Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 25 - Epigenetic Therapy of Human B Cell Malignancies

7. **Start and End Date of Research Project:** 9/1/2009 – 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Elliot Epner, MD, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 463,644

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Epner	Principal Investigator	16.6%	$79,682
Brucklacher	Core Facility Senior Gene Expression Analyst	50%	$52,956
Bixler	Core Facility Gene Expression Analyst	25%	$23,350
Stuart	Technician	100%	$43,140
Dong	Technician	47.5%	$15,219
Attia	Technician	100%	$14,424

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Shimko	Technician	100%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____X____ No_____

If yes, please indicate the source and amount of other funds:

Lymphoma Research Foundation: $125,000

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Combined epigenetic therapy for the treatment of follicular lymphoma	☐NIH ☐ Other federal (specify:) X Nonfederal source (specify: Lymphoma Research Foundation	02/13	$600,000	Pending
Combined epigenetic and monoclonal therapy for chronic lymphocytic leukemia	☐NIH ☐ Other federal (specify:) X Nonfederal source (specify: Lymphoma Research Foundation)	02/13	$250,000	Pending
Combined epigenetic therapy for the treatment of mantle cell lymphoma	☐NIH ☐ Other federal (specify) ☒ Nonfederal source (specify: Lymphoma Research Foundation)	2/13	$250,000	Pending

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

Future grants investigating the transcriptional role of Cyclin D1 in mantle cell lymphoma are

planned to be submitted to the NIH. Also, future trials incorporating other agents, such as the proteasome Bortezomib are being recruited, and funding will be sought for similar correlative/translational science.

12. Future of Research Project. What are the future plans for this research project?

We plan on submitting multiple publications based on this data. One will likely be clinical in nature, focusing on the SCR trial results along with some correlative science, possibly the G870A polymorphism. Another paper will deal with CD20 regulation, specifically by a SCR-resistant cell line created from one of the blastic patients on this study. A third paper will likely focus on cyclin D1's action as a transcription factor.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			2	
Female				
Unknown				
Total			**2**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			2	
Unknown				
Total			**2**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian			1	
Other				
Unknown				
Total			**2**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project expanded the scope of clinical studies related to cancer treatment at Penn State Hershey and thereby improved patient access to state-of-the-art experimental therapies.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

Two major collaborations are ongoing- one with Dr. Samir Parekh at the Albert Einstein College of Medicine involving high-throughput screening of methylation patterns and gene expression patterns of pre- and post-treatment patient lymphoma cells. The second is with Dr. Mirit Aladjem at the NCI, regarding a possible resistance mechanism to combination epigenetic and anti-CD20 monocolonal therapy in the form of downregulation of the CD20 protein.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

We have identified a potent, nontoxic combination therapy of cladribine and rituximab for B cell malignancies such as CLL and MCL. Our hypothesis is that cladribine is reactivating silenced genes in malignant B cells that potentiate rituximab's activity. We also have preliminary evidence that the addition of a histone deacetylase inhibitor potentiates the activity of this combination. We will further investigate the activity and mechanism of action of this epigenetic therapy in this proposal both in patient samples and in cell lines.

Our *long-term goal* is to develop new nontoxic combination therapies for the treatment of B cell malignancies. This goal is based on our discovery of the fact cladribine is an effective epigenetic agent particularly when used in combination with the monoclonal antibody rituximab.

The *objective of this project* is to develop, novel, nontoxic combination epigenetic and immunotherapies for the treatment of B cell malignancies and to understand their mechanism of action. The Penn State Cancer Institute in collaboration with Oregon Health Sciences University is doing a phase I/II clinical trial of combination therapy with vorinostat, cladribine, and rituximab in patients with B cell malignancies. However, the support for this clinical trial is not the goal of the current studies. Thus, the *central hypothesis* for these studies is that epigenetic therapy for B cell malignancies using this combination will be safe and effective. This discovery would be highly significant, demonstrating important therapeutic implications of combination epigenetic and immunotherapy for B cell malignancies. We formulated this hypothesis based on published and anecdotal studies showing that cladribine plus rituximab is an effective treatment for B cell malignancies. Published data also demonstrates that cladribine has a cryptic epigenetic activity.

The following *Specific Aims are proposed to accomplish this objective.*
The specific aims of this proposal are:
(1) Correlate global changes in DNA methylation and histone modifications with response to epigenetic therapy in B cell malignancies.
(2) Determine gene expression and miRNA profiles in patients before and after treatment with cladribine in combination with rituximab. Identify target genes and/or miRNAs activated by cladribine alone or in combination with the histone deacetylase inhibitor vorinostat.
(3) Validate the mechanism of action of cladribine plus rituximab in vitro in malignant B cell lines and further study the target genes and miRNAs identified in SCID mice using loss of function and overexpression studies.

Since, preliminary studies with vorinostat and DNA hypomethylating agents appeared to have limited activity, other combinations were considered. Cladribine is a purine analogue that has reported to have DNA hypomethylating effects through an alternate mechanism involving the methy donor SAM through the enzyme SAH. By this mechanism, cladribine could potentially inhibit histone methylation as well as DNA methylation. Cladribine is an active agent for MCL, particularly in the frontline setting and in combination with rituximab. We reasoned that if cladribine and rituximab were acting synergisticially through an epigenetic mechanism, addition of an HDACi might further potentiate antitumor activity. A phase II study conducted in conjunction with our lab evaluated the efficacy of vorinostat in combination with cladribine and rituximab (SCR) in 27 previously untreated MCL patients (Figure 1). The results were presented at the ASH conference in 2012. 25 patients (n = 27) had completed ≥ 2 cycles and were evaluable for response. The ORR was observed to be 100% (27/27) with 74% (20/27) achieving CR. Of those patients not attaining CR, 2 had blastic MCL, a very aggressive disease, and died and 2 had not completed active treatment at the time of analysis. 1 patient also withdrew consent after two cycles.

