

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE		State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number
5. APPLICANT INFORMATION		Organizational DUNS*: 004514360
Legal Name*: University of Pittsburgh Department: Office of Research Division: Street1*: 123 University Place Street2: City*: Pittsburgh County: State*: PA: Pennsylvania Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 15213-2303		
Person to be contacted on matters involving this application Prefix: Mr. First Name*: Brian Middle Name: Last Name*: Balich Suffix: Position/Title: Grants and Contracts Officer Street1*: 123 University Place Street2: B21 UCLUB City*: Pittsburgh County: State*: PA: Pennsylvania Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 15213-2303 Phone Number*: 412-624-7400 Fax Number: 412-624-7409 Email: offres@offres.pitt.edu		
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1250965591A6
7. TYPE OF APPLICANT*		X: Other (specify)
Other (Specify): Private Non-Profit State-related Educ Institution Small Business Organization Type <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged		
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).
<input checked="" type="radio"/> New <input type="radio"/> Resubmission <input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision		<input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration <input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify) :
Is this application being submitted to other agencies?* <input type="radio"/> Yes <input checked="" type="radio"/> No What other Agencies?		
9. NAME OF FEDERAL AGENCY*		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
National Institutes of Health		TITLE:
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*		
Oxygen impact on tumor metastasis dormancy and therapy		
12. PROPOSED PROJECT		13. CONGRESSIONAL DISTRICTS OF APPLICANT
Start Date*	Ending Date*	PA-014
07/01/2015	06/30/2020	

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Colin Middle Name: Henry Last Name*: Beckwitt Suffix:
 Position/Title: MD/PHD Candidate
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 State*: PA: Pennsylvania
 Province:
 Country*: USA: UNITED STATES
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15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* \$439,430.00
 b. Total Non-Federal Funds* \$0.00
 c. Total Federal & Non-Federal Funds* \$439,430.00
 d. Estimated Program Income* \$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
 DATE:
 b. NO PROGRAM IS NOT COVERED BY E.O. 12372; OR
 PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Jennifer Middle Name: E Last Name*: Woodward Suffix: PHD
 Position/Title*: Associate Vice Provost for Research Operation
 Organization Name*: University of Pittsburgh
 Department: Office of Research
 Division:
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 Street2:
 City*: Pittsburgh
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 State*: PA: Pennsylvania
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 Country*: USA: UNITED STATES
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Signature of Authorized Representative*

Brian Balich

Date Signed*

12/04/2014

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:1234-Coverletter_Beckwitt_Final.pdf

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Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Pittsburgh
Duns Number: 0045143600000
Street1*: 123 University Place
Street2:
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 15213-2303
Project/Performance Site Congressional District*: PA-014

File Name

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* <input checked="" type="radio"/> Yes <input type="radio"/> No	
1.a. If YES to Human Subjects Is the Project Exempt from Federal regulations? <input checked="" type="radio"/> Yes <input type="radio"/> No If YES, check appropriate exemption number: — 1 — 2 — 3 <input checked="" type="checkbox"/> 4 — 5 — 6 If NO, is the IRB review Pending? <input type="radio"/> Yes <input type="radio"/> No IRB Approval Date: Human Subject Assurance Number 00006790	
2. Are Vertebrate Animals Used?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
2.a. If YES to Vertebrate Animals Is the IACUC review Pending? <input type="radio"/> Yes <input type="radio"/> No IACUC Approval Date: Animal Welfare Assurance Number	
3. Is proprietary/privileged information included in the application?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
4.b. If yes, please explain: 4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? <input type="radio"/> Yes <input type="radio"/> No 4.d. If yes, please explain:	
5. Is the research performance site designated, or eligible to be designated, as a historic place?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
5.a. If yes, please explain:	
6. Does this project involve activities outside the United States or partnership with international collaborators?* <input type="radio"/> Yes <input checked="" type="radio"/> No	
6.a. If yes, identify countries: 6.b. Optional Explanation:	
7. Project Summary/Abstract*	Filename 1235- Project_Summary_Beckwitt_Final.pdf
8. Project Narrative*	1236- Project_Narrative_Beckwitt_Final.pdf
9. Bibliography & References Cited	1237-Bibliography_Beckwitt_Final.pdf
10. Facilities & Other Resources	1238-Facilities_Beckwitt_Final.pdf
11. Equipment	1239-Equipment_Beckwitt_Final.pdf
12. Other Attachments	1240- Additional_Educational_Information_Beckwitt_Final.pdf

