THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Randomized Phase II Double Blind Study of VPA vs Placebo to Shorten Time of Indwelling Pleural Catheter
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Core Protocol Information

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Which Committee will review this protocol?

◉ The Clinical Research Committee - (CRC)

Protocol Body

1.0 Background

1.0 Introduction

The intent of this protocol is to reduce the burden of pleural effusion in patients with metastatic breast cancer such that the time of indwelling catheter can be reduced. The hypothesis is that pleural effusion tumor burden is dictated by the amplification and expansion of progenitor cells that could be inhibited by pushing the stem/progenitor equilibrium back towards a more quiescent cancer stem cell using the histone deacetylase (HDAC) inhibitor, valproic acid (VPA). Patients who could benefit from an indwelling catheter based on MDACC algorithms for placement will be randomized to 10 weeks of valproic acid or placebo and a pleural catheter placed. Time to catheter removal based on MDACC algorithms will be the primary endpoint. Serial fluid samples will be examined to validate the translational hypothesis and ensure HDAC inhibition occurs.

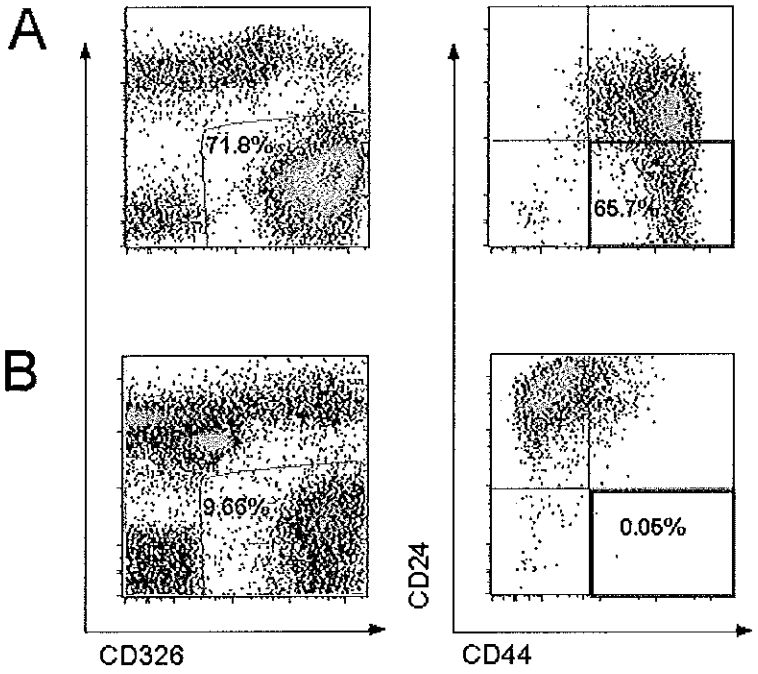
1.1 Background and Significance

Recurrent malignant pleural effusion (MPE) is a debilitating condition, which is associated with a significant morbidity and worsening of quality of life (QOL). Overall median survival of patients with MPE is poor and it changes slightly by histology: breast cancer, 7.4 months; non-small cell lung cancer, 4.3 months; and ovarian cancer, 9.4 months (1). Therapy of MPE is symptomatic and palliative, and consists of mechanical evacuation of the effusion to relieve pulmonary compression and dyspnea. There are no well-established methods of therapy, and the techniques used vary. They include repeated thoracentesis with small-bore catheters, tube thoracostomy, or video-assisted thoracic surgery for optimal drainage. Pleurodesis may be induced chemically by talc, tetracycline, doxycycline, bleomycin, or instillation of talc slurry(1). Patient selection is required since these techniques may be associated with complications (2). Several studies examined the utility of intrapleural drug administration for management of MPE. In a randomized trial, methylprednisolone did not delay reaccumulation of symptomatic pleural effusion compared to placebo (3). Immunotherapy with OK-432 had been shown to be feasible and active for MPE in a small-randomized trial (4). Recently a chronic indwelling pleural catheter has been introduced for the therapy of MPE. A randomized study examined its safety and efficacy versus tube thoracostomy and doxycycline sclerosis(5). Outpatient treatment with indwelling pleural catheter was found to be safe and equivalent in efficacy to treatment with tube thoracostomy(5). In addition, it was associated with fewer hospitalization days and was less expensive than inpatient methods(6). Our common practice in recent years had been placement of the indwelling pleural catheter in all patients who had reaccumulation of symptomatic malignant pleural effusion.

Tumor-Initiating Cells (TICs) in MPE

The cancer stem cell hypothesis purports that to eliminate cancer one must eliminate the small population of cancer stem cells that are self-renewing and capable of differentiating into the multiple lineages of the bulk tumor. In 2003 Al-Hajj and colleagues prospectively determined markers from pleural effusion-derived (metastatic) breast cancer cells that identified the subpopulation of cells that could selectively regenerate tumors when transplanted into the mammary fatpads of non-obese diabetic/severe immunocompromised mice (7). These pleural effusion-derived tumor-initiating cells (TICs, a cancer stem cell surrogate) were lineage marker-negative epithelial cells that expressed CD44+CD24-/lo cell surface markers on flow cytometry. Mixed clinical significance of these cells has been reported from analyses of primary

tissue where immunohistochemistry has been used to assess expression of CD44 and CD24 (8). In primary tumors, CD44+CD24-/lo cells were found in 69% of tumors: 100% of basal tumors and 52% of Her-2/neu overexpressing tumors (9). This phenotype would be predicted to correlate with poor outcome given the worse clinical outcomes associated with basal tumors (10, 11). Indeed, gene signatures derived from TICs correspond to the most undifferentiated breast cancers (12) and predict for worse disease free survival (13). Conversely however, Mylona et al report less lymph node positive disease among patients with significant CD44+CD24-/lo populations and a trend towards improved disease-free survival (14). This raises the question of whether this phenotype is simply not a robust, meaningful clinical marker, whether differences in the clinical cohorts examined account for the conflicting reports, or whether the markers for tumor-initiation in metastases such as pleural fluid cells may be different from those in primary disease.



Some data indicate that circulating tumor cells (15), and disseminated bone marrow cells (16), have CD44+CD24-/lo profiles, which is consistent with the hypothesis that these cells are responsible for tumor-initiation or metastasis. However, the published studies that support this hypothesis have examined relatively small samples and were not intended to report outcome.

In an IRB-approved laboratory study we cultured pleural effusion cells as spheres in tumor-initiating culture conditions (17, 18) and completed fresh flow cytometry analysis of the percentage of CD44+CD24-/lo epithelial cells (detected as CD45-CD326+ CD44+CD24-/lo cells) from 29 unselected patients with metastatic breast cancer who underwent pleurocentesis for symptomatic pleural effusion (Figure 1). In this exploratory analysis, we retrospectively examined the association between TICs and overall survival from the time of pleurocentesis and report that patients with higher percentages of TICs in the pleural fluid had significantly shorter overall survival than those with fewer TICs.

We analyzed fresh MPE samples from 29 pts with breast cancer for epithelial cells with a tumor-initiating cell phenotype (TIC, CD45-CD326+ CD44+CD24-/lo): CD45- (leukocyte

marker), CD326+ (also known as EpCAM an epithelial marker), and CD44+ and CD24-/lo (tumor-initiating markers), using multicolor flow cytometry on an IRB-approved protocol. Overall survival (OS) was measured from time of MPE assessment. Univariate analyses of survival were performed using Cox proportional hazards (CPH), with 1-year levels computed using the method of Kaplan-Meier. Multivariate analyses were performed using the CPH model. Median follow-up was 4.1 months, range 0.2 to 28.9. Eighteen patients have died of disease. Median percentage of CD45-CD326+ cells was 1.24% which contained a median TIC component of 9.5%. On univariate analysis, higher level of %TICs were significantly associated with differences in overall survival (Figure 2, P = 0.005). Actuarial 1-yr OS was 24% vs. 77% for patients with %TIC above (TIChi) and below (TIClo) the median (P = 0.016). Increasing percentage of TICs remained a significant predictor for worse OS on multivariate analysis (hazard ratio 1.25, confidence interval 1.06-1.49, P = 0.007).
An increased percentage of TICs predicted for decreased OS among patients with MPE consistent with the hypothesis that these cells mediate recurrence and death from breast cancer.

While these data support the cancer stem cell hypothesis suggesting that TICs are associated with poor outcome, accurate identification of TICs as a true cancer stem cells versus a progenitor undergoing transient amplification, a well described phenomenon in normal stem cell biology greatly impacts the interpretation and incorporation in rational clinical trial design. If indeed the TICs represent true cancer stem cells, it implies that in many of these patients the cancer stem cell population is not rare but abundant, and that treatment with a true cancer stem cell targeted agent will eliminate all TICS. Conversely, if TICs represent transiently amplifying cells, an expanded population of progenitor cells that bear the same markers and are capable of vast proliferation, multi-lineage differentiation but not self-renewal (a cancer stem cell hallmark), true targeted cancer stem cell agents may only target a small percentage of these metastatic cells, while the destructive progenitor cells continue to mediate outcome in this setting. **We hypothesize that TICs in pleural effusions are transiently amplifying progenitor cells and that a novel way to target these cells is to reverse the transient amplification, driving the cells back towards the quiescent stem cell disequilibrium using a safe relatively non-toxic drug in conjunction with standard anti-proliferative chemotherapy to target the differentiated cells.** Maintenance in this state may prevent MPE recurrence and improve overall survival. Subsequent treatment using sequential cancer stem cell agents would be expected to eradicate the cancer stem cell population herded into this disequlibrium state through dedifferentiation.

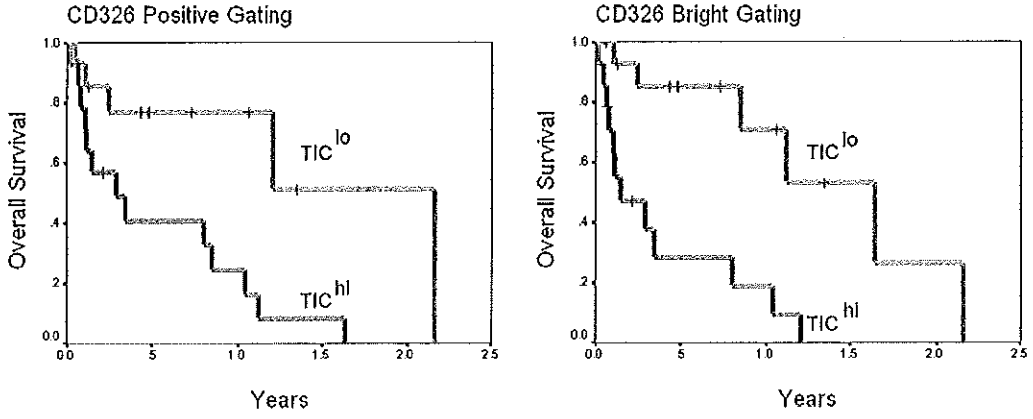


Figure 2. Kaplan Meier curves demonstrate a reduced overall survival is associated with an increased percentage of TICs regardless of gating strategy. %TIC assessed by gating $CD44^{+}CD24^{-/lo}$ on all $CD45^{-}CD326^{+}$ cells (left), and only the brightest $CD45^{-}CD326^{+}$ cells (right).

VPA is an HDAC inhibitor that dedifferentiates cancer progenitor cells

VPA is an established drug for the treatment of epileptic seizures, mania in bipolar disorder which has been reported to enhance proliferation and self-renewal of normal hematopoietic stem cells (HSCs) (19, 20). It has been suggested that differentiated cancer cells can *de* -differentiate into the cancer stem cell phenotype (Meyers et al., 2009; Gupta et al., 2009) and that Histone Deacetylase (HDAC) inhibitors, in particular VPA, enable efficient induction of pluripotent stem cells from adult fibroblasts (Huangfu et al., 2009). The finding that VPA was an effective inhibitor of HDACs surged from the observations that VPA was able to relieve transcriptional repression of a peroxisomal proliferation and activation of a glucocorticoid receptor (GR)–PPARϵ hybrid receptor and a RAR-dependent reporter gene expression system, suggesting that it acts on a common factor in gene regulation such as corepressor-associated HDACs rather than on individual transcription factors or receptors.

We hypothesized that HDAC inhibitors might increase the breast cancer stem cell population via dedifferentiation of differentiated cancer cells. To examine this hypothesis, triple negative, basal subtype Sum159 breast cancer cells were FACS-sorted based on stem cell surrogate, ALDH activity and subsequently treated with one of two HDAC inhibitors, VPA or SAHA (suberoylanilide hydroxamic acid). After sorting, ALDH-negative cells were treated either with VPA, SAHA, or vehicle. After a week, the percentage of ALDH-positive cells (passage 0, P0) was examined with flow cytometry while the remaining cells were passaged and incubated with and without VPA or SAHA for a week and the percentage of ALDH-positive cells again evaluated (P1). This was repeated for the third time (P2). Samples from each generation were also collected to examine the protein expression. On average, a 3-fold increase in ALDH positive cells was seen in VPA-treated cells (35.6% vs.12.6%) and a 1.5-fold increase in SAHA-treated cells (41% vs. 28%) compared to vehicle-treated controls. This effect was maintained through multiple passages. Moreover, the expression of β-catenin and EMT associated genes like vimentin, fibronectin, n-cadherin, which have been implicated in generating cancer stem cells was significantly increased with treatment in initial and passaged cells. Further functional endpoint studies are ongoing to validate these in vitro marker-based findings. Overall these data suggest that VPA has a differential effect on cancer stem cells and non-cancer stem cells, and that this effect may shift the equilibrium between stem cells, transient amplifying cells, and differentiated cells towards the stem cell state. Consistent with the hypothesis that this may be beneficial in the clinical setting if well understood, preclinical and clinical data demonstrate an anti-tumor effect in breast cancer.

Anti-tumor and treatment synergy effects of VPA

In recent studies using standard tissue culture techniques which arguable represent bulk, differentiated cancer cells(21), VA was found to have potent anti-tumor effects in breast, colon and prostate cancer cells (22-24). The anti-tumor activity of VPA was reported to be related to its ability to inhibit histone deacetylase 1 (HDAC1) (25-27). The effect of VPA in the

radiosensitization of cancer cells was investigated using adherent cultures of human glioma cell lines and in vivo tumor growth delay assays (28, 29). In those studies, VPA was shown to significantly enhance the in vitro and in vivo sensitivity of the cancer cells to radiation treatment. It can be argued that these in vitro assays use differentiation promoting adherent, serum containing culture conditions, and that tumor growth delay is not a measure of stem cell survival. Therefore, although VPA has been described as a radiosensitizer in standard, but non-stem cell assays (28-30), its effect on cancer stem cell radiosensitization was unknown. **A correlate to the hypothesis that VPA has a differential effect on stem and non-stem cells is that the effect of therapy on VPA treated cells would be different for cancer stem cells and non-cancer stem cells.**

To test this hypothesis, we used the MCF7 breast cancer cell line to examine the effect of pretreatment with VPA on radiation sensitivity of cells grown under stem cell promoting culture conditions (3D, mammosphere) and standard non-stem cell monolayer culture conditions (2D). We used the MCF7 breast cancer cell line grown under stem cell promoting culture conditions (3D, mammosphere) and standard non-stem cell monolayer culture conditions (2D) to examine the effect of pretreatment with VPA on radiation sensitivity in clonogenic survival assays and on the expression of embryonic stem cell transcription factors. 3D cultured MCF-7 cells express higher levels of Oct4, Nanog and Sox2. 3D passage enriched self-renewal and increased radioresistance in 3D mammosphere formation assays. VPA radiosensitized adherent cells but not 3D cells in single fraction clonogenic assays. Moreover, fractionated radiation sensitized VPA -treated adherent MCF7 cells, but did not have a significant effect in VPA -treated single cells grown to mammospheres. We conclude that VPA radiosensitizes differentiated cells over those expressing stem cell surrogates. Similar chemotherapy based studies using assays of differentiated cells have also shown synergy between VPA and systemic therapy agents in the treatment of breast cancer and suggest that in addition to shifting the progenitor equilibrium towards cancer stem cells, this drug has synergistic anti-tumor activity against differentiated cells.