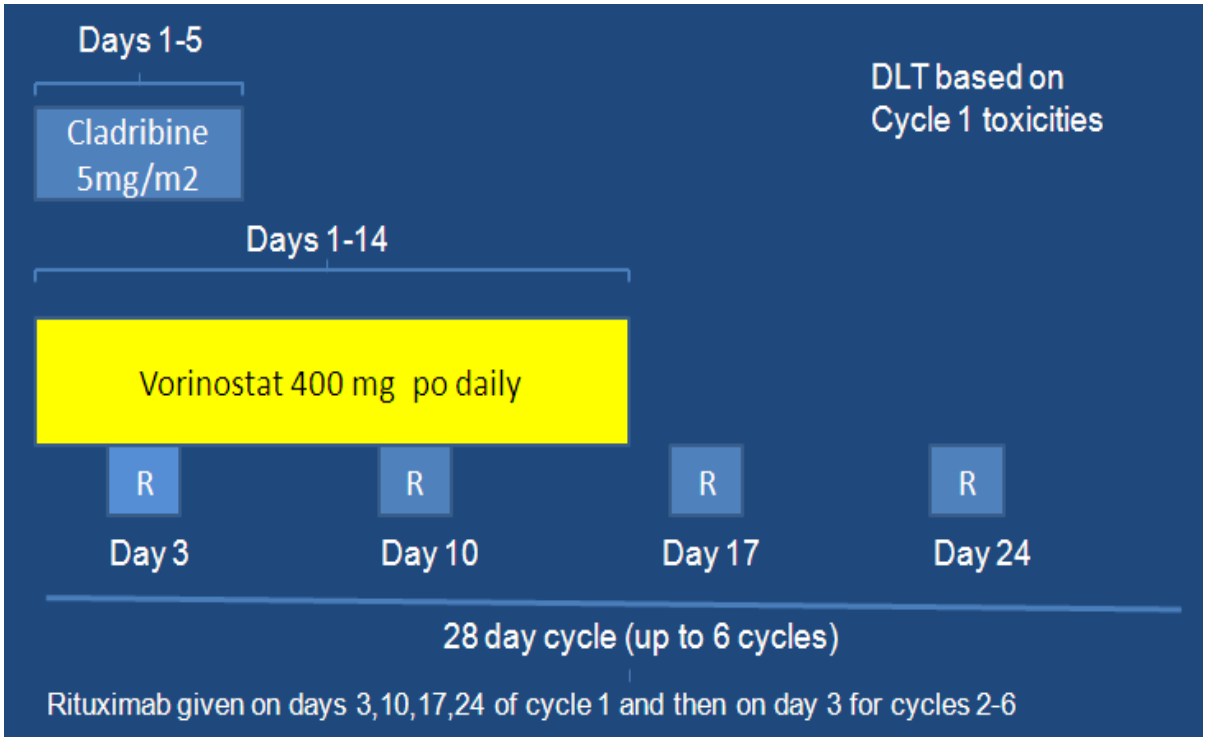
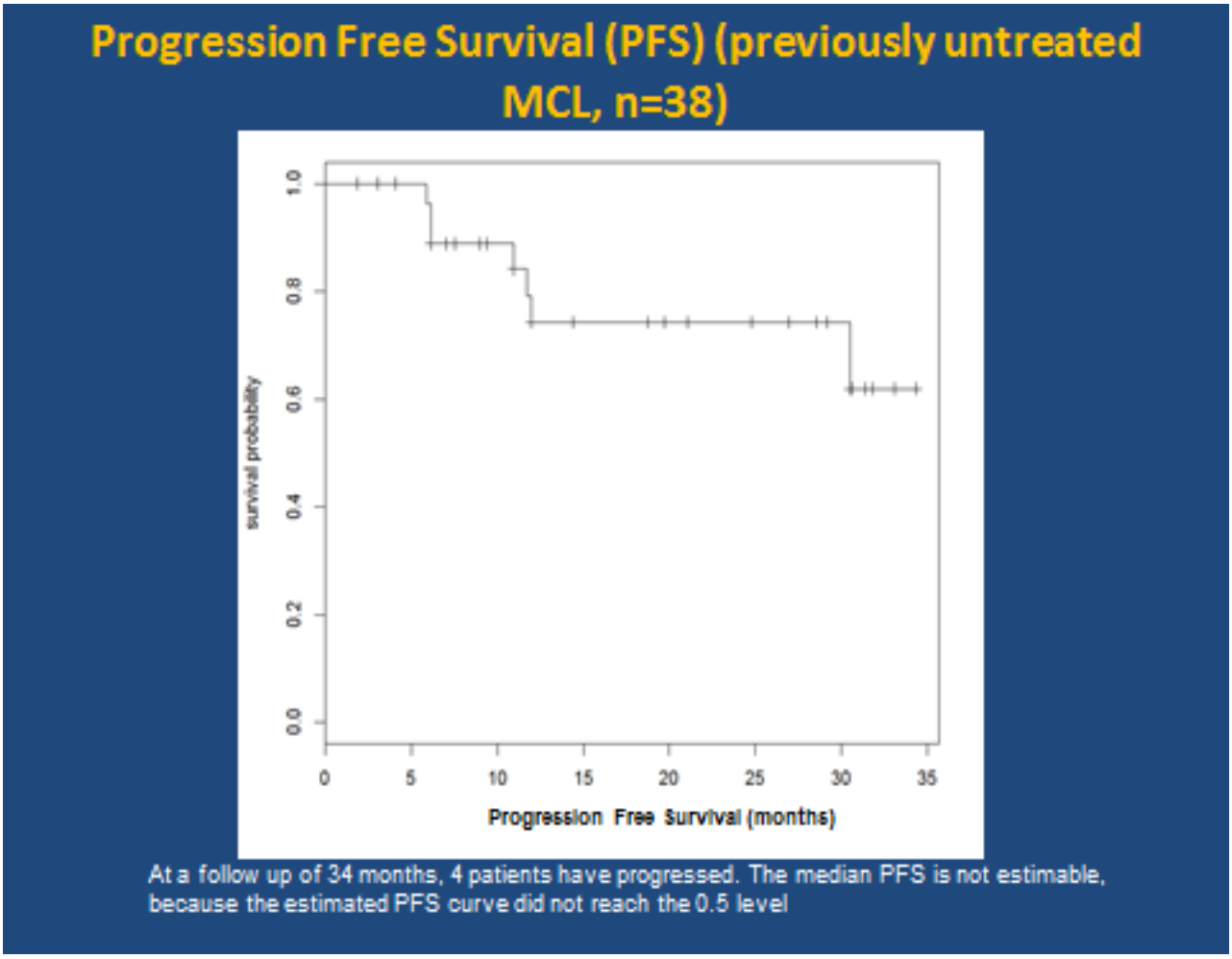


Figure 1: Treatment course of the SAHA Cladribine Rituxumab (SCR) trial.

At a median follow up of 18.2 months, 4 patients had relapsed and 2 had died. Of the relapsed patients, two had blastic MCL. None of the patients achieving a CR has relapsed. The estimated PFS curve did not reach the 0.5 level and therefore, median PFS could not be estimated (Figure 2). Toxicities primarily included neutropenia, thrombocytopenia, fatigue, anorexia, and dehydration. We found that cladribine hypomethylates DNA (6/6 pts) and potentially inhibits histone methylation *in vivo*. There were no upregulated genes common to untreated leukemic MCL patients; however, a number of changes in gene expression were observed. For example, upregulated genes included DUSP2 (3 patients), FOXO3 (2 patients), NOXA1 (2 patients), CEBPb (2 patients) and p53 (3 patients). UDP-glucuronosyltransferase 2B17 (UGT2B17) genotyping and its relationship to toxicity are ongoing. In conclusion, the SCR regimen has significant activity in previously untreated MCL and shows epigenetic activity in previously untreated MCL. Initial follow up results are promising, especially in patients without blastic MCL who continue with maintenance rituximab. Smaller numbers of patients with other B cell malignancies have been treated on and off trial with the SCR regimen with success. Blastic MCL patients have also been treated with this regimen both on and off trial. The use of ofatumumab for rituximab and the use of radiation therapy to resistant areas have produced durable CRs in several pts with blastic MCL. MCL cell lines treated with cladribine were isolated and Western blotting performed for methylated histones.

Figure 2



DNA methylation patterns in the mantle cell lymphoma genome have been analyzed using the HELP technique in collaboration with Dr. Samir Parekh's lab at the Albert Einstein College of Medicine. In the HELP (HpaII tiny fragment Enrichment by Ligation mediated PCR) assay, genomic DNA (gDNA) is digested using HpaII, a methylation sensitive enzyme and its iso-schizomer MspI, a methylation insensitive restriction enzyme to produce a differential digest of methylated and unmethylated gDNA. Digested products are then amplified using ligation mediated PCR and hybridized to a custom high density oligonucleotide array microarray having ~26000 elements covering more than 14,000 genes. Pre-processing and quality control procedures have been developed by the Einstein Bio-Informatics core. A measure of DNA methylation is obtained as the log (HpaII/MspI) ratio, where HpaII reflects the hypomethylated fraction of the genome and MspI represents the whole genome reference. Methylation ratios are also represented as an additional track on the UCSC genome browser for visual comparison between samples. The MspI track in HELP serves as an internal control for the procedure. This procedure was carried out on groups of patient samples with MCL (Figure 3) and CLL (Figure 4). Signifigantly ($p<0.05$)hypomethylated genes are shown as black circles.

