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 717-231-2825.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2014

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:** 4100054865

6. **Project Number and Title of Research Project:** 5. *Genome-wide Mapping of Transcriptional Factors Involved in Pathogenesis of LGL Leukemia*

7. **Start and End Date of Research Project:** 7/1/2011 – 6/30/2013

8. **Name of Principal Investigator for the Research Project:** Thomas P Loughran, MD
   The PI has left the institution.

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:

   $ 100,000

   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	% of Effort on Project	Cost
Olson Thomas	Graduate Assistant	63	33,221
Zhang Dan	Post-doctoral scholar	21	16,205
Dewey Alden	Technician	08	4,165.24
Loughran Thomas	Principal Investigator	<1%	998.52

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	% of Effort on Project
Hardison, Ross	Co-Investigator	5
Keller-Capone, Cheryl	Research Associate	1
Giardine, Belinda	Programmer/Analyst	1
Miller, Webb	Co-Investigator	1
Burhans, Richard	Computational Scientist	1

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
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____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 awarded:
Genomic Architecture of LGL Leukemia	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	10/2012	$3,238,809	Not funded

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:

We have submitted an R01 for which the goals of this project represent one specific aim. Though the grant application scored well it was not funded. We feel all criticisms of this grant application can be addressed in a satisfactory manner. We plan to resubmit this grant application in the next funding cycle.

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

The current plans are to expand the work completed in this proposal to other genome interactors, combine this with mutation data we are collecting as part of other projects and use both to build complex networks of interactions that lead to LGL leukemia. This project continues to be a key focus of our lab.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____X____ No_____

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

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				1
Unknown				
Total			**1**	**1**

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

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

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes_____ 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___X_____ No_____

If yes, please describe the collaborations: Indirectly yes.

Although the Principal Investigator of this grant has changed institutions to the University of Virginia, the collaboration with researchers at Penn State continues and it is anticipated that collaborative grant applications will continue to be submitted.

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.

Genome-wide Mapping of Transcriptional Factors Involved in Pathogenesis of LGL Leukemia – Large Granular Lymphocyte (LGL) leukemia is characterized by a clonal proliferation of antigen-primed terminally differentiated effector memory cytotoxic T cells (CTL). The overall objective of this project is to understand the pathogenesis of LGL leukemia by identifying the key genes which promote leukemic LGL survival. This project capitalizes on strengths of Penn State University by investigating the pathogenesis of a leukemia discovered by the PI utilizing a genomic approach in collaboration with colleagues at Penn State-University Park. T-bet is considered the master switch for the generation of antigen primed effector CTL. We previously showed that leukemic LGL are characterized by T-bet upregulation. Thus, LGL leukemia provides us with a unique model to study the functional role of T-bet in generalizing and maintaining CTL response.

The specific aims are:

1. To map genome-wide T-bet binding sequences from LGL leukemia samples.
2. To construct T-bet binding-partner network in LGL leukemia.

Sample Collection and Preparation for Sequencing. Preliminary work for this grant involved the collection and preparation of LGL patient and normal donor terminal effector memory cells (TEMRA). Approximately 10 LGL patient samples collected in accordance with Institutional Review Board protocols were obtained. Samples of high purity (greater than 85% LGL cells) were then prepared for chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq). The earliest samples were used to identify a high specificity antibody for T-bet bound to chromatin. Utilizing a real time PCR assay to determine the ability of antibodies to pull down the promoters of known T-bet target genes, a functional rabbit polyclonal antibody from Santa Cruz Biologicals was selected as being the best. 4 LGL patient samples were then prepared for sequencing. Briefly, samples were formalin fixed to crosslink T-bet to bound genomic regions, sheared by sonication into uniform sizes of DNA and then immunoprecipitated with antibodies to T-bet. This was followed by extensive washing to remove unbound material followed by reversal of crosslinks and extraction of the bound DNA. Both T-bet and no-antibody (input) controls were prepared for each patient sample. Significant work was expended in this area to optimize each of these steps leading to high quality samples for sequencing.

Sequencing of bound DNA. Immunoprecipitated DNA was prepared for next generation sequencing on the Illumina HiSeq 2000. This involved the ligation of specific sequencing

adapters and a short amplification step followed by gel extraction of DNA in the target range of 300-500 basepairs in length. Knowledge of the length of DNA sequenced, and the exclusion of longer and shorter reads is helpful for subsequent bioinformatic steps and to improve the resolution of chromatin regions that are bound. ChIP and input samples were barcoded to allow sequencing of each sample on one lane of the HiSeq 2000. Approximately 100 million reads, consisting of 38 nts from one end of the isolated DNA molecules, were collected for each lane. These sequences were then exported from the instrument and preprocessed to assign sequences to samples by barcode and to remove poor quality reads.

Bioinformatic analysis of T-bet bound regions. Data files were then uploaded to GALAXY which is a web platform for the implementation of many bioinformatic tools that is maintained by Penn State and Emory University. The Galaxy Project is supported in part by NSF, NHGRI, the Huck Institutes of the Life Sciences, the Institute for CyberScience at Penn State, and Emory University. Using this platform, sequence reads from input and T-bet ChIP samples were mapped to the human genome, build 19. A Model-based Analysis of ChIP-Seq (MACS) program was then used to identify T-bet binding sites. The basic interpretation of ChIP-Seq data is to determine which regions of the chromosomes are sequenced more often as this indicates that the antibody pulldown enriched these sequences. MACS improves upon this basic analysis by using the input sample to eliminate bias created by the fact that not all regions of the human genome are sequenced at the same rate. It also utilizes the estimated shear length of the prepared libraries to determine the interval size that should be created by reads that flank both sides of the bound region. The result of the analysis for 4 LGL samples resulted in 3 high quality samples with 4,500-9,000 T-bet binding sites being identified. The fourth sample had a low number of T-bet binding sites that may have been the result of a therapeutic intervention for neutropenia that was not reported at the time of sample donation.

Identification of T-bet binding sites in proximity to known genes. Figures 1 and 2 demonstrate the type and quality of data collected by the group so far. Of note, two canonical targets of T-bet, IFN-gamma and Granzyme B are shown to be bound in all three samples (Figure 1). The main thrust of this project is to identify binding sites that are critical to the T-bet induced expression of key genes in LGL leukemia and normal TEMRA. In support of this (Figure 2), two genes with known relevance to LGL leukemia, Fas ligand (FASL) and the Sphingosine-1-phosphate Receptor 5(S1PR5) show strong evidence of having T-bet bound in and around their promoter regions.

Preliminary pathway analysis of genes in proximity to predicted T-bet binding sites. The next funded year for this project sought to draw correlations between T-bet bound regions and genes that are differentially expressed in LGL leukemia and TEMRA. It is possible to take an initial look at genome-wide T-bet binding sites in our current data to see if they are consistent with the established roles of T-bet in T-cells. To achieve this, the 9,000+ binding sites from the single best patient sample were analyzed utilizing the Genomics Regions Enrichment of Annotations Tool (GREAT) hosted on the web by Stanford University. The tool works by assigning bound genomic regions to the nearest known gene and then analyzing the aggregate list of assigned genes represented to determine if enrichment of previously known pathways occurs in the gene list. Utilizing this tool, several pathways were identified as being enriched that are consistent with the known function of T-bet in T and other immune cells (Figure 3). We interpret this as

good preliminary evidence that data we are collecting is high quality. Now that we have verified that our protocol is identifying previously known targets, we will now shift towards identifying gene targets that do not have previously known associations with T-bet. These new targets will provide insight into the function of T-bet and may identify new points for therapeutic intervention in LGL leukemia.

