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:** Temple University

2. **Reporting Period (start and end date of grant award period):** 1/1/2012-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number: (**215) 204-7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 6: Nanoconjugates for Targeted Treatment of Acute Lung Injury

7. **Start and End Date of Research Project:** 1/1/2012-6/30/2012

8. **Name of Principal Investigator for the Research Project:** Laurie Kilpatrick, 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:

   $ 78,290.08

   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
Sun	Research Specialist	25%	$16,900
Romano	Research Assistant	25%	$15,531

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
Kilpatrick	PI	5%
Knight	Co-PI	5%
Kelsen	Co-PI	2%
Kennedy	Student	15%
Birabaharan	Student	10%

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:

Nanotechnology Institute, Ben Franklin Technology Partners of Southeastern PA. Grant award: $60,000

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:
Novel Protein Kinase C Targeted Therapy for Acute Lung Injury	X NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	11/2011	$1,912,500	$1,530,000 7/01/2012 – 6/30/2016
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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:

Plans are to resubmit an NIH grant R01 HL109521-01 entitled: Nanoconjugates for Targeted Treatment of Acute Lung Injury once we have sufficient data to address the reviewers concerns from the first submission

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

Continue to seek better targeting moieties for pulmonary endothelium and resubmit our NIH R01 grant entitled "Nanoconjugates for Targeted Treatment of Acute Lung Injury"

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_____ No__X_____

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

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

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

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

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_____ No____X_____

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

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_____ No_____X_____

If yes, please describe the collaborations:

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 <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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. <u>Provide detailed results of the project.</u> 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.

Project Overview

Acute respiratory distress syndrome (ARDS) is a major public health problem and a leading cause of death in intensive care units (ICU) (>200,000 cases/year). ARDS is characterized by an intense inflammatory response leading to neutrophil infiltration of the lungs and severe lung tissue damage. Treatment of ARDS is largely supportive and no specific pharmacologic therapies are available that protect the lung from neutrophil-mediated damage. We have identified the Protein Kinase C isotype-delta (δ-PKC) as a critical regulator of the inflammatory response controlling recruitment and activation of neutrophils in the lungs. Delivery of a δ-PKC TAT peptide inhibitor directly into the lungs of a rat model of ARDS had a dramatic anti-inflammatory and lung protective effect indicating this δ-PKC TAT peptide would offer a novel treatment option for ARDS. We have a proven δ-PKC peptide inhibitor which through linkage to a TAT peptide is taken up intracellularly to inhibit δ-PKC activity. To develop this δ-PKC TAT inhibitor into a viable therapeutic, a noninvasive mechanism for drug delivery is needed which selectively targets our δ-PKC inhibitor to the lung. We propose to use nanocarriers to transport our inhibitor directly to the lung. This nanoconjugate will optimize drug delivery, avoid immune cell activation, and protect the therapeutic from proteolytic degradation. Directed delivery of this highly specific δ-PKC-TAT inhibitor to the lungs would offer a protective treatment and could be administered in the emergency room or upon admittance to an ICU.

Aim 1: "Proof-of-concept" experiments to develop a series of nanoconjugate compounds which deliver the δ-PKC TAT inhibitory peptide directly to the lungs. Candidate nanoconjugate compounds will be screened using an in vitro model system of human cells. We will determine the effect of these drug delivery systems on δ-PKC inhibitor binding, intracellular uptake, inhibition of δ-PKC, and effect on neutrophil transmigration.

Aim 2: In vivo testing of radiolabeled (Tc-99m) candidate nanoconjugate compounds to determine uptake and biodistribution in our rodent model of ARDS. These studies will determine effectiveness of the drug delivery system in vivo and the concentration required to inhibit δ-PKC activity in the rat lung.

Summary of Research Completed

Aim 1: "Proof-of-concept" experiments to develop a series of nanoconjugate compounds which deliver the δ-PKC TAT inhibitory peptide directly to the lungs. Candidate nanoconjugate compounds will be screened using an in vitro model system of pulmonary microvascular endothelial cells (PMVEC).

The objectives of these in vitro experiments are: 1) to achieve sufficient loading of δ-PKC-TAT peptide into liposomes, 2) stable encapsulation of the peptide, and 3) intracellular delivery of the δ-PKC-TAT peptide into PMVEC.

Nanoconjugate design

To target the pulmonary endothelium we designed nanoconjugates composed of **4 components**: **1)** an inhibitory δ-PKC peptide, **2)** a cell permeable TAT peptide that facilitates intracellular uptake into cell cytosol, **3**) an endothelium targeting moiety, and **4**) a nanocarrier. The δ-PKC inhibitory peptide was composed of the following aa sequence: SFNSYELGSL. The δ-PKC inhibitory peptide was coupled via an N-terminal Cys-Cys bond to a membrane permeant peptide in the HIV TAT gene product (YGRKKRRQRRR). The use of a disulfide bond limits directed intracellular delivery of the δ-PKC TAT inhibitor to the cytoplasm. Some of the peptide was synthesized with a fluorescent tag at a noncritical site of the δ-PKC peptide, to enable tracking in vitro (5-carboxyfluorescein (5FAM-green label) or hydrazinonicotinate for ^{99m}Tc radiolabeling). The nanoconjugate used for these studies was a PEGylated liposome nanocarrier which encapsulated the δ-PKC-TAT inhibitory peptide into the hydrophilic interior of these liposomes. We successfully encapsulated 99mTc-δ-PKC-TAT peptide into PEGylated liposomes with a loading efficiency of 40-45% of 99mTc-HyNic-δPKC-TAT.

