be sure to retain successful fax acknowledgement
Email: drugsafety@sppirx.com

And

NCCN via fax at 215-358-7699 or e-mailed to ORPReports@nccn.org

In addition, the Investigator will adhere to the safety reporting requirements and timelines described in the Clinical Trial Agreement with National Comprehensive Cancer Network (NCCN).

The Investigator will provide full and timely cooperation with any requests from Allos, governing IRB, institution, or regulatory agency with any requests regarding reports of individual reports of adverse events.

Network Sites please see Appendix 2 for reporting information.

Additional Requirements for IND HOLDERS

Expedited IND Safety Reports:
Adverse events meeting certain criteria will require expedited reports to the FDA, IRB, and Allos Therapeutics. A case report must be one that is serious AND unexpected AND drug-related (to any degree) to be subject to regulations for expedited safety reports.

Any adverse event that meets criteria as an SAE (see section entitled "Types of Adverse Events") is SERIOUS.

Any adverse event not already described in the study Investigator Brochure is UNEXPECTED. If no formal Investigator Brochure exists, then the current, FDA-approved product label (package insert) will be used to determine expectedness of an event.

Causality assessment is described in the previous section entitled "Definition and Types of Adverse Events."

Types of Expedited Safety Reports

- *7-Calendar-Day FDA Telephone or Fax Report:*

The Investigator will directly notify the FDA within 7 calendar days of any adverse event that is ALL of the following:
-SERIOUS due to DEATH or FATALITY, or is immediately LIFE THREATENING
-UNEXPECTED
-CAUSALLY RELATED

Notification to the FDA will be made directly to the new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever was responsible for the review of the IND.

- *15-Calendar-Day FDA Written Report:*

The Investigator will directly notify the FDA within 15 calendar days of any adverse event that is ALL of the following:
-SERIOUS due to NON-fatal or NON-life threatening criteria
-UNEXPECTED
-CAUSALLY RELATED

Pregnancy Statement:

During the course of the trial, all patients of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition, a missed or late menstrual period should be reported to the treating physician. If a female patient, or an investigator, suspects that the female patient may be pregnant prior to administration of study drugs, the study drugs must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed the patient must not receive study medications and must be withdrawn from the study.

Throughout the entire pregnancy, additional contact should be made with the patient, and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.

If a male patient is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

Upon live-birth delivery, minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record abstraction, or a combination of these methods. All serious adverse event reports relating to the pregnancy, including spontaneous abortion, elective abortion and congenital anomalies, should be forwarded to the FDA and the NCCN and a copy sent to Allos Therapeutics.

17. Criteria for Response and Progression

The primary end-point of this phase II trial is the overall response rate (ORR) to combination pralatrexate and oxaliplatin in advanced EGC. This will be determined using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 guidelines every 8 weeks while on treatment.[52] Objective responses will be confirmed 4 weeks after first documentation of response.

17.1 Definitions:

RECIST Version 1.1 differs from the original version with respect to the following:

1. Number of lesions to be assessed decreased to a maximum of 5 (from 10) with a maximum of 2 per organ.
2. Assessment of pathological lymph nodes is now incorporated. Nodes that measure > 15mm in short axis are considered pathologic and measurable. This should be included in the calculation of tumor response, if applicable.
3. Greater stringency to the definition of progressive disease which requires and increase by 20% in sum of the target lesions AND a 5mm absolute increase.

Changes in only the largest diameter (unidimensional 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.

Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >10 mm with CT scan (CT scan slice thickness no greater than 5mm) or as >10 mm by clinical examination or > 20mm by chest X-ray. In addition, pathologic lymph nodes measuring ≥ 15mm in short axis are also considered measurable. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm using CT scan; LNs ≥ 10mm to < 15mm), are considered non-measurable disease. Blastic bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (identified by physical examination but not measurable by reproducible imaging techniques) are all non-measurable. For lytic bone lesions or mixed lytic plus blastic bone lesions that have an identifiable soft tissue component reproducibly measurable by standard criteria, definitions of measurability above will apply. For cystic lesions thought to represent malignant disease, similar criteria will apply, though it is preferable to use non-cystic lesions, if present, for the purpose of assessment and response.

Target lesions

All measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs, 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.

Non-target lesions

All other lesions (or sites of disease) 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 or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

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 4 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

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 aerated lung. However, CT is preferable.

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

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes,

subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

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

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

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

Response Criteria

Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions; any pathological lymph node (whether target or not) must have reduction in short axis to < 10mm

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. In addition, the sum must also demonstrate an absolute increase of at least 5mm.