RNA-Seq of LGL leukemia samples. There are many instances in which predicted binding sites are not in close proximity to known genes. Although we have readily available microarray data to correlate T-bet binding sites with gene expression, these data are incomplete in that they will not identify previously unknown transcripts that may be expressed due to the activity of T-bet. For this reason, we have extracted RNA from two of the ChIP-Seq patient samples and have prepared them for strand-specific next generation sequencing. These initial two samples yielded libraries of poor quality. After completion of this proposal, RNA-Seq of three LGL samples, two of which were ChIPed for T-bet was completed under other funding. This data is now viewable in display tracks on genome browsers similar to that of the ChIP-Seq tracks.

Isolation of normal TEMRA. We have succeeded in isolating over a dozen samples of normal TEMRA; however cell numbers from individual normal donors have been low. This would require us to pool as many as 4 to 5 individuals to achieve a cell count of 25 million. We are currently using 75 million cells from LGL patients for each ChIP-Seq. The requirement to pool as many as 15 normal donors to be sufficient for one ChIP-Seq sample may be problematic from the alignment aspect of the bioinformatic pipeline. However, we are continually working on the protocol to lower the cell number input requirement. Additionally, we believe the current samples will be more than sufficient to confirm individual binding sites in TEMRA that we identify as being of high importance in the LGL samples. After the isolation of the normal TEMRA samples we were also able to complete microarray analysis of 3 of these normal TEMRA samples. This is an improvement over previous array data which used CD8+ T cells or normal patient peripheral blood mononuclear cells (PBMCs) as controls.

Identification of STAT3 binding sites. Figure 4 demonstrates a sample of the data obtained in the second year. We see in one of three samples that STAT3 may be binding near its own transcriptional start site. One sample gave a much stronger signal than the other two and it was later determined that this patient had a Y640F mutation in STAT3 that has been shown to increase the transcriptional activity of STAT3 (Koskela, 2012 *NEJM*, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3693860/). A total of 1,900 regions potentially occupied by STAT3 were identified in this single sample. To increase the quality of the observed STAT3 ChIP-Seq signal, we have selected and prepared two additional patient samples with Y640F mutations. These will be sequenced using funds from another source but their preparation and isolation were completed under this project. Further analysis of the STAT3 data awaits these additional biological replicates.

Additional pathway analysis of genes in proximity to predicted T-bet binding sites. Additional analyses on T-bet data from the previous year were performed. Three samples were submitted to ChIP-Seq and significant overlap was observed between the three. One sample had more occupied sites than others (Figure 5). These additional sites clustered around those present in the other samples. This trend is currently of unknown significance. The 9,000+ binding sites from

this single best patient sample were analyzed utilizing the Genomics Regions Enrichment of Annotations Tool (GREAT) hosted on the web by Stanford University. As noted above, the tool works by assigning bound genomic regions to the nearest known gene and then analyzing the aggregate list of assigned genes represented to determine if enrichment of previously known pathways occurs in the gene list. We then exported the associated gene lists and overlaid them on existing signaling pathways utilizing Ingenuity IPA. Figure 6 is one example of a pathway with critical importance to LGL; T cell receptor signaling. Near complete coverage of this signaling pathway was observed indicating the high potential for T-bet to influence this pathway. The ability to overlay this and other pathways with additional data such as other bound factors and expression data will be crucial to our understanding of complex relationships among factors.

Discovery of additional factors that may interact with T-bet. We selected the largest dataset to determine what other DNA binding proteins may be associated with these occupied segments by looking for the enrichment of motifs. TBET bound intervals were shrunk to 50 basepairs on either side of their coordinate mean and submitted to DREME, which discovers short regular expression motifs. We found 28 statistically enriched motifs (E-value < 5E-04). We then used TOMTOM to quantify the similarity between our experimentally determined motifs and those of curated transcription factor binding motif databases. Examples of this are shown in Figure 7, but a total of 28 results were returned. Several interesting transcription factor binding sites, such as those bound by runt-related transcription factors (RUNX), were found to be consistent with the enriched motifs present in T-bet bound regions. This provided important input into what factors should be selected for future ChIP-Seq experiments, provided these factors are expressed in LGL. In the case of RUNX3, we found it to have 2.4 fold higher expression in LGL compared to normal CD8+ T cells. We have not previously investigated the role of RUNX in LGL leukemia.

Investigation of T-bet bound regions altered by mutation. The occupied segments for patient 1 and 3 were subtracted from patient 2 to yield those segments unique to patient 2. This yielded segments covering 608,507 basepairs of hg19. We determined coverage of these intervals by 30,780 small nucleotide variants (SNVs) that we identified in whole genome sequencing of paired normal and LGL samples of patient two in another project. Overall, 45 SNVs were detected in 24 intervals. Assuming a total genome size of 3 billion bases, these identified intervals had 7.2 times more SNVs than the average segment of the human genome (1 mutation was found for every 13,522 basepairs in the occupied segment versus 1 for every 97,466 basepairs in the total genome). Using the default basal plus extension (5kb upstream, 1kb downstream and a distal 1,000 kb until the nearest gene was encountered) settings of GREAT 22 regions were associated with 2 genes, 22 with 1 gene, and 1 region was not associated. It has been established that sequence variants can affect binding of transcription factors and subsequently transcription. We have created alignments of the reads supporting these altered segments to determine if there is a bias towards the mutated allele being bound which would indicate the variants are causing the site to become active. We are in the process of validating the impact of these individual mutations. Discovering these sites that are the result of mutation may be key to elucidating the pathogenesis of LGL leukemia and will help to distinguish how accurate a model LGL leukemia is for normal TEMRA.

Progress towards grant application and publication. Overall, we feel the T-bet data is of sufficient quality for publication. We are currently preparing a large number (24) of LGL patient

samples for RNA-Seq to determine which regions of the genome are transcribed. RNA samples corresponding to those used for ChiP-Seq will be included in that experiment and used to make a complete story with our previous data. This is being carried out under a different funding source. Our first attempt at RNA-Seq in this project was problematic in that large amounts of input RNA were required but we have since devised an improved method on three samples that we will soon expand under other funding. Large portions of the T-bet data were used as preliminary data in an R01 application. This application was fairly well received and was scored but not funded. We anticipate a resubmission of this grant with few changes in the next cycle. A key problem noted throughout the grant proposal was the need to acquire large numbers of cells for ChIP experiments. We have completed the new methods requiring 15 times less cellular input and have validated 7 of the T-bet bound regions in additional samples with this method. This lower input will allow us to measure bound regions of multiple factors in the same patient sample as well as to validate findings in the true normal counterpart to LGL. Although this was not funded by this grant, the knowledge gained by researchers funded by this grant was integral in bringing about this result.

