Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Institute for Cancer Research

2. **Reporting Period (start and end date of grant award period):** 1/1/2012 – 6/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Minko Gill

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100057660

6. **Project Number and Title of Research Project:** 02-Pre-BCR Function Selecting Novel B Cell Receptors in Chronic Lymphocytic Leukemia

7. **Start and End Date of Research Project:** 1/1/2012 – 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Richard R. Hardy, Ph.D.

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:

   $ 554,109

   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
Hardy, Richard	PI	10% years 1-2	$26,598.09
Shinton, Susan	Scientific Associate	70% year 1	$65,019.19
Bandi, Srinivasa	Postdoctoral Fellow	50% year 1	$23,165.61

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
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__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:
R01 - Mechanism of pre-BCR selection in development and transformation.	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	February 2014	$2,231,250	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:

I intend to re-apply for NIH funding of the application submitted in February 2014.

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

We will continue the research based on R01 support.

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				
Unknown				
Total				**1**

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

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

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

The project supported a postdoctotal fellow who carried out the work described in the project.

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.

B cell chronic lymphocytic leukemia (CLL) is the most common leukemia of adults in Western countries, characterized by usage of distinctive B cell receptor (BCR) heavy and light chain genes. In this project we analyzed BCR heavy chains for capacity to associate with surrogate light chain into a pre-BCR. Our hypothesis is that the striking biases seen in CLL heavy chains arise because their precursors come from a distinctive B cell development process that selects for weak association with surrogate light chain. We utilized cloning and staining procedures we developed in order to measure pre-BCR assembly. The work was divided into two specific Aims: *Aim 1 (Mouse CLL): Determine whether late-developing CD5$^+$ B cell leukemias in mice are enriched for Ig heavy chains that mediate weak pre-BCR signaling; Aim 2 (Human CLL): Determine if human chronic lymphocytic leukemias are enriched for Ig heavy chain VDJs that assemble weakly with surrogate light chain.* The work has been completed, but not yet submitted for publication. Therefore I will describe the project in detail in this report.

For Aim 1, we utilized a mouse model of CLL developed by Carlo Croce, that we characterized, a B cell targeted human TCL1 transgenic mouse line. These animals develop a lympho-proliferation of CD5$^+$ B cells by 5-8 months of age and eventually, by 8-16 months a clonal leukemia develops. We have been collecting leukemic cells for several years and so had a large number of samples (>40) for sequence analysis and cloning/expression. The approach we used was to design an IgH-mu expression vector with unique restriction endonuclease sites flanking the BCR heavy chain VDJ (the antigen recognition segment), which allowed insertion of different VDJs isolated from the TCL1 leukemia samples into the construct. We had already isolated the VDJs by PCR with a consensus VH primer and an IgH-mu antisense primer, so we next used similar primers but including appropriate restriction sites to facilitate subcloning into the expression construct (Figure 1). This cloning was done for all samples with distinct VDJ segments (some segments were repeated, due to independent identical VDJ rearrangement).

In order to analyze association of the BCR VDJ with surrogate light chain, we used retroviral transduction of a mouse Pro-B cell line. Pro-B cells are at a stage before expression of the BCR heavy chain, but already express the surrogate light chain (SLC). Thus, upon expression of the heavy chain it will assemble with the pre-existing SLC to form the pre-BCR complex. We can use immunofluorescence staining to detect both the IgH-mu heavy chain and the assembled pre-BCR, using two different monoclonal antibodies. The retroviral vector containing the specific BCR heavy chain couples expression to green fluorescent protein (GFP) through an IRES, so we can compare GFP levels with IgH-mu and pre-BCR levels using flow cytometry. An example of a heavy chain that assembles well into a pre-BCR and one that does not is shown in Figure 2. We can determine the mean fluorescent intensity of the GFP$^+$ cells for both IgH-mu (heavy chain) and pre-BCR, then calculate the ratio of pre-BCR to IgH-mu. This ratio is high for a BCR heavy chain that associates well with SLC (forming a large amount of pre-BCR), but low for certain IgH-mu that do not associate well with SLC (forming only low amounts of pre-BCR).

