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PRINCIPAL INVESTIGATOR: Michael Lilly, M.D.

CONTRACTING ORGANIZATION: Loma Linda University
Loma Linda, CA 92354

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14. ABSTRACT The contract supports studies to define the role of the PIM1 kinase in acquired resistance to chemotherapy by prostate cancer cells. Data to date for specific aim #1 define a signaling pathway induced by docetaxel, involving sequential steps of STAT3 activation, expression of PIM1, and activation of NFkB signaling. Blockade of this pathway by expression of dominant negative PIM1proteins blocks drug-induced upregulation of NFkB activity, and sensitizes cells to docetaxel. Other studies (specific aim #2) focus on identifying a mechanism through which PIM1 activates NFkB. We have unambiguously identified S937 as the major PIM1 phosphorylation site on the NFkB1/p105 precursor protein, through use of LCM/MS/MS analysis. Interestingly PIM2 is only a weak kinase for this site. Additional data (specific aim #3) have been generated to characterize a small molecule inhibitor of PIM1.					
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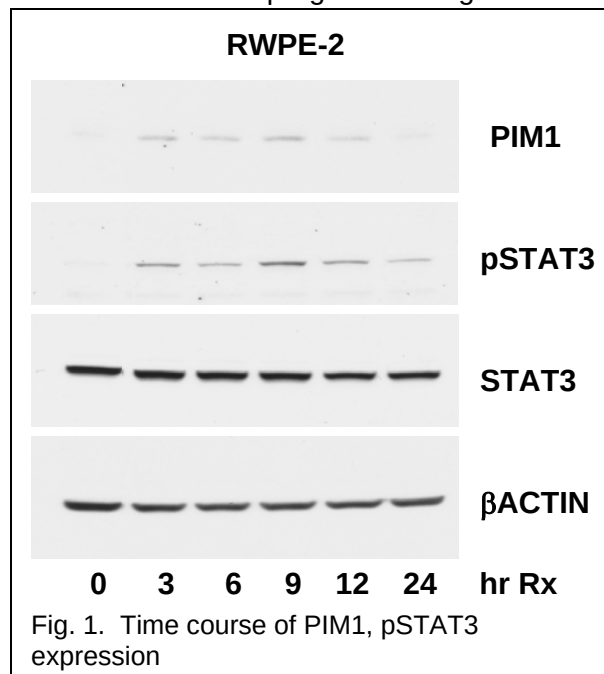
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INTRODUCTION

Studies under this funded activity are focused on characterizing the role of the PIM1 gene in acquired resistance to chemotherapy drugs, by prostate cancer cells. The proposal included three specific aims: 1) to define a novel signal transduction pathway activated by docetaxel, 2) to characterize the mechanism through which PIM1 activates and regulates NFkB signaling, and 3) to explore genetic and pharmacologic means of inhibiting PIM1 activity or expression to enhance the sensitivity of prostate cancer cells to docetaxel and other chemotherapy drugs. Substantial progress has been made in each of these areas during the 01 year of support.

BODY

We will outline our progress through reference to the specific aims described above. The first



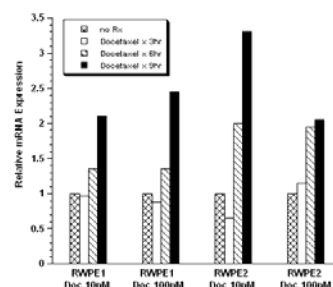
specific aim was to outline a signal transduction pathway activated by docetaxel and involving upregulation of PIM1 expression. This pathway has been substantially defined. Using RWPE1 and RWPE2 (not shown) prostate cells, we noted that docetaxel treatment rapidly leads to an increase in expression of the PIM1 serine/threonine kinase. Expression becomes apparent at 3hrs after drug addition, peaks at 9-12hrs, and returns to baseline by 24hrs (Fig. 1). This increase in expression is accompanied by an increase in *pim-1* mRNA, as shown by real time-PCR analysis (Fig. 2). Thus the effects of docetaxel are primarily transcriptional or post-transcriptional.

We next wanted to define mechanisms through which *pim-1* could be transcriptionally upregulated. Transcription of *pim-1* is known to be activated by STAT transcription factors and by NFkB transcription factors. We examined

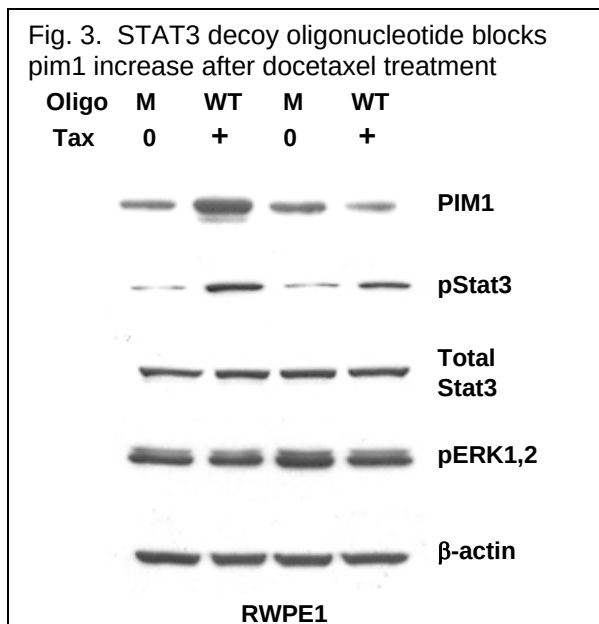
the time course of STAT3 activation after docetaxel treatment (Fig. 1), and noted that it paralleled the course of *pim-1* expression. We therefore suspected that docetaxel increased *pim-1* expression in a STAT3-dependent manner. This was directly demonstrated by use of decoy oligonucleotides (Fig. 3). Double-stranded DNA oligonucleotides matching a known STAT3 binding site blocked the drug-induced upregulation of *pim-1* expression, while a decoy based on a mutated (non-binding) STAT3 site did not. These data therefore establish a linear relationship among the following events: docetaxel treatment → STAT3 activation → *pim-1* expression.

