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:** Trustees of the University of Pennsylvania

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):** Gearline Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100054874

6. **Project Number and Title of Research Project:** 6-Therapeutic Response in Genetically Engineered Mouse Models

7. **Start and End Date of Research Project:** 7/1/2011 – 6/30/2014

8. **Name of Principal Investigator for the Research Project:** Lewis A. Chodosh, MD, PhD

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:

   $ 319,465.52

   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
Chodosh, Lewis	Professor	2% yr 2	$ 6,965
Brown, Eric	Associate Professor	2% Yr 2	$ 6,075
Chen, Chien-Chung	Mngr. Res. Proj	10.5% Yr 2	$ 9460.72
Ragland, Ryan	Postdoc. Fellow	5% Yr. 2	$ 5437.50
Alvarez, James	Res. Investigator	17% Yr. 2	$16,509.52

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
Xu, Minghua	Postdoctoral Researcher	1%
Belka, George	Mngr. Research Project	1%
Pickup, Stephen	Co-Investigator	1%
Schnall, Mitchell	Co-Investigator	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:
Breast cancer progression	☐NIH ☐ Other federal (specify:_ _) ☒ Nonfederal source (Breast Cancer Research Foundation)	6/2014	$250,000	$250,000
Preventing recurrence through surveillance tools and targeted interventions	☐NIH ☒ Other federal (Dept. of Defense) ☐ Nonfederal source (specify:_)	10/2014	$1,200,000	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 plan to apply for a Department of Defense Award to fund further studies aimed at identifying imaging biomarkers of early response to therapy.

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

We plan to publish the results described in this report, and will continue to pursue studies to identify and validate imaging biomarkers of early response to therapy, as possible based upon funding.

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 quality and scope of cancer imaging research, as well as research on DNA damage

response and genome integrity, was enhanced at the University as a result of the increased research activity in these areas that funding for this project permitted.

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.

Therapeutic Response in Genetically Engineered Mouse Models

The aims of this project were:

Aim 1: *To examine the ability of ATR-Chk1 pathway inhibition to synergistically elevate genomic instability and compromise cell viability when combined with oncogene expression and p53 or ATM deficiency.*

The tumor models in which we chose to examine the effects of ATR pathway suppression fulfill two main criteria for the proposed studies: 1) they are tractable for testing the effects of treatment while these malignancies are actively progressing to terminal disease; and 2) the cancers they model are particularly refractory to the current standard of care therapies in humans. The first qualification is critical, as a large number of studies have tested how suppression of particular genes effects initiation, but not the progression of these malignancies after full establishment of the disease. While the models have these characteristics, they have also proven to be more time consuming to produce than expected. The TetO-Kras4b^{G12D}CCSPrtTA system utilizes doxycycline (dox)-inducible expression of the Kras^{G12D} mutant, which drives tumor formation within 2-4 months after initiation of dox treatment. TetO-K-ras4b^{G12D} and CCSP-rtTA mice have been acquired from Dr. Harold Varmus and crossed with ATR$^{flox/flox}$ and ATR$^{+/seckel}$ and intercrossed to produce transgene homozygotes, as determined by real-time qPCR of genomic DNA. Unfortunately, our ability to produce sufficient numbers of the required homozygous parental mouse lines, ATR$^{flox/flox}$TetO-K-ras4b$^{G12D\text{-homozygous}}$ and ATR$^{+/seckel}$Cre-ERT2$^{-\text{homozygous}}$CCSP-rtTA$^{-\text{homozygous}}$, and sufficient numbers of ATR$^{flox/seckel}$ test and ATR$^{flox/+}$ littermate control mice was found to be limiting for reasons that were unclear, whether due to embryonic lethality of genotyping issues. As an alternate approach, ATR$^{flox/seckel}$p53-/- test and ATR$^{flox/+}$p53$^{-/-}$ control AML-ETO-expressing acute myeloid leukemias (AMLs) were generated through in vitro transformation, followed by primary and secondary transplantation. Secondarily

transplanted leukemias were then harvested and frozen for transplantation and testing of the effects of ATR suppression and Chk1 inhibition.

We successfully generated and imaged MLL-ENL and AML-ETO expressing lines by magnetic resonance imaging (MRI) and biofluorescent (GFP) imaging. Thus, for these models, these forms of imaging have been successfully validated.

Test the effect of antioxidants on oncogene-mediated ATR activation

In the past year, we have tested the following antioxidants on oncogene-induced ATR activation: N-acetyl cysteine (radical scavenger), ascorbic acid (radical scavenger), and ebselen (H_2O_2 scavenger). Unfortunately, each of these produced side-toxicities in our hands, including cell cycle effects and mild increases in cell death. These effects made the objective of their use to be difficult to obtain. However, we have since learned from Dr. Joan Brugge that Trolox has much less toxicity in her laboratory's experience. We now intend to test the use of this vitamin E derivative, as well as ectopically express key regulators of antioxidant levels, such as Nrf2 and Sestrins, to determine the effect of ROS suppression on ATR activation.

Progress was also made on an alternate approach to the proposed aims due to the development of a highly-specific ATR inhibitor. Using this inhibitor, ATR-45, we demonstrated that NIH3T3 cells transformed with $Hras^{G12V}$, $Kras^{G12D}$, or c-Myc are more sensitive to ATR inhibition than control cells (Figure 1). In addition, we completed studies examining the effect of ATR suppression on the progression of MLL-ENL AMLs. Our findings demonstrated that ATR hypomorphic reduction leads to a selective growth inhibition of MLL-ENL and $Nras^{G12D}$-expressing AMLs in comparison to normal bone marrow. These data indicate that the effects of oncogenic transformation cause a greater dependence on ATR for genome stability and cell viability than is required for normal tissue homeostasis (Figure 2). These findings validate the potential utility of ATR inhibition as a cancer therapeutic.