The stability of the nanocarriers was determined by measuring the release of the 99mTc-HyNic-δPKC-TAT from the interior of the nanocarrier. The nanocarriers were suspended in fresh buffer and incubated at 37°C in a Float-A-Lyzer dialysis unit with appropriate molecular weight cutoff. At select time points over 24 hrs, dialysis buffer was sampled and the release of δ-PKC-TAT peptide from the nanocarriers was determined by monitoring radioactivity to determine free peptide in solution as a fraction of the total. These nanocarriers were stable and showed low levels of δ-PKC-TAT peptide release over 24 hrs of 37°C dialysis as compared to free δ-PKC-TAT peptide (**Figure 1**).

Nanocarrier uptake into PMVEC

Encapsulation of the δ-PKC TAT peptide inhibitor into nanocarriers may alter the kinetics of peptide uptake by endothelial cells, intracellular targeting, and inhibitory activity. In this series of experiments we examined the uptake and internalization of the nanocarriers with encapsulated δ-PKC TAT into pulmonary microvascular endothelial cells (PMVEC). Human pulmonary microvascular endothelial cells (PMVEC) were purchased from Lonza (Walkersville, MD) and cultured in flasks using endothelial growth medium 2 (Lonza) with added bovine brain extract, vascular endothelial growth factor, epidermal growth factor, gentamycin, and hydrocortisone according to the manufacturer's specifications. Cells were obtained at passage 5 and used between passages 5 and 8. Cells were passed from T-25 flasks into experimental plates when 70–80% confluent and cultured in 96-well plates. Medium was changed on the monolayer every 48 hr. Rat pulmonary microvascular endothelial cells (PMVEC, (VEC Technology, Inc., Rensselaer, NY)) were also obtained, cultured to 70-80% confluency and then plated in fibronectin-coated 96 well plates. As shown in **Figure 2**, a fluorescently tagged δ-PKC TAT inhibitory peptide was encapsulated into nanocarriers. The nanocarriers were added to PMVEC and incubated for 20 min at 37°C, washed, and the δ-PKC inhibitory peptide binding to PMVEC was determined by fluorescence microscopy. These studies demonstrated δ-PKC peptide uptake but did not determine whether the δ-PKC peptide was released from the nanocarrier. The effectiveness of the encapsulated inhibitory peptide was next evaluated by determining the effect of encapsulation in nanocarriers on VCAM-1 expression in PMVEC.

Effect of δ-PKC TAT inhibitory peptide on VCAM-1 expression in PMVEC

The goal of these experiments was to test the hypothesis that encapsulated δ-PKC TAT peptide will be as effective as free peptide in modulating cytokine-mediated activation of pulmonary endothelial in vitro. We determined the effect of candidate nanoconjugates on cytokine-mediated expression of the adhesion molecule VCAM-1 on the surface of PMVEC. The surface expression of VCAM-1 was determined by a cell surface ELISA. PMVEC were grown to confluence in 96-well plates and then pretreated with buffer, free PKC-δ TAT peptide inhibitor (0.01-5 uM) or nanocarriers with encapsulated δ-PKC TAT (0.1uM-2 uM) for 1hr prior to the addition of IL-1 (10U/ml). The cells were incubated for 18 hrs, washed twice in washing buffer consisting of PBS with 0.5% BSA, 1mM $CaCl_2$ and 1mM $MgCl_2$, and then fixed for 15min at RT in 1% paraformaldehyde. The cell monolayers were washed and blocked for 1hr at 37°C in blocking buffer (washing buffer plus 2% BSA). The cells were incubated with anti-human VCAM-1 (0.25ug/ml, clone 51-10C9, BD Pharmingen) for 1hr, washed, and incubated with goat anti-mouse IgG conjugated to HRP (1:1000) for 1hr. The cell monolayers were washed, Tetramethylbenzidine (TMB) liquid substrate (Sigma-Aldrich) was added, and optical density (OD_{650}) determined. Mouse IgG1 was used as an isotype control at a concentration of 0.25ug/ml. The value obtained with mouse IgG1 was subtracted from the value obtained for VCAM-1 expression. We found that there was little VCAM-1 expression on PMVEC monolayers under basal or resting conditions. IL-1 stimulated a significant increase in VCAM-1 which was attenuated in a dose-dependent manner by incubation with either free δ-PKC TAT peptide inhibitor or by nanoconjugates with the encapsulated δ-PKC TAT peptide (**Figures 3 and 4**). Thus, δ-PKC selectively regulates activation of PMVEC and IL-1-induced cell surface expression of VCAM-1.

Furthermore, these studies indicate that the δ-PKC TAT peptide is still an effective inhibitor when delivered to the endothelium encapsulated in nanoconjugates.

