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:** 15 - Development of Nanoliposomal Therapeutics for Leukemia

7. **Start and End Date of Research Project:** 5/1/2009 to 6/30/2010

8. **Name of Principal Investigator for the Research Project:** David Claxton, MD

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:

   $ 195,778

   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
Keasey	Post Doc Fellow	5%	$1,409.00
Wang	Research Technologist	25%	$11,100.00
Shanmuga	Research Technologist	58%	$7,553.00
Bowerman	Research Technologist	12%	$2,893.96
Claxton, B	Wage Staff	100%	$1,392.00

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
Claxton, D	Professor	1%

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
Nitrogen Generator and Liquid Nitrogen Cryo Freezer	Long term cell storage	$27,832
Millipore Water Purication System	Generation of high-quality, pure water that is essential for all sensitive laboratory procedures and functions	$7,228.48
Fiberlite Rotor, adapters, and Microrotator	Enables ultra-centrifugal sub-cellular fractionation, purification of laboratory reagents such as lentiviral particles, and isolation of putative viral particles	$36,355.75
Participated in purchase of ABSciex 5600 Triple TOF	Proteomic, metabolomic, and lipidomic analyses	$62,389

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_____ No____X_____

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

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:
Sphingolipid Biology and the Transformation of Chronic Myelomonocytic Leukemia	■ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2012	$152,995	$0
Identifying Agents that Enhance the Anti-Leukemic Efficacy of Liposomal Ceramide	■ NIH ☐ Other federal (specify:__) ☐ Nonfederal source (specify:_)	June 2012	$420,744	$0
Targeted Sphingolipid Metabolisms for Treatment of AML - Core C	■ NIH ☐ Other federal (specify:__) ☐ Nonfederal source (specify: _)	November 2012	$3,564,457	$0
(PQB4)NADPH Oxidase-Mediated Aging Promotes Poor Prognosis Acute Myeloid Leukemia	■ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	December 2012	$2,776,239	$0

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:

As described below, funding for the "Targeted Sphingolipid Metabolisms for Treatment of AML" Program Project appears very likely. This significant funding will allow leveraging of the work to drive acquisition of data for other NIH and foundation applications.

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

Thanks to funding from this project, stable NIH funding seems very likely via the Program Project Grant "Targeted Sphingolipid Metabolisms for Treatment of AML" PIs T. Loughran and M Kester. This program recently received an outstanding score of **18**, making funding very likely. This will thus infuse $3,564,457 yearly into acute myelogenous leukemia research at PSHCI. In this programmatic application, Core C is led by Dr Claxton, who also serves as CoPI for Project 1. Project 1 is focused on optimization of ceramide nanoliposomal therapy for AML – thus extending the work performed in this project.

The work initiated by this project has also allowed recruitment of Dr Brian Barth. Dr Barth brings considerable sphingolipid expertise and industry to the efforts in therapeutic development in AML. Dr Barth has submitted a number of funding applications of his own in recent months.

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	1		1	
Female				1
Unknown				
Total	**1**		**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1		1	1
Unknown				
Total	**1**		**1**	**1**

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

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.

The following equipment is available for use by other investigators in the Cancer Institute: Nitrogen Generator, Liquid Nitrogen Cryo Freezer, Millipore Water Purication System, Fiberlite Rotor, adapters, and Microrotator.

The Triple TOF 5600 has provided extensive proteomic, metabolomic, and lipidomic analyses for more than 20 separate PIs with multiple projects ranging from obesity to cancer to diabetes to lung development.

Dr Barth has been hired as an instructor.

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:

Martin Tallman – Memorial Sloan-Kettering Cancer Institute (MSKCC)

.Ross Levine – MSKCC: Drs Tallman and Levine have been recruited as collaborators on the PO1 submission: "Targeted Sphingolipid Metabolism for Treatment of AML" _ PIs Loughran and Kester. They provide superb expertise in characterization of AML heterogeneity for drug development purposes.

Lenny Schultz – Jackson Laboratories: Has been recruited as a collaborator on the PO1 submission: "Targeted Sphingolipid Metabolism for Treatment of AML" _ PIs Loughran and Kester. He provides unique expertise in the development and extension of the NSG hAML xenograft model for drug development purposes.

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.

This project proposed the following specific Aims:

Specific Aim 1: Optimize nanoliposomal ceramide for therapy of leukemic mice. Nanoliposomal ceramide has been shown to be an effective anti-cancer therapeutic agent in multiple solid tumor models and shows *in vitro* activity against murine leukemia.

Specific Aim 2: Develop ceramide-cationic nanoliposomes containing anti-bcr-abl siRNA for therapeutic depletion of bcr-abl in vitro. The effect of anti-bcr-abl siRNAs will be assayed for ability to knock down the bcr-abl transcription and translation products in bcr-abl expressing cell lines when delivered in a ceramide-cationic liposomal formulation.

Specific Aim 3: Evaluate the anti-leukemic effect of anti-bcr-abl siRNA with and without ceramide in an in vivo mouse leukemia model. The material with the highest capacity to knock down bcr-abl, as identified in *Specific Aim 2*, will be tested for *in vivo* activity against a bcr-abl-dependent leukemogenic murine line. RNAi targeted to bcr-abl will be tested for the ability to decrease tumor burden and extend survival of leukemia-challenged mice.

