APPLICANT

TIMOTHY S. PARDEE

PROJECT DESCRIPTION

THE PROJECT DESCRIPTION IS LIMITED TO SEVEN PAGES, INCLUDING REFERENCES. THE RESEARCH PROJECT DESCRIPTION SHOULD BE PRESENTED IN THE SEQUENCE OUTLINED IN THE GUIDELINES & INSTRUCTIONS.

COMBINE ANY APPENDIX ITEMS, INCLUDING LETTERS OF COLLABORATION AND SIGNATURE PAGE, WITH THE COMPLETED PROJECT DESCRIPTION WITH BUDGET TEMPLATE, THEN UPLOAD AS A SINGLE PDF.

Project Title: "The Phospholipid Gemcitabine Conjugate, KPC34, is a Novel Treatment for Leukemia"

Leukemia in humans has 4 major subtypes, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL). It is estimated that over 40,000 Americans will be diagnosed with leukemia every year resulting in over 20,000 deaths. In leukemia targeted therapies have met with mixed results. In AML and ALL inhibition of a specific target or oncogenic pathway has led to only short term responses with frequent resistance and progression. In chronic leukemia it has been more successful with BCR-ABL inhibition in CML and Bruton's Tyrosine kinase inhibition in CLL making significant contributions to patient care. However even in these cases resistance is a problem and next line therapies, especially in CLL, are needed. KPC34 is a first-in class, novel phospholipid co-drug that is composed of 2 active moieties. It combines a classic DNA damaging agent with a kinase inhibitor. The head group is gemcitabine mono-phosphate and the backbone is a diacylglycerol mimetic that inhibits the classical Protein Kinase C (PKC) family members. It requires intracellular phospholipase C (PLC) to be converted into its two component parts adding an additional layer of tumor selectivity.

In this study we propose two **Specific Aims: 1) IND enabling toxicology studies in two species done under GLP conditions, 2) a phase 1 clinical trial of orally administered KPC34 in patients with relapsed leukemia** to determine the maximally tolerated dose and the relationship of KPC34 activity to PLC and/or PKC levels in the leukemia cells of treated patients. The hypothesis of these studies is that KPC34 will be well tolerated in clinical trial and its activity will correlate with protein kinase C and/or PLC levels. Our goal is to show safety and efficacy with KPC34 in leukemia patients and progress this novel agent toward FDA approval.

Scientific and Clinical significance of the work

The targeting of specific oncogenic pathways or tyrosine kinases in acute leukemia has led to brief responses only with very few exceptions. In AML the targeting of the commonly mutated tyrosine kinase FLT 3 has resulted in disappointing clinical results with most agents showing peripheral blood blast clearance only and few if any marrow responses[1, 2]. Next generation compounds with increased specificity have shown some improvement in response rates however duration of response remains a challenge[3]. In ALL the targeting of BCR-ABL has met with high initial response rates. However, when tyrosine kinase inhibitors (TKIs) are used as single agents responses are short-lived with rapid development of resistance[4]. This is likely a reflection of the intra-tumoral genetic heterogeneity contained within acute leukemia patients. In both of these examples responses have been made deeper and more durable by combining a targeted agent with chemotherapy. Indeed, the combination of a TKI with chemotherapy has now become the standard of care for patients with Philadelphia chromosome positive (Ph+) ALL[5]. Additionally, patients with AML containing a FLT3 mutation have now shown a significant benefit with the addition of the TKI

midostaurin in combination with traditional induction chemotherapy[6]. These data suggest that the combination of chemotherapy with targeted therapy inhibits the development of resistance.

Targeted therapy in chronic leukemia has been more successful but resistance is still a problem, especially in CLL. The advent of the Bruton's tyrosine kinase inhibitor ibrutinib represents a significant step forward in the clinical care of CLL patients[7]. Despite this most patients will ultimately develop resistance and require additional therapies. Of note is the fact that one of the main resistance mechanisms to ibrutinib is by mutation of PLC[8]. This mutation results in enhanced and constitutive activity of the enzyme[9]. As a result this group of patients would be predicted to be particularly susceptible to therapies such as KPC34 that utilize PLC activity.

