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PRINCIPAL INVESTIGATOR: Richard Fisher, M.D.

CONTRACTING ORGANIZATION: University of Rochester
Rochester, NY 14627

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Richard Fisher, M.D.

E-Mail: richard_fisher@urmc.rochester.edu

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14. ABSTRACT
This grant program encompasses two complimentary projects. The hypothesis that leukemia can be treated effectively by inhibition of putative cancer stem cells will be tested in project #1. This will be done by application of inhibitors of stem cells as a novel approach for eradication of leukemia tumor cells. Parthenolide (PTL)-based drugs and related drugs that inhibit nuclear factor kappa B (NF-κB) will be used. The effects of these drugs will also be tested on normal hematopoietic cells. In project 2, studies will investigate how standard therapies affect normal CNS stem cells, and will attempt to develop less toxic regimens for the treatment of brain cancers. To this end, studies will determine whether parthenolide or related drugs cause CNS damage in animals treated with these substances, and will assess whether parthenolide can function as a chemosensitizing agent for various conventional chemotherapy drugs.

15. SUBJECT TERMS
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Introduction

This grant is comprised of two complementary projects. For the purposes of this report, progress for each project will be described separately below.

Body

Project 1

The objective of this project is to develop a novel therapeutic agent that specifically targets human leukemia stem cells (LSC). While the concept of a critical leukemia stem cell in myeloid disease has been postulated for over three decades, to date no therapeutic agent has been identified that specifically and preferentially ablates LSC in vivo. Thus, the central premise of this grant is that direct targeting of LSC will yield more effective therapy for leukemia. Previously, we demonstrated that parthenolide (PTL) is highly cytotoxic to LSC in vitro, but does not significantly affect normal hematopoietic stem cells (HSC). However, solubility of PTL is limiting; thus we have generated a PTL analog, dimethyl amino parthenolide (DMAPT), that is much more soluble in water and retains the anti-leukemic activity of PTL. Using this agent, the tasks below were specified:

SOW task #1: To demonstrate that a parthenolide analog can be used for preclinical and clinical applications related to treatment of chronic leukemia (Months 1-36).

Progress: As noted in the year 1 report, we have tested DMAPT against a panel of primary chronic lymphocytic leukemias (CLL) and shown that the compound has significant activity. The LD50 was 3.0 micromolar, in good agreement with the studies of Hewamana et al (Blood, 2008, 111:4681) (These findings are further corroborated by the drug combination studies outlined below).

In collaboration with Dr. Chris Pepper at Cardiff University, we have begun to explore parthenolide based drugs in combination with other agents. Notably, we have demonstrated that DMAPT (clinical designation LC-1) in combination with the conventional agent fludarabine induces synergistic death of CLL cells (see figure 1)

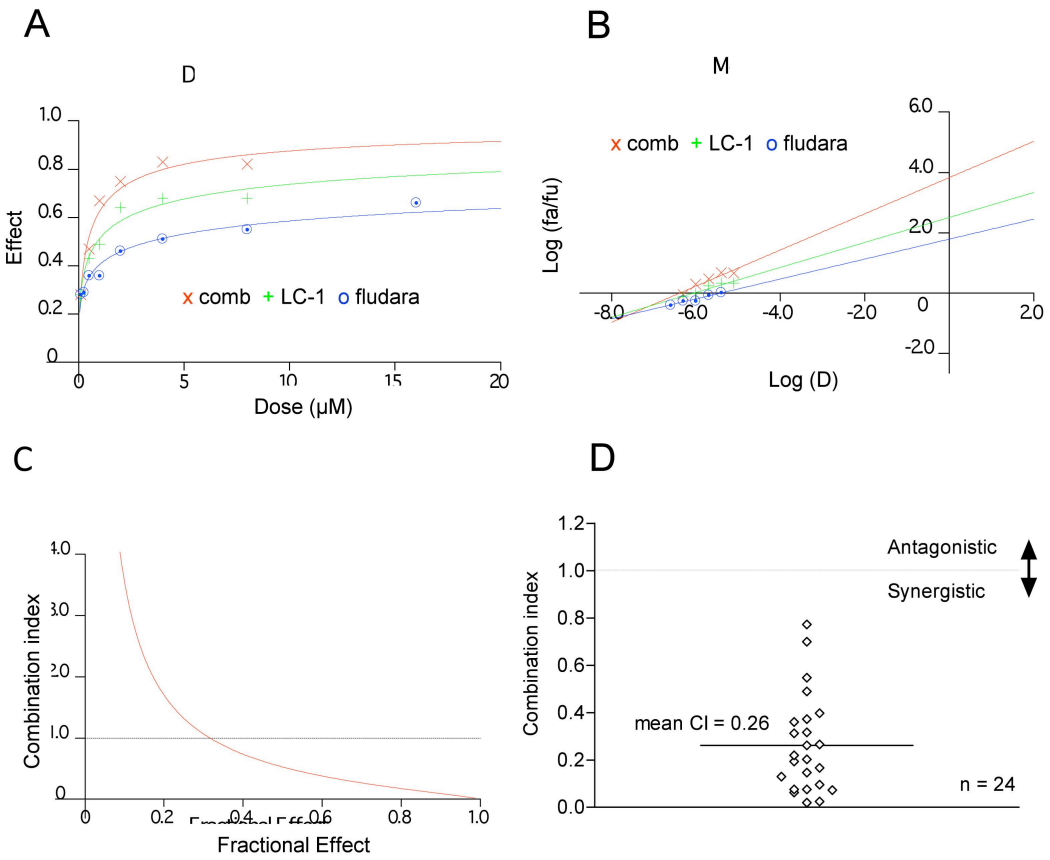


Figure 1. Synergistic effect of LC-1 with fludarabine in primary CLL cells. CLL cells were treated for 24h with LC-1 (0, 0.5, 1, 2, 4, 8μM) and / or fludarabine (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8, or 16μM) at a fixed molar ratio of 2:1. Cytotoxicity was quantified using an Annexin V/ propidium iodide assay. The median-effect plot was constructed using Calcusyn software where Fa = fraction affected and Fu = fraction unaffected. **(A)** Dose-response curve for CLL cells treated with LC-1 and/or with fludarabine. **(B)** Median-effect plot for CLL cells treated with LC-1 and/or with fludarabine. **(C)** The CI plot was constructed by computer analysis of the data in (B) using the conservative isobologram. Combination index values of less than 1 occurred at a wide range of Fa levels, indicating that synergy was produced by the combination. **(D)** Distribution of CI among 24 patients tested with mean value of 0.26 ± 0.20.

