

A Phase II Trial of Tetrathiomolybdate After Surgery for Malignant Mesothelioma: Final Results

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Background. Tetrathiomolybdate (TM) is an oral copper-depleting agent that has been shown to inhibit angiogenesis, and angiogenesis is a predictor of poor prognosis in malignant pleural mesothelioma. We hypothesized that cytoreduction of malignant pleural mesothelioma followed by TM will delay time to progression.

Methods. Between November 2000 and August 2003, 30 patients with malignant pleural mesothelioma received postoperative TM beginning 4 to 6 weeks after surgery at a dose adjusted to keep ceruloplasmin between 5 and 15 mg/dL. Time to progression was compared with the 55 stage I and II patients and 109 stage III patients previously treated with cytoreduction by one of us (H.P.).

Results. The 30 patients (25 men, 5 women; 13 stage I and II, 17 stage III), median age 67 years (range, 49–81 years), remained on TM a median of 14.9 months (range, 2 to 57 months). All patients reached target ceruloplasmin levels at a mean of 34 ± 2 days (95% confidence interval, 30 to 39 days), and vascular endothelial growth

factor levels at baseline (ceruloplasmin = 45.2 ± 2 mg/dL) decreased from $2,086 \pm 390$ pg/mL to $1,250 \pm 712$ pg/mL ($p < 0.002$) at target ceruloplasmin (13 ± 2 mg/dL; $p < 0.0001$ from baseline). The time to progression for all stage I or II TM patients was 20 months whereas that of 55 stage I or II non-TM-treated patients was 10 months ($p = 0.046$ versus TM). No differences in time to progression for the stage III TM patients from surgery were seen (7 months).

Conclusions. Tetrathiomolybdate has antiangiogenic effects in malignant pleural mesothelioma patients after resection of gross disease, and exhibits minimal toxicity and comparable efficacy to previous multimodality trials. Tetrathiomolybdate should be evaluated for efficacy in combination with standard malignant pleural mesothelioma regimens, as well as for postsurgical maintenance therapy.

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Malignant pleural mesothelioma (MPM) remains an orphan disease in search of novel but hopefully efficacious therapies. It is generally concluded that cytoreductive surgery in selected patients can be performed safely in the hands of experienced surgeons [1], and the addition of systemic or other local therapies to the surgery has been shown to be safe and to improve survival at least for patients with less bulky, node-negative mesothelioma [2–9]. Nonetheless, mesothelioma invariably progresses in 90% to 95% of the patients and, for the most part, is uniformly fatal [1].

Targeted therapies for MPM are beginning to exploit novel molecular genetic findings that predict that molecules which interfere with receptors for tyrosine kinase pathways or bind proangiogenic molecules or their receptors could impact tumor growth [10]. The copper chelator tetrathiomolybdate (TM), which quickly and effectively depletes copper stores, is under investigation

as an antiangiogenic agent [11]. Previous work demonstrating that thiomolybdate promoted copper deficiency suggested that TM might be a useful agent in the treatment of Wilson's disease. Taken with meals, TM forms a tripartite complex of TM, copper, and food protein thereby preventing copper absorption. Given between meals, TM is absorbed into the blood, and forms a tripartite complex with TM, albumin, and the freely available serum copper, making the thus complexed copper unavailable for cellular uptake, and the amount of free copper is rapidly reduced [12]. The induction of copper deficiency by TM can be easily monitored by measurement of serum ceruloplasmin (Cp).

Tetrathiomolybdate has been evaluated in a phase I trial of TM in 18 patients with a variety of metastatic cancers including breast, colon, lung, and prostate [13]. Toxicity was minimal and included mild anemia and neutropenia, and a proportion of these advanced patients had stabilization of disease. Results of a phase II trial of TM in 15 advanced kidney cancer patients revealed that

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4 patients (31%) had stable disease for at least 6 months; none had a partial or complete response [14]. Serum levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor, interleukin 6, and interleukin 8 measured at the onset of copper deficiency were significantly reduced compared with pretreatment levels.