Assembly of IgH-mu with SLC to make the pre-BCR complex is considered a key checkpoint in B cell development (Figure 3), but we have found that this is only the case for bone marrow localized development that takes place in adults (both in mice and in humans). During

embryonic life, B cell development instead occurs in the liver and we have found that much lower levels of pre-BCR are appropriate to foster B cell development in liver compared with the amounts required for bone marrow. Thus different VDJ rearrangements are "preferred" (i.e., selected for by pre-BCR formation) in these two sites during embryonic and adult B cell development, resulting in enrichment of different sets of VDJs in the BCR heavy chains found in B cells produced at these two different developmental times (embryonic and adult). Importantly, many embryonic generated B cells show self-reactivity that is eliminated during the process of tolerance in adult development, producing a distinctive B cell subset in mice (and probably humans) that expresses CD5. CD5 is found on all T cells but is absent from most B cells, except for a small subset that show this enrichment for self-reactivity. The recognition of self-determinants is thought to facilitate the clearance of dying cells and degraded cellular materials by antibody produced by these $CD5^+$ B cells (also known as B1 B cells).

However there is a long-standing connection between $CD5^+$ B1 B cells and leukemias or lymphomas. CD5 on B cells was first discovered on CLL B cells in humans and subsequently found on mouse lymphomas and leukemias. Importantly, in mice, we discovered normal (non-leukemic) B cells expressing CD5 and then others found the same BCR V_H and VL genes used in the normal and leukemic B cells, directly connecting the normal B1 pool with certain leukemic B cells. The consensus explanation is that self-reactivity results in constant re-stimulation of these cells and leads to some controlled proliferation throughout life as a normal feature of this type of B cells. However, occasionally clones develop where genetic or epigenetic changes disregulated normal growth controls and eventually allow the clone to expand, with further changes to become a leukemia or lymphoma. The TCL1 mouse, containing an expanded B1 population is an ideal model to study this process (Figure 4).

Thus BCRs serve to connect the autoreactive B1 B cell pool with B cell leukemias and autoreactive BCRs also to be a feature of human CLL. Over the past 30 years extensive analysis of BCRs in human CLL has shown that the disease can be subdivided into two subsets, one with extensive hypermutated BCRs (and a better prognosis) and the other with unmutated BCRs (and a worse prognosis). Further analysis has revealed repeated usage of identical heavy or light chain BCR segments in CLL, very surprising considering the great diversity of BCRs, and suggesting some kind of selection. Some CLL BCRs have also been shown to exhibit autoreactivity to common cellular determinants, making a connection between autoreactive B1 B cells and CLL in both mouse and humans. We hypothesize that B1 B cells in both mice and humans are generated by distinctive pre-BCR selection that enriched for certain BCR heavy chains that associate weakly with surrogate light chain (SLC), a marker for their novel development.

Thus, in this project, we generated a group of VDJ-mu retroviruses from TCL1-driven mouse leukemias and tested them for capacity to interact with SLC and form a pre-BCR, transducing a Pro-B cell line and then applying the staining assay described above. We have already analyzed normal $CD5^+$/B1 and $CD5^-$ B cells, finding that BCRs containing heavy chains weakly associating with SLC are relatively infrequent (<10%), but there is an enrichment in B1 B cells (>30%). We observed a similar enrichment in our analysis of TCL1 leukemia BCRs. We analyzed 73 samples, 33 of which are repeats. Of the 40 non-repeats, we see a clear enrichment of BCRs with heavy chain VDJs that associate weakly with SLC (Figure 5). All of these heavy chains were also tested in a growth assay in collaboration with Dr. Kyoko Hayakawa's