Methods

Peripheral blood samples from 42 patients with CLL were obtained with the patients' written informed consent (LREC # 02/4806). CLL was defined by clinical criteria as well as cellular morphology and the co-expression of CD19 and CD5 in lymphocytes simultaneously displaying restriction of light-chain rearrangement. Clinical information including treatment histories was available for all patients and none of the previously treated patients had received chemotherapy within 3

months prior to sample collection. Peripheral blood lymphocytes from 42 CLL patients were isolated from freshly collected blood samples by density centrifugation. The purity of the $CD5^+/CD19^+$ CLL cells was >96% as assessed by flow cytometry. After experimentally determining the optimal molar ratio for the combination of LC-1 and fludarabine (2:1), CLL lymphocytes were cultured in the presence of LC-1 (0.5 to 8 μM) and / or fludarabine (0.25 to 16 μM) for 24 and 48 hours. Control cultures were also carried out in which no drug was added. Apoptosis was assessed by dual-color immunofluorescent flow cytometry as described previously.[14;15] The novel compound LC-1 is a dimethylamino derivative of the naturally-occuring small molecule parthenolide. LC-1 (also known as DMAPT) was prepared from the reaction of parthenolide with dimethylamine, and the resulting dimethylamino analog was then converted to its water-soluble fumarate salt.

These studies described above were reported in a manuscript that was published in *Clinical Cancer Research*. However, due to an unfortunate error, this DOD grant was not cited. We apologize for this oversite, and will ensure that no such errors occur in the future.

SOW task #2: To demonstrate that a parthenolide analog can function as a chemosensitizing agent to enhance ablation of chronic leukemia cells (Months 37-60).

Progress: Not started yet

Project 2

The primary goal of this project is to investigate how standard therapies effect normal CNS stem cells and to develop less toxic regimens for the treatment of cancer:

SOW task #1: To determine whether parthenolide or parthenolide analogs cause CNS damage in animals treated with these substances, and to determine whether parthenolide or parthenolide analogs enhance the damage caused by cytarabine. (Months 1-24)

The toxicity for CNS progenitor cells in vitro revealed in our first year's work on parthenolide, and the toxicity seen for cytarabine, indicated that it was necessary to examine other relevant chemotherapeutic agents to try and identify a relatively non-toxic alternative that would provide a valuable control group for the combination of the parthenolide with other treatment modalities.

We have conducted in vivo studies on vincristine (an anti-mitotic agent that disrupts microtubule function, and is used in treatment of non-Hodgkin's

lymphoma, lymphoma, acute lymphoblastic leukemia and childhood leukemia), cyclophosphamide (a nitrogen mustard alkylating agent used in treatment of lymphomas and some forms of leukemia), etoposide (a topoisomerase II inhibitor used in the treatment of lymphoma and non-lymphocytic leukemia) and busulfan (an alkylating antineoplastic agent that earlier was a front-line agent for treatment of chronic myeloid leukemia and currently is used sometimes in treatment of chronic lymphocytic leukemia and more frequently as a conditioning drug for bone marrow transplantation).

We also have extended the regions of the CNS examined to initiate studies on the olfactory system. Loss of smell (anosmia) is a fundamental quality of life issue for cancer patients due to the loss of taste that accompanies loss of smell. While this seems a relatively minor issue, the loss of appetite in cancer patients is considered to be a serious quality of life issue.

Two other attractive features of including the olfactory system in our examinations is that nerve cells in both the olfactory epithelium of the nasal cavity in the CNS in the olfactory bulb occurs throughout life in both mice and humans. This allows us to consider toxic effects of treatment on additional precursor cells, including ones that migrate from the anterior region of the subventricular zone lining the ventricles in the cortex to the olfactory bulb. In addition, an understanding of toxicity in this area may eventually lead to the establishment of assays of physiological function that can be conducted in both patients and experimental animals.

Our in vivo studies have identified adverse CNS effects of all drugs tested except busulfan. Vincristine, cyclophosphamide and etoposide all compromise CNS precursor cell function in vivo.

In addition, we have now established expertise with the use of our spinning disk confocal microscope and associated software in the analysis of chemotherapy-induced toxicity to the CNS. This has greatly speed up our in vivo analyses.

Detailed results are presented for the analysis of vincristine and cyclophosphamide, as well as our preliminary results on the effects of cisplatin (another widely used chemotherapeutic agent) and these other agents on the olfactory system.

The subventricular zone (SVZ) is an area in the brain highly populated by progenitor cells, and has previous been observed to be sensitive to systemic cisplatin up to 6 weeks after treatment. One day after treatment, a consistent decrease with cisplatin was observed in the migrating progenitor cells (PSA-NCAM+/Ki67-), proliferating cells (Ki67+/PSA-NCAM-), and migrating proliferating cells (PSA-NCAM+/Ki67+) (Figure 1). These results are consistent with those seen in the olfactory epithelium. In this region, horizontal and basal dividing precursor cells were labeled with BrdU and counted. It was determined that cisplatin treatment significantly decreased BrdU+ cell numbers (Figure 2).