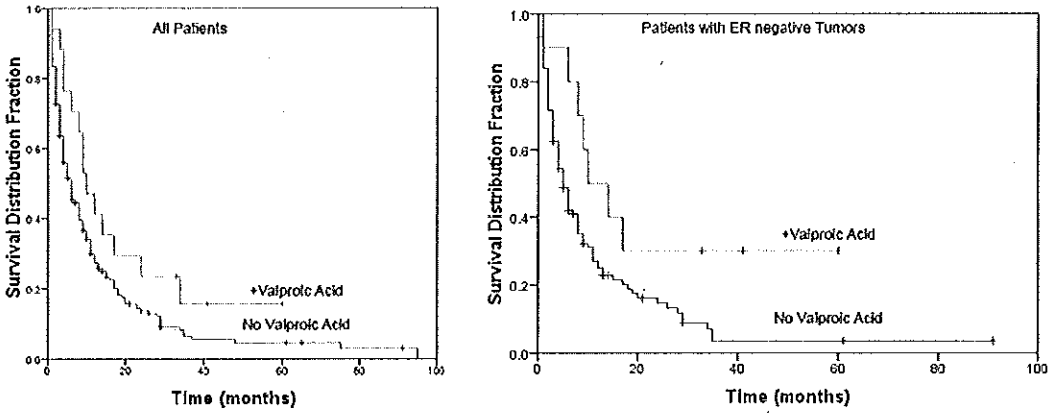
To that end, numerous preclinical studies have shown that VPA has anti-tumor activity against multiple tumor types cultured in standard non-stem cell conditions(23, 31-33). In breast cancer, VPA strongly reduces the growth of MCF-7 breast cancer cell line at a concentration of 0.23 mM, effect that seems independent of the estrogen receptor(23). VPA, in combination with retinoic acid and a DNA methyltransferase inhibitor leads to reactivation of the silenced tumor suppressor gene, *RARβ2*, in human breast cancer cells and also inhibits the proliferation of receptor positive and negative MCF-7 and MDA-231 cells either alone or in combination with retinoic acid and a DNA methyltransferase inhibitor(33) Interestingly, VPA potentiates the antiproliferative actions of tamoxifen, raloxifen, fulvestran and letrozol in breast cancer cell lines MCF-7, T47-D, and ZR-75-1 expressing the estrogen receptor alpha. The enhanced antiestrogen action is via apoptosis at least partially as a result of bik up-regulation. In addition, VPA increases the antitumor effect of tamoxifen in MCF-7 cells transfected with HER2. Unexpectedly, Ishikawa adenocarcinoma cells, which proliferate in response to tamoxifen are dramatically inhibited in their proliferation by VPA but it reverses the E2-like agonist activity of tamoxifen in these cells(31). In an *in vivo* model with MCF-7 xenografts, VPA at concentrations sufficient for histone hyperacetylation, produces down-regulation of heterochromatin maintenance proteins, chromatin decondensation and potently induces tumor regression when administered 48 h before a single dose of epirubicin(32).

Clinical data examining VPA in breast cancer patients

Interpreting the above data in the framework of the cancer stem cell hypothesis implies that VPA would worsen patient outcome by protecting breast cancer stem cells. Interestingly, the existing clinical data support our central hypothesis that TICs in pleural effusions can be targeted for improved outcome with VPA in spite the paradoxical effect increasing cancer stem cells in culture. Understanding this apparent paradox may contribute to optimally incorporating the cancer stem cell hypothesis into cancer therapy or of equal importance, possibly disproving the cancer stem cell hypothesis.

In a phase II clinical trial of VPA and the breast cancer regimen FEC100 (5-fluorouracil, epirubicin, cyclophosphamide 7/8 breast cancer patients had stable disease of partial response(34). Cell culture and xenograft studies suggest a synergistic interaction between histone deacetylase inhibitors (HDACi) and topoisomerase (topo) inhibitors, as well as other DNA targeting agents. In this phase I/II study, Munster et al determined the effects of escalating doses of VPA on the clinical efficacy and tolerability of epirubicin. The phase I part was open to patients will all solid tumors. A limited phase II part at the maximum tolerated dose (MTD) of VPA enrolled 10 breast cancer patients and incorporated the breast cancer regimen FEC100 (600/100/600 mg/m2). VPA was given on days 1-3 prior to epirubicin/FEC100 in 3-week cycles. HDAC expression, histone acetylation and topo II expression were evaluated in pre-and post-VPA peripheral blood mononuclear cells and tumor samples. Fifty-four (44 in phase I and 10 in phase II) patients [median age 55 (39-78)] received VPA (mg/kg/day): 15, 30, 45, 60, 75, 90, 100, 120, 140 and 160. Tumor types included: breast (10+10), melanoma (11), lung (6), sarcoma (2), GYN (2), GI (5) and others (8). Dose-limiting toxicities included somnolence, confusion and febrile neutropenia. No exacerbation of FEC100/epirubicin-related toxicities was observed. Objective responses in the phase I part 9/41 (22%) were seen across different tumor types despite a median number of 3 (0-6) prior regimens with stable disease/minor response in 16/41 (39%). In the breast-specific phase II part, partial responses to date were seen in 4/8 (50%) and stable disease in 2/8 (25%), progression in 1/8 (12.5%), 1/8 (12.5%) patients withdrew consent. All breast cancer patients with a response/stable disease received the maximal number of seven cycles. VPA plasma concentrations correlated with VPA dose. There was a positive correlation between histone acetylation and VPA dose as well as plasma levels in PBMC and further correlated with those in tumors. A sequence-specific combination of VPA and FEC100 in breast cancer is highly active without exacerbation of chemotherapy-induced toxicities. A neoadjuvant phase II trial using VPA (120 mg/kg) -> FEC100 in patients with early stage breast cancer is ongoing.

In addition to these encouraging data in breast cancer patients, we have previously published a cohort of patients who developed breast cancer which subsequently metastasized to the brain. We sought to examine the impact of VPA (VA) on overall survival among 254 women who developed CNS metastasis and also received whole brain irradiation (WBI). Initially, patients who did (17) and did not (237) receive VA were compared on the basis of several factors. Both groups display equal distribution among factors such as race, hormone receptor status, radiation dose, and the application of radiosurgery, although patients receiving VA were more likely to undergo neurosurgery to excise the metastatic lesion.



Overall survival (OS) was defined as the time between the diagnosis of brain metastases and death. Of 17 patients (6.6%) who received VPA (VA), OS was 24% at 2 years and 16% at 5 years post-diagnosis of metastatic to the brain. For patients who did not receive VA, OS was 14% and 5%, respectively (p=0.069, Figure). While not statistically significant (likely due to the small number of patients prescribed VA in this population), this data suggests that VA can improve overall prognosis in breast cancer patients with CNS involvement.

Subsequently, these patients were stratified by ER positivity as well as treatment with VA. For ER-positive patients, OS was identical for those taking and not taking VA (14% at 2 years for both, p=0.84, Figure). However, for ER-negative patients OS at 2 years was significantly improved in those who received VA (30%) as opposed to those who did not (16%, $p<0.05$, Figure). These findings suggest that VA may confer significant improvements in OS, especially in ER-negative disease. However, we were unable to observe any difference in outcome when stratifying on the use of VA and on Her2 status. We have already reported that Her2 positivity and Herceptin use improves OS in this population; however, VA seemed to modestly improve OS in both Her2-negative and –positive patient group. While the observation of improved survival is interpreted cautiously since limited by the retrospective nature, there is no evidence here that VPA was detrimental in this cohort.

1.2 Summary of Phase I/II studies and Adverse Events (AE) in VPA

A full summary of published phase I and II studies of VPA has been thoroughly reviewed (35) Section 1.2 is a direct transcription from this publication summarizing these data.

1.2.1 Phase I Studies

"In the first phase I study published, 12 patients with untreated cervical carcinoma were studied in the window from the diagnosis to the beginning of definitive treatment with chemoradiation. These patients had a macroscopic tumor accessible for punch biopsy and the study consisted in the administration of magnesium valproate in cohorts of four patients at 20 mg/kg, 30 mg/kg and 40 mg/kg from day 1 to 5. At day 6 tumor biopsies and blood samples were taken.

All patients completed the study medication and the mean daily dose for all patients was 1890 mg. According to dose level, mean daily dose for the 20-mg/kg dose level was 1245 mg (range 1000–1400 mg), while it was 2000 mg (1800–2100) for the 30 mg/kg and 2425 mg (1800–3300) for the 40-mg/kg dose level. Treatment in general was well tolerated. The most common side effects were somnolence and fatigue grade 2. Other side effects such as nausea, diarrhea, anorexia, and dizziness/lightheadedness were uncommon and mild. There were no changes in the values of non-hematological or hepatic parameters except by lymphopenia grade 1 in a patient receiving the lowest dose level. All toxicities disappeared within the ensuing week. Of note the pharmacodynamic evaluation of histone acetylation and HDAC inhibition was done in both, the primary and the peripheral blood at day 6. A clear increase in tumor H3 acetylation as evaluated by western blot was observed in 11 out of 12 patients whereas for H4, hyperacetylation was seen in seven patients. Both histones were hyperacetylated in six individuals. Further, evaluation of HDAC inhibition was observed in 8 of 12 (75%) patients whereas patients 3 and 8 (25%) had either no change or a mild increase in deacetylase activity. Unfortunately, the small sample size did not allow for establishing a correlation with H3 and H4 histone acetylation with HDAC inhibition. Nevertheless, two patients with no HDAC inhibition showed histone hyperacetylation. Histone hyperacetylation was observed in peripheral blood of the four patients that could be evaluated and correlated with hyperacetylation in tumors. These effects were achieved at plasma concentration of VPA that ranged from 73.6–170.49 ìg/mL, although there was lack of correlation between serum levels with dose level. Mean values for patients were 94.06 ìg/mL at 20-mg/kg, 123.46 ìg/mL at 30-mg/kg and 90.93 ìg/mL at 40 mg/kg. These data indicate that at the range of doses tested there is a plateau in the molecular response (100%, 50% and 50%) for the three tested doses(36).

The second phase I study of VPA was performed on patients with histologically confirmed progressive, advanced stage malignant disease without any options of standard treatment, who received at least one prior palliative chemotherapy. Patients were enrolled to the study in cohorts of three patients for each dose level which were 30, 60, 75, 90, 120, 180, 240, and 300 mg/kg at day by intravenous route on days 1–5 and days 22–26. The daily doses of VPA were divided into two equal parts and each part was given as an intravenous infusion of 60-min duration (VPA was dissolved in isotonic NaCl 0.9 or 5% glucose solution at a concentration of 900 mg/100 mL). The first infusion was administered in the morning between 08:00 and 10:00, and the second in the evening between 20:00 and 22:00. Dose escalation was performed as classical phase I studies for cytotoxics. The most common toxicity was neurological, occurring in almost all patients in a dose-dependent manner. Eight patients experienced confusion or disorientation being the DLT in seven of these patients. In addition, five had neurovisual or neuroacustical side effects, but of lower grade. Also grade 1 or 2 vertigo was observed in five out of 26 patients. Somnolence occurred in 21 out of 26 patients and was dose limiting in two patients (both in the 120 mg cohort). All neurological side effects, whether dose limiting or not, resolved completely after discontinuation of the treatment. Haematological and metabolic toxicities were rare and

mild. Five patients had fatigue, and in two patients, it was of grade 3 (DLT). Overall, the maximum tolerated dose was set at 60 mg/kg. Pharmacodynamic analysis performed by western blot in the peripheral blood showed increased histone hyperacetylation in 12 (75%) out of 16 patients, with either H3-hyperacetylation and/or H4-hyperacetylation. Of note, histone hyperacetylation did not seem to be dose dependent. Four patients had no detectable biological activity, two treated at 120 mg/kg, one at 30, and one at 60 mg/kg. HDAC2 levels were found downregulated in the four patients (at dose levels 30, 60, 90, and 120 mg/kg) tested. Interestingly, yet no objective responses were seen, two out of 26 patients (colon and lung carcinomas) had stable disease for 5 and 3 months respectively despite were rapidly progressing under prior chemotherapy(37).

These two studies are of different nature, the first one was a phase I study to find the "adequate" biological dose of magnesium valproate. The advent of the so called "targeted therapies" has led to reconsider the appropriateness of the traditional or classical designs for phase I trials which takes into account toxicity as the most important parameter for dose escalation. Instead some authors have proposed that the endpoint in phase I studies of targeted therapies should be a change in the level or activity of the target or enzyme or other surrogate marker(38). Because it is clear that the antiproliferative, differentiating and/or pro-apoptotic effects of HDAC inhibitors result from the histone hyperacetylation it seems logical to evaluate the acetylation status of H3 and H4 histones as the endpoint of the trial. On the other hand, not necessarily, the highest hyperacetylation should occur at the higher dose of the tested agent, being possible that a plateau in the effect can be seen in a range of doses that do not produce limiting toxicity. This issue is evident from both trials were no evidence consistent dose–effect was seen for histone hyperacetylation. In this sense, the concept of "adequate" instead of the "optimal" biological dose emerges because, by one hand, the number of patients required for finding the optimal dose may be to high for an agent that by itself (or as a single agent) is expected to produce a negligible clinical response rate or tumor shrinkage which equate to expose a larger number of patients to a potentially ineffective agent by its own(39, 40). Another issue for selecting adequate instead of optimal dose design is the fact that it allows investigators to quickly proceed to phase II trials (usually along with chemotherapy or radiation). Perhaps the only situation where an optimal dose design is preferred accounts when the study drug is planned to proceed to larger phase III trials(41).