Why target PKC? Protein kinase C is a family of at least 12 related proteins with diverse cellular functions whose dysregulation has been implicated in oncogenesis. The classic members (PKCα, β1, β2 and γ) require calcium and diacylglycerol (DAG) for activity. PKC lies downstream of multiple signaling pathways in leukemia. It is activated by the T cell receptor (TCR), B cell receptor (BCR) and BCR-ABL signaling. TCR signaling is important in T-cell ALL and BCR signaling is important in Ph negative B-cell ALL, as well as CLL. It is also downstream of the PI3 kinase pathway. Approximately 50% of AML patients show activation of the PI3K pathway[10]. The α and β family members are highly expressed in AML and ALL cell lines and primary patient samples and targeting these kinases is cytotoxic[11]. PKC isoforms have also been implicated in resistance to DNA damaging agents, including cytarabine in ALL and AML cells[12]. This is thought to occur via phosphorylation and activation of the anti-apoptotic protein BCL-2, and active PKCα and BCL-2 phosphorylation are associated with a poor prognosis in AML[13]. These data make protein kinase C an ideal target in leukemia.

Why incorporate gemcitabine? Nucleoside analogues have long been the backbone of chemotherapy for leukemia. Gemcitabine is a deoxycytidine analog with "self-potentiating" activities, including the inhibition of ribonucleotide reductase leading to increased DNA incorporation, chain termination after the addition of another nucleotide leading to "masked" chain termination resistant to the action of proofreading repair enzymes, inhibition of thymidylate synthase and prolonged cellular retention (reviewed in[14]). It is the only nucleoside analog that simultaneously inhibits these multiple pathways. These data suggest that gemcitabine is an ideal nucleoside analog for the treatment of leukemia. Despite these advantages gemcitabine is very similar in its requirements to other nucleoside analogues for tumor cell uptake and metabolism. Gemcitabine must enter the cell via an equilibrative nucleoside transporter (ENT-1), and needs to be activated by the action of deoxycytidine kinase (dCK). Consistent with this, down-regulation of ENT-1 and dCK confer a poor prognosis in leukemia[15, 16]. KPC34, however, does not require ENT-1 for transport into tumor cells and since gemcitabine monophosphate is formed by PLC cleavage this bypasses the need for dCK. This makes KPC34 an ideal agent for the treatment of nucleoside analog resistant leukemia.

Previous Studies/Preliminary Data

KPC34 induces apoptosis in leukemia cells. We initially tested the effect of exposure to increasing concentrations of KPC34 on a panel of human and murine leukemia cell lines. The IC_{50} values for all cell lines treated were in the nanomolar range (Table 1). To determine whether the observed decrease in viability was due to cytostatic or cytotoxic effects, the B6-ALL cell line was incubated with 25, 50 or 100 nM KPC34 for 48 hours and apoptosis was assessed by Annexin V/Propidium Iodide staining.

Table 1. IC_{50} (95% CI)	
Line:	**KPC34 nM**
HL60	17.52 (14.16-21.69)
MFL2	22.06 (12.62-38.55)
KG1a	42.59 (31.71-57.19)
B6-ALL	10.36 (8.448-12.7)
SUPB15	7.39 (6.25-8.73)
Jurkat	27.94 (22.88-34.12)
MOLT4	42.71 (32.59-55.96)

A dose-dependent increase in dual-stained populations was seen, demonstrating induction of apoptosis by KPC34. Induction of apoptosis was further confirmed by immunoblotting for cleaved caspase 3 in B6-ALL cells treated with KPC34 (data not shown). These findings demonstrate that KCP34 is a potent anti-leukemic agent that induces apoptosis in leukemia cells in a dose-dependent fashion.

