

Comprehensive Cancer Center of Wake Forest University
CCOP Research Base Protocol # 83403
A Phase II Study of Single Agent Depsipeptide (FK228) in Recurrent, Platinum Sensitive
Adeno-Carcinoma of the Ovary or Peritoneum

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NCI Supplied Agent: *Depsipeptide (NSC 630176; IND 51,810)*

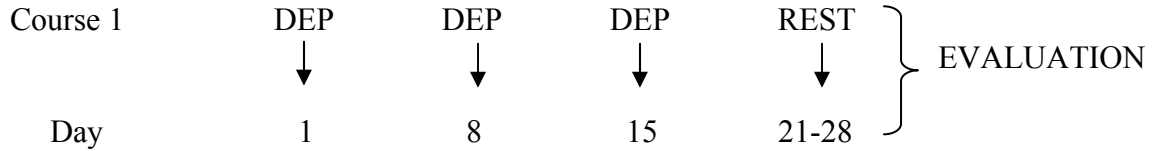
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SCHEMA



DEP = Depsipeptide

DEP is administered at a dose of 13 mg/m² as a 4-hour intravenous infusion in the outpatient setting.

One course is 28 day duration. Courses may be repeated every 28 days in the absence of intolerable toxicity or tumor progression.

Patients will be evaluated for response after two treatment courses

EKG monitoring during course 1: Day 1 EKG within 1 hour prior to administration of depsipeptide and within 1 hour following depsipeptide infusion; course 1 day 8 within 1 hour prior to depsipeptide administration; course 1 day 15 within 1 hour prior to administration of depsipeptide.

Eligibility Criteria

- Patients must have histologically or cytologically confirmed primary epithelial carcinoma of the ovary or peritoneum. Histologic confirmation of the recurrence is not necessary.
- Patients must have measurable disease following RECIST criteria, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques including palpation, plain x-ray, CT or MRI or as ≥ 10 mm with spiral CT scan. See section 9.2 for the evaluation of measurable disease.
- Patients must have had initial therapy with a platinum compound (cisplatin or carboplatin) and achieved a complete response to this therapy after a non-specified number of courses. The treatment may include conventional dose therapy, high dose therapy, consolidation therapy or extended therapy after surgical or non-surgical assessment.

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Patients who have not received paclitaxel or docetaxel as the initial therapy may have had a second regimen, which includes these compounds.

Patients must be regarded platinum sensitive: treatment free interval without evidence of progression of greater than 6 months, but shorter than 12 months after completion of a platinum based regimen.

Patients may not have received any additional chemotherapy for persistent or recurrent disease, including re-treatment with the original combination.

- Patients must have recovered from toxicities of previous treatments except grade 1 neuropathy.

All treatments directed against the tumor (chemotherapy, hormonal therapy, non-cytotoxic therapy) should be discontinued at least 4 weeks prior to initiation of protocol therapy. Estrogen replacement therapy can be continued.

- Age >18 years. Because no dosing or adverse event data are currently available on the use of depsipeptide in patients <18 years of age, children are excluded from this study.

➤ Life expectancy of greater than 6 months

➤ ECOG performance status #2 (Karnofsky $\geq 60\%$; see Appendix B).

➤ Patients must have normal organ and marrow function as defined below:

- leukocytes $\geq 3,000/\mu\text{L}$
- absolute neutrophil count $\geq 1,500/\mu\text{L}$
- platelets $\geq 100,000/\mu\text{L}$
- total bilirubin within institutional normal limits
- AST (SGOT)/ALT (SGPT) $\leq 2.5 \times$ institutional upper limit of normal
- creatinine within institutional normal limits

OR

- creatinine clearance $\geq 60 \text{ mL/min/1.73 m}^2$ for patients with creatinine levels above institutional normal
- potassium within institutional normal limits
- magnesium within institutional normal limits

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- Patients must be free of active infections requiring antibiotics.
- The effects of depsipeptide on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because histone deacetylase inhibitors are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
- Pregnant women are excluded from this study because depsipeptide is a histone deacetylase inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with depsipeptide, breastfeeding should be discontinued if the mother is treated with depsipeptide.
- Ability to understand and the willingness to sign a written informed consent document.

Sample Size: 46

Duration of Study: 2 years

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1. OBJECTIVES

- 1.1. To estimate the response rate of recurrent, platinum sensitive adenocarcinoma of the ovary or peritoneum to Depsipeptide.
- 1.2. To determine the toxicity of Depsipeptide (13 mg/m² i.v. for 3 out of 4 weeks) in this patient population.

2. BACKGROUND

2.1 Adenocarcinoma of the Ovary and Peritoneum

It is projected, that in 2003 about 25,400 women will be diagnosed with an ovarian malignancy and 14,300 will die from their disease (Jamal et al. 2003). Initial response to treatment with tumor reductive surgery and combination chemotherapy is as high as 70% (McGuire et al. 1996). However only 30% of patients with advanced disease remain long term cancer free (Jamal et al. 2003). Treatment for recurrent ovarian cancer is rarely curative. Higher response rates can be expected for patients, who reached a complete response after initial therapy and who remained disease free without therapy at least 6 months after completion of the initial treatment regimen. Re-treatment with taxanes and platinum leads to response rates as high as 70% (Dizon et al. 2002). Lower response rates are seen after single agent therapy such as topotecan RR 28% (Gordon et al 2001), pegylated liposomal doxorubicin RR 28% (Gordon et al 2001), or gemcitabine RR 19% (Lund et al. 2001). New agents for second line treatment are needed to increase the platinum free interval and form the basis for combination therapy.

2.2 Depsipeptide

Depsipeptide (FK 228, FR 901228), is a unique bicyclic peptide originally isolated from *Chromobacterium violaceum* strain 968 (Ueda *et al.* 1994a). It has a novel chemical structure composed of four amino acids (D-valine, D-cysteine, dehdrobutyrine, and L-valine) and a novel acid (3-hydroxy-7-mercapto-4heptenoic acid) configured in a cage-shaped bicyclic depsipeptide. Depsipeptide was discovered by Fujisawa Pharmaceuticals Co., Ltd., as part of a concerted search for novel compounds from natural products that would reverse the ras-transformed phenotype to normal.

Depsipeptide is also a potent inhibitor of the enzyme histone deacetylase. Deregulation of histone acetylation has been implicated in the development of several types of cancer. Genes that encode histone acetyltransferase enzymes are translocated, amplified, overexpressed and/or mutated in various cancers (Marks et al. 2001; Weidle et al. 2000). These findings suggest that deregulate acetylation of histones plays a role in the pathogenesis of hematological as well as solid tumors by changing the chromatin structure and transcription of genes involved in cell cycle control, differentiation or apoptosis. Consequently, there is considerable interest in

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histone deacetylase inhibition as a potential therapeutic modality in the treatment of hematologic malignancies and solid tumors.

2.2.1 Mechanism of Action

Results of early studies showed that depsipeptide inhibited the growth of the Ha-*ras*-transformed NIH3T3 clonal cell line, Ras-1, and induced reversion of the transformed morphology to normal within 1 day at a concentration of 2.5 ng/ml (Ueda *et al.*, 1994b).

While mRNA expression of the *c-myc* oncogene in Ras-1 cells was decreased in the presence of depsipeptide, Ha-*ras* mRNA expression was unaffected by 24-hour exposure to 2.5 ng/ml of depsipeptide. Depsipeptide blocked cell cycle transition from G₀/G₁ to S phase and induced nuclear quiescence. The course of *c-myc* suppression paralleled that of G₀/G₁ arrest and correlated with the morphologic reversion of the transformed cells. These results led to the proposal that the growth inhibition and G₀/G₁ arrest resulted from depsipeptide blocking the *ras*-mediated signaling transduction pathway (Fecteau *et al.*, 2002). Other investigations of the effect of depsipeptide on G₁ to S transition of the cell cycle showed that depsipeptide inhibits signal transduction through MAP kinase and causes p53-independent G₁ arrest (Sandor *et al.*, 1998). Depsipeptide has also been identified as a histone deacetylase (HDAC) inhibitor similar to trichostatin A based on its ability to cause arrest of the cell cycle at both G₁ and G₂/M phases, to induce internucleosomal breakdown of chromatin, and to inhibit intracellular HDAC activity resulting in an accumulation of marked amounts of acetylated histone species within M-8 cells (Nakajima *et al.* 1998). DNA methylation and histone deacetylase inhibition may act synergistically in the re-expression of genes silenced in cancer (Cameron *et al.*, 1999). This and other similar reports have generated considerable interest in combining a histone deacetylase inhibitor and a demethylating agent (such as decitabine) for synergistic activity in the treatment of hematologic malignancies and solid tumors. Depsipeptide, either alone or in combination with hypomethylating agents, has been shown to induce a number of cellular proteins that may have critical effects on apoptosis, proliferation and susceptibility to immunologic manipulation (Kitazono *et al.* 2001a, 2001b; Weiser *et al.*, 2001a, 2001b; Zhu *et al.*, 2001). The compound may also have antiangiogenic activity that contributes to antitumor efficacy (Kwon *et al.*, 2002).

2.2.2 Non-Clinical Studies

Antitumor Activity – *In vitro* and *in vivo*

Potent antitumor effects of depsipeptide have been demonstrated both *in vitro* and *in vivo* (Ueda *et al.*, 1994b, Rajgolikar *et al.*, 1998). *In vitro*, depsipeptide exerted antiproliferative activity against 12 human solid tumor cell lines (IC₅₀ ranged from 0.5 to 5.9 nM), but was less potent against cultured normal cells. It was found that the longer the duration of depsipeptide exposure, the lower the concentration of drug

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necessary to induce the antiproliferative activity. Similar IC_{50} values were found in a study of 13 lymphoid cell lines (Murata *et al.*, 2000). In non-small cell lung cancer (NSCLC) cells expressing wild-type or mutant p53, depsipeptide inhibited growth and induced apoptosis (Yu *et al.*, 2002).