We hypothesized that NFkB transcriptional activation would be a downstream event in this signal transduction pathway, because many chemotherapy drugs and other stressors are known to activate NFkB. We engineered RWPE2 cells to constitutively express a NFkB-dependent promoter/luciferase plasmid, and

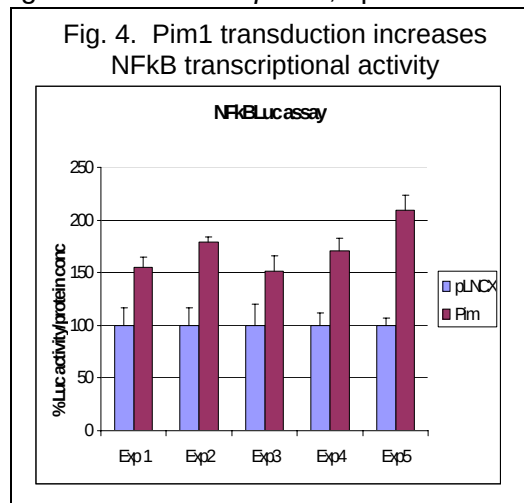
Fig. 2. Expression of *pim-1* mRNA after docetaxel treatment



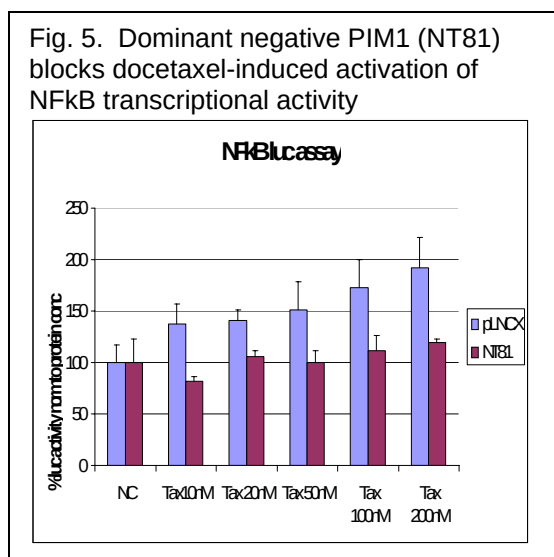
found that docetaxel treatment increased NFkB transcriptional activity. We then transiently infected these cells with a *pim-1*-encoding retrovirus. *Pim-1* expression also consistently increased NFkB transcriptional activity (Fig. 4). To determine if the drug-induced increase in NFkB activity occurred in a *pim-1*-dependent manner, we then infected the reporter cell line with a retrovirus encoding a dominant-negative form of *pim-1*, pimNT81. The



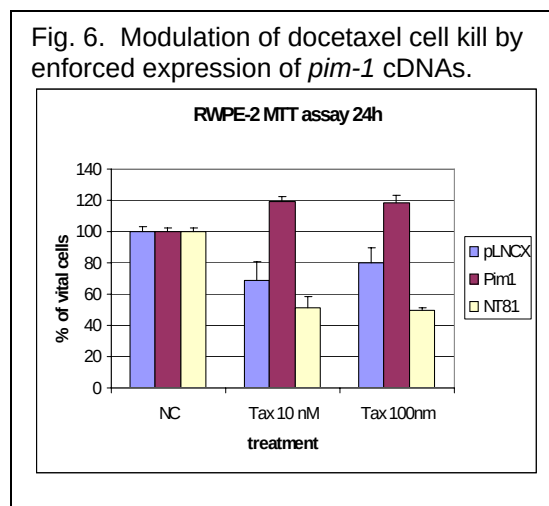
dominant negative *pim-1* cDNA completely blocked the drug-induced upregulation of NFkB activity, demonstrating that *pim-1* expression is a necessary upstream step in the drug-induced activation of NFkB (Fig. 5). In aggregate these studies establish a signal transduction pathway triggered by docetaxel treatment of RWPE2 prostate cancer cells.



To determine if this pathway modified drug toxicity, we examined the effects of enforced expression of wild-type or NT81 *pim-1* cDNAs of docetaxel cell kill (Fig. 6). Docetaxel produced dose-dependent cell kill in RWPE1, 2 cells. Enforced expression of wild-type *pim-1*



cDNA markedly reduced cell death. In contrast, expression of the dominant negative NT81 cDNA enhanced cell death after docetaxel treatment. These data demonstrate that *pim-1* expression can modulate drug-induced cell death, and demonstrate that the survival pathway described above is a legitimate target for pharmacologic intervention. These data will be presented at the 2006 AACR meeting in poster form (1).

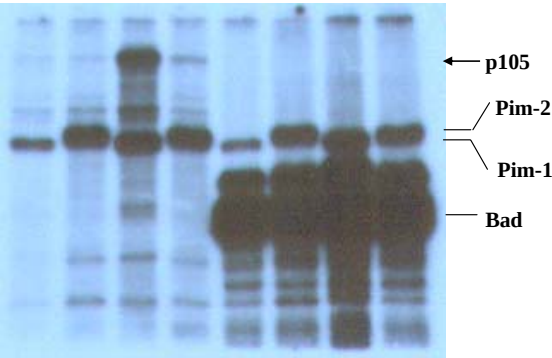


The goal of specific aim #2 was to define pathways through which the PIM1 kinase could activate NFkB transcriptional activity. We had hypothesized that PIM1 would phosphorylate the NFkB1/p105 precursor protein on serine-937, leading to proteolytic cleavage of the protein with release of active p50 protein as well as other sequestered NFkB components and the TPL2 kinase. A major goal of this specific aim was to identify the phosphorylation site on p105. We have used a variety of biochemical methods to accomplish the unambiguous identification. We initially expressed the full-length p105 protein in bacteria. This was reacted in a variety of *in vitro* kinase reactions with recombinant PIM1 or PIM2 enzymes. PIM1 strongly phosphorylated p105, but only in the presence of manganese, not magnesium. PIM2 was a much weaker kinase (Fig. 7).