KPC34 inhibits PKC activation. Cleavage of the phosphodiester bond on KPC34 by phospholipase C is predicted to produce a diacylglycerol mimetic capable of inhibiting signaling by the conventional PKC isoforms (α, βI, βII and γ). The classical forms of PKC require an auto-phosphorylation event to become catalytically active. To test KPC34's ability to inhibit PKC activation, we incubated both AML and ALL cells with 200 nM KPC34 for times ranging from 0.5-4 hours and collected cell lysates for Western blotting. We observed a decrease in phosphorylated PKC α/βII in cells treated with KPC34 (Figure 1 and data not shown). These findings demonstrate inhibition of PKC phosphorylation by KPC34.

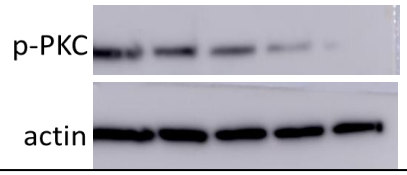

Figure 1. KPC34 inhibits PKC. OCI-AML 3 cells were incubated for 0, 0.5, 1, 2 or 4 hours with 200 nM KPC34 and blotted for phospho-PKC (p-PKC).

KPC34 is efficacious as a single agent against ALL in vivo. The *in vivo* efficacy of KPC34 was tested in a syngeneic immunocompetent orthotopic mouse model of Ph+ pre-B cell ALL [17] (Figure 2). Mice were injected with 10^6 B6 ALL cells and leukemic engraftment was confirmed six days later by bioluminescent imaging. Upon confirmation of engraftment mice were randomly assigned to receive four daily treatments with either vehicle control (PBS), 20 mg/kg of KPC34 by oral gavage, the maximally tolerated dose of cytarabine (100 mg/kg) intraperitoneal (IP) or equimolar (8.8 mg/kg) gemcitabine IP. KPC34 treatment resulted in a significant survival advantage (p = 0.0001) compared to saline, cytarabine and gemcitabine, with a median survival of 32 days compared to 14.5, 23 and 15.5, respectively (Figure 2). We next generated a resistant ALL model by injecting B6 ALL cells as above and treating engrafted animals with cytarabine until disease progression. Animals were then sacrificed and the leukemia cells harvested and injected into secondary recipients. These animals were then treated with four daily treatments of either control (PBS), KPC34 at 20 mg/kg, equimolar (8.8 mg/kg) gemcitabine IP or equimolar (7.1 mg/kg) cytarabine IP. Treatment with KPC34 resulted in a significant median survival benefit compared to control (24 vs 11 days), gemcitabine (24 vs 14) or cytarabine (24 vs 16). P value by log rank test was <0.0001 (data not shown). These data demonstrate that KPC34 is efficacious as a single agent against Ph+ ALL initially and following resistance to a traditional nucleoside analog, and is active when administered orally.

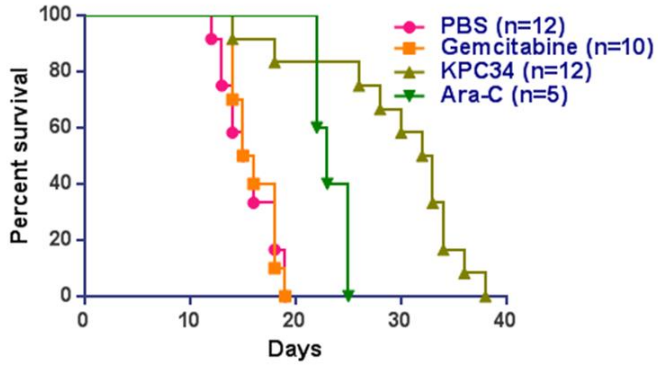

Figure 2. KPC34 is superior to gemcitabine and cytarabine against ALL (p= 0.0001).