The second issue to be discussed from the two trials is that, although limited, **preclinical evidence does suggest that like most "targeted therapies", the chronic administration of VPA is required to achieve the best results**(42, 43). This may have implications regarding the maximum tolerated dose, being possible that short-term administrations of VPA (three or five days) could allow to administer higher doses but for chronic administrations doses such as those encountered safe in the first study would be more suitable, particularly in combination therapies with other cytotoxic or cytostatic drugs. **In summary, it seems that doses between 20 and 60 mg/kg of VPA can be appropriate for further testing in solid tumors as H3 and H4 hyperacetylation in tumors is achievable in most patients.**

Phase I–II studies
A third study with VPA combined with epirubicin was recently published. This was a phase I–II study in patients with advanced solid tumor malignancies, an ECOG performance status of 0–2, and adequate organ function. Prior anthracyclines were permitted (doxorubicin ≤300 mg/m2 and epirubicin ≤600 mg/m2). In this study an intravenous loading dose of VPA was followed by five oral doses administered every 12 h beginning 1 h after the loading dose, though later in the study, the intravenous loading dose was replaced with an oral loading dose. Dose escalation started on 15 mg/kg up to 160 mg/kg. On day 3, 4 h after the last dose of VPA, epirubicin was administered by intravenous infusion in cycles of 21 days. The trial allowed for maximal cumulative doses of 750 mg/m2 for epirubicin (or epirubicin equivalent) or the use of a cardioprotectant in patients with clearly documented benefits beyond this dose.

A total of 174 cycles were administered to 44 patients, with a median number of four cycles. At 75 mg/kg of VPA, epirubicin was escalated to 100 mg/m2. Undesirable vestibular symptoms (mainly tinnitus and dizziness) as a result of rapid VPA infusion prompted a change from the intravenous loading to oral VPA loading. VPA doses were subsequently escalated to 160 mg/kg/d, which defined the maximum-administered dose (MAD). Dose adjustments for epirubicin and VPA occurred in 9% and 10% of all administered cycles, respectively, including the two patients at the MAD. At the 60-mg/kg VPA dose, one patient experienced febrile neutropenia in cycle 1. At the 75-mg/kg VPA dose, one patient experienced grade 3 somnolence, confusion, and dizziness. Multiple DLTs were seen in both patients receiving VPA 160 mg/kg. DLTs included somnolence, confusion, hallucinations, hearing loss, dizziness diarrhea and hyponatremia. The 140-mg/kg VPA dose level was expanded to six patients without further DLTs. However, because grade 2 neurovestibular toxicities were observed in all three patients who received more than two cycles, further dose escalations to 150 mg/kg were not pursued, rendering 140 mg/kg/d the recommended phase II dose when administered on days 1 through 3 before epirubicin 100 mg/m2 and repeated every 3 weeks. In regard, to response, the regimen proved to be quite active. Partial responses were observed in nine patients (22%) and patients (39%) stable disease (of these, 8 patients with minor response). Sixteen patients (39%) progressed within one or two cycles of treatment. Interestingly, responses were seen at all dose levels starting at 30 mg/kg, and four patients (10%) with stable disease and one patient (2%) with an objective response had been exposed to prior anthracyclines. Further, responses were seen across multiple tumor types, including breast, small-cell lung, pancreas, and prostate cancer (with liver involvement), as well as in tumor types thought to be anthracycline insensitive, such as melanomas and cervical cancer. As could be expect for the number of patients, there was a linear dose-dependent relationship in plasma levels of VPA as well as correlation between VPA levels with histone hyperacetylation. However, whether responses are related to histone hyperacetylation remains to be determined(44).

Phase II studies
A number of preclinical studies have consistently demonstrated that DNA demethylating

agents and HDAC inhibitors, either alone or in combination, are able to modulate gene expression(45, 46), and that both classes of agents synergize not only the antitumor effects(47-51) but also the extent of gene up-regulation(52, 53). We recently confirmed this synergy between hydralazine, a weak DNA methylation inhibitor(54-58) and VPA regarding global gene expression by studying colon carcinoma cell line SW480, in which hydralazine and VPA led to up-regulation of 153 and 178 genes, respectively, whereas the number of up-regulated genes increased to 352 when used in combination.

Valproate has been tested in combination with hydralazine as neoadjuvant therapy associated with standard doxorubicin cyclophosphamide in the basis of substantial evidence demonstrating that a number of critical tumor suppressor and growth regulatory genes are transcriptionally silenced in breast cancer(59).

Eligible patients for the study(60) were 18 years of age and older, ECOG performance status 0-2, and adequate organic function with histologically proven invasive T2-3, N0-2,, and M0 (stages IIB–IIIA) breast carcinoma. Additional eligibility requirements included ECOG performance status 0-2, and adequate organic function. Patients were treated with a daily dose of a slow-release formulation of hydralazine tablets containing either 182 mg for rapid-acetylators or 83 mg for slow-acetylators and magnesium valproate tablets of 700 mg at a dose of 30 mg/kg t.i.d. Both hydralazine and magnesium valproate were administered from day –7 until the last day of the fourth chemotherapy cycle which consisted on doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2 at day 1 every 21 days. Sixteen patients were included and all received four cycles of doxorubicin and cyclophosphamide plus hydralazine and magnesium valproate as planned. In addition, all patients were evaluated for clinical response and toxicity, and 14 for pathological response. There were five (31%) clinical complete responses and eight (50%), partials for an overall response rate of 81% and no one progressed. Among pathological responses, one (6.6%) had complete response, however, in 70% of cases the residual disease was <3 cm, 33% of cases had pathological negative lymph nodes, and no case had extranodal extension. The treatment was well tolerated but hematological toxicity was the most common side effect, with neutropenia grade 3/4 observed in 35% of cases and anemia grade 3/4 in 15% of cases. Among non-hematological toxicities drowsiness was the most frequent side-effect; it was observed in 31% of cycles, nonetheless in the vast majority, of grade 1 severity. Other side effects were tremor, edema, fatigue, nausea/vomiting, and headache, mainly grades 1 and 2. The pharmacodynamic evaluation showed a statistically significant reduction in the 5*m*C content from the DNA extracted from peripheral blood cells as well as a reduction in the enzymatic activity of HDAC. These effects were achieved at mean concentrations of VPA that varied from 78.5 lg/mL to 100.3 lg/mL, for an overall mean of 87.5 lg/mL. Noteworthy, although microarray analysis was intended to be done in all patients, adequate samples only sufficed for analysis in three patients (three pre-treatment biopsies and only one post-treatment). The number of genes up- or down-regulated by an at least threefold difference was 1091 and 89, respectively (GEO GSE6304). These data led authors to conclude that VPA and hydralazine exerts its proposed molecular effects of HDAC inhibition, DNA

demethylation, and gene reactivation in primary tumors of patients with breast cancer, and that despite the regimen containing epigenetic drugs with cytotoxics was apparently more myelotoxic it may have increased the efficacy of chemotherapy.

A second phase II study using the same combination of valproate and hydralazine was undertaken on the basis that chemotherapy resistance, either innate or acquired, requires expression changes in a large number of genes for its development; being possible that epigenetic-mediated changes could be the driving force responsible for chemotherapy resistance (61). Thus, it could be expected that agents targeting DNA methylation and histone deacetylation would, by reverting the epigenetic marker, overcome chemotherapy resistance Perez-Plasencia, 2006 #205.

Eligible patients were 18 years of age and older who have an ECOG performance status, 0-2; and adequate organ function with histologically proven malignant solid tumors who were receiving their second, third, or fourth line of palliative chemotherapy and who showed – at the second or third chemotherapy course – progressive disease as their maximum response according to Response Evaluation Criteria in Solid Tumors (RECIST), or Gynecologic Cancer Intergroup (GICG) CA125 criteria in the case of patients with ovarian cancer. **Schedules comprised cisplatin; carboplatin; paclitaxel; vinorelbine; gemcitabine; pemetrexed; topotecan; doxorubicin; cyclophosphamide, and anastrozole.** A total of 17 patients whose primary tumors sites were cervix (3), breast (3), lung (1), testis (1), and ovarian (7) carcinomas, were evaluable for toxicity, and 15 for response. Most patients were heavily treated, and had received two and three previous lines of treatment. After entering the protocol, of note, patients re-started the schedule employing the same dose they received previously. Clinical benefit (complete or partial response and disease stabilization) was observed in 12 (80%) of the 15 patients; four partial and eight stabilization of disease. Among partial responses, three were in ovarian cancer according to IGCG CA125 criteria, and a PR was observed in one patient with cervical cancer by MRI of a supraclavicular lymph node disease. Regarding patients with stable disease, four occurred in patients with ovarian cancer and one each in patients with cervix, lung, testis, and breast. The majority of patients who exhibited response or stabilization also demonstrated improvement in symptoms such as dyspnea, cough, and pain. Regarding evaluation of response according to total number of evaluable disease sites, there were 16 evaluable lesions in the eight cases excluding ovarian malignancies. Of these, one (6.6%) complete and three (18.75%) partial responses were achieved, whereas nine (56.2%) had disease stabilization. The treatment was well tolerated despite the study population was heavily pre-treated. The median number of weeks that patients received protocol therapy was 9.5 (range, 6–17 weeks) and the most significant toxicity was hematological. This hematological toxicity led to a decrease in dose intensity in nine (60%) patients as compared to that previous to entering the protocol.
Non-hematological toxicity, that could be attributed to valproate consisted on grade 3 drowsiness in three and delirium in one patient, respectively. These antitumor effects resulted in a median progression-free survival of 3.3 months (range 2.4–5.7 months) and median survival was 6.1 months (range 3.8–12.8 months). Regarding pharmacodynamic endpoints, there was a mean reduction of 23.3% in HDAC activity at

day 8 of treatment with the epigenetic agents. These effects were seen at a mean concentration of 86.3 ìg/mL of VPA levels in plasma. This study was not intended to have post-treatment samples of tumors, instead, the promoter methylation of three genes (*hMLH, RAR*â, and *DAPK*) were analyzed from serum DNA. Overall, of 15 informative methylation-specific PCR, reactions five (33.3%) showed promoter demethylation. As for patients, eight of nine were informative and four (50%) had demethylation of at least one gene. These results suggest that the response and disease stabilization rates observed with valproate and hydralazine may have resulted from the overcoming of epigenetic changes, mediating chemotherapy resistance (62).

In the most recent phase II study which is submitted to publication, the combination of valproate and hydralazine was added to standard cisplatin chemoradiation in FIGO stage IIIB patients under the rational that these drugs in combination show inhibitory growth effect *in vitro* and *in vivo*, chemosensitization, a synergistic effect upon global gene expression (36), and up-regulation of Human leukocyte antigen (HLA) class I antigen expression and antigen-specific Cytotoxic T-lymphocyte (CTL) response in cervical cancer cells without increasing human papillomavirus oncogene expression (63). Moreover, cervical cancer exhibits a number of cellular epigenetic alterations that include abnormal activity of DNMT1 and HDAC1 proteins, which are targets of the E7 oncoprotein during the HPV transformation process (64).

In this study, eligible patients were patients with cervical cancer staged as IIIB, aged more then 18 years, with an ECOG performance status, 0-2; and adequate organ function. Treatment included the combination of valproate hydralazine as above described starting 7 days before weekly cisplatin at 40 mg/m2 and external and intracavitary pelvic radiation. The results demonstrate that out of 18 patients evaluable for response, all have a clinical complete response at the end of external radiation and no side effects other than increased but manageable myelotoxicity in addition to grade 3 asthenia (9%), nausea/vomiting (14%), diarrhea (9%), proctitis (9%), hypoalbuminemia (9%), and headache (5%). Somnolence was observed in 73% of patients, but it was grades 1 and 2. Interestingly, a comparison between pre- and post-treatment biopsies (after the seven days of treatment with valproate and hydralazine) showed moderate-to-intense infiltration of tumor and stroma, composed mainly of lymphocytes as well as an increase in connective tissue with fragmentation of solid malignant nests, with a trabecular pattern, indicating that these drugs exert by their own a modest antitumor effect. The mean concentrations in plasma of VPA at weeks 1, 4 and 7 were 66.4, 63.7, and 63.5 ìg/mL, respectively, for an overall mean of 64.5 ìg/mL. Interestingly, in this study, 10 pairs of tumor samples before and after valproate hydralazine could be analyzed for global gene expression by microarray analysis. There were 964 significant up-regulated genes with a false discovery rate <5%. (GEO Submissions GSE8604)."

1.2.2 Safety Profile for VPA

Frequency of adverse effects by organ system:

>10%:

Central nervous system: Headache (·31%), somnolence (·30%), dizziness (12% to 25%), insomnia (>1% to 15%), nervousness (>1% to 11%), pain (1% to 11%)

Dermatologic: Alopecia (>1% to 24%)

Gastrointestinal: Nausea (15% to 48%), vomiting (7% to 27%), diarrhea (7% to 23%), abdominal pain (7% to 23%), dyspepsia (7% to 23%), anorexia (>1% to 12%)

Hematologic: Thrombocytopenia (1% to 24%; dose related)

Neuromuscular & skeletal: Tremor (·57%), weakness (6% to 27%)

Ocular: Diplopia (>1% to 16%), amblyopia/blurred vision (·12%)

Miscellaneous: Infection (·20%), flu-like syndrome (12%)

1% to 10%:

Cardiovascular: Peripheral edema (>1% to 8%), chest pain (>1% to <5%), edema (>1% to <5%), facial edema (>1% to <5%), hypertension (>1% to <5%), hypotension (>1% to <5%), palpitation (>1% to <5%), postural hypotension (>1% to <5%), tachycardia (>1% to <5%), vasodilation (>1% to <5%), arrhythmia

Central nervous system: Ataxia (>1% to 8%), amnesia (>1% to 7%), emotional lability (>1% to 6%), fever (>1% to 6%), abnormal thinking (·6%), depression (>1% to 5%), abnormal dreams (>1% to <5%), agitation (>1% to <5%), anxiety (>1% to <5%), catatonia (>1% to <5%), chills (>1% to <5%), confusion (>1% to <5%), coordination abnormal (>1% to <5%), hallucination (>1% to <5%), malaise (>1% to <5%), personality disorder (>1% to <5%), speech disorder (>1% to <5%), tardive dyskinesia (>1% to <5%), vertigo (>1% to <5%), euphoria (1%), hypoesthesia (1%)

Dermatologic: Rash (>1% to 6%), bruising (>1% to 5%), discoid lupus erythematosus (>1% to <5%), dry skin (>1% to <5%), furunculosis (>1% to <5%), petechia (>1% to <5%), pruritus (>1% to <5), seborrhea (>1% to <5%)

Endocrine & metabolic: Amenorrhea (>1% to <5%), dysmenorrhea (>1% to <5%), metrorrhagia (>1% to <5%), hypoproteinemia

Gastrointestinal: Weight gain (4% to 9%), weight loss (6%), appetite increased

(·6%), constipation (>1% to 5%), xerostomia (>1% to 5%), eructation (>1% to <5%), fecal incontinence (>1% to <5%), flatulence (>1% to <5%), gastroenteritis (>1% to <5%), glossitis (>1% to <5%), hematemesis (>1% to <5%), pancreatitis (>1% to <5%), periodontal abscess (>1% to <5%), stomatitis (>1% to <5%), taste perversion (>1% to <5%), dysphagia, gum hemorrhage, mouth ulceration

Genitourinary: Cystitis (>1% to 5%), dysuria (>1% to 5%), urinary frequency (>1% to <5%), urinary incontinence (>1% to <5%), vaginal hemorrhage (>1% to 5%), vaginitis (>1% to <5%)