In vivo, depsipeptide had moderate antitumor efficacy in various model systems, including murine B16 melanoma, M5076 sarcoma, Meth A fibrosarcoma, and colon 38 carcinoma plus several human tumor xenograft models, including stomach (SC-6), lung (Lu-65, LC-6, A549, PC-9, LX-1), breast (MX-1, MCF7), and colon (Colo201) (Ueda *et al.*, 1994c). Tumor inhibition in these studies ranged from 56% to better than 70%. Results from these animal studies indicated that an intermittent administration schedule (q4d x 3) of depsipeptide resulted in lower toxicity and greater efficacy than a daily schedule (qd x 5). The greater antitumor activity observed with an intermittent schedule may be attributable to the higher individual doses that were administered because of the greater tolerance to depsipeptide.

The intermittent schedule (q4d x 3) of depsipeptide administration was also better tolerated than daily treatment (qd x 5) in mice implanted subcutaneously (SC) with LOX IMVI melanoma. On the daily schedule, complete remissions were observed in three of ten mice in each of two groups given doses of 1.44 or 2.16 mg/kg/day (the LD_{40}). In contrast, administration of a 5.3 or 8 mg/kg/dose on the intermittent schedule (q4d x 3) resulted in complete remissions in 10 of 10 animals treated. In addition, the intermittent schedule gave 1.6- to 2-fold greater delays in tumor growth than the daily schedule. The MTD was estimated to be 1.44 mg/kg/day (4.32 mg/m²/day) for the daily schedule of depsipeptide treatment, while the total dose maximally tolerated dose (MTD) for mice treated on the intermittent schedule was 10.8 mg/kg (32.4 mg/m²).

In a *scid* mouse lymphoma model, male mice inoculated intraperitoneally (IP) with U-937 cells and treated with depsipeptide (0.1-1 mg/kg, IP) once or twice a week survived longer [median survival times of 30.5 days (0.56 mg/kg) and 33 days (0.32 mg/kg)], than saline-treated mice (20 days) (Sasakawa *et al.*, 2002). Two of 12 mice treated with 0.56 mg/kg depsipeptide survived past the observation period of 60 days.

Animal Toxicology

In a multidose toxicity study, male mice dosed IV with 10.8, 15.9, or 24 mg/m²/dose once or twice a week for 4 weeks developed no cardiac lesions. Other histopathological changes included testicular degeneration, inflammation of the injection site, bone marrow depletion/hyperplasia, hematopoietic cell proliferation in the spleen, splenic/thymic atrophy, and hematopoietic foci and fatty degeneration of the liver. In a second mouse study, animals treated with depsipeptide at doses ranging from 1.92 to 15.9 mg/m²/dose on a daily x 5 schedule had evidence of cardiotoxicity (increases in LDH and gross histopathologic lesions). The MTD of depsipeptide in mice is 15.9

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mg/m²/dose given once or twice a week for 4 weeks; the DLT on this schedule is injection site toxicity.

When depsipeptide was given to rats as a 4-hour infusion on days 1, 5, and 9, swelling in areas adjacent to the catheter was seen in animals given doses of 10 mg/m²/dose. Other toxicities in rats administered depsipeptide on this schedule included significant weight loss; leukopenia; thrombocytopenia; anemia; increased ALP, AST, ALT, and fibrinogen levels; and lesions in the bone marrow and thymus, lymph nodes, spleen, skin tissues (subcutaneous), and blood vessels (injection site). The MTD of depsipeptide in rats on this schedule of administration was 6 mg/m²/dose with a DLT of bone marrow toxicity.

Rapid, bolus (30-second) IV administration of 1.0 or 2 mg/kg (20 or 40 mg/m²) depsipeptide resulted in acute toxicity and death 2-8 days after drug administration in 5 of 14 dogs. Immediate dose-related elevations in LDH and CPK were observed (up to 500% of baseline). Animals that received a rapid, bolus dose of 0.5 mg/kg showed little or no toxicity. When the same doses were administered over a 4-minute period, increases in LDH and CPK were seen, but no deaths occurred. When the administration was prolonged to a 1-hour infusion, the same doses led to little acute toxicity. Histopathological lesions included lymph node/tissue depletion and/or necrosis, intestinal necrosis, pulmonary cellular infiltration, and necrosis/edema/hemorrhage at the site of injection. No cardiac lesions were seen.

When 1-hour infusions of 0.5, 1, or 2 mg/kg (10, 20, or 40mg/m²) depsipeptide were administered to dogs on once or twice a week schedules, one dog, dosed with 2 mg/kg twice a week for 4 weeks, died 6 days after completion of dosing. Clinical signs of gastrointestinal toxicity (i.e., diarrhea, emesis) occurred in all dose groups, and local injection site toxicity occurred primarily in dogs treated with depsipeptide twice a week for 4 weeks. Increases in CPK, LDH, and aspartate aminotransferase (AST) were seen in all dose groups on both schedules, but were not completely correlated with the administration schedule. Histopathological lesions included bone marrow depletion as well as lesions in the spleen, thymus, intestines, liver, lung, and site of injection in dogs in the 1 or 2 mg/kg/dose groups (both schedules). The MTD was 1 mg/kg (20 mg/m²) on the twice weekly schedule and 2 mg/kg/dose (40 mg/m²) on the once weekly schedule. The DLT was bone marrow, intestinal, and local toxicity.

Dogs were treated with 1 or 2 mg/kg (20 or 40 mg/m²) depsipeptide as a 4-hour infusion or 2 mg/kg as a 24-hour infusion on study days 1, 5, and 9 (q 4d x 3). All the dogs treated with 2 mg/kg as a 24-hour infusion died on day 2. One of four dogs treated with 2 mg/kg as a 4-hour infusion died on day 4. Severe signs of gastrointestinal toxicity (emesis and bloody diarrhea) occurred during and immediately after the infusion was completed. Less severe signs of gastrointestinal toxicity occurred after the infusion on days 5 and 9. Severe local toxicity (acute and/or chronic edema) occurred in dogs administered 1 and 2 mg/kg of depsipeptide as a 4-hour infusion. Lymph node/tissue

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atrophy and/or necrosis and intestinal necrosis were dose-related histopathological lesions and hypospermia, hepatic inflammation, and gallbladder necrosis were also present in dogs administered 1 or 2 mg/kg of depsipeptide as a 4-hour infusion. No cardiac lesions were seen. The MTD was 1 mg/kg (20 mg/m²) given as a 4-hour infusion on study days 1, 5, and 9. The DLT was local and intestinal toxicity.

Toxicity in general, specifically cardiotoxicity, appears to be related to the rate of drug administration in dogs and mice. A 4-hour infusion was better tolerated than a 30-second bolus or a 24-hour infusion. Lymph node/tissue atrophy and/or necrosis and intestinal necrosis were dose-related. Testicular hypospermia, hepatic inflammation, and gallbladder necrosis were also reported in dogs given 1 or 2 mg/kg depsipeptide as a 4-hour IV infusion. The maximum tolerated dose (MTD) in dogs was found to be 1 mg/kg (20 mg/m²) given over a 4-hour period on the intermittent schedule. Injection site reactions and intestinal toxicity were dose-limiting. The recommended starting dose for clinical trials was 2 mg/m² (1/10 MTD in dogs) given as a 4-hour infusion on days 1, 5, and 9.

2.2.3 Clinical Experience

Phase 1 Studies

The DCTD, NCI, is evaluating depsipeptide in several phase 1 trials in which the agent is administered as a 4-hour IV infusion on different schedules in patients with advanced cancer or hematologic malignancies, and one study is evaluating depsipeptide in combination with decitabine in patients with lung cancer. A starting dose of 1 mg/m² (one-third of the toxic dose low in rats) was used in the initial phase 1 trials, both of which evaluated intermittent schedules; one trial used a weekly schedule (**T95-0022**) and the other used a biweekly schedule (**T95-0077**). Because nonclinical toxicology data indicated cardiac injury in several animal species, particularly dogs, all patients on the initial phase 1 trials of depsipeptide were monitored closely for potential cardiac events. Based on these initial trials in advanced cancer patients, the MTD and recommended phase 2 dose for depsipeptide is 10 mg/m² when administered on a weekly schedule (or 13.3 mg/m² if an anti-emetic is administered before and after treatment) and 17.8 mg/m² when administered on a biweekly schedule.

The initial trial evaluating a weekly schedule of depsipeptide (days 1, 8, and 15 of a 4-week cycle) in patients with advanced cancer (**T95-0022**) accrued 33 patients at doses ranging from 1 to 17.7 mg/m². On this schedule, toxicity was generally dose-related with the most frequent events including nausea, fatigue, anorexia, anemia, and hyperglycemia; DLTs included fatigue, leukopenia, and weight loss. Seventeen of the 33 patients experienced grades 1 and 2 asymptomatic transient changes in EKG that did not appear to be dose related. There was also an increase in the incidence of decreased hemoglobin at doses greater than 5 mg/m² that was not clearly related to the dose.

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Minor responses were reported in two patients (ACTH-producing islet cell tumor and leiomyosarcoma).

A second early trial in patients with refractory neoplasms (**T95-0077**) evaluated a biweekly schedule of depsipeptide (days 1 and 5 of a 3-week cycle) and accrued 48 patients at doses ranging from 1 to 24.9 mg/m² (Sandor *et al.* 2002). At the highest dose, DLTs were atrial fibrillation, grade 4 thrombocytopenia, and grade 4 neutropenia (one patient each) plus grade 3 profound fatigue in several patients. The investigators noted that a significant proportion of patients at all dose levels were delayed in receiving their second dose of chemotherapy (day 5) because of toxicities. Objective responses were reported in 11 patients including 1 complete response (CR) at the 12.7 mg/m² dose level (peripheral T-cell lymphoma; PTCL) and 1 partial response lasting at least 9 months at a dose of 9.1 mg/m² (renal cell cancer); partial responses were also reported in 9 patients with cutaneous T-cell lymphoma who were treated at the 17.8 mg/m² dose level (Sandor *et al.*, 2002; Piekarz *et al.*, 2001; Piekarz *et al.*, 2002).