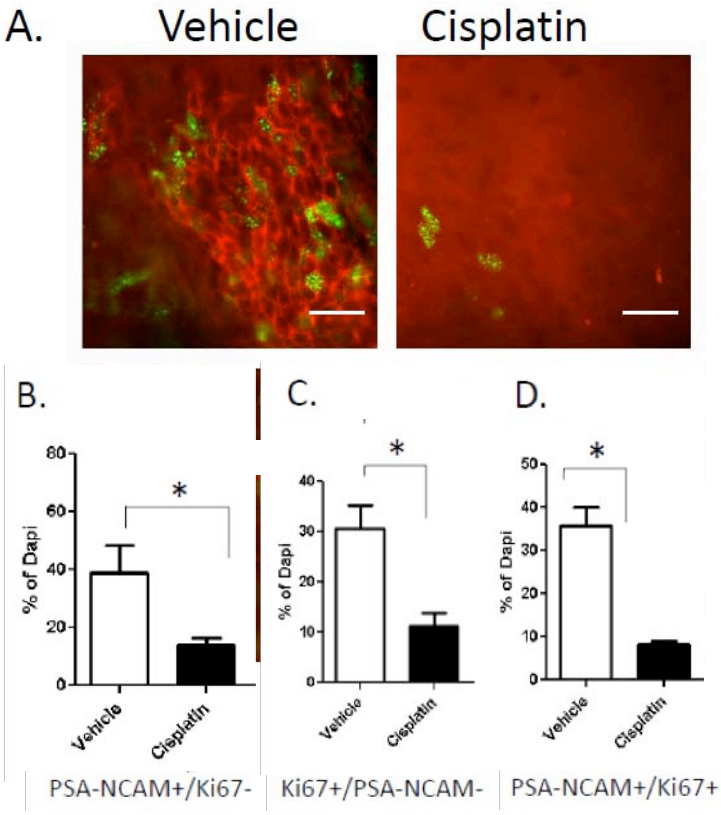


Figure 1. Cisplatin induced sensitivity in the SVZ. Mice treated with cisplatin were sacrificed 1 day after treatment. **A.** Representative confocal images of the SVZ. Sections were immunolabeled for PSA-NCAM (red) and Ki67 (green). **B.** With cisplatin there was a significant decrease in migrating progenitor non-proliferating cells (PSA-NCAM+/Ki67-), **C.** non-migratory proliferating cell population (PSA-NCAM-/Ki67+), **D.** migratory proliferating cell population (PSA-NCAM +/Ki67-) 1 day after treatment. n=5, Scale bar 25uM, *p<0.05.

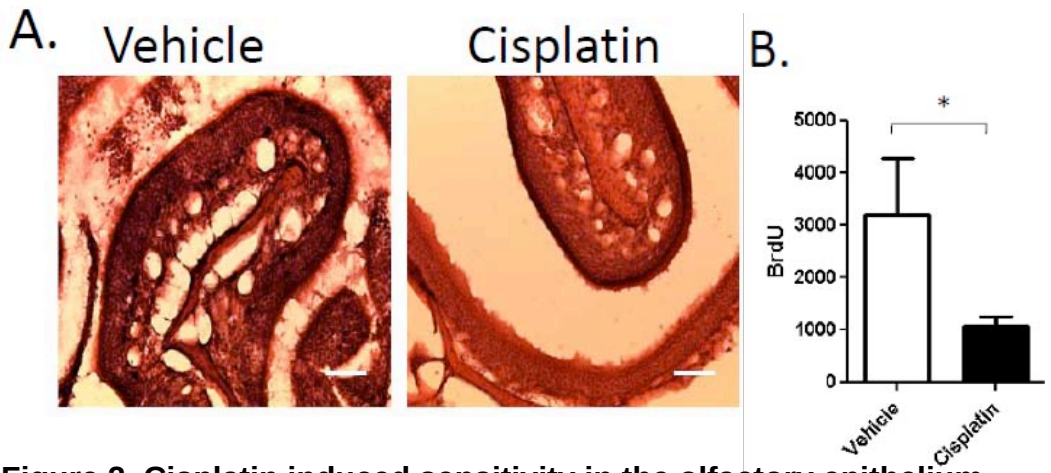
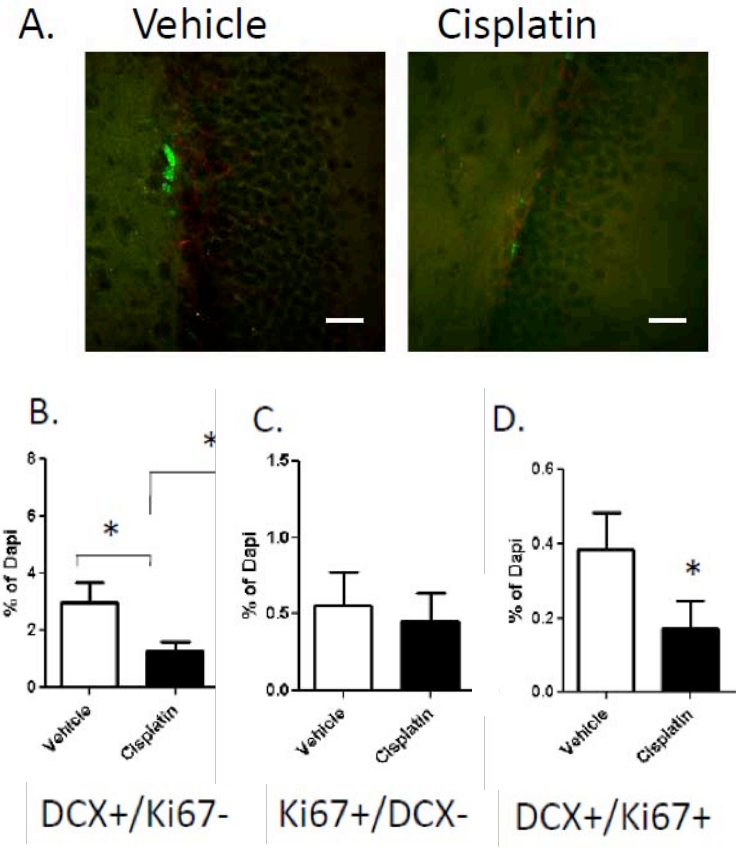


Figure 2. Cisplatin induced sensitivity in the olfactory epithelium. Mice treated with cisplatin were sacrificed 1 day after treatment. **A.** Representative images of the olfactory epithelium. Sections were stained for BrdU and hematoxylin and eosin. **B.** Cisplatin and cisplatin+erythropoietin treatment results in a significant decrease in BrdU+ cells in the olfactory epithelium. n=5, Scale bar 100uM, *p<0.05.