Hepatic: ALT increased (>1% to <5%), AST increased (>1% to <5%)

Local: Injection site pain (3%), injection site reaction (2%), injection site inflammation (1%)

Neuromuscular & skeletal: Back pain (·8%), abnormal gait (>1% to <5%), arthralgia (>1% to <5%), arthrosis (>1% to <5%), dysarthria (>1% to <5%), hypertonia (>1% to <5%), hypokinesia (>1% to <5%), leg cramps (>1% to <5%), myalgia (>1% to <5%), myasthenia (>1% to <5%), neck pain (>1% to <5%), neck rigidity (>1% to <5%), paresthesia (>1% to <5%), reflex increased (>1% to <5%), twitching (>1% to <5%)

Ocular: Nystagmus (1% to 8%), dry eyes (>1% to 5%), eye pain (>1% to 5%), abnormal vision (>1% to <5%), conjunctivitis (>1% to <5%)

Otic: Tinnitus (1% to 7%), ear pain (>1% to 5%), deafness (>1% to <5%), otitis media (>1% to <5%)

Respiratory: Pharyngitis (2% to 8%), bronchitis (5%), rhinitis (>1% to 5%), dyspnea (1% to 5%), cough (>1% to <5%), epistaxis (>1% to <5%), pneumonia (>1% to <5%), sinusitis (>1% to <5%)

Miscellaneous: Diaphoresis (1%), hiccups

<1%, postmarketing, and/or case reports (limited to important and/or life-threatening):

Aggression, agranulocytosis, allergic reaction, anaphylaxis, anemia, aplastic anemia, asterixis, behavioral deterioration, bilirubin increased, bleeding time altered, bone marrow suppression, bone pain, bradycardia, breast enlargement, cutaneous vasculitis, carnitine decreased, cerebral atrophy (reversible), coma (rare), dementia, encephalopathy (rare), enuresis, eosinophilia, erythema multiforme, Fanconi-like syndrome (rare, in children), galactorrhea, hematoma formation, hemorrhage, hepatic failure, hepatotoxicity, hostility, hyperactivity, hyperammonemia, hyperammonemic encephalopathy (in patients with UCD), hyperglycinemia, hypersensitivity reactions (severe, with multiorgan dysfunction), hypofibrinogenemia,

hyponatremia, hypothermia, inappropriate ADH secretion, intermittent porphyria, LDH increased, leukopenia, lupus, lymphocytosis, macrocytosis, menstrual irregularities, pancytopenia, parkinsonism, parotid gland swelling, photosensitivity, platelet aggregation inhibited, polycystic ovary disease (rare), psychosis, seeing "spots before the eyes," Stevens-Johnson syndrome, suicidal behavior/ideation, thyroid function tests abnormal, toxic epidermal necrolysis (rare), urinary tract infection

BOX WARNINGS:

Hepatotoxicity

Potentially fatal hepatic failure can occur.

Usually occurs during the initial 6 months of therapy. Children <2 years of age are at considerably increased risk of developing fatal hepatotoxicity, especially those receiving multiple anticonvulsants and those with congenital metabolic disorders, severe seizure disorders accompanied by mental retardation, or organic brain disease. Above this age group, the risk of fatal hepatotoxicity decreases considerably in progressively older patient groups. Serious fatal hepatotoxicity may be preceded by symptoms such as malaise, weakness, lethargy, facial edema, anorexia, and vomiting.

Monitor patients closely for development of any such changes.

Perform liver function tests prior to and at frequent intervals during therapy, especially during the first 6 months.

Fetal/Neonatal Morbidity and Mortality

Can produce teratogenic effects (e.g., neural tube defects such as spinal bifida).

Use in women of childbearing potential requires that potential benefits of therapy be weighed against the risk of fetal injury.

Pancreatitis

Life-threatening pancreatitis has occurred both in children and adults. Some cases described as hemorrhagic with rapid progression from initial symptoms to death. Can occur shortly after initial use as well as after several years of use. Warn patients and caregivers that abdominal pain, nausea, vomiting, and/or anorexia can be symptoms of pancreatitis that require prompt medical evaluation.

Usually discontinue the drug and initiate alternative therapy if pancreatitis is diagnosed.

1.3 Rationale

On the basis of the results from the studies cited above, we hypothesize that VPA may decrease pleural effusion production in cancer patients by inducing quiescence in there tumor cells through a dedifferentiation mechanism. To test this hypothesis, we propose to treat breast cancer patients who need an indwelling pleural catheter due to malignant pleural effusion with 10 weeks of VPA and compare to those treated with placebo. This compound inhibits histone deacetylase, and has shown a relatively safe profile at a dose of 30 mg/kg/day divided into three doses even in combination with second and third line chemotherapy drugs. This dose is expected to lead to hyperacetylation of H3 and H4 in most patients. Successful results of this study may improve the symptom

burden of end stage cancer patients with recurrent pleural effusion, and may reduce the expenses associated with their disease.

2.0 Objectives

2.0 Study Objectives

2.1 Research Design and Methods

Our goal is to better understand the biology of breast cancer stem cells and pleural effusion formation to improve therapy of malignant pleural effusion in breast cancer patients. Based on our preclinical studies described in the background section, a clinical trial is planned to answer specific questions regarding the role of histone deacetylation and effect of its inhibition on breast patients with MPE. In this study, eligible patients with recurrent symptomatic MPE who need an indwelling pleural catheter based on MDACC algorithms for placement will have one placed and will receive 10 weeks of VPA or placebo.

The first aim is to achieve a beneficial clinical effect of VPA on patients with MPE. The specific question is if VPA alters pleural effusion re-accumulation following placement of an indwelling pleural catheter to the extent that it will enable significant shortening of time to removal of the catheter compared to placebo. Additional aims will analyze the biology of histone deacetylation inhibition in MPE and will help to develop biomarkers of activity and noninvasive monitoring to such therapy:

The second aim is to analyze the amount of daily fluid drained with therapy. Patients will be provided a daily fluid drainage diary.

The third aim is to assess radiographic and clinical correlates using standard of care testing and assessment: Chest x-ray and CT to study effect on primary tumor burden and assessment of overall survival.

Our fourth aim is to study biological correlates. We will study the level of H3 and h4 hyperacetylation and inflammatory cytokines in the effusion and in the serum at baseline and at week 2. Fluid will be collected and stored if applicable from visits at baseline, at week 2, 6, and 10. We plan to study expression of stem cell markers on cells from effusion to demonstrate inhibitory effect of VPA on these cells. Urine will be collected and stored if applicable at baseline, weeks 2, 6, and 10 for correlative studies. Finally, we will study the effect of VPA therapy on level of circulating tumor cells.

Our fifth aim is to assess drug effect on patient symptoms, dyspnea and exercise tolerance using the EuroQOL, St George's respiratory questionnare and six-minute walk test respectively.

2.2 Primary Objective

2.2.1 To examine the effect of VPA on the time between placement of an indwelling pleural catheter and its removal due to successful treatment of pleural effusion.

2.3 Secondary Objectives

2.3.1 Measure the amount of pleural fluid drainage.
2.3.2 Study expression of inflammatory cytokines using the Luminex platform and the degree of H3 and H4 hyperacetylation in serum and pleural effusion cells.
2.3.3 Evaluate the effect of the study agent on tumor burden with chest CTs.
2.3.4 Evaluate the effect of the study agent on symptom burden,dyspnea and exercise tolerance
2.2.5 Measure level of circulating tumor cells in peripheral blood and pleural effusion.
2.2.6 Study expression of cancer stem cell markers on tumor cells in pleural effusion.
2.2.7 Record treatment related toxicity.
2.2.8 Record overall survival
2.2.9 Obtain urine samples for correlative studies

3.0 Study Plan and Overview

3.0 Study Plan and Overview

76 breast cancer patients with malignant pleural effusion will be recruited and treated with VPA or placebo for 10 weeks. Prior data suggest chronic low dose administration is ideal for HDAC inhibition. Since q3wk dosing has often been given an average of 4 cycles, 12 weeks, 10 weeks of daily treatment is in this range and is long enough to examine fluid at multiple endpoints while on treatment while minimizing the side effects and cost of the trial.

MD Anderson Cancer Center's IND office will provide monitoring for this protocol. Patients, the investigator and the research monitor will be blinded to treatment randomization.

3.1 Timeline

In the first two years of the proposed project, we will conduct the clinical trial and collect samples and information. Biological correlates and data analysis will be performed on fresh samples as the trial proceeds.

3.2 Patient Recruitment

Patients will be recruited for this study by working closely with Breast Medical Oncologists. Representatives from Radiation Oncology, Breast Medical Oncology and Pulmonary Medicine (i.e., the principal investigator, the co-principal investigator, co-investigators, and the research nurse) will screen and evaluate patients seen in the MD Anderson Breast Center to identify potential subjects.

When medically appropriate, catheters will be placed as per standard of care by a pulmonologist or their trained designee after signing the usual consent for this procedure. Monitering will be standard of care as directed by the MD responsible for

placing the catheter.

3.3 Pretreatment Evaluations

Patients deemed to require catheter placement based on standard of care algorithms and gleaned from the Electronic Medical Record, will be screened for protocol eligibility. (See Section 4.0) When medically appropriate, catheters will be placed as per standard of care by a pulmonologist or their trained designee after signing the usual consent for this procedure. Monitering will be standard of care as directed by the MD responsible for placing the catheter.

Unless otherwise noted, labs, physical exams, and screening are to be performed within 7 days of enrollment. Imaging studies are to be performed within 30 days of enrollment.

3.3.1 Sign an informed consent.

3.3.2 Complete physical exam, including measurement of vital signs, performance status, weight and medical history

3.3.3 Pretreatment laboratory tests:

- **Hematology:** CBC with differential
- **Chemistry:** Electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT or AST, LDH, total bilirubin, amylase, lipase, magnesium and ionized calcium.
- **Coagulation:** Prothrombin time (PT), partial thromboplastin time (PTT), and International Normalized Ratio (INR).
- **Serum or Urine Pregnancy test:** Required for females of child-bearing potential. Documented use of contraception, sexual abstinence, or amenorrhea for > 6 months is sufficient to document patient is not of child-bearing potential. Otherwise must be obtained prior to first dose of VPA and must be negative to enroll.

3.3.4 EQ-5D and St. George's respiratory questionnaire

3.3.5 Chest x-ray within 72 hours prior to catheter placement.

3.3.6 Chart will be reviewed for re-staging CT scan of the chest. One will be ordered if not performed since the development of the effusion.

3.4 On-Study Evaluations

On the day of indwelling intrapleural catheter placement the patient's pleural fluid will be drained, recorded and sent for cytology review. On the day of indwelling intrapleural catheter placement patients will receive VPA or **placebo** if cytology is positive. Patients will be provided a diary to record drainage from home.

Patients will be treated for the whole period of 10 weeks, and clinical course of disease will be monitored throughout this time. (Note: evaluations may be done within ± 7 business days of the date specified in the protocol).

Patients will initially receive daily oral VPA 15 mg/kg/day divided in three doses or placebo divided in three doses. Based on the patient's bmi, obese patient dosing will be based on the adjusted body weight calculated using the MD Anderson Clinical

Calculator Formula (http://inside2.mdanderson.org/apps/calculators/calc1.cfm). If the patient tolerates the reduced dose for 10 consectuive days then the VPA dose will increase to 30 mg/kg/day divided into three doses or placebo divided into three doses. The patient will be contacted by phone to assess tolerance and instruction to increase dose. Daily dose in mg will be divided by 250 mg and rounded down to a whole number of capsules to determine the number of VPA tablets or placebo tablets to be taken 3 times per day.

Evaluations may occur any time during weeks 2, 6, and 10.

3.4.1 Patients will document daily amount of pleural fluid drainage.
3.4.2 Patients will be evaluated at weeks 2, 6, and 10 with complete interim histories and physical examinations including measurement of vital signs, performance status, weight and medical history.
3.4.3 Patients will complete a EQ-5D and St. George's respiratory questionnaire, at weeks 2, 6, and 10 in person, via telephone or electronic media.
3.4.4 Blood samples for CBC with differential, platelet count, and PT/PTT/INR, serum and plasma banking, and optional CTC (if patient agrees) assessment will be collected prior to treatment and at weeks 2, 6 and 10.
3.4.5 Tests to measure serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, AST or ALT, LDH, total bilirubin, amylase, lipase, magnesium and ionized calcium will be performed at weeks 2, 6, and 10.
3.4.6 Optional urine (if patient agrees) will be collected prior to treatment and at weeks 2, 6 and 10.
3.4.7 Chest x-ray will be performed immediately after catheter placement and at weeks 2, 6, and 10.
3.4.8 CT scan will be performed at week10 or earlier, if clinically indicated, to determine amount of effusion and drug effect on tumor burden .
3.4.9 Pleural effusion will be collected at each clinic visit. Residual fluid may be stored for additional research studies (optional).

On-Study Evaluations beyond Week 10
The EQ-5D, St. George's respiratory questionnaire and the obtaining of pleural fluid samples for research purposes will cease at the end of Week 10. After the completion of 10 weeks of VPA or placebo the patient's medical record will be followed for the date of catheter removal or date of death, which ever comes first.

Study Parameters

Studies	Prior to catheter placement	Within 72 hours prior to catheter placement	Day of catheter placement	Weeks 2, 6 and 10[6*]	Week 10	Review EMR
Physical Exam including: v/s, wt	X[3]			X[3]		
Medical History	X			X		
Pregnancy Test if applicable	X[1,3]					
Ionized Calcium	X[3]					
Magnesium	X[3]					
Bloodwork	X[2,3]			X[2,3]		
ECOG PS	X			X		
St. George's questionnaire	X[3]			X[3]		
EQ-5D	X[3]			X[3]		
CXR PA and LAT		X	X	X		
Patient daily documentation of pleural fluid drainage			X	X		
Patient daily documentation of VPA or placebo administration			X	X		
optional pleural fluid			X	X		
optional blood			X	X		
optional urine			X	X		
CT chest	X[4]				X	
Serum valproic acid for grade 2 or greater AEs[5]						
Date of pleural catheter removal or death date						X

1. **Serum or Urine Pregnancy test:** Required for females of child-bearing potential. Documented use of contraception, sexual abstinence, or amenorrhea for > 6 months is sufficient to document patient is not of child-bearing potential. Otherwise must be obtained prior to first dose of VPA and must be negative to enroll.
2. **Hematology: CBC with differential Chemistry:** Electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT or AST, LDH, total bilirubin, amylase, lipase, magnesium and ionized calcium.
   Coagulation: Prothrombin time (PT), partial thromboplastin time (PTT), and International Normalized Ratio (INR) at baseline only.
3. Within 7 days of enrollment
4. Baseline CT chest completed after development of effusion and within 4 weeks of enrollment.
5. Draw serum valproic acid level for any grade 2 or greater adverse events.
6. Evaluations may occur any time during weeks 2, 6, and 10.

* If the patient tolerates the reduced dose for 10 consecutive days then the VPA dose will increase. This will be assessed at the 2 week visit. The patient will be contacted by phone to assess tolerance and instruction to increase the dose.