KPC34 is efficacious against CNS leukemia. ALL involves the central nervous system (CNS) in ~10% of patients at diagnosis and up to 30% at relapse. As a result all patients receive CNS directed therapy either as prophylaxis to prevent or active treatment to eradicate CNS leukemia. Given the unique lipophilic structure of KPC34 we thought it possible that it would cross the blood brain barrier and treat CNS disease. Our B6 ALL model has a high rate of CNS involvement at progression of disease and hind limb paralysis is a main indication for euthanasia in this model. To assess KPC34 activity against CNS disease we injected mice with B6 ALL cells as before and treated the mice with a combination of cytarabine and doxorubicin. Upon onset of hind limb paralysis we re-treated the same animals with KPC34 orally at 20 mg/kg. Treatment with KPC34 rapidly reversed (within 24 hours) hind limb paralysis and prolonged survival to median of 50 days (see supplemental video and data not shown). These data demonstrate the efficacy of KPC34 against CNS leukemia as an oral agent and proves it's tolerable by mice already treated with chemotherapy.

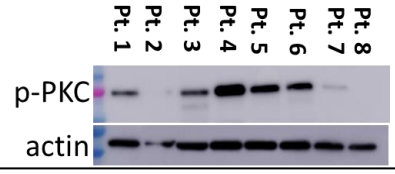

Figure 3. Phosphorylated PKC is detectable in the majority of AML patient samples. Primary patient samples were blotted for p-PKC.

Phosphorylated PKC is detectable in the majority of AML patient samples. Previous studies have suggested that p-PKC is active in ~50-60% of AML. In order to confirm this we performed Western blots for the phosphorylated forms of PKCα and βII. Consistent with these studies five of eight patient samples show strong phosphorylated PKC signals (Figure 3). These data suggest that a majority of AML patients express a KPC34target and that phosphorylated PKC may serve as a molecular marker of patients most likely to benefit from it.

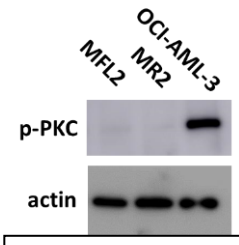

Figure 4. Phosphorylated PKC is not detectable in several AML cell lines.

KPC34 is efficacious as a single agent against phosphorylated-PKC expressing AML in vivo. To assess the efficacy of KPC34 in AML we initially utilized two syngeneic mouse models driven by MLL-ENL and either Flt3 ITD (MFL2) or $NRas^{G12D}$ (MR2) expression. Neither model expresses detectable levels of phosphorylated PKCα or βII (Figure 4). KPC34 was not efficacious against either model with no significant survival benefit seen compared to control treated animals (data not shown). In order to see if a model with phosphorylated PKCα/βII would respond differently we used an orthotopic mouse model of human AML. In this model NSG-tg mice were injected with 10^6 luciferase tagged OCI-AML3 cells that exhibited phosphorylated PKC (Figure 4) and leukemic engraftment was confirmed ten days later by bioluminescent imaging. Upon confirmation of engraftment mice were randomly assigned to receive four daily treatments with either vehicle control (PBS), 10 mg/kg of KPC34 by oral gavage or the maximally tolerated dose of cytarabine (50mg/kg) IP. KPC34 treatment

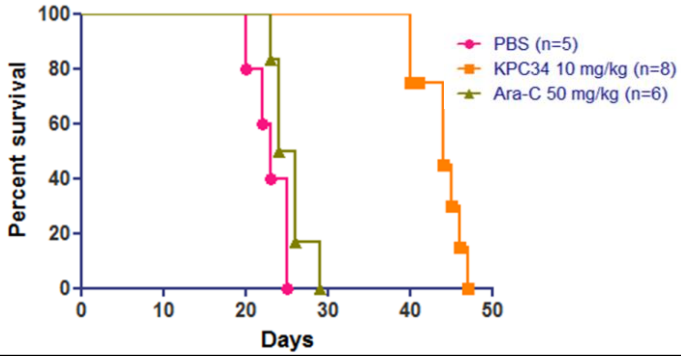

Figure 5. KPC34 is superior to gemcitabine and cytarabine against AML (p= 0.0068).

resulted in a significant survival advantage ($p = 0.0068$) compared to both saline and cytarabine, with a median survival of 44 days compared to 23 and 25, respectively (Figure 5). These data demonstrate that KPC34 is efficacious as a single agent against AML with detectable phosphorylated PKCα/βII.

