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:** Lehigh University

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

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Nicole M. Corali, MBA, CRA

4. **Grant Contact Person's Telephone Number:** 610-758-4585

5. **Grant SAP Number:** 4100057665

6. **Project Number and Title of Research Project:** 1- Automated Analysis of Microtubule Dynamics to Study Cytoskeleton-Targeted Chemotherapies

7. **Start and End Date of Research Project:** 1/1/2012-6/30/2014

8. **Name of Principal Investigator for the Research Project:** Lynne Cassimeris, 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:

   $ 40,769.55

   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
Ciccone, Daniel	Research Technician	8% Yr 1	$2,423.38
Molnar, Cody	Graduate Student	10% Yr 1 20% Yr 2	$12,873.75
Yankovskaya, Lyubov	Graduate Student	2.5% Yr 1	$738.75

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
Molnar, Cody	Graduate Student	25% Yr 2; 50% Yr 3
Cassimeris, Lynne	Principle Investigator	10% Yr 1-3

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

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X____

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

11. Leveraging of Additional Funds

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

Yes_____ No_____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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

Yes____X_____ No_____

If yes, please describe your plans:
(1) The adoption of automated tracking methods will contribute to applications proposing to study cytoskeletal regulatory proteins that are potential chemotherapeutic targets. (2) A small NIH award (R21 or R15) would allow us to pursue possible mitotic functions of p53 beyond the regulation of microtubule assembly dynamics explored here.

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

    The results from this project are not publishable on their own. Additional studies will be necessary, but funding is not yet in place.

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

    Yes_____X____ No_____

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

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

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

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

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

Yes_____ No_____X_____

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

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

Yes_____ No____X_____

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

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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 Overview

The gene for p53, encoding a critical tumor suppressor protein, is mutated in at least 50% of all human cancers and lack of p53 is thought to facilitate cancer cell survival and tumor progression. Several functions have been ascribed to p53 in addition to its role as a transcription factor, including regulating microtubule assembly dynamics (Galmarini et al.). This latter function is of interest because cells harboring mutations in, or loss of, p53 are often more susceptible to Taxol (Wah et al.), a chemotherapeutic drug that prevents microtubule disassembly (Jordan et al.). While we know much about how Taxol stabilizes microtubules, we still do not know how Taxol treatment ultimately causes cell death and why Taxol appears to be much more effective in cells lacking p53. This project addressed one possible explanation for p53-dependent response to Taxol - that p53's contribution to microtubule dynamics makes these microtubules more responsive to Taxol.

Specific Aims:
Aim 1: Adapt automated particle tracking methods developed by others. Two research groups have developed automated tracking methods to detect the position of all GFP- EB1-marked

microtubule ends within an image obtained from a living cell. Each group built their analysis system in Matlab and each group freely shares their software with the research community. Our first goal is to optimize image acquisition conditions to best suit computer-based image recognition methods and to determine which algorithm provides the best analysis tools for both interphase and mitotic cells.

Aim 2: Does p53 regulate microtubule dynamics and antagonize Taxol? Our major goal is to examine microtubule assembly in cells manipulated to vary p53 expression and to examine how the cell's response to Taxol (at the level of microtubule assembly) depends on its p53 status. We will use transient expression of EB1-GFP in several cell lines and manipulate p53 as we have described previously.

Results

Introduction and rationale: Microtubules are polymers of tubulin subunits found within all eucaryotic cells that function to localize intracellular organelles, define cell shape, and move chromosomes at each cell division. This latter function has made microtubules a popular and successful target for chemotherapies; disrupting microtubule structure or function blocks cells in mitosis and ultimately leads to cell death. Vinblastine, vincristine and their derivatives prevent microtubule polymers from forming. These drugs are used to treat several cancers including Hodgkin's lymphoma, testicular cancer, non-small cell lung cancer, breast cancer, Kaposi's sarcoma, neuroblastoma and several others. Paclitaxel (tradename Taxol) and derivatives such as docetaxel have the opposite effect on microtubules, preventing their depolymerization. These microtubule stabilizing drugs also block cells in mitosis, leading to cell death. Normally, microtubules continually assemble and disassemble by a process called dynamic instability (shown below in Figure 1), a process vital for chromosome movement during mitosis. Drugs such as the vinca alkaloids or the taxanes disrupt this normal dynamic turnover and lead to mitotic defects and cell death.

Taxol and its derivatives, the taxanes, are used to treat breast, metastatic prostate, gastric and several other cancers. Susceptibility to the taxanes varies among different cancers and in many cases drug combination therapies are needed to control cancer cell proliferation. In addition, some cancers evolve resistance to the taxanes. The taxanes are also used in drug-eluting stents, where slow release of paclitaxel significantly limits scar tissue formation after angioplasty. Understanding how the taxanes disrupt microtubule functions, during both the mitotic and interphase times of the cell cycle, should allow design of novel drug combinations for cancer treatment and/or new uses for these drugs, such as the current use in drug-eluting stents.