Methylation changes after Cladribine treatment in MCL patients

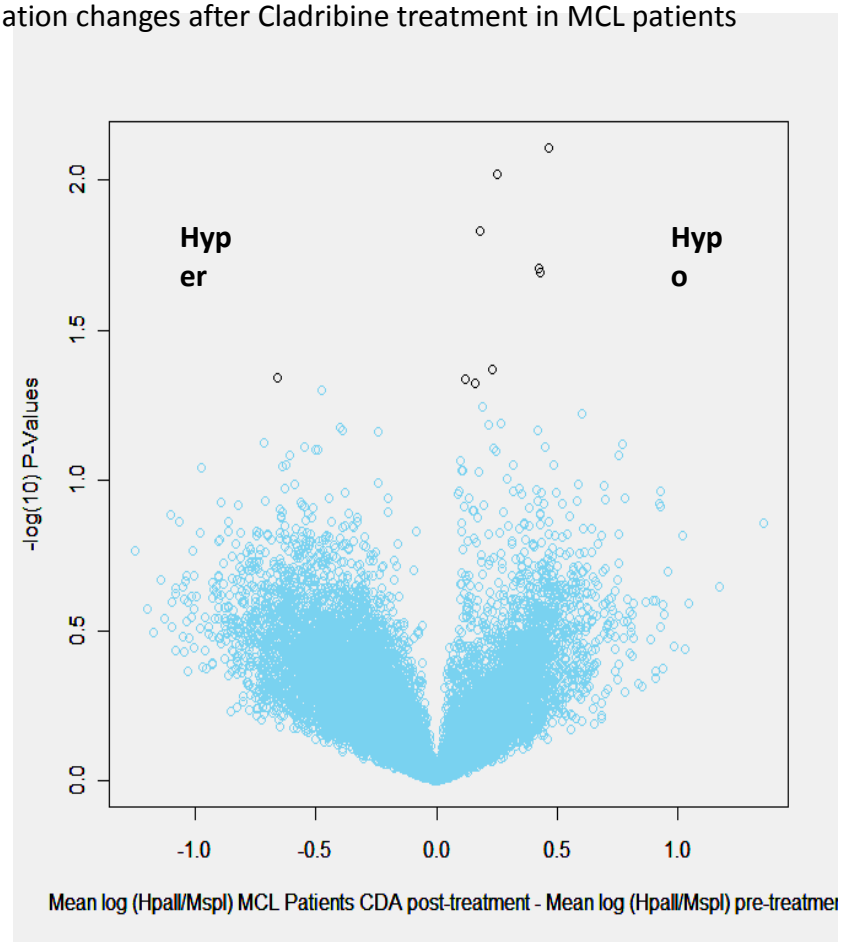


Figure 3: Volcano plot of methylation difference (X axis) vs. Significance (on Y axis) showing selected differentially methylated loci between patients after treatment with Cladribine (CDA) as compared to before it. Probe sets that were differentially methylated are marked in grey ($p<0.05$). Total 6 patients.

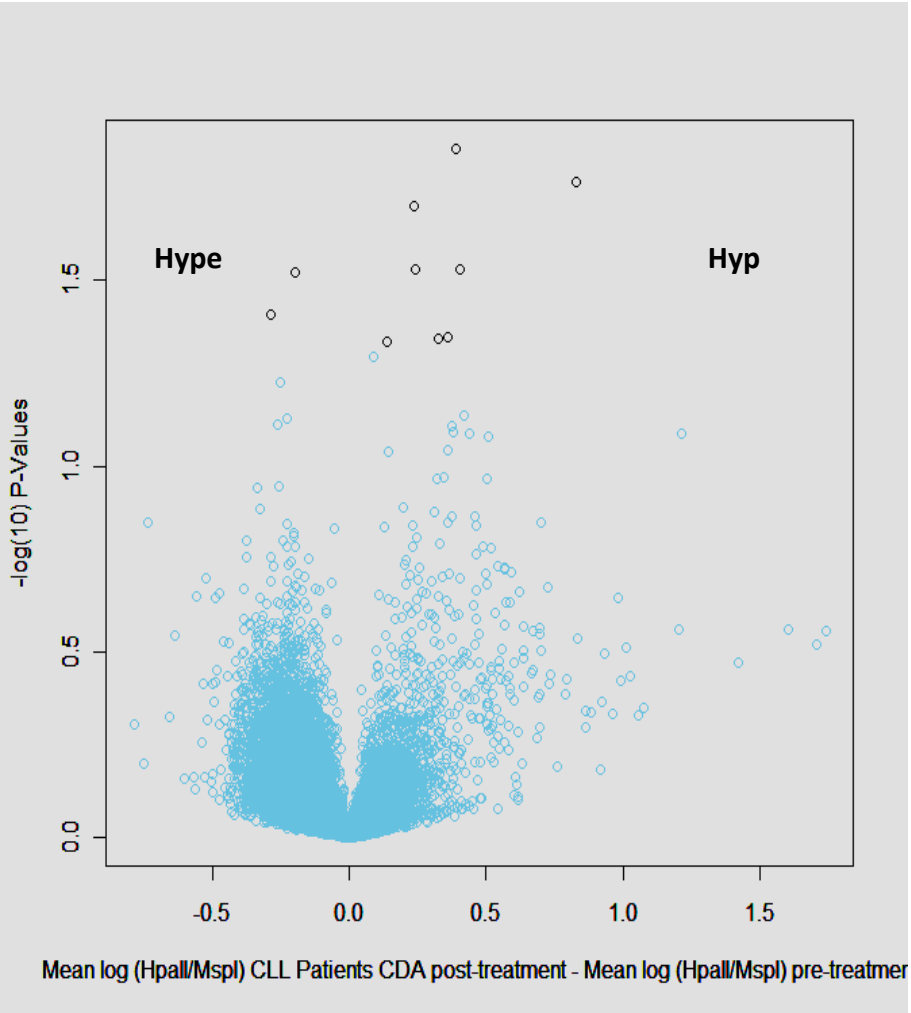


Figure 4: Volcano plot of methylation difference (X axis) vs. Significance (on Y axis) showing selected differentially methylated loci between CLL patients after treatment with Cladribine (CDA) as compared to before it. Probe sets that were differentially methylated are marked in grey ($p<0.05$). Total 3 patients.

Analysis of the HELP assay results (Figure 5) produced a number of potential target genes. Among the genes that showed signifigant downregulation was DUSP2- a p53-regulated phosphatase that negatively regulates members of the MAP kinase superfamily, such as MAPK/ERK, SAPK/JNK, p38. In this role, DUSP2 may serve as a tumor suppressor which is inappropriately methylated in some cases of MCL.

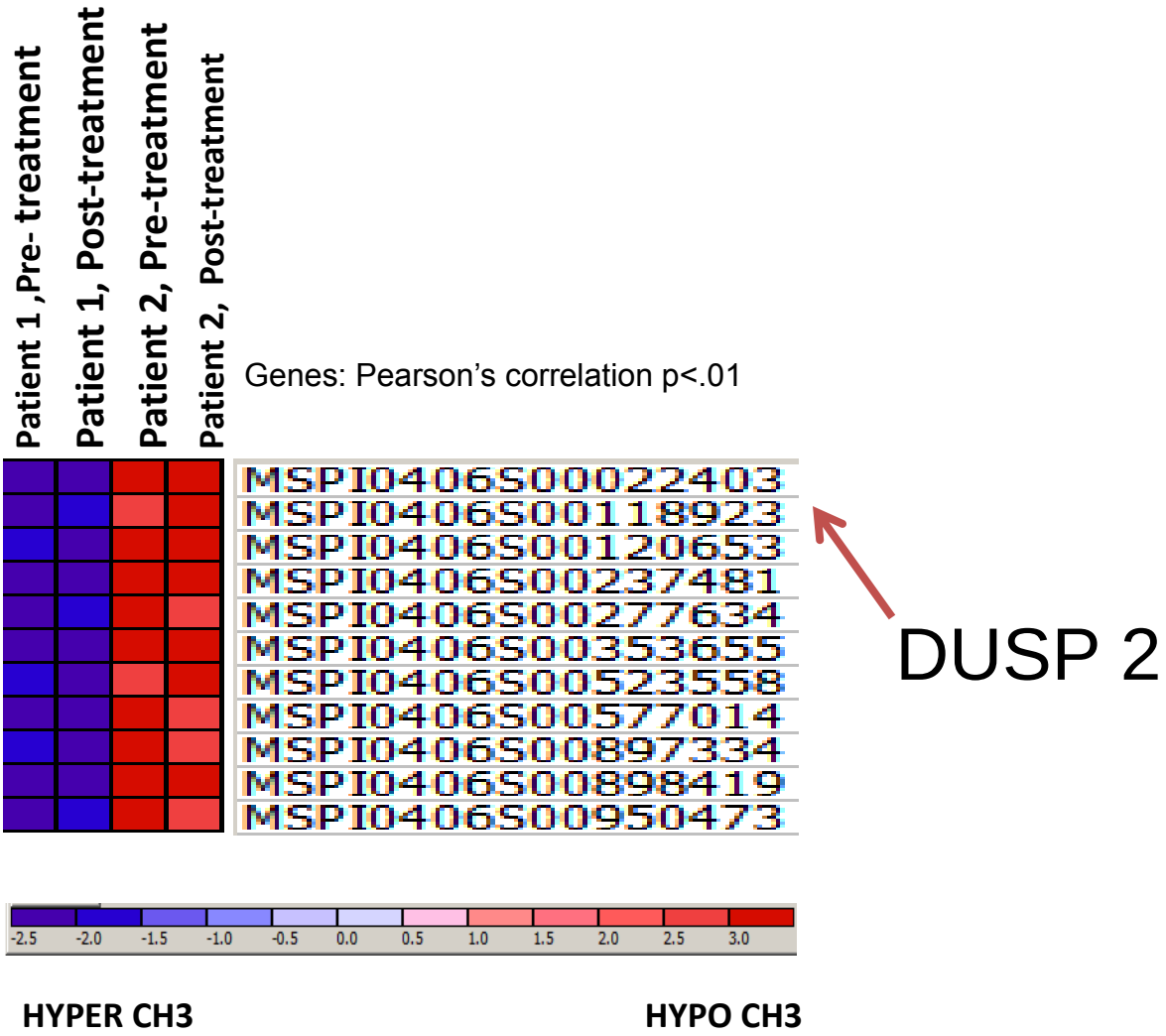


Figure 5: Hierarchical clustering of MCL and CLL patients samples before and after Cladribine treatment using Pearson's correlation (methylation data)

DUSP2 was then assayed in an array of patient samples by qPCR. mRNA extracted from peripheral blood lymphocytes before and after treatment (typically day 1 and day 5 of the regimen pictured in Figure 1) was analyzed by RT-PCR to determine the effects of epigenetic therapy on gene expression (Figure 6).