laboratory, verifying that low expression of the pre-BCR does not stimulate bone marrow Pro-B cell proliferation, but does allow ongoing fetal liver Pro-B cell proliferation to continue.
For Aim 2, we asked the same question, but instead analyzed BCR heavy chain VDJs from unmutated human CLL samples. Some of these were obtained locally from our pathology laboratory at Fox Chase Cancer Center and some were obtained from the CLL Consortium with help from our collaborator, Dr. Thomas Kipps at the Moores Cancer Center, UCSD. We designed a human IgH-mu expression construct, as with the mouse construct containing unique restriction sites flanking the VDJ. Instead of cloning by PCR with a consensus V primer, we used 5'-SMART-RACE with an antisense C-mu primer. We previously had made libraries of BCR heavy chain VDJs from naïve (unmutated) $CD5^{-}$ human B cells to serve as a control for the CLL samples. We analyzed over 40 of each set of VDJs, first cloning into the expression vector, next subcloning into a retrovirus, then transducing a human Pro-B cell line, RS(4:11), with the retroviral supernatant. We have two monoclonal antibodies, one that recognizes human IgH-mu and another that binds the assembled pre-BCR complex. Thus we calculate a value for pre-BCR expression exactly as with mouse, described above.

We found that naïve unmutated human B cells showed a low number of BCR heavy chain VDJs that associated weakly with surrogate light chain (about 10%), whereas this value was much higher in human CLL VDJs (>40%), as shown in Figure 6. This is a striking result and we plan on publishing it by the end of the year.

We are extending this work by asking whether there are any differences between the BCRs that contain heavy chains showing strong SLC association and the others that show weak association. We are taking two approaches to this work. First we selected 5 strong and 5 weak BCRs from unmutated CLLs. We cloned the light chains from all of these by 5'-SMART-RACE. We then generated single chain FVs (scFVs) by synthesizing the V_H-V_L segment (including a flexible linker), inserting the resulting DNA segment into a prokaryotic expression vector. Transfected bacteria secrete the scFV which is then purified on a nickel column (the vector contains a his tail). Purity is assessed by SDS PAGE analysis. Another approach we are using is cloning both the VH and VL into separate heavy and light chain eukaryotic expression vectors, then transfecting HEK293T cells. Cell supernatant contains the Ig which can then be purified. Both types of Ig will be tested for autoreactivity by binding assays, including anti-nuclear staining immunofluorescence and ELISA binding for ssDNA and dsDNA. We are also planning to carry out a bead-binding assay for a number of autoantigens with the help of Gregg Silverman at NYU Langone Medical Center, New York, NY.

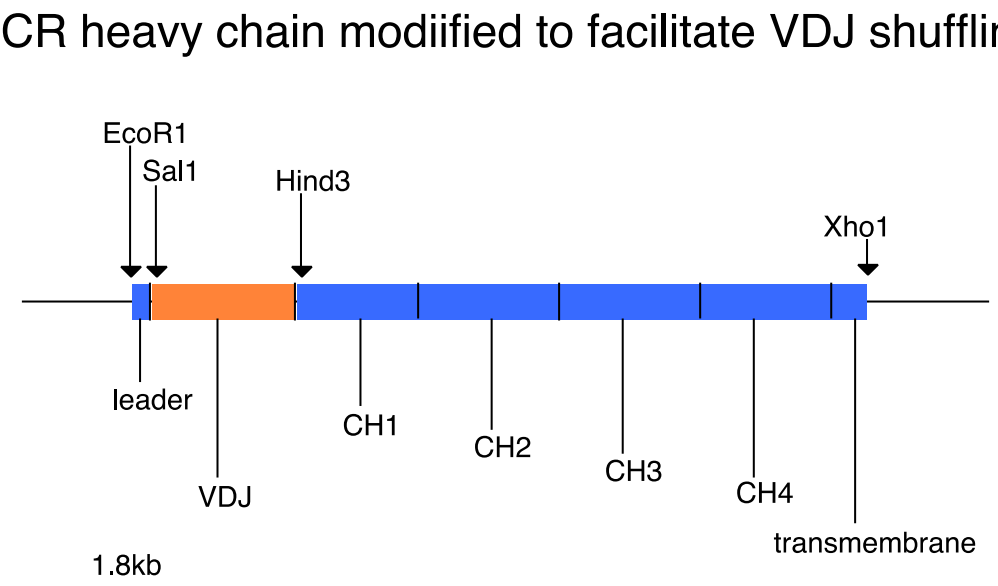

Figure 1. Mouse BCR IgH-mu heavy chain expression vector.