The most relevant cytokines and growth factors targeted by TM in angiogenic pathways are also active in mesothelioma and include the interleukin 6, interleukin 8, fibroblast growth factors, and VEGF [15-18]. Given the data that have emerged about the proangiogenic pathways in MPM as well as the ongoing investigations of TM as an antiangiogenic strategy, a phase II trial that investigated the impact of TM on time to recurrence after surgical cytoreduction was launched in November 2000. The specific aims of the study were to investigate whether TM could be given to MPM patients in an adjuvant setting safely on a long-term basis and maintain moderate copper deficiency as defined by serum Cp levels, and also to document time to progression and ultimate survival after surgery. In selected patients, serum VEGF levels were measured to document any effect of the drug on angiogenic cytokines.

Patients and Methods

Patient Populations

After approval by the Karmanos Cancer Institute and Wayne State University Human Investigation Committees, this study was launched in November 2000. Between November 2000 and August of 2003, 55 patients had exploration for mesothelioma at the Karmanos Cancer Institute. Seven of these patients had had preoperative chemotherapy, rendering them ineligible for the protocol. Of the other 48, 8 patients were found to have unresectable disease, 3 patients refused the protocol and were lost to subsequent follow-up, 1 patient sustained a postoperative nonfatal myocardial infarction, and 1 patient died in the postoperative period. One other patient was found to have an elevated calcium postoperatively as a result of a parathyroid cancer and was treated for this secondary malignancy. Four patients (3 biphasic, 1 epithelial) had histologic and radiographic progression postoperatively before study entry. None of these TM-ineligible patients were considered in the analysis of the historical group. This left 30 patients eligible and able to be evaluated (55%) for postoperative copper-reduction therapy during this period.

The cohort of 164 pathologic stage I through III patients having cytoreductive surgery for mesothelioma in the authors' previous experience from February 1990 to April 2005 was used to serve as an internal reference to compare to the TM cohort. Specifically, time to progression from surgery was used as a primary intermediate end point with survival as a secondary end point.

Surgical Eligibility

Patients with histologically documented malignant mesothelioma who could tolerate pleurectomy or extrapleu-

ral pneumonectomy, both performed as a maximal cytoreduction along with a mediastinal lymph node dissection, were eligible for the trial if they had not had any previous therapy. All patients were surgically staged using the International Mesothelioma Interest Group staging system [19], incorporating the surgeon's personal observations (H.I.P.), operative, and postoperative pathology reports. After recovery from surgery, patients returned for induction of copper deficiency.

Induction of Copper Deficiency

TETRATHIOMOLYBDATE. Tetrathiomolybdate was provided for this study by the University of Michigan in bulk lots suitable for human administration (Sigma-Aldrich Chemical Company, St. Louis, MO, and Milwaukee, WI). The TM was stored in 100-g lots under argon and was placed in 20-mg gelatin capsules for patient use. No other treatment including cytotoxic chemotherapy, molecularly targeted therapy, or radiation therapy was allowed while the patients were taking TM.

MONITORING OF COPPER DEPLETION. The serum Cp level was used as a surrogate measure of total-body copper status. As total-body copper was reduced, the serum Cp level proportionately decreased. The objective was to reduce Cp to 5 to 15 mg/dL of baseline, and to maintain this level for the course of the study. In previous trials, there appeared to be no untoward clinical effects from this degree of copper reduction that was termed "chemical copper deficiency."

INDUCTION OF COPPER DEFICIENCY. Four to six weeks after cytoreduction, patients returned for follow-up examination and baseline Cp measurement, complete blood count, and liver and renal function tests. Patients were then started on an induction dose of TM of 180 mg/day, given as a dose of 40 mg with meals three times a day, and then 60 mg away from food, generally at bedtime. Ceruloplasmin levels were monitored every week for the first 8 weeks of therapy (for out-of-state patients, the blood was drawn at home and sent in a overnight delivery postage-paid container), and then every 2 weeks thereafter. After achieving the target Cp, in most patients the first maintenance dose was 20 mg twice daily with meals and 40 mg at bedtime. Further modifications were performed aimed at maintaining Cp within a target window of 5 to 15 mg/dL and to prevent Cp values of less than 5 mg/dL. Ceruloplasmin levels were then performed every 2 weeks with monitoring for necessary dose adjustment. Patients were seen for history and physical examinations and toxicity evaluations every 2 months in follow-up.