The broad goals of this project are to develop new tools for study of microtubule assembly dynamics in living cells and to apply those tools to understand why cells harboring mutations in p53 are more susceptible to the taxanes. Several models could explain the greater susceptibility of p53 mutant cells to taxol treatment including: (1) differences in microtubule dynamic instability between p53 wild type and p53 deficient cells; (2) differences in response to cell cycle errors (for example, cells lacking p53 cannot induce expression of p21, a protein responsible for a G1 cell cycle arrest) Jordan et al.; Giannakakou et al.); and (3) differences in centrosome functions (recent data has indicated that p53 regulates activation of key enzymes driving mitotic

entry (Nam and van Deursen). Here we specifically examined whether p53 status changes microtubule dynamic instability, as reported previously in one study (Galmarini et al.), and whether p53 status changes the microtubule response to 10 nM taxol. This low dose of taxol is in line with concentrations present in patients undergoing chemotherapy (Yeung et al.).

Many previous studies compared cell lines differing in p53 level. A complication of this type of analysis is that each tumor cell line may differ in a number of ways. For example different cell lines may have polymorphisms in genes encoding the tubulins (subunits of microtubules) that make those microtubules more or less sensitive to Taxol. Such polymorphisms have been documented by others (e.g. Haber et al.). Additionally, cell lines are known to differ in the activity of their multi-drug resistance channel, a protein that actively pumps drugs out of cells. To avoid possible cell line differences in response to Taxol, we used Hela cells for our studies. This cell line lacks detectable p53 protein because they were transformed by human papillomavirus (HPV) genome integration. The HPV E6 protein targets p53 for destruction and keeps the p53 level low in these cells. Depletion of E6 allows restoration of p53 expression.

Results of Aim 1 - Implement automated image analysis methods: A major goal of the proposed research was to adopt and implement automated computer-based methods for image analysis for measurements of microtubule assembly dynamics. At the start, we compared several user-generated programs and settled on one initially developed by Dr. Torsten Wittman and colleagues at the University of California, San Francisco (published in Nature Methods. 2010. 7: 761-768). In initial studies, we optimized algorithms for feature detection to provide the best detection of individual moving objects in sequential image frames. In an iterative process, the microscope, laser and camera settings were also adjusted to maximize contrast within fluorescent images and reduce background noise, while at the same time preserving living cell health over the course of at least 60 minutes. These optimizations required trial-and-error approaches, but have led to a set of conditions for image acquisition and analysis that match well with published data analyzed by non-automated "hand-tracked" image series. These automated tracking methods make use of a fluorescent biosensor that my lab introduced 10 years ago as a microtubule end marker for high resolution imaging. EB1 fused to GFP appears as a 1 µm long dash at the tip of each microtubule. Because these proteins bind only to growing microtubule tips, they appear to move as a comet-like shape across the inside of the cell. The movement of the comet actually reflects polymerization (growth) of a microtubule polymer. The automated tracking methods apply computer algorithms to identify each comet within a single frame and then identify that comet in sequential frames. Examples from region of single cells are shown in Figures 1, 2.