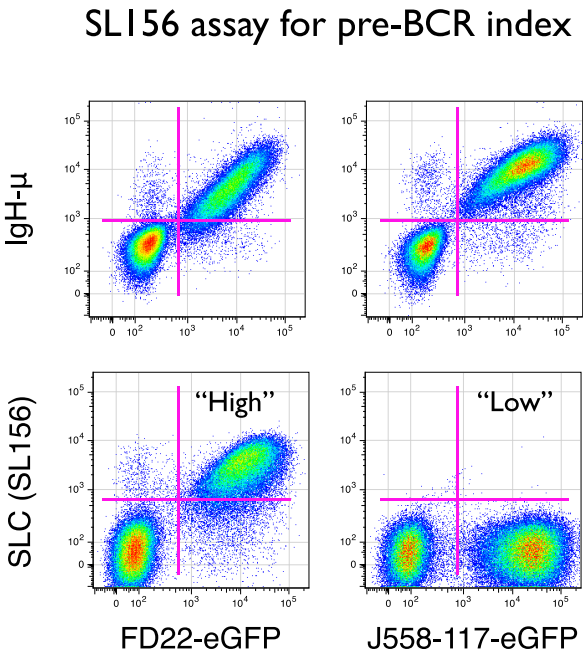

Figure 2. Pre-BCR assay using the SL156 monoclonal antibody.

Surrogate Light Chain, the pre-BCR, and IgH-μ selection

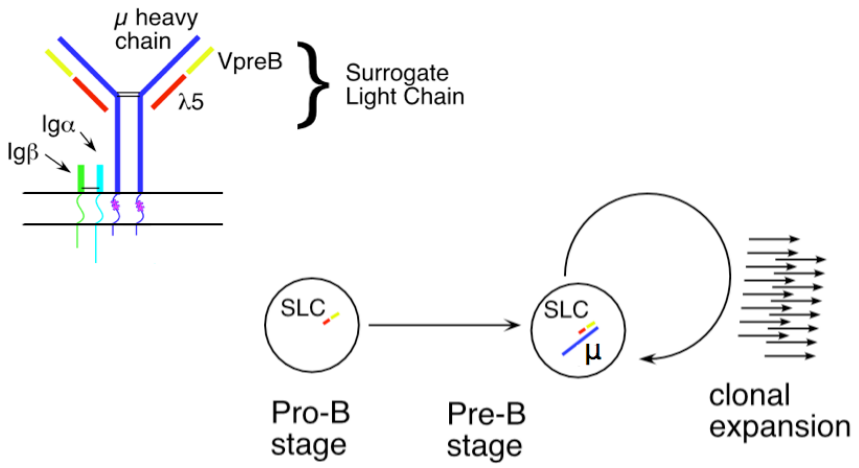

Figure 3. Pre-BCR structure and pre-BCR checkpoint in bone marrow B cell development.