Selection of targeting moiety

The pulmonary endothelium targeting moiety has been the rate limiting step in these studies. To design a nanoconjugate which would target pulmonary endothelial cells, we tested a series of ICAM-1 and PECAM-1 antibodies and Lisinopril. We also had a cyclo [CGSPGWVRC] peptide synthesized, a putative pulmonary endothelial targeting ligand identified from phage-display libraries. Both anti-PECAM-1 and anti-ICAM-1 have successfully targeted other nanocarriers to lung endothelium both in vitro and in animal studies. Antibody binding characteristics were determined in vitro using cultured pulmonary microvascular endothelial cells. For binding experiments, rhodamine –tagged ICAM-1 or PECAM-1 antibodies were added to PMVEC monolayers and incubated for 1 hr at 37°C. The cell monolayers were washed and fluorescence measured using a fluorescent microplate reader. In vitro, we found that PECAM-1 antibodies bound to rat PMVEC with greater affinity than ICAM-1 antibodies, the cyclic peptide or Lisinopril in resting as well as cytokine-activated endothelial cells. In particular, a mouse anti-Rat PECAM-1 [CD31] (Clone TLD-3A12)-Millipore) antibody bound to rat lung endothelial monolayers in a dose-dependent manner at concentrations > 0.01 ug/ml (**Figure 5**). We next tested the binding affinity of rhodamine-tagged nanoconjugates coupled to these targeting moieties. Two different PECAM-1 antibodies, the cyclopeptide, and Lisinopril were coupled to fluorescent-tagged nanoconjugates and binding to PMVEC was compared to IgG-labeled nanocarriers and unlabeled nanocarriers. Specificity of binding was determined by pretreatment with 100-fold excess of unlabeled targeting antibody, peptide or Lisinopril prior to addition of fluorescently-tagged nanoconjugates. Two anti-Rat PECAM-1 (Clone TLD-3A12 and Clone 164.1) antibodies bound to rat lung endothelial monolayers in a dose-dependent manner. Using these antibodies, there was significantly increased association of anti-PECAM-1-coupled nanocarriers with rat lung endothelial monolayers as compared to IgG-tagged nanocarriers controls indicating increased targeting and association of the nanocarriers with the PECAM-1 antibody (**Figure 6**).

Summary of Aim 1

We were able to accomplish the objectives of this aim. First, we successfully encapsulated 99mTc-δ-PKC-TAT peptide into PEGylated liposomes and achieved a loading efficiency of 40-45% of 99mTc-HyNic-δPKC-TAT. Second, the encapsulation of the δ-PKC-TAT peptide into the nanocarriers was stable and demonstrated low levels of peptide release over 24 hrs of 37°C. Third, we demonstrated uptake of the encapsulated δ-PKC-TAT peptide into pulmonary microvascular endothelial cells in vitro. The encapsulated inhibitory peptide was active and decreased cytokine-mediated expression of the adhesion molecule VCAM-1in endothelial cells. Lastly, we tested a series of targeting moieties and found increased association of anti-PECAM-1-coupled nanocarriers with rat lung endothelial monolayers in vitro as compared to IgG-tagged nanocarriers controls indicating increased targeting and association of the nanocarriers with the PECAM-1 antibody.

Aim 2: To test radiolabeled (Tc-99m) candidate nanoconjugates to determine in vivo uptake and biodistribution in rats.

In Vivo Targeting Experiments

In vivo targeting experiments were carried out using adult Sprague Dawley rats (225-250 g). Animals were anesthetized with isoflurane and injected intravenously (IV) in the tail vein with (^{99m}Tc) labeled free inhibitor, antibodies, cyclic peptide, or targeted nanocarriers. The animals were imaged by gamma camera at 15 min intervals. At times up to 1 hr post injection, animals were euthanized, and tissue and blood samples collected (Temple Univ. Approved ACUP #3300). Radioactivity was monitored in blood, lung, heart, liver, kidney, spleen, muscle and fat tissue. The radioactivity in each tissue was counted in comparison with a standard of the administered dose in order to determine the percent of the administered dose per organ and per gram of tissue (% ID/g).

Intravenous (IV) administration of the free radiolabeled δ-PKC-TAT peptide resulted in rapid excretion and peptide was found principally in the kidneys, liver and bladder with little localization to lung tissue indicating that IV delivery of the δ-PKC-TAT peptide alone to the lungs is inefficient. This is in contrast to animals in which the δ-PKC-TAT was administered intra-tracheally, in which most of the dose was delivered to the lungs and exhibited little systemic absorption or exhalation (**Table A**). Our goal was to identify a carrier formulation for optimal delivery of δ-PKC-TAT to the lungs by IV administration. Table B shows the targeting ability of various molecules which were under consideration for coupling to the delivery vehicle for use as lung-specific targeting agents. The specificity of the molecules was evaluated by the ratio of delivery to lung (% ID/g lung) versus other organs (kidney or liver). A lung:liver ratio greater than 1 indicates preferential delivery to lung, and higher numbers indicate enhanced targeting. As shown in **Table B**, PECAM-1 antibody 2 had better lung specificity than the other two targeting moieties. This antibody was then coupled to the nanocarriers, radiolabeled with 99mTc, and administered IV to rats for imaging. Control experiments were carried out using nanocarriers coupled to a non-specific IgG moiety. **Table C** shows that 99mTc-targeted nanocarriers coupled to anti-PECAM-1 had better specificity for the lung than the same delivery vehicle containing a nonspecific molecule (nontargeted). There is a difference in the biodistribution at early (20-25 min) and later (1 hr) time points, suggesting breakdown of the delivery vehicle during this timeframe. We next examined the tissue distribution of the ^{99m}Tc-δ-PKC-TAT peptide when encapsulated in the targeted carrier as compared to free peptide given IV or IT. **Table A** shows that when ^{99m}Tc-δ-PKC-TAT peptide was administered IV without a carrier, it had very low targeting of the lung compared to kidney and liver. Encapsulation of the ^{99m}Tc-δ-PKC-TAT into a targeted delivery vehicle coupled to anti-PECAM-1 had improved lung: kidney and lung:liver ratios. *However, this targeted nanoconjugate did not achieve the high level of targeting to the lung that is achieved with intratracheal (IT) delivery of the peptide.*

Biodegradable albumin particles

In order to more efficiently target the lungs, we investigated biodegradable albumin particles that are designed to be trapped by the lung capillaries and arterioles (size range desired is 10-90 microns diameter). We prepared aggregated serum albumin particles that are coated with a surfactant moiety to achieve a hydrophilic coat. The particles were crosslinked with glutaraldehyde to maintain stability. Our optimal particles should remain stable during storage, but since physical trapping in the lungs takes place very quickly, the particles should then degrade quickly in blood upon delivery to the lung vasculature in order to release the

drug locally. We found the albumin particles ranged in size from 10-70 microns (>90% between 20-50 um) (See **Figure 7**) and are readily re-suspended in aqueous buffer. These particles degraded completely in 0.01 mg/ml trypsin following overnight incubation.