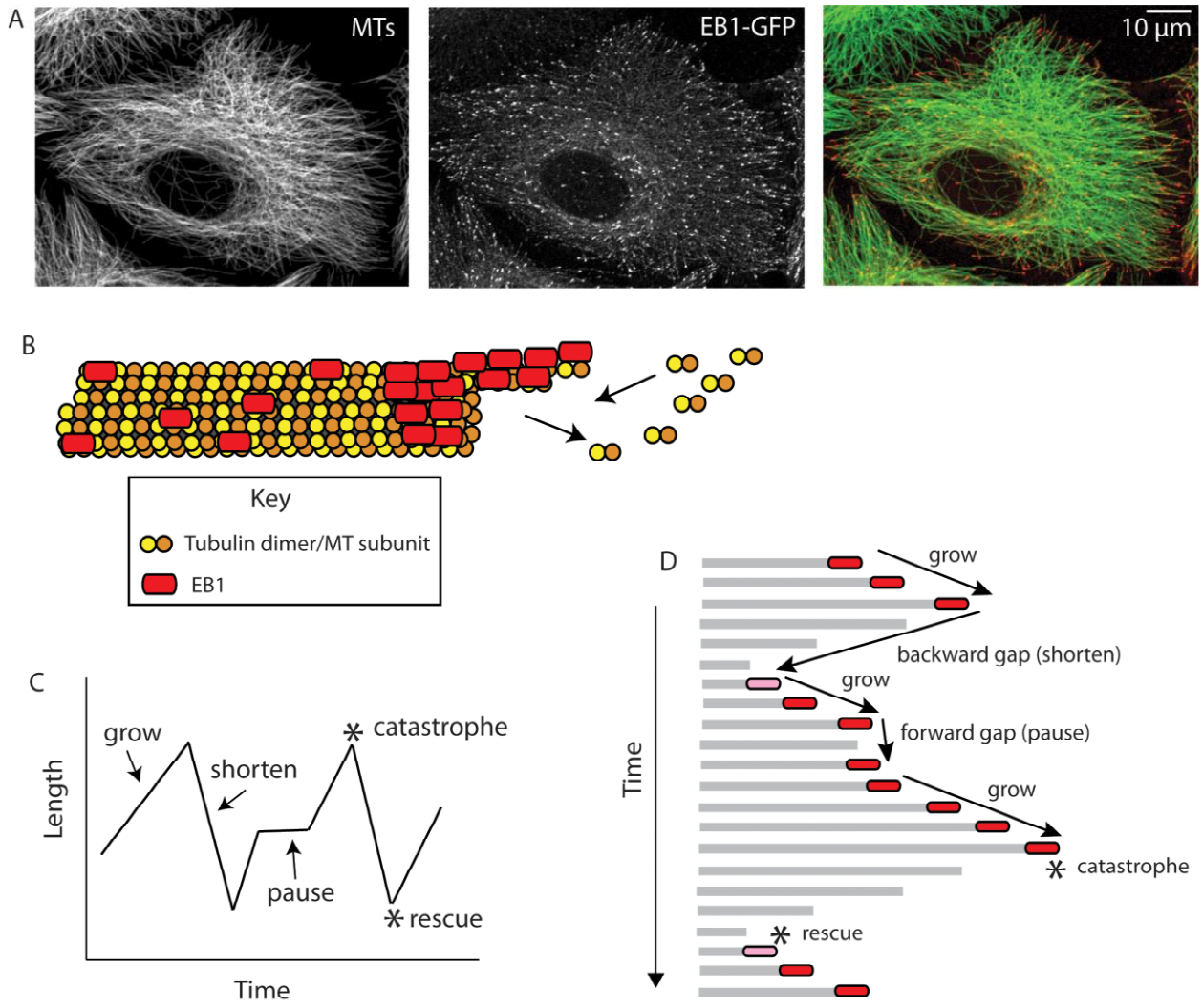

Figure 1: Microtubule and EB1 localizations, microtubule turnover by dynamic instability and outline of dynamics parameters measured by TipTracker software. (A) Microtubules (MTs) and EB1-GFP localization in a Hela cell after fixation and staining of tubulin subunits. Overlay image shows microtubules in green and EB1-GFP in red (pseudocolors selected for contrast). (B) Schematic showing polymerization of tubulin dimers (yellow/orange peanut shapes) into a microtubule polymer. EB1 (red rectangles) proteins bind predominately to the ends of polymerizing microtubules. (C) Schematic representing the constant turnover (polymerization and depolymerization) of a microtubule undergoing the state-switches characteristic of dynamic instability. Microtubule ends can exist in one of three states (growth, shortening or pause) and can switch abruptly between these states during molecular events termed catastrophe and rescue (marked by *'s). (D) Schematic showing EB1 (red rectangle) bound to the tip of a growing microtubule. The underlying microtubule is unlabeled in our experiments but represented here by the grey lines. Automated tracking of EB1-GFP over time allows direct measurement of microtubule growth velocity and extrapolation of other parameters as outlined in the schematic. The pink rectangles represent newly forming concentrations of EB1-GFP as a microtubule begins a growth phase.

Figure 2

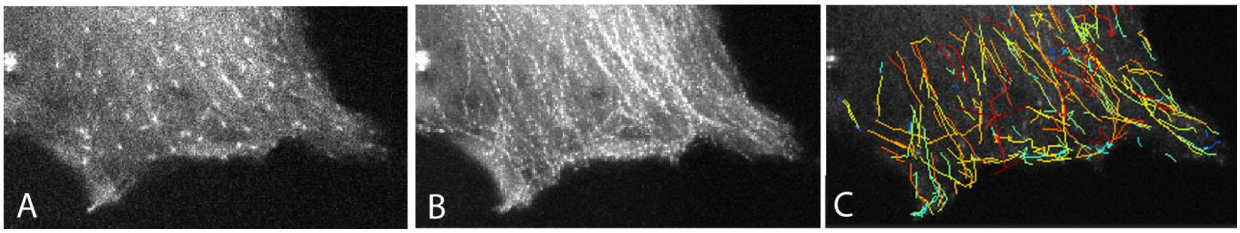

Figure 2: Example of automated microtubule tip tracking. (A) single frame from the edge of a Hela cell expressing EB1-GFP. (B) Overlay of 26 sequential images, collected at 2 second intervals, for the same region shown in (A). The growth of each microtubule is detectable as a "dashed line." (C) The same 26 frames were analyzed by Tip Tracker software. Each colored line represents the growth of an individual microtubule. The rate of polymerization is determined from these tracks. Much other information can be retrieved, including the directions of growth across the cell and the rate of turnover of individual microtubules. The 26 images shown here represent only a small region from a single cell and are only a small portion of a 5 minute time series (150 total images).