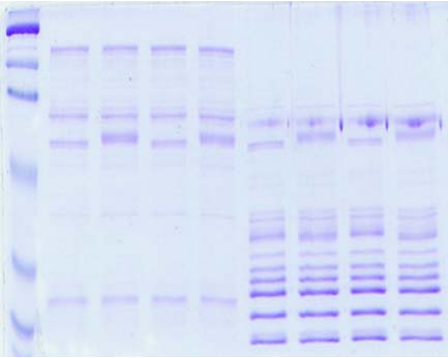
To demonstrate the site of phosphorylation we used mass spectroscopy of tyropsin-digested fragments of p105 that had been phosphorylated *in vitro*. We had previously demonstrated that PIM1-dependent phosphorylation happens exclusively on serine. Fragments were separated by LC/MS/MS analysis and mass/charge ratios were determined. The predicted peptide fragment that would result from phosphorylation at serine-937 was recovered, with a mass of 1016 (Fig. 8). Since there are several potential phosphorylation sites within this peptide, we proceeded to

Fig. 7. PIM1 phosphorylates p105

Autoradiogram

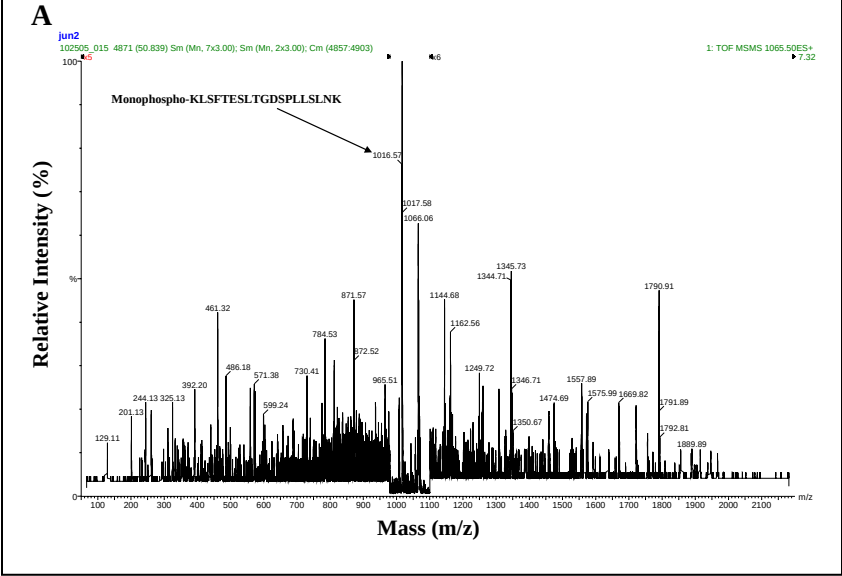


Protein staining



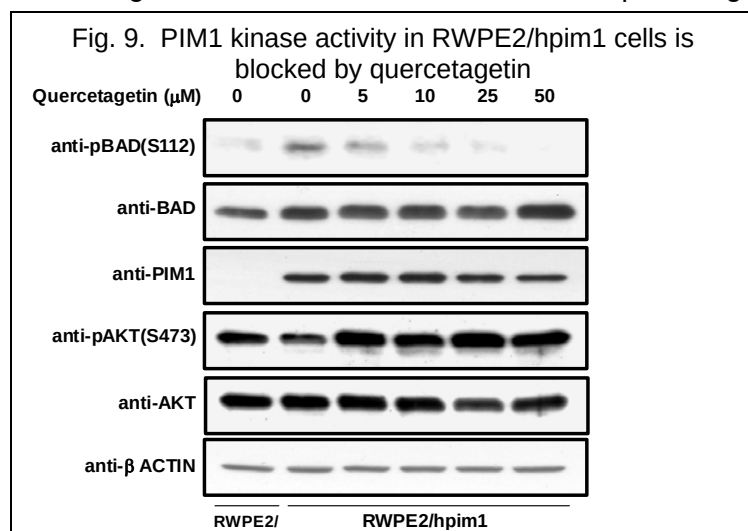
Pim:	1	2	1	2	1	2	1	2
Buffer:	Mg2+	Mn2+	Mg2+	Mn2+	Mg2+	Mn2+	Mg2+	Mn2+
	p105				Bad			

Fig. 8. Mass spectroscopy of p105 phosphorylated by PIM1



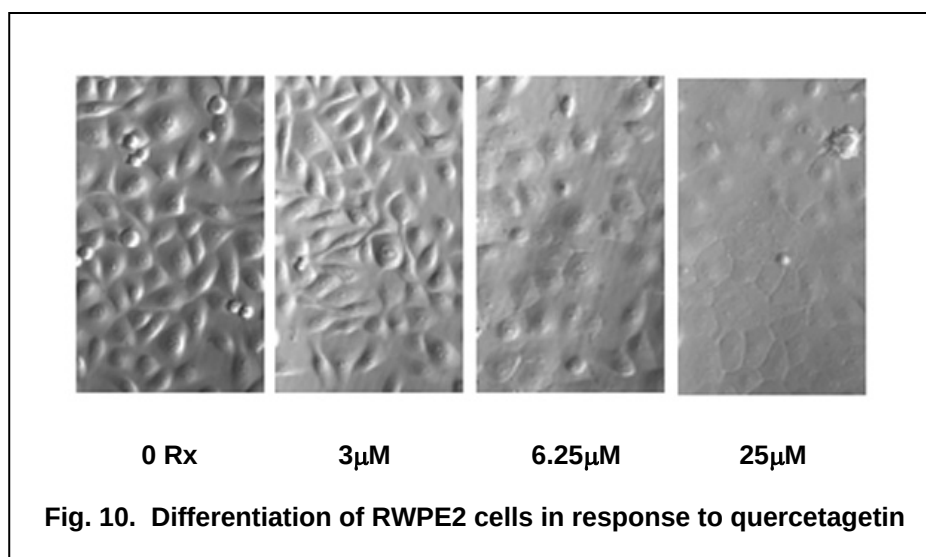
sequence the peptide with mass spectroscopy. Only the fragment corresponding to phosphoserine-937 was not recovered. These data unambiguously demonstrate that the major phosphorylation site of PIM1 on p105 is serine-937. We also found evidence by MALDI-MS that serine-851 may also be phosphorylated by PIM1. These data have not yet been confirmed by LC/MS/MS analysis.