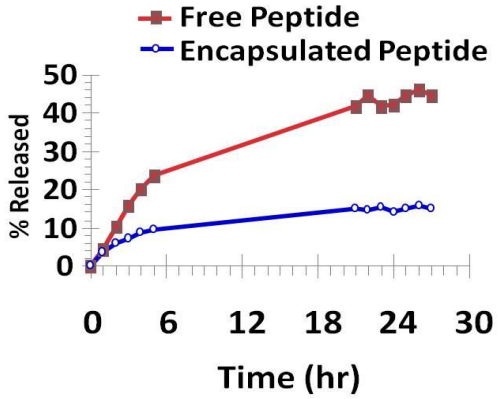
Loading of the δ-PKC TAT inhibitory peptide into the albumin particles was tested using the fluorescently-tagged δ-PKC TAT peptide inhibitor. We achieved a very high loading of the δ-PKC TAT inhibitory peptide into the albumin particles, with less than 5% unincorporated drug. Release of drug (δ-PKC peptide inhibitor)-loaded albumin particles was determined by suspending the particles in buffer and incubating with 0.01 mg/ml trypsin at 37°C. Maximal release of the peptide in this formulation amounted to 60% based on mass balance estimates. The maximal release was achieved by 1 hr incubation (see **Figure 8**) which is consistent with the rapid release of drug that we intend in vivo. Future experiments are planned to test peptide-loaded albumin particle uptake into PMVEC and biodistribution in vivo following IV injection in our animal model.

Summary of Aim 2

The goal of this aim was to determine uptake and biodistribution of candidate nanocarriers when administered intravenously into rats by tail vein injection. Based on our in vitro studies in Aim 1, we tested a number of targeting moieties and found that 99mTc-targeted nanocarriers coupled to anti-PECAM-1 had better specificity for the lung than the same delivery vehicle containing a nonspecific molecule (nontargeted). In addition, the encapsulated δ-PKC peptide inhibitor in a nanocarrier had better delivery to the lung as compared to free peptide alone. However, intravenous administration of this PECAM-1-targeted nanoconjugate did not achieve the high level of targeting to the lung that is achieved with intratracheal (IT) delivery of the peptide. Preliminary studies suggest that peptide loaded albumin may offer better lung targeting. Thus, further studies are required to fully achieve the goals of this aim.

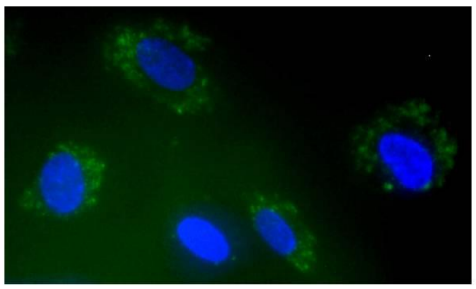
Figures for Research Project 6: Nanoconjugates for Targeted Treatment of Acute Lung Injury

Figure 1: Release of the δ-PKC TAT Peptide from Nanoconjugates



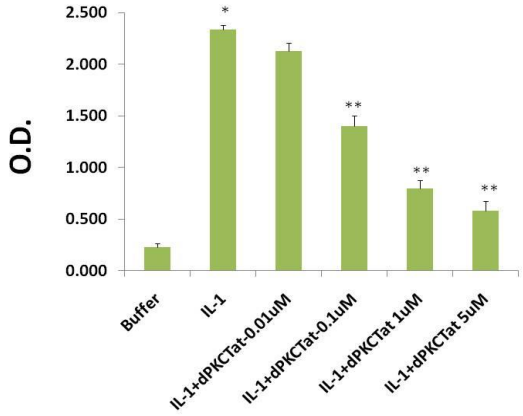
^{99m}Tc-∂-PKC-TAT peptide (free or encapsulated in nanoconjugates) was dialyzed (20 kDa MWCO) against pH 7.2 buffer at 37°C. Samples (1 ml) of dialysate were counted.

Figure 2: Binding of FAM-tagged δ-PKC peptide encapsulated in a Nanocarrier to PMVEC



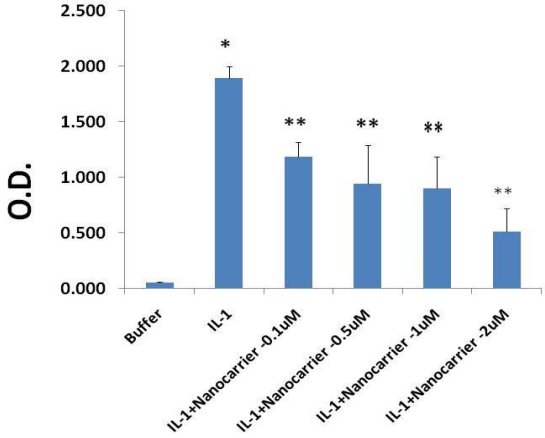
FAM (green)-tagged δ-PKC TAT inhibitory peptide encapsulated nanocarriers were incubated with rat PMVEC monolayers in chamber slides for 20 min at 37°C. The slides were washed and mounted in Vectashield with DAPI (blue) and imaged by fluorescence microscopy.