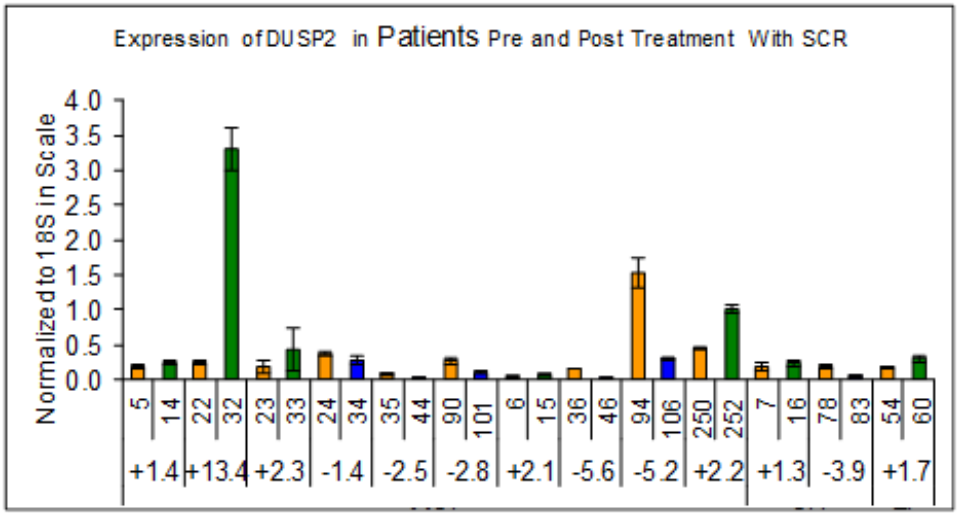


Figure 6: Expression of DUSP2 in SCR patients, pre and post therapy.

Alterations in expression of specific genes such as p53, DUSP1, DUSP2, NOXA1, FOXO3, DAPK1,and CEPBb were identifiend in specific responding patients. There were no common upregulated genes identified. (Figures 7-12) Further pathway targeted analysis is being considered.