In summary, our preliminary data demonstrate that the novel "co-drug" KPC34 delivers simultaneously a targeted therapy and a DNA damaging agent. KPC34 is active as an oral agent, treats CNS leukemia, is more effective than its parental drug gemcitabine or the currently used cytarabine even when treated with maximal doses. The presence of detectable phosphorylated PKCα/βII may predict those patients most likely to benefit. Finally, there is very strong IP protection of KPC34 with a composition of matter patent issued in 2012 (patent number US 8,138,200 B2). We believe our extraordinary preclinical data, combined with an institutional commitment (see support letters in appendix) to aid in the development of this agent when combined with the resources of this award will allow for the rapid translation of this agent to the clinic.

Research Methods

Aim 1. Carryout IND enabling toxicology studies in two species done under GLP conditions. Prior to the approval of an IND the FDA mandates toxicology studies be carried out in two animal models under good laboratory procedure (GLP) conditions. Methods: Utilizing our support from Wake Innovations we have contracted with PharmAgra Inc. who has completed a scale up synthesis protocol and generated GMP quality KPC34 for use in toxicology studies. We will obtain single and repeat dose toxicology in rats and dogs using the services of a CRO. We have previously obtained pharmaco-kinetics of KPC34 in mice (see appendix) but we will need ADME studies including plasma protein binding, CYP450 inhibition studies, and human liver S9 fraction studies. We will also obtain pharmacology safety studies including determination of potential inhibition of Ether-a-Go-Go-Related Gene (hERG) channels. While these data are being generated, in collaboration with Wake Forest School of Medicine's FDA liaison and IND specialist Heather Hatcher (see support letter in appendix) we will conduct a pre-IND phone call with the FDA to ensure all required studies are completed. Following the completion of all required studies an IND will be filled with the FDA.

Aim 2. Complete a phase 1 clinical trial of orally administered KPC34 in patients with relapsed leukemia. Upon receipt of IND approval we will conduct a traditional phase I dose escalation study in patients with relapsed or refractory leukemia. This phase I trial will be supported by the Comprehensive Cancer Center of Wake Forest University (see support letter from Cancer Center director, Dr. Boris Pasche). The study will be a 1-3-6 dose escalation study. The starting dose will be $1/10^{th}$ the human equivalent dose of the no observed adverse event level (NOAEL) determined from the toxicology studies. The schedule is based on the preclinical efficacy studies and will be as follows: Cycle 1, days 1-4, one daily dose followed by 2 doses weeks 2-4 of a 28 day cycle. The plan will be for each patient to receive one cycle of therapy. If patient's are judged to have benefited by their treating physician they can continue. All subsequent cycles will have 2 daily doses per week. For acute leukemia patients response will be assessed by bone marrow biopsy after cycle 1. For patients continuing on therapy repeat biopsies will be done quarterly until a complete remission (CR) is obtained or stable disease is seen on 3 consecutive biopsies, then biannually until a loss of response. In patients with CLL response will be assessed on peripheral blood lymphocyte count unless criteria for a CR are met and then a bone marrow biopsy will be performed. The primary endpoint of the study will be safety and to find the maximally tolerated dose. Additionally, PK samples will be obtained just prior to and 0.5, 1, 2, 4, 6, 8, 24, 48, 72 and 96 hours after the first dose of KPC34. Plasma KPC34 levels will be determined using the validated electrospray ionization mass spectroscopy method routinely

used in the Kucera laboratory. We will also analyze baseline blood or marrow samples for detectable phosphorylated PKCα/βII as well as PLC by Western blot. CLL patients who have failed ibrutinib will have their PLC gene sequenced.

