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):** 1/1/2011 – 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:** 4100054848

6. **Project Number and Title of Research Project:** 06-Use of Somatic Cell Nuclear Transfer to Generate TCR Monoclonal Mice

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

8. **Name of Principal Investigator for the Research Project:** Dietmar Kappes, 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:

   $ 328,219

   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
Kappes, Dietmar J	PI	5% year 2	$9,802.24
Lee, Hyung-Ok	Research Associate	46% year 1; 39% year 2	$67,789.93

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
XYCLONE 20x Laptop	Used by Transgenic Facility to improve efficiency of ES cell injection into mouse blastocysts	$34,417
Olympus Inverted Microscope	Used by Transgenic Facility to improve efficiency/capacity for mouse pronuclear and blastocyst injection for generation of transgenic and knockout mice	$47,081
LSR II Special Order Laser Option	Used by Flow Cytometry Facility to enhance detection capacity of LSR II flow cytometer. Ability to detect additional fluorochromes allows more detailed analysis of tumors and other cell populations	$70,470

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 - Dissecting the role of ThPOK in thymic development and T cell differentiation	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	July 2013	$2,231,250	$1,356,600

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No___X_____

If yes, please describe your plans:

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

Further functional analysis of OB11 and related mouse lines generated with CURE funds will be pursued as part of a recently funded NIH R01 grant, entitled "Dissecting the role of ThPOK in thymic development and T cell differentiation". As outlined below, preliminary analysis of CD4 T cells from these mice delineates novel roles for ThPOK in regulating multiple cytokine and cytokine receptor genes, including IL-9 and IL2R. These mouse models will allow us to ask important questions about ThPOK gene regulation, as well as the function of ThPOK in CD4 cell development and effector differentiation, which could not so far be addressed.

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

Yes_____ No____X_____

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

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

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

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

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

Yes_____ No___X_____

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

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

Yes___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 ability to introduce manipulated genes and delete or modify endogenous genes into the

germline of animals is a major technological advance in biology, enabling investigators to answer questions about gene function, which must be analyzed in a whole animal model system. The new laser drill and microscope purchased with CURE funds have been essential in upgrading capacity of the Fox Chase Transgenic Facility to generate new transgenic and knockout mouse models for Fox Chase investigators of all programs. New mouse lines have been used to probe mechanisms of developmental gene regulation, cellular interactions within the immune system, and the effect of oncogenes on growth and differentiation. During the last fiscal year the Transgenic Mouse Facility created transgenic and knockout mouse models for all Fox Chase Cancer Center (FCCC) Research Programs. The Transgenic Mouse Facility plays a critical role in the continuing study of oncogenesis at FCCC by providing mammalian model systems for human cancer. The study of these models leads to the development of new strategies for cancer treatment. The Facility completed 72 transgenic requests and knockout requests in 2013. Among the novel strains recently generated are mice with novel mutations in Erk2, a conditional Bap1 knockin, ThPOK variant alleles, and several GFP reporter knockin lines.

Multi-parameter flow cytometry is a powerful technology that measures expression levels of multiple proteins simultaneously on the surface of the same cell. This is extremely useful to distinguish phenotypically distinct cell populations within a given organ, cell culture or tumor. CURE funds were used to add a green laser and upgrade the red laser on an existing BD LSR II flow cytometer. This increased the number of fluorescence detectors from 10 to 13 and improved signals on all the PE and PE-tandem channels (the green laser excites PE and PE-tandems much more efficiently than the existing blue laser). The green excitation also conferred capacity to detect several additional red-fluorescent proteins that could not be utilized before. This has resulted in significant improvement of the flow analysis infrastructure, and was, for instance, essential to assess developmental and functional outcomes for the novel OB11 mouse line described below.

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.

Project goals, objectives and specific aims

A key approach in experimental immunology is to generate mice that express single T or B cell receptors (TCR, or BCR) in order to examine their specificity, function, and effect on development. Somatic cell nuclear transfer (SCNT) is a promising technique for the rapid

generation of such TCR monoclonal mice, which involves replacing the oocyte's nucleus with a donor nucleus from a T cell. The nucleus of transferred T cells is reprogrammed, but nevertheless, retains the rearranged DNA encoding the TCR subunits and antigen specificity of the original T cell. The current study has two aims: 1) To improve the efficiency of SCNT specifically for mouse T lymphocytes, and 2) To utilize SCNT to efficiently generate a number of TCR transgenic mouse lines from ThPOK deficient and ThPOK overexpressing mice, which will facilitate characterization of their developmental and functional capacities and elucidate the mechanism of CD4/CD8 lineage commitment.

