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.

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

2. **Reporting Period (start and end date of grant award period):** 11/26/2012 - 12/31/2013

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

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

5. **Grant SAP Number:**4100050904

6. **Project Number and Title of Research Project:** 48. Targeting Survival Pathways in Treatment of Large Granular Lymphocyte (LGL) Leukemia

7. **Start and End Date of Research Project:** 11/26/2012 - 12/31/2013

   Name of Principal Investigator for the Research Project: Jun Yang, MD, The PI has left the Institution.

8. **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:

   $ 81,049

   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, First Name	Position Title	Institution	% of Effort on Project	Cost
Yang Jun	Assistant professor	Penn State Hershey Cancer Institute	100%	$55,199

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, First Name	Position Title	Institution	% of Effort on Project	Cost
None				

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_____ No____X_____

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

11. Leveraging of Additional Funds

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

Yes_____ No____X_____

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:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: Fundation_ _____)		$	$
	☐NIH ☐ Other federal (specify:_____		$	$

	_____) ☐ Nonfederal source (specify: _____)			
	☐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_____ No____X_____

If yes, please describe your plans:

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

In addition to the constitutive activation of NF-κB pathways, results from the current study showed evidence of other pathways that are also involved in mediating/sustaining survival of leukemic LGLs. IL-6, INF-g, IL-8 and RENTES are known to activate JAK/STATs pathways, MAPK3/JNK and p38MAPK pathways in different cancers. Therefore, our future research will focus on the cytokine/chemokine related survival signaling pathway activation in LGL leukemia. We also want to continue the studies for the mechanisms of PDGF-RTK inhibition-related cell death and restoration of Fas/FasL sensitivity in LGL leukemia of T and NK subtypes.

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

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

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

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

Large granular lymphocyte (LGL) leukemia is a disorder of clonal expansion of cytotoxic lymphocytes resulting from dysregulation of cell death, clinically characterized by severe/symptomatic neutropenia or anemia. No standard treatment for patients with LGL leukemia is available because of the absence of prospective clinical trials. We discovered that overexpressed PDGF-BB and IL-15 are the key molecules that trigger multiple downstream survival pathway activations in leukemic LGL. We also found that the TRAIL/DcR2 pathway plays a decisive role in mediating NF-κB pathway constitutive activation. The activated NF-κB pathway is an upstream regulator, and regulates multiple downstream transcript expressions such as Mcl-1, c-FLIP and survivin and multiple cytokines/lymphokines such as IL-8, IL-18, INF-r, Fas/FasL and TRAIL, which are all implicated in the pathogenesis of LGL leukemia. We observed that inhibition of IL-15, PDGF-RTK and downstream NF-κB and ERK/survivin pathways resulted in an elevated level of apoptosis in LGL leukemia cells but not in normal counterpart cells.

Hypothesis: We hypothesize that the targeted therapy with multiple drugs listed above will eliminate the pathologic LGL clones via inhibition of NF-κB activation, resulting in downregulation of overexpressed anti-apoptotic proteins and the dysregulated cytokines/lymphokines production that are known to be responsible for initiating/sustaining leukemia cell survival.

Specific Aim 1: To determine clearance of LGL pathologic clones using targeted drugs listed above in PBMCs from patients with LGL leukemia and LGL leukemia cell lines. We will evaluate the tumor selective cytotoxicity of these targeted drugs in LGL leukemia cells versus primary $CD3^+/CD8^+CD57^+$ T cells or $CD3^-/CD56^+CD16^+$ NK cells from normal donors.

Specific Aim 2: To determine the mechanisms of LGL leukemic clonal elimination in response to the drugs as it correlates with NF-κB activity in leukemic cells. Correlative studies proposed here are designed to determine the regulatory mechanisms of NF-κB activity by inhibition of upstream IL-15 pathway, PDGF-RTK phosphorylation or direct targeted inhibition of NF-κB. We also propose to evaluate the activities of other transcription factors, including Stat-3 and T-bet which are constitutively activated in leukemic LGL as alternatives.

Specific Aim 3: To determine the contributions of NF-κB pathway in regulating cyokine/lymphokine production in LGL leukemic cells. Correlative studies proposed here are designed to determine the transcriptional and translational regulatory mechanisms of key cytokine/lymphokine production by leukemic cells.

The following specific aims were accomplished or in progress for this period:
Research goal 1: As proposed in the specific aim 1, we finished the experiments for testing the therapeutic efficacies for the drugs/compounds we proposed in this grant using LGL leukemia cell lines and PBMC from patients with LGL leukemia of T and NK subtypes.
Research goal 2: As proposed in the specific aim 2, NF-κB activity inhibition with the drugs that were found to have the selective anti-cancer effects was studied. NF-κB downstream target genes such as c-FLIP, McL-1, Bcl-1 and survivin expression were determined at transcription level and translational level.

Research goal3: As proposed in the specific aim 3, we performed experiments designed for the determination of NF-κB pathway derived cytokine/lymphokine production using PBMC from patients with LGL leukemia of T and NK subtypes

Work Performed:
PBMC Isolation:
Total RNA Isolation from Experiment Samples:
Real-Time Quantitative Polymerase Chain Reaction (PCR):
Protein Isolation and Western Blot Assay:
Immunocytochemistry Staining (ICC staining)
Electrophoretic Mobility Shift (EMSA) Assay
Multiplex Cytokine/Chemokine Bio-Plex (Luminex) Assay:
Apoptosis assay and TRAIL receptor detection assay by flow cytometric assay.

Results and Conclusions:
Three milestones were achieved in progress for this period:

Milestone 1: Completion of the studies in testing the candidate drugs proposed in this project for the selective cytotoxicity in LGL leukemia cells using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and apoptosis assay (Annexin-V/7AAD staining).
Milestone 2: Completion of the studies of TRAIL-mediated constitutive activation of NF-κB pathway, and NF-κB inhibition with Bortezomib and Ixazomib (MLN9708) mediates a selective cytotoxicity in LGL leukemia cells from patients with T and NK -LGL leukemia.
Milestone 3: PDGF-RTK inhibition with Imatinib and Nilotinib mediates a significant increase of apoptosis and the restoration of Fas/CD95 sensitivity in PBMC from patients with T and NK -LGL leukemia through an NF-κB independent pathway.
Milestone 4: Partial completion of the experiments in determination of NF-κB-derived cytokine/Chemokine production in PBMC from patients with T and NK -LGL leukemia patients.

PBMC Isolation- PBMC from eleven LGL leukemia patients (Leukemia patients with T-LGL n=9, NK-LGL n=6) and 6 normal donors were isolated by Ficoll-Hypaque gradient separation.