Figure 3: Effect of free δ-PKC TAT on VCAM-1 Expression in PMVEC



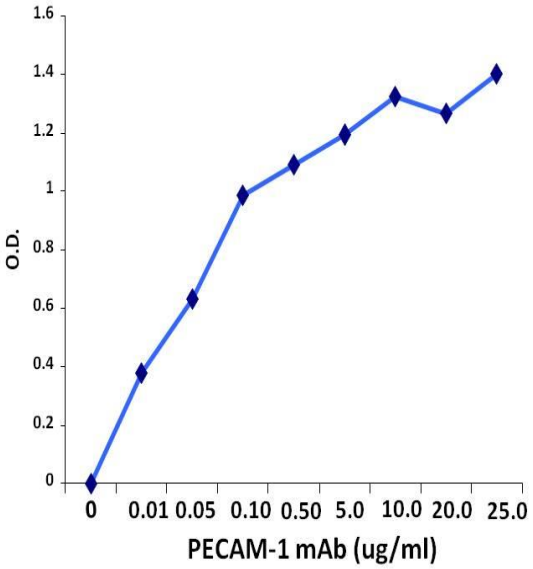
PMVEC were treated overnight with buffer or IL-1±δ-PKC TAT peptide (0.01-5uM). VCAM-1 expression in PMVEC monolayers was determined by ELISA (N=4) *p<0.01 buffer vs. IL-1, **p<0.01 IL-1 vs. IL-1+ δ-PKC TAT (0.1-5uM)

Figure 4: Effect of δ-PKC TAT encapsulated in a nanocarrier on VCAM-1 Expression in PMVEC



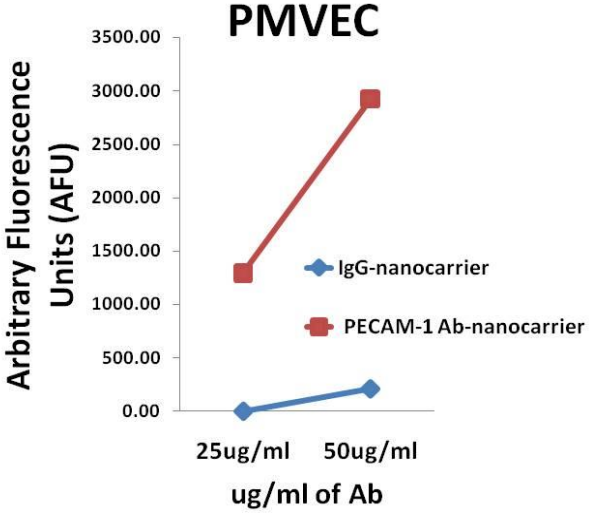
PMVEC were treated overnight with buffer or IL-1±δ-PKC TAT peptide encapsulated in a nanocarrier (0.1-2uM). VCAM-1 expression in PMVEC monolayers was determined by ELISA (N=4) *p<0.01 buffer vs. IL-1, **p<0.05 IL-1 vs. nanocarriers (0.1-2uM)

Figure 5: PECAM-1 Ab Binding to Rat Lung Microvascular Endothelial Cells



Anti-PECAM-1 (0-25ug/ml) binding to rat lung endothelial cell monolayers was determined by a cell surface ELISA. Representative binding curve from 3 separate experiments.

Figure 6: PECAM-1 Coupled Nanocarrier Binding to Rat PMVEC



Rhodamine-labeled nanocarriers were coupled to PECAM-1 Ab or isotype control Ab (IgG1). Rat PMVEC cultured in 96 well plates were incubated with the nanocarriers for 1hr at 37°C, washed and fluorescence measured in a plate reader. N=2 separate experiments (triplicates).

Table A. Effect of encapsulation of δ-PKC-TAT peptide into delivery vehicle on lung targeting

	^{99m}Tc-δ-PKC-TAT peptide	*^{99m}Tc-δ-PKC-TAT peptide in delivery vehicle –PECAM-1 antibody*	*^{99m}Tc-δ-PKC-TAT peptide*
	IV	*IV*	*IT*
Time post injection	1 hr	1 hr	15 min
Lung:kidney ratio*	0.16	0.69	57.04
Lung:liver ratio§	0.30	0.97	276.41

Table B. Lung specificity of targeting molecules

	^{99m}Tc-Targeting cyclic peptide	*^{99m}Tc-Targeting PECAM-1 antibody 1*	*^{99m}Tc-Targeting PECAM-1antibody 2*
Time post injection	1 hr	1 hr	1 hr
Lung:kidney ratio	0.02	0.74	4.44
Lung:liver ratio	0.67	0.84	2.78

Table C. Lung specificity of delivery vehicle with and without targeting PECAM-1 Antibody

	^{99m}Tc-Delivery Vehicle – PECAM-1 Ab		*^{99m}Tc-Nonspecific Delivery Vehicle*	
Time post injection	20 min	1 hr	25 min	1 hr
Lung:kidney ratio	1.31	0.21	0.16	0.30
Lung:liver ratio	1.54	2.82	2.35	0.16

* Lung:kidney ratio = % ID/g lung / % ID/g kidney
§ Lung:liver ratio = % ID/g lung / % ID/g liver
% ID/g = percent of injected dose per gram of tissue

Figure 7: Size distribution of aggregated human serum albumin microparticles determined by light microscopy