Aim 2: *To test the utility of ATR-Chk1 pathway suppression as treatment for chemotherapy-resistant acute myeloid leukemias (AML) and p53- or ATM-deficient lung adenocarcinomas.*

The goal of this aim was to perform genome stability and cell proliferation on transformed cells following ATR suppression. As described (Schoppy et al., J. Clin Invest, 2012), a first analysis of the effects of oncogene expression on sensitivity to ATR-CHK1 pathway inhibition has been performed in murine fibroblasts. To explore this relationship in human breast epithelium and in breast cancer cells, MCF10A mammary epithelial cells were virally transduced to express HRASG12V, KRASG12B, MYC, and overexpress EGFR (Figure 3). These cells were then exposed to CHK1 inhibitor (PF477736) or CHK1 inhibition in combination with complete replication stalling (aphidicolin treatment). According to our model, oncogenic stress emulates replication stalling by increasing helicase-polymerase uncoupling, thereby increasing reliance on ATR-CHK1 function to prevent replication fork collapse into DNA double strand breaks (DSBs). Results from these experiments are consistent with this model (Figure 3). Although CHK1 inhibition in MCF10A cells expressing the control vector led only to a weak double-stranded break response, as detected by H2AX phosphorylation, CHK1 inhibition in MCF10A cells expressing oncogenes or overexpressing wild-type EGFR significantly elevated H2AX phosphorylation (Figure 3). This level was in some cases equivalent to the level observed with

aphidicolin treatment. Because the combination of ATR-CHK1 pathway inhibition with aphidicolin treatment is invariably lethal, these data indicates that oncogene or EGFR overexpression will be synthetically lethal with ATR/CHK1 inhibition. This result is potentially clinically relevant, as a large fraction of triple negative breast cancers overexpress EGFR.

We also tested whether a similar genome-destabilizing effect would be observed upon CHK1 inhibition in human triple negative breast cancer cells lines. MCF10A cells were again used as a control. In both triple negative cell lines, a substantial increase in DSB generation was observed upon CHK1 inhibition (Figure 3B). Notably, CHK1 inhibition had a more destabilizing effect than ATR inhibition (Figure 3B), a result we plan to follow up to determine the mechanism of selectivity. In aggregate, these data substantiate the effect of oncogenic stress on sensitivity to ATR-CHK1 and demonstrate that CHK1 inhibition has a substantial genome destabilizing effect on human breast cancers that are typically refractory to standard therapies.

Aim 3: *Use diffusion MRI to measure changes in the apparent diffusion constant following blockade of an oncogenic pathway in vivo.*

Diffusion weighted MRI imaging has been shown to be sensitive in monitoring early response to therapy and is sensitive to changes in extracellular water. In this aim, the apparent diffusion constant (ADC) was quantified in a panel of mammary tumors arising in a conditional bitransgenic system for mammary tumor development before, and at increasing intervals after, oncogene down-regulation and tumor regression.

Studies on tumor progression typically necessitate that animals be sacrificed to perform tissue and molecular analysis. This prevents researchers from observing in vivo the natural or perturbed evolution of the processes under study. As a consequence, repetitive studies of tumors using functional, molecular, and morphologic quantitative imaging techniques has the potential to serve as an important tool for providing data about biochemical, genetic and pharmacological processes in the same animal over time in vivo. As such, an additional aim of this project was to address this need by determining whether serial MRI can be used to measure tumor response to therapy.

Although breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related death in women, the mechanisms of human breast cancer progression are largely unknown. Understanding the molecular, cellular, and pathophysiological events that contribute to his process is therefore essential. To achieve this goal, our laboratory has developed a novel series of inducible transgenic mouse lines that mimic the natural course of breast cancer development in humans, including the development of resistance to therapy, metastasis, and recurrence. These mouse lines permit oncogenes relevant to human breast cancer to be turned “on” and “off” in mammary glands of mice in response to doxycycline administered in their drinking water. Following oncogene induction with doxycycline, mammary epithelial cells become increasingly abnormal and eventually form invasive cancers that metastasize. When doxycycline is withdrawn, oncogene expression is halted and many tumors regress, often to a nonpalpable state. In some ways, the ability of downregulation of oncogenes to result in tumor regression can be viewed as being analogous to the effects of a molecularly targeted therapy. In

this manner, downregulation of oncogene expression in intact tumors achieved by doxycycline withdrawal represents a genetic approach to modeling the effects of targeted pharmacologic agents directed against the oncogene pathway.

During cancer treatment, it is desirable to accurately monitor and quantify tumor early response to therapy, which may guide therapeutic decisions and yield prognostic information. Non-invasive imaging, particularly magnetic resonance imaging (MRI), has become a powerful tool in detecting and monitoring tumor response to therapy. Recent studies in human and animals reveal that diffusion weighted MRI can identify rapid changes in tumor water diffusion values after successful therapeutic intervention in solid tumors. In general, water diffusion (mobility) is reduced in the restricted environment of cellular-dense tissues relative to cellular-sparse tissues that exhibit high diffusion properties. Successful treatment of a tumor results in significant damage to the tumor cells with a subsequent reduction in tumor cell density and an increase in the diffusion of water within the damaged tumor tissue.

Diffusion MRI can detect relatively small changes in tissue structure at the cellular and sub-cellular level by assessing tumor water diffusion or mobility with the measurements of apparent diffusion coefficient (ADC) values. In addition, some studies demonstrate that ADC values correlate with T2 values, though not universally. Tissue T2 value is related to the tissue water content and higher in free water than in water bound to macromolecules, such as proteins and myelin lipids. As such, important factors influencing T2 are the macromolecular environment in which water exists, beyond water content itself. T2-weighted MRI generally provides sufficient contrast to highlight tumors and then can apply to evaluate changes in tumor size relying on comparison of tumor anatomical images acquired before and after therapeutic treatment.

In these studies, we present a study of using diffusion MRI to detect mammary tumor early response to oncogene downregulation in transgenic mouse models for breast cancer. We observed a statistically significant increase of ADC values in solid mammary tumors within a short time period of 2 or 3 days after oncogene deinduction with doxycycline withdrawal, accompanied by a slight decrease in T2 or tumor size. These results demonstrate that diffusion MRI analysis can provide an early biomarker for mammary tumor response to oncogene downregulation.

Materials and Methods

Animal Models

We studied two bitransgenic mouse models for breast cancer: MMTV-rtTA;TetO-MYC (MTB/TOM) and MMTV-rtTA;TetO-Akt1 (MTB/tAKT), which conditionally express the human oncogenes c-MYC and AKT1, respectively, in the mammary gland in response to doxycycline treatment.

Starting at age of 6 weeks, bitransgenic MTB/TOM and MTB/tAKT mice were administered doxycycline 2mg/mL with 5% sucrose and 0.1mg/mL, respectively, in their drinking water. The administration of doxycycline induced transgene expression in a mammary-specific manner. Doxycycline bottles were changed approximately once a week. Mice were monitored twice per week for tumor formation. Palpable tumors were identified approximately 22 or 15 weeks after

starting induction in MTB/TOM or MTB/tAKT mice, respectively. Calipers were used to measure tumor size. Protocol for animal research was approved by University of Pennsylvania the Institutional Animal Care and Use Committee (IACUC). All animal experiments were conducted humanely.