Summary of Research Completed

1. Towards improving the efficiency of SCNT for T lymphocytes. Establishing somatic cell nuclear transfer (SCNT) for T lymphocytes has proven highly challenging, due to the inherently difficult and inefficient nature of this procedure for lymphocytes, as noted in our original application. As previously reported, we have been unsuccessful at carrying out direct somatic cell nuclear transfer (SCNT) to insert T lymphocyte nuclei into mouse oocytes. Specifically, we carried out experiments to test whether injection of oocytes with mRNAs encoding reprogramming factors would lead to improved embryo survival after T lymphocyte nuclear transfer. In each case, transfers were carried out with >100 enucleated oocytes, but no viable embryos were obtained. Continuous review of the pertinent literature up to the present, shows that no other laboratory has reported effective methodologies for direct transfer of lymphocyte nuclei into oocytes to date, attesting to the inherent difficulties of this procedure.

2. Developmental and functional characterization of ThPOK-independent T cell subsets. We have previously shown that almost all cells belonging to the CD4 or CD8 subsets in ThPOK-deficient and ThPOK transgenic mice, respectively, are redirected to the opposite lineage. However, small subsets of the affected cells persist in both strains of mice. We previously reported that the rare remaining CD4 T cells in ThPOK-deficient mice and CD8 T cells in ThPOK transgenic mice exhibit distinct patterns of V region usage, consistent with either alternate thymic selection/development or selective expansion in the periphery. To distinguish these 2 possibilities for ThPOK-deficient T cells, we have utilized the microinjection equipment purchased with CURE funds to generate a novel mouse line in which ThPOK is selectively turned off in peripheral T cells (so-called OB11 mice). For this purpose, we mutated a key transcriptional element of the endogenous ThPOK gene, so that thymic expression of ThPOK is regulated normally, while peripheral expression is extinguished. Specifically, we used a site-specific Zn finger nuclease (ZFN)-mediated strategy to delete a 90 bp region of the endogenous ThPOK silencer element. Real-time polymerase chain reaction (RT-PCR) analysis indicated that homozygous OB11 mice displayed a dramatic reduction of ThPOK mRNA in peripheral CD4 T cells, although not in thymocytes. Thus this mutation selectively extinguishes ThPOK transcription in peripheral CD4 T cells. Because thymocyte expression of ThPOK is unaltered, development of MHC class II-restricted thymocytes proceeds normally. Fluorescence-activated cell sorting (FACS) analysis of peripheral T cell subsets of homozygous mutant mice revealed that a significant proportion of CD4 cells had downmodulated surface CD4 expression and/or upmodulated CD8 expression, resulting in atypical CD4lo and DP (CD4+8+) subsets, in

addition to phenotypically normal CD4hi cells. This is consistent with an established function of ThPOK in regulating transcription of the CD4 and CD8 genes. Backcrossing OB11 mice to transgenic lines bearing class I- or class II-restricted TCR transgenes, demonstrated that CD4hi, CD4lo and DP populations were MHC class II-restricted. Because OB11 mice selectively downmodulate ThPOK after thymic selection, unusual MHC class II-restricted CD4lo and DP T cells must develop from mature CD4hi T cells in the periphery. Because these mice selectively lack ThPOK expression in peripheral CD4 T cells, they provide an ideal model to assess whether the distorted TCR repertoire of residual CD4 lymphocytes observed in ThPOK-deficient mice results from post-thymic expansion of a fraction of cells with particular TCR specificities. To assess this, we are currently backcrossing the OB11 silencer mutant mice, which were generated on a mixed C57BL/6-C3H strain background, to derive mice with a genetically uniform C57BL/6 background. This is necessary to preclude strain-dependent variation in TCR usage, which would otherwise confound analysis of the TCR repertoire in these mice. In the meantime, we have achieved 5 backcross generations to C57BL/6.