MTT assay and apoptosis assay- MTT assay was performed using CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit. The number of viable cells from each assay was acquired by plotting the ODs to the standard curve of each 96-well plate. Apoptosis assay was carried out in the samples by staining the cells with Annexin-V/7AAD (7-Aminoactinomycin D). Apoptosis cells were determined using flow cytometric assay. All data were standardized in each experimental sample by using the equation: *Total Apoptosis = (Number of apoptotic cells of drug treated sample – Number of apoptotic cells in vehicle treated sample) X 100 / (100 – number of apoptotic cells in vehicle treated sample).*

TRAIL receptor detection assay- TRAIL receptor1-4 were detected by incubating PBMC samples with mouse anti-human TRAIL receptor antibodies with specificity against TRAIL-R1, R2, R3 and R4 molecules (Alexis Biochemicals). The primary antibodies were then identified by incubating PBMC with anti-mouse IgG antibody conjugated with FITC fluorochrome. Cell

surface expression of each TRAIL receptor was determined by flow cytometric assay. Anti-mouse IgG antibody was used as antibody isotype control.

RNA Isolation- Total RNA was isolated from experimental samples using Trizol reagent. Total RNA samples were then reverse-transcribed into c*DNA* using random hexamer primers for semi-quantitative and real-time PCR reactions.

*Whole Cells Lysate and Nuclear protein, Cytosol Protein Prepara*tion- Whole cell lysates from experimental samples were prepared using Ripa buffer for Western blot assays. Protein samples of nuclear fraction and cytosol fraction from experimental samples were isolated using NE-PER Nuclear and Cytoplasmic Extraction Reagents. Nuclear protein samples were used for electrophoretic mobility shift assays (EMSA), or for the determination of target protein nuclear translocations by Western blot assay.

EMSA Assay- NF-κB nuclear translocation and DNA binding activity was determined using the LightShift Chemiluminescent EMSA Kit following the manufacture protocol (Thermo Scientific).

Western Blot Assay- Western blot assays were performed using antibodies against NK-κB family proteins and their downstream target molecules in samples collected from LGL leukemia patients and the age-matched healthy donors. Blots were washed and developed with chemiluminescence following the manufacturer's instructions. Semi-quantitative densitometry analysis was performed by using ChemDox XR (Bio-rad) to determine the volume and the densities of the protein bands on the Western blot films.

Semi-Quantitative PCR and Real-Time PCR- cDNA was prepared from total RAN samples collected from either leukemia cells or normal PBMC. The expression of NF-κB downstream targets was determined by Semi-quantitative PCR or Real-time PCR.

Immunocytochemistry (ICC) Staining- ICC staining for TRAIL and NF-κB family protein such as p50 and p65 and Iκ-B-α was carried out in the purified LGL leukemia cell and their counterpart cells from normal donors using polyclonal antibodies against human TRAIL and NK- κB family proteins such as p50, p65 as well as Iκ-B alpha.

Bio-Plex Assay- Two customized Bio-Plex Assay kits were designed using Bio-Plex Assay Builder software (Bio-Rad Life Science Research) to query the target genes of interest. The following NF-κB downstream transcripts were included in the company defined Group I and Group II human cytokine/chemokine assay kits. Group I: IL-1β, Il-2, IL-4, IL-6, IFN-γ, MIP-1α, MIP-1β, PDGF-BB, RANTES and TNF-a; Group II: INF-α, IL-1α, IL-2Rα, IL-18, and TNF-β. Cytokine/chemokine concentrations in tissue culture supernatants were determined using Bio-Plex 200. Data analysis was performed using the Bio-Plex system in combination with the Bio-Plex Manager software (BIO-RAD Life Science Research).

Results and conclusions

In specific aim 1 we planned to evaluate the potential therapeutic efficacies of multiple targeted drugs proposed in this study in LGL leukemia cells of both T and NK subtypes. To accomplish

this plan, we tested the following reagents which include HuMax-IL15 (human IgG1 anti–IL-15 monoclonal antibody), Platelet growth factor receptor tyrosine kinase (PDGF-RTK) inhibitor Imatinib and Nilotinib, proteasome inhibitor Ixazomib (MLN9708) and ERK/Survivin pathway inhibitors LCL161 in LGL leukemia TL1 (T-LGL leukemia cells), NKL (NK-LGL leukemia cells) and PBMC from patients with T and NK -LGL leukemia as well as PBMC from age- and genda-matched healthy donors (normal controls) using MTT assay and apoptosis assay. Based on the data generated from this study, the following results and conclusions can be drawn:

1] *Ixazomib (MLN9708) Induces Increase Apoptosis in LGL Leukemia Cells*- We previously reported that the proteasome inhibitor Bortezomib (Velcade, PS-341) mediated a significant increase of apoptosis by inhibition of the mitochondrial protein Bid proteasome degradation in PBMC from patients with LGL leukemia of T and NK subtypes when compared to that observed in PBMC from normal controls (Cancer Research, 2008). In this study, we focused on exploring the potential therapeutic efficacy of the second-generation oral proteasome inhibitor Ixazomib in treatment of leukemic LGLs. Our data shows that MLN9708 induced a significant increase of apoptosis in PBMC from patients with T and NK -LGL leukemia at nanomolar level compared with the apoptosis observed in PBMC from normal controls (data not shown) (Figure 1-A and -B). The effective doses for MLN9708 in mediating apoptosis in LGL leukemia cells are between 50-400nM. This dose range correlates to the doses reported to test its effectiveness of proteasome inhibition and anti-cancer activity in *in vitro* studies in different cancer cells.

2] *PDGF-RTK Inhibitor Mediates Elevated Levels of Apoptosis and Restores Fas/CD95 Sensitivity in Leukemic LGLs*- Imatinib (Gleevec) and Nilotinib (AMN107, Tasigna) are tyrosine kinase inhibitors which are widely used in treatment of Philadelphia (Ph) chromosome-positive chronic myeloid leukemia (CML). Both drugs have shown the potent inhibitory effectiveness on RTK activities in different cell types with the doses that we tested. We previously reported that PDGF-RTK is constitutively activated in LGL leukemia cells of both T and NK subtypes via an autocrine activation loop. Data from our current study indicate that Imatinib induces a significant increase of apoptosis in a time and dose-dependent fashion in PBMC from patients with T and NK -LGL leukemia. More importantly, Imatinib treatment sensitizes the LGL primary leukemia cells to Fas/CD95-mediated apoptosis (Figure 2-A and -B). Similar to Imatinib, the second generation RTK inhibitor Nilotinib (AMN107) significantly decreased the number of viable cells in LGL leukemia cell line TL1 (AH8.3) and NKL cells (Figure 3-A and -B) and primary cells (Figure 3-C and -D) in a dose- and time-dependent manner. Furthermore, Nilotinib induced significant increases of apoptosis in PBMC from patients with T and NK -LGL leukemia (Figure 3-E and -F, data from normal control data was not shown). These data strongly suggest that the apoptosis induction in the leukemic LGLs is the mechanism for the reduction of cell viability observed in the MTT assays. The effective dose range in induction of apoptosis is between 5-10uM for Imatinib and Nilotinib. Importantly, we observed that both drugs are able to restore Fas/CD95 sensitivity in PBMC from patients in T and NK -LGL leukemia cells, specifically when low doses of these drug treatments (2.5-5.0uM) were applied. These results point to a potential therapeutic application for these drugs in treatment of LGL leukemia due to elevated systemic levels of Fas and Fas ligand in LGL leukemia patients that are not observed in normal healthy individuals.