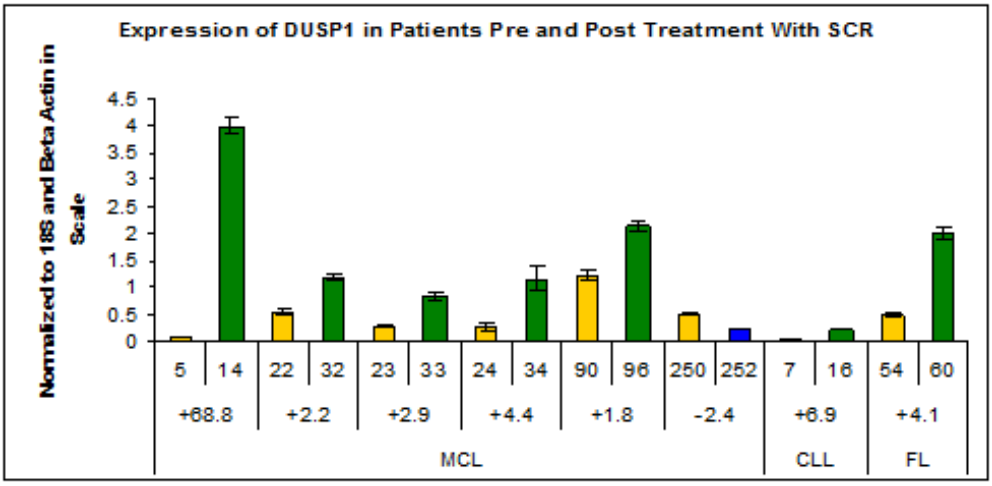


Figure 7

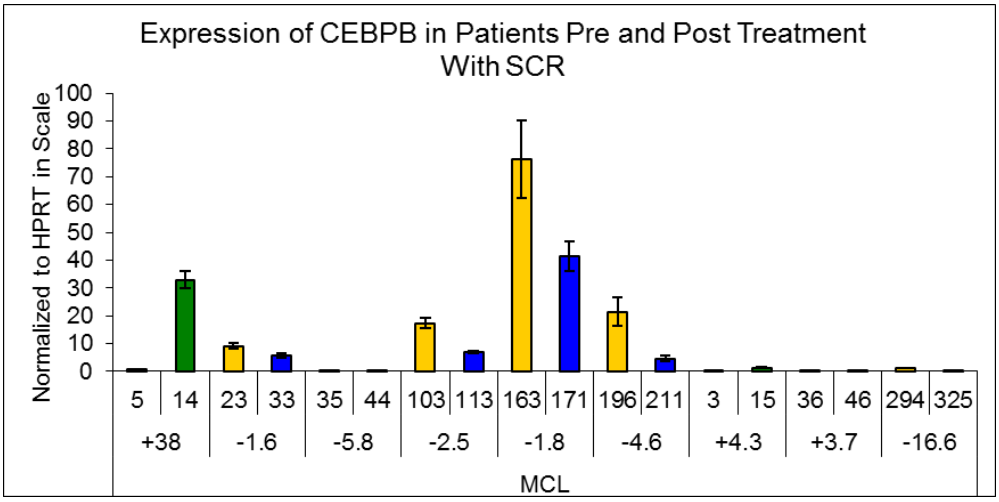


Figure 8

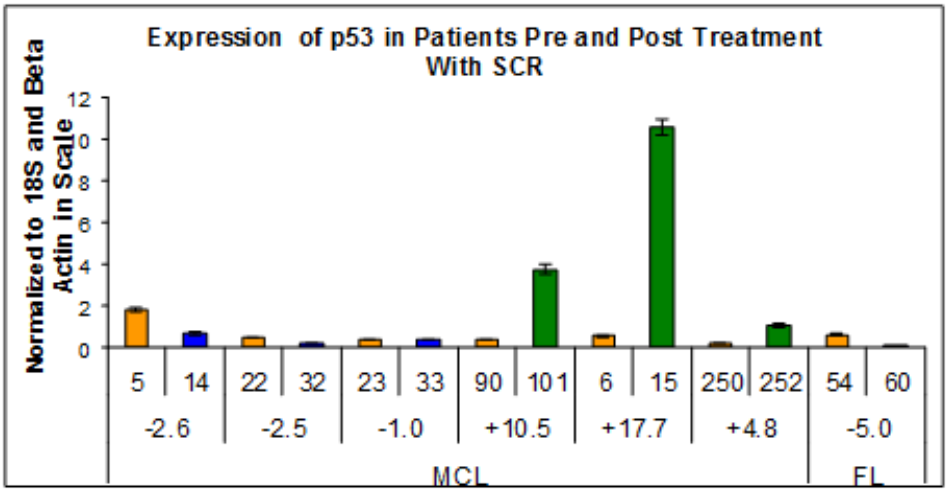


Figure 9

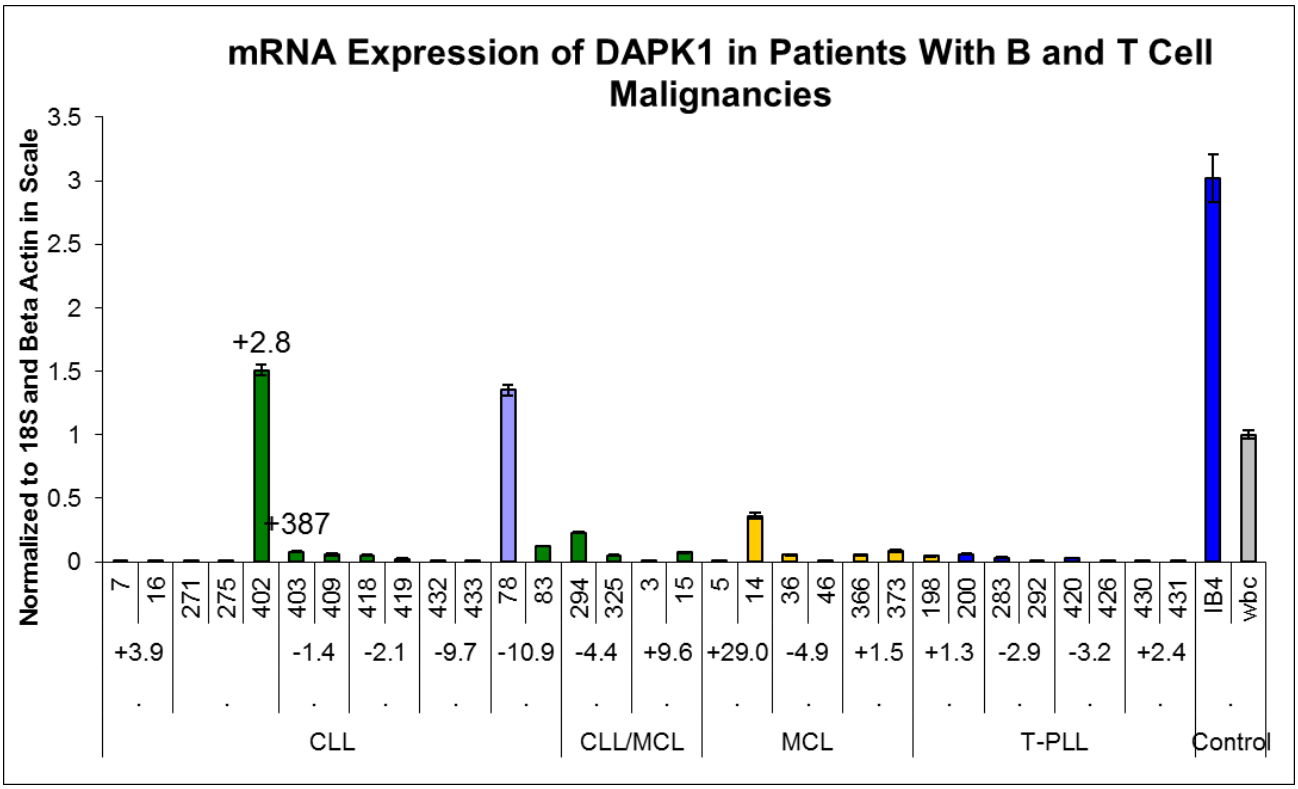


Figure 10

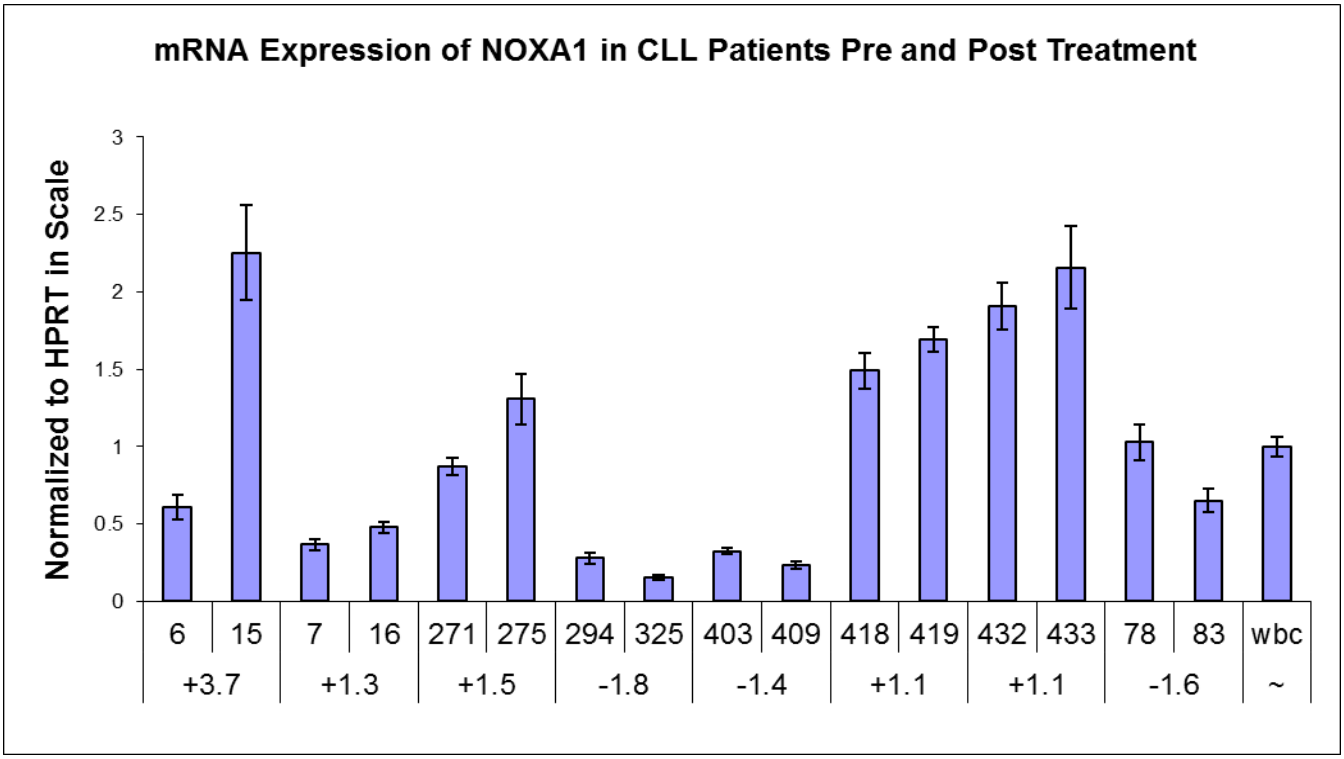


Figure 11

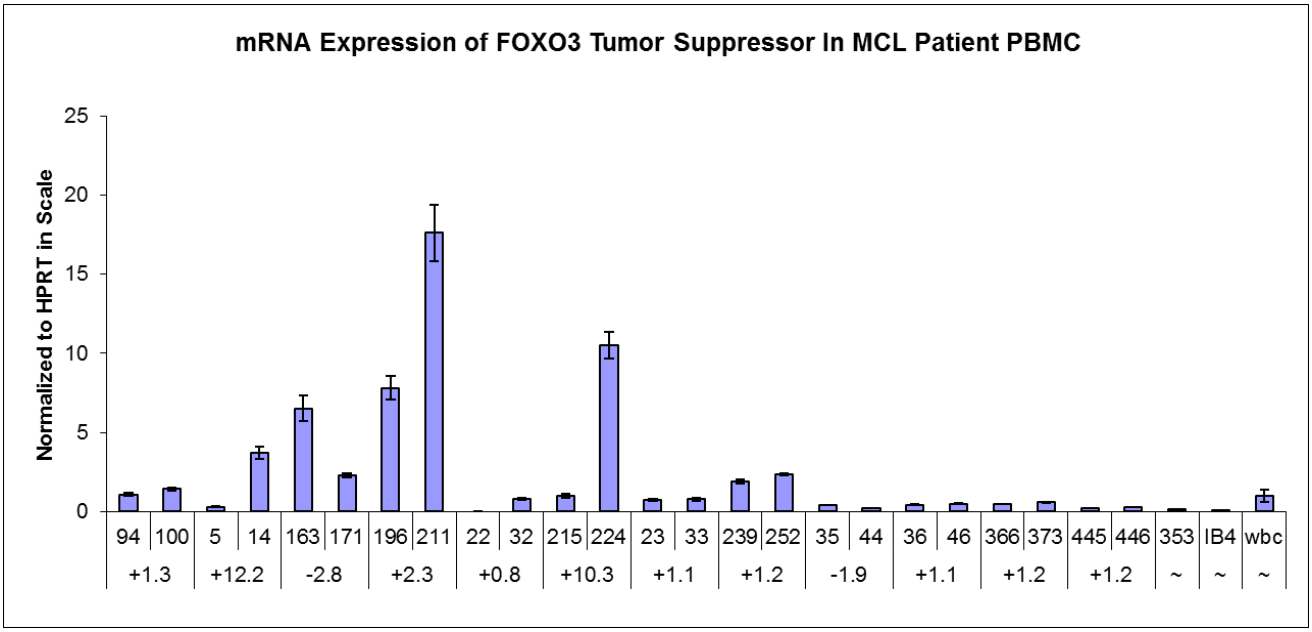


Figure 12

In order to investigate the mechanism at work, and verify cladribine's activity as a hypomethylating agent, experiments were performed on MCL cells in vitro to examine histone methylation in response to cladribine treatment. Western blot analysis shows that cladribine decreases H3 methylation, specifically trimethylation of lysine 9 and 27, both epigenetic markers of repressed chromatin (figure 13). Further, analysis of MCL patient samples showed similar, albeit less pronounced decreases in trimethyl histone residues in 2 out of 3 patients examined.