To test whether the OB11 mutation blocked ThPOK transcription in peripheral CD4 cells by relieving ThPOK silencer activity, we further mutated the Runx factor binding motifs of the ThPOK silencer, which are essential for silencing and which are located immediately 3' to the OB11 deletion. Thus we generated a new knockin mouse that both lacked the 90bp OB11 region and the adjacent Runx binding sites (OB11-ΔRunx knockin mice). Indeed, mice bearing both mutations on the same allele failed to repress ThPOK in peripheral CD4 T cells, indicating that the OB11 region is necessary to antagonize silencing (data not shown). Because OB11 mice selectively lack ThPOK expression in peripheral CD4 T cells, they provide an ideal model to assess the role of ThPOK in mature T cell function separately from its role in development. The fact that thymic development is normal precludes the possibility that changes in T cell function result from developmental reprogramming in the thymus rather than a direct role of ThPOK in peripheral T cells.

As discussed above, OB11 mice possess atypical MHC II-restricted CD4lo and DP T cell subsets, in addition to phenotypically normal CD4hi cells. To assess the role of ThPOK in cytokine response of these populations, sorted cells were cultured in the presence or absence of anti-TCR stimulation. CD4hi cells from OB11 mice showed relatively normal induction of all cytokines examined, while CD4lo and DP subsets showed significantly altered expression patterns, indicating that underlying ThPOK-dependent gene regulation was relatively perturbed in the latter subsets. Two effects are particularly noteworthy. First, IL-17 production is markedly increased in CD4lo and DP subsets. This was not due to misregulation of RORγt, the Th17 master regulator, which was comparable to wt CD4 cells for all subsets. Secondly, IL-9 was expressed by CD4lo and DP cells even in the absence of TCR stimulation and decreased after TCR stimulation, while CD4hi cells showed normal TCR-dependent induction. Indeed, several other cytokines were found to be expressed in CD4lo and DP cells upon exposure only to IL-2, including also IL-17 and IFNγ, but not IL-4 or IL-22. Overall, our preliminary data indicate that absence of ThPOK renders certain cytokine expression pathways hyper-responsive to TCR as well as IL-2 signaling, probably in part due to increased IL2R expression. Significantly, this effect is restricted to particular cytokines, suggesting that ThPOK may play a differential role in controlling alternate

cytokine responses, which would be novel and interesting. In future work, we propose to use OB11 mice to precisely dissect the molecular basis for the role of ThPOK in effector T cell responses.

In summary, it has become clear that establishing SCNT for T lymphocytes is significantly more difficult than anticipated, so that progress in this regard has been minimal.

Nevertheless, establishing this capacity at Fox Chase remains an important long-term goal, and efforts will continue using equipment purchased with CURE funds. On the other hand, progress in generating knockin and knockout mice using the new microinjection equipment provided by CURE funds has been substantial and significant. In addition to the ThPOK silencer element mutant described above, the Fox Chase Transgenic Facility has generated >40 new mouse knockin lines using the new equipment.

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 to date				☐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:

Studies described above employing novel ThPOK knockin mice will result in at least 3 publications. Manuscripts are currently in preparation.

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

None

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

None

23. Inventions, Patents and Commercial Development Opportunities.

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

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

If yes, indicate date patent was filed:

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

If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

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

If yes, how many licenses were granted?_____

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

If yes, describe the commercial development activities:

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

Yes_____ No____X____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages..

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Kappes, Dietmar J., Ph.D.	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) DKAPPES	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Harvard University, Boston, MA	B.S.	06/80	Biology, Chemistry
Harvard University, Boston, MA	Ph.D.	06/88	Biology

A. PERSONAL STATEMENT

My laboratory employs genetic approaches to elucidate the signaling and transcriptional pathways that regulate thymic selection and T lymphocyte lineage commitment, as well as the molecular basis of lymphomagenesis. We have a particular interest in the control of CD4/CD8 lineage choice, arising from our identification of the transcription factor ThPOK as the "master regulator" of CD4 commitment. In my additional function as Director of the Transgenic Facility at FCCC, I take a lead role in integrating important new technologies into the facility's services, including most recently ZFN-mediated gene targeting.