7. PROJECT SUMMARY/ABSTRACT

Following dissemination from a primary lesion, a few surviving carcinoma cells establish residency in distant sites (micrometastases). Many micrometastases assume a dormant (quiescent) cellular phenotype, characterized by a G0/G1 arrest that may persist years to decades before outgrowth to form clinically evident metastases; these emergent tumors almost inexorably lead to death. Unfortunately, micrometastases often appear to be refractory to therapeutic agents, even ones that show success against the primary tumors from which they derive. This situation is particularly daunting in breast cancer that can lie dormant for a decade or more before recurring.

Signals from the tumor microenvironment have been shown to play a critical role in maintaining this dormant phenotype on the one hand or facilitating emergence from cellular quiescence on the other. Culture of breast cancer cell lines in a novel all human liver microphysiological system (MPS) our lab employs has been shown to reliably induce cancer cell dormancy. Traditionally, rapidly proliferating cancer cells resort to glycolysis in order to accumulate the massive amounts of metabolic intermediates required for cell division ("Warburg Effect"). However, the phenotypically dormant cancer cells may exhibit a different metabolic profile, which may be essential to both maintaining quiescence and successful novel treatment approaches. *Our foundational model is that dormant micrometastases adapt a low metabolic and proliferative state concordant with chemoresistance and reduced glycolytic flux. We hypothesize that oxygen tension levels will dictate the switch between oxidative phosphorylation and glycolytic metabolism, and thereby impinge on dormancy.*

This model will be tested by measuring the basal glycolytic and oxidative phosphorylation fluxes of dormant cancer cells by quantifying glucose uptake, glycolytic enzyme expression, and intracellular ATP concentration through cell culture experiments and immunofluorescence. A novel hemoglobin-based oxygen carrier (HBOC) will be introduced to the system to modulate the oxygen tension to which the cancer cells and liver tissue are exposed. Sensitive ruthenium oxygen sensors will be used to determine the oxygen tension to which the cells are exposed and the trans-tissue oxygen gradient. The impacts of oxygen tension on tumor dormancy phenotype and cell metabolism will be determined. Finally, select anti-tumor agents, including the chemotherapeutics doxorubicin and cisplatin, will be incorporated into the liver MPS to determine whether therapeutic efficacy is dependent on cancer cell metabolic state and additionally monitor toxicities to the liver tissue.

Understanding the influence of oxygen tension on dormant micrometastasis phenotype, metabolism, and treatment susceptibility is expected to yield insights into basic tumor biology and promote translational studies into improving the efficacy of current treatments (drug rescue) and the discovery of novel therapies.

8. PROJECT NARRATIVE

Tumor metastasis to the liver, one of the most common manifestations of advanced cancers, commonly establishes dormant, treatment refractory micrometastases and that herald mortality from the disease. The metabolic activity of these dormant tumor cells will be studied along with the impact of altering the oxygen tension of the tumor microenvironment on cancer cell metabolism, dormancy phenotype, and treatment potentiation. Understanding these processes will provide novel insights into the basic tumor biology of metastases, promote translational studies into improving the efficacy of existing agents (drug rescue), and motivate the development of new adjuvant therapies for this mortal stage of cancer progression.

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10. FACILITIES

All necessary facilities and equipment are in place at the present time.