Toxicity

Toxicities were evaluated using the National Cancer Institute Common Toxicity Criteria 2.0. The first indication of true clinical copper deficiency is a reduction in blood cell counts, primarily anemia, as copper is required for heme synthesis as well as cellular proliferation. Thus, the copper deficiency objective of this trial was to reduce the Cp to 5 to 15 mg/dL, without decreasing the patient's hematocrit or white blood cell to below 80% of the

baseline value at entry. To maintain the Cp target and to prevent absolute Cp values less than 5 mg/dL, TM doses were adjusted as soon as possible once the Cp level was known. Tetrathiomolybdate was discontinued temporarily until hematocrit was restored to acceptable levels. If the hematocrit did not recover within 5 to 7 days of stopping the drug, the patient was transfused. Tetrathiomolybdate was discontinued and the dose adjusted if patients in the second stage of copper deficiency had acceptable hemopoietic indices (ie, >80% baseline) but had other grade 3 toxicities.

Vascular Endothelial Growth Factor Measurements

Blood was collected in a serum separator tube and was allowed to clot for 30 minutes before centrifugation at 1,000 g for 10 minutes. Serum was immediately frozen (-80°C) in aliquots of 1.2 mL in microcentrifuge tubes. Human VEGF enzyme-linked immunosorbent assay was performed as directed by the manufacturer (R&D Systems, Minneapolis, MN).

Disease Monitoring

All patients had pre-TM induction and follow-up computerized tomograms performed at the Karmanos Cancer Institute. Specifically, the computerized tomograms were performed within 1 week of starting TM (first postoperative computerized tomogram), when the target Cp level was reached, and then every 4 months as long as the patient was on the study. Any patient having physical examination or symptoms suggesting recurrent disease had radiographic or biopsy documentation at that clinic visit if it was between the 4-month follow-up period. Patients with documented recurrence had their TM discontinued and were counseled regarding other treatment options.

Table 1. Characteristics of the Tetrathiomolybdate and Historic Cohort

Patient Demographics	TM	Historic Cohort
Mean age (range)	66 ± 2 (49-84 y)	60 ± 1 (34-80 y)
Sex (male/female)	25/5	129/35
Histology		
Sarcomatoid	1	116
Biphasic	10	40
Epithelial	19	108
IMIG stage		
I	7	21
II	6	34
III	17	109
Lymph node status (involved/uninvolved)	10/20	66/98
Operation (pleurectomy/EPP)	11/19	72/92

EPP = extrapleural pneumonectomy; IMIG = International Mesothelioma Interest Group; TM = tetrathiomolybdate.

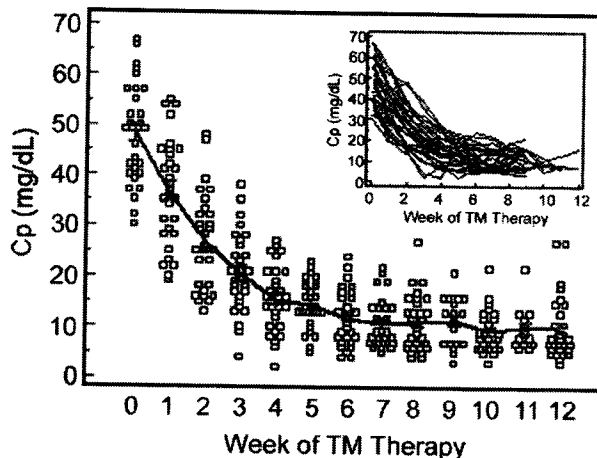


Fig 1. Mean ceruloplasmin (Cp) levels at inception of trial initiation and through induction. Inset, values for all patients during the induction period.

Statistical Analysis

The purpose of the trial was to determine whether the time to progression of patients after cytoreductive surgery could be significantly increased over the historic data for median time to progression for patients receiving surgery for mesothelioma determined by one of us (H.P.). If the median time to progression in the first 10 patients was not longer than this historic data, the study would have been closed. Survival time and time to progression was calculated from date of surgery until progression, death, or last follow-up as appropriate. All data are current to December 2007. The probability of survival or progression was calculated using the Kaplan-Meier method, and the significance of the difference between pairs of Kaplan-Meier curves was calculated using the Mantel-Haenszel procedure. Paired Student's *t* test evaluation of patients having both serum VEGF and Cp levels was performed using serum obtained before induction of TM and at the time of reaching the target Cp level.