MR Imaging

MR experiments in MTB/TOM mice were initiated when tumors reached a diameter of ~10 mm or less. Tumors were imaged at 3 time-points: baseline with oncogene induction, and 2 and 5 days after oncogene deinduction. After the completion of baseline studies (Day 0), the initiating oncogene was deinduced through removing doxycycline from the water supply on Day 0. Tumors were further imaged on Day 2 and Day 5 after oncogene deinductio.

Studies on MTB/tAKT mice were performed with a refined imaging protocol. MR experiments were initiated when the tumors reached a size of ~6 mm. Tumors were imaged at 3 time-points: baseline with oncogene induction, and 1 and 3 days after oncogene deinduction. The baseline scan was performed on or 1 day prior to Day 0. After the completion of baseline studies, the initiating oncogene was deinduced through removing doxycycline from the water supply on Day 0. Tumors were further imaged on Day 1 and Day 3 after oncogene deinduction.

All MR experiments were performed in the Small Animal Imaging Facility at University of Pennsylvania, with a Unity INOVA console (Varian, Palo Alto, CA) interfaced to a 4.7 T horizontal bore magnet and a 12 cm gradient insert capable of generating magnetic field gradients of up to 25 G/cm. A home-built 5× 12 cm linearly polarized birdcage RF coil was used for all studies.

During MR experiments, mice were sedated with 1-2% isoflurane delivered in oxygen at a flow rate of 1.0 L/min through a nose cone. Animal core temperature was continuously monitored using a rectal thermometer with an MR-compatible small animal monitor system (SA Instruments, Bayshore, NY), which also regulated a warm air source blowing warm air into the magnet core to maintain the animal's core temperature at 36.9 ºC (±0.1) during the course of imaging experiments.

Coronal scout images were obtained to determine the position and size of tumors. T2-weighted MR imaging was then carried out using a conventional Spin-Echo Multi-Slice (SEMS) pulse sequence, typically with the following acquisition parameters: repetition time (TR)=2000ms, echo time (TE)=13,23,33,43, 53 and 63ms, image field-of-view (FOV)=40mm×20mm, matrix size=256×128, slice thickness=1mm. In initial imaging studies of MTB/TOM mice, only one or several slices with 1-mm slice gap covering the middle and/or more regions of the tumors were imaged. All other MTB/TOM mice and all the MTB/tAKT mice were imaged typically with 15 (or less, depending tumor size) transverse slices without slice gap continuously covering the entire tumor volume. In the data acquisition, we shuffled the order of slice excitations such that adjacent slices were not excited sequentially to reduce possible crosstalk effect. In addition, respiratory gating was used to reduce motion artifacts. Animal respiration was tracked by computer, which was connected to the above small animal monitoring system. MRI data acquisition was restricted to the expiratory phase. For example, 15 slices were shuffled into five

expiration periods in the following arrangement: slice 1, 6 and 11 were acquired in the first expiration, slice 2, 7, and 12 in the second expiration, etc.

After the completion of T2-weighted imaging, diffusion-weighted MR imaging was performed on the same slices using a Spin-Echo Multi-Slice Diffusion Weighted (SEMSDW) imaging sequence. Acquisition parameters were the same as used in SEMS sequence, with the exception that TE was not arrayed, but a fixed value between 13 and 53 ms. The SEMSDW sequence was set to acquire images at a set of diffusion sensitivities, i.e., b-values= 221, 442, 663, 884, 1105, 1326 s/mm^2 along all three orthogonal directions: x, y, and z axes.

MR Image Analysis

All T2-weighted and diffusion MR images were spatially co-registered slice by slice. This allowed us to manually draw the region of interest (ROI) based on both T2 and diffusion contrast.

Tumor size was assessed using T2-weighted MR images. For a round or elliptical tumor, two orthogonal diameters (width and length) or three orthogonal diameters (width, height, and length), were measured from ROIs manually drawn on images. Tumor volume was then computed based on an elliptical model: volume=width×width×length×π/6 for two dimensional measurements and volume=width×height×length×π/6 for three dimensional measurements. For the entire tumor covered by multiple slices, tumor volume was quantified as the product of slice-to-slice separation and the sum of areas from manually drawn tumor ROI on images. Then, average tumor diameter, as the indicator of normalized tumor size, was calculated by the cube root of (6×volume/ π).

Tumor mean T2 values, in the early study of MTB/TOM mice, were generated by whole ROI fitting, fitting the sum of signal intensity in tumor ROI to a single exponential decay exp(-TE/T2) as a function TE. For all other MTB/TOM mice and all the MTB/tAKT mice, T2 maps were first generated by the pixel fitting, fitting the signal intensity of each pixel to an exponential decay exp(-TE/T2), and then the mean T2 values in ROIs were calculated. Although the value obtained from whole ROI fitting might differ slightly from the mean value for ROI obtained by pixel fitting, the difference is negligible for homogeneous tumors. Moreover, the pixel fitting method requires high signal-to-noise ratio, while the whole ROI fitting is more tolerable with noise.

Tumor ADC values, in the initial study of MTB/TOM mice, were derived by fitting the sum of signal intensity in tumor ROI to a single exponential decay exp(-b×ADC) as a function b-values. For all other MTB/TOM and all the MTB/tAKT mice, ADC maps were first generated by fitting the signal intensity of each pixel to an exponential decay exp(-b×ADC) for all three orthogonal directions: x, y, and z axes respectively. Then, ADC values in pixels were calculated as the quantity ADC = (ADCx + ADCy + ADCz)/3, which represents a scalar invariant of the diffusion tensor normally used to fully characterize diffusion in an anisotropic system. Tumor mean ADC values for ROIs were further calculated. Histograms of ADC values in tumor ROIs were also generated. A paired sample t-test was used to determine statistical significance with a p value < 0.05.

Histologic Analysis
MTB/tAKT mice were sacrificed in groups before oncogene deinduction and 3 days after oncogene deinduction for histologic analysis. In a separate study, primary tumors in MTB/TOM mice were first biopsied before oncogene deinduction and then mice were sacrificed 3 days after oncogene deinduction for histologic analysis. Mammary tissues were harvested, fixed overnight in 4% paraformaldehyde, and transferred to 70% ethanol. Fixed mammary tissues were further embedded in paraffin and sectioned for histological staining as described. Sections (5 μm) were cut, mounted on glass slides and stained with Hematoxylin and Eosin (H&E). Randomly selected 200× fields were captured and digitized. Hematoxylin stained images were separated by color deconvolution using Ruifrok & Johnston's method.