Specific Aim 1.
Ceramide nanoliposomes have been explored in multiple doses and schedules and in combination with a variety of putatively synergistic compounds in multiple models.

<u>In vitro data:</u> Data has shown that C6 ceramide nanoliposomes (Lip-C6) are active *in-vitro* in a number of leukemias – cell lines and primary cells. – Figure 1, 2, 4 – below.

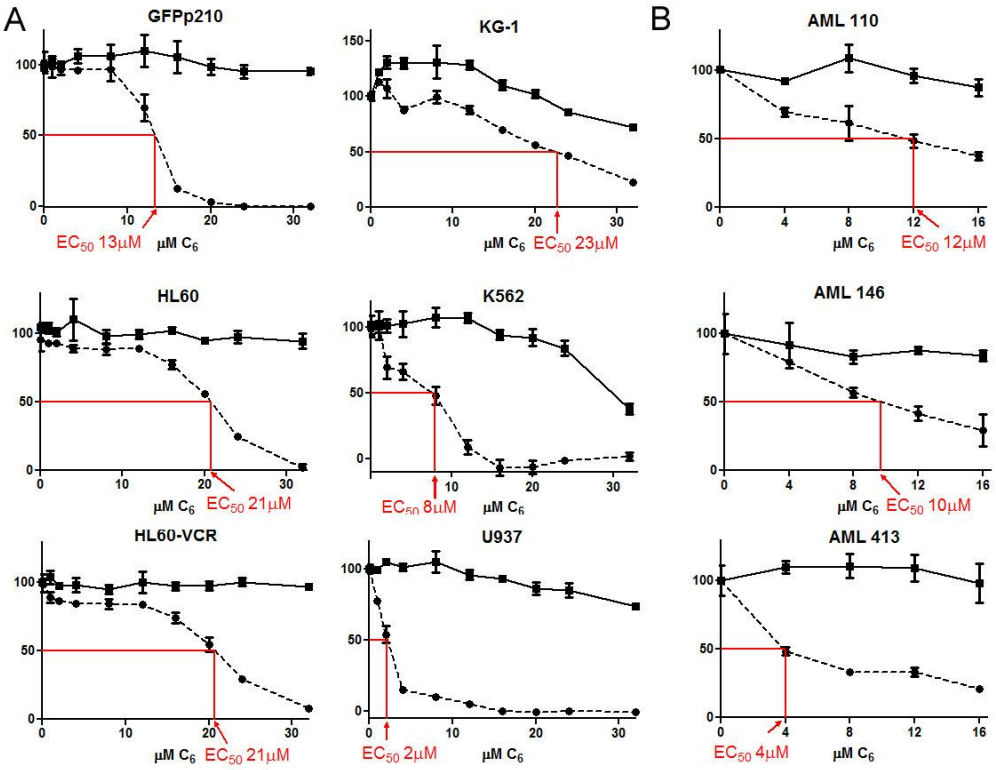


*Figure 1. Ceramide affects viability of leukemia cell lines (A) and primary leukemia samples (B) in vitro. Cells were exposed to indicated concentrations of ceramide nanoliposomes (dotted line) or ghost nano*liposomes (solid line) for 48 hours, followed by an MTS assay. Average signal for wells containing no drug were normalized to 100% (y-axis). Mean (n = 3) and standard deviation are shown. Nonparametric regression method was used to estimate the dose of ceramide required to evoke a 50% response (EC_{50}).