Figure 3. Cisplatin treatment in the dentate gyrus. Mice treated with cisplatin were sacrificed 1 day after treatment. **A.** Representative confocal images of the dentate gyrus. Sections were immunolabeled for doublecortin (DCX, red) and Ki67 (green). **B.** With cisplatin or there was a significant decrease in neuro-progenitor non-proliferating cells (DCX+/Ki67-) **C.** No changes were observed between treatment groups in the proliferating cell population (DCX-/Ki67+). **D.** Cisplatin treatment significantly affected the neuro-progenitor proliferating cell population (DCX +/Ki67-) 1 day after treatment. n=5, Scale bar 25uM, *p<0.05.



Other areas of proliferation also were examined. Neural progenitor cells migrate from the subgranular zone to the dentate gyrus where they differentiate into granule neurons. One day after cisplatin treatment, the neuroblast non-proliferating (DCX+/Ki67-) and neuroblast proliferating (DCX+/Ki67+) populations were significantly decreased compared to vehicle. The non-neuroblast proliferating population was unaffected (Figure 3).

We next examined the effects of vincristine and cyclophosphamide, drugs which are commonly used in treatment of both adult and childhood cancers. To expand our data set so as to include information on the vulnerability of younger treatment cohorts, 3 week old mice instead of 8 week old mice were used.

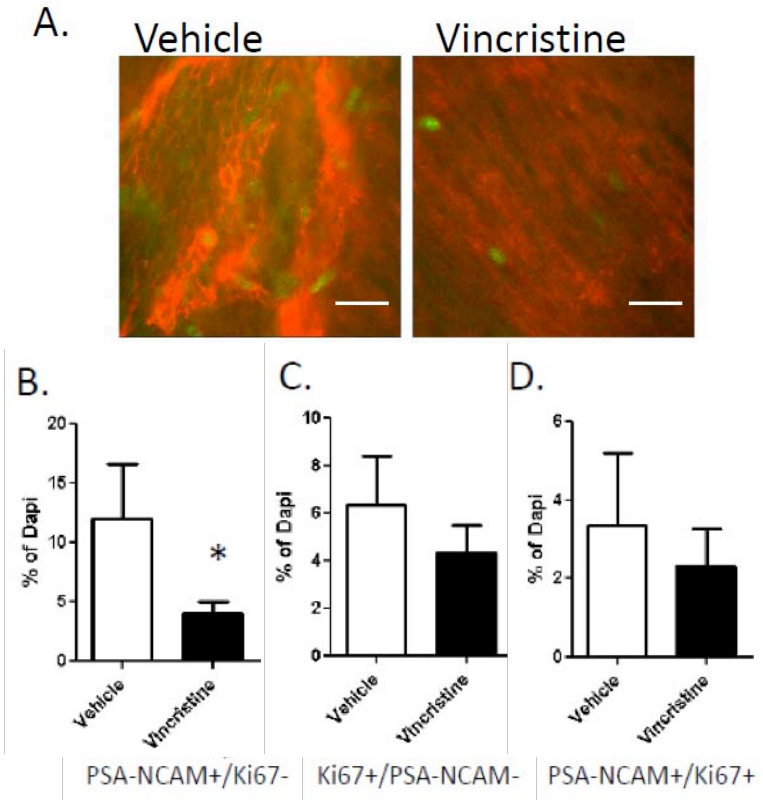


Figure 4. Vincristine induced damage in the SVZ. Mice treated with vincristine were sacrificed 1 day after treatment. **A.** Representative confocal images of the SVZ. Sections were immunolabeled for PSA-NCAM (red) and Ki67 (green). **B.** With vincristine there was a significant decrease in migrating progenitor non-proliferating cells (PSA-NCAM+/Ki67-) **C.** With treatment, there were no changes in the non-migratory proliferating cell population (PSA-NCAM-/Ki67), **D.** nor the migratory proliferating cell population (PSA-NCAM +/Ki67-) 1 day after treatment. n=5, Scale bar 25uM, *p<0.05.

The subventricular zone was first examined, and in mice treated with vincristine there was a significant decrease in migrating non-proliferating progenitors (PSA-NCAM+ /Ki67-). Vincristine did not reduce the proliferating (Ki67+/PSA-NCAM-) nor migrating proliferating (PSA-NCAM+/Ki67+) progenitors (Figure 5).