Results
We first wished to examine histological sections of solid MTB/tAKT or MTB/TOM tumors obtained before and after oncogene deinduction. As shown in Figure 4, we observed an increase in tumor extracellular space after oncogene deinduction. There was also increase in cells with the characteristic morphologic features of apoptosis. These results clearly indicated that significant cell death occurred in tumors after oncogene deinduction. These tissues are therefore suitable for studying the response of tumors to oncogene down-regulation using MRI.

We next wished to compare diffusion values of tumors with other tissue. MR images obtained at baseline before oncogene deinduction from a representative MTB/tAKT mouse are shown in Figure 5. As shown in this example, fat tissue had very lower water diffusion (ADC $<0.2\times10^{-3}$ mm^2/s) since less water content was in fat. Brain tissue had low water diffusion (ADC $\sim0.6\times10^{-3}$ mm^2/s) since it was solid and dense. Muscle had relatively high water diffusion (ADC = $1\sim1.5\times10^{-3}$ mm^2/s) due to its high water content. Bladder had the highest water diffusion (ADC $\sim2.1\times10^{-3}$ mm^2/s) because most inside it was free water.

We next imaged MTB/tAKT mice at three time-points: baseline before oncogene deinduction, 1 day and 3 days after oncogene deinduction. Figure 6 shows sequential T2-weighted images (Figure 6A), corresponding ADC maps (Figure 6B) and ADC histograms in tumor ROIs (Figure 6C) from a representative solid tumor. The T2-weighted images indicated that the tumor regressed following oncogene deinduction, where tumor ROIs are enclosed by black lines and indicated by arrows. The serial ADC histograms shown for this representative tumor (Figure 6C) indicated a right shift (higher mean diffusion values) in tumor water diffusion.

We next quantified the mean diameter (Figure 7A), T2 relaxation time (Figure 7B), and ADC (Figure 7C), for 3 MTB/tAKT mammary tumors over time. Before oncogene deinduction, these tumors reached a size of about 6 mm. Mean ADC values at baseline were relatively low and around $0.7x10^{-3}$ mm^2/s, far less than free water diffusion ($\sim2x10^{-3}$ mm^2/s). Tumor mean sizes were decreased on day 3 after oncogene deinduction, as shown in Figure 7A. Tumor mean T2 values were also slightly decreased from 42 ms at baseline to 35 ms on day 3 after oncogene deinduction as shown in Figure 7B. Tumor mean ADC values were significantly ($p < 0.03$) elevated from 0.7 to 0.9 ($\times10^{-3}mm^2/s$), overtime after oncogene deinduction, as shown in Figure 7C. Mean ADC values of individual tumors were all increased after oncogene deinduction.

We next imaged MTB/TOM mice by ADC at three time-points: baseline before oncogene deinduction, 2 days and 5 days after oncogene deinduction. The mean diameter (Figure 7D), T2 relaxation time (Figure 7E), and ADC (Figure 7F), are shown for 6 MTB/TOM mammary tumors over time. As shown in Figure 7D, the tumor sizes for this group at baseline were in the range of 6 mm to 11 mm, and the mean sizes were decreased after oncogene-deinduction with a significant standard deviation due to biological variations. Mean T2 values were approximately 44 ms at baseline before deinduction, and slightly decreased on Day 2 or 5 after oncogene deinduction, as shown in Figure 7E. Mean ADC values were significantly ($p<0.003$) increased from 0.6 to 1.0 ($\times 10^{-3} mm^2/s$), after oncogene deinduction, as shown in Figure 7F. Mean ADC values of individual tumors all increased following oncogene deinduction.

For MTB/TOM mice, the mean ADC values of mammary tumors at baseline were all close to 0.6×10^{-3} mm^2/s, which was similar to the ADC values measured in solid tissues such as brain. This result indicated that MTB/TOM primary mammary tumors were relatively solid and dense. This is consistent with the previous finding based on histological analysis. For MTB/tAKT transgenic mice, it has been suggested that mice might accumulate a milk-like substance in the lumens of their mammary glands following doxycycline administration. This milk-like substance would be anticipated to display high diffusion properties because of its high water content. Consistent with this, the mean ADC values of mammary tumors at baseline for the MTB/tAKT tumors varied from a low value of 0.6×10^{-3} mm^2/s to a high value of 0.8×10^{-3} mm^2/s. Among them, the mean value and the distribution of ADC in tumor #1 were very close to the brain's, which indicated tumor #1 had low diffusion and was solid and dense. In tumor #3, there was a partially high diffusion region, according to its ADC histogram, which consisted of two parts: one with low diffusion and the other with high diffusion. This high diffusion part could potentially result from the excreted milk-like substance inside the tumor.

For both mouse models, a statistically significant increase of tumor water diffusion following oncogene downregulation was observed in all mammary solid tumors. The average increase was approximately 30%, compared to the mean values of ADC obtained on Day 2 or 3 after oncogene deinduction with the baseline ADC values obtained before oncogene deinduction. A slight decrease in tumor size on Day 2 or 3 after oncogene deinduction was also observed. These results were consistent with the fact that oncogene deinduction resulted in the increase of cell death in oncogene-induced tumors, and consequently the increase in cell death increaesd the extracellular space and membrane permeability, resulting in greater water mobility, which displayed higher ADC values by the measurement of diffusion MRI. The significant ADC increase in tumors only 2 or 3 days after oncogene deinduction indicates that anticancer treatment targeting cellular pathways involved in tumor growth regulation could be effective in treating cancers. In addition to the ADC increase observed following oncogene deinduction, a corresponding slight decrease of T2 values in both tumor models was also observed. This result indicates the existence of some inverse correlation between T2 value and ADC diffusion, as reported in other studies.

Compared to the other tissues, mammary tumors had medium water diffusion and their ADC values were between ADC values observed for solid tissues, such as brain, and ADC values observed for muscle. The sensitivity of diffusion MRI for detection of therapeutic-induced changes depends on the possible overall dynamic range of water diffusion observed by ADC

measurements. For example, potentially due to a milk-like substance within the epithelium, MTB/tAKT tumor #3 had a relatively high mean ADC value at baseline. This would decrease the sensitivity of diffusion MRI for detection of slight ADC changes in response to therapy. Additionally, mammary tumors in mouse models may have central necrosis after attaining a large size (>15 mm in diameter), resulting in some regions of tumors with high extracellular water contents that may elevate the ADC values at baseline and reduce the sensitivity of diffusion MRI for detection of ADC changes to therapy. Therefore, to achieve high detection sensitivity for diffusion MRI in the study of tumor response to oncogene deinduction or targeted therapy in mouse models, the best strategy, as adopted in this study, would be to limit tumor size to minimize central necrosis. An alternate solution to this problem would be to exclude known regions with necrosis and only compare regions that are present in both the pre-therapy and post-therapy tumor volumes. Nevertheless, how to segment the overlap regions from the images obtained on different days remains a challenge because mammary tumors can be easily deformed by pressure in experiments.