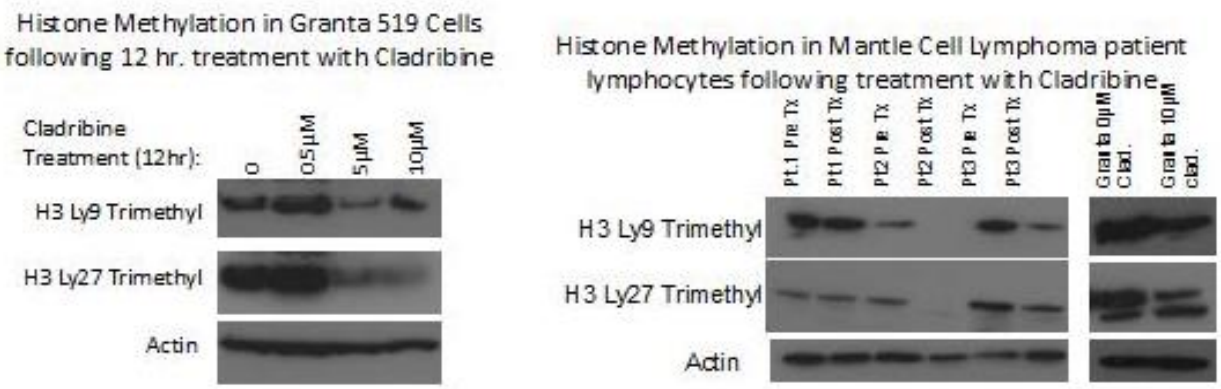


Figure 13 (right) Western blot of Granta cells treated with indicated concentrations of cladribine. (left). Western blot of patient cells. Antibodies to methlated histones and actin were used.

Further analysis of histone modification in granta cells showed a decrease methylation levels in many different histone residues, supporting cladribine's role as a hisotne methylation inhibitor. (Fig 14)

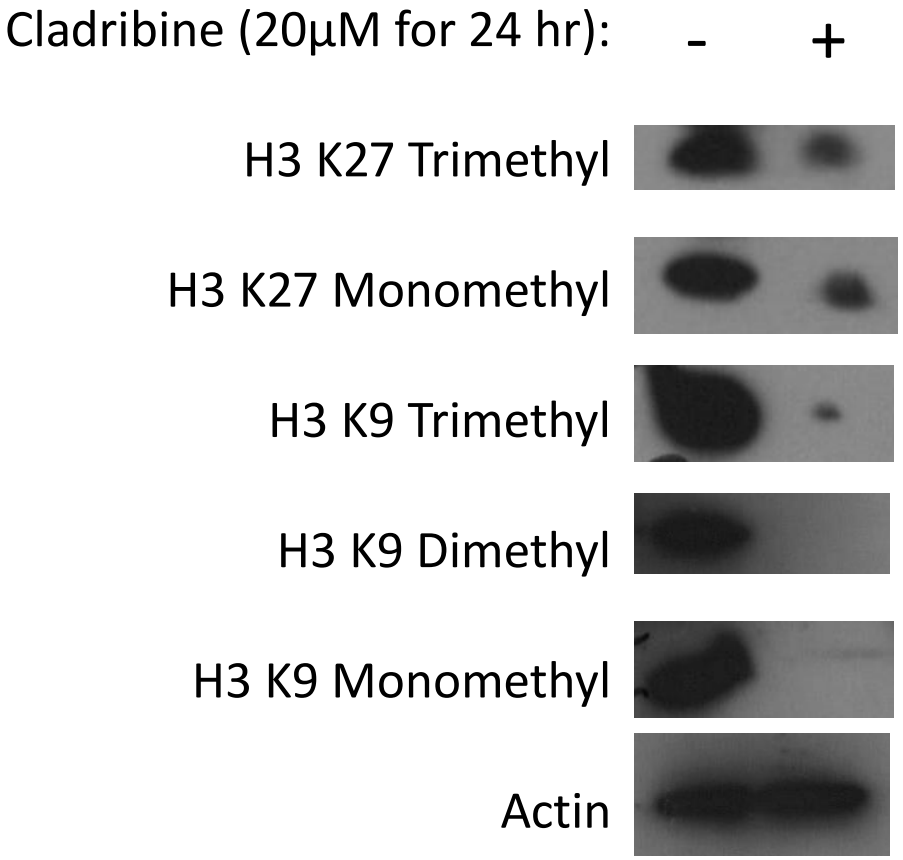


Figure 14: Histone methylation in granta 519 cells following cladribine treatment

When DUSP 2 protein is assayed in the same granta cells treated with cladribine, we found a slight increase in protein expressed, supporting DUSP2's possible role in cladribine-driven cell death (Figure 15). To determine whether changes in histone methylaiton or DNA methylaiton were the cause of increased DUSP2 expression, chromatin immunoprecipitation was performed on the proximal promoter of DUSP2 using antibodies to both H3 Trimethyl Lysine 27 and to 5-methyl Cytosine, which detects methylated DNA. The results show a signifigant decrease of methylated H3 Ly27 at the DUSP2 promoter, while methylated DNA remains unchanged and very low (Figure 16). These results indicate inappropriate DUSP2 methylation may be a contributing factor to a subset of MCL, which can be potentially corrected by cladribine treatment.

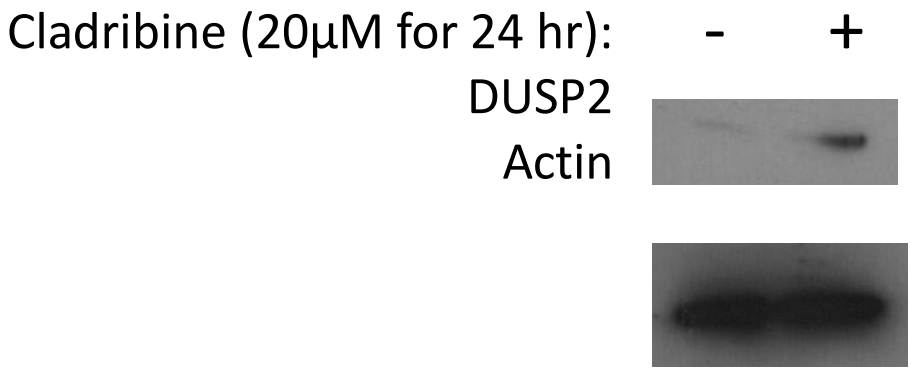


Figure 15: DUSP2 expression in granta cells following cladribine treatment

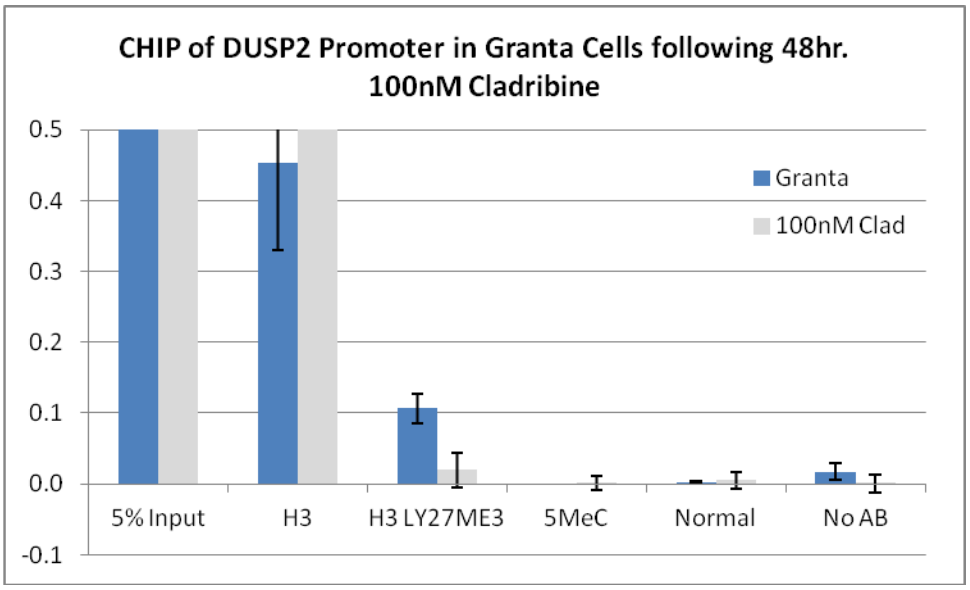


Figure 16: Chromatin Immunoprecipitation of proximal DUSP2 promoter in Granta cells following cladribine treatment