The third specific aim proposed to use small molecule inhibitors of the PIM1 kinase as molecular probes to determine their effect on docetaxel sensitivity. We have submitted a report describing one such molecule, the flavonol quercetagenin (2). We have demonstrated that



quercetagenin in a moderately potent ($\text{IC}_{50} = 340\text{nM}$, specific, and cell-permeable inhibitor of PIM1 activity in prostate cancer cells. Key data include the demonstration that quercetagenin is competitive with ATP. A crystal structure of PIM1 in complex with quercetagenin, or with three other flavonoids, has been determined. We have also shown that quercetagenin is able to inhibit the activity of the PIM1 kinase in prostate cancer cells at an IC_{50} of about $5.5\mu\text{M}$. Interestingly the activity of the AKT kinase is not inhibited at all under these

conditions (Fig. 9). RWPE2 prostate cancer cells treated with quercetagenin develop morphologic changes consistent with differentiation or senescence, accompanied by profound growth inhibition, at concentrations that inhibit PIM1 kinase activity (Fig. 10).



KEY RESEARCH ACCOMPLISHMENTS

- Definition of a novel survival pathway activated by docetaxel treatment, and involving sequential activation or expression of STAT3, PIM1, and NFkB components.
- Identification of serine-937 as the major phosphorylation site for PIM1 on the p105/NFkB1 precursor protein
- Identification of quercetagenin as a moderately potent and specific, cell-permeable PIM1 kinase inhibitor
- Abstract accepted for presentation at the annual AACR meeting, Washington DC, April, 2006

REPORTABLE OUTCOMES

None in 01 year

CONCLUSIONS

Our data demonstrate that PIM1 is a critical component of a survival/stress pathway activated by docetaxel treatment of prostate cancer cells. This pathway leads to activation of NFkB-dependent transcription, possibly by phosphorylation of p105/NFkB1 by PIM1 at serine-937. Targeting PIM1 kinase activity with quercetagenin, or other PIM1 kinase inhibitors, may lead to additive or synergistic cell kill following docetaxel treatment.

REFERENCES

1. Zemskova M, Sahakian E, Lilly M: The PIM1 kinase is a critical component of a survival pathway that protects prostate cancer cells from docetaxel-induced death (abstract #2777), approved for presentation at 97th Annual Meeting of AACR, Washington, DC, April 2006.
2. Holder S, Zemskova M, Bremer R, Neidigh JW, Lilly MB: Characterization of a potent and selective small-molecule inhibitor of the PIM1 kinase (submitted to Molecular Cancer Therapeutics, 2006).

APPENDIX

Research data are presented throughout the body of this report.
The appendix contains two items:

1. AACR abstract #2777, approved for presentation at the 97th Annual Meeting, April, 2006, entitled "The PIM1 kinase is a critical component of a survival pathway that protects prostate cancer cells from docetaxel-induced death" by M. Zemskova, E. Sahakian, M. Lilly.
2. Curriculum vitae for Michael Lilly, MD

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Control/Tracking Number: 06-AB-2777-AACR Activity: Abstract Submission Current Date/Time: 11/11/2005 1:35:09 AM			
The PIM1 kinase is a critical component of a survival pathway that protects prostate cancer cells from docetaxel-induced death.			
Short Title: PIM1 and docetaxel-induced death			
Author Block: <u>Marina Zemskova, Eva Sahakian, Michael B. Lilly</u> , Loma Linda University, Loma Linda, CA			
The PIM1 serine-threonine kinase is a true oncogene that mediates survival and proliferation signals in human neoplasms, including leukemias and prostate adenocarcinomas. Enforced expression of PIM1 has been shown to enhance resistance of cells to cytotoxic agents and ionizing radiation. To examine potential pathways through which PIM1 produces drug resistance, we examined RWPE1 and RWPE2 prostate epithelial cells treated with docetaxel, a taxane used for treatment of prostate cancer. Cells treated with docetaxel demonstrated an increase in PIM1 protein and pim-1 mRNA 3-12 hrs after drug exposure. We then sought to establish upstream and downstream effectors of PIM1 expression. Docetaxel also induced expression of phosphoSTAT3 with similar kinetics. Drug-induced upregulation of PIM1 expression was abolished when cells were transfected with STAT3 decoy oligonucleotides, demonstrating that STAT3 activation by docetaxel is required for drug-induced upregulation of PIM1. Docetaxel treatment, and infection with a PIM1 expressing retrovirus, both induced activation of NFkB transcriptional activity in RWPE2 stably transfected with an NFkB/luciferase reporter plasmid. However, when a dominant negative PIM1 protein (NT81) was introduced by retroviral transduction, drug induced activation of NFkB activity was abolished. Retroviral transduction of wild-type PIM1 or dominant-negative (NT81) PIM1 increased or decreased survival of RWPE1 and RWPE2 cells treated with docetaxel. These data establish a survival pathway (drug → STAT3 → PIM1 → NFkB) induced by docetaxel and capable of impairing drug cytotoxicity. Targeting the PIM1 kinase, along with STAT3 and NFkB, may be a viable approach to enhancing cell kill by cytotoxic drugs such as docetaxel. Author Disclosure Block: M. Zemskova, None; E. Sahakian, None; M.B. Lilly, None. Additional Disclosures (Complete): The presenter of this abstract will discuss commercial products, devices, or technology in this presentation, as outlined below. : No - [Type "none" in the first box below] 1. Generic Name: : none I anticipate discussing an OFF-LABEL use of a commercial product/device in this educational activity. : No - [Type "none" in the box below] If you selected "Yes" above, you must indicate the product/device and describe its intended use in the box below. If you selected "No" above, you must type "none" in the box below: : none Investigational Use (Not approved by the FDA for any purpose): I anticipate discussing an INVESTIGATIONAL use of a commercial product/device in this educational activity. : No - [Type "none" in the box below] If you selected "Yes" above, you must indicate the product/device and describe its intended use in the box below. If you selected "No" above, you must type "none" in the box below: : none I agree with the declaration statement above. : True Name: : Marina Zemskova Date [mm/dd/yyyy]: : 11/10/2005 Category and Subclass (Complete): CB02-07 Survival factors Keywords/ Indexing (Complete): Kinases ; Resistance ; Docetaxel ; Prostate Sponsor (Complete): 2006 Travel Awards (Complete): Payment (Complete): Your credit card order has been processed on Friday 11 November 2005 at 1:34 AM. Status: Complete			