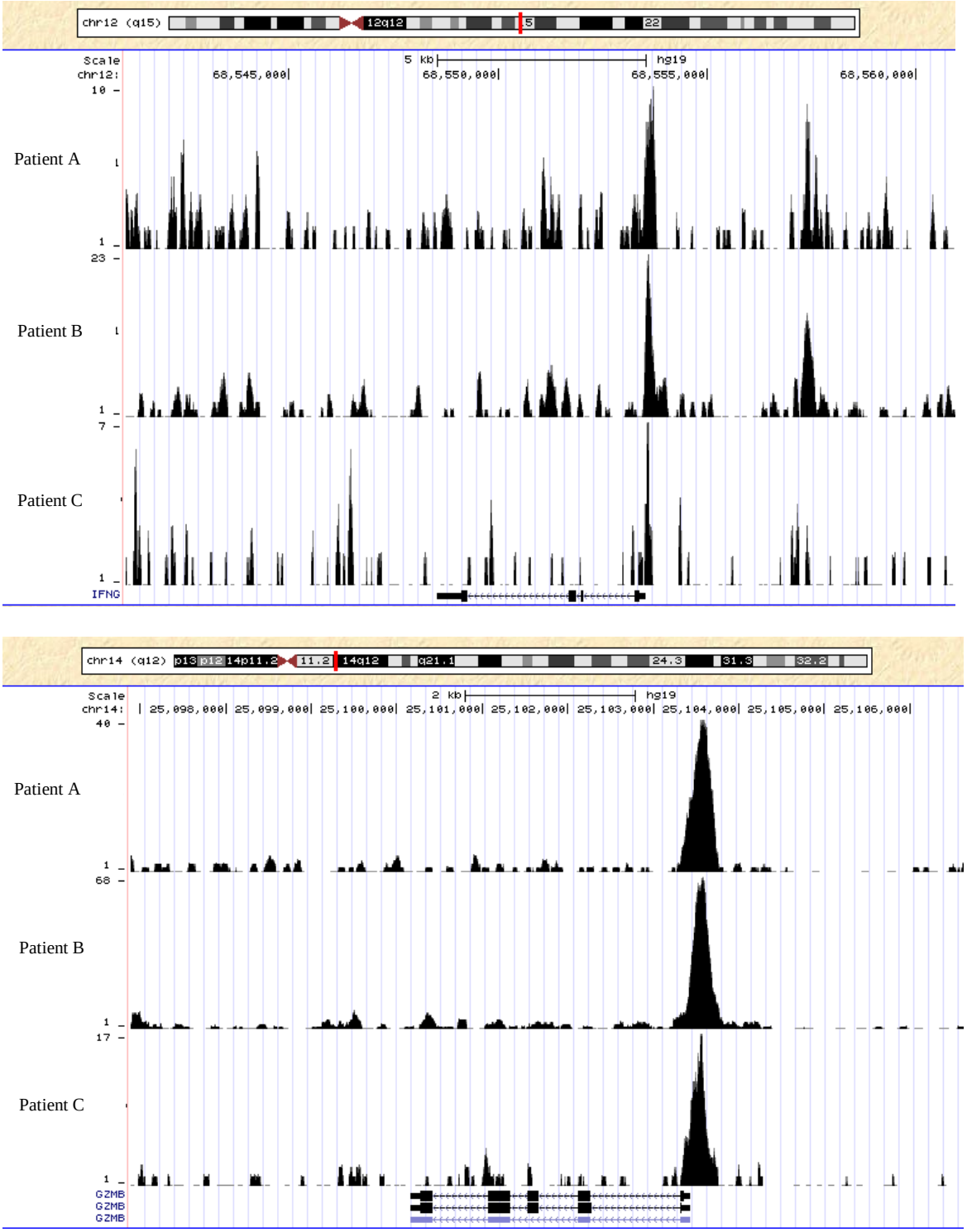

Figure 1. Big-Wig files of genomic regions bound by T-bet from 3 separate LGL patient samples displayed in relation to two known gene targets of T-bet, Interferon-gamma (IFNG, top panel) and Granzyme B (GZMB, bottom panel). Peaks represent a pile-up of the number of times each genomic region was sequenced indicating enrichment by the T-bet antibody.