University of Pittsburgh Department of Pathology (Wells lab)

Laboratory: Wells has ~2000 sq ft of general laboratory space in Scaife Hall, 7th floor. This building is connected via walkways to BST, in which Dr. Stolz (the imaging doyen) and across the street from Salk Hall, in which Dr. Venkataramanan is housed. This space includes 16 workstations of 3 linear feet, plus 9 desk areas. A fume hood, equipped for radioactive work is in place. In the labs are located -80°C and -20°C freezers. The laboratories are equipped with small equipment suitable for all proposed molecular biology, cell biology and protein chemistry experiments. In the labs are an inverted microscope (a second one fitted with an Eppendorf semi-automated microinjector system is under order) and an Olympus B-MAX 40 dual view microscope, all with digital photomicrography capabilities. The Olympus B-MAX 40 is fitted with a mercury arc lamp and appropriate filters for fluorescence studies. Two three-color cooled-CCD cameras are fitted to the Olympus B-MAX 40 and inverted microscope. These microscopes are fitted with time-lapse image capture and analysis capabilities by analog bright-field camera fitted to a MacIntosh G5 with image capture capabilities. There is a separate tissue culture room, equipped with two laminar flow hoods and two doublet banks of CO₂ incubators. Wells has 500 sq ft of general laboratory space in the VA Medical Center Research wing. This is located 300 ft from the VAMC animal care facility. The space is equipped for rodent necropsy and histology as well as PCR-based identification of transgenic mice.

The laboratory spaces includes a dedicated tissue culture room in which a CO₂ incubator has already been specially modified for bioreactor use, with access ports and mounted with control elements. Oxygen level gas mixers and specific temperature controls are in place to maintain this physiological aspect of the experimentation. These specialized equipments are used for the current generation bioreactor with the next generation bioreactor being adapted to use these same control manifolds.

Animals: Univ Pittsburgh and the Pittsburgh VAMC, of which Wells is a staff physician with funded a research program on cancer progression, both contain federally registered animal facilities that provide for the housing and care of the rodents as well as having facilities for small animal surgery, animal quarantine, and all other manipulations proposed.

Computers: Wells possesses Macintosh computers (G5s and G4s) networked together via ethernet connections and to LaserPrinters and the Internet for real time communication with collaborators. In addition, there is a 1200dpi color scanner and color printers. Most software necessary to process and analyze data (statistical, graphics and image analysis programs) are purchased and installed. Image analyses for cell morphometry and motility utilize proprietary software for the CCD cameras and the DIAS3 and MetaMorph programs. Access to Medline and GenBank is through the network.

Offices: Wells has a newly renovated office, equipped with furnishings and computer network lines. A fellows office houses the graduate and post-doctoral trainees (including Dr. Shao). A desk is available for a visiting trainee. Departmental conference room and library are located on the floor and in adjacent buildings. Secretarial help is available for manuscript preparation. FAX machine and copiers are in this room, also, and are available through departmental funds.

Other key facilities: The Center for Biological Imaging, run by Drs. Simon Watkins and Donna Stolz, is located in an attached building (BST 2nd floor). The University of Pittsburgh tumor and tissue banks are located on the same floor at Scaife directed by Dr. Rajiv Dhir, a collaborator on a complementary project. The Core Protein Facility, run by Dr. Nathan Yates, is located in BST. The clinical pharmacology facility is across the street in Salk Hall, run by Dr. Raman Venkataramanan. Other core facilities provide oligonucleotides and DNA sequencing. University of Pittsburgh provides a major medical library, medical illustration services, animal care facilities, various maintenance shops, and both Radiation Safety and Health and Safety offices.

11. EQUIPMENT

University of Pittsburgh Department of Pathology (Wells lab)

Major Equipment: Departmental common equipment rooms are located on the floor. They contain two superspeed and two ultra-centrifuges, with appropriate rotors for each, a gamma counter, and a beta scintillation counter. Floor common equipment include a fully equipped bacterial, yeast and insect cell culture facility and a cold room. There is a floor dark room with a scanning densitometer.

Specialty Equipment:

Scaife 7 A BioSpherix Controlled Environment setup, complete with imaging and culture incubation within the controlled environment, is available and being used on Scaife 7 for the physiological and hypoxia conditions. A four color BD FACSCaliber for single cell quantitative analyses, a Millipore Milliplex Luminex instrument for multiplex quantitative analyses of signaling molecules, a Nikon Eclipse Ti for multiple single live cell imaging, and a HP GC/MS for protein and metabolome analyses are also available on Scaife 7; all these shared instruments are under the oversight of Wells and are used without recharges.