Parameters describing dynamic instability were calculated based on automated image analysis. EB1-GFP bound at microtubule tips has a length of about 1 µm, consistent with measurements by others (Seetapun et al.). This size equates to approximately 4 - 10 pixels in length using our imaging protocol. The fluorescence intensity of 4 pixels is sufficient to allow detection against the weak background fluorescence from the pool of EB1-GFP that is unbound to microtubules. Microtubules do not bend over sharp angles and therefore we found that limiting the automated search by a forward angle of 30° maximum and a backward angle of 10° maximum was sufficient for the algorithm to follow a single "comet" in successive images. We also established that a time window of 13 seconds was optimal for allowing "comets" to come in and out of focus, while still being detected by the algorithm as representing the same microtubule. A small number for this time window minimizes false positives for pause (undetectable growth or shortening), while larger time windows provide a more accurate tracking of microtubules as they shift in space along the Z axis. The 13 second time window provides the best compromise, as determined by analysis of test samples over a range of time windows from 2 - 30 seconds. Other parameters used by the algorithm did not have a significant impact on the numbers generated and thus the algorithm is fairly robust and largely independent of most parameters used to follow individual fluorescent "comets." Nevertheless, we settled on a set of algorithm parameters after trial and error and used that set throughout all subsequent experimental analyses.

Our selected algorithm parameters yielded values consistent with previously published measurements of microtubule dynamics as shown in Table 1. Here we measured values in cells transfected with a non-targeting siRNA (control) and DMSO (vehicle for most drugs). The table also includes comparison to data reported by Applegate et al. In general, our measurements match well with previously published analyses.

Table 1

Parameter	non-targeting siRNA + DMSO	Applegate et al.
Growth Rate (µm/min)	21.07 ± 3.75	21.7 ± 9.8
Growth lifetime (sec)	9.23 ± 1.28	12.9±11.3
Growth Length (µm)	3.078 ± 0.682	5.3±6.0
Forward Gap (pause) Lifetime (sec)	13.99 ± 2.14	13.1 ± 6.2
Forward Gap (pause) Rate per Micron	0.565 ± 0.201	0.17
Backward Gap (shortening) Rate (µm/min)	38.42 ± 7.78	26.3 ± 5.4
Backward Gap (shortening) Lifetime (sec)	6.57 ± 1.91	12.0 ± 4.9
Backward Gap (shortening) Length ((µm)	4.13 ± 1.36	5.2

For subsequent work it is imperative that EB1-GFP binds microtubule ends in the presence of the experimental treatment since this binding is our marker for microtubule ends. Therefore we asked whether Taxol or E6 depletion negatively impacted EB1-GFP binding to microtubules (Figure 3). In cells fixed and stained for tubulin, neither Taxol incubation nor E6 depletion disrupted EB1-GFP binding to microtubules. In addition, neither Taxol treatment nor E6 depletion changed the latency time for formation of EB1-GFP accumulation at newly growing microtubule ends. Latency time was calculated as described by Thoma et al. and is depicted schematically in Figure 1 by the light pink comets.

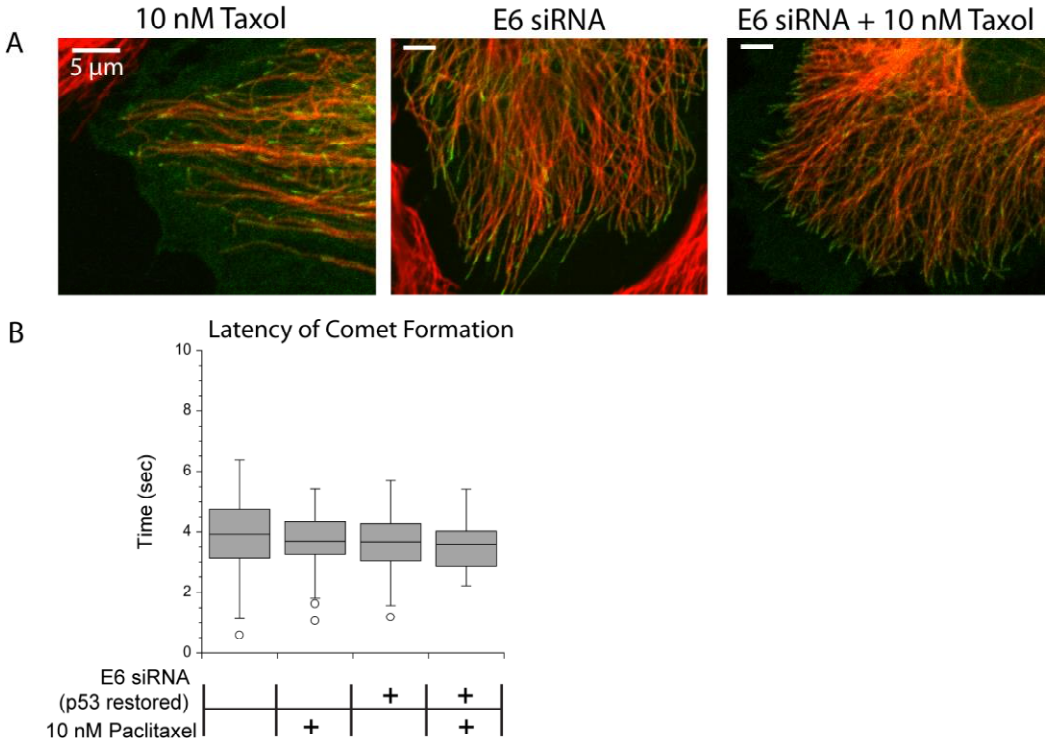

Figure 3. Neither Taxol nor E6 depletion disrupts EB1-GFP binding to microtubule ends. (A) immunofluorescence images of cells fixed and stained for tubulin (red). Cells were transfected with a plasmid encoding EB1-

GFP 24 - 48 hr before fixation. EB1-GFP (shown in green) is bound to microtubule ends under all experimental conditions. (B) The time required for growing microtubules to form an aggregate of EB1-GFP at their tip was consistent across treatments. No significant differences in latency were observed.