MICHAEL BRIAN LILLY, MD

Updated 1/2006

Personal Data:	Birth: 26 December, 1950, Atlanta, GA Citizenship: USA
Office:	Center for Molecular Biology and Gene Therapy Loma Linda University School of Medicine Loma Linda, CA 92354 Phone (909) 558-8777 Fax (909) 558-0177 e-mail: mlilly@llu.edu
Home:	511 West Olive Avenue Redlands, CA 92373 (909) 792-5573
Education:	Southern Adventist University, Collegedale, TN 1967-1971, B.A. (biology, chemistry) Loma Linda University, Loma Linda, CA 1971-1975, M.D.
Postgraduate Training	Internal Medicine residency University of Alabama at Birmingham Birmingham, AL 1975-1978 Hematology-Oncology Fellowship University of Alabama at Birmingham 1978-1981
Faculty Positions:	6/81-6/82 Instructor in Medicine UAB School of Medicine 6/82-10/88 Assistant Professor of Medicine, UAB School of Medicine 6/82-10/88 Associate Scientist, Lurleen Wallace Institute, Birmingham, AL
Tumor	

**Faculty Postions
(cont'd):**

6/89-9/98 Associate Professor of
Medicine, University of Washington School
of Medicine, Seattle, WA

4/96-10/96 Visiting Scientist, The Walter and Eliza
Hall Institute for Medical Research, Melbourne,
Victoria, AUSTRALIA

9/98 – present Professor of Medicine & Microbiology
Director, Center for Molecular Biology & Gene
Therapy Loma Linda University School of Medicine,
Loma Linda, CA

Hospital Positions:
Alabama

1981-1988 Attending Physician, University of
Hospitals and Clinic

1981-1988 Staff Oncologist, Birmingham VA Medical
Center, Birmingham, AL

1989-1998 Staff Oncologist, Seattle
VA Medical Center, Seattle, WA

1998-present Attending Physician, Loma Linda
University Medical Center, Loma Linda, CA

Honors:

1974 Alpha Omega Alpha

1980 National Research Service Fellow

1981 Fellow, American College of Physicians

Board Certification:

1979 American Board of Internal Medicine

1980 ABIM Subspecialty Exam, Hematology

1981 ABIM Subspecialty Exam, Med. Oncology

Licensure:

Alabama Medical License #7730 (3/77-12/91)

Washington State License #27864 (12/91 – 12/00)

California Medical License #G84932 (12/98 – present)

Organizations:

Fellow, American College of Physicians

Member, American Society of Hematology

Member, American Society for Bone Marrow
Transplantation

Member, American Society for Gene Therapy

**National
Professional
Responsibilities**

Member, ad hoc study sections for NIH:

1987 Diagnostic Radiology

1988 Experimental Therapeutics

Member, site visit team for program project

**Special Local
Responsibilities**

Dr. George Hahn, PI; Stanford University
1988, 1989
Member, site visit team for program project
Dr. Bayard Clarkson, PI, Memorial-Sloan
Kettering Inst., 1997
Member, Scientific Review Subcommittee
SVAMC, 1993, 1994, 1997
Member, Research & Development Committee
SVAMC, 1994, 1995
Member, Hospice Advisory Committee
SVAMC, 1994, 1995
Board Development Committee, Leukemia &
Lymphoma Society (Southern California Chapter),
2003

Consultant

Cetus Corporation (1986)
EncorePharma (2001-present)
Myriad Genetics (2002-present)
Exelixis Pharmaceuticals (2005-present)

GRANTS & CONTRACTS (PRINCIPAL INVESTIGATOR) *Note: This listing does not
include multicenter clinical trials in which Dr. Lilly was the local principal investigator.*

National Institutes of Health F32CA27980 *Hyperthermia of animal and human tumors;*
7/80-6/82

National Institutes of Health R01CA18138-11 *Prediction of thermal tolerance by in vivo*
NMR spectroscopy; 7/82-6/83

National Institutes of Health R01CA36790 *Assessment of hyperthermia by in vivo ³¹P-*
NMR spectroscopy; 9/84-9/87

Cetus Corporation *Characterization of a human granulocyte CSF; 7/85-6/86*

National Institutes of Health R01CA45672 *Cytokine signaling in myeloid leukemia;*
9/87-10/98

VA Merit Review Award *Non-protein hematopoietic agents; 10/90-4/97*

March of Dimes Birth Defects Foundation *Characterization of a 28kd protein related to*
G-CSF; 7/93-6/96

Lymphoma Research Foundation of America *Mechanism of action of the pim-1*
oncogene; 7/95-7/96

Roche Pharmaceuticals *Preclinical study of Roferon and bryostatin 1 in a melanoma model*; 1/98-12/99

Department of Defense, National Medical Technology Testbed #76-FY99: *Cell-permeable proteins for cell regulation*. 12/99 – 7/02

Leukemia Society of American Translational Award *Propionic Acid Analogues for CLL*. 9/1/01 – 8/31/05

Celgene Corporation, *Phase I-II trial of combined GM-CSF (sargramostim) and thalidomide for hormone-refractory prostate cancer* (5/02-5/04).