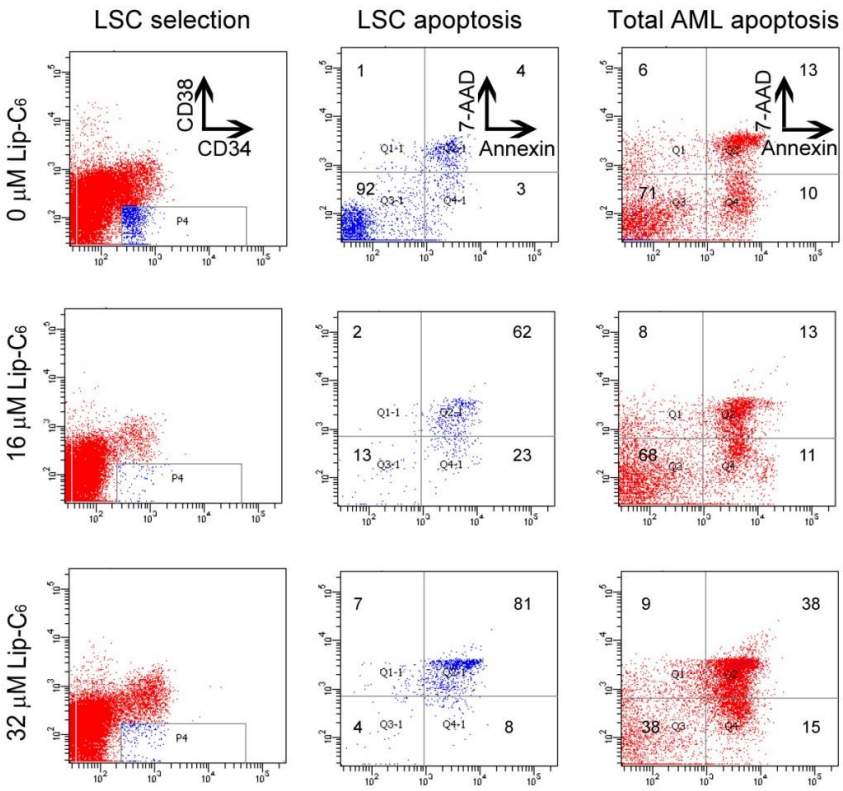


Figure 2. Lip-C6 induces apoptosis in LSCs and bulk AML. Fresh primary AML cells were exposed to the indicated concentration of ceramide nanoliposomes for 48 hours, followed by flow cytometric analysis using Annexin V and 7-AAD. Black, viable; dark gray, early apoptosis; light gray, late apoptosis; white, necrosis. 3B Fresh human AML cells were exposed for 48 hours to the indicated concentration of Lip-C_6 followed by flow cytometric analysis for LSC and apoptotic markers. Apoptosis of LSCs (blue, CD34+CD38-) and total AML cells is shown. Quadrant

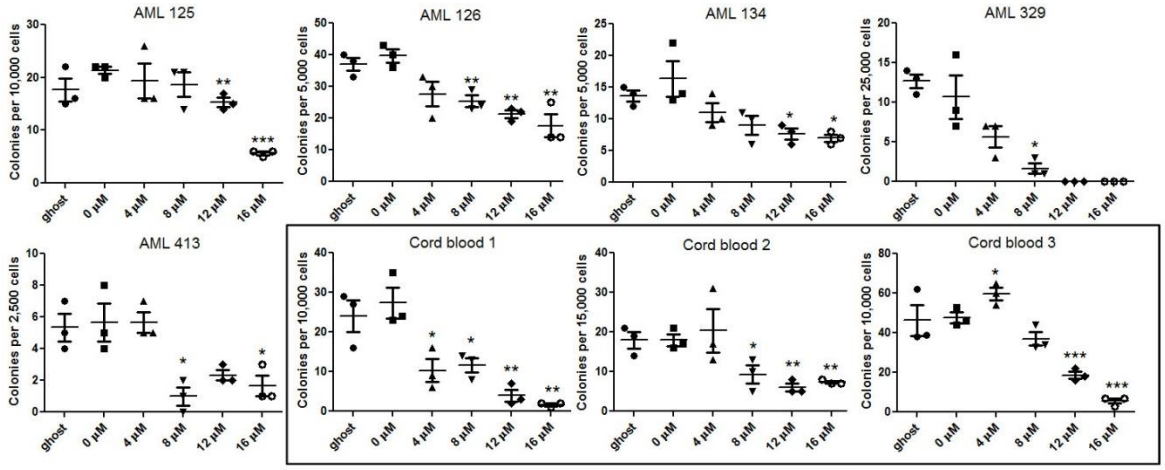


Figure 3. Primary human AML cells (n=5) or cord blood control cells (n=3, in box) were grown for 10-12 days in semi-solid medium containing the indicated concentration of ceramide nanoliposomes or ghost nanoliposomes volume-matched to the highest ceramide treatment. Ceramide inhibits colony formation of both AML cells and cord blood controls in a dose-dependent manner while ghost nanoliposomes have no effect. Each treatment group is compared to 0 μM control (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Figure 4 below, further demonstrates that LipC6 triggers apoptosis via Caspase 8 and 9 dependent mechanisms.

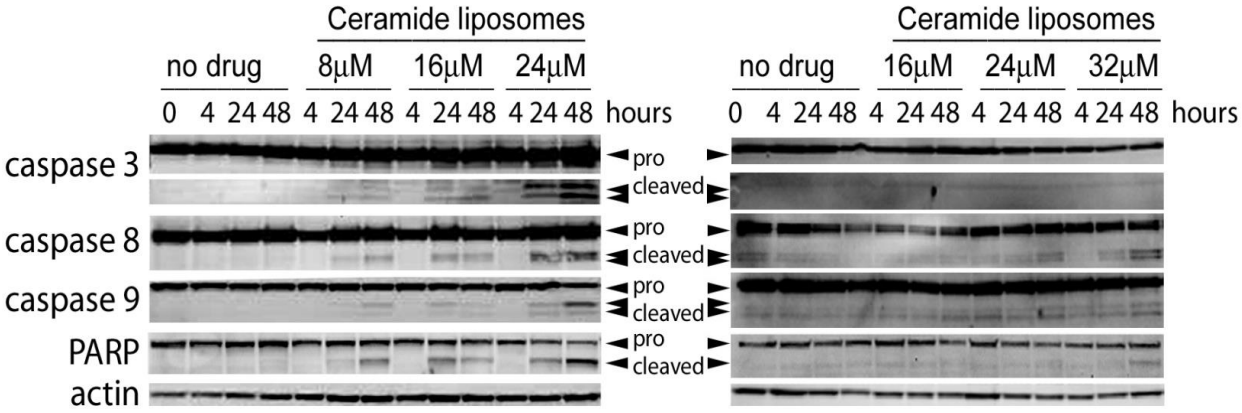


Figure 4. Study of dose and time dependent caspase and parp cleavage in K562 (A –left) and KG1 cells (B-right)K562 cells (left) and KG-1cells (right) exposed to ceramide undergo dose- and time-dependent cleavage of caspase 3, 8, and 9 and PARP.