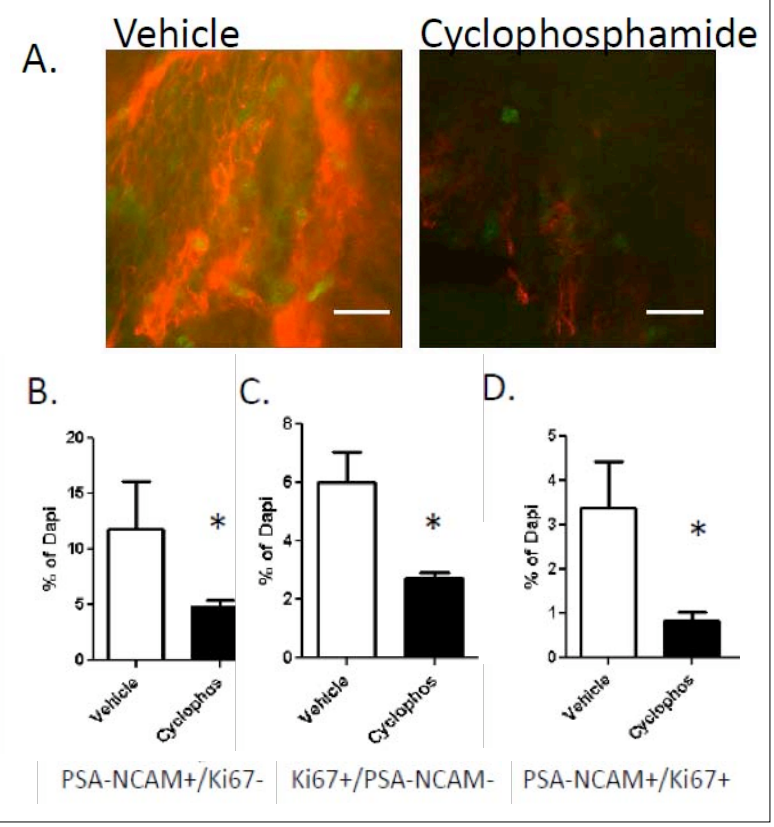


Figure 5. Cyclophosphamide induced damage in the SVZ. Mice treated with cyclophosphamide were sacrificed 1 day after treatment. **A.** Representative confocal images of the SVZ. Sections were immunolabeled for PSA-NCAM (red) and Ki67 (green). **B.** With cyclophosphamide there was a significant decrease in migrating progenitor non-proliferating cells (PSA-NCAM+/Ki67-) **C.** Cyclophosphamide significantly decreased the non-migratory proliferating cell population (PSA-NCAM-/Ki67), **D.** and also the migratory proliferating cell population (PSA-NCAM +/Ki67-). n=5, Scale bar 25uM, *p<0.05.

Cyclophosphamide treatment also caused a decrease in the migrating non-proliferating progenitor population (PSA-NCAM+/Ki67-). Cyclophosphamide additionally negatively affected the proliferating (Ki67+/PSA-NCAM-) and migrating proliferating (PSA-NCAM+/Ki67+) progenitor cell populations(Figure 6).

The effects of vincristine and cyclophosphamide were next examined in the dentate gyrus and subgranular zone of the hippocampus. Systemic vincristine treatment resulted in a significant decrease in the non-proliferating neural progenitor cells (DCX+/Ki67-). No significant changes were observed in the proliferating non-neural progenitor cell population (Ki67+/DCX-), nor in the proliferating neural progenitor cell population (DCX+/Ki67+). Similar results were seen in the dentate gyrus of young mice treated with cyclophosphamide. Here, the neural progenitor non-proliferating cell population (DCX+/Ki67-) were negatively affected. Similar to vincristine treatment, no changes were determined in both proliferating cell populations: the non-neural progenitor cells (Ki67+/DCX-) and also the neural progenitor cells (DCX+/Ki67+).

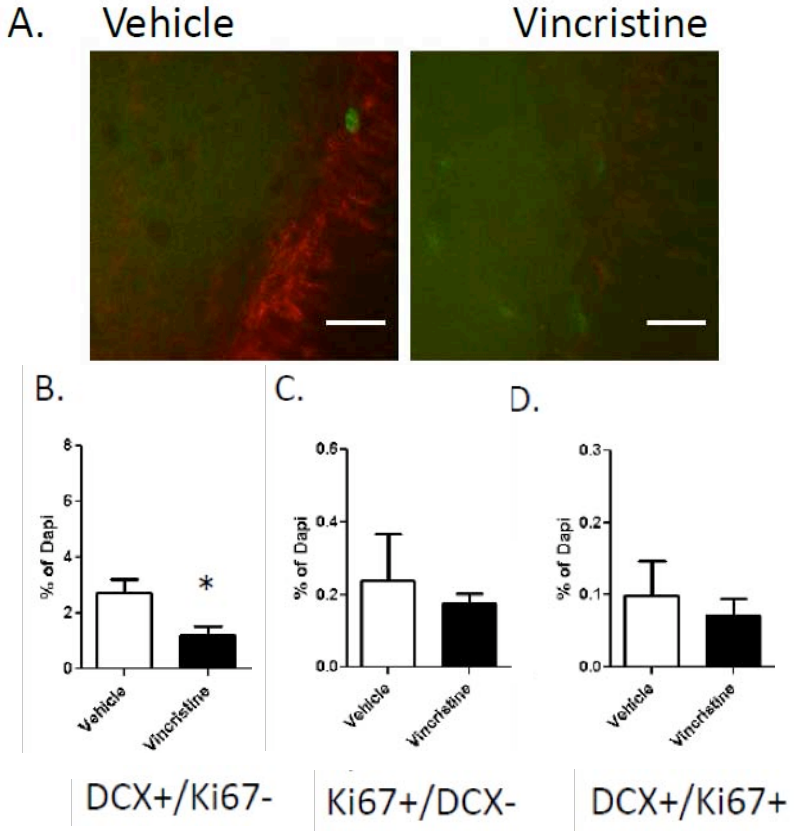


Figure 6. Vincristine treatment in the dentate gyrus. Mice treated with vincristine and/or EPO were sacrificed 1 day after treatment. **A.** Representative confocal images of the dentate gyrus. Sections were immunolabeled for doublecortin (DCX, red) and Ki67 (green). **B.** With vincristine there was a significant decrease in the neuro-progenitor non-proliferating cells (DCX+/Ki67-) **C.** In the proliferating non neuroprogenitor cell population (DCX-/Ki67+), vincristine treatment caused no significant changes. **D.** Vincristine treatment did not affect the neuro-progenitor proliferating cell population (DCX +/Ki67-) 1 day after treatment. n=5, Scale bar 25uM, *p<0.05.