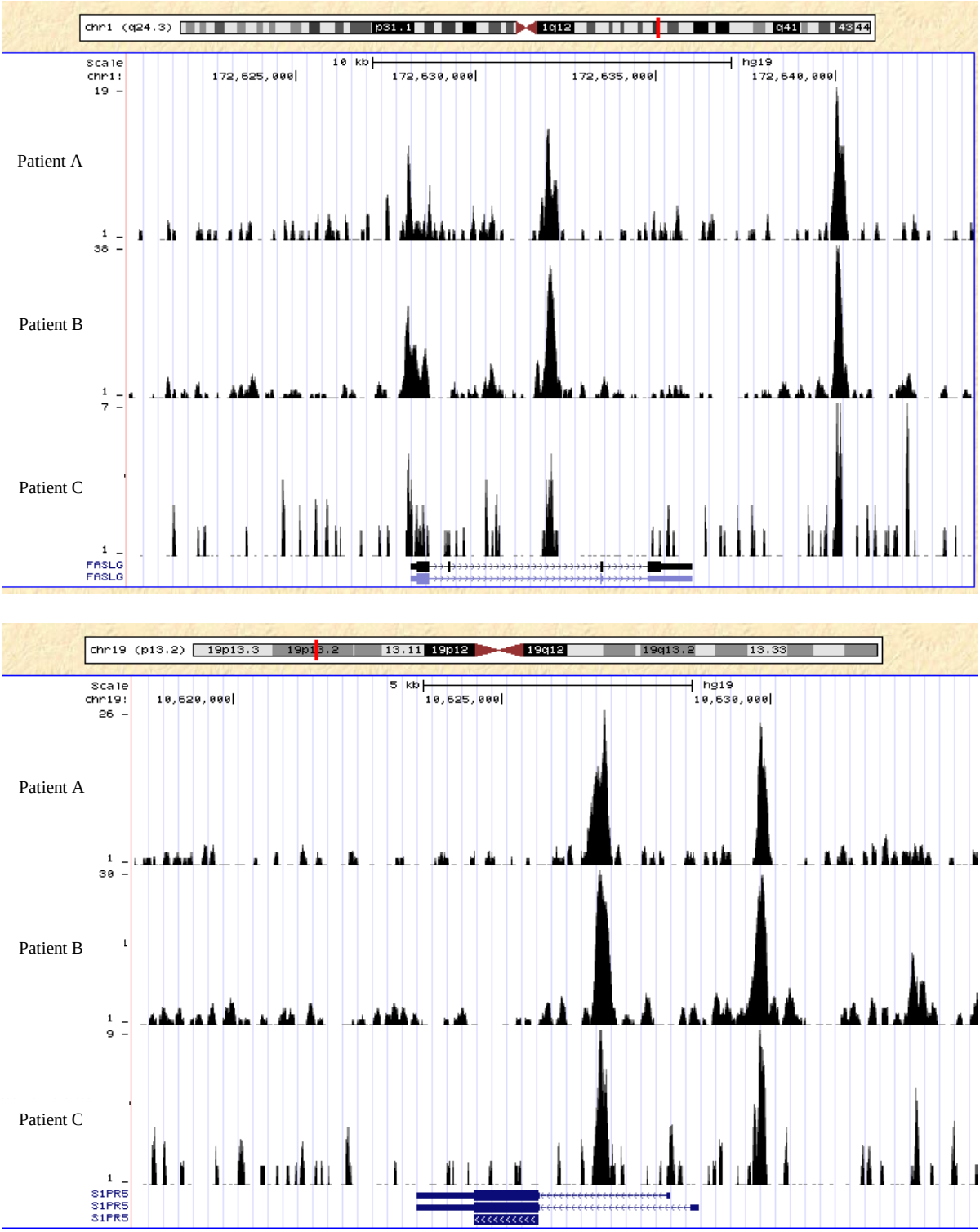

Figure 2. Demonstration of T-bet binding in and around the promoter to two known genes with high relevance to LGL leukemia, Fas ligand (FASL, top panel) and the Sphingosine-1-phosphate receptor 5 (S1PR5, bottom panel). Peaks represent a pile-up of the number of times each genomic region was sequenced indicating enrichment by the T-bet antibody.

Gene Ontology Term Name	Binomial Rank	Binomial Raw P-Value	Binomial FDR Q-Value
regulation of immune response	4	8.51E-97	1.86E-93
leukocyte activation	5	3.78E-84	6.62E-81
lymphocyte activation	6	3.76E-73	5.49E-70
immune response-activating cell surface receptor signaling pathway	7	5.13E-67	6.42E-64
immune response-regulating cell surface receptor signaling pathway	9	2.28E-66	2.22E-63
antigen receptor-mediated signaling pathway	10	6.42E-65	5.63E-62
immune response-activating signal transduction	12	1.32E-61	9.65E-59
immune response-regulating signaling pathway	13	7.25E-60	4.89E-57
positive regulation of immune response	15	1.15E-55	6.70E-53
leukocyte migration	17	4.78E-55	2.46E-52
activation of immune response	19	9.17E-54	4.23E-51
regulation of cytokine production	20	4.87E-52	2.14E-49
T cell receptor signaling pathway	24	2.78E-49	1.02E-46
T cell activation	30	1.97E-45	5.74E-43
regulation of immune effector process	33	8.65E-44	2.30E-41
lymphocyte differentiation	39	1.26E-39	2.83E-37
regulation of B cell activation	42	5.38E-39	1.12E-36
regulation of response to biotic stimulus	46	5.71E-36	1.09E-33
regulation of leukocyte mediated immunity	53	8.98E-35	1.48E-32
interferon-gamma-mediated signaling pathway	76	1.38E-29	1.59E-27

Figure 3. Gene Ontology pathways associated with T-bet bound regions. Analysis was completed utilizing GREAT version 2.01 with the following setting: species assembly hg19, Association rule: Basal+extension: 5000 bp upstream, 1000 bp downstream, 1000000 bp max extension, curated regulatory domains included.

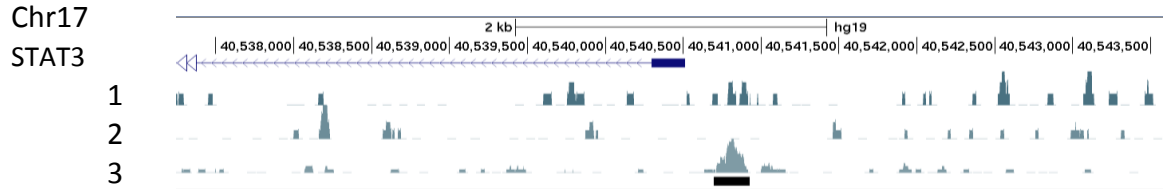

Figure 4. Big-Wig plots of ChIP-Seq data from three LGL patient samples.
This figure shows the transcriptional start site of STAT3 which may indicate a mechanism of positive feedback. Of note only the third sample appears to produce a strong signal. This sample contains a strongly activating mutation in STAT3. hg19= human genome build 119 Chr17= chromosome 17