In vivo studies

In vivo studies of Lip-C6 activity were carried out in multiple murine models as proposed. C3H/HeJ mice were rendered leukemic by tail vein injection of 32D Bcr/Abl-GFP cells. These allowed flow cytometric assessment of blood leukemia burden as shown below in figure 5. LipC6 showed activity in diminishing this burden, but this activity was modest in this model.

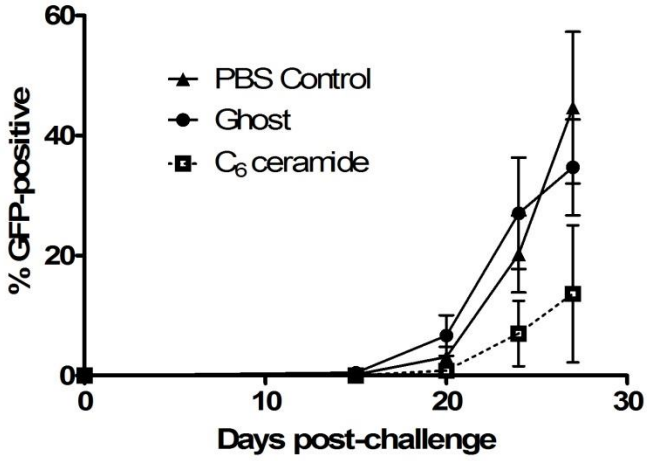


Figure 5. C3H/HeJ mice were rendered leukemic by tail vein injection of 10^6 32D Bcr/Abl-GFP cells. Mice were treated every 2-3 days with tail vein injections of C_6 ceramide liposomes (36 mg/kg), or ghost liposomes (liposomes containing all lipids except C_6 ceramide). Flow cytometry reveals a slower tumor progression trend in ceramide treated mice relative to PBS or ghost liposome treated mice. (mean and standard error of the mean are shown, n = 5)

Survival of C3HHEJ leukemic mice was modestly prolonged by LipC6 as shown in figure 6 below.

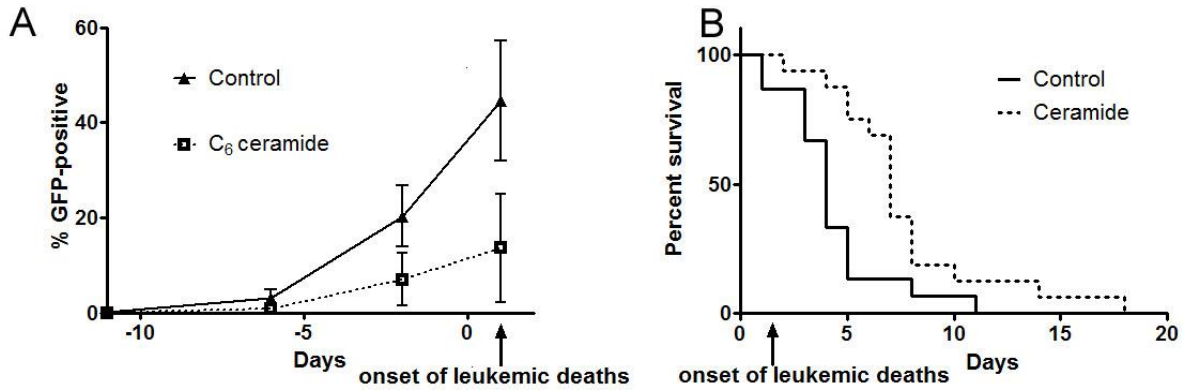
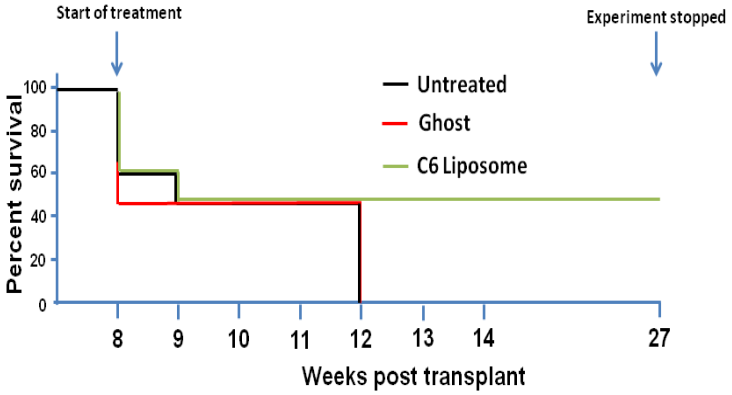


Figure 6. *Nanoliposomal ceramide slows leukemic engraftment and improves survival of C3H/HeJ mice during myeloid leukemia challenge.* C3H/HeJ mice were rendered leukemic by tail vein injection of 10^6 32D-Bcr/Abl-GFP cells. Mice were treated every 2-3 days with tail vein injections of C_6 ceramide liposomes (36 mg/kg) or PBS. A. Flow cytometry reveals a slower tumor progression trend in ceramide treated mice relative to PBS treated mice. (mean and standard error of the mean are shown, n = 5). Repeated ANOVA model analysis indicates that leukemic burden in the blood of ceramide treated mice progresses more slowly than in PBS treated mice ($p=0.031$). Ghost liposome was indistinguishable from PBS control (data not shown). B. Survival is extended in ceramide-treated mice over PBS control. A Kaplan-Meier survival plot is shown from three pooled experiments where the onset of leukemic deaths is normalized to day 1($p< 0.01$).