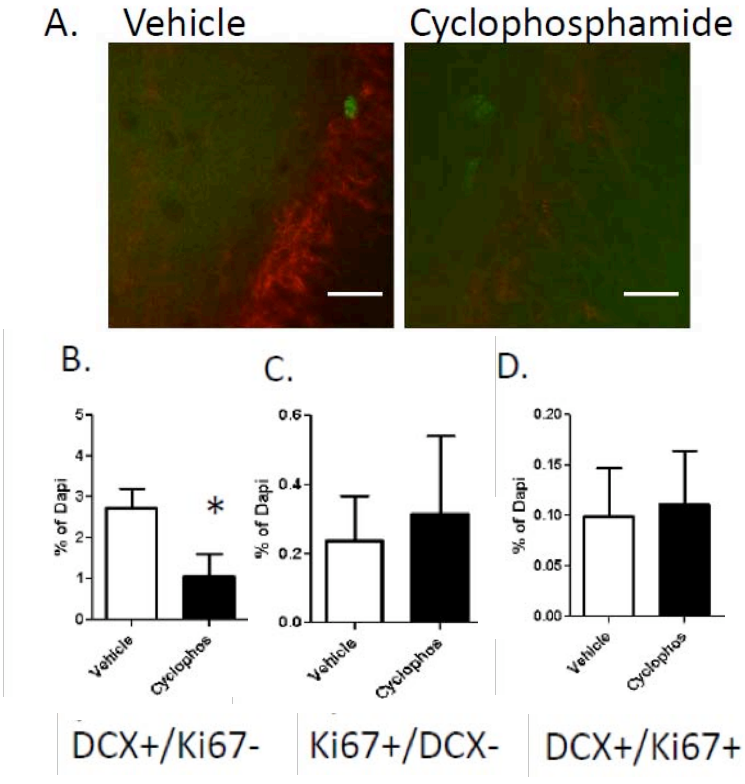


Figure 7. Cyclophosphamide treatment in the dentate gyrus. Mice treated with cyclophosphamide and/or EPO were sacrificed 1 day after treatment. **A.** Representative confocal images of the dentate gyrus. Sections were immunolabeled for doublecortin (DCX, red) and Ki67 (green). **B.** Cyclophosphamide caused a significant decrease in neuroprogenitor cells (DCX+/Ki67-) **C.** In the proliferating non neuroprogenitor cell population (DCX-/Ki67+), there were no significant changes. **D.** Cyclophosphamide treatment did not affect the neuro-progenitor proliferating cell population (DCX +/Ki67-) 1 day after treatment. n=5, Scale bar 25uM, *p<0.05.

Busulfan, in contrast, thus far has no detectable adverse effects in vivo on CNS

progenitor cells. Based on the lack of adverse effects of busulfan in vivo, we examined its activities in vitro and found it also to be relatively non-toxic for purified populations of CNS precursor cells – thus making this compound the only chemotherapeutic agent (out of ten examined thus far) that is not overtly toxic for these cells at clinically relevant exposure levels.

The lack of toxicity of busulfan in vitro is particularly striking in that it does not even increase the toxicity of other physiological stressors. For example, one of the clearest cases in which busulfan is applied in the presence of another known physiological stressor of the CNS is in a genetic leukodystrophy called Krabbe disease. This disease is known to be due to an enzymatic deficiency in the CNS, and current treatments involve ablating the bone marrow of affected children (with chemotherapy) and then carrying out a bone marrow transplant.

As an example of the lack of toxicity of busulfan, Figure 8 shows data from an experiment examining busulfan either alone or in combination with 10 μM psychosine, a concentration of this lipid that kills 70-75% of oligodendrocyte progenitor cells. Even at concentrations of busulfan up to 100 μM there is no killing of progenitor cells, nor any enhancement of killing by psychosine.