Cardiac testing done during the depsipeptide biweekly administration trial (**T95-0077**) included an EKG at 2 hours post-infusion, 24 hours, and daily for five additional days (Sandor *et al.* 2002). Asymptomatic, transient EKG changes including ST-T wave flattening and inverted T waves were noted. These changes were usually evident by 24 hours after completion of drug infusion (not usually at the 2 hour post-infusion time point) and typically resolved before drug administration on day 5. The EKG changes in several patients were more pronounced following the day 5 infusion. One episode of atrial fibrillation was observed in a patient who was experiencing severe nausea and vomiting from the investigational drug, but there were no other symptomatic changes in patients receiving depsipeptide. One patient had a 5-beat run of asymptomatic ventricular tachycardia, and another patient had a short run of atrial bigeminy, but these episodes were not clearly drug related. No cardiac changes were noted during drug infusion, nor were elevations of cardiac enzymes (CK-MB or troponin) noted following drug administration. MUGA scans obtained pretreatment and at various time points after initiation of therapy did not identify any significant decrement in cardiac functional capacity.

Two ongoing phase 1 studies are designed to determine the *in vivo* biologic effects of depsipeptide in patients with hematologic malignancies using weekly (**27**) or biweekly (**1715**) schedules. There was no evidence of impaired myocardial contractility or cardiovascular toxicity in a preliminary study of 11 patients treated on the weekly schedule (Marcucci *et al.*, 2002; Sooraj *et al.*, 2002). Criteria for clinical responses were not met in 20 evaluable patients on this study, although one chronic lymphocytic leukemia (CLL) patient had a 46% decrease in lymphadenopathy, one had tumor lysis syndrome and seven CLL patients had improvement in peripheral lymphocyte counts. One acute myeloid leukemia (AML) patient developed tumor lysis syndrome and several patients had transient declines in peripheral blast counts. The median increase in histone acetylation of peripheral blood mononuclear cells was 40% and 100% for H3

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and H4 acetylation, respectively. One CR has been reported on the trial of biweekly depsipeptide administration (**1715**). Response data are not available from the depsipeptide/decitabine combination trial in lung cancer (**5270**) because it is too early in the accrual process.

Pharmacokinetics

Pharmacokinetic (PK) data from the advanced cancer study of weekly depsipeptide administration (**T95-0022**) indicate that steady-state plasma concentrations of the drug in the microgram/mL range are reached by the end of the 4-hour infusion and that the drug is rapidly cleared (Chassaing *et al.*, 1998). PK data from all dose levels on the biweekly administration trial (**T95-0077**) support these observations (Sandor *et al.* 2002). The area under the curve (AUC) profiles of depsipeptide generated indicate that the drug declined in a bi-exponential manner. The mean terminal $t_{1/2}$ was approximately 8 hours at the MTD of 17.8 mg/m², and the mean clearance was approximately 12 L/hr/m². The data suggest that the PK parameters were linear over the ranges investigated, and that depsipeptide is both extensively distributed and rapidly cleared from the body. In addition, it appeared that the PK parameters did not change appreciably with repeated administration. Preliminary PK data on 10 patients with CLL or AML treated on the weekly schedule (**27**) have been published (Bruner *et al.*, 2002; Marcucci *et al.*, 2002). These studies demonstrated a mean AUC of 3,252 ng/mL × hour, mean C_{max} of 749 ng/mL, and $t_{1/2}$ of 3.39 hours. Of interest, the highest values of AUC (7,269 ng/mL×hour) and concentration at steady state (C_{ss}) (1,750 ng/mL) were observed in a patient with AML who eventually developed tumor lysis syndrome (Marcucci *et al.*, 2002).

Other Clinical Trials

A phase 2 study of depsipeptide given on the biweekly schedule to 19 patients with lung cancer (16 non-small cell; 3 small cell) has been completed (**1053**); there were no objective responses. Among 26 patients in a phase 2 study in PTCL or cutaneous T-cell lymphoma (CTCL), 2 CRs (CTCL) and 2 PRs (1 CTCL; 1 PTCL) have been reported. Several other single-agent phase 2 studies are ongoing in CLL, AML, and non-Hodgkin's lymphoma. In addition, new single agent studies evaluating other schedules of administration are underway as well as phase 1 studies in pediatric patients (**ADV10212**).

Toxicities

Toxicities, expected and attributed to depsipeptide include anemia, leukopenia, neutropenia, thrombocytopenia, fatigue, anorexia, nausea, vomiting, increased SGOT/SGPT, increased CPK, asymptomatic EKG changes (ST-T wave flattening and inverted T waves, prolonged QTc interval), supraventricular arrhythmias (SVT/atrial fibrillation/flutter), hypoalbuminemia, hypocalcemia, hyperuricemia, and tumor lysis syndrome. Fatigue, leukopenia, thrombocytopenia, vomiting, cardiac arrhythmia, and

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weight loss were the DLTs reported for the two DCTD, NCI-sponsored clinical trials of depsipeptide. Depsipeptide and warfarin can cause an increased coagulable state to occur.

2.3 Rationale

Many of the pathways mentioned above are also important in ovarian cancer.

1) The Raf – Akt – PI-3K signaling cascade is important for growth and chemo-sensitivity of ovarian cancer. Although K-ras mutations do not occur frequently in epithelial ovarian and peritoneal carcinoma, activated ras is frequent (Garrett, 2000). Using immuno-histochemistry c-Raf expression was seen in 49 of 53 ovarian adenocarcinomas (McPhillips, 2001). Raf-1 kinase activity predicts paclitaxel resistance in ovarian cell lines with p53 mutations (Britten et al. 2000). Decreased resistance to paclitaxel in ovarian cancer cell lines was noted after blocking the Akt-Raf-1 pathways as well as the PI-3K pathways (Mabuchi et al. 2002). In a mouse ovarian cancer model inhibition of PI-3K leads to enhanced paclitaxel-induced apoptosis (Hu et al. 2002). Akt-2 activity is increased in 36% of ovarian epithelial tumors (Yuan et al. 2000). P38 has been implicated in the activation of VEGF in ovarian cancer cell lines, even independent of HIF-1 α (Duyndam et al. 2003).

2) Depsipeptide inhibits induction and activity of HIF-1 in response to hypoxia (Lee, 2003). Using PCR expression of HIF-1 α and VEGF was noted in 71% of ovarian cancers, independent of stage, tumor type and patient age. Expression was higher in grade III tumors. HIF-1 α may stimulate angiogenesis via VEGF (Nakayama et al. 2002). HIF-1 α however may also promote tumor growth through other pathways independent of VEGF (Ryan et al. 2000). Birner determined HIF-1 α , Bcl-2, p53 and MVD in ovarian cancer using immuno-histochemistry (Birner et al. 2001). HIF-1 α was expressed in 68% of the ovarian tumors, but was not an independent prognostic factor although patients with HIF-1 α and p53 over-expression were noted to have a significantly shorter survival.

3) Depsipeptide leads to reduced expression of mutant, but not wild type p53 as well as reduced binding of mutant p53 to Hsp90. Over-expression of p53 is seen in 47% of ovarian cancers (Sagarra et al. 2002). Immuno-histochemically detected p53 mutations were noted to have a negative prognostic impact in a meta-analysis of 6 studies (Thames et al. 2002).

Depsipeptide affects several signaling cascades important for ovarian cancer growth and therefore is likely to lead to a response in recurrent ovarian cancer as a single agent; it may also be suitable for combination therapy. An anti-angiogenic compound could be used to disrupt the blood supply to the tumor and the addition of a HIF inhibitor will then disrupt the tumors ability to adapt to hypoxia (Semenza et al. 2002). This may be an important treatment option, especially in the presence of low tumor volume. In breast cancer cells inhibition of HDACs restored active estrogen receptors (Yang et al. 2001); this mechanism could also be used in ovarian cancer, where response to hormonal therapy is low around 10% (Markman et al. 1996). Finally depsipeptide has been shown to increase the toxicity of paclitaxel in vitro and thus could render initial chemotherapy more effective.

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3. PATIENT SELECTION

3.1 Eligibility Criteria

- 3.1.1 Patients must have histologically or cytologically confirmed primary epithelial carcinoma of the ovary or peritoneum. Histologic confirmation of the recurrence is not necessary.
- 3.1.2 Patients must have measurable disease following RECIST criteria, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques including palpation, plain x-ray, CT or MRI or as ≥ 10 mm with spiral CT scan. See section 9.2 for the evaluation of measurable disease.
- 3.1.3 Patients must have had initial therapy with a platinum compound (cisplatin or carboplatin) and achieved a complete response to this therapy after a non-specified number of courses. The treatment may include conventional dose therapy, high dose therapy, consolidation therapy or extended therapy after surgical or non-surgical assessment.

Patients who have not received paclitaxel or docetaxel as the initial therapy may have had a second regimen, which includes these compounds prior to protocol entry.

Patients must be regarded platinum sensitive: treatment free interval without evidence of progression of greater than 6 months, but shorter than 12 months after completion of a platinum based regimen.

Patients may not have received any additional chemotherapy for persistent or recurrent disease, including re-treatment with platinum compound and/or taxanes.

- 3.1.4 Patients must have recovered from toxicities of previous treatments except grade 1 neuropathy.