Another avenue of inquiry concerning the mechanisms behind MCL involves cyclin D1- the oncogene which is overexpressed following the characteristic t(11;14) translocation present in approximately 90% of MCL. A common polymorphism in cyclin D1, G870A, creates a splice variant, resulting in a truncated protein that lacks a nuclear export signal, resulting in

an accumulation of nuclear cyclin D1. The AA genotype has been associated with a poor prognosis or early onset in many different types of human cancer. We hypothesize that this polymorphism may influence the course of MCL, possibly through cyclin D1 acting as a transcription factor.

To investigate this possibility, we genotyped the MCL patients on the SRC study for this G870A polymorphism and compared genotype with clinical response. (Figure 17). GG is a predictor of more likely complete remission as well as a negative predictor of blastic (more aggressive) phenotype.

genotype	CR	blastic
AA	5/8	9
AG	7/9	2
GG	10/10	_

Figure 17: Patient 8/70A Genotype compared with complete response rates and blastic MCL rates

The mechanism for this poor prognosis may have something to do with the emerging hypothesis that cyclin D1 acts as a transcription factor when in the nucleus. Previous work in our lab suggested an inverse relationship between cyclin D1 and cyclin D3, in which cyclin D1 actively represses cyclin D3 expression. To test this, we conducted chromatin immunoprecipitation assays on different promoter regions of the cyclin D3 gene precipitating with antibodies against cyclin D1. While we were unable to enrich for the full length transcript (Figure 18 a, primer set CD3A), primers for a known cyclin D3 regulatory site showed cyclin D1 binding in MCL cell lines posessing the AA phenotype (Granta), but not the GG phenotype (NCEB, not shown). Signifigant cyclin D1 binding was also seen in 2 MCL patients with the AA phenotype, but not in cyclin D1 knockout cells (G4) or cyclin D1 negative MCL patient cells. Further, cyclin D1 was found to bind to the promoters of other genes previously reported to be regulated by Cyclin D1 .

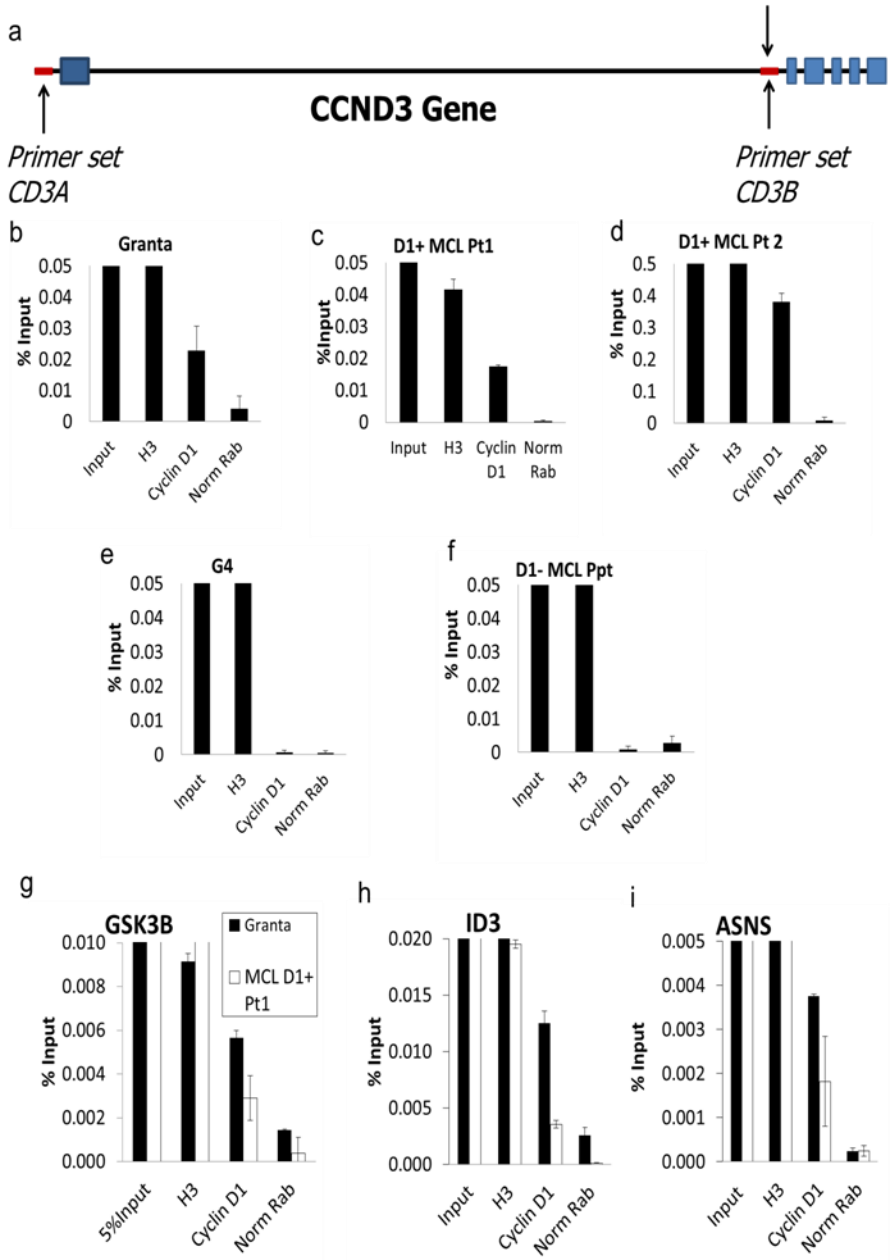


Figure 18 Cyclin D1 binds to the cyclin D3 promoter, as well as the promoters of 3 putative cyclin D1 target genes. (a) A map of the cyclin D3 gene, CCND3, indicating primer sets CD3A and CD3B. (b-f) ChIP assay in Granta 519 cells (b), two cyclin D1 positive MCL patients(c,d), G4 cells(e) and one cyclin D1 negative patient(f), using primer CD3B. Primer CD3A showed no enrichment. (g-i) ChIP assays in Granta and patient 1 (cyclin D1 positive) for the promoter regions of 3 potential cyclin D1 regulated genes, GSK3 β(g), ID3(h) and ASNS(i).

Abstracts and Meeting Posters:

1.Stephen E Spurgeon, Craig Y. Okada, Gundula Palmbach, Nan Subbiah, Jing Huang, John D. Phillips, **Elliot M. Epner**. Targeting the epigenome in B-cell malignancies: A phase I/II study of vorinostat (SAHA), cladribine (2-CdA), and rituximab (R) in B-cell malignancies. American Society of Clinical Oncology Annual Meeting June 2010 (poster)

2.Talia Pindyck, Stephen E.F. Spurgeon, Marc M. Loriaux, Craig Okada, Yiyi Chen, Zunqiu Chen, Elana Mater, **Elliot M. Epner**. Cladribine plus rituximab for the initial treatment of mantle cell lymphoma. American Society of Clinical Oncology Annual Meeting June 2010 (poster)

3.Ghai V, Shamra K, Abbi KS, Shimko S, **Epner E**, Combination therapy with cladribine, vorinostat and ofatumuab achieves complete hematological remission in refractory classical hairy cell leukemia American Society for Hematology Annual Meeting Abstracts 2011 118: Abstract 2440

4.Stuart A, Huang J, Shimko S, **Epner E**. Crosstalk Between Cyclins D1 and D3 in Mantle Cell Lymphoma at the Transcriptional and Post-Transcriptional Levels. American Society for Hematology Annual Meeting Abstract 1373 December 2011

5.Ghai V, Sharma K, Hasanali Z, Shimko S, Stuart A, **Epner E** A retrospective study analyzing activity of ofatumumab in blastic variant mantle cell lymphoma American Society of Clinical Oncology 2012 Annual Meeting *ASCO Proceedings:*e18500