In conclusion, these studies demonstrate that diffusion MRI analysis provides an early biomarker for solid mammary tumor response to oncogene downregulation. The mean values of ADC in mammary tumors are significantly increased rapidly following oncogene deinduction, and were accompanied by slight decreases in T2 values and tumor size. These findings indicate that combining inducible transgenic mouse models for breast cancer and diffusion MRI will allow visualization and analysis of mammary tumor response to anticancer therapy targeting specific oncogene pathways.

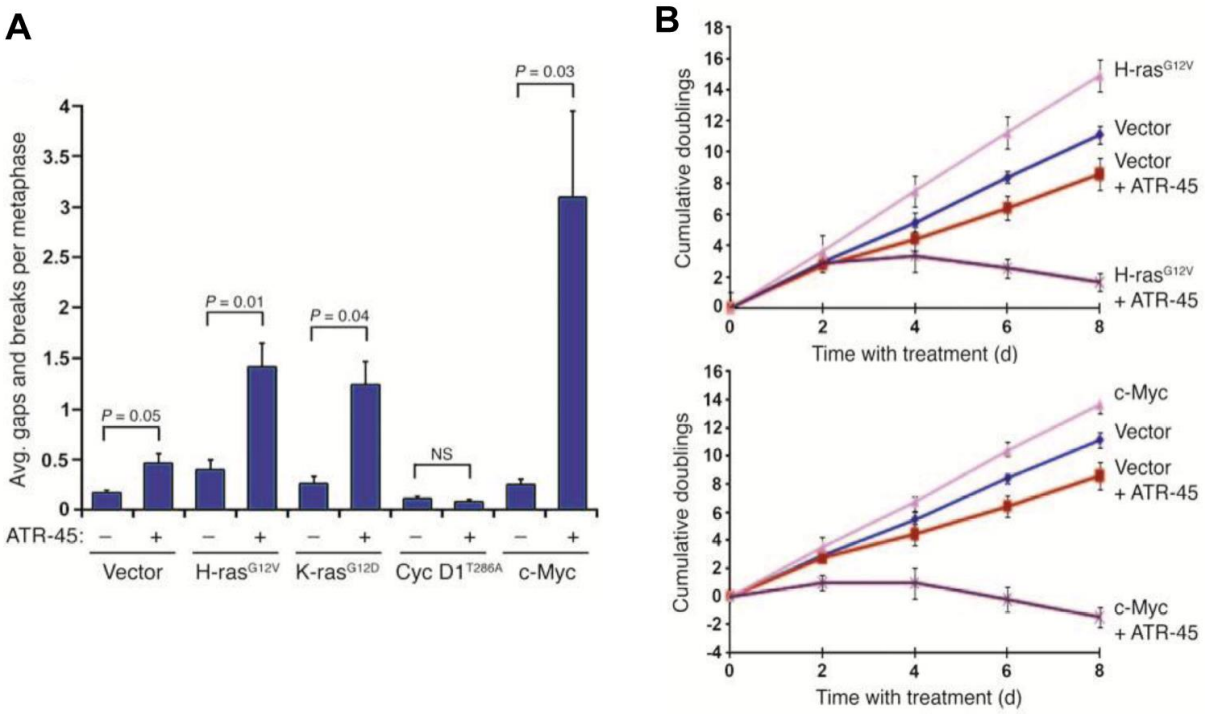


Figure 1. ATR inhibition synergizes with H-ras^{G12V}, K-ras^{G12D}, and c-Myc overexpression to cause increased genomic instability and cell synthetic lethality. A. Chromatid breaks following short-term ATR inhibition in oncogene expressing and control cell lines. Cell lines were treated with 2 μM ATR-45 inhibitor for 7 hours, as described in **A**, were harvested for mitotic spreads, and chromatid breaks were quantified. Nocodazole (0.5 μM) was added 4 hours prior to harvest. Data represent mean ± SEM. **B**. Effects of ATR inhibition on the proliferation of oncogene-expressing and control cell lines. Asynchronously growing cell lines were treated with ATR inhibitor (2 μM); medium and inhibitor were replaced at replating every 2 days. Cells were counted at replating, and cumulative doublings were quantified. Data represent mean ± SEM.

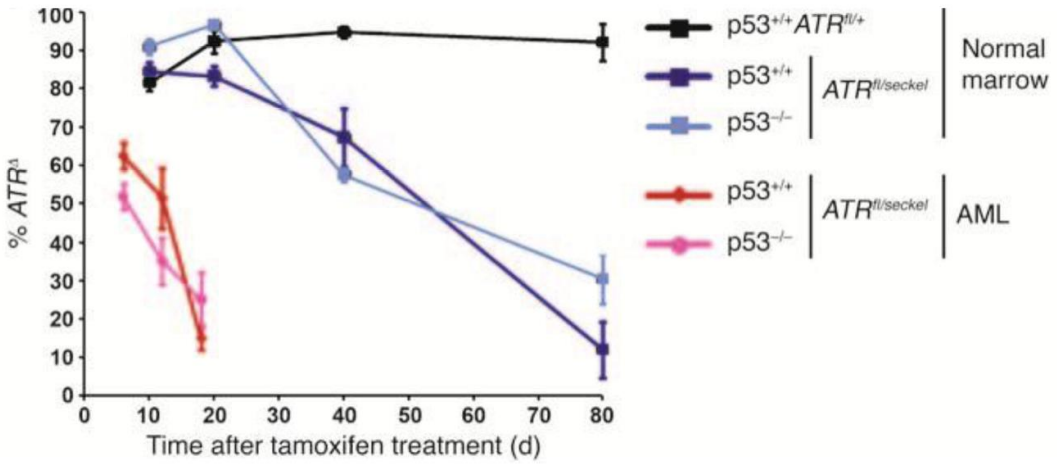


Figure 2. ATR hypomorphic suppression is more limiting to the growth of MLL-ENL– and N-ras^{G12D}–transformed AMLs than to normal bone marrow cells. Tamoxifen-treated secondary (AML) recipient mice were sacrificed at various days following initiation of treatment. Genomic DNA was isolated from leukemic bone marrow, and Cre-mediated *ATR*^{fl} recombination (*ATR*$^{\Delta}$) was quantified through qPCR. For comparison, bone marrow from systemically treated *ATR*$^{fl/seckel}$ mice was quantified similarly for the persistence of *ATR*$^{\Delta/seckel}$ cells. No significant differences were observed between *p53*$^{+/+}$ and *p53*$^{-/-}$ backgrounds. Data represent mean ± SEM.