In summary, we achieved the major goals of Aim 1 by developing procedures for GFP-tagged protein expression, image acquisition and image analysis to best allow automated detection of fluorescently labeled microtubule tips. We also optimized various parameters within the software to insure accurate and reproducible detection of labeled microtubule tips, without picking up signal from fluorescence background signals. We had initially proposed to analyze microtubule dynamics within both interphase and mitotic cells. The density of microtubules, even when using EB1-GFP to only mark microtubule ends, made it difficult to image microtubules during this cell cycle stage. Therefore, we limited our analyses to interphase cells. While Taxol (below) is thought to induce cell death due to defects in mitosis, it also stabilizes microtubules during interphase.

Results from Aim 2 - Using our established imaging conditions and optimized analysis algorithms, we collected the large data sets needed to detect even small changes to individual microtubule turnover rates. Using these methods we followed the dynamics of thousands of microtubules, where previously researchers had to make conclusions based on analysis of 25 - 50 microtubules per condition. We applied these methods to cells expressing different levels of p53, a tumor suppressor protein that has been reported to regulate microtubule dynamics, among its other functions. Figure 3 shows an immunoblot demonstrating our ability to restore p53 expression in Hela cells. Here, we depleted Hela cells of E6, a protein encoded by the human papillomavirus (HPV) which targets p53 for destruction. By depleting Hela cells of HPV protein E6, we restore p53 protein level to that typical of epithelial cells.

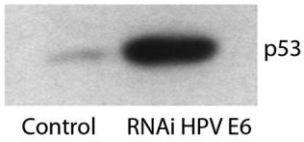

Figure 3. Immunoblot demonstrating manipulation of p53 protein level. P53 is restored in Hela cells by depleting them of the HPV viral protein E6. Normally in these cells, E6 targets p53 for destruction. Depleting cells of E6 allows p53 to return to a level typical of epithelial cells. These conditions were used to probe p53-dependent changes to the microtubule cytoskeleton.

Using automated microtubule tracking algorithms, we measured the dynamic turnover of over 14,000 -16,000 microtubules in each experimental condition, compared to previously reported results from <35 microtubules per condition. Two regions within each cell were also analyzed separately: the cell edge, defined as the region within 5 µm of the plasma membrane and the cell interior, defined as the remainder of the cytoplasm. Others had previously demonstrated that microtubule polymerization dynamics can differ between the cell edge and the cell interior (Wittmann et al.), and we did not want to miss possible p53-dependent changes by pooling data from microtubule ends located at all locations throughout the cell.

The data are presented in Figure 4 and in Tables 2 and 3 below. Briefly, treating Hela cells with 10 nM Taxol resulted in more stable microtubules at the cell edge and the cell interior;

microtubules polymerize at a slower rate and spend more time in a growth state, they pause for longer durations and they shorten at a slower rate compared to DMSO treated cells. Restoring p53 to Hela cells resulted in relatively minor effects on microtubule dynamics. Cells with restored expression of p53 also responded to Taxol in much the same ways as did Hela cells (low p53). Taken together, our data demonstrate that Taxol stabilizes microtubules independent of p53 status.