National Institutes of Health R03CA107820 *Molecular Targets of NSAIDs in Prostate Cancer*; (5/1/04 – 4/30/07)

Department of Defense, CDMRP Prostate Cancer Program PC040635 *Pim-1: A Molecular Target to Modulate Cellular Resistance to Therapy in Prostate Cancer* (10/04 – 10/07)

Pharmion Corporation, *Use of azacytidine to reverse silencing of GST-p1 in early prostate cancer*. (10/05 – 10/07)

GRANTS and CONTRACTS (Co-investigator)

National Institutes of Health R01CA097043 *Molecular pathology of 2-deoxy-5-azacytidine*; L. Sowers, PI; Michael Lilly, co-investigator (10% FTE). 7/1/03 – 6/30/08

PUBLICATIONS IN PEER-REVIEWED JOURNALS

1. Brezovich I, **Lilly M**, Durant J, Richards D: A practical system for clinical radiofrequency hyperthermia. *Int J Rad Onc Biol Phys* 7:423-430, 1981
2. Ng T, Evanochko W, Hiramoto R, Ghanta V, **Lilly M**, Lawson A Corbett T, Durant J, Glickson J: ³¹P-topical NMR spectroscopy of *in vivo* tumors. *Mag Res* 49:271-286, 1982.
3. **Lilly M**, Brezovich I, Chakraborty D, Atkinson W, Durant J, Ingram J, McElvein R: Hyperthermia with implanted electrodes: *in vitro* and *in vivo* correlations. *Int J Rad Onc Biol Phys* 9:373-382, 1983.
4. Evanochko W, Ng T, **Lilly M**, Kumar N, Durant J, Glickson J: *In vivo* ³¹P-NMR studies of the effect of cancer therapy on a murine mammary carcinoma. *Proc Natl Acad Sci USA* 80:334-338, 1983.

5. **Lilly M**, Ng T, Evanochko W, Kumar N, Elgavish G, Durant J, Hiramoto R, Ghanta V, Glickson J: *in vivo* ³¹P-NMR study of hyperthermia tumor treatment. *Cancer Res* 44:663-638, 1984.
6. Hiramoto R, Ghanta V, **Lilly M**: Reduction in tumor burden in murine osteosarcoma by hyperthermia and cyclophosphamide. *Cancer Res* 44:1405-1408, 1984.
7. Brezovich I, Atkinson W, **Lilly M**: Local hyperthermia with interstitial techniques. *Cancer Res* 44:46752s-4756s, 1984.
8. **Lilly M**, Brezovich I, Atkinson W: Hyperthermia with thermally self-regulating ferromagnetic implants. *Radiology* 154:243-244, 1985.
9. **Lilly M**, Katholi C, Ng T: Direct relationship between high-energy phosphate content and blood flow in thermally treated tumors. *JNCI* 75:885-889, 1985.
10. **Lilly M**, Omura G: Clinical pharmacology of oral intermediate dose methotrexate with or without probenecid. *Cancer Chemo Pharm* 15:220-222, 1985.
11. **Lilly M**, Carroll A, Prchal J: Lack of association between glutathione content and development of thermal tolerance in human fibroblasts. *Radiation Res* 106:41-46, 1986.
12. Tucker K, **Lilly M**, Heck L, Rado T: Characterization of a new human diploid myeloid leukemia cell line (PLB985) with granulocytic and monocytic differentiating capacity. *Blood* 70:372-378, 1987.
13. Devlin J, Devlin P, Myambo K, **Lilly M**, Rado T, Warren K: Isolation and expression of a cDNA encoding a human granulocyte colony-stimulating factor. *J Leukocyte Biol* 41:302-306, 1987.
14. **Lilly M**, Devlin J, Devlin P, Rado T: Production of granulocyte colony-stimulating factor by a human melanoma line. *Exp Hematol* 15:966-971, 1987.
15. Barton J, Parmley R, Butler T, Williamson S, **Lilly M**, Gualtieri R, Heck L: Differential staining of neutrophils and monocytes: surface and cytoplasmic iron-binding proteins. *Histochem J* 210:147-155, 1988.
16. Csepreghy M, Yielding A, **Lilly M**, Scott C, Prchal J: Characterization of a new G6PD variant: G6PD Central City. *Am J Hematol* 28:61-62, 1988.
17. **Lilly M**, Kraft A: Leukemia-differentiating activity expressed by the human melanoma cell line LD1. *Leukemia Res* 12:213-218, 1988
18. Prchal J, Hauk M, Csepreghy M, **Lilly M**, Berkow R, Scott C: Two apparent G6PD variants in normal XY man: G6PD Alabama. *Am J Med* 84:517-523, 1988.

19. Bailey A, **Lilly M**, Bertoli L, Ball E: An antibody which inhibits in vitro bone marrow proliferation in a patient with system lupus erythematosus and aplastic anemia. *Arthritis and Rheumatism* 32:901-905, 1989.
20. Kraft A, Williams F, Pettit R, **Lilly M**: Variable response of human myeloid leukemia lines and fresh cells to differentiating activity of bryostatin 1. *Cancer Res* 49:1287-1293, 1989.
21. Everson M, Brown C, **Lilly M**: IL6 and GM-CSF are candidate growth factors for chronic myelomonocytic leukemia cells. *Blood* 74:1472-1476, 1989.
22. Nemunaitis J, Andrews F, Mochizuki D, **Lilly M**, Singer J: Human marrow stromal cells: response to IL6 and control of IL6 expression. *Blood* 74:1693-1699, 1989.
23. Brezovich I, **Lilly M**, Meredith R, Weppleman B, Brawner W, Henderson R, Salter M: Hyperthermia of pet animal tumors with self-regulating ferromagnetic thermoseeds. *Intl J Hyperthermia* 6:117-130, 1990.
24. **Lilly M**, Tompkins C, Brown C, Pettit R, Kraft A: Differentiation and growth modulation of chronic myelogenous leukemia cells by bryostatin 1. *Cancer Res* 50:5520-5525, 1990.
25. **Lilly M**, Brown C, Pettit R, Kraft A: Bryostatin 1: a potential cytotoxic agent for chronic myelomonocytic leukemia cells. *Leukemia* 5:282-287, 1991.
26. Andrews D, **Lilly M**, Tompkins C, Singer J: Sodium vanadate, a tyrosine phosphatase inhibitor, affects expression of hematopoietic growth factors and extracellular matrix RNAs in SV40-transformed human marrow stromal cells. *Exp Hematol* 20:391-400, 1992.
27. **Lilly M**, Le T, Holland P, Hendrickson S: Expression of the pim-1 kinase is specifically induced in myeloid cells by growth factors whose receptors are structurally related. *Oncogene* 7:727-732, 1992.
28. Takahashi G, Andrews D, Tompkins C, Montgomery R, **Lilly M**, Singer J, Alderson M: Effect of granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin 3(IL3) on interleukin 8 (IL8) production in neutrophils and monocytes. *Blood* 81:357-364, 1993.
29. Polostkya A, Zhao C, **Lilly M**, Kraft A: A critical role for the intracellular domain of the alpha chain of the GM-CSF receptor in cell cycle transition. *Cell Growth & Diff* 4:525-531, 1993