4.0 Patient Eligibility

4.0 Eligibility Criteria

4.1 Inclusion Criteria

4.1.1 Patients with symptomatic pleural effusion requiring placement of an IPC.

4.1.2 Pathologic documentation of breast cancer.

4.1.3 Performance status 0 to 3 (ECOG scale).

4.1.4 Signed informed consent prior to the indwelling pleural catheter placement.
4.1.5 Subject must be female or male age 18 years or over.
4.1.6 At least one prior line of chemotherapy in the metastatic setting
4.1.7 Positive effusion cytology

4.2 Exclusion Criteria

4.2.1 Palliative radiation to the chest. (Palliative radiotherapy will be allowed to extra thoracic sites.)
4.2.2 No other prior malignancy is allowed except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer from which the patient has been disease-free for at least two years.
4.2.3 Laboratory results sustained at: Neutrophils less than 1.5 × 109/L ; Serum bilirubin >1.5 x the upper limit of reference range (ULRR); Serum creatinine >1.5 x ULRR or creatinine clearance < 30 mL/minute (calculated by Cockcroft-Gault formula).
4.2.4 Ionized calcium or magnesium out of normal range despite supplementation; Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2.5 × ULRR or alkaline phosphatase (ALP) >2 x ULRR, or > 4x ULRR if judged by the investigator to be related to liver metastases.
4.2.5 Serious underlying medical condition that would impair the ability of the patient to receive protocol treatment, specifically cardiac diseases, uncontrolled hypertension or renal diseases.
4.2.6 Diagnosis of an infection requiring IV antibiotics 14 days prior to registration.
4.2.7 Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule.
4.2.8 Women who are currently pregnant or breast feeding.
4.2.9 Known hypersensitivity to VPA, valproate sodium, disodium valproate, or any ingredient in the respective formulation.
4.2.10 Known urea cycle disorders based on history.
4.2.11 Known HIV infection based on history.
4.2.12 Active or recent pancreatitis (within last 6 months).
4.2.13 Any of the following interventions on the affected hemithorax: prior IPC, prior chest tube placement, history of chemical or mechanical pleurodesis, history of thoracotomy within 4 weeks and incompletely healed surgical incision before randomization.
4.2.14 Evidence of empyema or history of empyema of the affected hemithorax
4.2.15 Non-correctable bleeding diathesis
4.2.16 Clinical evidence of skin infection at the potential site of IPC placement.
4.2.17 Patients currently taking valproic acid.
4.2.18 History of hepatitis or liver disease
4.2.19 The following drugs will not be administered concurrently with VPA.

- Carbapenem antibiotics
- Clonazepam
- Topiramate
- Felbamate
- Lorazepem

- Barbiturates
- CarBAMazepine
- ChlorproMAZINE
- Ethosuximide
- GuanFACINE
- LamoTRIgine
- Methylfolate
- OXcarbazepine
- Paliperidone
- Phenytoin
- Primidone
- Protease Inhibitors
- Rifampin
- Risperidone
- Rufinamide
- Salicylates
- Temozolomide
- Tricyclic Antidepressants
- Vorinostat
- Zidovudine

4.2.20 History of seizures

4.3 Restrictions

Because of the teratogenic nature of VPA, female patients must be six months postmenopausal, surgically sterile, or using an acceptable method of contraception. Female patients must agree to continue using an acceptable method of contraception for a period of three months after study medication administration. Acceptable methods of contraception are oral contraceptives, barrier methods, approved contraceptive implant, long-term injectable contraception, intrauterine device or tubal ligation.

5.0 Treatment Plan

5.0 Treatment Plan

Chronic dosing has been suggested to be more effective for targeted therapies in pre-clincial studies. As such a dosing regimen found to be relatively non-toxic has been selected for chronic administration with concurrent standard agents (details above). Patients will initially receive daily oral VPA 15 mg/kg/day divided in three doses or placebo divided into three doses. Based on the patient's bmi, obese patient dosing will be based on the adjusted body weight calculated using the MD Anderson Clinical Calculator Formula (http://inside2.mdanderson.org/apps/calculators/calc1.cfm). Body mass index is a measure of body fat based on height and weight (applies to both men and women adults)

BMI	Weight Status

Below 18.5	Underweight
18.5 – 24.9	Normal
25.0 – 29.9	Overweight
30.0 and Above	Obese

If the patient tolerates the reduced dose for 10 consecutive days then the VPA dose will increase to 30 mg/kg/day divided into three doses or placebo divided into three doses. The patient will be contacted by phone to assess tolerance and instruction to increase dose. Daily dose in mg will be divided by 250 mg and rounded down to a whole number of capsules to determine the number of VPA tablets or placebo tablets to be taken 3 times per day. Depending on the time of indwelling pleural catheter placement, on day 1 of treatment the patient may take 1, 2, or 3 doses. The research team will instruct the patient on the number of doses to take on day 1. Patients will be instructed to take the VPA at approximately the same time each day. Patients will be instructed to take the pills with food. Study drug compliance will be verified by a returned pill diary. Patients will be treated for 10 weeks. We anticipate this study will be activated in January 2014 and will be completed in January 2016.

5.1 Study Drug

5.1.1 Accountability

The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. Valproic acid will be dispensed from commercial stock. All study drug must be accounted for, including study drug accidentally or deliberately destroyed. All discrepancies between amounts of study drug dispensed and amounts returned must be documented. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

5.1.2 Storage

A description of the appropriate storage and shipment conditions are specified on the product label and brochure. The stored study drug supplies must be accessible to authorized staff only. The storage area must also have adequate control of temperature in order to maintain stability and potency of study drug supplies. The capsules should be stored in the original pack until use. For further information, investigators should refer to the product label.

5.1.3 Mechanism of Drug Destruction

Dispensed unused drug will be returned to the study nurse for accounting purposes. Unused drug will be destroyed per MDACC's Drug Destruction Policy for Clinical Research Protocols.

5.1.4 Possible Adverse Effects

The most frequent adverse effects following initiation of therapy for seizure disorders

are: nausea, vomiting, and indigestion. Eructation, fecal incontinence, gastroenteritis, glossitis, flatulence, hematemesis, periodontal abscess, tooth disorder, dry mouth, stomatitis, and constipation may occur.

Somnolence, asthenia, dizziness, and tremor generally are the most frequently reported adverse nervous system effects. In humans with cancer receiving 30mg/kg/day with other systemic chemotherapy agents bone marrow suppression was the primary toxicity.

Frequency of adverse effects by organ system:

>10%:

Central nervous system: Headache (·31%), somnolence (·30%), dizziness (12% to 25%), insomnia (>1% to 15%), nervousness (>1% to 11%), pain (1% to 11%)

Dermatologic: Alopecia (>1% to 24%)

Gastrointestinal: Nausea (15% to 48%), vomiting (7% to 27%), diarrhea (7% to 23%), abdominal pain (7% to 23%), dyspepsia (7% to 23%), anorexia (>1% to 12%)

Hematologic: Thrombocytopenia (1% to 24%; dose related)

Neuromuscular & skeletal: Tremor (·57%), weakness (6% to 27%)

Ocular: Diplopia (>1% to 16%), amblyopia/blurred vision (·12%)

Miscellaneous: Infection (·20%), flu-like syndrome (12%)

1% to 10%:

Cardiovascular: Peripheral edema (>1% to 8%), chest pain (>1% to <5%), edema (>1% to <5%), facial edema (>1% to <5%), hypertension (>1% to <5%), hypotension (>1% to <5%), palpitation (>1% to <5%), postural hypotension (>1% to <5%), tachycardia (>1% to <5%), vasodilation (>1% to <5%), arrhythmia

Central nervous system: Ataxia (>1% to 8%), amnesia (>1% to 7%), emotional lability (>1% to 6%), fever (>1% to 6%), abnormal thinking (·6%), depression (>1% to 5%), abnormal dreams (>1% to <5%), agitation (>1% to <5%), anxiety (>1% to <5%), catatonia (>1% to <5%), chills (>1% to <5%), confusion (>1% to <5%), coordination abnormal (>1% to <5%), hallucination (>1% to <5%), malaise (>1% to <5%), personality disorder (>1% to <5%), speech disorder (>1% to <5%), tardive dyskinesia (>1% to <5%), vertigo (>1% to <5%), euphoria (1%), hypoesthesia (1%)

Dermatologic: Rash (>1% to 6%), bruising (>1% to 5%), discoid lupus erythematosus (>1% to <5%), dry skin (>1% to <5%), furunculosis (>1% to <5%), petechia (>1% to <5%), pruritus (>1% to <5), seborrhea (>1% to <5%)

Endocrine & metabolic: Amenorrhea (>1% to <5%), dysmenorrhea (>1% to <5%), metrorrhagia (>1% to <5%), hypoproteinemia

Gastrointestinal: Weight gain (4% to 9%), weight loss (6%), appetite increased (·6%), constipation (>1% to 5%), xerostomia (>1% to 5%), eructation (>1% to <5%), fecal incontinence (>1% to <5%), flatulence (>1% to <5%), gastroenteritis (>1% to <5%), glossitis (>1% to <5%), hematemesis (>1% to <5%), pancreatitis (>1% to <5%), periodontal abscess (>1% to <5%), stomatitis (>1% to <5%), taste perversion (>1% to <5%), dysphagia, gum hemorrhage, mouth ulceration

Genitourinary: Cystitis (>1% to 5%), dysuria (>1% to 5%), urinary frequency (>1% to <5%), urinary incontinence (>1% to <5%), vaginal hemorrhage (>1% to 5%), vaginitis (>1% to <5%)

Hepatic: ALT increased (>1% to <5%), AST increased (>1% to <5%)

Local: Injection site pain (3%), injection site reaction (2%), injection site inflammation (1%)

Neuromuscular & skeletal: Back pain (·8%), abnormal gait (>1% to <5%), arthralgia (>1% to <5%), arthrosis (>1% to <5%), dysarthria (>1% to <5%), hypertonia (>1% to <5%), hypokinesia (>1% to <5%), leg cramps (>1% to <5%), myalgia (>1% to <5%), myasthenia (>1% to <5%), neck pain (>1% to <5%), neck rigidity (>1% to <5%), paresthesia (>1% to <5%), reflex increased (>1% to <5%), twitching (>1% to <5%)

Ocular: Nystagmus (1% to 8%), dry eyes (>1% to 5%), eye pain (>1% to 5%), abnormal vision (>1% to <5%), conjunctivitis (>1% to <5%)

Otic: Tinnitus (1% to 7%), ear pain (>1% to 5%), deafness (>1% to <5%), otitis media (>1% to <5%)

Respiratory: Pharyngitis (2% to 8%), bronchitis (5%), rhinitis (>1% to 5%), dyspnea (1% to 5%), cough (>1% to <5%), epistaxis (>1% to <5%), pneumonia (>1% to <5%), sinusitis (>1% to <5%)

Miscellaneous: Diaphoresis (1%), hiccups

<1%, postmarketing, and/or case reports (limited to important and/or life-threatening):

Aggression, agranulocytosis, allergic reaction, anaphylaxis, anemia, aplastic anemia, asterixis, behavioral deterioration, bilirubin increased, bleeding time altered, bone marrow suppression, bone pain, bradycardia, breast enlargement, cutaneous vasculitis, carnitine decreased, cerebral atrophy (reversible), coma (rare), dementia, encephalopathy (rare), enuresis, eosinophilia, erythema multiforme, Fanconi-like syndrome (rare, in children), galactorrhea, hematoma formation, hemorrhage, hepatic failure, hepatotoxicity, hostility, hyperactivity, hyperammonemia, hyperammonemic encephalopathy (in patients with UCD), hyperglycinemia, hypersensitivity reactions (severe, with multiorgan dysfunction), hypofibrinogenemia, hyponatremia, hypothermia, inappropriate ADH secretion, intermittent porphyria, LDH increased, leukopenia, lupus, lymphocytosis, macrocytosis, menstrual irregularities, pancytopenia, parkinsonism, parotid gland swelling, photosensitivity, platelet aggregation inhibited, polycystic ovary disease (rare), psychosis, seeing "spots before the eyes," Stevens-Johnson syndrome, suicidal behavior/ideation, thyroid function tests abnormal, toxic epidermal necrolysis (rare), urinary tract infection

BOX WARNINGS:
Hepatotoxicity
Potentially fatal hepatic failure can occur.
Usually occurs during the initial 6 months of therapy. Children <2 years of age are at considerably increased risk of developing fatal hepatotoxicity, especially those receiving multiple anticonvulsants and those with congenital metabolic disorders, severe seizure disorders accompanied by mental retardation, or organic brain disease. Above this age group, the risk of fatal hepatotoxicity decreases considerably in progressively older patient groups. Serious fatal hepatotoxicity may be preceded by symptoms such as malaise, weakness, lethargy, facial edema, anorexia, and vomiting.
Monitor patients closely for development of any such changes.
Perform liver function tests prior to and at frequent intervals during therapy, especially during the first 6 months.
Fetal/Neonatal Morbidity and Mortality
Can produce teratogenic effects (e.g., neural tube defects such as spinal bifida).
Use in women of childbearing potential requires that potential benefits of therapy be weighed against the risk of fetal injury.
Pancreatitis
Life-threatening pancreatitis has occurred both in children and adults. Some cases described as hemorrhagic with rapid progression from initial symptoms to death. Can occur shortly after initial use as well as after several years of use. Warn patients and caregivers that abdominal pain, nausea, vomiting, and/or anorexia can be symptoms of pancreatitis that require prompt medical evaluation.
Usually discontinue the drug and initiate alternative therapy if pancreatitis is diagnosed.

5.1.5 Precautions for Treatment
VPA should not be administered to pregnant women. A negative pregnancy test should be confirmed before administration of VPA.

5.1.6 Concomitant Treatment
Use of VPA at this dose has been documented in patients receiving FEC chemotherapy and is routinely used as an anti-seizure medication among breast cancer patients receiving chemotherapy for metastatic disease. Patients currently receiving hormonal or systemic chemotherapy for metastatic disease will be eligible. Toxicity will be assessed after half of the accrual is met, and early stopping rules will be employed.

5.2 Dose Modification Summary
Attributions will be made using clinical judgment and assigned as definite probably possible etc...as per the human subjects research manual Chapter 15.001 (inside MDA).

Valproic acid serum levels will be drawn where indicated and for grade II toxicity not possibly related to other systemic treatments. Results will be reviewed by the study research nurse. Abnormally high values (increased above upper limit normal) will be reported to the treating physician, will be considered in the clinical attribution, and the patient will become unblinded and IND monitor notified. Subsequent serum monitoring will be at the discretetion of the treating physician.