Results

Characteristics of the patient populations are seen in Table 1. All patients in the trial recovered from surgery such that they were able to begin the induction dose of TM within 40 ± 2 days (range, 16 to 57 days) with no differences between patients having extrapleural pneumonectomy (40 ± 2 days) or pleurectomy (41 ± 2 days).

Once the induction dose of TM was started, all patients reached the level of copper deficiency as defined in this study at a median of 4.9 ± 0.3 weeks (range, 2 to 9 weeks; Fig 1). The Cp level at the start of the study was 48 ± 2 mg/dL (range, 30 to 67 mg/dL), and the mean Cp at 5 weeks was 14 ± 1 mg/dL (range, 2 to 23 mg/dL). Twenty-seven patients were maintained on the TM regimen until documented progression (range, 2 to 30 months), whereas 3 patients who did not have a

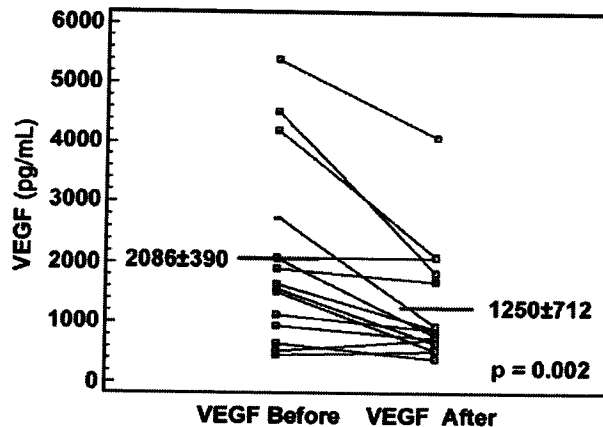


Fig 2. Influence of tetrathiomolybdate on serum vascular endothelial growth factor (VEGF) levels. Serum levels were analyzed before tetrathiomolybdate initiation and when target ceruloplasmin levels were reached.

recurrence had the TM discontinued when the protocol was closed to follow-up (61, 59, and 51 months from study entry).

Toxicity of Induced Copper Deficiency

All patients were able to be evaluated for toxicity, and for the most part, the TM was well tolerated. Dizziness unrelated to blood pressure or posture was a commonly seen adverse event (16 patients, 53%) during the induction phase to which the patients became acclimated. Grade 1 or 2 fatigue was reported by 90% of the patients. Sixteen (53%) of the patients had the TM held or reduced, most frequently because of grade 3 granulocytopenia (12 patients, 40%) with or without grade III anemia (4 patients, 13%) or thrombocytopenia (1 patient, 3%). The granulocytopenia reversed within 1 week of holding the TM in all cases, and the patients were then restarted on the drug at a reduced dose. There were no episodes of febrile neutropenia. Seven patients (4 extrapleural pneu-

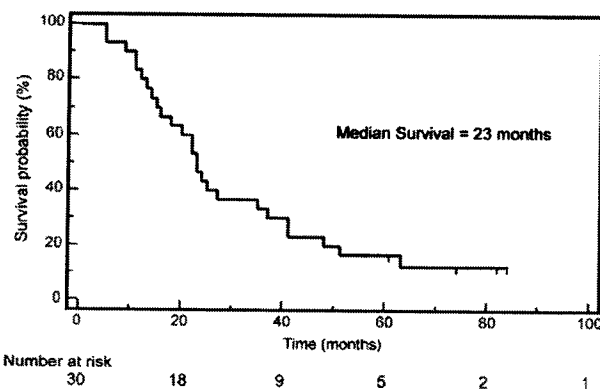


Fig 3. Survival from surgery for all tetrathiomolybdate patients. Survival of all 30 patients receiving tetrathiomolybdate and reaching ceruloplasmin levels of 5 to 15 mg/dL.