3] IAP inhibitor LCL161 showed a mild cytotoxic effect on LGL leukemia cells. A synergistic effect in samples treated with combination of LCL161 and Nilotinib was not observed. YM155 was not tested because of the low expression levels of IAP family genes in Leukemic LGLs.

4] HuMax-IL15 failed to induce cell death/apoptosis in LGL leukemic cells of both T and NK subtypes.

Collectively, based on the data collected from the experiments of Specific Aim 1, we observed that RTK inhibitors Imatinib and Nilotinib, as well as the proteasome inhibitor Ixazomib exhibit potent activities in induction of apoptosis in LGL leukemia cells. Therefore subsequent studies will be focused on exploring the mechanisms of NF-κB constitutive activation and the regulatory effects of PDGR-RTK inhibitors and proteasomes inhibitors on NF-κB pathway in LGL leukemia cells.

In Specific Aim 2 we proposed to determine the correlative mechanisms of leukemic LGL clonal elimination with the NF-κB activity inhibition by the proposed drugs or compounds. Based on the data collected from current studies, the following results and conclusions can be drawn:
1] *Autocrine Activation Loop Sustains NF-κB Pathway Constitutive Activation*- We previously found systemic high levels of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, Apo-2 ligand) in LGL leukemia patients of both T and NK subtypes. We also observed that TRAIL receptor 4 (TRAIL-R4, DcR2) was overexpressed in T-LGL leukemia cells while other receptor expression levels are identical to the levels seen in PBMC from normal donors (data not shown). It is known that the interaction of TRAIL and DcR2 leads to activation of NF-κB pathway. In the current study, we observed that: 1) High expression level of TRAIL in PBMC from patients with T and NK -LGL leukemia but not in PBMC from normal controls (Fig 4-A, -B and -C), 2) An elevated expression level of DcR2 in NK-LGL leukemia cells was observed (data not shown), 3) Recombinant human TRAIL (rh-TRAIL) mediates an increase of NF-κB nuclear translocation and DNA binding activity in LGL leukemia cells but not in PBMC from normal controls (Fig. 5-A, EMSA, 5-B ICC staining), and finally, 4) rh-TRAIL treatment induced significant upregulation of cellular expressions of NF-κB downstream targets such as TRAIL (Fig 6-A and -B), McL-1, c-FLIP and BcL-2 (data not shown) in PBMC from patients with T-LGL,

2] *Bortezomib and Ixazomib Inhibit rh-TRAIL-Induced NF-κB Activation*- Data from the *in vitro* studies carried out in PBMC from patients with T and NK -LGL leukemia demonstrated: 1) Bortezomib and MLN9708 dramatically blocked rh-TNF-α and rh-TRAIL mediated NF-κB activation (Figure 6-C and 6-D), 2) Bortezomib effectively inhibited TNF-α and rh-TRAIL-mediated multiple NF-κB pathway downstream transcript expressions including TRAIL (Figure 6-B), c-FLIP, McL-1 and Bcl-2 at gene and protein levels (data not show).

3] *PDGF-RTK Inhibitors-* Data from the *in vitro* studies carried out in the PBMC from patients with T-LGL indicated that 1) Imatinib and Nilotinib showed no effect on inhibition of NF-κB activity. The mechanisms of these drugs in induction of apoptosis, and the ability of restoration of Fas/CD95 sensitivity remain unknown and further investigation is warranted.

Collectively, data generated from the experiments designed for specific aim 2 showed the evidence of TRAIL/DcR2 interaction axis in mediating and sustaining NF-κB pathway activation. Proteasome inhibitors Bortezomib and Ixazomib dramatically blocked NF-κB nuclear translocation and DNA binding activity. Furthermore, both Bortezomib and Ixazomib abolished or blunted the TRAIL-mediated NF-κB downstream target expressions. Evidently, the interaction of TRAIL/DcR2 plays an important role in mediating and sustaining constitutive activation of NF-κB pathway, which subsequently regulates downstream pro-survival/anti-apoptotic molecule expressions, such as c-FLIP, McL-1, BcL-2 and TRAIL.

In Specific Aim 3, we proposed to determine the contributions of NF-κB pathway in regulating cyokine/lymphokine production in LGL leukemic cells. Correlative studies proposed here are designed to determine the transcriptional and translational regulatory mechanisms of key cytokine/lymphokine production by leukemic cells. The experiments designed for this specific aim were only partially completed due to the relocation of our research laboratory. As proposed in this grant, the experiments that designed to study NF-κB pathway derived cytokine/lymphokine production were carried out. Concentrations of NF-κB regulated cytokines/chemokines were determined using the customized Bio-Plex Luminex kits as described before. The data generated from this study led to the following results and conclusions:

1] *High Expression Levels of Multiple Cytokines/Chemokines in T and NK -LGL Leukemia* - Data from this study showed that T and NK -LGL leukemia patient cells share similar cytokine/chemokine expression profiles. The results of this experiment are outlined below. High protein concentrations of PDGF-BB, IL-8, MIP-1β and RANTES were observed in the tissue culture medium. Concentrations of other targets were either relatively low or undetectable in comparison to the product's standard curves generated for each target. Additionally, data from this current study closely correlates to our previous observations concerning LGL leukemia cells, sera and plasma samples from patients with T and NK -LGL leukemia subtypes (Yang, et al; *Blood*, 2010, Vol 115, pp51-60, Kothapalli/Nyland, et al; *Int J. Oncology*, 2005, Vol 26 pp529-535). The table below details some of the proteins expressions in LGL leukemia.

Concentration (pg/ml)	IL-8	PDGF-BB	RANTES	MIP-1β	IL-6	IL-2	IFN-γ	IL-2Rα	IL-18
T-LGL Pt. # 1	124.2	269.6	2109.5	230.3	6.3	3.1	17.7	2.8	1.64
T-LGL Pt. # 2	746.0	46.3	2385.0	560.3	18.7	23.0	0.0	32.8	6.95
T-LGL Pt. # 3	596.0	75.4	1261.0	92.7	3.3	0.0	0.0	5.72	1.55
NK-LGL Pt.	96.9	567.3	701.2	152.2	0.56	7.5	9.3	0.0	0.76

IL-8, MIP-1β, RANTES and PDGF-BB are NF-κB downstream transcripts (Boston University Biology, www.bu.edu/nf-kb/gene-resources/target-genes/) and highly expressed in leukemic LGLs. Our data from the current study suggest a rational link between NF-κB activity and the transcript expression elevations in LGL leukemia cells.