Accordingly other models were examined for their leukemic sensitivity to LipC6. With the collaboration of Dr Paulson of the Univeristy Park campus, an MLL-AF9 retroviral leukemic model has been studied. Results are shown here in figure 7, showing prolonged survival and potential cure of some of these animals via intravenous therapy with Lip-C6.

Figure 7. C6-ceramide nanoliposomes administered retro-orbitally extend the lifespan of the MLL-AF9 mice as compared to untreated controls or mice who received equal concentrations of liposome without C6-ceramide (ghost).

Development of the Human AML NSG model and application to Ceramide Nanoliposomal therapy of human AML.

The murine leukemia models described above, while providing reproducible and rapid

readout of efficacy of therapeutic compounds, do not truly replicate AML as it develops in patients. The triple knockout NOD/SCID/IL2rγ^{null} (NSG) mice however provide a convenient platform for propagation of human AML (hAML) in an immunodeficient host.

AML Sample #	Cytogenetic Category (2010 MRC classification)	Mutations	Integrated Genetic Risk Profile	# Mice Engrafting (> 0.5%) / Total # Mice Evaluated	Max % Primary Engraftment	Max % Secondary Engraftment (Blood)
146	Intermediate (Normal Karyotype)	FLT3-ITD, DNMT3a R882H	Poor	1/1	30(Blood)	55
202	Intermediate (Normal Karyotype)	FLT3-ITD, IDH2 R140Q, DNMT3A R882P	Poor	2/2	13.5 (Marrow)	NA
329	Unfavorable (inv3, -7)	NA	Poor	4/4	63 (Blood)	52
370	Intermediate (Normal Karyotype)	FLT3-ITD, ASXL1 E1102D	Poor	2/2	2 (Marrow)	NA
436	Intermediate (Normal Karyotype)	FLT3-ITD	Intermediate	2/2	61 (Blood)	23.9
441	Intermediate (Normal Karyotype)	FLT3-ITD	Intermediate	4/4	44 (Blood)	48
448	Favorable (inv16)	NA	Good	1/2	58 (Blood)	4.9
452	Intermediate (der12, t8;12)	FLT3-ITD	Intermediate	2/4	42 (Marrow)	1.3
476	Unfavorable (Complex Karyotype)	NA	Poor	1/3	3.4 (Marrow)	NA
481	Intermediate (t9;11, +8)	FLT3 D835	Intermediate	5/6	17 (Marrow)	0
482	Intermediate (del9)	NPM1, DNMT3A R882H	Intermediate	2/2	74 (Marrow)	0
489	Intermediate (+19)	IDH2 R140Q	Intermediate	0/1	0 (Blood)	NA
541	Intermediate (Normal Karyotype)	FLT3-ITD, NPM1, IDH1R132H	Intermediate	3/3	1.5 (Blood)	NA
555	Intermediate (t9;11)	none	Intermediate	2/2	12.9 (Blood)	NA

With support through this funding mechanism, we have studied a series of human AMLs collected under IRB informed consent here at Penn State Hershey Cancer Institute (PSHCI), and have examined their propagation in NSG mice. This work has extended the observations of other investigators suggesting that most hAMLs will grow in this host. We have also studied the efficacy of Lip-C6 – liposomal C6 ceramide with and without liposomal Vinblastine (VBL) in this model. This effort, with collaboration through Dr L. Shultz (above) was instrumental in the successful program project application described above, allowed engraftment of 13/14 hAMLs in NSG hosts as shown in the table below. Also shown in the table are genotyping of the various hAMLs as performed via the collaboration with Drs Levine and Tallman outlined above. Successful Lip-C6 therapy is shown in figure 8 below.

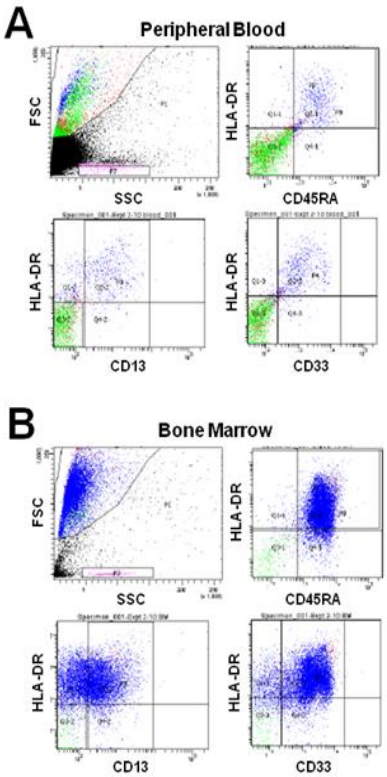
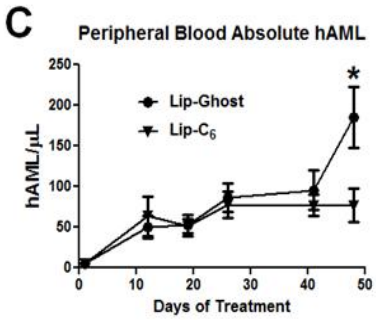