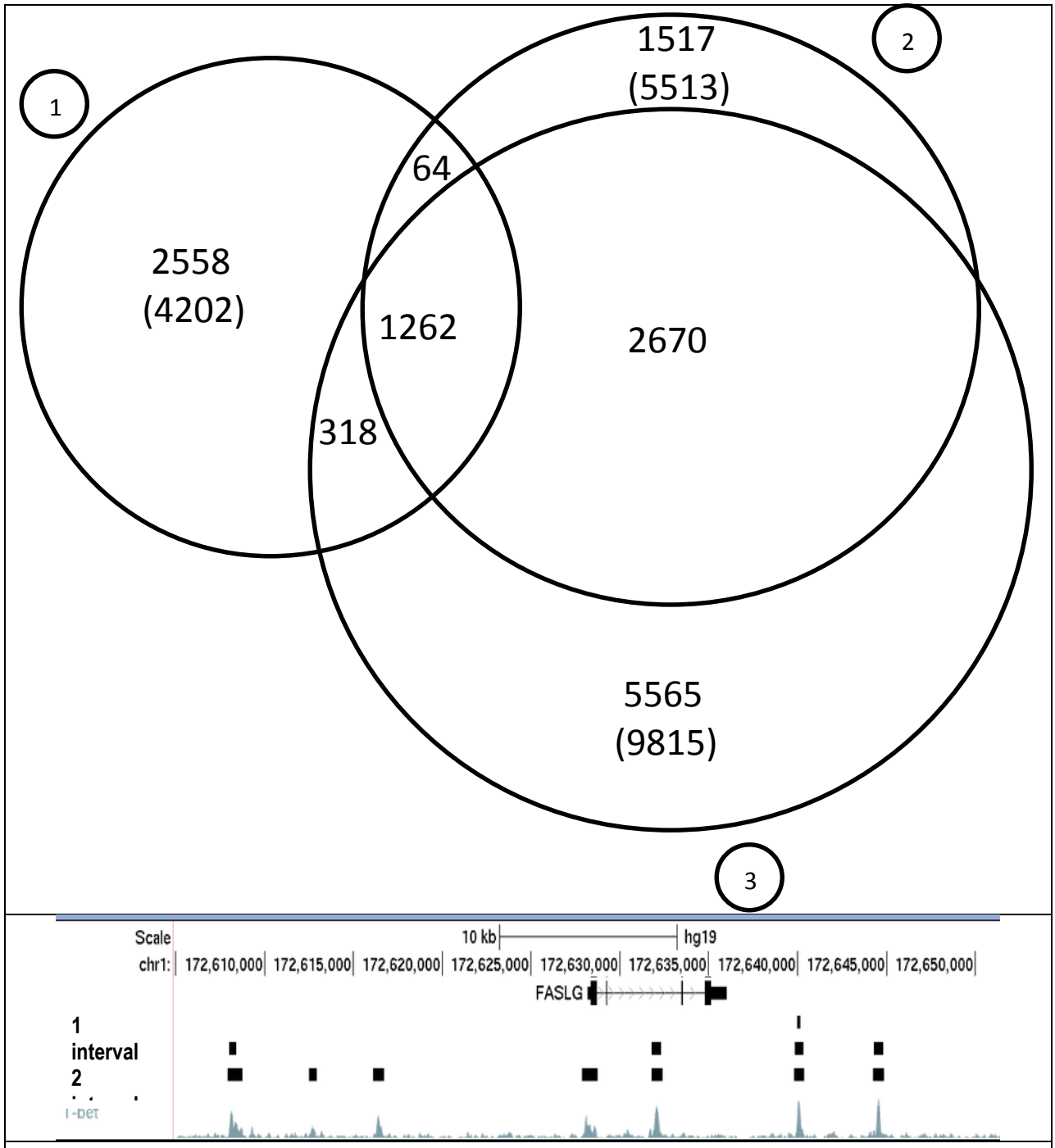

Figure 5: Initial Assessment of ChIP-Seq Datasets from Thee Patients
The top panel indicates overlap between intervals in each of three datasets. The size of circles representing datasets is exactly proportional, but overlapping regions are not. Parenthetical values are the total number of intervals for each sample. The bottom panel shows intervals for binding in all three datasets and peak data for sample three around the Fas Ligand gene (FASLG). Peak height is scaled to approximately 50 reads at the highest peak. Similar visualizations for these data are available to our lab across the whole of hg19 to facilitate further hypothesis generation.

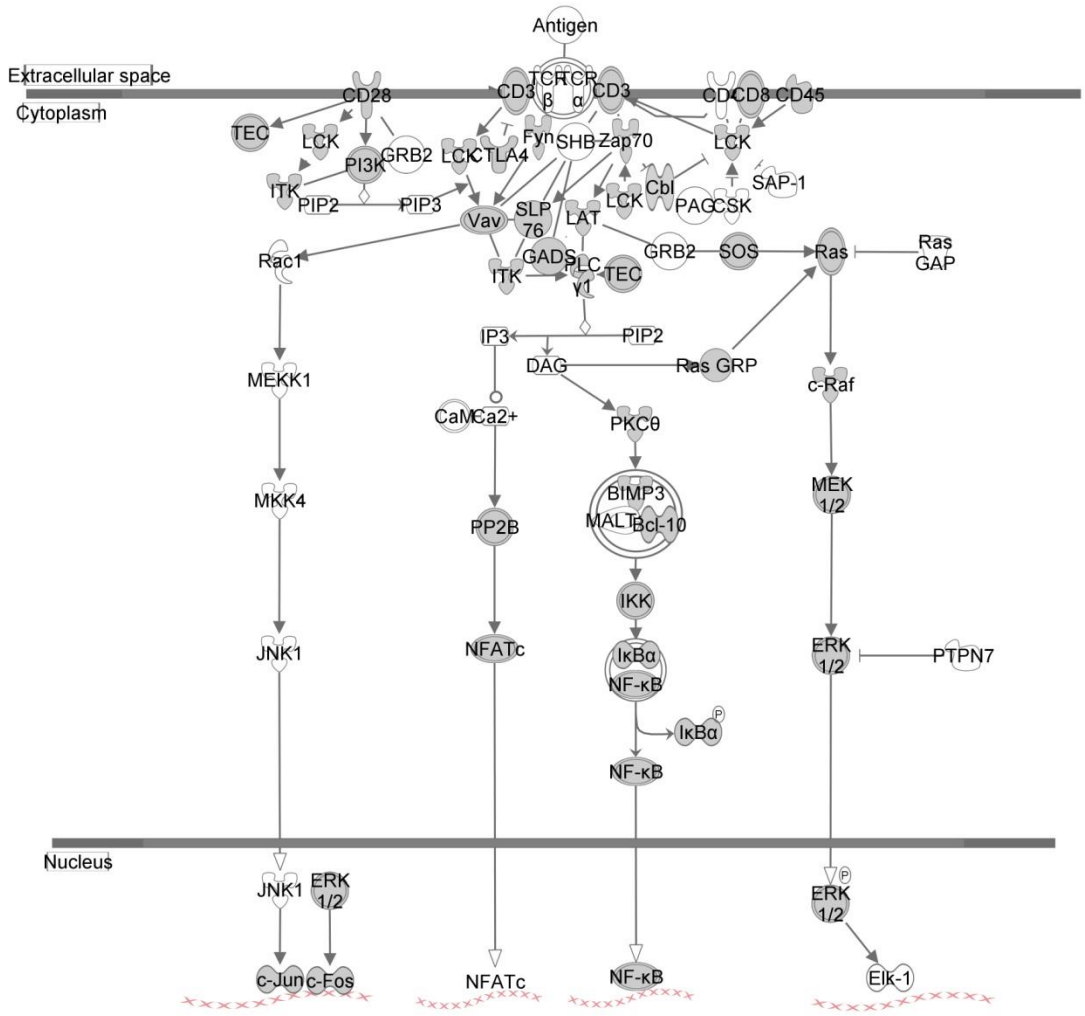

Figure 6: Coverage of TCR Signaling Network by Genes in Proximity to TBET bound regions.
Genes in proximity to TBET occupied segments are shaded in grey. Genes with double rings represent groups with multiple members. Pathway was generated with Ingenuity IPA.