2] *rh-TRAIL Induces Elevated Multi-Cytokine/Chemokine Production*- Rel or NF-κB comprise a family of structurally-related eukaryotic transcription factors molecules include class1 members of p50/p105, p52/p100, p105 and Relish, and class 2 of RelA (p65), RelB c-Rel, Dorsal and Dif. NK-κB family molecule forms homo- or heterodimers in the process of activation. Individual

dimer binds to the distinct DNA binding site to exert the specific regulatory role in controlling downstream target expressions. Consequently, the downstream molecules executed their tasks in regulating immune functions, tumor genesis, cell proliferation and survival. In turn, the highly expressed transcripts such as INF-γ, IL-6, IL-8 and RANTES are important for NF-κB and JAK/STAT3, MAPK3/JNK and p38MAPK pathway activations. Data generated from this *in vitro* study strongly support our hypothesis of NF-κB activation via TRAIL/TRAIL-DcR2 interactions. Upon measuring the protein concentrations for the downstream targets of interest in the supernatants, we observed that IL-6, IFN-γ, soluble IL-2 receptor alpha (sIL-2Rα), IL-2, and IL-18 were significantly upregulated in the rh-TRAIL-treated T-LGL leukemia PBMC (Fig. 7). The expression levels of these proteins were either low or undetectable in samples with no treatment or in vehicle control samples. Similar results were observed in tissue culture supernatants of PBMC treated with rh-TNF-α at 10ng/ml concentration. Rh-TNF-α was widely used as a positive control for the canonical NF-κB pathway activator in different cell types. Importantly, the proteasome inhibitors Bortezomib (10nM) and Ixazomib (200nM) effectively downregulated cytokine/chemokine productions as measuring the protein concentrations in tissue culture supernatants. In contrast to the results observed in T-LGL leukemia cells, sIL-Rα remained undetectable in NK-LGL leukemia samples regardless of whether they were treated with rh-TRAIL, with rh-TNF-α, or with vehicle. Treatment with the second generation proteasome inhibitor Ixazomib (MLN9708) dramatically eliminated the TRAIL-mediated increases of IL-1β and RANTES, and downregulated IL-18 and IL-6 productions (Fig.8). Ixazomib showed no efficacy in downregulating the expression of IL-2, INF-γ, IL-8 and PDGF-BB. Collectively, data from this study strongly supports our hypothesis that TRAIL/TRAIL-DcR2 interaction plays a decisive role in triggering and sustaining NF-κB pathway activation in T-LGL leukemia cells. Importantly, our work with NK-LGL leukemia patient specimens also provided preliminary data, which lays a valuable foundation for directing our future studies.

Large granular lymphocyte (LGL) leukemia is a disorder of clonal expansion of cytotoxic lymphocytes resulting from dysregulation of cell death. We previously reported that the constitutively activated platelet-derived growth factor-receptor tyrosine kinase (PDGF-RTK) pathway regulated Src family kinase (SFK) and PI3K pathways, and NF-κB pathway play important role in mediating cell survival and proliferation in T and NK -LGL leukemia cells. Inhibition the downstream targets of these pathways resulted in an elevated level of apoptosis in LGL leukemia cells but not in normal counterpart cells (Yang, et al; *Blood*, 2010, Vol 115, pp51-60, Zhang R. et al. *PNAS*, 105(42):16308-13. 2008). It is known that PDGF-RTK activity can lead to NF-κB pathway activation through PKB/AKT-mediated IκB phosphorylation pathway (Leendert et al. *Invest Ophthalmol Vis. Sic*. 2010;51:1002-1007). Therefore, we hypothesized that NF-κB is the key regulatory element, which plays a crucial role in pathogenesis of leukemic LGLs. Owning to lack of standard treatment for patients with LGL leukemia available because of the absence of prospective clinical trials. Searching for the new therapy is urgently needed. In this current proposal, we aimed to investigate the therapeutic efficacy and the correlated regulatory mechanism of NF-κB pathway using drugs or compounds that are FDA approved either for pre-clinical trials or clinical use to speed up the process of searching new therapies.

As we proposed in this grant, we screen the compounds and drugs for the efficacy of LGL leukemia pathologic clonal elimination. Our data suggested the Tyrosine Kinase inhibitor (TKI) Imatinib mesylate and Nilotinib mediated increased levels of apoptosis in PBMC from patients

with T and NK -LGL leukemia but not in the counterpart cells from normal controls. The doses of these two tested TKIs (10uM) are closely correlated to the systemic concentrations observed in the cancer patients who received these drug treatments at clinical settings. Similar to Imatinib and Nilotinib, Dasatinib is a broad-spectrum BCR/Abl tyrosine kinase and SFK inhibitor approved for the first line used in the patients with chronic myelogenous leukemia (CML) and Ph+ALL. Desatinib has been reported to cause clonal lymphocytosis with the phonotypical and morphological features of T or NK -LGL (Mustjoki S. et al. Leukemia 2009;23:1398-05). Noticeably, this side effect of mediating LGL-like clonal expansion has not been reported for Imatinib and Nilotinib. One of the pathologic features of LGL leukemia is that LGL leukemia cells express high levels of Fas and Fas ligand and yet are resistant to Fas/CD95-mediated apoptosis. Bothe Imatinib and Nilotinib showed the effectiveness of restoration of Fas sensitivity in LGL leukemia cells. This finding may be beneficial to use these drugs to treat LGL leukemia patients since the high expression levels of Fas/FasL has already existed in the leukemic LGLs. Both imatinib and Nilotinib failed to block rhTRAIL-induced NF-κB activation in T and NK leukemia cells which suggests that the RTKIs-mediated apoptosis is NF-κB independent.