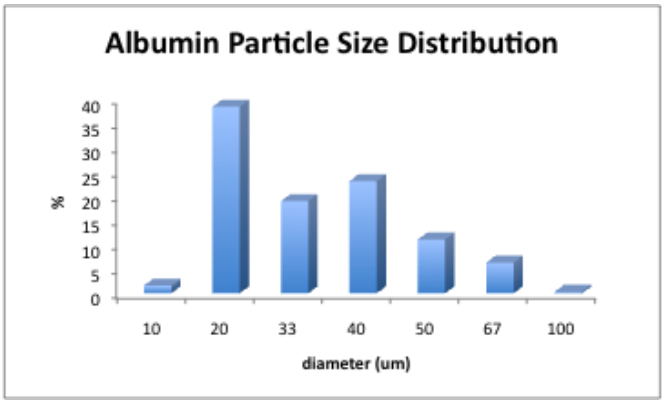
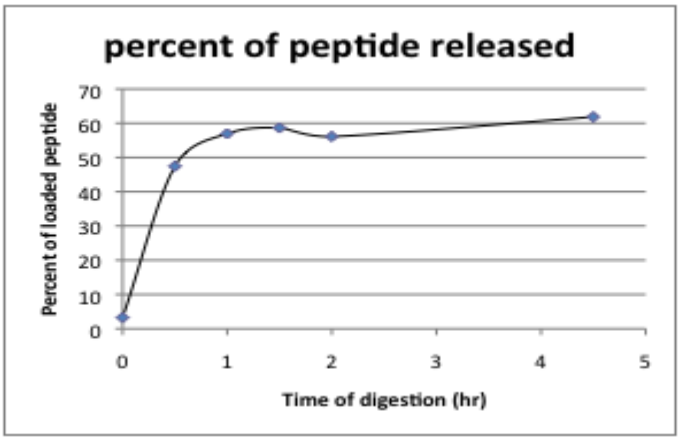


Figure 8: The rate of inhibitor peptide released from albumin microparticles during incubation in PBS containing 0.01 mg/ml trypsin at 37°C. At each time point, remaining particles were separated from the supernatant by centrifugation, and peptide content in the supernatant determined.



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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐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:

We plan to continue our experiments in nanoconjugate development and report on pulmonary endothelial targeting and cellular uptake with different types of targeting moieties.

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.*

See attached BioSketches

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Laurie E. Kilpatrick, PhD	Associate Professor of Physiology
eRA COMMONS USER NAME (credential, e.g., agency login)	
Kilpatrick	

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
Hampshire College, Amherst, MA	BA	1976	Biology
University of Pennsylvania, Philadelphia, PA	Ph.D.	1983	Biochemistry
University of Pennsylvania, Philadelphia, PA	Post-doctoral Fellow	1983-1986	Pharmacology

Positions and Honors

Positions and Employment

1986-1993 Research Assistant Professor, Department of Pediatrics, University of Pennsylvania
1987-1993 Adjunct Faculty, Department of Biochemistry and Biophysics, University of Pennsylvania
1987-2008 Medical/Dental Staff Member of Children's Hospital of Philadelphia
1994-2008 Associate Member, Joseph Stokes, Jr. Research Institute, Children's Hospital of Phila
2003-2008 Research Associate Professor, Department of Pediatrics, University of Pennsylvania
2009-present Associate Professor, Department of Physiology, Temple University School of Medicine
2009-present Associate Professor, Center for Inflammation, Translational and Clinical Lung Research (CILR), Temple University School of Medicine
2009-present Associate Professor, Thrombosis Research Center, Temple University School of Medicine

Other Experience and Professional Memberships

National Advisory Boards:

2003 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, Denver, CO 12/3/03-12/05/03
2004 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, ZGM1 TB-6 (01) Bethesda, MD 11/30/04-11/31/04
2005 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, ZGM1PPBC-5 Newark, NJ 11/9/05-11/10/05
2006-2010 Shock Society – Awards and Honors Committee
Shock Society Selection Committee for the 2009 Distinguished Service Award and Scientific Achievement Award
Reviewer: Shock Society Research Fellowship for Early Career Investigations
2009 Challenge Grant Reviewer- Special Emphasis Panel, ZRG1 CVRS B (58) R
2009 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, ZGM1 PPBC-5 (TB) 11/19/09
2010 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, ZGM1 PPBC-5 (TB) 12/2/10
2010-2014 Basic Science Councilor- Shock Society
2010 National Institute of General Medical Sciences, Special Emphasis Panel Meeting, Loan Repayment Program (LRP) Scientific Review Group 2011/08 ZGM1 BRT-1 (LR) 05/05/2011
2011-2015 Editorial Board – journal SHOCK
2012 NIH CSR Special Emphasis Panel ZRG1 F15-P20L, Fellowship: Surgical Sciences, Biomedical Imaging and Bioengineering, 07/05/2012

2012 Ad Hoc Member- NIH CSR Surgery, Anesthesiology and Trauma (SAT) Study Section, 10/3/2012-10/4/2012

Selected Peer-reviewed Publications (Selected from 57 peer-reviewed publications)

1) **Kilpatrick, L.E.**, Standage, S.W., Li, H., Raj, N.R., Korchak, H.M., Wolfson, M.R. and Deutschman, C.S.: Protection against Sepsis-induced Lung Injury by Selective Inhibition of Protein Kinase C-delta (δ-PKC). *Journal of Leukocyte Biology* 89:3-10, 2011. PMCID: PMC 20724665 . **(Published in the "*Spotlight on Leading Edge Research"* section of the journal with an accompanying editorial)**

2) **Kilpatrick, L**., Sun, S., Li., H., Vary, T. and Korchak, H.M.: Regulation of TNF-Induced Oxygen Radical Production in Human Neutrophils: Role of δ-PKC. *Journal of Leukocyte. Biology* 87: 153-164, 2010. PMCID: PMC 19801500.