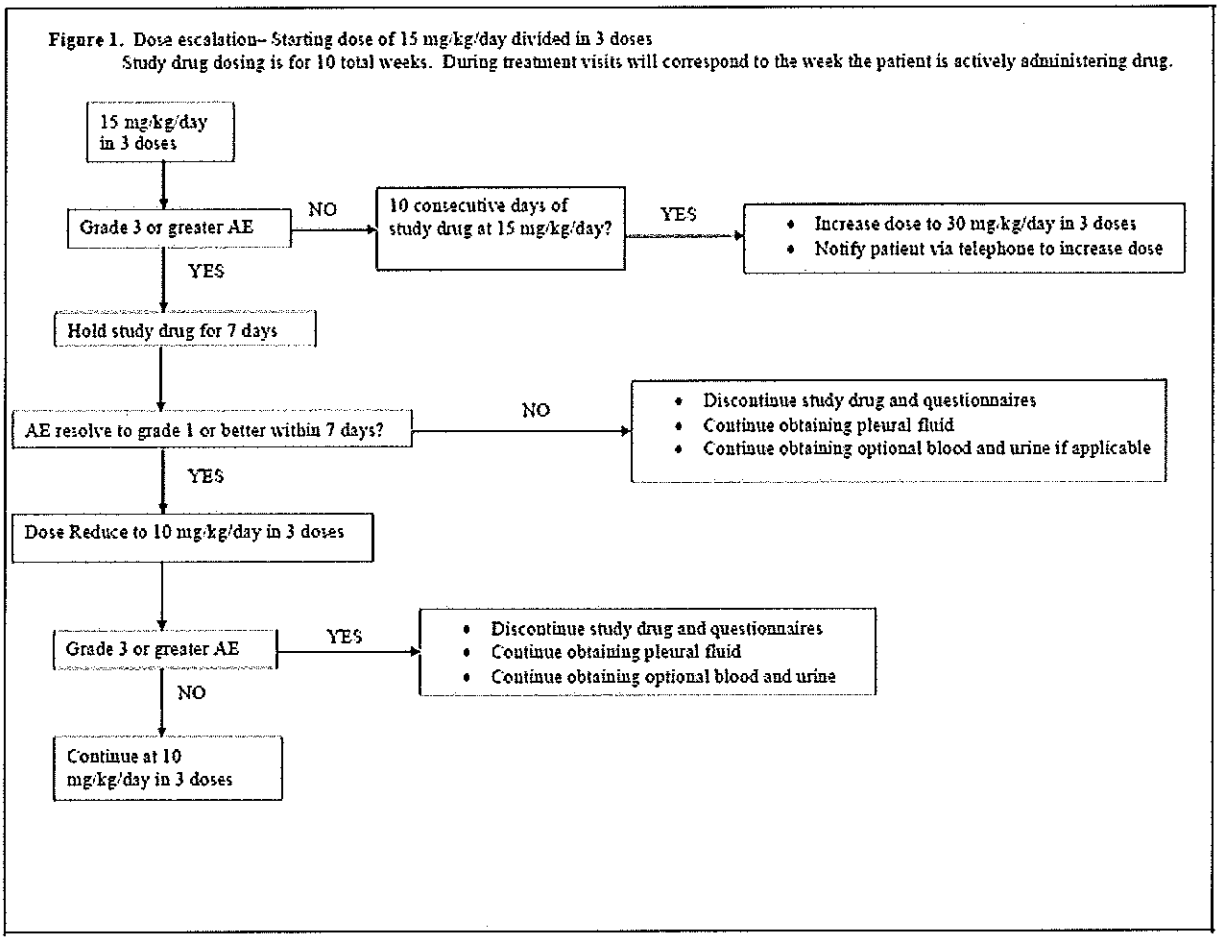
2. Attribution changes to above category based on best judgement with ongoing clinical evaluation and data.

Table 1 - Starting Dose Reduction Example

Dose	Toxicity (see 5.2 and 5.3)	Dose reduced by 5mg/kg/day	Calculation Example	Number of capsules
Starting dose	Dispense 250 mg capsule e.g. (70 kg pt) per day	Reduced dose	Dispense 250 mg capsule per day	Number of capsules to be taken approx. every 8 hours
15 mg/kg/day	Dispense 4 capsules 15mg/70kg/day 15 x 70 = 1050 Rounded down to 1000 mg = 4 capsules	10 mg/kg/day	Dispense 2 capsules 10 mg/70kg/day 10x70 = 700 Rounded down to 500 mg = 2 capsules	Take one capsule every 12 hours (if this scenario were to occur this is not a protocol violation as this is an appropriate dose for such a patient)
10 mg/kg/day	Dispense 2 capsules 5 mg/70kg/day 10 x 70 = 700 700 mg = 2 capsules	5 mg/kg/day	Dispense 1 capsule 5mg/70kg/day 5 x 70 = 350 Rounded down to 250 mg = 1 capsule	Take one capsule once a day (if this scenario were to occur this is not a protocol violation as this is an appropriate dose for such a patient)

Table 2 - Dose Reduction Example Full Dose

Dose	Toxicity (see 5.2 and 5.3)	Dose Reduced 10mg/day	Calculation Example	Number of Capsules
Original Dose	Dispensed 250 mg capsules (ie 70 Kg pt) per day	Reduced dose	Dispensed 250 mg capsules per day	Number of capsules to be taken approx. every 8 hours
30 mg/kg/day	8 (30 x 70 = 2100, rounded down to 2000mg = 8 capsules)	20mg/kg/day	5 (20 x 70 = 1400, rounded down to 1250 = 5 capsules)	Dose 1 = 2 capsules Dose 2 = 2 capsules Dose 3 = 1 capsule
20mg/kg/day	5 (20 x 70 = 1400, rounded down to 1250 = 5 capsules)	10mg/kg/day	2 (10 x 70 = 700, rounded down to 500 = 2 capsules	Dose 1 = 1 capsule Dose 2 = 1 capsule Dose 3 = 0 capsules



5.3 Guidance on the management adverse events

Excluded reporting and recording of adverse events:

1. Alopecia
2. Differential component of CBC except WBC, ANC, platelet count and hemoglobin

Baseline reporting of adverse events prior to protocol treatment will not be recorded or reported. All other baseline adverse events will be reviewed and if worsen will be captured in Figures 2-9.

The eligibility criteria provide an extensive list of concomitant medication as exclusions. Non-Oncologic concomitant medications will not be reported.

5.3.1 Guidance on dose reduction for specified alterations in amylase, lipase, or liver enzymes.

Figure 2. CNS Adverse Event Management
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.

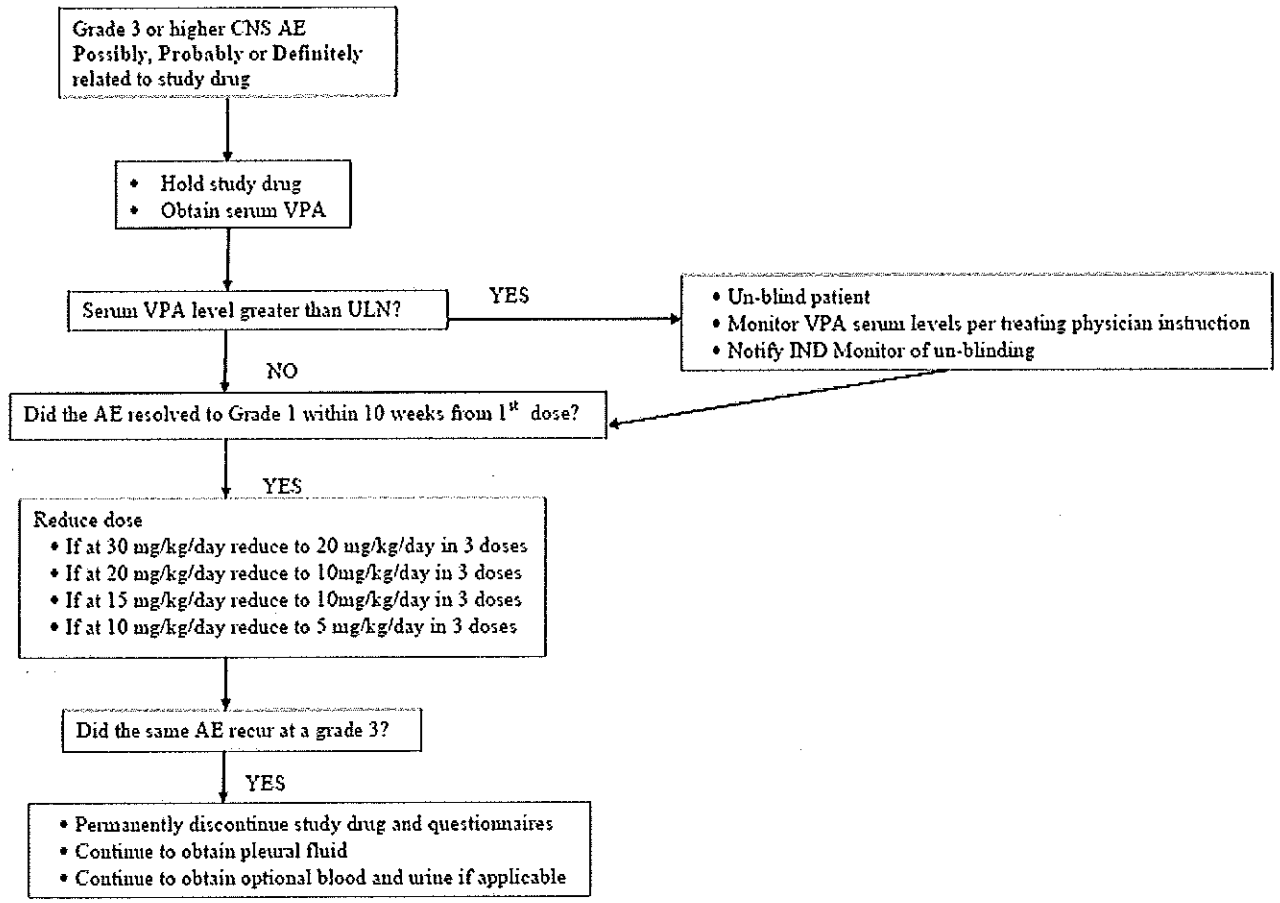


Figure 3. Elevated Amylase and Lipase at any dose
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.

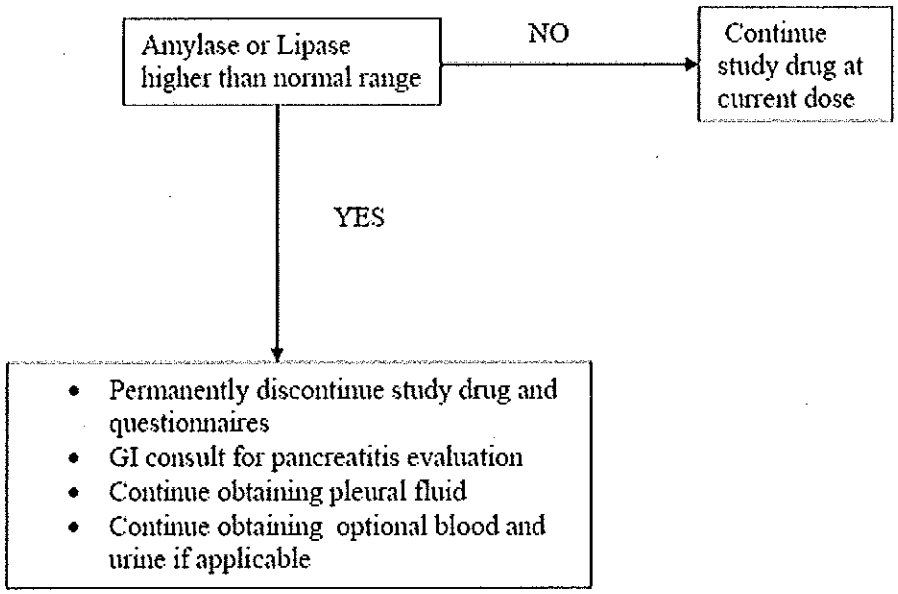
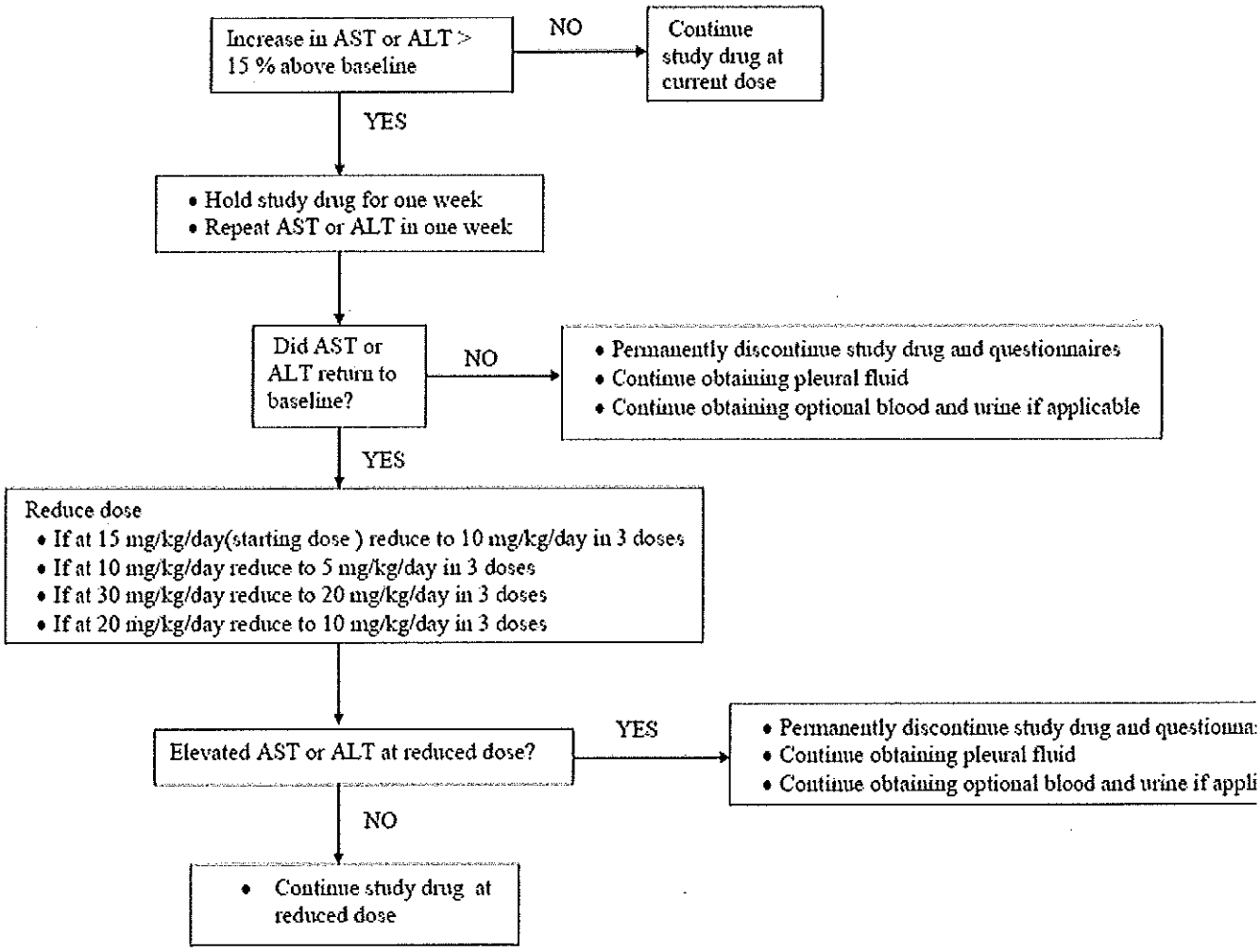


Figure 4. Management of elevated AST or ALT
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administer



5.3.1.1 Guidance for all other Adverse Events

Figure 5. Non AST, Non ALT, Non Amylase, Non Lipase And Non CNS Adverse Event (All other adverse events) Management
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.