We hypothesize that the interaction between TRAIL and TRAIL-R4/DcR2 plays a decisive role in mediating NF-κB pathway activation in leukemia LGLs. The activated NF-κB regulates the expressions of multiple downstream transcripts such as Mcl-1, c-FLIP and Bcl-2 and multiple cytokines/lymphokines such as TRAIL, Fas/FasL, IL-6, IL-8, IL-18, MIP-1β, INF-γ and RANTES, which are implicated in the pathogenesis of LGL leukemia. TRAIL (Apo-2 ligand) consists of 281 amino acids and is a type II transmembrane protein having its greatest amino acid identity to Fas ligand (28%). TRAIL is a member of the TNF family of cytokines that are capable of inducing apoptotic cell death in a variety of tumor cells by engaging specific death receptors, while having low toxicity towards normal cells. Therefore, TRAIL-based therapy is widely evaluated in clinical studies as a tumor cell selective pro-apoptotic approach. However, besides activating canonical caspase-dependent apoptosis, TRAIL ligand can activate non-canonical cell survival pathways in TRAIL resistant tumor cells through the interactions with the same receptors. Interestingly, LGL leukemia cells are found to be resistant to TRAIL-mediated cell death. There are five receptors identified that can bind to their receptors with identical affinity except for the receptor named as osteoprotegerin (OPG). TRAIL activates apoptosis via two membrane receptors designated TRAIL-R1 (DR4) and -R2 (DR-5) whereas TRAIL-R3 (DcR-1), -R4 (DcR-2) and circulating osteoprotegerin (OPG) have been proposed to function as decoy receptors that sequester TRAIL, causing the suppression of apoptosis (LeBlanc HN et al. *Cell Death Differ* 2003;10:66-75). The two death receptors, TRAIL-R1/DR4 and TRAIL-R2/DR5, contain a cytoplasmic death domain thereby triggering apoptosis signals upon TRAIL binding. In contrast, three other receptors that do not have a functional death domain may act as decoy receptors. TRAIL-R3 (DcR1, TRID) lacking a death domain and TRAIL-R4 (DcR2, TRUNDD) containing a truncated death domain do not transduce apoptosis signals. Despite its lack of a complete death domain, TRAIL-R4 encodes what appears to be a functional cytoplasmic sequences signaling region. In several other members of TNFR family the cytoplasmic region also contain sequences involved in activation of NF-κB. Transient overexpression of TRAIL-R4 in the cells normally sensitive to TRAIL-mediated killing confers complete protection (Mariapia A, et al. *Immunity* 1997;7:813-20). Therefore, resistance to apoptosis may involve activation of NF-κB to the induction of pro-survival genes. We previously observed that TRAIL concentrations in serum samples from patients with T and NK -LGL

leukemia are significantly higher than the serum levels observed in the age- and gender-matched normal controls (unpublished data). Data from this current study showed that TRAIL expressions in PBMC from patients with LGL leukemia of T and NK subtypes are significantly higher than the expression levels observed in PBMC from normal controls by Western blot assay. Identical results are observed in $CD8^+$ cells isolated from T-LGL leukemia patients and normal controls by ICC staining. Given that, data generated form this current study indicated that LGL leukemia cells are the major sources of TRAIL production in LGL leukemia patients. In addition, we also observed that TRAIL-R4/DcR2 expressions in PBMC from patients with T and NK -LGL leukemia are statistically higher than the expressions observed in PGMC from normal control (p value are < 0.005 for both T and NK LGLs vs. normal control). Identical low expression levels of TRAIL death receptors DR4, DR 5 and DcR1receptor are observed in the PBMC from LGL leukemia patients and normal controls. Therefore, it is rational to believe that the over expressions of TRAIL and TRAIL-R4/DcR2 in leukemic LGLs play are responsible for NF-κB activation in leukemic LGLs. Supportively, data from this study showed that the NF-κB activation can be induced in leukemic LGLs by rhTRAIL or rhTNF-α (EMSA assay). NF-κB subunit RelA (p65) nuclear translocation are observed in the $CD8^+$ cells from T-LGL leukemia patients that were treated with either rhTNF-a or rhTRAIL (ICC staining). Furthermore, the excessive expression of DcR2 receptor in combination with the low expressions of the death receptor DR4 and DR5 in leukemic LGLs offers a greater chance for TRAIL to engage to the predominant DcR2 than the chance to engage the death receptors. On the other hand the TRAIL/DcR2-mediated NF-κB activation and its downstream anti-apoptotic transcript gene expressions are more likely to be responsible for promoting LGL leukemia cell long term survival and resistant to other pro-apoptotic molecule-mediated cell death such as TNF, Fas/Fas ligand. Both TRAIL-R1 and TRAIL-R2 were found to activate NF-κB in a TRADD- and RIP1-dependent pathway (Schneider P, et al. *Immunity* 1997; 7:831-36). NEMO (IKK-γ) was found to be part of the secondary complex, able to recruit IKKα/β to the signaling complex causing the phosphorylation and subsequent proteasomal degradation of the inhibitor of κB (IκB), leading to release and accumulation of NF-κB. NF-κB then translocates to the nucleus where it can activate the transcription of the anti-apoptotic genes such as c-FLIP, Bcl-xL, Mcl-1 and cIAPs (Henson ES, et al. *J Cell Biochem* 2003;89:1177-92). Data from this current studies demonstrated that multiple cytokines/lymphocytes were overexpressed in leukemic LGLs, such as TRAIL, Fas/FasL, IL-6, IL-8, IL-18, MIP-1β, INF-γ and RANTES. In this proposal, we aimed to investigate the mechanisms of NF-κB activity in regulating this atypical cytokine/chemokine expression in LGL leukemia cells. In leukemic LGLs TRAIL expression is closely regulated by NF-κB. Data form this study showed that rhTRAIL treatment induced a time dependent increase of TRAIL gene expression with the expression peak at 2hr time point, and this TRAIL gene upregulation was completely blocked by Bortezomib at 2hr treatment. Collectively, we conclude that proteasome inhibitor Bortezomib inhibits TRAIL gene expression may be possible due to the effect of NF-κB inhibition. In addition, the intracellular synthesis of IL-6, INF-γ, IL-2, and IL-18 by leukemic LGLs were significantly enhanced by rhTRAIL and rhTNF-α treatments. Proteasome inhibitors Bortezomib and Ixazomib, that are known to be capable of inhibiting NF-κB activity in leukemic LGL, effectively blocked or downregulated these cytokine/lymphokine productions as measured the concentrations in the supernatant samples. High expression of soluble IL-2 receptor alpha (sIL-2Rα) was observed in the supernatant samples from T-LGL leukemia which has been implicated to activate STAT5 pathway by forming a complex with IL-2. Increasing evidence from us and other researchers have shown that NF-κB regulated

downstream transcripts are important activators or co-activators for multiple signaling pathways such as JAK/STAT pathways, MAPK3/JNK/SAPK pathways and p38MAPK pathways. Data from this current study lays a valuable foundation for our future studies.

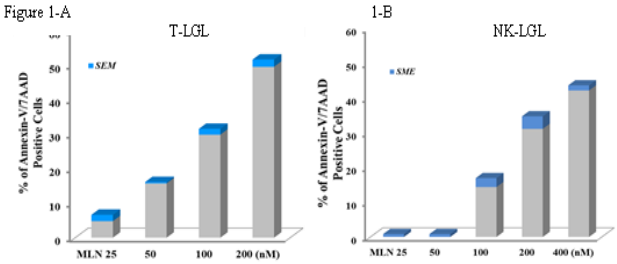


Fig 1. Potency of proteasome inhibitor MLN9708 on induction of apoptosis in PBMC from patients with T- and NK-LGL leukemia was determined at 24hr time point. Data are normalized by using DMSO controls. MLN 9708 dose response curves are shown for 25 to 200 nM in of T-LGL (A) and 25 to 400 nM in of NK-LGL (B**).** (Representative figures of three patient samples tested).