Other Pathology Shared Instrumentation: On Scaife 7 there are an Affymetrix microarray scanner and a HiSeq 2000 for deep sequencing. On adjacent floors in Pathology, we have two confocal microscopes for imaging, as well as a second Milliplex. All are available for just reagent costs.

Center for Biological Imaging (<http://www.cbi.pitt.edu/>), in an adjoining building at the University of Pittsburgh, is one of the premier facilities in academe for cellular imaging. This includes live cell tracking, fixed cell, and various electron microscopes. These are available free of recharge as Wells has joint grants with faculty in CBI.

University of Pittsburgh facilities: The Clinical Proteomics and Research Proteomics facilities have various MS instruments with ion trap for protein (and metabolomics) analyses. The Department of Clinical Chemistry has MS and HPLC for small molecule/metabolomic analyses. The Genome Core facility is being used for sequencing and oligo design and generation. The Flow Cytometry Core has cell sorting capabilities. These are all available for minimal recharge costs. Wells has utilized all these facilities in previously published works.

12. Additional Education Information

The MSTP Director describes the program and my progress through and toward milestones below.

Structure of the Pittsburgh MSTP

MSTP students in Pittsburgh complete a MSTP-specific enrichment curriculum beyond the standard courses in medical and graduate school. This consists of three summer professional development courses, a three-semester weekly journal club featuring research papers consistent with the coincident SOM curriculum, a 4-week case-based ethics course, a monthly program-wide workshop, a 40-week longitudinal clinical clerkship during the graduate years and yearly special events such as the 3-day MSTP Scientific Retreat.

Goals of the enrichment program remain: •Understanding the research basis of medical knowledge; •Build skills in presenting literature to peers; Critically evaluate literature & design experimentation; Write and critique papers and grants with peers; Identify and learn from role models; Learn and discuss biomedical ethics and the responsible conduct of research; •Identify strategies for choosing the best mentor and laboratory; Anticipate and prepare for career path; Learn how to engage in professional networking. There are four parallel narratives entwined in the program structure: (1) laboratory research, (2) professional development, (3) biomedical and ethical expertise, and (4) clinical/research integration.

Laboratory research rotations: Research rotations begin the summer prior to the start of medical school (see diagram). These are front-loaded in order to optimize breadth of student exposure to high caliber researchers, providing students with the opportunity to return in their 3rd summer rotation to a laboratory that had been a good match in order to bolster progress in this laboratory as a thesis lab choice. In addition to developing manuscripts and presenting at scientific meetings based on their rotation results, all students turn in a written scientific report that is reviewed by MSTP leadership and present their work at the annual MSTP scientific retreat. These activities augment the evaluation of student accomplishments by the laboratory mentor, offering an additional platform for constructive feedback, building student momentum. The choice of thesis laboratories by students is informed by their rotation history and by discussion with their individual Career Advisors (who follow them longitudinally in the program).

Professional development: Students take three successive 8-10 week long Professional Development Courses during summers prior to starting graduate school. Objectives are included in Figure 2. In general, the first course (PD1) focuses on scientific writing and introduces students to biomedical software and to key methods used by different disciplines to approach scientific problems; PD2 focuses on scientific design and career development strategies, and PD3 focuses on grant review and ethics and grant writing.

Biomedical and ethical expertise: Students build biomedical knowledge through a 3-semester MSTP literature review course in which students present papers after formal consultation with local faculty experts in the field of that paper. Prior to class, non-presenting students complete homework assignments that analyze the paper and are graded per rubric with formative feedback. Separate concurrent classes are held for MS1 and MS2 MSTP students. During the G1 (or rarely, G2) year of graduate school, MSTP students take a month-long, weekly, case-based research ethics course. Throughout the tenure of students in both medical school and graduate school, all MSTP students meet monthly for seminars (arranged by students) that pose scientific, logistical, clinical and/or ethical dilemmas. These workshops are presented by students and/or guest faculty experts. Students are grouped at tables such that students from every year are represented at each table and work together.