Figure 8: Monitoring the efficacy of ceramide nanoliposomes in NSG mice engrafted with human AML. NSG mice were irradiated prior to engraftment with hAML via tail veins. Engraftment was evaluated by flow cytometry of the blood (A), and bone marrow (B), using anti-human CD13, CD33, HLA-DR, and CD45.



(C) Anti-AML efficacy of ceramide nanoliposomes (Lip-C_6), or control nanoliposomes (Lip-Ghost), was evaluated using NSG mice engrafted with a poor prognosis human AML sample (inv3, -7), and leukemia burden was routinely monitored by analysis of blood collected from tail vein prick (*$p<0.05$, 2-way ANOVA, n=3-5 mice per group).

From in-vitro and in-vivo studies of Lip-C6 we conclude it has significant, novel activity vs human myeloid leukemias but that this activity varies considerably, and may be too low to be clinically useful in many cases. Optimization is thus of great interest.

Specific Aim 2. *Develop ceramide-cationic nanoliposomes containing anti-bcr-abl siRNA for therapeutic depletion of bcr-abl in vitro.* The effect of anti-bcr-abl siRNAs will be assayed for ability to knock down the bcr-abl transcription and translation products in bcr-abl expressing cell lines when delivered in a ceramide-cationic liposomal formulation.

We examined the efficacy of a series of abl specific siRNAs in inhibition of survival and growth after liposomal transfection in cells lines. The bcr-abl dependent lines K562 and 32D-Bcr/Abl were both examined.

Encouraging results were optioned in the K562 line as shown below in figure 9.

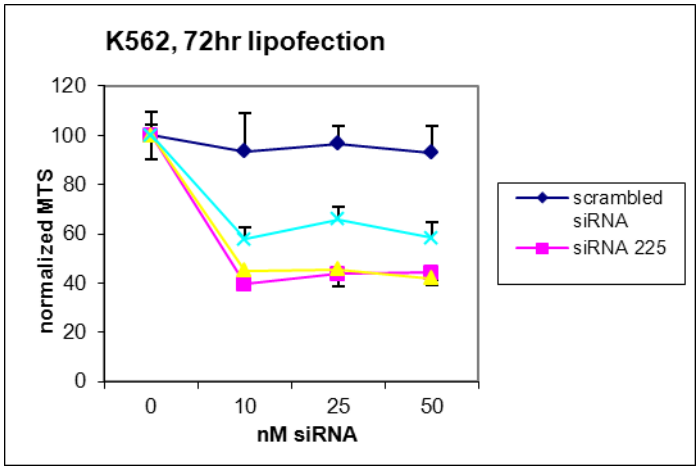


Figure 9. K562 cells were lipofected with three siRNA molecules directed against the Bcr-Abl fusion, or a scrambled control. Following a 72-hour incubation, an MTS assay was performed. Each anti-Bcr-Abl siRNA decreased MTS signal at the concentrations tested.

Unfortunately repeated efforts to confirm activity in the 32D-Bcr/Abl line, expected to be bcr-abl dependent and thus sensitive, were unsuccessful.

Specific Aim 3: Evaluate the anti-leukemic effect of anti-bcr-abl siRNA with and without ceramide in an in vivo mouse leukemia model. Given the lack of transfectability of our 32D-Bcr/Abl line, we were unable to progress to this phase of work, but elected instead to pursue alternative optimization strategies for of Lip-C6.

Inhibitors of autophagy for enhancement of Lip-C6 activity

Given the integral involvement of the process of autophagy in sphingolipid metabolism, it was rationalized that inhibitors of this cellular process might be potent enhancers of ceramide therapeutic efficacy. The first such inhibitor we examined was the cytotoxic agent vinblastine (VBL). Data is shown below in figure 10. VBL showed in-vitro synergy with

Lip-C6 in KG1 cells (0therwise very insensitive to Lip-C6). Similar supradditive efficacy was seen in primary human AML 441 in a colony forming assay, and in-vivo in the NSG mouse using hAML case 329.