30. Polostkya A, Zhao C, **Lilly M**, Kraft A: Mapping the intracytoplasmic regions of the alpha granulocyte-macrophage colony-stimulating factor receptor necessary for cell growth regulation. *J Biol Chem* 269:14607-14613, 1994
31. Takahashi G, Montgomery B, Stahl W, Crittenden C, Thorning D, **Lilly M**: Pentoxifylline inhibits tumor necrosis factor-alpha mediated cytotoxicity and activation of phospholipase A2 in L929 murine fibrosarcoma cells. *Intl J Immunopharm* 16:723-736, 1994
32. Sensebe L, Li J, **Lilly M**, Crittenden C, Herve P, Charbord P, Singer J: Non-transformed colony-derived stromal cell lines from normal human marrows. I. Growth requirements, characterization ,and myelopoiesis-supportive ability. *Exp Hematol* 23:507-513, 1995
33. Asiedu C, Biggs J, **Lilly M**, Kraft A: Inhibition of leukemic cell growth by the protein kinase C activator Bryostatin 1 correlates with the dephosphorylation of cyclin-dependent kinase 2. *Cancer Res* 55:3716-3720, 1995
34. **Lilly M**, Vo K, Lee T, Takahashi G: Bryostatin 1 acts synergistically with interleukin 1 to promote the release of G-CSF and other myeloid cytokines from marrow stromal cells. *Exp Hematol* 24:613-621, 1996.
35. Matsuguchi T, Zhao Y, **Lilly MB**, Kraft AS: The cytoplasmic domain of the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α subunit is essential for both GM-CSF-mediated growth and differentiation. *J Biol Chem* 272:17450-17459, 1997.
36. **Lilly M**, Kraft A: Enforced expression of the 33kd pim-1 kinase enhances factor-independent growth and inhibits apoptosis in murine myeloid cells. *Cancer Res* 57:5348-5355, 1997.
37. Matsuguchi T, **Lilly MB**, Kraft AS: Cytoplasmic domains of the human granulocyte-macrophage colony-stimulating factor receptor β chain (h β c) responsible for human GM-CSF-induced myeloid cell differentiation. *J Biol Chem* 273:19411-19418, 1998.
38. Frankel A, **Lilly M**, Kreitman R, Hogge D, Beran M, Freedman MH, Emanuel PD, McLain C, Hall P, Tagge E, Berger M, Eaves C: Diptheria toxin fused to granulocyte-macrophage colony-stimulating factor is toxic to blasts from patients with juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. *Blood* 92:4279-4286, 1998.
39. **Lilly M**, Kiskonen P, Sandholm J, Cooper JJ, Kraft AS: The Pim-1 kinase prevents apoptosis-associated mitochondrial dysfunction, and supports cytokine-independent survival of myeloid cells in part through regulation of *bcl-2* expression. *Oncogene* 18:4022-4031, 1999.

40. **Lilly M**, Zemskova M, Frankel AE, Salo J, Kraft AS: Distinct domains of the human GM-CSF receptor alpha subunit mediate activation of JAK/STAT signaling and differentiation. *Blood* 97:1662-1670, 2001.
41. Wu X, Daniels T, Molinaro C, **Lilly MB**, Casiano C: Caspase cleavage of the nuclear autoantigen LEDGF/p75 abrogates its prosurvival function: implications for autoimmunity in atopic disorders. *Cell Death Differentiation* 9:915-924 (2002).
42. Lombano F, Kidder MY, **Lilly M**, Gollin YG, Block BS: Recurrence of microangiopathic hemolytic anemia after apparent recovery from the HELLP syndrome: A case report. *J Reprod Med* 47:875-877 (2002)
43. Frankel AE, Powell BL, **Lilly MB**. Diphtheria toxin conjugate therapy of cancer. *Cancer Chemother Biol Response Modif.* 2002;20:301-13. Review
44. Ionov Y, Le X, Tunquist BJ, Sweetenham J, Sachs T, Ryder J, Johnson T, **Lilly MB**, Kraft AS: Nuclear localization of the pim-1 protein kinase is necessary for its biologic effects. *Anticancer Res* 23(1A):167-78 (2003).
45. Yan B, Zemskova M, Holder S, Chin V, Kraft AS, Koskinen PJ, **Lilly MB**: The PIM-2 kinase phosphorylates BAD on serine-112 and reverses BAD induced cell death. *J Biol Chem* 278:45358-45367 (2003)
46. Aho TLT, Sandholm J, Peltola KJ, Mankonen H, **Lilly M**, Koskinen PJ: Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the Ser¹¹² gatekeeper site. *FEBS Letters* 571:43-49 (2004).
47. Fodor I, Timiryasova T, Denes B, Yoshida J, Ruckle H, **Lilly M**: Vaccinia virus-mediated p53 gene therapy of bladder cancer in an orthotopic murine model. *J Urology* 173, 604-609 (2005).
48. Kim K-T, Baird K, Ahn J-Y, Meltzer P, **Lilly M**, Small D: Pim-1 is upregulated in constitutively activating FLT3 mutants and plays a role in FLT3-mediated cell survival. *Blood* 105(4), 1759-1767 (2005).
49. Chen WW, Chan DC, Donald C, **Lilly MB**, Kraft AS. Pim family kinases enhance tumor growth of prostate cancer cells. *Mol Cancer Res.* 2005 Aug;3(8):443-51.
50. Zemskova M, Wechter W, Yoshida J, Ruckle H, Reiter RE, **Lilly MB**: Gene expression profiling in R-flurbiprofen-treated prostate cancer: Identification of prostate stem cell antigen as a flurbiprofen-regulated gene. (submitted, 2006).
51. Holder SL, Zemskova M, Bremner R, Neidigh J, **Lilly MB**: Identification of specific, cell-permeable small molecule inhibitor of the PIM1 kinase. (submitted, 2006)