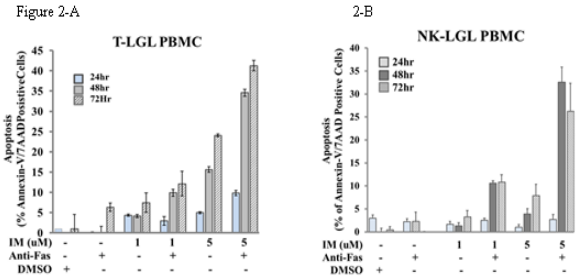
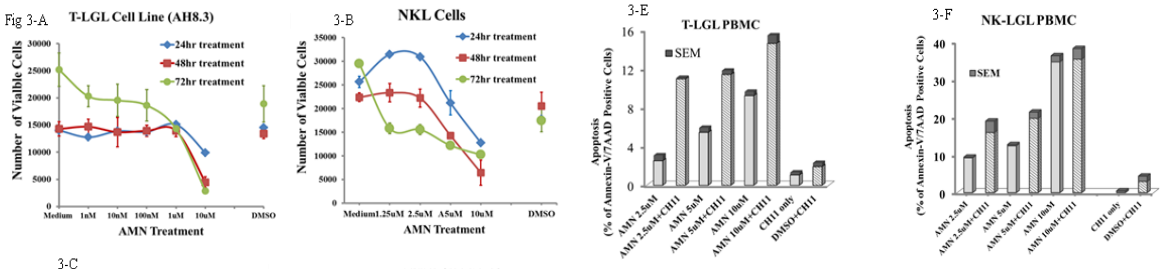


Fig 2. Time and dose dependent apoptosis induced by Imatinib (IM) in patients cells of T-LGL (A) and NK-LGL (B) was determined in the presence or absence of anti-Fas antibody (CH11) treatment. (Representative figures of three patient samples tested).



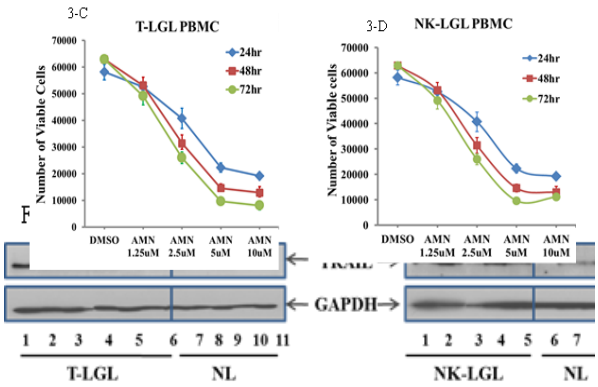
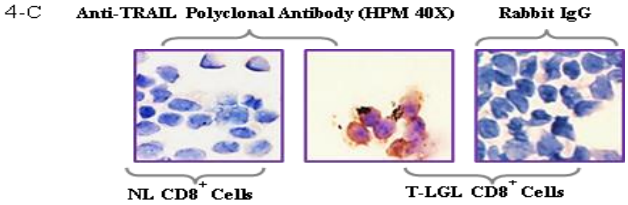


Fig 3 Number of viable cells in Nilotinib (AMN) treated LGL leukemia cell lines of TL1 (AH8.3) cells (A), NKL cells (B) and primary PBMC from patients with T- (C) and NK-LGL leukemia (D) were determined by MTT assay. AMN-induced apoptosis in PBMC from patients with T- (E) and NK-LGL leukemia (F) was determined at 24hr time point in presence or absence of anti-Fas antibody (CH11). DMSO was used as treatment and vehicle controls. SEM: standard error of mean.



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expressions in purified CD8+ cells from T-LGL leukemia patients and normal donors were detected by IHC stained with anti-TRAIL polyclonal antibody. Anti-Rabbit IgG AB was used as isotype antibody control.

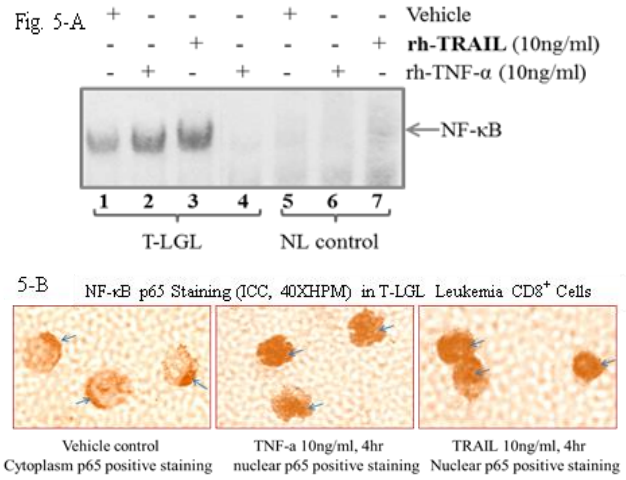

Fig 5 (A) rhTRAIL-induced NF-κB nuclear translocation and DNA binding activity in PBMC from patients with T-LGL and normal controls were detected using AMSA assay. (B) NF-κB p65 subunit unclear translocation in $CD3^+/CD8^+$ cells from T-LGL leukemia patients was detected by ICC staining using anti-TRAIL polyclonal antibody. Anti-Rabbit IgG was used as isotype antibody control. rhTNF-a serves as positive control.