All treatments directed against the tumor (chemotherapy, hormonal therapy, non-cytotoxic therapy) should be discontinued at least 4 weeks prior to initiation of protocol therapy. Estrogen replacement therapy can be continued.

- 3.1.5 Age >18 years. Because no dosing or adverse event data are currently available on the use of depsipeptide in patients <18 years of age, children are excluded from this study.
- 3.1.6 Life expectancy of greater than 6 months
- 3.1.7 ECOG performance status #2 (Karnofsky $\geq 60\%$; see AppendixB).

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3.1.8 Patients must have normal organ and marrow function as defined below:

- leukocytes $\geq 3,000/\mu\text{L}$
- absolute neutrophil count $\geq 1,500/\mu\text{L}$
- platelets $\geq 100,000/\mu\text{L}$
- total bilirubin within institutional normal limits
- AST (SGOT)/ALT (SGPT) ≤ 2.5 X institutional upper limit of normal
- creatinine within institutional normal limits

OR

- creatinine clearance ≥ 60 mL/min/1.73 m² for patients with creatinine levels above institutional normal

3.1.9 Patients must have a potassium level and a magnesium level within institutional normal limits.

3.1.10 Patients must be free of active infections requiring antibiotics.

3.1.11 The effects of depsipeptide on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because histone deacetylase inhibitors are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.12 Pregnant women are excluded from this study because depsipeptide is a histone deacetylase inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with depsipeptide, breastfeeding should be discontinued if the mother is treated with depsipeptide.

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

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3.2 Exclusion Criteria

- 3.2.1 Patients should not have had prior therapy with depsipeptide and may not be receiving any other investigational agents; or drugs known to have HDI activity such as sodium valproate.
- 3.2.2 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- 3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to depsipeptide.
- 3.2.4 Significant cardiac disease including congestive heart failure that meets New York Heart Association (NYHA) class III and IV definitions (see Appendix B), history of myocardial infarction within one year of study entry, uncontrolled dysrhythmias, or poorly controlled angina.
- 3.2.5 History of serious ventricular arrhythmia (VT or VF, ≥ 3 beats in a row), QTc ≥ 500 msec.
- 3.2.6 Patients may not be co-medicated with an agent that causes QTc prolongation (see Appendix D).
- 3.2.7 Uncontrolled electrolyte abnormalities (hypokalemia and hypomagnesemia)
- 3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.2.9 Pregnant women are excluded from this study because depsipeptide is a histone deacetylase inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with depsipeptide, breastfeeding should be discontinued if the mother is treated with depsipeptide.
- 3.2.10 Patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy. Therefore, HIV-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with depsipeptide.

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- 3.2.11 Patients who have received radiation to more than 25% of marrow-bearing areas.
- 3.2.12 Patients who have received non-cytotoxic therapy for recurrent disease such as monoclonal antibodies, cytokines or signal transduction inhibitors.
- 3.2.13 Patients with other invasive malignancies, with the exception of non-melanoma skin cancer, who had any evidence of cancer present during the past 5 years.

3.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial.

		Race/Ethnicity				
Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Hispanic	Asian Pacific Islander or	Unknown	Total
Male	0	0	0	0	0	0
Female	35	6	4	1	0	46
Total	35	6	4	1	0	46

3.4 Research Eligibility Evaluation

Complete medical history and history of previous treatment

Complete physical examination and pelvic examination

Chemistry panel to include:

Sodium, Potassium, Chloride, Carbon dioxide, Glucose, Total Protein,
LDH, Bun, Creatinine or Creatinine Clearance, Phosphorus, Albumin,
Calcium, Alkaline Phosphatase, Total Bilirubin

SGOT, SGPT

Mag⁺⁺

CBC, differential, platelet count

Urinalysis

β HCG for women of child-bearing potential

Serum CA-125

If Applicable, PT/INR

EKG

Baseline CT or MRI of abdomen and pelvis

Baseline chest x-ray or CT or MRI

Optional: VEGF and Tissue samples

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3.5 Patient Registration

Patients may be registered by calling the Comprehensive Cancer Center of Wake Forest University Protocol Registrar at (336) 713-6767 between 8:30 AM and 4:00 PM, Monday - Friday. Patients must be registered before initiation of treatment in order to be eligible for the study. The eligibility criteria/registration form and pre-study flow sheet should be completed prior to telephone registration and faxed to the registrar (336) 713-6772. If the patient meets all eligibility criteria and treatment must be started over a weekend or during a holiday, a maximum of 72 hours may elapse between the initiation of treatment and registration of the patient.

A Form 310 and an IRB approved Consent form must be received by the Research Base Data Management Center at the time of patient registration, or prior to patient registration.

Sites must order drug. See section 6.4 for ordering instructions.

4. TREATMENT PLAN

Study Design: This is a phase II study to determine the response rate of ovarian or peritoneal adenocarcinoma to depsipeptide. A two-stage design as proposed by Simon will be used for evaluation. For details see section 11. Laboratory correlates will be performed as described in section 7.

4.1 Depsipeptide Administration

Depsipeptide will be administered on an outpatient basis at a dose of 13 mg/m² as a 4-hour infusion on days 1, 8, and 15 of each 28-day cycle. A treatment course of therapy will be defined as 28 days.

Depsipeptide will be administered via a central venous catheter, PICC line (peripherally inserted central catheter) or peripheral IV line.

4.1.2 Prophylactic antiemetics are mandatory (see Section 4.2).

4.1.3 First treatment cycle: Patients must have a baseline EKG with a rhythm strip performed prior to administration of depsipeptide (within 1 hour), immediately following the infusion (hour 4) and within 1 hour prior to administration of depsipeptide on days 8 and 15 during the first cycle of treatment. If the EKG is normal it does not have to be reviewed immediately. In the case of abnormal EKG findings (QTc \geq 500 msec, prolongation of QTc from baseline by 33%, new ST depressions of \geq 2mm or new arrhythmia) a cardiology consultation will be obtained to determine whether further cardiac evaluation is necessary and whether treatment should be delayed or discontinued.

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Subsequent cycles:

If no alerting findings during the first cycle were noted no further monitoring is necessary. After the 1st cycle of Depsipeptide, even if dose changes occurred, cardiac monitoring will be at the treating physician's discretion.

- 4.1.4 Patients must have a potassium level ≥ 4.0 mmol/L and a magnesium level ≥ 2.0 mg/dL prior to each treatment.

Reported adverse events and potential risks are described in Section 6.

Appropriate dose modifications of depsipeptide are described in Section 5.

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

4.2 Supportive Care Guidelines

- 4.2.1 Antiemetics will be given because depsipeptide is moderately emetogenic. An 5HT3 antagonist (*e.g.* ondansetron 32mg PO and dexamethasone 8mg PO) needs to be given before each infusion of depsipeptide in combination with other anti-emetics as needed. An antiemetic (*e.g.* metoclopramide 20mg PO) will be administered after completion of the infusion and then as needed.
- 4.2.2 Magnesium and potassium levels must be checked prior to administration of depsipeptide and replaced appropriately to maintain high upper limits of normal levels.

Potassium level must be > 4.0 mmol/L before Depsipeptide can be given

Potassium supplementation guidelines

Potassium > 4.0 mmol/L	Requires no Potassium to be given
< 4.0 and > 3.5 mmol/L	40 meq. Potassium po and/or IV routes
< 3.5 mmol/L	80 meq. Potassium divided between po and IV routes. (40 meq. po and 40 meq. IV)

Magnesium level must be > 2.0 mg/dl (0.85 mmol/L) before Depsipeptide can be given

Magnesium supplementation guidelines

Magnesium > 2.0	0.85 mmol/L	Requires no Magnesium to be given
1.9	0.79 mmol/L	1 gram MgSO ₄ IV (8.12 meq.)
1.8	0.75 mmol/L	2 grams MgSO ₄ IV (16.24 meq.)
1.7	0.70 mmol/L	3 grams MgSO ₄ IV (24.36 meq.)
< 1.6	0.70 mmol/L	4 grams MgSO ₄ IV (32.48 meq.)

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- Maximum dose is 4 grams of MgSO₄ (32.48 meq.)
- Each individual institution is cautioned to follow their own guidelines for administration of these electrolytes.
- Electrolyte infusion must be completed before initiation of the Depsipeptide infusion.
- Electrolyte levels must be rechecked and adequate levels achieved prior to Depsipeptide administration.

If patient requires supplemental electrolytes, a repeat EKG needs to be done within 1 hour prior to administration of depsipeptide.

- 4.2.3 Prophylactic (oral) antibiotics and (oral) antifungals such as fluconazole are permitted in neutropenic patients (ANC <1000).
- 4.2.4 G-CSF use is allowed in patients with neutropenic fever according to ASCO guidelines (www.asco.org;))

In general, other concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed. Their use should be documented in the patient records and study specific flow sheets (this includes blood/platelet transfusions for patients with anemia and thrombocytopenia). The administration of other anti-neoplastic agents including chemotherapy, radiation therapy and biologic agents is not permitted on this study (except as described above). The use of other investigational agents is not allowed during this trial.

4.3. Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

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

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5. DOSING DELAYS/DOSE MODIFICATIONS

Hematologic, hepatic, and renal toxicities should be evaluated prior to each infusion of depsipeptide and dose modified and/or delayed according to the table below (Table 5.1 and 5.1.4). Cardiac toxicities are evaluated and the treatment modified according to Table 5.2 (below). Cardiac monitoring guidelines as described in appendix L have to be followed

Patients who develop grade 3 or 4 non-hematologic toxicity that has not resolved by the time the next treatment is due should have treatment delayed by at least 1 week. They may be re-treated at a reduced dose according to the dose de-escalation schema described below only when their toxicity has resolved to \leq grade 2. Exceptions to this will include grade 3 fatigue, nausea and asymptomatic hypocalcemia where treatment may continue after discussion with the study chair.