BOOKS AND CHAPTERS:

Singer J, Slack J, **Lilly M**, Andrews D: Marrow stromal cells: response to cytokines and control of gene expression (in) *The Hematopoietic Microenvironment*. M. Wicha and M. Long, eds. Johns Hopkins Press, Baltimore, (1993).

RECENT ABSTRACTS:

Hromas R, Collins S, Bavisotto L, Hagen F, Raskind W, **Lilly M**, Kaushansky K: HEM-1, a potential transmembrane protein, is restricted to, yet ubiquitous in, hematopoietic cells. *Blood* 75:98a, 1990

Bianco J, Nemunaitis J, Andrews D, **Lilly M**, Shields A, Singer J: Combined therapy with pentoxifylline, ciprofloxacin, and prednisone reduces regimen related toxicity and accelerates engraftment in patients undergoing bone marrow transplantation. *Blood* 78:237a, 1991.

Lilly M, Sensebe L, Singer J: Characterization of cell-associated granulocyte colony-stimulating factor in human marrow stromal cells. *Blood* 78:261a, 1991 (oral presentation)

Takahasi G, **Lilly M**, Bianco J, Crittenden C, Singer J: Pentoxifylline inhibits tumor necrosis factor-alpha cytotoxicity and activation of phospholipase A2 in murine fibrosarcoma cells. *Blood* 78:323a, 1991.

Kirshbaum M, **Lilly M**: Multiple growth factors induce expression of the Bcl-2 protein in 32D murine hematopoietic cells, but differ in their ability to inhibit apoptosis. *Blood* 84:423a, 1994.

Lilly M, Pettit G: Identification of the cephalostatins as potent cytotoxic agents for myeloid leukemia cells. *Blood* 86:517a, 1995 (poster presentation)

Lilly M, Kraft A, Rotman E: Enforced expression of the human 33kd Pim-1 kinase enhances autonomous proliferation and tumorigenicity in factor-dependent murine FDCP1 cells. *Blood* 86:588a, 1995 (oral presentation).

Lilly M, Cooper JJ: Enforced expression of the human 33kd Pim-1 kinase prevents apoptosis-associated mitochondrial dysfunction and upregulates *bcl-2* mRNA expression in murine myeloid cells. (oral presentation, ASH 12/97)

Wu X, Molinaro C, **Lilly M**, Casiano C: Caspase-mediated cleavage of the transcription co-activator p75 during apoptosis (abstract #993). *Proc AACR* 41:155 (2000).

Quiggle DD, **Lilly M**, Murray ED, Gibson K, Leipold D, Gutierrez I, Loughman B, Wechter W: PK guided multi-dose, tolerance, and safety of E-7869 in prostate cancer patients (abstract #3874). *Proc AACR* 41:609 (2000)

Lilly M, Frankel AE, Salo J, Kraft AS: Distinct domains of the human GM-CSF receptor alpha subunit mediate activation of Jak/Stat signaling and differentiation (abstract #2455). *Blood* 96:572a (oral presentation, ASH 12/00)

Chen CS, **Lilly MB**, Wang FS, Howard FD, Houwen B: Rapid monitoring of peripheral blood stem cells (PBSC) mobilization by using cell membrane phospholipid content correlates well with CD34+ measurements, successful harvest and engraftment (abstract #1642). *Blood* 96:380a (poster presentation, ASH 12/00)

Lilly M, Ruckle H, Quiggle D, Gutierrez I, Murray D, Gibson K, Leipold D, Wechter W, Loughman B: Multi-dose phase I-II trial of E-7869 in prostate cancer patients: safety and time to PSA progression (TPSAP). *Proc AACR* 42:142 (2001)

Kastaros EP, Casiano C, Colburn KK, **Lilly M**, Weisbart RH, Kim J, Green LM: Lupus associated anti-guanosine antibodies: potential pathogenic effects. *Arthritis & Rheumatism* 44:S99 (2001)

Yan B, Zemskova M, Kraft AS, Koskinen PJ, **Lilly MB**: The pim-2 kinase phosphorylates Bad on serine-112 and reverses Bad induced cell death. (abstract #2919). *Blood* (poster presentation, ASH 12/02).

Lilly MB, Thorn S, Oberg K, Bashirova S, Zemskova M: The pim-1 serine/threonine kinase is primarily expressed in granulocytes and macrophages in inflamed tissues (abstract #981). *Blood* 102:276a (poster presentation, ASH 12/2003)

Neidigh J, Holder S, **Lilly MB**: Using docking to improve comparative modeling predictions: applications to pim-1 kinase. (poster presentation, *Structure-Based Drug Design 2004*, Boston, MA, April 26-28, 2004)

Chen CS, Zemskova M, Reiter R, **Lilly MB**: Gene expression profiling in R-flurbiprofen-treated prostate cancer: Identification of prostate stem cell antigen as a flurbiprofen-regulated gene. (poster presentation, *AACR 3rd Annual Conference on Frontiers in Cancer Prevention*, Seattle, WA; October 2004).

Lilly MB, Wechter W, Puuvula L, Henry H: R-Flurbiprofen (RFB) a non-steroidal anti-inflammatory drug (NSAID) with anti-tumor activity, inhibits the expression of CYP24 in murine prostate carcinomas. (poster presentation at *Biennial Vitamin D Conference "Vitamin D and Cancer Chemoprevention"*, NIH, Bethesda, MD, November 2004)