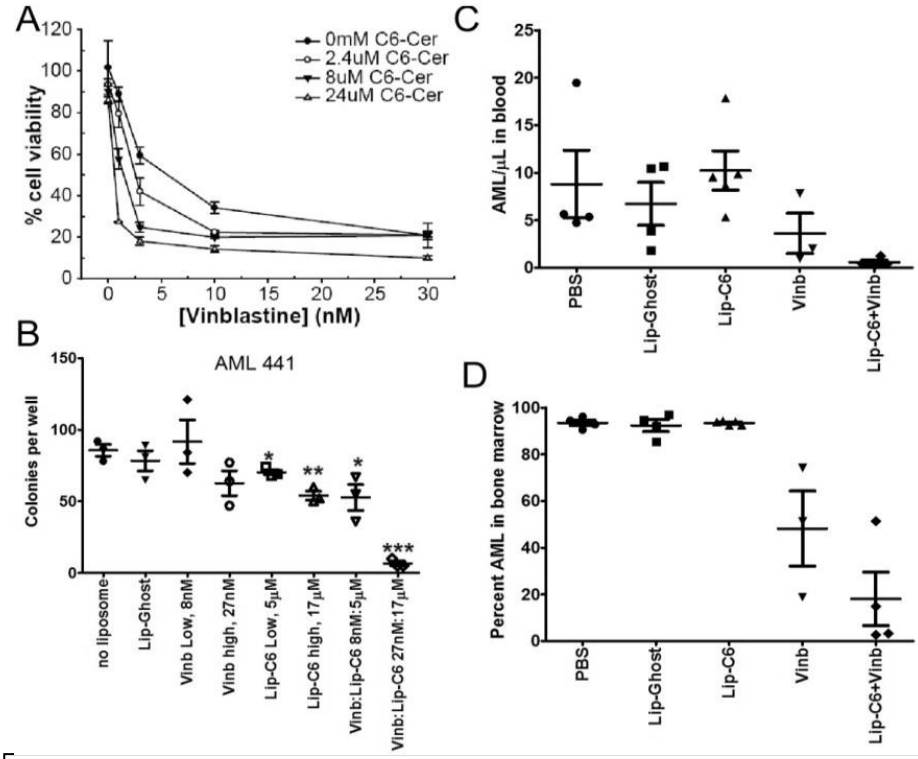


Figure 10. Enhanced efficacy of Lip-C_6 and vinblastine *in vitro* (A,B) and *in vivo* (C.D) Vinblastine potentiates the inhibitory activity of C6 ceramide nanoliposomes upon growth and survival of KG1 cells in culture (A) and primary human AML of poor prognosis (#441) in semisolid media for clonogeneic growth (B) respectively. *In Vivo* therapeutic activity of C6-VBL ("combo") nanoliposomes against poor prognosis hAML #329 growing in NSG mice. C) Blood burden of human AML (in 1000cells/ul of blood) showing effect of compound C6-VBL vs control PBS or ghost nanoliposomes (p-0.0007 and 0.0022 respectively). D) Similar results demonstrated for marrow replacement by hAML 329. C6, Ghost, and PBS are all significantly different than Compound C6-VBL (p=0.0009, 0.0016, and 0.0015 respectively).

This work has been central in the successful program project application: "Targeted Sphingolipid Metabolisms for Treatment of AML".

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. None				☐Submitted ☐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:

Two manuscripts are in preparation:

1) "Engraftment of Human Acute Myeloid Leukemia Defined by Integrated Genetic Profiling in NOD/SCID/IL2rγ^{null} Mice for Preclinical Therapeutic Evaluation"

2) "Nanoliposomal Ceramide and Inhibitors of autophagy for therapy of Myeloid Leukemia"

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.

This funding has provided support for the recognition that 1) Lip-C6 is active for human myeloid leukemias and for 2) its optimization via co-targeting of autophagy in these leukemias.

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 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 **Claxton, David F.**	POSITION TITLE Professor of Medicine
eRA COMMONS USER NAME DCLAXTON	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
McGill University, Montreal, Quebec	College Diploma	05/73	Medicine
McGill University, Montreal, Quebec	MD	05/78	Medicine

A. Personal Statement

I am Professor of Medicine, Director of the Hematological Malignancies Group, Penn State Hershey Cancer Institute, Hershey, PA. My research interests focus on developmental therapeutics for myeloid leukemias and myelodysplastic syndrome. These activities include laboratory investigation in collaboration with basic scientists, and clinical investigation. I have authored over 100 articles, book chapters, abstracts and editorials. I am fully supportive of this application, and will facilitate its goals in every way possible.

B. Positions and Honors

Positions and Employment

1978 – 1979 Rotating Internship, Royal Jubilee Hospital, Victoria, B.C.
1979 – 1980 General Practice – Emergency Room, Hotel Dieu St. Jerome, Rue Principale, St. Jerome, Quebec
1981 – 1984 Internal Medicine Residency, Royal Victoria Hospital, Montreal, Quebec
1984 – 1987 Hematology Fellowship, Royal Victoria Hospital, Montreal, Quebec
1987 – 1990 Fellow, Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas
1990 – 1992 Faculty Associate, The University of Texas M.D. Anderson Cancer Center, Houston, Texas
1992 – 1998 Assistant Professor, Department of Hematology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas
1998 – 2000 Associate Professor, Department of Blood and Marrow Transplantation, The University of Texas M.D. Anderson Cancer Center, Houston, Texas
2000 – 2005 Associate Professor with Tenure, Department of Medicine, Division of Hematology/ Oncology, Milton S. Hershey Medical Center, Hershey, Pennsylvania
2005 – Present Professor of Medicine, Division of Hematology/Oncology, Milton S. Hershey Medical Center, Hershey, Pennsylvania