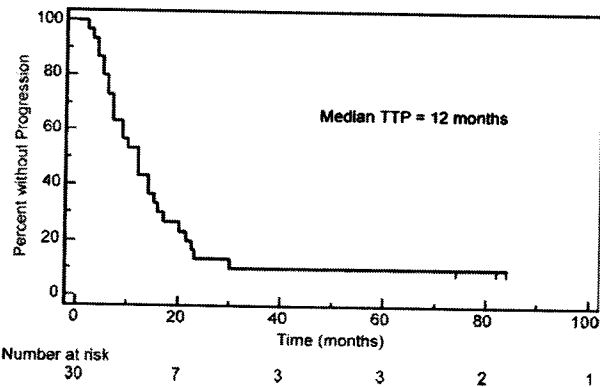


Fig 4. Progression from surgery for all tetrathiomolybdate patients. Time to progression (TTP) of all 30 patients receiving tetrathiomolybdate and reaching ceruloplasmin levels of 5 to 15 mg/dL.

monectomy, 3 pleurectomy) required red blood cell transfusion for the grade III anemia combined with grade II fatigue.

Vascular Endothelial Growth Factor Levels

Matching serum VEGF levels were measured after surgery, before institution of TM, and from the serum of 15 patients at the time of reaching their prescribed Cp level. A significant decrease in the serum VEGF levels was noted on reaching moderate copper deficiency (Fig 2).

Time to Progression and Survival

The median survival for all TM patients from surgery in the series regardless of stage was 23 months (1 year, 80%; 2 year, 43%; 3 year, 36%; Fig 3), and the median time to progression was 12 months (Fig 4). Four patients remain alive a median of 64 ± 2 months from surgery. Three of these 4 patients are alive without disease. The time to

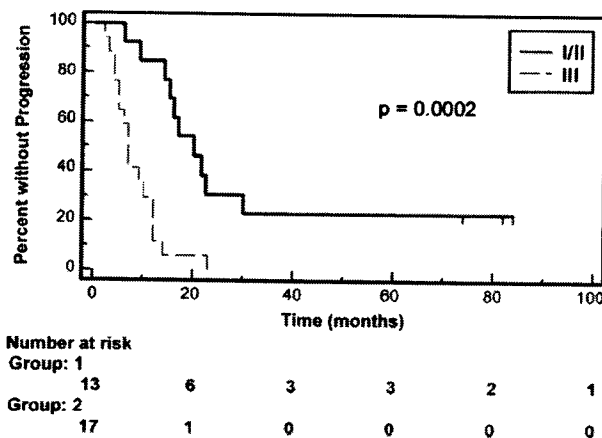


Fig 5. Progression from surgery for all tetrathiomolybdate patients, stage I or II (group 1; blue line) versus stage III (group 2; maroon line). Survival of all 30 patients receiving tetrathiomolybdate and reaching ceruloplasmin levels of 5 to 15 mg/dL by stage.

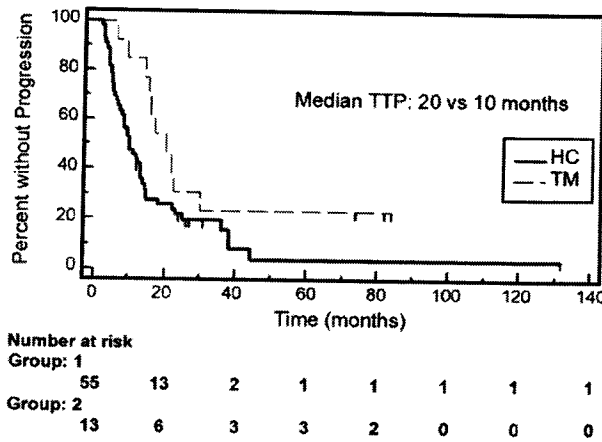


Fig 6. Progression from surgery for tetrathiomolybdate (TM; group 2; maroon line) versus historic cohort, stage I or II (HC; group 1; blue line). Time to progression (TTP) of stage I or II patients receiving tetrathiomolybdate and reaching ceruloplasmin levels of 5 to 15 mg/dL compared with historic controls.

progression for all stage I or II patients was 20 months, whereas the time to progression for the stage III patients from surgery was 7 months (Fig 5). In the historic cohort, the median time to progression for the 55 stage I or II non-TM-treated patients, whether they received adjuvant therapy at some point or not, was 10 months ($p = 0.0046$ versus TM; Fig 6). In the evaluation of the data, 1 sarcomatoid patient occurred in the TM stage I or II group whereas there were 10 sarcomatoid patients in the stage I or II historic group. When the sarcomatoid patients were eliminated from evaluation of both groups, there was a significant increase in time to progression from 11 months to 17 months favoring the TM group. There was no significant difference in time to progression for the 17 stage III TM patients compared with the 109