6.Spurgeon S, Sharma K, Claxton DF, Ehmann CW, Gallagher C, Shimko S, Stuart A, Parekh S, Leshchenko V, Chen Y, **Epner E** Vorinostat (SAHA), Cladribine, and Rituximab in Previously Untreated Mantle Cell Lymphoma: Updated Results From a Phase I/II Trial 2012 ASH Annual Meeting and Exposition, Atlanta, GA. Poster Presentation

7. Zainul Hasanali BS, Kamal Sharma DO, Stephen E. Spurgeon,MD, David F. Claxton, MD, W. Jason Liao PhD, Samir S. Parekh, MD, Violetta Leshchenko, PhD, Motomi Mori, PhD, Sara Shimko, BA, August Stuart, MS, Elliot M. Epner, MD PhD Combined epigenetic and immunotherapy produces dramatic responses in 100% of newly diagnosed mantle cell lymphoma patients American Association of Cancer Researchers 2013 meeting- late breaking abstract

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__ No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Oncogene Addiction/Substitution In Cyclin D1 Deregulated B Cell Malignancies	August Stuart, Jing Huang, Anna Ragland, Hui Liu, Sara Shimko, Kamal Sharma and Elliot Epner	PLOS One	Jan 2012	XSubmitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We plan on submitting multiple publications based on this data. One will likely be clinical in nature, focusing on the SCR trial results along with some correlative science, possibly the G870A polymorphism. Another paper will deal with CD20 regulation, specifically by a SCR-resistant cell line created from one of the blastic patients on this study. A third paper will likely focus on cyclin D1's action as a transcription factor.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Elliot Epner	Professor of Medicine
eRA COMMONS USER NAME (credential, e.g., agency login)	
epnere	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Wesleyan University, Middletown, CT	B.A.	05/76	Biology
Columbia University, New York, NY	M.D.	05/83	Medicine
University of California, Irvine, CA	Ph.D.	06/85	Human Genetics
University of Washington/Fred	Intern/ Residency	08/86	Internal Medicine
Hutchinson Cancer Center, Seattle WA	Fellowship	04/89	Medical Oncology

A. Positions and Honors

Positions and Employment

2008-	Professor of Medicine, Penn State Hershey Medical Center, Hershey PA
2004-2008	Associate Professor of Medicine, Oregon Health Sciences University, Portland OR
1998-2003	Assistant Professor of Medicine, University of Arizona Cancer Center, BMT program, Tucson AZ
1994-1998	Assistant Professor of Medicine, University of Washington, Seattle WA
1993-1998	Assistant Member, Clinical Research Division FHCRC, Seattle WA
1989-1992	Associate in Clinical Research, Attending Physician Marrow Transplant Unit FHCRC, Seattle WA

Honors

1972	New York State Regents Scholarship, National Merit Finalist
1976	Magna cum laude with honors in biology
1976-83	Medical Scientist Training Program Fellowship (NIH)
1981	Society of Blood Fellow's nite award
1983	Alfred Steiner Dean's Day Award in Medical Research
1983	Upjohn Research Award
1989-91	McDonnell Fellow in Molecular Oncology
1999	Louis J Kettel Faculty Mentor Award, Outstanding Basic Sciences Mentor
2000	Mentor, AACR Young Investigator Scholar Award
2003	Mentor, AACR Young Investigator Scholar Award

B. Selected Peer Review Publications

1. **Epner, E**, Rifkind, R, Marks, PA. (1981) Replication of α-and β-globin DNA sequences occurs during early S phase in murine erythroleukemia cells. Proc. Natl. Acad. Sci. USA 78:3058-3062. PMCID: PMC319499
2. Aladjem, M, Groudine M, Brody, L, Dieken, E, Fournier, REK, Wahl, G., Groudine, M, **Epner, E.** (1995) Initiation of DNA replication in the human β--globin locus requires the locus control region. Science 270:815-819. PMID: 7481774
3. Dieken, E, **Epner, E**, Fournier, R.K, Groudine, M. (1996). Efficient modification of human chromosomal alleles using recombinant proficient chicken/human microcell hybrids. Nature Genetics 12:174-182. PMID: 8563756
4. **Epner,E,** Reik, A, Cimbora, D, Telling, A, Fiering, S, Keller,G, and Groudine, M. (1998). The beta-Globin LCR is not Required for an Open Chromatin Structure or Low Level Transcription of the Mouse β -Globin Locus. Molecular Cell, 2:447-455. PMID: 9809066
5. Liu, H, Wang J, and **Epner, E.** (2004). Cyclin D1 activation in B cell malignancy: association with changes in histone acetylation, DNA methylation and RNA polymerase binding to both promoter and distal sequences. Blood 104:2505-13. PMID: 15226187
6. Wang J, Liu H, Lin CM, Aladjem MI, **Epner E**. (2005). Targeted deletion of the chicken beta –globin regulatory elements reveals a cooperative gene silencing activity. J Biol Chem. 280:23340-8. PMID: 15824098
7. Fisher, R, Bernstein S, Kahl, B, Djulbegovic, B, Robertson M, De Vos S, **Epner, E,** Krishnan A, Leonard J,Lonial S, Stadtmauer E, Oconnor O, Shi, H, Boral A, and Goy A. (2006). A Multicenter Phase II Study of Bortezomib in Patients with Relapsed or Refractory Mantle Cell Lymphoma. Journal of Clinical Oncology.24:467-74. PMID: 19074748
8. Liu, H, Huang, J, Wang, J, Jiang, S, Bailey, AS, Welcker, M, Bedell, V, Slovak, M, Clurman, B, Thayer, M, Fleming, WH, and **Epner, E**. Transvection Mediated by the Translocated Cyclin D1 Locus in Mantle Cell Lymphoma. J Exp Med, 2008, 205:1843-58. PMCID: PMC2525596
9. Spurgeon, S, Yu, M, Phillips, J, and **Epner, E.** Cladribine: Not Just Another Purine Analog. Expert Opinion On Investigational Drugs, 2009 18(8):1169-81. PMID: 19604118
10. Spurgeon, S, Pindyck, T, Okada, C, Chen, Y, Chen, Z, Mater, E, Abbi, K and **Epner E,** Cladribine plus rituximab is an effective therapy for newly diagnosed mantle cell lymphoma. Leuk Lymphoma 2011 52(8); 1488-94. PMID: 21623691
11. Hasanali, Z Sharma K, **Epner E**. Flipping the cyclin D1 switch in mantle cell lymphoma. Best Prac Res Clin Heamatol 2012 Jun25;(2) 143-52 PMID: 22687450
12. Abbi KK, Zhu J, Ehmann WC, **Epner E**, Carraher M, Mierski J, Talamo G, Lucas K, Rybka W, Claxton D. G-CSF mobilized vs conventional donor lymphocytes for therapy of relapse or incomplete engraftment after allogeneic hematopoietic transplantation. Bone Marrow Transplant 2012 Aug6. [Epub ahead of print] PMID: 22863726
13. Ghai V, Sharma, K, Abbi KK, Shimko S, **Epner EM**. Current approaches to epigenetic therapy for the treatment of mantle cell lymphoma. Adv Exp Med Biol 2013 779:257-66 PIMD 23288643

C. Research Support

Completed Research Support

1999-2004 PI National Institutes of Health R01 DK56798-01 Public Health Service Grant "Genetic Analysis of the Chicken Beta-Globin Locus"

2000-2001 PI International Myeloma Foundation Junior Research Grant Award "Cyclin D1 Deregulation in Multiple Myeloma"

2002 PI International Myeloma Foundation 2002 Brian D. Novis Sr. Research Award "Genetic Analysis of Gene Deregulation in Multiple Myeloma"

2005-2008 PI Lymphoma Research Foundation "Turning cyclin D1 on and off in mantle cell lymphoma"

2009-2011 PI Lymphoma Research Foundation "Combined Epigenetic and Immunotherapy for Folicular Lymphoma"

2009-2011 PI PA tobacco Settlement Fund (TSF) "Epigenetic Therapy of Human B Cell Malignancies"

Ongoing Research Support

Pending	PI National Institutes of Health R21 "Combined epigenetic and monoclonal therapy for the treatment of T cell malignancies"
Pending	PI National Institutes of Health R21 "The Role of Cyclin D1 in Transcriptional Regulation of Mantle Cell Lymphoma"