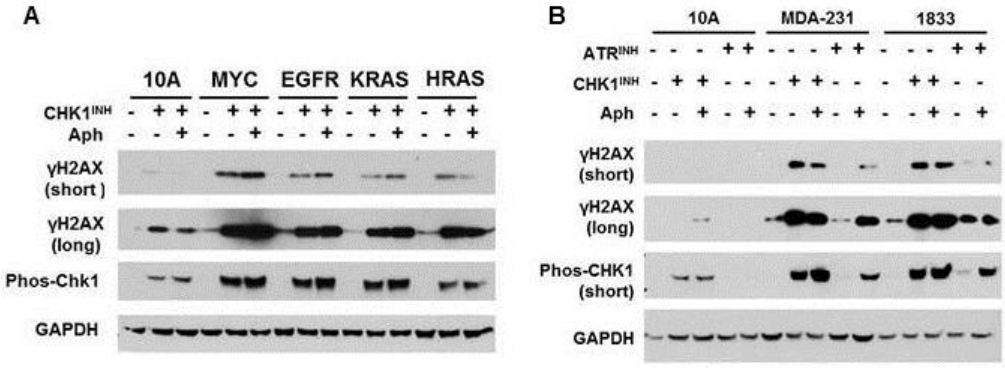


Figure 3. Oncogenic stress and transformation generates and increase sensitivity to CHK1 inhibition. A. The effect of oncogene expression on the ability of CHK1 inhibition to induce replication fork collapse. A western blot detected for the proteins indicated is shown. **B.** Sensitivity of two triple-negative breast cancer lines to CHK1 inhibition.

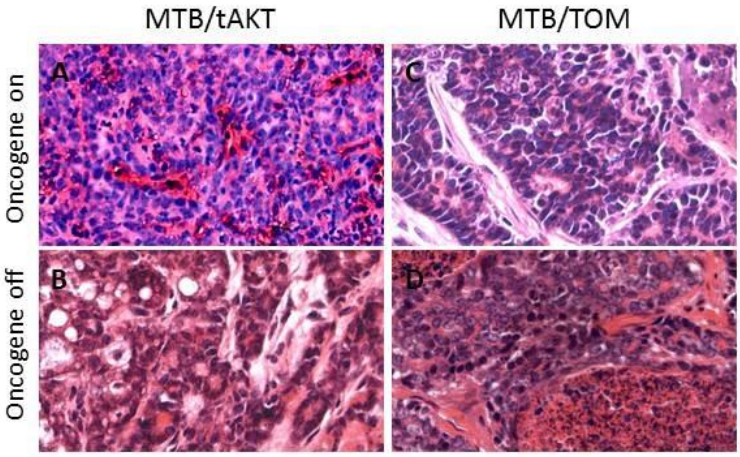
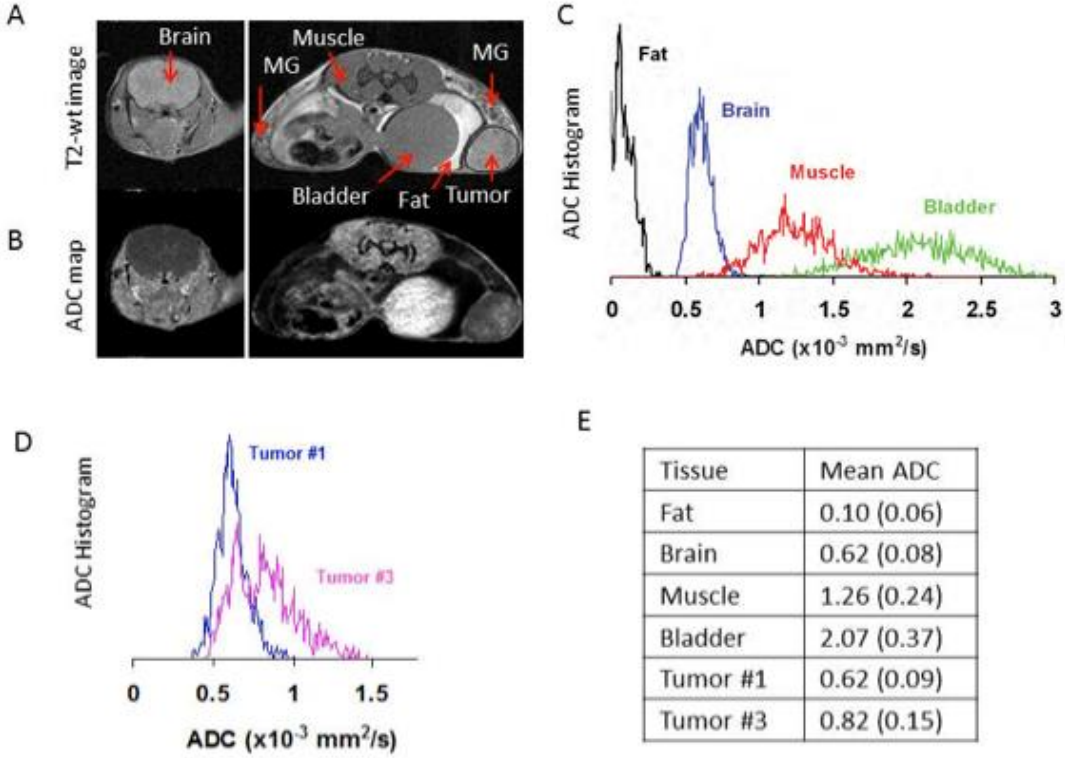


Figure 4. Examples of histopathologic changes in H&E stained sections of MTB/tAKT (A and B) and MTB/TOM (C and D) tumors obtained before and after oncogene deinduction.