Interaction with other Investigators

Dr. Pardee has worked closely with Dr. Kucera on developing the preclinical studies and clinical development of KPC34 and their offices are approximately 20 feet apart. They meet to discuss data on a daily basis. Dr. Yohannes, the associate director of product innovation and commercialization services with Wake Innovations has worked closely with both Drs. Pardee and Kucera since the funding of their original Spark Application (2013) and continues to have regular meetings with the team. Dr. Pardee is the co-leader of the Hematologic Malignancy Disease Oriented team and meets weekly with the leukemia clinicians at Wake Forest.

Resources and Environment

Scientific Environment: The Comprehensive Cancer Center of Wake Forest University provides not only an outstanding scientific infrastructure for conducting the proposed studies but also brings together the requisite expertise for maximizing the use of these facilities and translating KPC34 into the clinic. Dr. Pardee's laboratory consists of 900 sq. ft. on the 3rd floor of the Hanes Research Building and is equipped for molecular biology, tissue culture of mammalian cells, and in vivo studies. Dr. Pardee's equipment includes among other things, a biosafety cabinet, tissue culture hoods, two Napco series 8000 DH tissue culture incubators and an Olympus NAO inverted microscope. His lab contains all the necessary equipment to carry out the proposed studies.

Clinical Environment: The Comprehensive Cancer Center of Wake Forest University is an NCI designated comprehensive cancer center. It is a free standing cancer hospital with over 180 acute care and observation beds making it the largest cancer hospital in the region. It was ranked as the #1 Cancer center in North Carolina by US News & World Report in 2015 and was ranked #20 in the nation. It has dedicated clinical study teams for a variety of malignancies including a dedicated hematologic malignancy team. In 2013 the cancer center was visited by over 200 leukemia patients and has a strong track record of leukemia clinical trials accruals. In addition to Dr. Pardee there are four other physicians dedicated to the treatment of leukemia including Drs. Powell, Howard, Ellis and Berenzon. We have conducted and completed multiple leukemia clinical trials including several phase I studies. The cancer center has dedicated pharmacy personnel experienced in the handling and administration of experimental agents. Additional clinical trial support includes dedicated biostatistician's, safety oversight committee, study management and data entry personnel. In summary it is the ideal place to conduct the proposed phase I clinical trial.

Time Line:

Quarter	Year 1	Year 2	Year 3
Q1	IND enabling toxicology	GMP Manufacture of KPC34, ADME studies	GMP manufacture of KPC34, sample analysis
Q2	Pre-IND call with FDA	File IND with FDA	Sample analysis
Q3	IRB approval of protocol	Start of Phase I trial	Sample analysis
Q4	Start of GMP synthesis	Bank PK and baseline patient samples	Completion of trial and PK/Sample analysis

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DETAILED BUDGET FOR INITIAL BUDGET PERIOD

Personnel			Dollar Amount Requested		
Name	**Role on Project**	**% Effort On Project**	**Salary Requested**	**Fringe Benefits**	**Total**
Timothy Pardee	PI	0	0	0	0
Greg Kucera	Co-PI	0	0	0	0
Peter Alexander	Post-Doc	0	0	0	0
				Total	0

Consultant Costs

Total Consulting Costs

Equipment *(Itemize)*

Total Equipment

Supplies *(Itemize by category)*

Total Supplies

Travel

Total Travel

Patient Care Costs

Total Inpatient

Total Outpatient

Other Expenses *(Itemize)*

Total Other Expenses 180,018

DIRECT COSTS FOR INITIAL BUDGET PERIOD	180,018
Indirect Costs for initial budget period (capped at 11.1% of requested direct costs)	19,982

TOTAL INITIAL BUDGET	200,000

SUMMARY BUDGET FOR ENTIRE THREE YEAR PROJECT

	Year 1	**Year 2**	**Year 3**	**Total**
Personnel	0		57,318	57,318
Consultant Costs				
Equipment				
Supplies			32,700	32,700
Travel				
Patient Care Costs				
Other Expenses	180,018	180,018	90,000	450,036
Total Direct Costs				540,054
Total Indirect Costs				59,946
TOTAL BUDGET				600,000