Figure 4

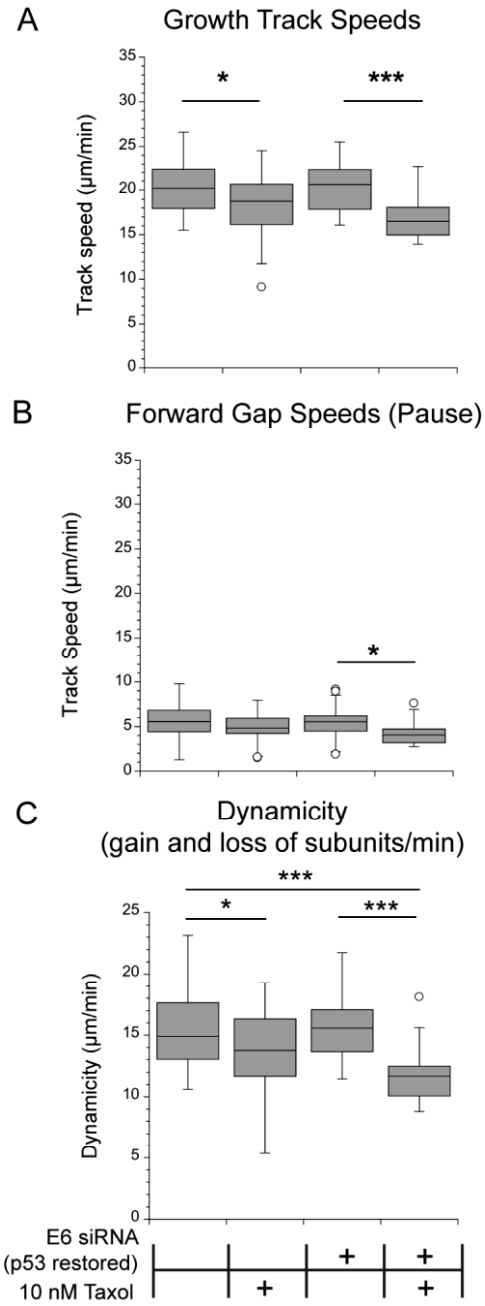

Figure 4: Microtubule dynamics in response to p53 level and/or 10 nM Taxol. Cells were incubated in 10 nM Taxol for 4 hours prior to imaging. Dynamicity is a sum of the gain and loss of tubulin subunits per unit time and is a convenient measure for comparison between treatments. Note that microtubule polymers contain 1624 tubulin dimers/µm, so dynamicity values of 15 µm/min represent the gain and loss of tens of thousands of subunits each minute. Addition of Taxol slows microtubule growth and reduces dynamicity independent of p53 status. Values shown here represent data from the entire cell volume.

Table 2 provides a list of values for all parameters measured at the edge of the cell and compares 4 cell treatments: Control siRNA transfectants, E6-depleted (p53 restored), Control siRNA transfectants + 10 nM Taxol, E6-depleted (p53 restored) + 10 nM Taxol.

Table 2: Microtubule Dynamics Parameters Measured at the Cell Edge

	Control siRNA + DMSO	E6 siRNA (p53 restored)	% change vs. control siRNA + DMSO	Control siRNA + 10 nM Taxol	% change vs. control siRNA + DMSO	E6 siRNA (p53 restored) + 10 nM Taxol	% change vs. E6 depletion alone
Growth Rate (µm/min)	21.07 ± 3.75	19.61 ± 2.78		17.28 ± 3.48	-18.0***	16.90 ± 2.61	-18.0***
Growth lifetime (sec)	9.23 ± 1.28	10.09 ± 1.27	+7.9*	9.68 ± 1.02	-	10.14 ± 1.06	-
Growth Length (µm)	3.078 ± 0.682	3.11 ± 0.602		2.65 ± 0.684	-13.9***	2.68 ± 0.565	-14.1**
Pause Lifetime (sec)	13.99 ± 2.14	13.49 ± 1.52		13.69 ± 1.26	-	14.21 ± 0.983	-
Pause Rate per Minute	0.151 ± .0284	0.153 ± 0.0154		0.153 ± 0.0160	-	0.154 ± 0.0173	-
Pause Rate per Micron	0.565 ± 0.201	0.689 ± 0.351		0.767 ± 0.334	+35.6***	0.800 ± 0.236	-
Shortening Rate (µm/min)	38.42 ± 7.78	36.43 ± 5.78		32.85 ± 6.01	-14.5***	32.47 ± 5.42	-17.1***
Shortening Lifetime (sec)	6.57 ± 1.91	6.41 ± 1.46		6.42 ± 1.51	-	5.79 ± 1.44	-14.9***
Shortening Length (µm)	4.13 ± 1.36	3.84 ± 1.01		3.47 ± 1.06	-15.8*	3.06 ± 0.776	-28.4***
Rescue Rate per Minute	0.129 ± 0.069	0.158 ± 0.0647		0.136 ± 0.0699	-	0.144 ± 0.0683	
Rescue Rate per Micron	0.391 ± 0.227	0.536 ±0.275		0.578 ± 0.411	+47.6*	0.556 ± 0.284	
Catastrophe Rate per Minute	0.160 ± 0.0164	0.152 ± 0.0108	-4.3*	0.157 ± 0.00885	-	0.152 ± 0.00809	
Catastrophe Rate per Micron	0.746 ± 0.367	0.735 ± 0.159		0.947 ± 0.521	+26.8*	0.847 ± 0.192	
Percent Time Spent in Growth	73.90 ± 8.48	77.55 ± 6.26	+5.1*	77.2 ±6.83	+4.5*	77.27 ± 4.69	
Percent Time Spent in Pause	24.7 ± 8.38	21.01 ± 5.15	-15.4*	21.63 ± 5.87	-12.4*	22.04 ± 4.39	
Percent Time Spent in Shortening	1.40 ± 1.35	1.44 ±2.30		1.17 ± 1.43		0.689 ± 0.654	
Number of Cells	49	33		41		23	

* denotes p<0.05, ** denotes p<0.01, *** denotes p<0.001.

Table 3 contains the same data, but for microtubules located within the cell interior.