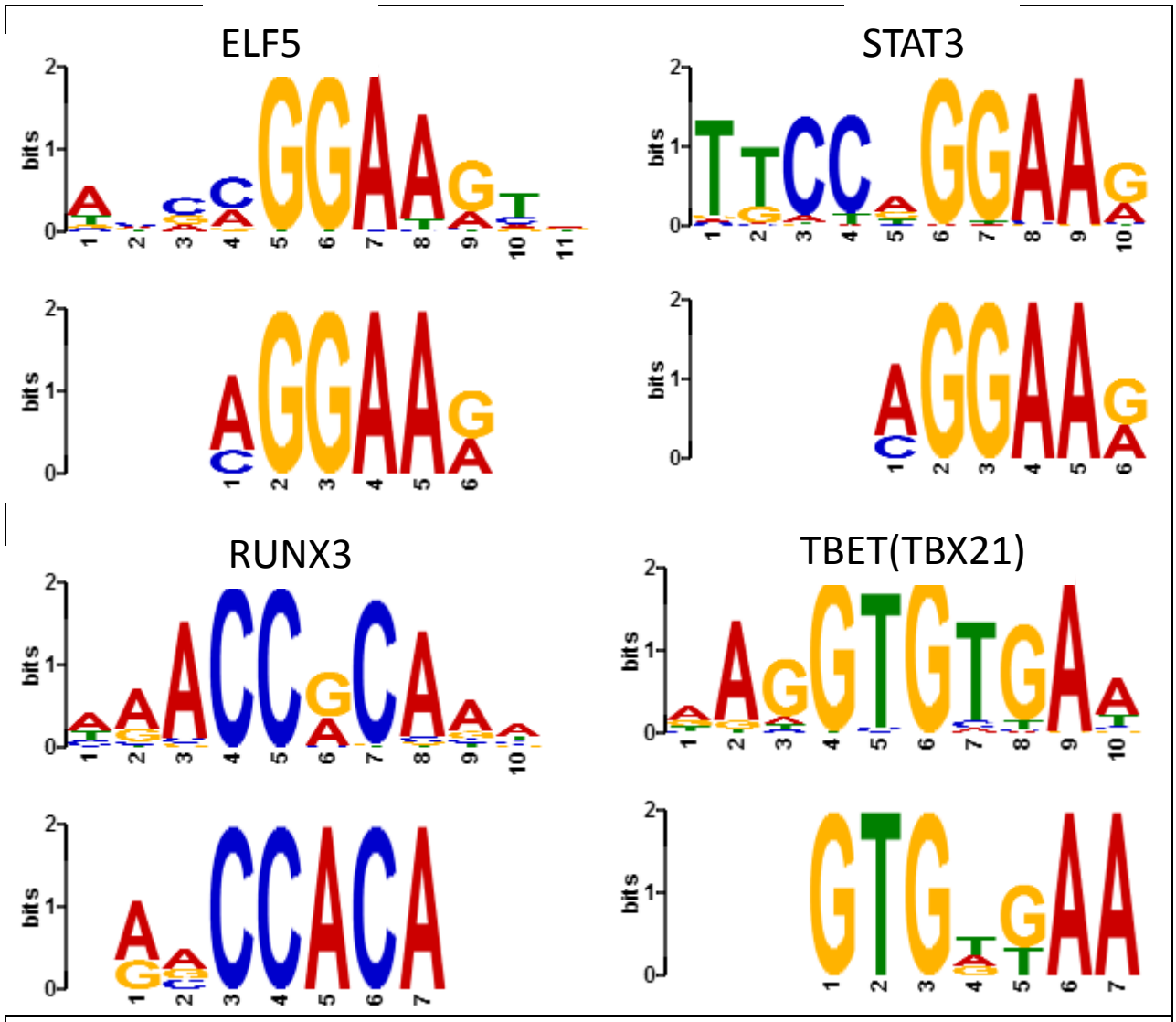

Figure 7: Motifs Enriched in Sites Occupied by TBET.
Correlates made by TOMTOM of enriched motifs and known binding sequences of transcription factors determined by SELEX (Jolma, http://www.sciencedirect.com/science/article/pii/S0092867412014961). Total column height represents the amount of conservation of that base position. Fractional bases are proportional to the amount of representation of each base in the bound sequence where more than one base is tolerated. The top logo represents that sequence which is known for the binding site while that underneath is the enriched motif from the TBET bound region.

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, 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. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We plan to complete a manuscript detailing the unique binding sites of T-bet in Temra, highlighting its differential function in these compared to other immune cells. Our success in reducing the number of cells needed for quality ChIP in LGLL cells makes the normal cell equivalent accessible and therefore necessary to complete and compare in order for publication. We are committed to completing that project under other funding with the next 6 months.

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.

Although our research may lead to enhanced treatment modalities in the future, the results of this proposal did not have any direct impact on the disease at this time.

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.

Previous reports have shown T-bet and Eomes (a similar Tbox protein) to be critical to the effector function of cytotoxic lymphocytes. Our data identifies T-bet binding sites upstream of these effector molecules, many of which were not previously recognized as such. The action of these effector molecules on blood components such as red blood cells and neutrophils are the primary cause of symptoms in LGL Leukemia, anemia and neutropenia respectively. Identifying these molecules is the first step in elucidating the mechanisms of LGL pathology so that effective treatments can be designed.

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.

NAME	POSITION TITLE
Loughran, Jr., Thomas Patrick	Director, University of Virginia Cancer Center

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Ursinus College, Collegeville, PA	BA	06/75	Biology
Hahnemann Medical School, Philadelphia, PA	MD	06/79	Internal Medicine

A. Personal Statement

I am currently the Director of the Cancer Center at University of Virginia, after having served as the founding Director of Penn State Hershey Cancer Institute (PSHCI) in 2003. I had excellent training at the Fred Hutchinson Cancer Research Center and served on its faculty for many years. I was the Leader of the Hematologic Malignancies Program at the H. Lee Moffitt Cancer Center and Research Institute. My career path has been a testament to translational research. Indeed, the circular route of bedside to bench to bedside, beginning with the clinical discovery of LGL leukemia, has been the foundation of my career.

B. Positions and Honors

Positions and Employment

1979-1982 Internal Medicine Internship and Residency; Thomas Jefferson University Hospital; Philadelphia, PA

1982-1985 Senior Oncology Fellow; Fred Hutchinson Cancer Research Center; University of Washington; Seattle, WA

1983-1985 Transplantation Biology Division; Fred Hutchinson Cancer Research Center; Laboratory of Dr. Rainer Storb

1985-1986 Associate in Clinical Research; Fred Hutchinson Cancer Research Center; Seattle, WA

10/86-1/87 Visiting Scientist, National Cancer Institute-Frederick Cancer Research Facility, Laboratory of Dr. Francis Ruscetti; Frederick, MD

1986-1990 Assistant Member, Clinical Research; Fred Hutchinson Cancer Research Center; Seattle, WA

1990-1992 Associate Member, Clinical Research; Fred Hutchinson Cancer Research Center, Seattle, WA

1992-1996 Professor of Medicine and Microbiology and Immunology
Associate Director, Bone Marrow Transplant Program SUNY Health Science Center and Chief of Hematology, Veterans Administration Medical Center; Syracuse, NY

1996-2003 Program Leader, Hematologic Malignancies, H. Lee Moffitt Cancer Center and Research Institute, and Professor of Medicine, University of South Florida; Tampa, FL

2003-2013 Director, Penn State Hershey Cancer Institute, and Professor of Medicine, Penn State Milton S. Hershey Medical Center, Penn State College of Medicine; Hershey, PA

2013-present Director, University of Virginia Cancer Center; F. Palmer Weber-Smithfield Foods Professor of Oncology Research; Professor of Medicine; and Co-Director, Mellon Institute, University of Virginia

Honors

1975	B.S. Cum Laude, Departmental Honors (Biology), Ursinus College Sigma Xi National Research Honor Society; Cub and Key Honor Society, Ursinus College
1976	Honors for Basic Sciences curriculum (top 10% of class), Hahnemann Medical School
1979-1980	Intern of the Year, Thomas Jefferson University Hospital
1983-1984	American Cancer Society Clinical Fellow
1985-1987	Leukemia Society of America Fellow
1987-1990	Special Fellow of the Leukemia Society of America
2010	Ursinus College Alumni Award
2011	First Annual Distinguished Alumni Lecture Thomas Jefferson University