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

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

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.

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

Notes:

- 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.

Confirmatory Measurement/Duration of Response

Confirmation

This is required in the current trial as the primary end-point is response rate. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed *4 to 6 weeks* after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 to 8 weeks.

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.

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.

Response Review

This is performed by the Response Review Committee at the Roswell Park Cancer Institute.

18. Ethical and Regulatory Standards

18.1 This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each patient (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the patient is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the patient log and patient records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining patient authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the patient is treated, in accordance with the Declaration of Helsinki, Good Clinical Practice, and according to the guidelines in this protocol, including attached appendices.

18.2 Informed Consent

The Investigator is responsible for obtaining written consent from each patient or the patient's legally authorized representative in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the patient according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The patient should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the information sheet and of the signed consent form to the patient and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the patient file. At any stage, the patient may withdraw from the study and such a decision will not affect any further treatment options.

19. Study Responsibilities

19.1 Data Collection

Data entry into the database is to be completed in a timely fashion (approximately within 28 days) after the patient's clinic visit. If an adverse event is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Form, which is handled in an expedited fashion (see Section 16)

Data management activities will be performed using eRT. eRT is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate it's development by the eRT Information Technology team. Once the database design is approved by the PI, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs (electronic CRFs).

eRT is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the CRC and PI. Once all queries have been resolved, the data can be released to the statistician for analysis.

20. Maintenance of Study Documents

Essential documents should be retained per RPCI policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI. It is the responsibility of RPCI to inform the investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to RPCI upon written agreement between the Investigator and RPCI.

21. Administrative Rules

21.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

21.2 Termination of the Study

It is agreed that, for reasonable cause, either the Investigators or the Sponsor, RPCI may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of patients enrolled in the study.

21.3 Confidentiality

All information provided to the Investigator by RPCI including preclinical data, protocols, CRFs, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting this study, and no disclosure shall be made except in accordance with any right of publication granted to the Investigator. This information may be related in confidence to the IRB/ERC or other committee functioning in a similar capacity. No report or information about the study will be provided to anyone not involved in the study without consent of RPCI except if required by law.

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Appendix: 1: Diary for Folic acid medication

PATIENT INSTRUCTIONS: **Use this calendar to record your daily dose of Folic Acid**

☐ *Take 1 tablet daily* ***Patient Initials (Last, F, M)*** ☐ ☐ ☐ ***Patient MR #:_____***

Time Period From: ☐☐ / ☐☐ / ☐☐☐☐ ***To:*** ☐☐ / ☐☐ / ☐☐☐☐
M M D D Y Y Y Y ***M M D D Y Y Y Y***

DAY Of WEEK ***Sunday***	***DAY OF WEEK*** ***Monday***	***DAY OF WEEK*** ***Tuesday***	***DAY OF WEEK*** ***Wednesday***	***DAY OF WEEK*** ***Thursday***	***DAY OF WEEK*** ***Friday***	***DAY OF WEEK*** ***Saturday***
☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***	☐ ***Day of cycle*** ***# of tablets taken:*** ***AM _____*** ***PM _____***
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Notes:

Be sure to bring this calendar and the pill bottle with you for all of your return appointments.
Return the calendar and the pill bottle to your Clinical Research Coordinator. Thank you.

Date: 09.01.15

Appendix 2: Network Sites

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:
Roswell Park Cancer Institute
CRS Network Office
ASB K 102B
Buffalo, New York 14263

Telephone:
716-845-8084 or 716-845-1203 - M-F; 7:00 AM to 4:30 PM
716-845-2300 - After hours, weekends and holidays: request the RPCI Principal Investigator
Fax:
716-845-8743

2. INFORMED CONSENT

- Informed Consent must be obtained by the **Investigator** from any patients wishing to participate, **prior to any procedures or change in their treatment.**
- An informed consent template is provided by RPCI and can be amended to reflect institutional requirements.
- All consent changes **must** be reviewed by Roswell Park Cancer Institute Network Office prior to submission to the site IRB.
- The informed consent must be IRB approved.
- Always check that you are using the correct date and version of the IRB approved consent.

3. SUBJECT REGISTRATION

Phase II protocol registration instructions:

The **Subject Enrollment Log** must be faxed to the CRS Network Office within 24 hours of the date the patient is consented. Once the Principal Investigator has determined that eligibility has been met, complete the **Subject Registration Form and fax it** to the RPCI Network Office at (716) 845-8743.

Note: The subject completes the **Gender, Race, and Ethnicity form** and this is placed in the study binder.