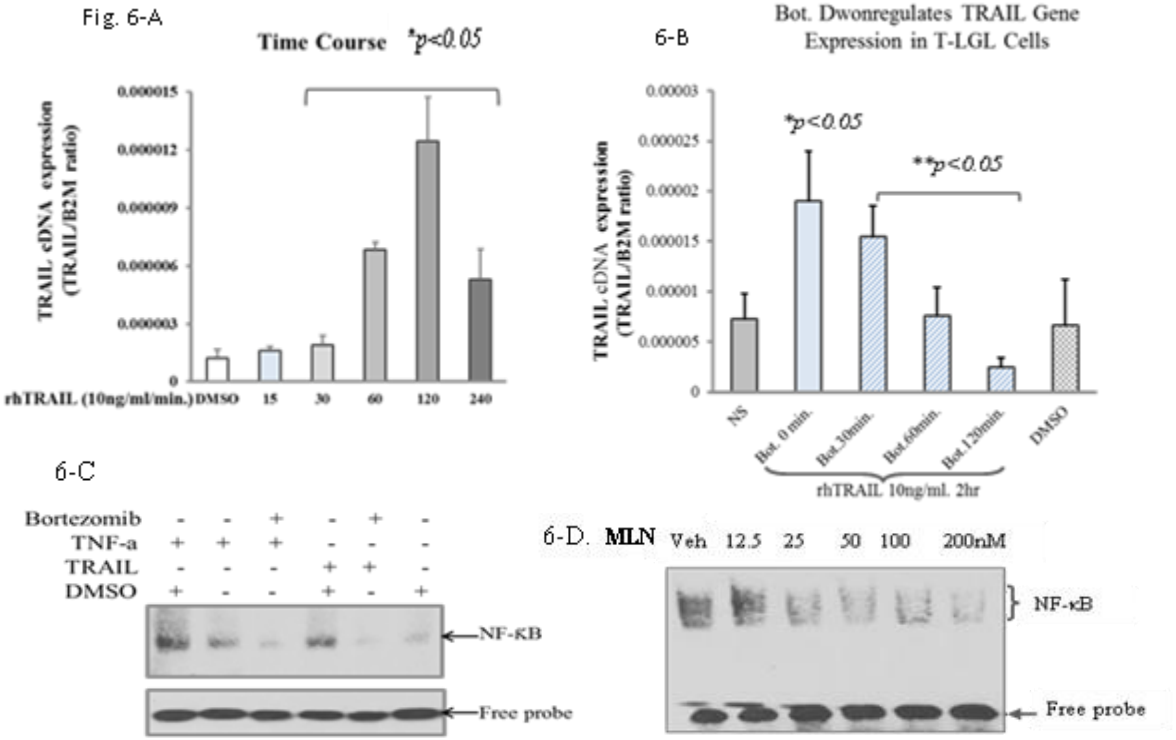

Fig 6. TRAIL gene expression in T-LGL leukemia cells was determined by Real-Time quantitative PCR. B2M was used as an endogenous control. Ratio of the cDNA copies of TRAIL and B2M indicate the expression level of the target genes. DMSO was used as vehicle control. (A) TRAIL expression time course. (B) Bortezomib treatment time course for TRAIL expression in T-LGL leukemia cells. NF-κB nuclear translocation and DNA binding activities in PBMC treated with Bortezomib and MLN9708 in PBMC from patients with T-LGL (C) and NK-LGL (D) leukemia. DMSO treatment was used as vehicle controls.

Fig 7.

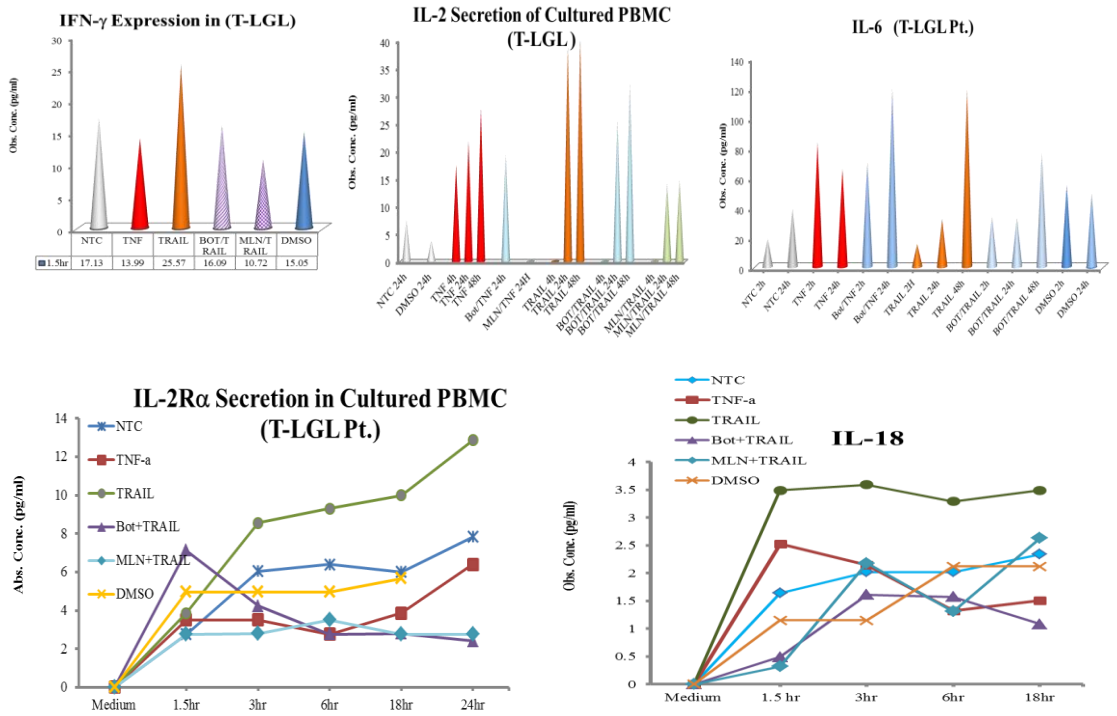

Fig. 7 Cytokine/chemokine expressions in T-LGL PBMC tissue culture supernatants with rhTRAIL or rhTNF-α a dose of 10ng/ml concentration in presence or absence of RTKIs. Representative graphs for three repeated experiments.

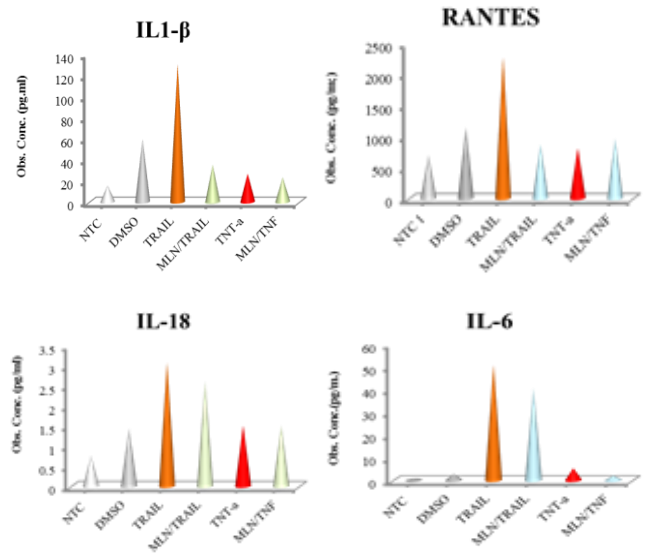

Fig 8. PBMC from a NK-LGL patient were pretreated with Ixazomib (200nM) or DMSO for 2hr followed for additional 4hr treatment of rhTRAIL or rhTNF-α at a dose of 10ng/ml. Supernatants were harvested, cytokine/chemokine protein concentrations were determined using Bio-Plex Luminex assay.

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. None

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, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

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:

I will write a paper entitled "TRAIL/TRAIL-DcR2 Interaction forms An NF-κB Autocrine Activation Loop in T and NK -LGL leukemia" (The title of the paper make be modified). The target journal of this manuscript is Blood.

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.