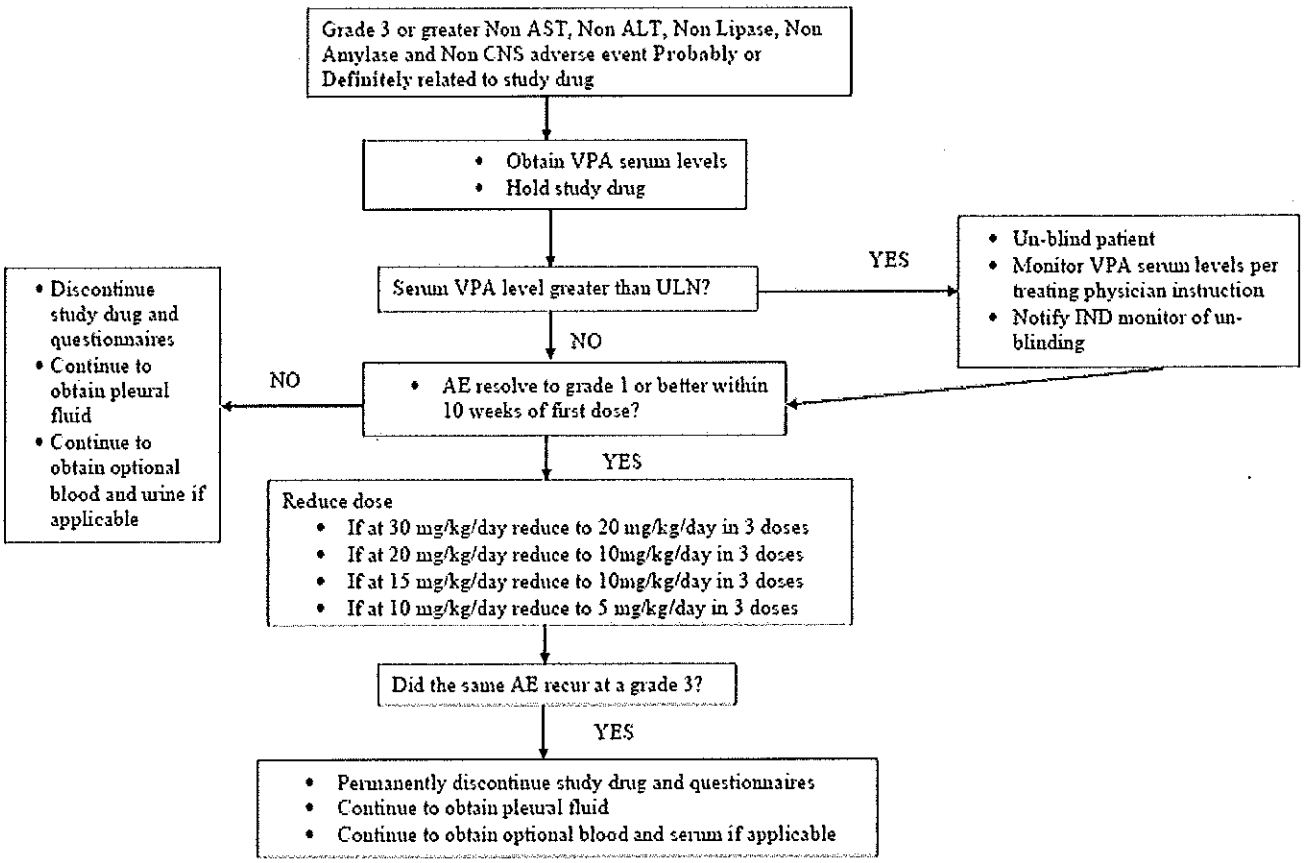


Figure 6. Grade 3 Non CNS Adverse Event Management Not assessed under Figure 4
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.

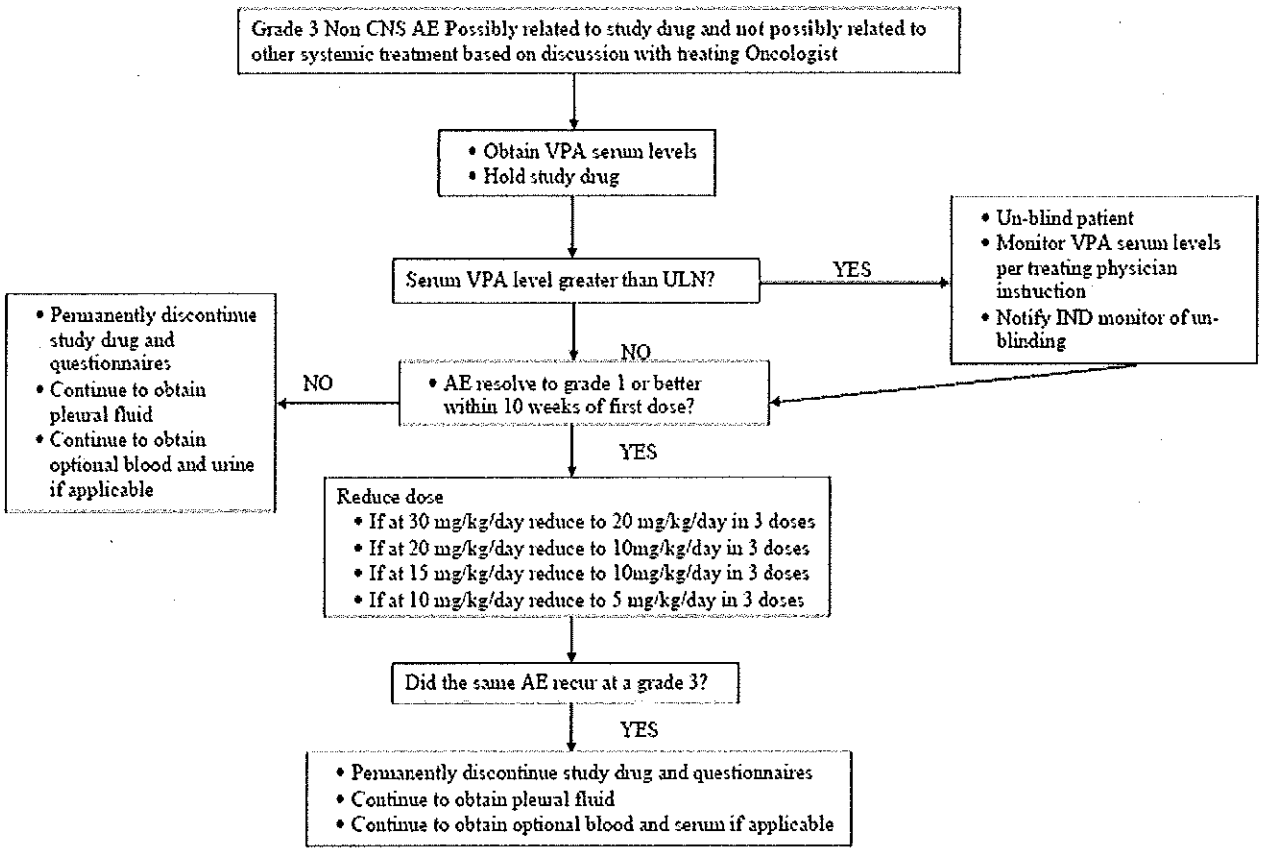
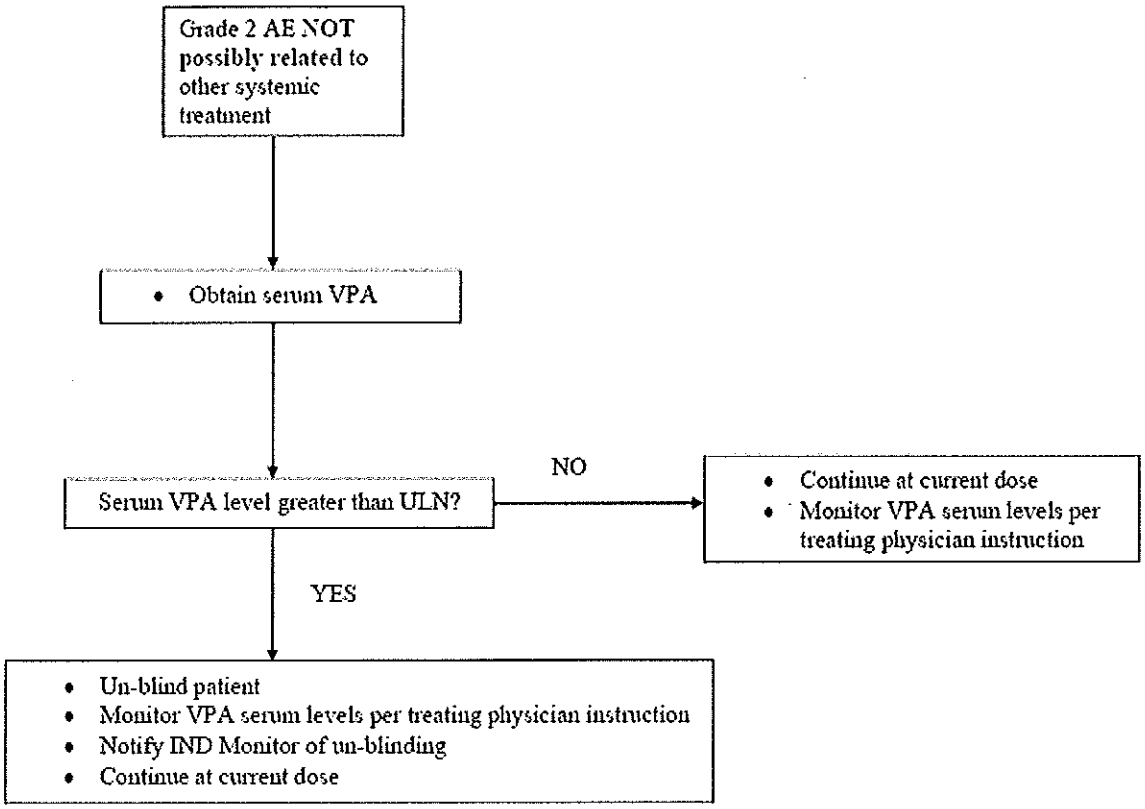


Figure 7. Any Grade 2 deemed NOT possibly related to other systemic treatment



Do Not report or record alopecia and components of the CBC except WBC, ANC, platelet count and hemoglobin.

Figure 8. CNS Adverse Even Management NOT related to study drug (possible, probable or definite attribution)
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.

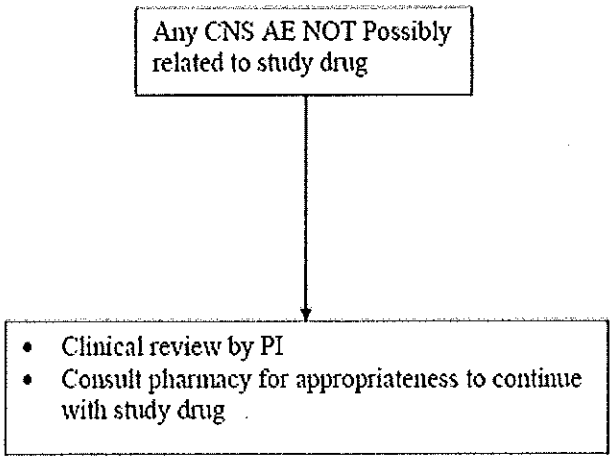
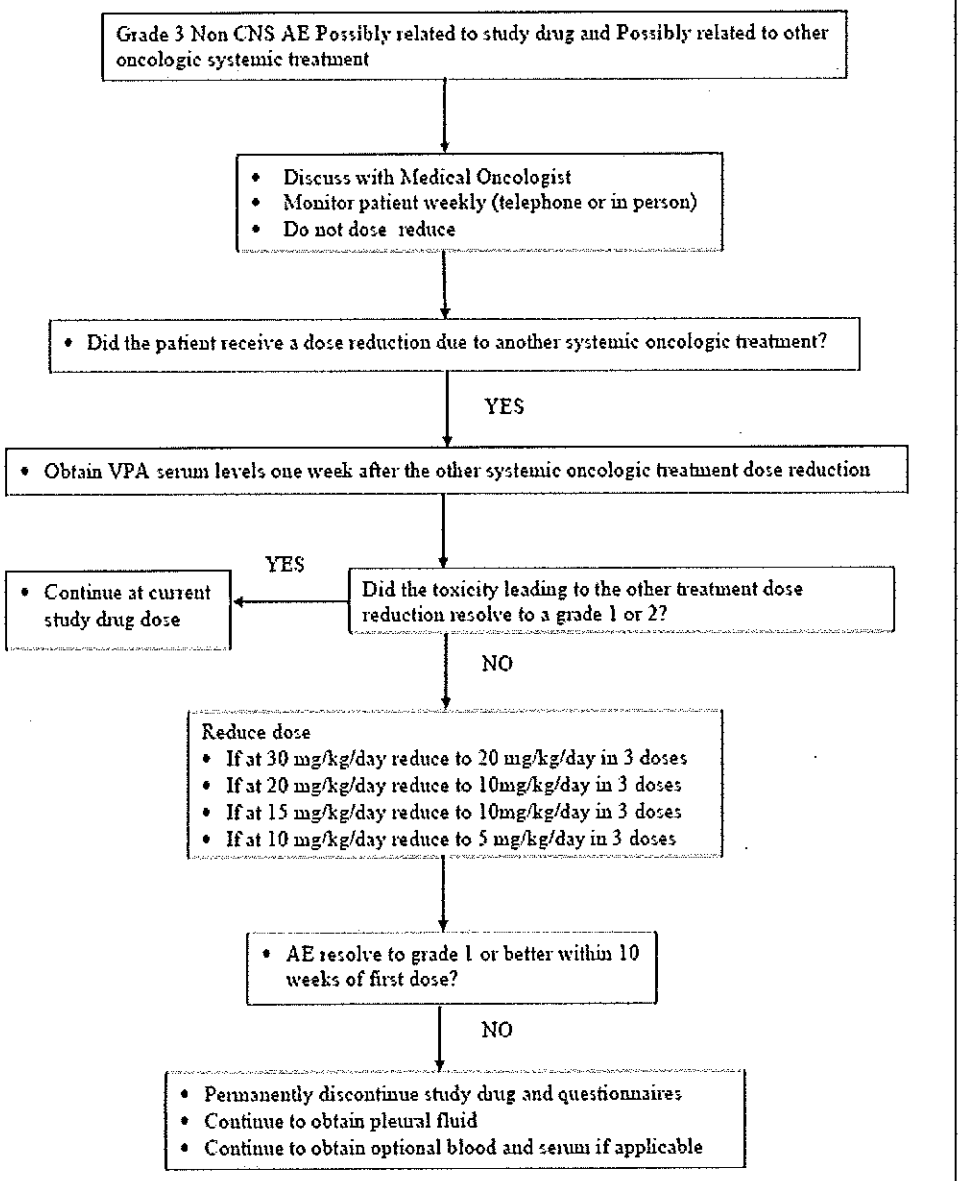


Figure 9. Grade 3 Non CNS Adverse Event Management Possibly related to study drug and Possibly related to other oncologic systemic treatment
Study drug dosing is for 10 total weeks. During treatment visits will correspond to the week the patient is actively administering drug.