Table 5.1.1: **Dose Modifications Day 1** (for toxicities other than cardiac)

Adverse Event	Action	Depsipeptide Dose (mg/m²)
Hematology		
Nadir ANC \geq 1000 and PLT < 100,000	Delay treatment until ANC \geq 1500 and platelets \geq 100,000	13
Nadir ANC \leq 1000 and/or PLT \leq 50,000	Delay treatment until ANC \geq 1500 and platelets \geq 100,000	13
Febrile neutropenia (Nadir ANC \leq 1000 and temp > 38°C or 101°F) and/or PLT \leq 50,000	Delay treatment until ANC \geq 1500 and platelets \geq 100,000 Growth factors are allowed if recurrent febrile neutropenia develops after dose reduction	10
Anemia	No dose reduction; patients supported per treating physician's discretion	13
SGOT and Bilirubin		
\leq 3x ULN and \leq 1.5 x ULN	None	13
\geq 3x ULN and/or \geq 1.5 x ULN	Delay infusion until SGOT < 3 x ULN and Bilirubin < 1.5 x ULN	10
Creatinine		
\leq 1.5 x ULN	None	13
> 1.5 x ULN	Delay infusion until creatinine is < 1.5 x ULN	10
Gastrointestinal		
Nausea, grade 3	Treatment delayed until grade 1, then may continue after discussion with PI	10
Nausea and/or vomiting, grade 1 and 2	Control with adequate antiemetics	13
Nausea or vomiting with metabolic consequences	Treatment delayed until grade 1 then may continue after discussion with PI	13
Constitutional Symptoms		
Fatigue, grade 3	Treatment delayed until grade 1, may continue after discussion with PI	10
Metabolic		
Asymptomatic hypocalcemia	Treatment may continue after discussion with PI	13

5.1.2 ** Once the dose is reduced to the lower level, it is used for all subsequent depsipeptide infusions. No dose escalation and no further decrease are allowed.

5.1.3 **Treatment day 1 may be delayed for up to 2 weeks. If a longer interval is needed for resolution of toxicity the patient is removed from the study

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Table 5.1.4: **Dose Modifications Day 8 and 15** (for toxicities other than cardiac)

Blood Counts	Action	Depsipeptide Dose (mg/m ²)
Hematology		
ANC ≥ 1000 and/or PLT ≥ 75,000	None, continue treatment as planned	13
ANC ≤ 1000 and/or PLT ≤ 75,000	Omit treatment	0

5.1.4 **If a treatment on day 8 or 15 cannot be given, it will not be re-scheduled, but omitted.

Table 5.2: **Dose Modifications for Cardiac Toxicity**

Parameter / Symptoms	Change **	Action	Measurement / Continuation
Sinus tachycardia	Pulse >140/min after recumbency	Hold further dosing, consult local cardiologist and treat appropriately	<u>Not resolved</u> : Take off study
Atrial dysrhythmia (SVT, atrial fibrillation or atrial flutter)	New occurrence		
Prolongation of QTc compared to baseline	To ≥ 500 msec		<u>Resolved</u> : Restart depsipeptide at reduced dose of 10 mg/m ²
	Increase by ≥ 50 msec		
T-wave morphology***	Inversion of > 4 mm		
ST-segment***	Depression of ≥ 2 mm		
Ventricular arrhythmia (VT or VF)	≥ 3 beats in a row	Confirm with local cardiologist and treat appropriately	
Troponin I*	Above upper limit of normal value	Hold further dosing and <u>verify as soon as possible</u>	<u>Confirmed</u> : Off study <u>Not confirmed</u> : Restart depsipeptide at 13 mg/m ²
LVEF	Decrease to a value of ≤ 40%	Hold further dosing and verification by repeat MUGA	<u>Confirmed</u> (repeat MUGA): Off study <u>Not confirmed and resolved</u> : Restart depsipeptide at 13 mg/m ²
	Decrease by ≥ 25% from but not to ≤ 40%		
A subsequent episode of any of the above, despite dose reduction			Take off study

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Parameter / Symptoms	Change **	Action	Measurement / Continuation
Sinus tachycardia	Pulse >140/min after recumbency	Hold further dosing, consult local cardiologist and treat appropriately	<u>Not resolved</u> : Take off study <u>Resolved</u> : Restart depsipeptide at reduced dose of 10 mg/m ²
Atrial dysrhythmia (SVT, atrial fibrillation or atrial flutter)	New occurrence		
Prolongation of QTc compared to baseline	To ≥ 500 msec		
	Increase by ≥ 50 msec		
T-wave morphology***	Inversion of > 4 mm		
ST-segment***	Depression of ≥ 2 mm		
Ventricular arrhythmia (VT or VF)	≥ 3 beats in a row	Confirm with local cardiologist and treat appropriately	Take off study
Troponin I*	Above upper limit of normal value	Hold further dosing and <u>verify as soon as possible</u>	<u>Confirmed</u> : Off study <u>Not confirmed</u> : Restart depsipeptide at 13 mg/m ²
LVEF	Decrease to a value of ≤ 40%	Hold further dosing and verification by repeat MUGA	<u>Confirmed</u> (repeat MUGA): Off study <u>Not confirmed and resolved</u> : Restart depsipeptide at 13 mg/m ²
	Decrease by ≥ 25% from but not to ≤ 40%		
A subsequent episode of any of the above, despite dose reduction			Take off study

* If there is an alert finding on the ECG tracing after end of infusion, the subsequent dose should be reduced to 10 mg/m² even if the pre-dose ECG has returned to normal.

** In some patients, ST segment and T-wave morphology changes may recur despite a dose reduction to 10 mg/m². In such cases, further treatment should be held until the ECG changes resolve. If the patient experiences no concomitant clinical events, treatment may be resumed at the reduced dose of 10 mg/m².

Cardiac monitoring guidelines as described in appendix L have to be followed

6. PHARMACEUTICAL INFORMATION

6.1 Depsipeptide (NSC 630176)

Chemical Name: N-(3-hydroxy-7-mercapto-1-oxo-4-heptenyl)valylcysteinyl-2,3-didehydro-2-aminobutanoylvaline- γ -lactone, cyclic-1→2-disulfide; (E)(1S,4S,10S,21R)-7-[(Z)-ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo [8,7,6]-tricos-16-ene-3,6,19,22-pentanone

Other Names: FR901228, FK228

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Classification: HDAC inhibitor

Molecular Formula: C₂₅H₃₆N₄O₆S₂ **M.W.:** 541

Description: Bicyclic peptide

How Supplied: Depsipeptide is supplied by the DCTD, NCI in a dual pack with special diluent.

Active Drug: Sterile, single use vial containing 10 mg of lyophilized depsipeptide and 20 mg povidone, USP.

Special Diluent: Sterile vial containing 2 mL of a solution of 20% ethanol USP, in propylene glycol, USP.

Preparation: Withdraw 2 mL of Special Diluent from the vial; add to the depsipeptide vial. Swirl until contents of the vial are free from visible particles. This provides a 5 mg/mL solution. Dilute further with 0.9% Sodium Chloride Injection, USP to a final drug concentration in the range of 0.02 to 0.1 mg/mL. The dilute solution is compatible with both glass bottles and PVC IV infusion bags and is chemically stable for at least 24 hours when stored at room temperature.

Storage: Store the dual pack in the refrigerator (2-8°C).

Stability: Formal shelf-life surveillance of the drug dual pack is on-going. Vials of formulated drug show no loss in potency up to twelve months when stored at 50°C.

CAUTION: The single-use lyophilized dosage vial contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded 8 hours after initial entry.

Route of Administration: Intravenous.

6.2 **Reported Adverse Events and Potential Risks:**

Decreased hemoglobin, leukopenia, neutropenia, thrombocytopenia, asymptomatic ST and T wave changes, prolonged QTc interval, supraventricular arrhythmias (SVT/atrial fibrillation/flutter), cardiac ischemia/infarction, fatigue (lethargy, malaise, asthenia), anorexia, nausea, vomiting, hypoalbuminemia, infection with normal ANC or Grade 1 or 2 neutrophils, increased SGOT/SGPT, increased CPK, hyperuricemia, hypocalcemia, tumor lysis syndrome, and drug interaction between warfarin and depsipeptide may result in increased PT/INR.

Also reported on depsipeptide trials but with the relationship to depsipeptide still undetermined: hyperglycemia, fever, gastritis, diarrhea, abdominal pain, dehydration, weight loss, headache, joint pain, muscle pain, blurred vision, cataract, nail changes, constipation, taste alteration, pneumonia,

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depression, limb edema, wound infection, mucositis/stomatitis (functional/symptomatic) hypophosphatemia, hyponatremia, hypokalemia, hypomagnesemia, syncope, hypoxia, pleural effusion, hypotension, myocardial ischemia, increased cardiac troponin I, increased cardiac troponin T, ventricular tachycardia, sinus bradycardia, sudden death, confusion, increased creatinine, increased bilirubin, increased alkaline phosphatase, cranial neuropathy, sensory neuropathy, infection, injection site reaction, melena/GI bleed, hemorrhage, vaginal bleeding, hemolysis.

6.3 Availability

Depsipeptide is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Depsipeptide is provided to the NCI under a Cooperative Research and Development Agreement between Gloucester Pharmaceuticals, Inc. and the DCTD, NCI (see Section 10.4).

6.4 Agent Ordering

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designees) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that the agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). Completed Clinical Drug Requests (NIH-986) should be submitted to the PMB by fax (301) 480-4612 or mailed to the Pharmaceutical Management Branch, CTEP, DCTD, NCI, 9000 Rockville Pike, EPN Rm. 7149, Bethesda, MD 20892.

6.5 Agent Accountability

The Investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form.