Other Experiences and Professional Memberships

6/08 – 9/09 Interim Chief, Division of Hematology/Oncology, Milton S. Hershey Medical Center, Hershey, Pennsylvania

Honors

1972 Academic Scholarship, McGill University
1987 – 1988 Clifton D. Howe Award for Clinical Excellence in Medical Oncology
1989 – 1990 Upjohn Research Award for Outstanding Contributions in Cancer Research

C. Selected Peer-Reviewed Publications (selected from a list of 129, in chronological order)

Most Relevant to Current Application

1. Choudhury A, Liang JC, Thomas EK, Flores-Romo L, Xie QS, Agusala K, Sutaria S, Sinha I, Champlin RE, **Claxton DF**: Dendritic cells derived in vitro from acute myelogenous leukemia cells stimulate autologous, anti-leukemic responses. Blood 1999; 93: 780-786.
2. **Claxton DF**, McMannis J, Champlin R, Choudhury A. Therapeutic potential of leukemia-derived dendritic cells: Preclinical and clinical progress. Critical Reviews in Immunology 2001; 21:147-155.
3. **Claxton DF**, Ehmann WC, Rybka W. Control of advanced and refractory acute myelogenous leukemia with sirolimus based non-myeloablative allogeneic stem cell transplantation. Brit J Haematol 130; 256-264, 2005.
4. Popova EY, **Claxton DF**, Lukosova E, Bird PI, Grigoryev SA. Epigenetic heterochromatin markers distinguish terminally differentiated leukocytes from incompletely differentiated leukemia cells in human blood. Exp Hematol, 34(4):453-62, 2006.
5. Bayerl MG, Bruggeman RD, Conroy EJ, Hengst JA,King TS, Jimenez M, **Claxton DF**, Yun JK. Sphingosine kinase 1 protein and mRNA are overexpressed in non-hodgkin lymphomas and are attractive targets for novel pharmacological interventions. Leuk Lymphoma, 49(5):948-954, 2008. PMID: 18452097
6. von Reyn Cream L, Ehmann WC, Rybka W, **Claxton DF**. Sirolimus in unmanipulated haploidentical cell transplantation. Bone Marrow Transplant 42: 765-6, 2008.
7. O'Brien SM, **Claxton DF**, Crump M, Faderl S, Kipps T, Keating MJ, Viallet J, Cheson BD. Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. Blood 113(2): 299-305, 2009. PMID: 18931344
8. Kantarjian HM, Erba HP**, Claxton D**, Arellano M, Lyons RM, Kovascovics T, Gabrilove J, Craig M, Douer D, Maris M, Petersdorf S, Shami PJ, Yeager AM, EckertS, Abichandani R, Faderl S. Phase II study of clofarabine monotherapy in previously untreated older adults with acute myeloid leukemia and unfavorable prognostic factors, J Clin Oncol. 2009 Dec 21. PMID: 20026805
9. Keasey N, Herse Z, Chang S, Liggitt DH, Lay M, Fairman J, **Claxton DF.** A non-coding cationic lipid DNA complex produces lasting anti-leukemic effects. Cancer Biol Ther. 2010 Sep 13;10(6). PMIC: 20647744
10. **Claxton D**, Erba HP, Faderl S, Arellano M, Lyons RM, Kovacsovics T, Gabrilove J, Huebner D, Gandhi PJ, Kantarjian H. Outpatient consolidation treatment with clofarabine in a phase 2 study of older adult patients with previously untreated acute myelogenous leukemia. Leuk Lymphoma. 2011 Oct 24. [Epub ahead of print] PubMed PMID: 21877883
11. Barth BM, Altinoğlu E, Shanmugavelandy SS, Kaiser JM, Crespo-Gonzalez D, DiVittore NA, McGovern C, Goff TM, Keasey NR, Adair JH, Loughran TP Jr, **Claxton DF**, Kester M. Targeted indocyanine-green-loaded calcium phosphosilicate nanoparticles for in vivo photodynamic therapy of leukemia. ACS Nano. 2011 Jul 26;5(7):5325-37. Epub 2011 Jul 8. PubMed PMID: 21675727
12. **Claxton DF**. Out foxing bcr-abl. Cancer Biol Ther. 2011 Apr 15;11(8):769-70. Epub 2011 Apr 15. PubMed PMID: 21358259
13. **Claxton D**, Erba HP, Faderl S, Arellano M, Lyons RM, Kovacsovics T, Gabrilove J, Huebner D, Gandhi PJ, Kantarjian H. Outpatient consolidation treatment with **clofarabine** in a phase 2 study of older adult patients with previously untreated acute myelogenous leukemia. Leuk Lymphoma. 2011 Oct 24. [Epub ahead of print]
14. Karch J, Zhu J, Ehmann WC, **Claxton D.** Clofarabine and CY do not yield reliable engraftment of hematopoietic stem cells. Bone Marrow Transplant. 2011 Nov 14. doi: 10.1038/bmt.2011.224. [Epub ahead of print]

15. Joudeh J, **Claxton** D. Obatoclax mesylate : pharmacology and potential for therapy of hematological neoplasms. Expert Opin Investig Drugs. 2012 Mar;21(3):363-73