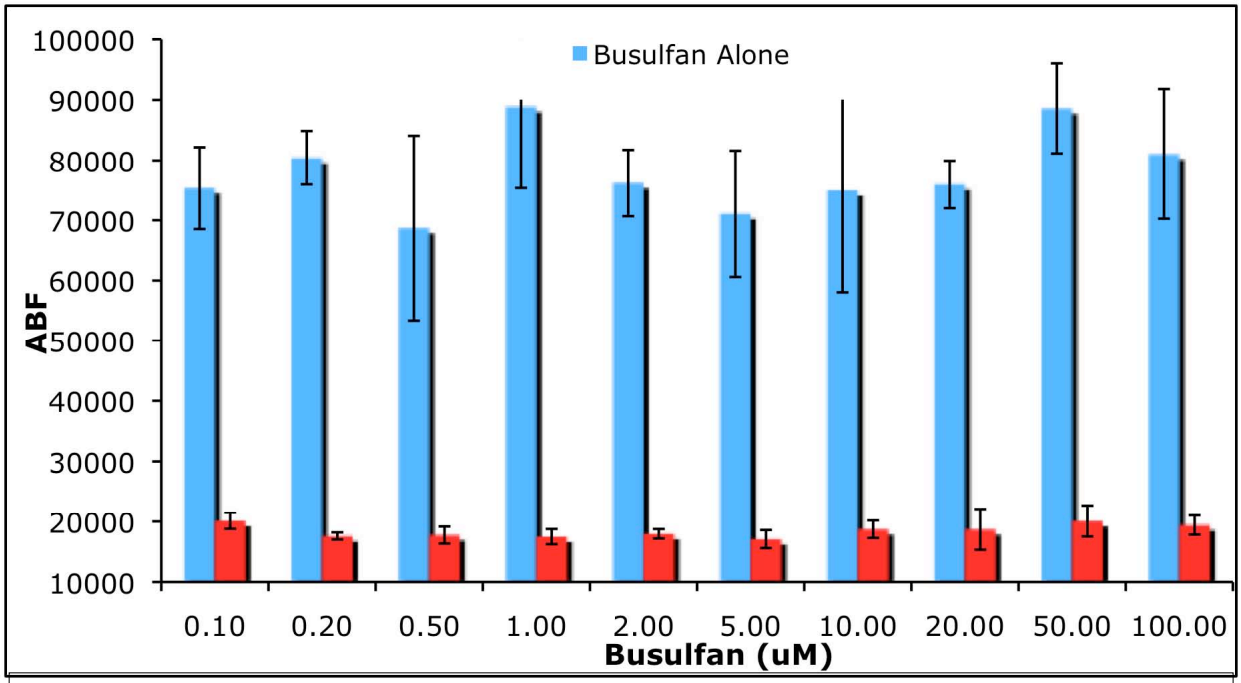


Figure 8. Busulfan is not toxic for oligodendrocyte precursor cells, nor does it enhance the toxicity of psychosine. Even when psychosine was applied at a concentration of 10 μM, which kills 70-75% of oligodendrocyte progenitor cells, the additional presence of busulfan at concentrations from 0.10 to 100 μM was not able to increase this toxicity. Similar results were obtained when progenitor cells were exposed to 1 μM, except that this concentration of psychosine was not toxic and the additional presence of busulfan did not lead to any toxicity reactions.

The discovery of the relative lack of toxicity of busulfan both in vivo and in vitro provides us with a vital tool for the examination of parthenolide in vivo, both by itself and in combination with other agents. If [busulfan + parthenolide] together are not toxic this will enable us to determine that neurotoxicity is not an inevitable outcome of the use of established chemotherapeutic agents with parthenolide. In contrast, if the combination of these agents is enough to increase parthenolide toxicity, this will encourage us to focus all remaining efforts on learning how to ameliorate such toxicity.

The baselines established thus far enable us to now properly test the hypothesis that parthenolide is safe for the CNS but only when used in conjunction with agents that do not open the blood brain barrier or otherwise cause CNS toxicity.

Methods

In vivo analysis: 6-8 week male C57/Bl6 mice were treated with three intraperitoneal doses of 5mg/kg cisplatin, 40mg/kg 5-fluorouracil or 0.9% NaCl vehicle control once every 48 hours. Animals were then sacrificed one day, 6 weeks, and 6 months after treatment, as in previous studies [1, 2]. 3-4 hours before sacrifice, mice were injected intraperitoneally with 20mg/kg BrdU. Mice were perfused under deep anesthesia using sodium pentobarbital 40mg/kg first with 2U/ml heparin then with 4% paraformaldehyde (PFA). The cortex was then dissected and submerged in 4% PFA for 2 hours and cryoprotected in 30% sucrose. The right or left hemisphere of the cortex was randomly chosen and sectioned sagittally into 50µm sections that were obtained using a microtome and placed free floating in PBS with 0.02% NaN3.

In addition to the analysis of the CNS, the nasal cavity was dissected after perfusion and submerged in 4% PFA. After 2 hours, the nasal cavity was placed in 0.5M EDTA and rocked at 4C for 1 week when it was placed in increasing concentrations of sucrose to a maximum concentration of 30% sucrose. The tissue was then placed in optical cutting temperature compound (OCT)(Tissue Tek) and frozen using chilled isopentane, and sectioned to expose the olfactory epithelium on the cryotome into 12µm sections, and mounted onto superfrost plus microscope slides (VWR).

Immunofluorescence, TUNEL staining, and BrdU incorporation: Free floating 50um sections were rinsed twice in TBS (0.9% NaCl\0.1M Tris-HCl pH 7.5) then permeabilized for 30 minutes using 0.5% Triton X-100 in TBS+ (TBS/0.1% Triton X 100/3% donkey serum). Sections were then immunolabeled with antibody in TBS+, with the dilution was determined empirically to optimize the signal to background ratio. Antibodies used were anti-PSA-NCAM (1:500 Chemicon MAB326) a marker of migrating progenitor cells, Ki67 (1:500 BD 550609) a proliferation marker, DAPI (1:2000 Molecular Probes D1306) a nuclear marker,

anti-Olig2 (1:500 Chemicon AB 9610) a marker of oligodendrocyte precursors and mature oligodendrocytes, CC1 (1:500 Calbiochem OP80) a marker of mature oligodendrocytes, anti-doublecortin (1:250 Cell Signaling 4604) a marker of neural precursors, and anti-GFAP (1:500 Cell Signaling 3670) a marker of astrocytes and type B stem cells. *You should have references for each of these markers. Samples were set at 4C on a rocker and incubated over 2 nights. The sections were then washed 3 times with TBS and blocked with TBS+ for 30 min then incubated at RT with the respective secondary antibodies for 4 h in TBS+. All secondary antibodies, generated in donkey or goat (anti-rat, anti-rabbit, and anti-mouse) were coupled to Alexa 488, Alexa 594 or Alexa 680 for *in vivo* staining and were used according to the species of the primary antibody. After 6 washes with TBS, sections were mounted on VWR superfrost microslides and coverslipped using Prolong Antifade (Molecular Probes).

A terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay was used to detect DNA fragmentation, a hallmark of apoptosis, using the ApopTag Fluorescein Direct In Situ Apoptosis Detection Kit (Millipore S7160). Sections were rinsed in TBS then dehydrated using increasing concentrations of ethanol (50%, 70%, 90% and 100%), then rehydrated with decreasing concentrations of ethanol and then water. The sections were rinsed in TBS 3 times and washed in the provided equilibrium buffer for 1 min, and immersed in the reaction buffer with the TdT enzyme for 1 h at 37C. The TdT enzyme acts by recognizing and labeling the broken 3'-OH DNA strands with modified nucleotides, detecting single and double stranded DNA breaks. The TdT enzymatic reaction was terminated using the provided Stop Buffer for 10 min and then the sections were rinsed 3 times in TBS. The fragmented DNA was then detected using the anti-digoxigenin-FITC antibody.