B. POSITIONS

Teaching Assistant, Introductory Biology and Membrane Biology, Harvard University, Boston, MA	1982-1985
Head Teaching Fellow for undergraduate and advanced graduate courses in immunology, Harvard University, Boston, MA	1984-1987
Postdoctoral Fellow, Damon Runyon-Walter Winchell Cancer Research Fund in the group of Susumu Tonegawa, Massachusetts Institute of Technology, Cambridge, MA	1988-1992
Associate Member, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA	1992-1999
Director, Transgenic Mouse Facility, Institute for Cancer Research, Fox Chase Cancer Center	1994-present
Member with Tenure, Institute for Cancer Research, Fox Chase Cancer Center	1999-2007
Professor, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA	2007-present
Member, Blood Cell Development and Cancer Keystone Program, Fox Chase Cancer Center	2008-present

C. SELECTED PEER-REVIEWED PUBLICATIONS

1. Davé, V.P., Allman, D., Keefe, R., Hardy, R.R., Kappes, D.J. HD mice: a novel mouse mutant with a specific defect in the generation of CD4+ T cells. Proc. Natl. Acad. Sci. U.S.A. 95:8187-8192, 1998.
2. Davé, V.P., Allman, D., Wiest, D.L., Kappes, D.J. Limiting TCR expression leads to quantitative but not qualitative changes in thymic selection. J. Immunol. 162:5764-5774, 1999.
3. Keefe, R., Davé, V.P., Allman, D., Wiest, D., Kappes, D.J. Regulation of lineage commitment distinct from positive selection. Science 286:1149-1153, 1999.

4. Kappes, D.J., Lawrence, D.M., Vaughn, M.M., Davé, V., Belman, A.R., Rall, G.R. Protection of CD3δ knockout mice from lymphocytic choriomeningitis virus-induced immunopathology: implications for viral neuroinvasion. Virology 269:248-256, 2000.
5. Delgado, P., Fernández, E., Davé, V., Kappes, D.J., Alarcón, B. CD3δ couples T-cell receptor signalling to ERK activation and thymocyte positive selection. Nature 406:426-430, 2000.
6. Hayes, S.M., Laky, K., El Khoury, D., Kappes, D.J., Fowlkes, B.J., Love, P.E. Activation-induced modification in the CD3 complex of the γδ T cell receptor. J. Exp. Med. 196:1355-1361, 2002.
7. He, Xiao, He, Xi, Davé, V.P., Zhang, Y., Hua, X., Xu, W., Roe, B.A., Kappes, D.J. The zinc finger transcription factor TH POK regulates CD4 versus CD8 T lineage commitment. Nature 433:826-833, 2005.
8. Haks, M.C., Lebebvre, J.M., Lauritsen, J.P., Carleton, M., Rhodes, M., Miyazaki, T., Kappes, D.J.*, Wiest, D.L.* Attenuation of γδ TCR signaling efficiently diverts thymocytes to the αβ lineage. Immunity 22:595-606, 2005. *Denotes authors contributed equally.
9. He, X., Park, K., Wang, H., Zhang, Y., Hua, X., Li, Y., Kappes, D.J. CD4-CD8 lineage commitment is regulated by a silencer element at the ThPOK transcription-factor locus. Immunity 28:346-358, 2008.
10. Lauritsen, J.P.H., Wong, G.W., Lee, S.Y., Lefebvre, J.M., Ciofani, M., Rhodes, M., Kappes, D.J., Zúñiga-Pflücker, J.C., Wiest, D.L. Marked induction of the helix-loop-helix protein Id3 promotes the gammadelta T cell fate and renders their functional maturation Notch independent. Immunity 31:565-575, 2009.
11. Park, K., He, X., Lee, H. O., Hua, X., Li, Y., Wiest, D., Kappes, D.J. TCR-mediated ThPOK induction promotes development of mature (CD24-) γδ thymocytes. EMBO J. 29:2329-2341, 2010.
12. Engel, I., Hammond, K., Sullivan, B.A., He, X., Taniuchi, I., Kappes, D., Kronenberg, M. Co-receptor choice by V alpha14i NKT cells is driven by Th POK expression rather than avoidance of CD8-mediated negative selection. J. Exp. Med. 207:1015-1029, 2010.
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