Table 3: Microtubule Dynamics Parameters Measured at the Cell Interior

	Control siRNA + DMSO	E6 siRNA (p53 restored)	% change vs. control siRNA + DMSO	Control siRNA + 10 nM Taxol	% change vs. control siRNA + DMSO	E6 siRNA (p53 restored) + 10 nM Taxol	% change vs. E6 depletion alone
Growth Rate (µm/min)	20.38 ± 2.99	20.62 ± 2.72		18.60 ± 3.42	-8.8**	16.24 ± 2.79	-18.1***
Growth lifetime (sec)	9.40 ± 1.50	9.86 ± 1.18		9.45 ± 1.19		10.14 ± 1.37	
Growth Length (µm)	2.99 ± 0.671	3.19 ± 0.56		2.79 ± 0.605		2.65 ± 0.671	-15.7***
Pause Lifetime (sec)	14.32 ± 2.35	13.43 ± 1.80		13.72 ± 1.69		13.91 ± 1.20	+5.9*
Pause Rate per Minute	0.149 ± 0.0422	0.152 ± 0.0157		0.155 ± 0.0184		0.152 ± 0.0163	
Pause Rate per Micron	0.716 ± 0.731	0.594 ± 0.208		0.694 ± 0.264		0.796 ± 0.310	+29.7***
Shortening Rate (µm/min)	38.58 ± 7.76	35.96 ± 5.34		32.68 ± 7.83	-10.7*	30.52 ± 5.09	-10.0*
Shortening Lifetime (sec)	6.85 ± 2.04	5.86 ± 1.39	-14.6*	6.67 ± 1.99		5.44 ± 0.936	
Shortening Length (µm)	4.21 ± 1.67	3.48 ± 0.964	-17.0*	3.52 ± 1.20		2.77 ± 0.754	
Rescue Rate per Minute	0.128 ± 0.0780	0.146 ± 0.0628		0.125 ± 0.0690		0.119 ± 0.0517	
Rescue Rate per Micron	0.455 ± 0.339	0.684 ± 0.731		0.552 ± 0.410		0.555 ± 0.378	
Catastrophe Rate per Minute	0.160 ± 0.0165	0.158 ± 0.0146		0.161 ± 0.0136		0.152 ± 0.0119	-3.9*
Catastrophe Rate per Micron	0.746 ± 0.306	0.741 ± 0.163		0.796 ± 0.231		0.972 ± 0.659	-14.4*
Percent Time Spent in Growth	76.9 ± 8.49	78.79 ± 5.97		78.4 ± 7.34		77.33 ± 4.02	
Percent Time Spent in Pause	21.8 ± 7.75	20.07 ± 5.51		20.37 ± 6.40		21.94 ± 3.84	
Percent Time Spent in Shortening	1.26 ± 1.58	1.14 ± 1.65		1.23 ±1.37		0.720 ± 0.543	
Number of Cells	49	33		41		23	

* denotes $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$.

Our data indicate that some of the previously reported p53-dependent changes to microtubule assembly dynamics are not detectable in very large samples (with greater statistical power). Indeed, almost all parameters describing microtubule dynamic instability were unchanged by restoring p53 protein. We found that shortening microtubules were more likely to switch back into a growth phase when p53 is present, but parameters such as growth and shortening velocities were unchanged. We also found that p53 expression level had little impact on the microtubule response to Taxol. These data make it unlikely that p53 expression dictates cellular response to Taxol simply via microtubule dynamics. Instead, downstream consequences, such as mitotic errors and loss of mitotic checkpoint control are likely responsible for cell-type differences in response to Taxol.

References Cited

Applegate et al. 2011. PlusTipTracker: quantitative image analysis software for the measurement of microtubule dynamics. *Journal of structural biology* 176: 168-184.

Galmarini et al. 2003. Drug resistance associated with loss of p53 involves extensive alterations in microtubule composition and dynamics. *British journal of cancer* 88: 1793-1799.

Giannakakou et al. 2001. Low concentrations of paclitaxel induce cell type-dependent p53, p21 and G1/G2 arrest instead of mitotic arrest: molecular determinants of paclitaxel-induced cytotoxicity. Oncogene. 20: 3806-3813.

Haber et al. 1995. Altered expression of M beta 2, the class II beta-tubulin isotype, in a murine J774.2 cell line with high expression of taxol resistance. J. Biol. Chem. 270:31269-31275.

Jordan et al. 1996. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death." *Cancer research* 56: 816-825.

Nam and van Deursen. 2014. Cyclin B2 and p53 control proper timing of centrosome separation. Nature Cell Biology. 16: 535-546.

Matov et al. 2010. Analysis of microtubule dynamic instability using a plus end growth marker. Nature Methods. 7: 761-768.

Seetapun et al. 2012. Estimating the microtubule GTP cap size in vivo. Curr. Biol. 22: 1681-1687.

Thoma et al. 2010. Quantitative image analysis identifies pVHL as a key regulator of microtubule dynamic instability. J. Cell Biol. 190: 991-1003.

Wahl et al. 1996. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nature Medicine. 2: 72-79.

Wittmann et al. 2003. Regulation of leading edge microtubule and actin dynamics downstream of Rac1. J. Cell Biol. 161: 845-851.