5.3.2 On-treatment pregnancy

Positive blood or urine Hcg will result in immediate discontinuation of the drug and referral to gynecology.

6.0 Statistical Considerations

6.0 Statistical Considerations

Sample Size

The primary outcome for this study is time to pleural catheter removal because it is no longer needed to drain the pleura of fluid. Patients who have their catheter removed for other reasons will be censored as of the date of catheter removal. Patients who die before having their catheter removed will be censored on the date of death.

Preliminary data from 58 breast cancer patients with ECOG performance status 0, 1, 2, or 3 revealed a median time to catheter removal of 110 days with 95% confidence interval 56 to 328 days. Twenty-nine of these 58 patients had their catheters removed because they were no longer needed to drain the pleura of fluid.

Patients will be randomized to active treatment (valproic acid) or control. A sample size of 76 patients (38 on each arm) will yield 80% power to detect an improvement in median time to catheter removal from 56 days in the control arm to 28 days (hazard ratio = 2.0) in the active treatment arm with a 2-sided significance level of 0.05. We expect to enroll 3 patients per month. We will be able to detect a hazard ratio of 2.0 (control vs. experimental), regardless of the median for the control group. I fthe medican for the control group is actually 110 days, then we will be able to detect a significant difference if the median in the experimental group is 55 days.

This sample size calculation includes an interim analysis for futility and for efficacy once 36 patients have had their catheters removed using the methods of Lan and DeMets. The interim analysis for efficacy will employ an O'Brien-Fleming stopping boundary with a nominal significance level of 0.0031, while the interim analysis for futility will employ a Pockock stopping boundary with a nominal significance level of 0.3217. The final analysis will use a nominal significance level of 0.049 and will be conducted after all patients have had their catheters removed. These sample size calculations were performed with East 5.0 (Copyright © 2007, Cytel Inc., Cambridge, MA). Sample size was calculated based on 50% reduction in median time to catheter removal secondary to improved fluid in the control.

Randomization

Patients will be randomized to active treatment or control stratified by lung re-expansion (≥80% lung re-expansion, <80% lung re-expansion), ECOG performance status (0-1, 2, 3), using the Clinical Trial Conduct website (https://biostatsitics.mdanderson.org/ClinicalTrialConduct), which is housed on a secure server at MDACC and maintained by the MDACC Department of Biostatistics. Access to the website will be gained through usernames and passwords provided by the MDACC Department of Biostatistics to personnel responsible for enrolling patients and reviewing and analyzing patient data. Training on the use of the Clinical Trial Conduct website to randomize and enroll patients on the study will be provided by the study statistician. Investigation Pharmacy will have access the Clincial Trial Conduct website

to review the patient assignment. The IND office will be notified if an event occurs leading to the un-blinding of the randomization.

Toxicity Monitoring

We will use the methods described by Thall et al. (1995) to monitor the rate of toxicity leading to dose reduction for patients on active treatment. Because the study is blinded to the investigators, the study statistician will monitor the toxicity monitoring rule for the active treatment arm. Toxicity data leading to dose reduction will be forwarded to the study statistician as they accrue in the form of an email with the patient study identifier and a description of the toxicity leading to dose reduction.

If the rate of toxicity leading to dose reduction for patients on active treatment is more than 25% the study will be stopped. We will enroll a minimum of 10 and a maximum of 40 patients on active treatment. We will stop the study early if P(toxicity leading to dose reduction for patient on active treatment > 25% | data from the study) > 0.975. That is, given the toxicity outcomes from the patients on active treatment, if we determine that there is more than a 97.5% chance that the rate of toxicity leading to dose reduction is more than 25% we will top the study. This decision rule gives the following stopping rule. We assume a uniform prior distribution for the rate of toxicity leading to dose-reduction. Stop the study if

[# of pts on active treatment with toxicity leading to dose reduction / # of pts evaluated]

> 6/10, 8/15, 9/20, 11/25, 13/30, 14/35, 16/40

The operating characteristics of this study design are shown in the table below.

Operating Characteristics of Toxicity Monitoring Rule

Rate of Toxicity Leading to Dose Reduction	Probability of Stopping Early	Sample Size		
		P_{25}	P_{50}	P_{75}
0.20	0.020	40	40	40
0.25	0.071	40	40	40
0.30	0.205	40	40	40
0.35	0.407	20	40	40
0.40	0.636	15	30	40
0.45	0.827	10	20	35

Analysis

We will use summary statistics to describe the demographic and clinical characteristics of patients on each treatment arm.

Primary Outcome

Time to catheter removal will be measured from the date of placement of the catheter to the date it is removed. Removal of the catheter because it is no longer needed to drain the pleura will be considered an event. Removal of the catheter for other reasons will be considered competing risks. Patients who die before their catheter is removed will be considered censored on the date of death. We will use the methods described by Gooley et al. (1999) to estimate the cumulative distribution of time to catheter removal stratified by treatment arm and randomization strata.

We will use Cox (1972) proportional hazards regression to model time to catheter removal as a function of treatment arm, cytology, and lung re-expansion, as well as other potential prognostic factors, including ECOG performance status, the number of circulating tumor cells in the peripheral blood and in the pleural effusion.

Secondary Outcomes

We will use summary statistics to describe the amount of pleural fluid drainage by treatment arm, and we will use a two-sample t-test to compare treatment arms with respect to the mean amount of pleural fluid drainage. If the usual normality assumptions do not hold we will use the Mann-Whitney test to compare treatment arms with respect to the median amount of pleural fluid drainage.

We will compare treatment arms with respect to tumor burden using a Cochran-Mantel-Haenszel chi-square test stratified by concomitant therapy. Tumor burden will be assessed with chest cat scan during week 10.

We will use summary statistics to describe symptom burden dyspnea and exercise tolerance changes and dyspnea as measured by the EQ-5D and St George's respiratory
questionnaire at baseline and again at weeks 2, 6, and 10. We will use repeated measures analysis of variance methods to assess differences between treatment arms with respect to QOL, dyspnea and exercise tolerance

Toxicity

We will estimate the rate of toxicity leading to dose reduction for patients on the active treatment with a 90% credible interval. If we have toxicity leading to dose reduction in 5 of the 40 patients on the active treatment, then our 90% credible interval for the rate of toxicity leading to dose reduction will be 6.6% to 23.6%. We will also report the posterior probability that the rate of toxicity leading to dose reduction for patients on the

active treatment is more than 25%.

We will tabulate adverse events by treatment arm, grade, and relationship to study drug.

Translational Studies
For CTC studies using the adnagen approach .3 ng/ml in GA733-2 or muc-1 will be considered positive and both conversion to negative and change from baseline (using continuous data) will be assessed. Descriptive statistics will be applied. Using the Veridex system 5 CTCs will be considered positive for all dichotomous analyses and conversion to negative and change from baseline will be assessed as above. Statistically significant conversion to negative in the VPA arm in either test will be considered a significant change. CTC studies will be performed only if funds are available and will be done by Dr. James Reuben who has extensive experience with both tests.

For quiescence and stem cell analysis flow cytometry will be performed for cell cycle, CD44+CD24-CD45-Epcam+ and aldefluor. Samples will be divided into three replicates to address run variability. Total percent quiescent (G1/G0) epithelial cells will be the primary metric for correlative evaluation although quiescence within stem cell populations will be assessed and reported. All data are continuous variables. Dichotomous analysis will be based on values > or </= to the median for the entire dataset for each variable. All biomarker analyses related to outcome will be performed according to REMARK guidelines incorporating time to metastases, extent of metastatic disease (multiple vs. effusion only), triple negative vs. not, extent of prior therapy, and KPS. Analysis will include correlation between percent quiescent cells and time to catheter removal in both arms and change in percent quiescent cells from baseline in both arms. Given there will be significant variation in the baseline G0/G1 measurement we will consider a 30% relative increase in G0/G1 from baseline significant.

7.0 Criteria for Removal from the Study

7.0 Criteria for Discontinuation on Study

Patients will be removed from the study for any of the following reasons:

1. Patient requests to withdraw.
2. The patient is unwilling or unable to comply with study requirements.
3. There is an unrelated intercurrent illness that will affect assessment of clinical status to a significant degree as determined by the principal investigator or the treating physician.
4. The treating physician or investigator must discontinue study if he/she thinks that the patient's health or well-being is threatened by continuation on study.
5. Identification of recurrent or new cancer.

If any safety parameters show a clinically significant change from baseline that warrants early termination of treatment, the patient will continue with the scheduled study-related procedures. Appropriate safety monitoring will continue until the patient is discharged from the study. The reason for and date of the discontinuation will be obtained.

Participants have the right to withdraw from study at any time. If a participant chooses to withdraw, he/she should contact the principal investigator and/or research nurse who will provide any necessary instructions for finalizing removal from study. If the patient requests to withdraw his/her samples (i.e. residual pleural fluid, blood), appropriate personnel will be notified and samples will be withdrawn and destroyed as per UTMDACC Institutional Laboratory Guidelines. If a patient is non-compliant or lost to follow-up, the research nurse or his/her designee will make three attempts to call the patient over the period of one month. These attempts will be documented. If the research nurse or his/her designee is unable to make contact with either the patient or a family member after three phone calls, then a letter will be sent to the patient's last known address. If after one month from the letter's sent date there is no reponse from the patient or family member then the patient will be withdrawn from study.

Precautions for Treatment
VPA should not be administered to pregnant women. A negative pregnancy test should be confirmed before administration of VPA.

Concomitant Treatment
Use of VPA at this dose has been documented in patients receiving FEC chemotherapy and is routinely used as an anti-seizure medication among breast cancer patients receiving chemotherapy for metastatic disease. Patients currently receiving hormonal or systemic chemotherapy for metastatic disease will be eligible. Toxicity will be assessed after half of the accrual is met, and early stopping rules will be employed.

7.1 Criteria for Intrapleural Catheter Removal

If a patient experiences the following criteria:
Once the amount of daily drainage is $\leq$150 ml for three consecutive days, pleural fluid will drained every other day. While draining every other day, if the amount of fluid increases >150 ml, daily drainage will be restarted. If the amount of fluid drained every other day is $\leq$ 150 ml on three consecutive occasions (total of 6 days), patients will be evaluated clinically and with chest radiographs and the indwelling intrapleural catheter will be removed if there is no evidence of fluid reaccumulation.

Treatment failure:

A. Patients meeting the criteria below will be considered failures as they require removal of their catheters:

- Death while catheter in place.
- Fluid loculation with a non functioning catheter that does not meet the definition of pleurodesis.
- Blockage of IPC requiring removal of the catheter. Blockage of IPC not requiring removal but use of any unblocking therapeutic manouvers will be documented as a complication but not considered as treatment failure.
- Infection - tunnel infection, empyema or systemic infection. Exit site infection

will be reported as complication but not as a treatment failure unless it progresses to tunnel infection, empyema or systemic infection.
- Intractable pain.
- Dislodged cuff requiring removal of the catheter.

B. IPC in place >180 days without meeting catheter removal criteria.

C. Any other IPC removal that does not meet the criteria established in the treatment plan.

D. Non compliance.

E. Secondary failure. Patients requiring any additional procedure to remove pleural fluid from the affected hemithorax, to alleviate respiratory symptoms during the first 6 months after IPC has been removed following the treatment plan guidelines.

Symptomatic pleural effusion was defined as the radiological presence of pleural fluid regarded as the cause of the dyspnea by the treating physician.

Time to pleurodesis was calculated as the days between IPC insertion and removal.

Adequate lung re-expansion is defined as at least 80% pleurae apposition on the affected hemithorax as determined by visual estimation on radiological studies of the chest while IPC is in place.

Unexpandable lung (non-reexplandable lung) is the inability of the lung to expand to the chest wall allowing for normal visceral and parietal pleural apposition. The lung could be trapped or entrapped.

Pleurodesis

A. **Successful pleurodesis:** After IPC removal, long term relief of symptoms related to effusion with absence of fluid reaccumulation on chest radiographs for the remainder of the patients life or until last follow up (6 months after IPC removal).
B. **Partially successful pleurodesis:** After IPC removal, diminution of dyspnea related to the effusion, with 50% or less reaccumulation of fluid when compared to pre-procedure chest radiographs but without the need for further therapeutic thoracentesis for the remainder of the patients life or until last follow up (6 months after IPC removal)

IPC related infections are defined as follows:

A. **Local infection -** temperature <38 ·C, no rigors, no purulent pleural fluid, no evidence of systemic infection. Local infections are divided in:
   i. Exit site infection - erythema, tenderness and induration only at the exit site.

ii. Tunnel infection - erythema, tenderness and induration overlying tunnel tract, and extending more than 2 cm from exit site.

B. **Empyema or systemic infection** - fever > 38 C or rigors, purulent pleural fluid or evidence of systemic infection.

Other complications during or after a pleural procedure were defined as:
A. Bleeding causing hemodynamic derangement or requiring blood transfusion.
B. Presence of pneumothorax.
C. Persistent ipsilateral chest pain after IPC insertion

Catheter obstruction - catheter blockage requiring removal with an additional palliative procedure.

Non Compliance - patient's inability or refusal to comply with study procedure after documented counseling.

Completion of Study - > 180 days with catheter in place, > 180 days after removal of IPC, or considered a treatment failure.

7.2 Replacement of Subjects

Participants who withdraw from the study prior to completion of the study treatments for reasons other than serious adverse events, unacceptable toxicity or progressive disease will be defined as dropouts and will be replaced. Replacement participants will be assigned the next sequential number.

5.1.5 Precautions for Treatment

VPA should not be administered to pregnant women. A negative pregnancy test should be confirmed before administration of VPA.

5.1.6 Concomitant Treatment

Use of VPA at this dose has been documented in patients receiving FEC chemotherapy and is routinely used as an anti-seizure medication among breast cancer patients receiving chemotherapy for metastatic disease. Patients currently receiving hormonal or systemic chemotherapy for metastatic disease will be eligible. Toxicity will be assessed after half of the accrual is met, and early stopping rules will be employed.

8.0 Adverse Events

8.1 Serious Adverse Event Reporting (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- **Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.**

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office. -

- **Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.**
- **Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.**

- **Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.**

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

8.2.1 PDMS/CORe

PDMS/CORe will be used as the electronic case report form (CRF) for this protocol and all protocol specific data will be entered into PDMS/CORe.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

8.3 Emergency Procedures

Procedures in Case of Pregnancy

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study. Should pregnancy occur during a subject's trial participation, the subject will immediately be discontinued from the trial and followed-up per protocol. All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. All outcomes of pregnancy must be followed-up and documented.

8.4 Procedures in Case of Overdose

There is no specific treatment for an overdose of VPA. In case of overdose, therapy may be interrupted, and any adverse reactions treated symptomatically.

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