We proposed to study the therapeutic efficacy of multiple drugs and compounds, which are approved either for pre-clinical trials or used at clinical settings as anticancer drugs by the FDA, in PBMC from patients with LGL leukemia and from normal controls. The major discoveries for the completed studies are summarized as following: 1) Targeting platelet-derived growth factor receptor tyrosine kinase (PDGF-RTK) pathway using RTK inhibitor Imatinib and Nilotinib induce an elevated level of apoptosis in leukemic LGLs of T and NK subtypes via a NF-κB independent pathway. 2) PDGF-RTK inhibition sensitized leukemic LGLs to Fas/CD95-mediated apoptosis. The mechanisms of this PDGF-RTK inhibition-mediated Fas/CD95 sensitivity restoration remain unclear. 3) Rh-TRAIL induces NF-κB activation in leukemia cells from patients with T and NK -LGL leukemia but not in their count part cells from normal controls. 4) The constitutively activated NF-κB pathway in leukemic LGLs is responsible for the overexpression of downstream transcript TRAIL in LGL leukemia cells. The interactions between TRAIL and the overexpressed TRAIL receptor-4 (DcR2) in LGL leukemia cells forms an autocrine activation loop to mediate and to maintain NF-κB constitutive activation. 5) Proteasome inhibitor Bortezomib and Ixazomib induced a significant increase of apoptosis in leukemic LGLs via blocking NF-κB nuclear translocation and DNA binding activities which consequently eliminate or downregulate NF-κB downstream transcripts expressions. 6) Bortezomib and Ixazomib treatment blocked or downregulate multiple anti-apoptotic proteins and cytokines/chemokines such as c-FLIP, McL-1, BcL-2, TRAIL, IL-2Rα IL-8, IL-6 MIP-1β, RANTES and IFN-γ. 5) Data from this current study point out the potential clinical applications for future treatment of LGL leukemia.

23. Inventions, Patents and Commercial Development Opportunities.

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

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

If yes, indicate date patent was filed:

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

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

If yes, how many licenses were granted?_____

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

If yes, describe the commercial development activities:

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

Yes_____ No____X_____

If yes, please describe your plans:

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

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Jun Yang (PI)	Assistant Professor of Medicine

EDUCATION/TRAINING			
BEGIN WITH BACCALAUREATE OR OTHER INITIAL PROFESSIONAL EDUCATION, SUCH AS NURSING DEGREE, AND INCLUDE POSTDOCTORAL TRAINING.			
INSTITUTION AND LOCATION	**DEGREE** *(if applicable)*	**YEAR CONFERRED**	**FIELD OF STUDY**
Kunming Medical College, Yunnan, P. R. of China	M.D.	1984	Medicine
Surgery Department, College of Medicine, The University of South Florida, 2900 Bruce B. Downs Blvd. Tampa, FL 33613	M.D.	Aug.1995-Dec. 2003	Acute servere pancreatitis
Penn State Hershey Cancer Institute, College of Medicine, Penn State University 500 University Drive, P. O. Box 850, Cancer Institute Building, Room T4421. Hershey, PA 17036	M.D.	Dec.2003-Sept. 2013	Hematology oncology, LGL leukemia
Universith of Virginia, College of Medicine, Cancer Cernter. University of Virginia Carter-Harrison Research Building (MR6), Room G707A 345 Crespell Drive, P.O. Box 801378, Charlottesville, VA 22908	M.D.	Oct. 2013-now	Hematology Oncoloty, LGL leukemia

RESEARCH AND PROFESSIONAL EXPERIENCE

CONCLUDING WITH CURRENT POSITION, LIST PREVIOUS RESEARCH POSITIONS, PROFESSIONAL EXPERIENCE AND HONORS

2013. 10-Now : Assistant professor, University of Virginia, Cancer Center. Department of Medicine, College of Medicine, University of Virginia.

2003. 12- 2013.09: Assistant professor, Penn State Cancer Institute. Hershey PA. Department of Medicine, College of Medicine, Penn State University,

2003. 06-2003.12: Assistant professor. Surgery Department of USF Tampa, FL

2001.07-2003. 06: Senior Biological Scientist. Surgery Department of USF, Tampa FL.

1996.07-2001.06: Biological Scientist. Surgery Department, USF, Tampa, FL.USA.

1995.08 –1996. 07: Visiting Scholar (Courtesy Research Associate), Surgery Department, UAF, Tampa, FL.

1992.06 - 1995.08: Attending Doctor (General Surgeon), Assistant Professor. Surgery Department of KMC. First Affiliated Hospital of KMC. Major in pancreatic diseases, liver, biliary tract diseases and Laparoscopic surgery (Inflammatory and Oncologic diseases).

Awards and Other Professional Activities

University of South Florida*26th Annual Association of VA Surgeon's Meeting*, Best Resident Basic Science presentation, April 2002

University of South Florida Health Science Center, Annual Research Day-Superior Presentation, Dept. of Surgery, February 14,2002
Florida committee on Trauma, ACS-Resident Paper Competition, first runner-up, November 200, *Southeastern Surgical Congress.* Gold Medal Forum 1997
Kunming Medical College Exchange Scholar Program
The University Visiting Scholarship of Yunnan Province (China) 1995-1996

Personal Statement

I have nine years surgical oncology experience and nearly 18 years bench work experience in the fields of inflammatory disease and cancer research. My research activities have focused on the investigation of pathogens and the pathologic mechanisms of large granular lymphocyte (LGL) leukemia since late 2003. In this project, I mainly focused on the studies of the potential therapeutic efficacy of NF-κB and PDGF-RTK targeting drugs or compounds that are now either tested in the preclinical trails or they are currently used at clinical settings in treatment of LGL leukemia. This project will lay a solid foundation for the future studies as well as for search the standard treatments in LGL leukemia patients.

SELECTED PEER-REVIEWED PUBLICATIONS

LIST IN REVERSE CHRONOLOGICAL ORDER COMPLETE CITATIONS FOR THE LAST THREE (3) YEARS OF PROJECT LEADER'S PUBLICATIONS RELEVANT TO THE PROPOSED RESEARCH, AND REPRESENTATIVE EARLIER PUBLICATIONS THAT ARE RELEVANT TO THE PROPOSED RESEARCH.

1. Ren T**, Yang J,** Broeg K, Liu X, Loughran TP Jr., Hua Cheng**.** *Developing an in vitro model of T cell type of large granular lymphocyte leukemia. Leukemia Research*. 3013;37:1737-1743.

2. **Yang J**, Loughran TP, Jr. *Potential clinical application of Imatinib Mesylate in patients with leukemic Large Granular Lymphocytes*. *Leukemia & Lymphoma*. 2011 Oct; 52(10):1834-5

3. Liao A, Broeg K, Fox T, Tan SF, Walters R, Shah MV, Zhang LQ, Li Y, Ryland L, **Yang J,** Loughran TP, and Liu X. *Therapeutic efficacy of FTY720 in a rat model of NK-cell leukemia*. Blood. 2011Sep.8:118(10):2793-800

4. Liu X, Ryland L, **Yang J**, Liao AJ, Aliaga C, Watts R, Tan SF, Kaiser,J,

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5. **Yang J**, Liu X, Nyland SB, Zhang R, Ryland LK, Broeg K, Baab KT, Jarbadan NR, Irby R, Loughran TP Jr. *Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway*. *Blood.* 2010 Jan 7;115(1):51-60.

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