Tissue	Mean ADC
Fat	0.10 (0.06)
Brain	0.62 (0.08)
Muscle	1.26 (0.24)
Bladder	2.07 (0.37)
Tumor #1	0.62 (0.09)
Tumor #3	0.82 (0.15)

Figure 5. MR images of various tissues in a MTB/tAKT mouse before oncogene deinduction. **A.** Representative T2 weighted images (TR/TE = 2000/13 ms). MG: mammary gland. **B.** Quantitative apparent diffusion coefficient (ADC) maps. **C.** Normalized ADC histograms in indicated tissues. **D.** ADC histograms of tumor #1 and tumor #3. Tumor #1 is shown in Figure 6 at baseline, and tumor #3 is shown in **A** and **B** of this figure. **E.** Mean ADC values for various tissues, presented at mean (and STD) ($\times 10^{-3} mm^2/s$).

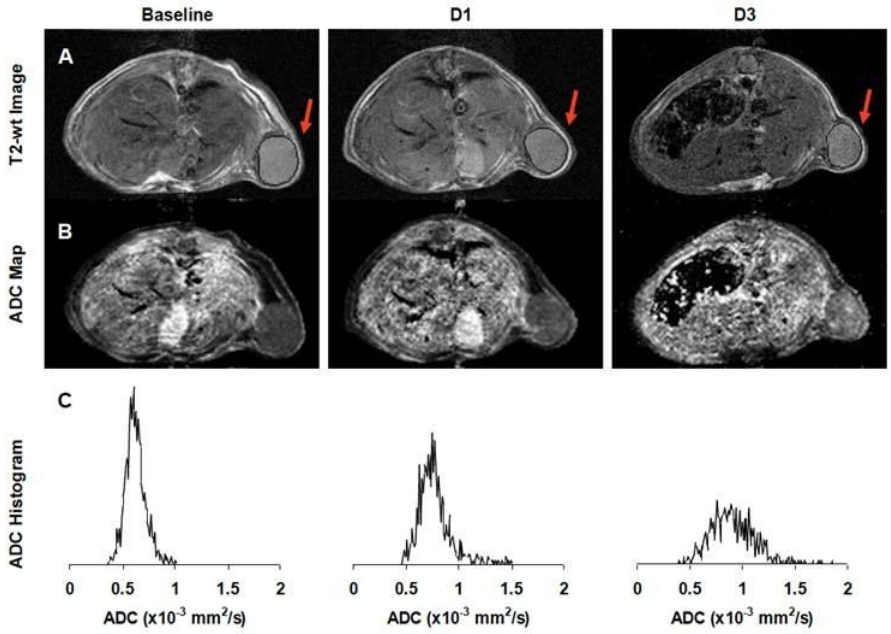


Figure 6. ADC imaging of an MTB/tAKT mammary tumor A. Representative T2 weighted images (TR/TE = 2000/13 ms) obtained 1 day before starting oncogene deinduction (Baseline), and 1 day (D1) and 3 days (D3) after oncogene deinduction, respectively. Tumor ROIs were drawn manually as enclosed by black lines and indicated by arrows. The T2 images illustrated the slight tumor shrinkage over time after oncogene deinduction. **B.** Quantitative apparent diffusion coefficient (ADC) maps at the same time intervals. **C.** ADC histograms in tumor ROIs at the same time intervals. Vertical axis represents voxel numbers in ROIs. Both the tumor ADC maps and histograms in tumor ROIs revealed increase diffusion or water mobility after oncogene deinduction.

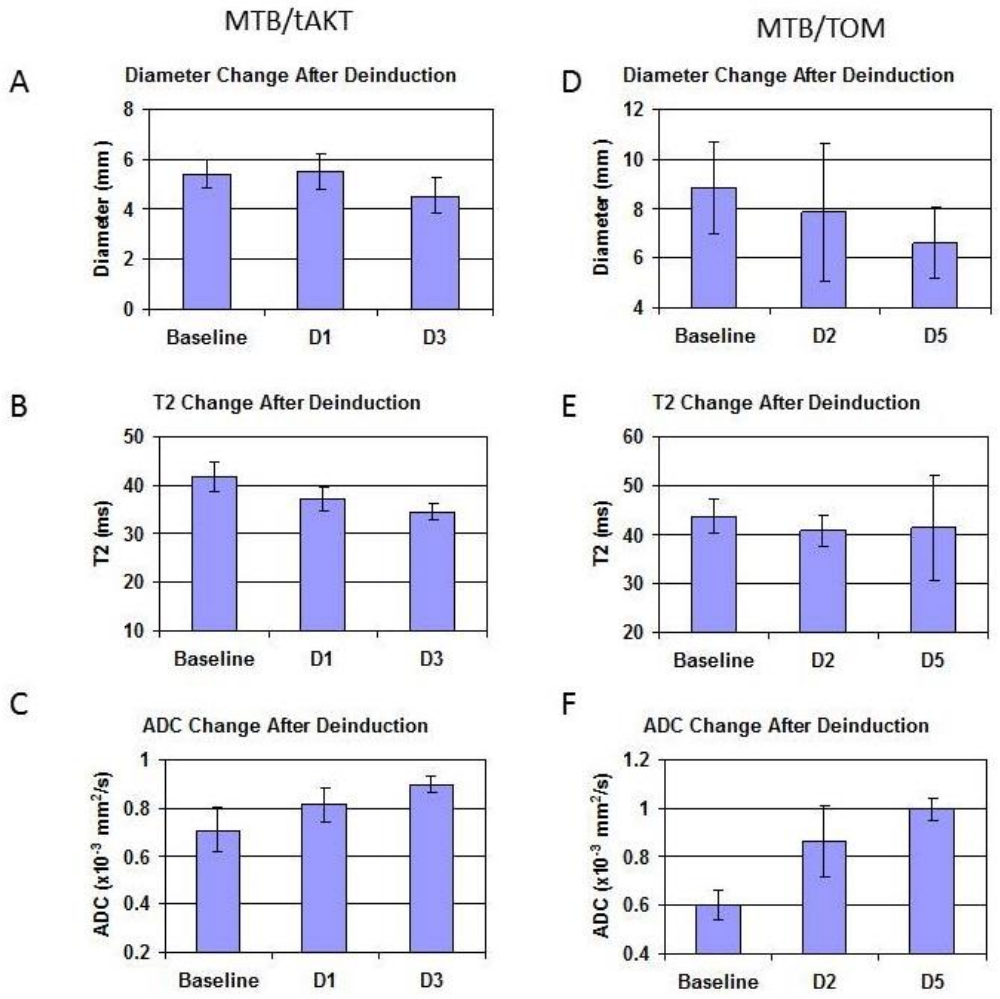


Figure 7. Mean values of diameter (**A, D**), T2 relaxation time (**B, E**), and ADC (**C, F**), for MTB/tAKT (A-C) and MTB/TOM (D-F) mammary tumors over time. Data are presented as mean ± STD. Baseline: 1 day prior to or the day of starting oncogene deinduction after MR imaging and n=3. D1-5 denotes the number of days after oncogene deinduction.