C. Selected Peer-reviewed Publications (Selected from 245 peer-reviewed publications)

1. **Loughran TP**, Jr, Kadin ME, Starkebaum G, Abkowitz JL, Clark EA, Disteche C, Lum LG, Slichter SJ: Leukemia of large granular lymphocytes: Association with clonal chromosomal abnormalities and auto-immune neutropenia, thrombocytopenia and hemolytic anemia. Ann Intern Med, 102:169-175, 1985. PMID: 3966754
2. Epling-Burnette PK, Liu JH, Catlett-Falcone R, Oshiro MM, Kothapalli R, Li XX, Jove R, **Loughran TP, Jr**: Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes (LGL) and decreased Mcl-1 expression. J Clin Invest 107:351-362, 2001. PMID: 11160159
3. Shah MV, Zhang R, Irby R, Kothapalli R, Liu X, Arrington T, Frank B, Lee NH, **Loughran TP, Jr**: Molecular profiling of LGL leukemia reveals role of sphingolipid signaling in survival of cytotoxic lymphocytes. Blood 2008; 112(3):770-81. PMCID: PMC2481553
4. Zhang R, Shah MV, Yang J, Nyland SB, Liu X, Yun JK, Albert R, **Loughran TP, Jr**: Network model of survival signaling in large granular lymphocyte leukemia. Proc Natl Acad Sci U S A 2008; 105(42):16308-13. PMCID: PMC2571012
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; 115:51. PMID: 19880494
6. Lamy T, **Loughran TP Jr**: How I treat LGL leukemia. Blood 2011; 117:2764-74. PMID: 21190991.
7. Saadatpour A, Wang RS, Liao A, Liu X, **Loughran TP**, Albert I, Albert R: Dynamical and structural analysis of a T cell survival network identifies novel candidate therapeutic targets for large granular lymphocyte leukemia. PLOS, Computational Biology, 7: e1002267, 2011. PMID: 22102804
8. Koskela H, Eldfors S, Ellonen P, van Adrichem A, Kuusanmäki H, Andersson E, Lagström S, Clemente M, Olson T, Jalkanen S, Majumder MM, Almusa H, Edgren H, Lepistö M, Mattila P, Guinta K, Koistinen P, Kuittinen T, Penttinen K, Parsons A, Knowles J, Saarela J, Wennerberg K, Kallioniemi O, Porkka K, **Loughran T**, Heckman C, Maciejewski J, Mustjoki S: Somatic STAT3 Mutations in Large Granular Lymphocyte Leukemia. N Engl J Med 366:1905-1913, 2012. PMID: 22591296

NAME	POSITION TITLE
Ross C. Hardison, Ph.D.	T. Ming Chu Professor of Biochemistry and Molecular Biology, The Pennsylvania State University

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Vanderbilt University, Nashville, TN	B.A.	05/1973	Chemistry
University of Iowa, Iowa City, IA	Ph.D.	08/1977	Biochemistry
California Inst. of Technology, Pasadena, CA	Post-Doc	01/1980	Molecular Biology

A. Personal Statement

The long-term goal of my research is to understand molecular mechanisms and evolution of gene regulation in mammals. I have worked on many projects in pursuit of these goals for four decades, beginning with my undergraduate research. The projects include fundamental studies of chromatin structure, developing methods for isolating molecular clones of genomic DNA from mammals (the beginnings of genomics), determining structure and expression profiles of globin genes during development, using interspecies sequence comparisons to illuminate genome evolution and predict regulatory regions, and using second generation sequencing methods for genome-wide mapping of transcripts, histone modifications and transcription factor binding in search of global mechanisms of gene regulation. This diversity of approaches has been enabled by excellent workers in my lab and by several long-term collaborations. For over 20 years, I have worked with Dr. Webb Miller on generating alignments of large genome sequences, predicting gene regulatory function from patterns in those alignments, and testing those predictions in the laboratory. For the past 10 years, I have collaborated with Drs. Mitch Weiss, Gerd Blobel, and James Taylor to apply high throughput genomics methods, including massively parallel short read sequencing on the Illumina platform, to determine transcriptomes and to map sites of occupancy by key transcription factors and the landscape of histone modifications during differentiation of multiple hematopoietic lineages in mouse. Drs. David Bodine, Yu Zhang, and Feng Yue joined this collaboration over the past few years. We have been members of the ENCODE Project Consortium since its inception. My own laboratory has produced over 100 datasets released through our work in the mouse ENCODE consortium (where I was a PI) and as an on-going collaboration with Dr. Richard Myers at HudsonAlpha and Dr. Barbara Wold (Caltech). I have been active in integrative analysis throughout all phases of the ENCODE project. I have enjoyed a strong collaboration with Drs. Tom Loughran and Tom Olson on the LGL leukemia project. In summary, I bring extensive, diverse experience in biochemical, genetic, and evolutionary approaches to studies of genome function and gene regulation, all of them enabled by sophisticated bioinformatics. Our current research aims not only to illuminate critical issues in gene regulation during hematopoiesis, but to also serve as a paradigm for the integrative analysis of the flood of epigenomic data *in a way that is meaningful to working researchers*.

B. Positions and Honors

Positions

1980-1986 Assistant Professor of Biochemistry, The Pennsylvania State University
1986-1991 Associate Professor of Biochemistry, The Pennsylvania State University
1988 Visiting Professor, Biomedical Research Center, Univ. of British Columbia, Canada
1991-present Professor of Biochemistry, The Pennsylvania State University
1992-1997 Director, Center for Gene Regulation, The Pennsylvania State University

1999-2000 Visiting Scientist, Fred Hutchinson Cancer Research Center, Seattle, WA
2008-2010 Director, Huck Institute for Genomics and Bioinformatics, Penn State University
2003-present Director, Center for Comparative Genomics and Bioinformatics, Penn State University

Other Experience and Professional Memberships

1980-present Member of American Society for Biochemistry and Molecular Biology, American Chemical Society, American Society for Microbiology, American Society for Human Genetics, Society for Molecular Biology and Evolution
1984-present *Ad hoc* member of many NIH study sections
1990-1993 Editorial Board of **Molecular Biology and Evolution**
1991-1998 Editorial Board, **Molecular Phylogenetics and Evolution**
1992-2005 Associate Editor of **Genomics**
2004-2010, 2014-present Editorial Board of **Genome Research**
2011-2013 member, **National Advisory Council**, National Human Genome Research Institute, NIH
2008-present Founding Editorial Board and Associate Editor of **Genome Biology and Evolution**
2014-present Editorial Board of **Journal of Biological Chemistry**
2014-present Scientific Advisory Board (chair), GENCODE project, Sanger Centre and EBI
2014-present Scientific Advisory Board, University of California at Santa Cruz Genome Browser

Honors

1978 C. P. Berg Award, Department of Biochemistry, University of Iowa
1977-1979 Jane Coffin Childs Memorial Fund for Medical Research, California Institute of Technology
1987-1992 NIH Research Career Development Award, Pennsylvania State University
2000 Faculty Scholar Award (with Dr. Webb Miller) Penn State University
2005-present T. Ming Chu Professor of Biochemistry and Molecular Biology, Penn State University

C. Selected peer-reviewed publications (total of 210 publications; over 31,800 citations according to Google Scholar; h-index = 61; i10-index = 154)

1. The Mouse ENCODE Consortium, including Weisheng Wu (co-first author with 10 others), Cheryl A. Keller, Christapher S. Morrissey, Tejaswini Mishra, Deepti Jain, Nergiz Dogan, Robert S. Harris, Gerd A. Blobel, Mitchell J. Weiss, James Taylor and Ross C. Hardison (co-corresponding author with 7 others) last author Bing Ren (2014) An Integrated and Comparative Encyclopedia of DNA Elements in the Mouse Genome, **Nature** *515*:355-364. PMID: 25409824 PMCID in progress.
2. Yong Cheng (co-first author), Zhihai Ma (co-first author), Bong-Hyun Kim, Weisheng Wu, Philip Cayting, Alan P. Boyle, Vasavi Sundaram, Xiaoyun Xing, Nergiz Dogan, Jingjing Li, Ghia Euskirchen, Shin Lin, Yiing Lin, Axel Visel, Trupti Kawli, Xinqiong Yang, Dorrelyn Patacsil, Cheryl A. Keller, Belinda Giardine, The Mouse ENCODE Consortium, Anshul Kundaje, Ting Wang, Len A. Pennacchio, Zhiping Weng, Ross C. Hardison (co-corresponding author), Michael P. Snyder (co-corresponding author) (2014) Principles of Regulatory Information Conservation Revealed by Comparing Mouse and Human Transcription Factor Binding Profiles. **Nature** *515*:371-375. PMID: 25409826 PMCID in progress.