<http://ctep.cancer.gov/requisition/storage.html>

7. CORRELATIVE/SPECIAL STUDIES

7.1 Depsipeptide interferes with several intracellular signaling cascades. The enzyme pattern within the tumor cell may be important for the response to therapy. We will try to identify a tumor profile predicting response to depsipeptide by evaluating relevant markers in tumor tissue removed at the original surgery or preferably when available, tumor samples removed immediately prior to initiation of treatment with depsipeptide:

- 1) Since preclinical data indicate that depsipeptide may act through inhibition of Akt signaling, HIF-1 α synthesis or VEGF syntheses and that PTEN has a modulating effect, these parameters will be measured using immuno-histochemistry in paraffin embedded blocks.
- 2) Depsipeptide has been shown in lung cancer cells to deplete mutant p53 and stabilize wild type p53. Overexpression of p53 is seen in about 60% of ovarian cancers, is related

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to poor prognosis, and may be related to the effect of depsipeptide. Immunohistochemistry will be used to evaluate the tumor for overexpression of p53.

- 3) In lung cancer cells, depsipeptide depletes ErbB1 and 2 oncoproteins. The effect of the drug is more pronounced in cells, which express high levels of ErbB. Only 17% to 36% of ovarian cancers express ErbB.
- 4) Serum VEGF levels will be measured using ELISA and will be followed as a surrogate marker for tumor response and will be determined before each treatment and at time of progression.

7.2 Handling of the specimen

*Please contact Andrea Rice at (336) 716-2573 or by email at arice@wfubmc.edu to order lab specimen collection kits prior to enrolling patients.

7.2.1 Blood samples

Collect 10cc of blood by peripheral venipuncture. (1) 4cc yellow top serum separator tube and (1) 4.5cc EDTA purple top tube. Allow to clot for 30 minutes then centrifuge at 1000g X 15 minutes. Place serum from yellow top tube and plasma from EDTA purple top tube into separate transfer vials. Both blood specimens should be labeled with indelible ink with the patient's first and last initials, date and time of specimen collection and patient protocol ID number. Completely fill out VEGF and tissue sample form and submit with specimens. Place both samples in an insulated cooler and transport at room temperature between 50-70 degrees F with the biohazard label on the front to Dr. Miller's laboratory for processing. Specimens may be stored at -20degrees for up to 3 days. If a longer period of storage is needed, both specimens should be stored at 70°C.

** CCOP/non-CCOP Instructions for Handling of Specimens. See Appendix M.**

Samples will be sent by Federal Express to Andrea Rice, Wake Forest University Health Sciences, 4th Floor Hanes Building Room 4012, Medical Center Blvd, Winston-Salem, NC 27157, phone (336) 716-2573 for storage at the laboratory of Dr. Larry Daniel, phone (336) 713-7216. Samples should be sent Monday – Friday and not to arrive on Saturday, Sunday, or a holiday. Samples will be identified only with the patient initials and the protocol ID number and will be kept locked to ensure privacy.

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7.2.2 Tissue samples

Ten tissue sections of 4mm thickness will be cut from paraffin embedded blocks and put on slides. Samples will be sent in regular slide containers by Express Mail to Andrea Rice, CCCWFU Research Base Core Lab, Wake Forest University Health Sciences, Medical Center Blvd, Hanes Building, 4th Floor, Room 4012, Winston-Salem, NC 27157, phone (336) 713-2573 for storage at the laboratory of Dr. Larry Daniel. Samples should be sent to arrive Monday – Friday and not on Saturday, Sunday, or a holiday. Samples will be identified only with the patient initials and the protocol ID number and will be kept locked to ensure privacy. Samples may be stored and sent in batches.

7.3 Correlative Studies

Background: Depsipeptide interferes with cancer cell metabolism on several different levels. As described below a variety of factors are suppressed leading to down-stream effects such as apoptosis, decreased angiogenesis and cell cycle arrest at the G0/G1 phase. It is not known, if a "biochemical" profile of the tumor can be determined, which would be related to a higher response rate to depsipeptide. The tumor most sensitive to depsipeptide may show overexpression of Akt, HIF-1, p53 and erbB-2 and have a high level of glutathione. In addition a tumor with a high content of Akt may respond better to therapy with depsipeptide in combination with cisplatin and paclitaxel. Lastly PTEN influences p53 function and inhibits the PI3-kinase/Akt pathway; as some of the effects are similar to depsipeptide a reduced response may be expected in tumors high in PTEN.

The PI3-kinase/Akt pathway may play an important role in development and progression of ovarian cancer. Akt activity is elevated in 36% to 88% of ovarian cancers (Yuan, 2000). Elevated Akt levels are related to resistance to cisplatin (Yuan, 2003) and paclitaxel (Page, 2000). Depsipeptide reduces the Akt protein content in cell lines (Fecteau, 2002) leading to apoptosis. A tumor with a high content of Akt may respond better to therapy with depsipeptide.

HIF-1 protein expression is elevated in 68% of ovarian cancers, in combination with p53 overexpression it is a poor prognostic factor (Birner, 2001). Depsipeptide inhibits the induction and activity of HIF-1 as well as angiogenesis in response to hypoxia (Lee, 2003). The effect would be greatest in a tumor with high protein expression of HIF-1.

Overexpression of p53 is observed in 54% of long-term ovarian cancer survivors and in 80% of short term survivors (Goff, 1998), it is more frequent in serous tumors, 63%, than in mucinous tumors, 22% (Morrita, 2000). In Lung cancer cell lines depsipeptide has been shown to deplete mutant p53 and stabilize wild type p53. It is not known, if p53 overexpression is related to the response to depsipeptide.

ErbB-2 overexpression is seen in 31% of epithelial ovarian cancers. Overexpression may be of prognostic importance (Meden, 1997) although this has not been confirmed by others (Goff, 1998). Depsipeptide depletes lung cancer cells of erbB contributing to inhibition of p53 transcription (Yu, 2002).

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Lastly PTEN is one of the most frequently mutated tumor suppressor genes besides p53 and these genes interact on multiple levels: for example PTEN increases the half-life of p53, on the other hand an increase in p53 protein also enhances PTEN transcription. PTEN also effectively antagonizes the PI3/Akt pathway by dephosphorylation of PIP3 (Trotman, 2003, Godberdhan, 2003). In ovarian cancer loss of PTEN expression is linked to elevated pAkt levels (Kurose, 2001). Using immuno-histochemistry to detect PTEN expression intense staining was seen in 22%, reduced staining in 51% and no staining in 25% of epithelial ovarian carcinomas. PTEN mutations are rare in ovarian cancer (Maxwell, 1998).

In conclusion we hypothesize that a tumor sensitive to depsipeptide would have high levels of Akt, HIF-1, p53, erbB-2 and low levels of PTEN. We therefore plan to evaluate tumor samples for these parameters. Patients in this protocol are treated for their initial recurrence and are still regarded as platinum sensitive. It is therefore probable, that tumor biology has not changed significantly since the initial surgery. Tumor blocks from the initial surgery, or if available from a procedure done immediately before starting treatment with depsipeptide will be available in most cases. The tissue will be paraffin embedded in the routine pathology laboratory. Detection methods will have to be confined to techniques applicable to these samples. There may be differences related to tissue preservation in different pathology laboratories, however, it is impossible to control for this in a retrospective study. On the other hand, this is the only way to try and evaluate tumor specimens as a prospective study will be impossible due to logistic difficulties. Tumor profiling will only be worth while for clinical decision making, if it can be accomplished on routinely fixated specimens. We elected immunohistochemistry as the best technique when available, as it is adequate to determine the protein level in the tumor and allow for exact correlation of the protein to tumor histology. Patients showing moderate to high staining levels will be compared to those with absent or low staining levels regarding the response to depsipeptide therapy.

In advanced ovarian cancer elevated serum levels of Vascular Endothelial growth Factor (VEGF) seem to reflect tumor progression and ascites formation (Gadducci, 2000) and may even be of prognostic importance (Chen, 1999). Evaluation as a tumor marker for ovarian cancer revealed a sensitivity of 71% and a specificity of 65% (Oehler, 1999). In cancer cell lines treatment with depsipeptide resulted in suppression of VEGF due to histone acetylation of the promoter regions. It seems, that this effect is important for the anti-tumor activity of depsipeptide (Sasakawa, 2003). Monitoring of the serum VEGF levels may correlate with the effect on angiogenesis and the response to depsipeptide.

METHOD

Immunohistochemistry

There are multiple publications about the use of immuno-histochemistry for the detection of the above mentioned parameters in the literature. The following is only a selection: Akt: Yuan, 2000, HIF-1: Birner, 2002 ; p53: Goff, 1998, Morita, 2000 ; erbB-2: Meden, 1997, Goff 1998; PTEN: Kurose, 2001. A laboratory specializing on immuno-histochemistry, director Mark

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Willingham MD, is available at Wake Forest University to perform the staining procedures. Kim Geisinger MD from the Department of Pathology, Wake Forest medical School, has experience with immuno-histochemistry for gynecologic malignancies and will supervise the staining procedures and the evaluation. Tissue evaluation will take place after all available samples are collected in as few batches as possible to reduce inter-assay variability.

For staining 4 micron sections are cut, deparaffinized and incubated in buffer. Slides are immersed in 1.5% hydrogen peroxide/methanol for 15 minutes and exposed to 1.5% normal goat serum for 30 minutes to block endogenous peroxidase and nonspecific binding sites. Immunostaining is performed using specific antibodies as stated below. The reaction is then developed using biotinylated secondary antibodies, an avidin-biotin peroxidase complex and ACE staining. One slide will be H&E stained to confirm presence of tumor.

Slide evaluation will follow routine guidelines. Slides will be evaluated qualitatively under the microscope by two independent examiners. The epithelial area with the highest staining will be selected for evaluation. Three hundred consecutive cells within the tumor will be counted. The number of cells stained will be described in four categories: 0 = staining absent, 1 = weak staining, 10% or less epithelial cells stained, 2 = moderate staining, 11%-50% of epithelial cells stained, 3 = strong staining, over 50% of cells stained. The staining intensity will be described in 4 categories: 0 = no stain, 1 = weak stain, 2 = moderate stain and 3 = intense stain.