***PROJECT BUDGET JUSTIFICATION* -- Do not exceed two pages.**

Personnel Justification

Timothy S. Pardee, M.D., Ph.D (PI): Dr. Pardee is an Associate Professor of Medicine in the Section on Hematology and Oncology and the Director of Leukemia Translational Research at the Comprehensive Cancer Center of Wake Forest University. He is an expert in the treatment of acute and chronic leukemia and has extensive experience in clinical trials and translational science. He will supervise all aspects of the project including the design and execution of the phase I clinical trial, all experiments, reporting of all data, and personnel management. Dr. Pardee is currently supported by NCI 1K08CA169809-03, as the initial 2 years of the award the bulk of the project will be conducted by fee for service companies we are only requesting in year 3, 0.6 months of salary support.

Gregory Kucera, PhD (Co-PI): Dr. Kucera is an Full Professor of Medicine in the Section on Hematology and Oncology and the associate director of the tumor bank for the Comprehensive Cancer Center of Wake Forest University. He has expertise in the design of lipid-nucleoside conjugates and their detection by electrospray ionization mass spectroscopy. He is named on multiple patents including the composition of matter patent for KPC34. For the reasons listed above we are only requesting 1.2 months of salary support during year 3.

Peter Alexander, PhD (Lab Personnel): Dr. Alexander is currently a post-doctoral fellow in the Pardee lab and has extensive experience with KPC34. He has generated nearly all of the preliminary data in this application and will carry out the analysis of patient samples for PCK and PLC. For the reasons listed above we are only requesting 6 months of salary support during year 3.

Fee for Service Justification

IND enabling toxicology studies must be conducted under GLP conditions. For this reason we will utilize the services of a CRO for the studies listed in Aim 1. Dr. Yohannes has extensive experience in dealing with CROs for this type of work and is currently in negotiations with several local organizations. In year one the entire direct costs will be used to obtain single and repeat dose toxicology in rat and dog species. In year 2 ~$90,000 will be used for completion of toxicology studies as well as additional ADME required studies.

GMP quality drug will be needed to conduct the phase I trial. PharmAgra Inc has developed a GMP compatible synthesis process. They have agreed to make 100 gram lots of GMP quality KPC34 for $90,000 each. In year 2 we will obtain the first 100 gram lot. In year 3 we will obtain a second 100 gram lot. We believe we can complete a ~20 patient study with a total of 200 grams of drug. This is based on the conservative assumption that the highest starting dose the FDA would allow is the tolerable and effective dose used in our mouse studies of 20 mg/kg. The human equivalent dose is 1.6 mg/kg which is equal to ~60 mg/m^2. A patient with an average BSA of 1.8 m^2 would need ~1gm for a complete cycle of 10 doses (4 doses week 1, 2 doses weeks 2-4). Using a modified Fibonacci dose escalation scheme and 3 patients per cohort 118 grams will be needed for 6 dose escalations. The remaining drug would be used for cohort expansion and patients receiving additional cycles.

Supplies justification

Funds will be needed for the collection, storage and analysis of patient samples. Reagents are needed for the PK analysis as well as the analysis of PKC and PLC in patient samples.

Leukemia & Lymphoma Society | Signature Page

APPLICANT: _____

Role: Principal Investigator	
Name	
Institution	
Title	
Division	
Department	
Telephone	
Email	
Signature	

Role: Financial Officer	
Name	
Institution	
Title	
Division	
Department	
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Role: Research Administrator	
Name	
Institution	
Title	
Division	
Department	
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Email	
Signature	

Role: Institutional Signing Official	
Name	
Institution	
Title	
Division	
Department	
Telephone	
Email	
Signature	

Role: Technology Transfer Official	
Name	
Institution	
Title	
Division	
Department	
Telephone	
Email	
Signature	

Role: Co-Principal Investigator	
Name	
Institution	
Title	
Division	
Department	
Telephone	
Email	
Signature	