Yeung et al. 1999. The mode of action of taxol: apoptosis at low concentration and necrosis at high concentration. *Biochemical and biophysical research communications* 263: 398-404.

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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

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

Yes_____X____ No_____

If yes, please describe your plans: The small project funded here addresses one possible mechanism underlying cell sensitivity to Taxol. Additional experiments are necessary to address other possible mechanisms.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Lynne Cassimeris	Professor of Biological Sciences
eRA COMMONS USER NAME (credential, e.g., agency login) CASSIMERIS	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Springfield College, Springfield, MA	B.S.	1980	Biology
Univ. North Carolina, Chapel Hill, NC	Ph.D.	1988	Cell Biology
Univ. Pennsylvania, Philadelphia, PA	Post-doc	1988-1992	Cell Biology

A. Positions and Honors

Positions and Employment

1992 – 1996 Assist Prof, Molecular Biology, Biological Sciences, Lehigh University
1996 – 2004 Associate Professor, Biological Sciences, Lehigh University
2000 Visiting Associate Professor, Dept. Cell Biology, Duke University, Durham, NC
2004 - Professor, Biological Sciences, Lehigh University

Other Experience and Professional Activities

Editorial Boards:

Cytoskeleton (2008 - present)
Cellular and Molecular Bioengineering (2008 - 2012)

Study Sections:

Dept. of Defense. Breast Cancer Research Program.	Member (1998)
American Cancer Society. Cell Cycle and Growth Control	Member (2004-2008)
NIH Biology 2	Member (1999)
NIH CDF4	Member (2000, 2002)
NICH Cell dev. and function (NRSA)	Member (2005; 2007)
NIH NICHD R03	Member (2005 -06)
NIH ZRG-1-CB-N02	Member (2008)
NSF. Cell Biology	Panel Member (2000, 2009)
NIH. Intercellular Interactions	Member (2013)

Honors (1992 - present)

1992 – 1996 Dana Foundation Faculty Fellow
1999 – 2001 Distinguished Associate Professor, Endowed by Lehigh Univ. Class of ‘61
2002 Distinguished Graduate Alumnus, University of North Carolina, Chapel Hill
2004 – 2007 Keith R. Porter Fellow
2006 Eleanor and Joseph F. Libsch Research Award, Lehigh University

2011-2012 New Directions Fellowship. Lehigh University College of Arts and Sciences

B. Selected peer reviewed publications (of 80 total; h-factor = 39 calculated on May 6, 2014 via Google Scholar) Members of my group are underlined.

N.T. Thomas, R. Thamkachy, K.C. Sivakumar, S.K. Jayakumari, X.L. Louis, S.A. Thomas, R. Kumar, K.N. Rajasekharan, L. Cassimeris and S. Sengupta. 2014. Reversible Action of Diaminothiazoles in Cancer Cells is Implicated by the Induction of a Fast Conformational Change of Tubulin and Suppression of Microtubule Dynamics. Mol. Cancer Ther. 13: 179-189.

V. Caruso Silva and L. Cassimeris. 2013. Stathmin and microtubules regulate mitotic entry in Hela cells by controlling activation of both Aurora Kinase A and Plk1. Mol. Biol. Cell. 24: 3919-3931.

L. Cassimeris, L. Guglielmi, V. Denis, C. Larroque and P. Martineau. 2013. Specific in vivo labeling of tyrosinated a-tubulin and measurement of microtubule dynamics using a GFP tagged, cytoplasmically expressed recombinant antibody. PLoS One. 8:e59812.

S. Nakamura, I. Grigoriev, T. Nogi, T. Hamaji, L. Cassimeris and Y. Mimori-Kiyosue. 2012. Dissecting the nanoscale distributions and functions of microtubule-end-binding proteins EB1 and ch-TOG in interphase HeLa cells. PLoS One. 7: e51442.

Bruce K. Carney, V. Caruso Silva and L. Cassimeris. 2012. The microtubule cytoskeleton is required for a G2 cell cycle delay in cancer cells lacking stathmin and p53. Cytoskeleton. 69: 278-289.

Blake-Hodek, K.A., L. Cassimeris and T.C. Huffaker. 2010. Regulation of microtubule dynamics by Bim1 and Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. Mol. Biol. Cell. 21: 2013 - 2023.

B.K. Carney and L. Cassimeris. 2010. Stathmin/oncoprotein 18, a microtubule regulatory protein, is required for survival of both normal and cancer cell lines lacking the tumor suppressor, p53. Cancer Biol. and Therapy. 9: 699-709.

M. Murphy and L. Cassimeris. 2006. A novel cancer therapy approach targeting microtubule function. Cancer Biol. Ther. 5: 1721 – 1723.

Piehl, M. U.S. Tulu, P. Wadsworth and L. Cassimeris. 2004. Centrosome maturation: Measurement of microtubule nucleation throughout the cell cycle using GFP tagged EB1. Proc. Natl. Acad. Sci. 101: 1584-1588.

M. Piehl and Cassimeris, L. 2003. Organization and dynamics of growing microtubule plus ends during early mitosis. Mol. Biol. Cell. 14: 916 - 925.