We plan to use the following antibodies:

Parameter	Method	Antibody
Akt	Immunohistochemistry	F-7, sc-5270, Santa Cruz
HIF-1	Immunohistochemistry	28b, sc-13515, Santa Cruz
P53 overexpression	Immuno-histochemistry	DO-1, sc-126, Santa Cruz
erbB-2 overexpression	Immuno-histochemistry	9G6, sc-08, Santa Cruz
PTEN	Immuno-histochemistry	26H9, #9556, Cell Signaling

Serum VEGF

VEGF levels in serum will be determined in the laboratory of David Sane MD, Section on Cardiology, Wake Forest University Medical School, using the commercially available ELISA kit available from R&D Systems, #DVEOO.

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8. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to registration. Scans and x-rays must be done 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

		C1D1			C2D1				C3D1				Off Study (k)	
	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	
Depsipeptide (a)		X	X	X		X	X	X		X	X	X		
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X-----X												
Physical exam	X	X		X		X		X		X		X		X (k)
Pelvic exam	X					X				X				X (k)
Vital signs	X	X		X		X		X		X		X		X(k)
Height	X													
Weight	X	X		X		X		X		X		X		X(k)
Performance status	X	X		X		X		X		X		X		X(k)
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X	X	X	X	X(k)
Serum chemistry (b), (c)	X	X	X	X	X	X	X	X	X	X	X	X	X	X(k)
Serum Creatinine or Creatinine clearance (c)	X	X	X	X	X	X	X	X	X	X	X	X	X	X(k)
PT/INR (h)	X	X	X	X	X	X	X	X	X	X	X	X	X	X(k)
Urinalysis	X													
EKG (as indicated) (i)	X	X (j)	X	X										
Adverse event evaluation		X-----X												X (k)
Tumor measurements Palpable lesions	X	Tumor measurements are repeated every <u>4</u> weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X (k)
Radiologic evaluation CXR, CT, MRI	X	Radiologic measurements should be performed every <u>8</u> weeks.												X (e) (k)
β-HCG (d)	X (e)													
Serum Ca125	X					X				X				X (k)
VEGF (f) (optional)	X									X				
Tissue samples (g) (optional)	X													

a: Depsipeptide: Dose as assigned; as a 4-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle.
b: Sodium, potassium, chloride, carbon dioxide, glucose, total protein, LDH, Bun, creatinine or creatinine clearance, phosphorus, albumin, calcium, alkaline phosphorus total bilirubin. (Must include Mag++ level)
c: Serum Creatinine within institutional normal limits or Creatinine clearance ≥ 60 ml/min/1.73 m² for patients with Creatinine levels above institutional normal.
d: Serum pregnancy test (women of childbearing potential).
e: Off-study evaluation. **Two consecutive measurements taken 4 weeks apart must be used to document progressive disease if the patient is removed from study for this reason.**
f: VEGF levels will be obtained pre-study and day 1 of every other course, i.e., prior to 3rd cycle, prior to 5th cycle, until patients has progression. See Section 7.0 for further details of Correlative/Special Studies.
g. Tissue samples (optional) should be from original path or biopsy from recent procedure. See section 7.0 for further details of correlative/special studies.
h. (If applicable). Patient on warfarin sodium due to possible drug interaction with depsipeptide.
i. EKG within 1 hour prior to administration of depsipeptide and immediately after treatment on C1D1. EKG within 1 hour prior to administration of depsipeptide on C1D8 and C1D15. (Additional EKG's per Toxicity Guidelines)
j. Pre- and post-study
k. Every 2 months for 1 year, then every 3 months for another year, then every 6 months until 5 years are completed.

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9. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be reevaluated for response every 4 weeks. In addition to a baseline scan, confirmatory scans should also be obtained every 8 weeks following initial documentation of objective response.

9.1 Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. 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.

9.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques (CT, MRI, x-ray, as well as superficial lesions noted on physical exam, which are measurable by photography or with the use of a ruler) or as ≥ 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.2 Non-measurable disease

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

9.1.3 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 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.

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9.1.4 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.

9.2 Guidelines for Evaluation of Measurable Disease

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

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

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.

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 or subcutaneous lesions. 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 and therefore they will not be used to assess tumor response in this trial. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

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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. Specific additional criteria for standardized usage of CA-125 response in support of clinical trials are being developed.

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

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

9.3 **Response Criteria**

9.3.1 **Evaluation of target lesions**

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

9.3.2 **Evaluation of non-target lesions**

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

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Although a clear progression of “non-target” lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair).

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

9.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1 and 9.4.1.).

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

Note:

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

9.4 Confirmatory Measurement/Duration of Response

9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4 weeks after the criteria

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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 8 weeks (see section 9.3.3).

9.4.2 Duration of overall response

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

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

9.4.3 Duration of Stable Disease

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

9.5 Progression-Free Survival

Progression free survival (PFS) and survival will be secondary clinical outcome measures. PFS is defined as the time from the date of first treatment until the date of progression. Survival is defined as the time from first treatment until death or the last date of contact.

9.6 Response Review

All radiological images will be reviewed by Dr. Robert Bechtold, Department of Radiology, 2nd Floor Meads Hall, Medical Center Boulevard, Winston-Salem, NC 27157 (336) 716-2471.

Dr. Bechtold is a radiologist at Wake Forest University Medical School, who has extensive experience in evaluations using RECIST criteria.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

list Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following of AEs (Section 10.1) and the characteristics of an observed AE (Section 10.2) will determine whether the event requires expedited (via ADEERS) reprint **in addition** to routine (via CTMS or CDUS) reporting.

10.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system.

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In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (the ASAEL) contains events that are considered ‘expected’ for expedited reporting purposes only. Refer to the “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” (<http://ctep.cancer.gov/reporting/adeers.html>) for further clarification. The CAEPR does not provide frequency data; refer to the Investigator’s Brochure for this information.

The Comprehensive Adverse Event and Potential Risks List:

Decreased hemoglobin, leukopenia, neutropenia, thrombocytopenia, asymptomatic ST and T wave changes, prolonged QTc interval, supraventricular arrhythmias (SVT/atrial fibrillation/flutter), cardiac ischemia/infarction, fatigue (lethargy, malaise, asthenia), anorexia, nausea, vomiting, hypoalbuminemia, infection with normal ANC or Grade 1 or 2 neutrophils, increased SGOT/SGPT, increased CPK, hyperuricemia, hypocalcemia, tumor lysis syndrome, and drug interaction between warfarin and depsipeptide may result in increased PT/INR.

Also reported on depsipeptide trials but with the relationship to depsipeptide still undetermined: hyperglycemia, fever, gastritis, diarrhea, abdominal pain, dehydration, weight loss, headache, joint pain, muscle pain, blurred vision, cataract, nail changes, constipation, taste alteration, pneumonia, depression, limb edema, wound infection, mucositis/stomatitis (functional/symptomatic) hypophosphatemia, hyponatremia, hypokalemia, hypomagnesemia, syncope, hypoxia, pleural effusion, hypotension, myocardial ischemia, increased cardiac troponin I, increased cardiac troponin T, ventricular tachycardia, sinus bradycardia, sudden death, confusion, increased creatinine, increased bilirubin, increased alkaline phosphatase, cranial neuropathy, sensory neuropathy, infection, injection site reaction, melena/GI bleed, hemorrhage, vaginal bleeding, hemolysis.

10.1.1 The Agent Specific Adverse Event List

Neutrophils, leukocytes, platelets, hemoglobin, supraventricular and nodal arrhythmia, prolonged QTc interval, fatigue (lethargy, malaise, asthenia), nausea, vomiting, anorexia, SGOT, SGPT, CPK, hypoalbuminemia, hypocalcemia, hyperuricemia, tumor lysis syndrome.

10.2 **Adverse Event Characteristics**

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov>).

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- **“Expectedness”**: AEs can be ‘Unexpected’ or ‘Expected’ (*see section 10.1 above*) for expedited reporting purposes only. ‘Expected’ AEs (the ASAE) are ***bold and italicized*** in the CAEPR (Section 10.1.1)
- **Attribution** of the AE:
 - Definite: The AE *is clearly related* to the study treatment.
 - Probable: The AE *is likely related* to the study treatment.
 - Possible: The AE *may be related* to the study treatment.
 - Unlikely: The AE *is doubtfully related* to the study treatment.
 - Unrelated: The AE *is clearly NOT related* to the study treatment.

10.3 Expedited Adverse Event Reporting

- 10.3.1 Expedited AE reporting for this study must use AdEERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page (<http://ctep.cancer.gov>). The reporting procedures to be followed are presented in the “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” which can be downloaded from the CTEP home page (<http://ctep.cancer.gov>). These requirements are briefly outlined in the table below (Section 10.3.3)

In the rare occurrence when Internet connectivity is lost, an AE report may be submitted using CTEP’s Adverse Event Expedited Report-Single Agent or Multiple Agent paper template (available at <http://ctep.cancer.gov>) and faxed to 301-230-0159. A 24-hour notification is to be made to CTEP by telephone at 301-897-7497, only when Internet connectivity is disrupted. Once Internet connectivity is restored, an AE report submitted on a paper template or a 24-hour notification phoned in must be entered electronically into AdEERS by the original submitter at the site.

- 10.3.2 AdEERS is programmed for automatic electronic distribution of reports to the following individuals: Data Management Coordinator of CCCWFU CCOP Research Base, Principal Investigator, and the local treating physician. AdEERS provides a copy feature for other e-mail recipients.
- 10.3.3 **Expedited Reporting Guidelines** – AdEERS Reporting Requirements for Adverse Events that occur within 30 days¹ of the last dose of the Investigational Agent on Phase 2 and 3 trials.