Roswell Park Cancer Institute does not grant exceptions to eligibility criteria.

4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this should be reported to the RPCI Network, site IRB and any other regulatory authority involved in the trial.
- ANY study deviation will be recorded on the Study Deviation Log.

- Subjects who are inadvertently enrolled, with significant deviation(s) from the study-specified criteria, will be removed from the study.
- Notify RPCI of any early subject withdrawal and appropriately document the discontinuation and the reason why.

5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The auditor must be able to read what has been deleted.
    - Do NOT use white-out, magic marker, scratch-outs.
    - Do NOT erase entries.
- Use only black ink for documentation on the accountability form and any other study forms.

6. DRUG ACCOUNTABILITY

Drug accountability will be strictly maintained by recording quantities of study drug received, dispensed to patients and wasted, lot number, date dispensed, patient ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.

- Responsibility rests solely with the Principal Investigator but can be delegated as appropriate (e.g. to pharmacy personnel).
- Records must be maintained regarding receipt, dispensing, return, waste and disposition of all investigational agents.
- Study drug supply should only be used in accordance with the IRB approved study.
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.
- Any discrepancies shall be documented and explained.
- An inventory count should be performed with each transaction.
- Drug accountability forms shall be stored with study related documents.
- Each medication provided for this study and each dosage form and strength must have its own Drug accountability.
- Do NOT “transfer”, “borrow” or “replace” supplies between studies.
- Dispensing the wrong study supply is considered a medication error.
- Never replace investigational agents with commercial product.

7. SERIOUS ADVERSE EVENT REPORTING:

The Investigator will utilize the MedWatch Form for the reporting of adverse events and follow up information to those events.

The site Investigator or designated research personnel will report all serious adverse events, whether related or unrelated to the investigational agent(s) to the IRB in accordance with their local institutional guidelines.

All serious adverse events regardless of severity or relationship must be reported to Allos within 24 hours of the investigational staff's knowledge.

"Spectrum Pharmaceuticals
157 Technology Drive
Irvine, CA 92618
Phone: (949) 788-6700
Fax: (949) 788-6706; be sure to retain successful fax acknowledgement
Email: drugsafety@sppirx.com"

And

NCCN via fax at 215-358-7699 or e-mailed to ORPReports@nccn.org

The site will notify the CRS Network Office within one business day of being made aware of the SAE. A preliminary written report must follow within 24 hours (1 business day) of the oral notification using the following forms:

- SAE report form
- MedWatch 3500A

A complete follow-up report must be filed within 10 working days

8. UNANTICIPATED PROBLEM REPORTING:

An Unanticipated Problem (UAP) is any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given:
   (a) the research procedures that are described in the study- related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data;
   (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized;

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention the participating physician or delegated research staff from each site will notify **their local IRB in accordance with their local institutional guidelines**. The site must also notify the CRS Network Office within 24 hours of being

made aware of the Unanticipated Problem by completing the **RPCI Unanticipated Problem Report Form** and faxing it to the CRS Network office.

9. SAMPLE SHIPPING INSTRUCTIONS

MicroRNA

- Formalin-fixed paraffin embedded tissue block or 5-10 unstained formalin-fixed paraffin embedded sections on appropriate slides and one Hematoxylin and Eosin stained slide of the primary tumor
- Labeled with Patient initials, Subject ID (provided by RPCI), Protocol number I 169210 and date and time of collection
- Shipment Notification email to Eric.Kannisto@roswellpark.org
- Ship samples overnight (Monday through Thursday)

SHIPPING ADDRESS:
Roswell Park Cancer Institute
Eric Kannisto/ Dr. Yendamuri
Medical Research Complex (MRC) - Room 207
RE: I 169210 Specimens
Elm & Carlton Streets
Buffalo, NY 14263
716-845-8364

Genotype DNA Samples:

- After collection in EDTA tubes, blood sample should be decanted into 5 mL cryovials and stored at -70 to -80 C
- Labeled with Patient initials, Subject ID (provided by RPCI), Protocol number I 169210 and date and time of collection
- Shipment Notification: Huayi.huang@RoswellPark.org
- Shipment frozen aliquots overnight on dry ice (Monday through Thursday)

SHIPPING ADDRESS:
Roswell Park Cancer Institute
RE: I 169210 PG Specimens
Attn: Huang Huayi (c/o Dr. Araba Adjei)
Dept of Pharmacology & Therapeutics
Grace Cancer Drug Center Room G127
Elm & Carlton Streets
Buffalo, New York 14263
(716) 845-7656