Mouse model connecting B1 B cells with disregulated growth

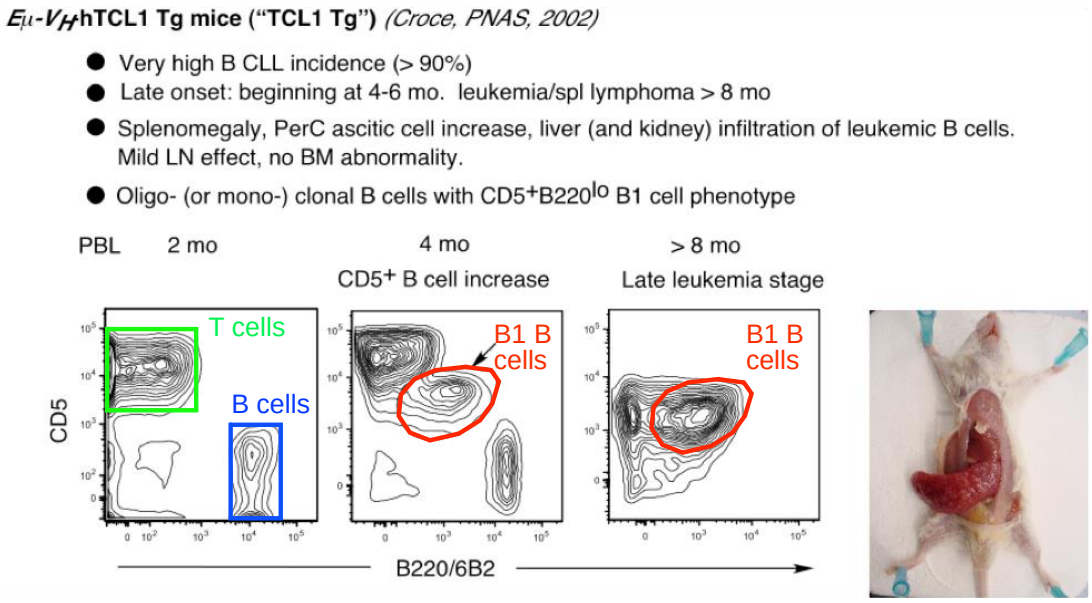

Figure 4. The TCL1 mouse model of human B-CLL.

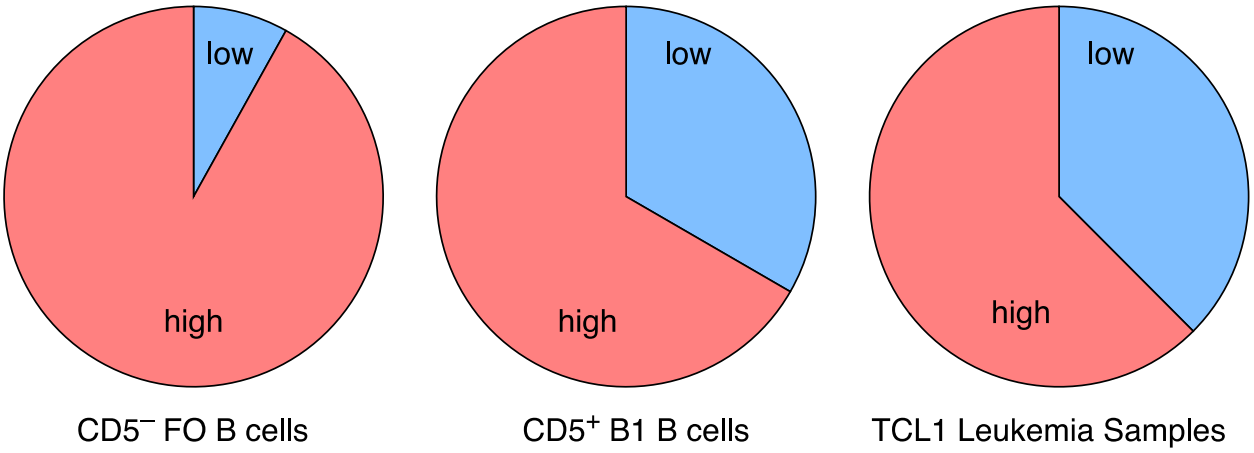

Figure 5. Frequency of mouse samples with heavy chain BCRs that have low or high surrogate light chain association. Comparison of Follicular (FO), B1, and TCL1 leukemia samples.