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 submit a manuscript based upon these studies, entitled "Detection of mammary tumor early response to oncogene downregulation in transgenic mouse models for breast cancer by diffusion MRI", with authors Xu M, Belka GK, Li L, Pickup S, Schnall MD and Chodosh LA. We anticipate submitting this to an imaging journal in mid to late 2015.

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 at the present time. However, we anticipate that the application of non-invasive imaging techniques, as pursued in this application, will enable the serial analysis of critical aspects of tumor progression and response to therapy that cannot be provided by longitudinal cross-sectional analysis of tumor-bearing populations. As such, we believe that this research

project provided additional support for the use of non-invasive imaging markers of early response to therapy in the identification of effective molecularly targeted agents, which would thereby facilitate the application of personalized medicine approaches to human cancers.

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 Lewis A. Chodosh	POSITION TITLE Professor and Chairman of Cancer Biology
eRA COMMONS USER NAME (credential, e.g., agency login) chodoshl	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Yale University	B.S	1981	Biochemistry
Harvard Medical School	M.D.	1989	Medicine
Massachusetts Institute of Technology	Ph.D.	1989	Biology, Dr. Phillip Sharp
Massachusetts General Hospital	Medicine	1991	Medicine Residency
Massachusetts General Hospital	Endocrinology	1994	Endocrine Fellowship
Harvard Medical School	Postdoc	1994	Breast Cancer, Dr. Philip Leder

A. Personal Statement

I am a physician-scientist in the Departments of Cancer Biology and Medicine in the Division of Endocrinology, Diabetes and Metabolism. I have extensive experience in the use of molecular, cellular, *in silico*, and animal model systems to study the function of key regulatory molecules in cancer biology, including the generation and analysis of transgenic and knockout mouse models of breast cancer, protein biochemistry, molecular biology, molecular genetics, cell biology, endocrinology, animal physiology, small animal imaging, genomics and computational biology. As a physician, I have expertise in internal medicine and endocrinology. I serve as Chairman for the Department of Cancer Biology, Associate Director of the Abramson Cancer Center, and Director of Cancer Genetics for the Abramson Family Cancer Research Institute. In addition, I am Editor-in-Chief of *Breast Cancer Research*, an advisor to the Nurses' Health Studies I and II, and Principal Investigator for an NCI UO1 that supports one of the sites of the NCI Mouse Models of Human Cancers Consortium. I have trained numerous graduate students and postdoctoral researchers in scientific methods and approach, and routinely teach undergraduates, graduate students, medical students and medical residents.

B. Positions and Honors

Current Positions and Employment

2005- Director, Cancer Genetics, Abramson Family Cancer Research Institute
2005-2012 Program Leader, Breast Cancer Program, Abramson Cancer Center
2006- Professor, Departments of Cancer Biology, Cell & Developmental Biology, and Medicine
Division of Endocrinology, Diabetes & Metabolism, Univ. of Pennsylvania School of Medicine
2006- Associate Director for Basic Science, Abramson Cancer Center
2008- Chairman, Department of Cancer Biology, University of Pennsylvania School of Medicine
2012- Co-Director, 2-PREVENT Breast Cancer Translational Center of Excellence, ACC

Honors (selected)

2002-	American Society for Clinical Investigation
2002	AACR-Sidney Kimmel Symposium for Cancer Research Scholar Award
2003-2009	Co-Chair, AACR Special Conference on Advances in Breast Cancer Research
2008-2012	J. Samuel Staub Professor of Cancer Biology
2008-	Association of American Physicians
2013	Department of Cancer Biology, Teaching Award

C. Selected Peer-reviewed Publications (selected from 128 peer-reviewed publications)

29. Magnitsky S, Belka GK, Sterner C, Pickup S, Chodosh LA and Glickson JD. Lactate detection in inducible and orthotopic Her2/neu mammary gland tumours in mouse models. *NMR in Biomedicine*, 48:353-64, 2012.
30. Chen CC, Stairs DB, Boxer RB, Belka GK, Horseman ND, Alvarez JV and Chodosh LA. Autocrine prolactin induced by the Pten-Akt pathway is required for lactation initiation and provides a direct link between the Akt and Stat5 pathways. *Genes & Development*, 26:2154-68, 2012.
31. Yeh ES, Belka GK, Vernon AE, Chen CC, Jung JJ and Chodosh LA. Hunk negatively regulates c-myc to promote Akt-mediated cell survival and mammary tumorigenesis induced by loss of Pten. *Proceedings of the National Academy of Sciences USA*, 110:6103-8, 2013.
32. Alvarez JV, Pan TC, Ruth J, Feng Y, Zhou AY, Pant D, Grimley JS, Wandless TJ, Demichele A and
Chodosh LA. Par-4 down-regulation promotes breast cancer recurrence by preventing multinucleation following targeted therapy. *Cancer Cell,* 24:30-44, 2013.
33. Feng Y, Pan TC, Pant DK, Chakrabarti KR, Alvarez JV, Ruth JR and Chodosh LA. SPSB1 promotes breast cancer recurrence by potentiating c-MET signaling. *Cancer Discovery,* 4:790-803. 2014.
34. Lee JV, Carrer A, Shah S, Snyder NW, Wei S, Venneti S, Worth AJ, Yuan ZF, Lim HW, Liu S, Jackson E, Aiello NM, Haas NB, Rebbeck TR, Judkins A, Won KJ, Chodosh LA, Garcia BA, Stanger BZ, Feldman MD, Blair IA and Wellen KE. Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. *Cell Metabolism,* 20:306-319, 2014.
35. Payne AW, Pant DK, Pan TC and Chodosh LA. Ceramide kinase promotes tumor cell survival and mammary tumor recurrence. *Cancer Research*, 21:6352-63, 2014.
36. Alvarez JV, Belka GK, Xu M, PanTC, Sudarshan S, Chen CC, Young B, Notarfrancesco K, Circiumaru V, Alavi A, Karp J, Acton P and Chodosh LA. Oncogene pathway activation in mammary tumors dictates [^{18}F]-FDG-PET uptake. *Cancer Research*, 2014 [Epub ahead of print].