BrdU is a nucleoside analogue of thymidine and is incorporated into DNA during S phase. It is used here to mark proliferating cells in the olfactory epithelium and also in a BrdU pulse chase assay to identify the progeny of proliferating cells. 2 weeks after the BrdU pulse, the cells that continue proliferating will have diluted the BrdU that was incorporated into their DNA, while those that undergo differentiation will not undergo such serial dilutions. The BrdU dose administration protocol is described below. For cellular BrdU unmasking, the DNA was denatured and sections were incubated for 2 hr in 50% formamide/ 2x SSC (0.3M NaCl and 0.03M sodium citrate) at 65C, rinsed for 8 min in 2x SSC, incubated for 30min in 2 N HCl at 37C, and then for 10 min in 0.1M borate buffer pH 8.5. The sections were then washed in 6 changes of TBS and incubated in TBS+ for 30 min and incubated with rat anti-BrdU antibody (Accurate Chemicals OBT0030 1:500) over 2 nights rocking at 4°C. For the BrdU pulse/chase assay, cells were double-labeled using the above antibodies.

For the olfactory epithelium, BrdU unmasking and antibody labeling was carried out as above. As a secondary antibody, Donkey anti-rat-biotin was incubated with the slides for 2 hours, rinsed 3 times in TBS then incubated with Avidin:biotinylated enzyme complex (ABC Elite, Vector Labs) for 1 hour. After rinsing 5 times with TBS, slides were incubated with DAB (10mg tablet Diaminobenzidine /0.009%H2O2 /0.04%NiCl) then rinsed 3 times in H2O and 2

times in TBS. Slides were then immersed in Hematoxylin for 20 seconds, and rinsed in H_2O 5-10 times, and once in 0.3% Sodium Borate. After washing 5-10 times in H_2O, slides were dipped in Eosin for 20 seconds and rinsed in H_2O 5-10 times. Slides were then dehydrated in 95% ETOH 2 times, 10 seconds each and then 99% ETOH 2 times for 10 seconds each, then xylene for 10 seconds twice. After drying, coverslips were mounted with permount.

Quantification of cell proliferation and cell death: Antibody labeling was detected using an Olympus BX51WI Disk Spinning Unit Confocal Microscope. Images were obtained with a Hammamatsu EM-CCD Digital Camera and processed using Adobe Photoshop CS3 Imaging Software. Cells were counted in every sixth section (50µm) from the sagittal series, using StereoInvestigator (Microbrightfield Systems, Willington VT), and the included Optical Fractionator Workflow using a 60x oil immersion objective lens, and 40x objective lens for analysis of the cortex. For the subventricular zone, rostral migratory stream and the dentate gyrus an exhaustive count was done. For the olfactory bulb and the corpus callosum a systematic random sample set was generated with each grid measuring 200µm x 200µm and the optical fractionator counting frame measuring 100µm x 100µm. The thickness of each section following processing was measured using the Stereoinvestigator system XYZ stage controller with the average thickness being reduced to 60% of the initial thickness

For all cell counting, the contour of the area of interest was first traced using DAPI staining at 4X and the top of the section was set for every other counting frame by manual focusing, with a guard zone of 2µm given on the top and bottom of each section. Using the Acquire SRS Image Series Workflow, images from each counting frame were acquired with the use of a computer driven motorized stage to shift through the region of interest. For all regions, cells were counted on a work station computer using the Optical Fractionator Workflow probe. Total cell number was counted using DAPI to identify cell nuclei, and was counted separately due to differences in immunofluorescence intensity. For the regions where a non-exhaustive count was done, total cell number was estimated using the optical fractionator formula: N=1/section sampling area x 1/area sampling section (area sampled/total area of the region of interest) X 1/height sampling fraction X ΣQ (total number of cells counted).

BrdU Pulse/Chase Assay: C57/Bl6 male 8 week old mice were given 3 intraperitoneal doses of vehicle (0.9% NaCl), cisplatin (5mg/kg) or 5-FU (40mg/kg) with one dose every 48 hours. 1 week after the last dose, mice were given 20mg/kg BrdU daily for 5 days and 2 weeks later the mice were sacrificed and perfused as described above. The cortical tissue was sectioned 50µm. To visualize BrdU-DNA incorporation, the tissue was processed following the BrdU processing protocol as described above.

SOW task #2: Demonstrate that mice in which purified cells are more oxidized in vitro will exhibit more extensive damage from cytarabine, parthenolide (or

parthenolide analogs) or the combination of these agents, than those in which purified cells are intrinsically more reduced (Months 25-48).

Progress: Not started yet.

SOW task #3: To initiate identification of potential prognostic indicators to detect individuals at greater risk for adverse side effects of therapy with cytarabine, parthenolide (or parthenolide analogs) or the combination of these agents, and begin testing to provide proof of principle for protective strategies that involve administration of N-acetyl-L-cysteine (alone or in combination with Vitamins E and/or C) as an anti-oxidant to protect against oxidative damage (Months 49-60).

Progress: Not started yet.

Key Research Accomplishments

Analysis of multiple chemotherapeutic agents used in the treatment of lymphomas and .leukemias leading to identification of a single agent (busulfan) that is not associated with CNS toxicity. This discovery provides the crucial control condition needed for our analysis of parthenolide by providing a drug that can be used in combination with parthenolide but which by itself does not compromise CNS precursor cell function. In addition, we have increased our knowledge of the profile of toxic agents and added additional regions of the CNS (olfactory epithelium, olfactory bulb and rostral migratory stream) that give us a still more detailed understanding of the toxicity of chemotherapy.

Reportable outcomes

Pending

Conclusions

Parthenolide-based drugs appear promising as agents for treatment of CLL, but identification of possible combinatorial strategies may be needed to optimize treatment while minimizing toxicity.