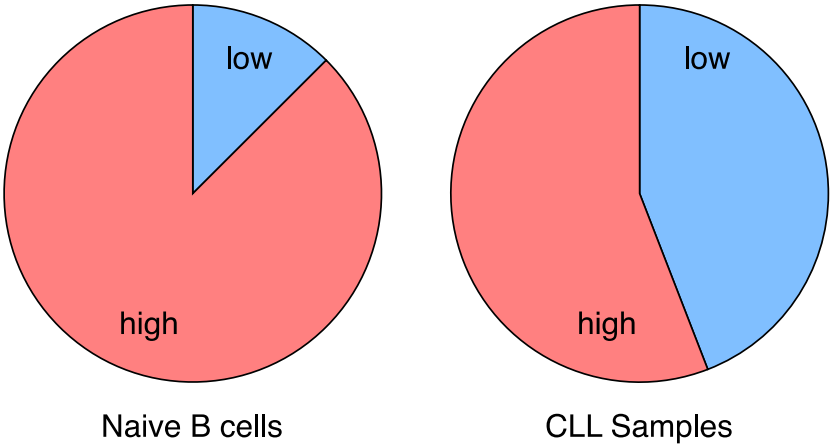

Figure 6. Frequency of human samples with heavy chain BCRs that have low or high surrogate light chain association.

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:

I plan to report this work in a peer reviewed journal.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of

the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

If yes, indicate date patent was filed:

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

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

If yes, how many licenses were granted?_____

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

If yes, describe the commercial development activities:

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

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key

investigators. In place of narrative you may insert the NIH biosketch form here.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Richard R. Hardy, Ph.D.	Professor
ERA COMMONS USERNAME rrhardy	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Illinois Institute of Technology, Chicago, IL	B.S.	05/74	Chemistry
California Institute of Technology, Pasadena, CA	Ph.D.	03/80	Biochemistry

A. Personal Statement

My research interest is the regulation of mouse B cell development, both in fetal liver and in bone marrow of adult mice, utilizing careful delineation and characterization of intermediate stages in development, identified by differential expression of cell surface proteins as measured by high-dimensional flow cytometry. Specific projects examine growth regulation of B cell precursors mediated by microenvironmental interactions and signaling through the pre-B cell receptor. I also investigate the relationship between pre-BCR/BCR structure and entry into distinct peripheral B cell populations, with emphasis on the self-reactive CD5+ B-1 B cell subset, of particular interest because of its similarity to B cell leukemias in the mouse and chronic lymphocytic leukemia (CLL) in humans.

B. Positions and Honors

Undergraduate Research Assistant, Illinois Institute of Technology, Chicago, IL	1973-1974
Research/Teaching Assistant, California Institute of Technology, Pasadena, CA	1974-1980
Postdoctoral Research Affiliate, Department of Genetics, Stanford University, Palo Alto, CA	1981-1984
Visiting Scientist, Institute for Molecular and Cellular Biology, Osaka University, Japan	1984-1987
Associate Member, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA	1987-1993
Director, Cell Sorting Facility, Fox Chase Cancer Center, Philadelphia, PA	1987-date
Director, DNA Sequencing Facility, Fox Chase Cancer Center, Philadelphia, PA	1992-date
Member, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA	1993-1998
Senior Member, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA	1998-date

<u>Honors</u>

Chemistry Department Achievement Award, Illinois Institute of Technology	1974
Herbert Newby McCoy Award, Department of Chemistry, California Institute of Technology	1980
American Cancer Society, California Division, Junior Fellowship/ Senior Fellowship	1982-1984