3) Bynagari-Settipalli, Y.S., Chari, R., **Kilpatrick, L.E**. and Kunapuli, S.P.: Protein Kinase C – possible therapeutic target to treat cardiovascular diseases. *Cardiovascular & Hematological Disorders-Drug Targets,* 10:292-308, 2010 20678065.

4) Chernova, I., Lai, J.-P., Li, H., Schwartz, L., Tuluc, F., Korchak, H.M., Douglas, S.D., and **Kilpatrick L.E**.: Expression of the Truncated Form of the Neurokinin-1 receptor in Human Monocytes: Enhancement of CCL5-mediated Chemotaxis and Intracellular Signaling by Substance P (SP). *J. Leukocyte Biol.,* 85:154-164, 2009. PMCID: PMC 18835883. (Journal Cover).

5) Tuluc, F., Lai, J.-P**., Kilpatrick, L.E.,** Evans, D.L. and Douglas, S.D.: Neurokinin 1 receptor isoforms and the control of innate immunity. *Trends in Immunology*. 30:271-276, 2009. PMCID: PMC 19427266.

6) Lai, J.-P., Lai, S., Tuluc, F., Tansky, M.F., **Kilpatrick, L.E.,** Leeman, S.E. and Douglas, S.D.: Differences in the Length of the Carboxyl Terminus Mediate Functional Properties of Neurokinin-1 Receptor. *Proc. Natl. Acad. Sci*., 105:12605-12610, 2008. PMCID: PMC 18713853.

7) Korchak, H.M., Dorsey, L.B., Li, H., Mackie, deM., and **Kilpatrick, L.E.**: Selective roles for α-PKC in positive signaling for O_2^- generation and calcium mobilization but not elastase release in differentiated HL60 cells. *BBA-Molecular Cell Research,* 1773:440-449, 2007. PMCID: PMC 17157935.

8) Lai, JP, Ho, WZ., **Kilpatrick, L.E**., Wang, X., Tuluc, F., Korchak, H.M. and Douglas, S.D.: Full length and truncated neurokinin-1 receptor (NK-1R) expression and function during monocyte-macrophage differentiation, *Proc. Natl. Acad. Sci*. 103:7771-7776, 2006. PMCID: PMC 16675550.

9) **Kilpatrick, L.E**., Sun, S., Mackie, deM., Baik, F., Li, H. and Korchak, H.M: Regulation of TNF mediated anti-apoptotic signaling in human neutrophils: role of δ-PKC and ERK1/2. *Journal of Leukocyte Biology,* 80:1512-1521, 2006. PMCID: PMC 17138860.

10) **Kilpatrick, L.E.,** Sun, S. and Korchak, H.M.: Selective regulation by δ-PKC and PI 3-kinase in the assembly of the anti-apoptotic TNFR-1 signaling complex in neutrophils. *Amer. J. Physiol.: Cell Physiol* 287:C633-C642, 2004. PMCID: PMC 15115707.

Ongoing Research Support

Agency: NIH/NHLB R01HL0111552-01A1 PI: Kilpatrick 7/1/12- 6/30/16
Novel Protein Kinase C Targeted Therapy for Acute Lung Injury
The goal of this study is to test the hypothesis that infection activates δ-PKC and this kinase plays an important role in inflammation and the development of tissue damage in sepsis-associated lung injury. This study uses a δ-PKC TAT peptide inhibitor to examine the effects of δ-PKC inhibition.

Agency: NIH/NIGMS R41GM103193-01 MPI: Swindle and Kilpatrick 9/1/12-8/31/13
A Novel Therapeutic Strategy for Acute Lung Injury: Inhibition of PKC-delta

The goal of this study is to test the efficacy of a new small molecule PKCδ selective inhibitor (CGX1037) as a therapeutic for the treatment of sepsis-induced lung injury, and to determine the pharmacokinetic and pharmacodynamic properties of CGX1037 in a clinically-relevant rat model of ALI/ARDS.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Linda C. Knight	Professor of Radiology
eRA COMMONS USER NAME (credential, e.g., agency login)	
LINDA_KNIGHT	

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
University of California, Davis, CA	BS	06/72	Chemistry (honors)
Washington University, St. Louis, MO	MA	06/74	Nuclear&Radiochemistry
Washington University, St. Louis, MO	PhD	12/76	Nuclear&Radiochemistry
Washington University, St. Louis, MO	postdoc.	10/77	Nuclear&Radiochemistry

A. Personal Statement

I have a longstanding interest in developing radiotracers for targeting vascular receptors in thrombosis, angiogenesis and other diseases. My training and most of my work has been in attaching radiolabels to biomolecules and cells, for the purpose of tracking them in vivo by scintigraphic imaging. For several decades I have been evaluating and optimizing radiotracers for imaging thrombi by targeting molecular receptors in platelets and fibrin. I have taken protein- and peptide-based radiopharmaceuticals from the bench to clinical trials. My work has involved selecting the targeting agent and the conjugation/radiolabeling approach, performing the preclinical studies in vitro and in vivo, writing the Investigative New Drug (IND) application and preparing doses for human subjects. My laboratory has all of the facilities needed to prepare conjugates and radiolabeled products.