NAME Olson, Thomas Lynn	POSITION TITLE Postdoctoral Fellow		
INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Nebraska-Lincoln	B.S.	12/2000	Biology
Quest Diagnostics	M(ASCP)	11/2003	Microbiology
The Pennsylvania State University	Ph.D.	08/2013	Molecular Medicine
University of Virginia	Postdoctoral	Current	Cancer Biology

A. Personal Statement

I have been working on LGL Leukemia for 8 years under the guidance of Dr. Thomas Loughran, the discoverer of the disease, beginning at Penn State and now continuing at the University of Virginia. I have been involved in the publication of numerous highly collaborative works that are the first descriptions of mutational causes of LGL leukemia. I was also the principle person designing and carrying out clinical correlative studies (serum cytokine, microarray, and mutational status) in the first prospective clinical trial of immunosuppressives in LGL leukemia. In this paper I was able to make correlations between mutational status and treatment response. The collaborative nature of these projects combined with the ambitiousness of those that I am currently focusing on has delayed the release of personal projects. I am therefore highly motivated to generate first author publications from this proposal as well as other current projects. I was instrumental in setting up the collaborations required for the generation of the data in this application, which is remarkable in that our lab had no prior experience with next generation sequencing. I have broad knowledge of next generation sequencing and bioinformatics along with intimate understanding of LGL leukemia biology that is required to properly plan and execute these types of experiments.

B. Positions and Honors

Positions and Employment

1995-1999 Workstudy, University of Nebraska-Lincoln, Lincoln, NE

2001-2005 Technologist of Microbiology, Quest Diagnostics, Lincoln, NE

2013- Postdoctoral Fellow, Cancer Training Grant, University of Virginia, Charlottesville, VA

Honors

2008 Judy S. Finkelstein Memorial Student Research Award, Penn State University, Hershey, PA

C. Select Peer-reviewed publications.

1. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmäki H, Andersson EI, Lagström S, Clemente MJ, Olson T, Jalkanen SE, Majumder MM, Almusa H, Edgren H, Lepistö M, Mattila P, Guinta K, Koistinen P, Kuittinen T, Penttinen K, Parsons A, Knowles J, Saarela J, Wennerberg K, Kallioniemi O, Porkka K, Loughran TP Jr, Heckman CA, Maciejewski JP, Mustjoki S. (2012) Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med. 366(20):1905-13.

2. Loughran TP Jr, Zickl L, Olson TL, Wang V, Zhang D, Rajala HLM, Hasanali Z, Bennett JM, Lazarus HM, Litzow MR, Evens AM, Mustjoki, S, Tallman MS. (2014) STAT3 Y640F Mutation Identifies Favorable Response Group to Immunosuppressive Therapy of LGL

Leukemia: Results of a Prospective Multicenter Phase II Study of Initial Treatment with Methotrexate by the Eastern Cooperative Oncology Group (E5998). Accepted at Leukemia

3. Ratan A, Olson TL, Loughran TP Jr, Miller W. Identification of indels in next-generation sequencing data. In revision at BMC Bioinformatics.

4. Olson TL, Giardine BL, Keller-Capone C, Hardison R, Loughran TP Jr. (2014) Defining the Role of the Transcription Factor T-bet in Terminal Effector Memory Cells Via ChIP-Seq of Large Granular Lymphocytic Leukemia. In preparation

5. Rajala HLM, Olson T, Clemente M, Lagström S, Ellonen P, Lundan T, Zaman S, Lopez Marti JS, Andersson EI, Jerez A, Porkka K, Maciejewski JP, Loughran TP Jr, Mustjoki S. (2014) The analysis of clonal hierarchy and therapy responses using STAT3 mutations as a molecular marker in large granular lymphocytic leukemia. Haematologica. 2014 Oct 3. pii: haematol.2014.113142 [Epub ahead of print]

6. Jerez A, Clemente MJ, Makishima H, Koskela H, Leblanc F, Peng Ng K, Olson T, Przychodzen B, Afable M, Gomez-Segui I, Guinta K, Durkin L, Hsi ED, McGraw K, Zhang D, Wlodarski MW, Porkka K, Sekeres MA, List A, Mustjoki S, Loughran TP, Maciejewski JP. (2012) STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood 120(15):3048-57.

7. Rajala HL, Eldfors S, Kuusanmäki H, van Adrichem AJ, Olson T, Lagström S, Andersson EI, Jerez A, Clemente MJ, Yan Y, Zhang D, Awwad A, Ellonen P, Kallioniemi O, Wennerberg K, Porkka K, Maciejewski JP, Loughran TP Jr, Heckman C, Mustjoki S. (2013 Apr 17) Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. Blood. 2013 May 30;121(22):4541-50.

8. Jerez A, Clemente MJ, Makishima H, Rajala HLM, Gómez-Seguí I, Olson T, McGraw K, Przychodzen B, Kulasekararaj AG, Afable MG, Husseinzadeh HD, Hosono N, LeBlanc F, Lagström S, Zhang D, Ellonen P, Lichtin AE, Wodnar-Filipowicz A, Mufti GJ, List AF, Mustjoki S, Loughran TP, Maciejewski JP (2013) STAT3-Mutations Indicate the Presence of Subclinical Self-Reactive Cytotoxic T Cell Clones in Aplastic Anemia and Myelodysplastic Syndromes. Blood. 2013 Oct 3;122(14):2453-9.

9. Andersson EI, Rajala HLM, Eldfors S, Ellonen P, Olson T, Jerez A, Clemente MJ, Kallioniemi O, Porkka K, Heckman C, Loughran TP, Maciejewski JP, Mustjoki S (2013) Novel Somatic Mutations in Large Granular Lymphocyte Leukemia affecting the STAT-pathway and T-cell activation. Blood Cancer J. 2013 Dec 6;3:e168

10. Bedoya-Reina OC, Ratan A, Burhans R, Giardine B, Riemer C, Olson TL, Loughran TP, Perry GH, Schuster SC, Miller W (2013) Galaxy Tools to Study Genome Diversity. Gigascience. 2013 Dec 30;2(1):17.

11. Yan Y, Olson TL, Nyland SB, Feith DJ, Loughran TP Jr (2014) Emergence of a STAT3 Mutated NK Clone in LGL Leukemia. Accepted at Leukemia Research Reports.