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

¹ Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:
 AdEERS 24-hour notification followed by complete report within 5 calendar days for:
 • Grade 4 and Grade 5 unexpected events
 AdEERS 10 calendar day report:
 • Grade 3 unexpected events with hospitalization or prolongation of hospitalization
 • Grade 5 expected events

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

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

- Expedited AE reporting timelines defined:
 - “24 hours; 5 calendar days” – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
 - “10 calendar days” - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

10.3.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

- For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting

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Guidelines and do not require expedited reporting (i.e., AdEERS). The following AEs must be reported through the routine reporting mechanism (*Section 10.4*):

CTCAE Category	Adverse Event***	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Bone/Bone Marrow	Neutrophils, Leukocytes, Platelets, Hemoglobin	1-4	Grades 1-4 excluded	Related Unrelated	
Cardiac Arrhythmia	Supraventricular and nodal arrhythmia, Prolonged QTc Interval ¹	1-4	No exclusions	Related Unrelated	Asymptomatic ST and T wave changes
Constitutional	Fatigue (Lethargy, Malaise, Asthenia)	1-4	Grades 1-3 excluded	Related Unrelated	
Gastrointestinal	Nausea, Vomiting, Anorexia	1-4	Grades 1-4 excluded	Related Unrelated	
Infection		1-4	Grades 1-3 excluded	Related Unrelated	
Metabolic/Laboratory	SGOT, SGPT, CPK, Hypoalbuminemia, Hypocalcemia, Hyperuricemia	1-4	Grades 1-3	Related Unrelated	Must report all Grade 3-4 bilirubin
Syndromes	Tumor Lysis Syndrome	3	Grade 3 excluded	Related Unrelated	
*** Reporting requirements of events that occur as a result of an excluded AE listed above are the same as for the listed AE, e.g., grade 3 dehydration resulting from grade 3 vomiting need not be reported through AdEERS.					
¹ From dose modification table.					

10.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine (CTMS or CDUS) study data submissions. **AEs reported through AdEERS must also be reported in routine study data submissions.**

All Adverse events should be reported to the Comprehensive Cancer Center Research Base Data Management Center of Wake Forest University, Medical Center Blvd, Winston-Salem NC, 27157; phone (336) 713-3172, fax (336) 713-6476. The Data Management staff will follow the mandatory CROC SAE Notification Procedure (Revised 05/04/04).

10.4.1 Data Reporting

10.4.1.1 This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. The CCCWFU CCOP Research Base Data Management Center will submit cumulative CDUS data quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP web site (<http://ctep.cancer.gov/reporting/cdus.html>).

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10.4.1.2 The registration forms will be faxed to the Comprehensive Cancer Center of Wake Forest University CCOP Research Base Protocol Registrar at 336-713-6767 between 8:30 AM and 4:00 PM, Monday - Friday at fax 336-713-6772, phone 336-716-4536. (See Section 3.5 Patient Registration)

10.4.1.3 Data Management Forms. The Treatment Form (Appendix F), the Toxicity Form (Appendix G), and the Tumor Evaluation Form (Appendix H) will be submitted within one month after each cycle or evaluation. The follow-up form will be submitted within 2 weeks after the examination. All forms will be sent to Rhonda Kimball, Department of Radiation Oncology, Wake Forest University Health Sciences, Medical Center Blvd. Winston-Salem, NC 27157 fax number (336) 713-6565 .

10.4.1.4 Submission for Data Management Forms

Data Forms

Submission Schedule

Eligibility Criteria/	
Registration Form	Within two weeks of registration
Addenda Sheet (as applicable)	Within two weeks of registration
Treatment Form	Within 1 month after each treatment or evaluation
Toxicity Reporting	Within 1 month after each treatment or evaluation
Tumor Evaluation	Within 1 month after each treatment or evaluation
Treatment Completion	Within 1 month after completion of treatment
Off Treatment follow-up form	Within two weeks after follow-up exams

10.5 Secondary AML/MDS

Investigators are required to report cases of secondary AML/MDS occurring on or following treatment on NCI-sponsored chemotherapy protocols using the NCI/CTEP Secondary AML/MDS Report Form. This form can be downloaded from the CTEP web site (<http://ctep.cancer.gov>). Refer to the “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” (available at <http://ctep.cancer.gov>) for additional information about secondary AML/MDS reporting.

10.6 CTEP Multicenter Guidelines

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, the following guidelines must be followed.

Responsibility of the Protocol Chair

The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the

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protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.

The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.

The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.

The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The CCCWFU CCOP Research Base Data Management Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.

Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO and CCCWFU CCOP Research Base Data Management Center.

The CCCWFU CCOP Research Base Data Management is responsible for central patient registration. The CCCWFU CCOP Research Base Data Management Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.

The CCCWFU CCOP Research Base Data Management Center is responsible for the preparation of all submitted data for review by the Protocol Chair.

The CCCWFU CCOP Research Base Data Management Center will maintain documentation of AE reports. Participating institutions may report directly to CTEP with a copy to the Coordinating Center. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.

Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the CCCWFU CCOP Research Base Data Management Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

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Agent Ordering

Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

10.7 Cooperative Research and Development Agreement (CRADA)

The agent(s), supplied by CTEP, DCTD, NCI, used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA) between Gloucester Pharmaceuticals, Inc. [hereinafter referred to as ACollaborator(s)@] and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the AIntellectual Property Option to Collaborator@ contained within the terms of award, apply to the use of Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient participating on the study or patient's family member, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from <http://ctep.cancer.gov>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):
 - a. NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
 - b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.
 - c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use

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the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

3. Clinical Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
6130 Executive Boulevard, Suite 7111
Rockville, MD 20852
FAX 301-402-1584
E-mail: anshers@ctep.nci.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

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This study is designed to assess the effectiveness of depsipeptide for women with platinum sensitive recurrent ovarian cancer. 'Activity' will be quantitated using tumor response, which will be estimated as the proportion of patients with complete or partial reduction in tumor burden. Time to progression, survival and toxicity will be secondary clinical outcome measures. Secondary correlative endpoints are described below. Time to progression is defined as the time from first treatment until the date of progression. Survival is defined as the time from first treatment until death or the last date of contact. Toxicity will be assessed throughout the study using the CTCAE version 3.0. The secondary outcomes will be evaluated in all patients, whether or not they are evaluable for response.

The optimal two-stage phase II design proposed by Simon (1989) will be used to test the null hypothesis $H_0: r \leq .3$ versus the alternative hypothesis $H_1: r > .3$ with a type I error of 10% and a power of 90% or more for alternative response rates of 50% or more. A maximum sample size of 46 evaluable patients will be needed; 22 of these will be accrued during the first stage, and, if necessary, the remaining 24 during the second stage. If seven or fewer of the first 22 evaluable patients respond to therapy, the study will be terminated and the null hypothesis 'accepted'. However, if 8 or more respond, the additional 24 patients will be accrued. If 17 or fewer of the 46 evaluable patients respond, the null hypothesis will be 'accepted'. Otherwise, the null hypothesis will be rejected, and the study regimen will be accepted as active therapy. If the true response rate is 30%, the actual sample size for the study will be 22 with probability .67 and 46 with probability .33. If the true response rate is 50%, the actual sample size for the study will be 22 with probability .07 and 46 with probability .93.

Descriptive statistics (means, standard deviations, frequencies, etc.) will be presented for pretreatment patient characteristics and the outcome measures mentioned. Each of the outcomes mentioned above will be analyzed and reported separately. Chi-squared and Wilcoxon rank-sum tests will be used to assess the univariate associations of patient characteristics with response. The proportion of responders will be compared to the cut points described above during interim and final analyses. Logistic regression will be used to determine which covariates are jointly predictive of response. Kaplan-Meier methods will be used to estimate the time to progression and survival distributions. Logrank tests will be used to assess which covariates are univariately predictive of these outcomes, and Cox's proportional hazards regression model will be used to assess the joint effects of the various covariates. In addition, the frequency and severity of all side effects and toxicities will be tabulated and analyzed using categorical techniques when the sample size warrants.

11.2 **Sample Size/Accrual Rate**

Assuming that 10% of the patients will not be evaluable for response (i.e., they fail to have a necessary repeat evaluation, they expire of other causes prior to repeat evaluation, etc.), we will need a maximum of 51 patients. At Wake Forest University Comprehensive Cancer Center 10 patients were enlisted on protocols for this patient population in 2002. At Piedmont Hematology Oncology Associates, who participate in Wake Forest Comprehensive Cancer Center trials about 20 patients with this disease status are seen every year, about 50% of who would be eligible and willing to participate. In addition the CCCWFU CCOP Research Base

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includes 28 Community Cancer Centers. Thus the first component of the study could be completed in less than a year; the entire study should be completed in less than 2 years

11.3 Stratification Factors

No patient stratification factors will be included.

11.4 Analysis of Secondary Endpoints

Akt signaling, PTEN, HIF-1 α , ErbB1, and p53, will be measured from tumor tissue at baseline, and serum VEGF and CA 125 levels will be measured before each treatment and at time of progression. Logistic regression will be used to assess the association between these measures and tumor response. It is likely that there will be too few responses to support complex multivariate models. However, if the number of response warrant, smaller multivariate models consisting of subsets of the correlative measures will be evaluated. Cox's proportional hazards regression models will be used to assess the univariate and joint (again probably limited to subsets of the covariates) effects of the correlative measures on time to progression and survival. Mixed models analysis of covariance will be used to model the longitudinal serum measures of VEGF and CA 125, and to assess the effect of the patient characteristics and correlative measures on these parameters.

11.5 Reporting and Exclusions

11.5.1 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with depsipeptide.

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

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

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these

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subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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