B. Positions and Honors.

Positions and Employment

1977-1985 Research Asst Prof, Thrombosis Research Center, Temple Univ School of Medicine
1985-present Research Assoc Prof, Thrombosis Research Center, Temple Univ School of Medicine
1981-1985 Research Asst Prof, Dept. of Diagnostic Imaging, Temple Univ School of Medicine
1985-1990 Research Associate Prof, Dept. of Diagnostic Imaging, Temple Univ School of Medicine
1990-1997 Research Professor, Dept. of Diagnostic Imaging, Temple Univ School of Medicine
1997-present Professor, Dept. of Diagnostic Imaging, Temple Univ School of Medicine
1998 Granted tenure, Professor, Dept. of Diagnostic Imaging, Temple University
2006-present Adjunct Professor, Graduate School, Mechanical Engineering, Temple University

Honors

1971 Phi Kappa Phi Honor Society, American River College
1972 Bachelor's Degree with Honors, Univ. of California, Davis
1989 Outstanding Researcher/Inventor, Temple University
1989 Honorary faculty member, doctoral dissertation committee, University of Leiden
2004-07 Member, Million Dollar Research Award Club, Temple University

Other Experience and Professional Memberships

6/1994 Ad hoc member of Diagnostic Radiology Study Section, NIH
1994-98 Regular member of Diagnostic Radiology Study Section, NIH
1998-99 Member of Expert Panel to forecast future demand for medical isotopes, US Dept of Energy
1998-2001 Member of Nuclear Energy Research Advisory Committee, US Dept of Energy
2/2000 Ad hoc member of Diagnostic Radiology Study Section, NIH

1/2002	Invited Contributor to Round Table Workshop on Emerging R&D Needs, US Dept of Energy
2/2002	Member of Special Emphasis Study Section, ZRG1 SSS-7, NIH
2/2004	Ad hoc member of Diagnostic Radiology Study Section, NIH
3/2005	Member of Special Emphasis Study Section, Small Business Medical Imaging Technologies, NIH
7/2005	Member of Special Emphasis Study Section, NCI, review of Centers of Cancer Nanotechnology Excellence, NIH
6/2008	Ad hoc member of MEDI Study Section, NIH
9/2008	Chair of ZRG1 MEDI-A 09 Study Section, NIH
2/2009	Chair of ZRG1 MEDI-A 09 Study Section, NIH
10/2009	Member of ZRG1 SBIB V-(50)-R Special Emphasis Panel/Scientific Review Group, NIH
6/2011	Member of Clinical Molecular Imaging & Probe Development Study Section (CMIP), NIH
6/2011	Member of ZRG-1 SBIB W (55) Study Section, NIH

C. Selected peer-reviewed publications

Knight LC, Maurer AH, Ammar IA, Epps LA, Dean RT, Pak KY, and Berger HJ. ^{99m}Tc-Antifibrin Fab' fragments for imaging venous thrombi: Evaluation in a canine model. Radiology 1989; 173: 163-169.

Knight LC, Radcliffe R, Maurer AH, Rodwell JD, and Alvarez VL. Thrombus imaging with technetium-99m synthetic peptides based upon the binding domain of a monoclonal antibody to activated platelets. J Nucl Med 1994; 35: 282-288.

Knight LC, Baidoo KE, Romano JE, Gabriel JL, and Maurer AH. Imaging pulmonary emboli and deep venous thrombi with ^{99m}Tc-labeled bitistatin, a platelet-binding polypeptide from viper venom. J Nucl Med 2000; 41: 1056-1064.

McQuade P, Knight LC, Welch MJ. Evaluation of ^{64}Cu and ^{125}I-Radiolabeled Bitistatin as Potential Agents for Targeting $\alpha_v\beta_3$ Integrins in Tumor Angiogenesis. Bioconjugate Chemistry 2004; 15:988-996.

Baidoo KE, Knight LC, Lin K-S, Gabriel JL and Romano, JE. Design and Synthesis of a Short-Chain Bitistatin Analogue for Imaging Thrombi and Emboli. Bioconjugate Chemistry 2004; 15:1068-1075.

Knight LC, Romano JE. Functional Expression of Bitistatin, a Disintegrin with Potential Use in Molecular Imaging of Thromboembolic Disease. Protein Expression and Purification 2005; 39: 307-319

Knight LC, Romano JE, Bright LT, Agelan A, Kantor S, Maurer AH. Platelet Binding and Biodistribution of ^{99m}Tc-rBitistatin in Animal Species and Humans. Nuclear Medicine and Biology 2007; 34: 855-863.

Knight LC, Romano JE, Cosenza SC, Iqbal NM, and Marcinkiewicz C. Differences in Binding of ^{99m}Tc-Disintegrins to Integrin $\alpha v\beta 3$ on Tumor and Vascular Cells. Nuclear Medicine and Biology 2007; 34: 371-381.

Pattillo CB, Venegas B, Donelson FJ, Del Valle L, Knight LC, Chong P L-G and Kiani MF. Radiation-Guided Targeting of Combretastatin Encapsulated Immunoliposomes to Mammary Tumors. Pharmaceutical Research 2009; 26 (5): 1093-1100.

Knight LC, Romano JE, Krynska B, Faro S, Mohamed FB and Gordon J. Binding and internalization of iron oxide nanoparticles targeted to nuclear oncoprotein. Journal of Molecular Biomarkers and Diagnosis 2010; 1:102. Doi: 10.4172/2155-9929.1000102

D. Research Support

Agency: NIH/NHLBI R01HL0111552-01A1 PI: Kilpatrick 7/1/12- 6/30/16
Novel Protein Kinase C Targeted Therapy for Acute Lung Injury-The goal of this study is to test the hypothesis that infection activates δ-PKC and this kinase plays an important role in inflammation and the development of tissue damage in sepsis-associated lung injury. This study uses a δ-PKC TAT peptide inhibitor to examine the effects of δ-PKC inhibition.
Role: Co Investigator