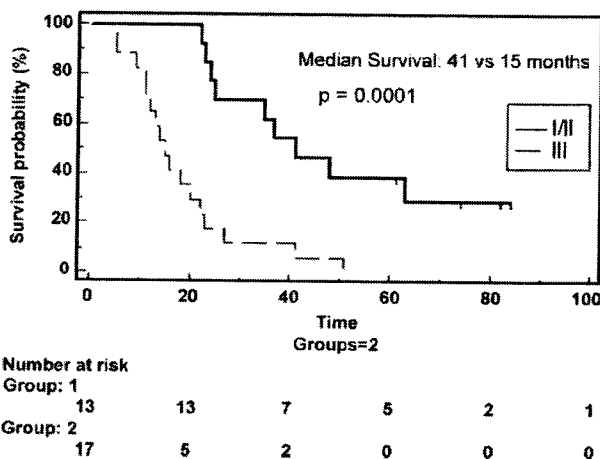


Fig 7. Survival from surgery for all tetrathiomolybdate patients, stage I or II (group 1; blue line) versus stage III (group 2; maroon line). Survival of stage I or II tetrathiomolybdate patients and stage III tetrathiomolybdate patients.

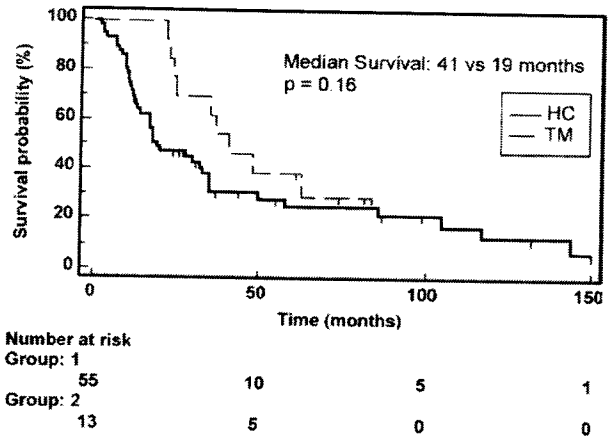


Fig 8. Survival from surgery for tetrathiomolybdate (TM; group 2; maroon line) versus historic cohort (HC; group 1; blue line), stage I or II. Survival of stage I or II patients receiving tetrathiomolybdate and reaching ceruloplasmin levels of 5 to 15 mg/dL compared with historic controls.

stage III historic cohort patients (both 7 months). The most common site of first recurrence was the ipsilateral chest ($n = 14$), and other sites of progression included the abdomen with ascites ($n = 7$), thoracoscopy or thoracotomy site ($n = 3$), parenchyma of lung ($n = 2$), and abdominal lymph nodes ($n = 1$). All of the stage III patients experienced recurrence, whereas 3 of the stage I or II patients remain free of disease (1 biphasic, 74 months; 1 sarcomatoid, 84 months; 1 epithelial, 82 months).

For the patients treated with TM, the median survival for all stage I or II patients was 41 months (1 year, 100%; 2 years, 77%; 3 year, 57%) whereas that for stage III patients was 15 months (1 year, 65%; 2 year, 18%; 3 year, 12%; Fig 7). There were two non-mesothelioma-related deaths: 24 months after surgery in a 78-year-old stage I patient who died of a stroke 9 months after progressing and a 73-year-old stage III patient who died in an automobile accident 28 months after progression. There was a nonsignificant trend for decreased median survival (19 months; $p = 0.16$ versus TM; Fig 8) for the 55 stage I or II non-TM-treated patients, whether they received adjuvant therapy at some point or not. No differences in survival were seen in stage III patients treated with or without TM.

Influence of Postprogression Adjuvant Therapy

All patients who progressed were given the option of receiving first-line mesothelioma chemotherapy, ie, Alimta and cisplatin or gemcitabine and cisplatin. Three patients remain alive without evidence of disease without receiving adjuvant chemotherapy or radiation in addition to their TM. Of the 27 patients who experienced recurrence, 16 received adjuvant therapy with Alimta and cisplatin ($n = 16$; stage I or II, 9; stage III, 7), and