C. Selected Peer-reviewed Publications

1. Hardy, R.R., Carmack, C.E., Shinton, S.A., Kemp, J.D,, Hayakawa, K. Resolution and characterization of Pro-B and pre-Pro-B cell stages in normal mouse bone marrow. J. Exp. Med. 173:1213-1225, 1991.
2. Spanopoulou, E., Roman, C.A.J., Corcoran, L., Schlissel, M., Silver, D.P., Storb, U., Nemazee, D., Nussenzweig, M., Shinton, S.A., Hardy, R.R., Baltimore, D. Expression of functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1 deficient mice. Genes Dev. 8:1030-1042, 1994.
3. Li, Y.-S., Wasserman, R., Hayakawa, K., Hardy, R.R. Identification of the earliest B lineage stage in mouse bone marrow. Immunity 5:527-535, 1996.
4. Wasserman, R., Li, Y.-S., Shinton, S.A., Carmack, C.E., Manser, T., Wiest, D.L., Hayakawa, K., Hardy, R.R. A novel mechanism for B cell repertoire maturation based on response by B cell precursors to pre-B receptor assembly. J. Exp. Med. 187:259-264, 1998.
5. Hayakawa, K., Asano, M., Shinton, S.A., Gui, M., Allman, D., Stewart, C.L., Silver, J., Hardy, R.R. Positive selection of natural autoreactive B cells. Science 285:113-116, 1999.
6. Chumley, M.J., DalPorto, J.M., Kawaguchi, S., Cambier, J.C., Nemazee, D., Hardy, R.R. A VH11VK9 BCR drives generation of CD5+ B cells both in vivo and in vitro. J. Immunol. 164:4586-4593, 2000.
7. Allman, D., Lindsley, R.C., DeMuth, W., Rudd, K., Shinton, S.A., Hardy, R.R. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. J. Immunol. 167:6834-6840, 2001.
8. Bichi, R., Shinton, S.A., Martin, E.S., Koval, A., Calin, G.A., Cesari, R., Russo, G., Hardy, R.R., Croce, C.M. Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression. Proc. Natl. Acad. Sci. U.S.A. 99:6955-6960, 2002.
9. Hayakawa, K., Asano, M., Shinton, S.A., Gui, M., Wen, L.-J., Dashoff, J., Hardy, R.R. Positive selection of ATA B-1 cells and natural serum autoantibody production independent from BM B cell development. J. Exp. Med. 197:87-99, 2003.
10. Maier, H., Ostraat, R., Gao, H., Fields, S., Shinton, S.A., Medina, K.L., Ikawa, T., Murre, C., Singh, H., Hardy, R.R., Hagman, J. Early B Cell factor cooperates with Runx1 and mediates epigenetic changes associated with mb-1 transcription. Nat. Immunol. 5:1069-1077, 2004.
11. Wen, L., Brill-Dashoff, J., Shinton, S.A., Asano, M., Hardy, R.R., Hayakawa, K. Evidence of marginal-zone B cell positive selection in spleen. Immunity 23:297-308, 2005.
12. Rumfelt, L., Zhou, Y., Rowley, B.M., Shinton, S., Hardy, R.R. Lineage specification and plasticity in CD19-negative early B-cell precursors. J. Exp. Med. 203:675-687, 2006.
13. Kitaura, Y., Jang, I.K., Wang, Y., Han, Y.C., Inazu, T., Cadera, E.J., Schlissel, M., Hardy, R.R., Gu, H. Control of the B cell-intrinsic tolerance programs by ubiquitin ligases Cbl and Cbl-b. Immunity 26:567-578, 2007.
14. Amin, R.H., Cado, D., Nolla, H., Huang, D., Shinton, S.A., Zhou, Y., Hardy, R.R., Schlissel, M.S. Biallelic, ubiquitous transcription from the distal germline IgK locus promoter during B cell development. Proc. Natl. Acad. Sci. USA 106:522-527, 2009.
15. Painter, M.W., Davis, S., Hardy, R.R., Mathis, D., Benoist, C. Transcriptomes of the B and T lineages compared by multiplatform microarray profiling. J. Immunol. 186:3047-3057. 2011.
16. Cooper C.L., Hardy R.R., Reth M., Desiderio, S. Non-cell-autonomous hedgehog signaling promotes murine B lymphopoiesis from hematopoietic progenitors. Blood 119:5438-5448, 2012.