Clinical/Research Integration. This is a central focus of our MSTP, in order better to model the physician scientist career. **Clinical activities during the research years:** Prior to starting graduate school, all of our MSTP students complete 8 weeks of required clinical core clerkships. This allows flexibility in their clinical schedule once they return from graduate school to medical school.

All MSTP students are required to complete a minimum of two “Longitudinal Clinical Clerkships” during the research years, to be completed no later than the semester prior to their thesis defense. Each LCC is 20 weeks long. For each LCC, students have ½ day each week of one-on-one clinical mentoring by a clinician scientist in a clinical area of interest to and chosen by the student with guidance from the MSTP LCC director, Andy

Nowalk MD PhD. The student frames clear clinical learning objectives for the rotation and writes a report of the outcomes of the rotation that is available to all other MSTP students. Students are graded by their LCC mentor.

Activities to ease transition from the graduate to the clinical years of the dual-degree program. In addition to the 40 weeks of LCC rotation, the transition back to the clinic is facilitated by our MSTP Clinical Reentry elective that all returning students have elected to take. In this elective, a master clinician mentors the returning MSTP students once weekly over the month prior to reentry as they examine, discuss, diagnose and plan treatment for surrogate patients presenting with common outpatient or inpatient ailments (abdominal pain, chest pain, dizziness, infection etc). This reentry course has garnered glowing evaluations from students who consider it to have effectively facilitated their transition into MS3. Students return to MS3 either in May, September, or January. Consequently, the number of students reentering at any time is small and the Clinical Reentry class can focus closely on the participating students.

Research activities during clinical years: In addition to the research-focused MSTP classwork that takes place during preclinical School of Medicine years, our students continue their research focus after re-entry to medical school after defending their PhD thesis. This happens in three ways:

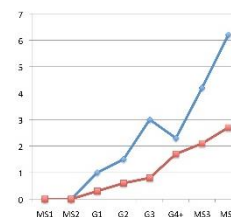
--MS3 and MS4 students remain engaged in the MSTP enrichment curriculum and plan and execute MSTP Workshop sessions that feature research topics and research challenges to be discussed with MSTP peers.

--Twice yearly meetings with Career Advisors (often physician scientists in the students area of burgeoning clinical interest). Students complete formal reflective and goal-oriented self-assessment evaluations that are shared with these advisors.

--*Ongoing research productivity during the clinical years.* Our students average 2.8 new publications during the MS3 and MS4 years (at least one first authored; in recent years, students have an average of 5-7 papers upon graduation, see figure 1 on right).

--Research months during the clinical years. A high proportion of our students elect to take 1-2 months during their MS4 year to undertake MSTP Research elective. This has been used both to extend findings of thesis work or to build skillsets in a translational area.

Avg total and 1st au pubs/student by MSTP year



Another novel feature of our MSTP, the MSTP Postdoctoral Fellowship, was initiated in 2009, providing support in coordination with the Dean’s office for 5 months of postdoctoral research prior to residency for MSTP students graduating in December. Applications (at the end of MS3) address research hypotheses and aims, career development aims, expected deliverables, mentor fit and technical and intellectual goals.

•Over the past 2 years, 8 of 28 MSTP graduates undertook MSTP Postdoctoral Fellowships. The students were highly productive and viewed the Fellowship as extremely valuable. Paper output and some exemplary comments from students who did the Fellowship in 2013 and 2014 are represented in Figure 2 on the left.

Mini-postdoc outcomes

	Graduation	Paper published during fellowship 1 st au* 2 nd au	Paper submitted during fellowship 1 st au* 2 nd au	Writing completed during fellowship 1 st au* 2 nd au	Additional Publications in 2014 (year after postdoc)	Presentations during fellowship
1	2013	1	3*	2*	2 first authored 2 co-authored	
2	2013	0	2*	1*	1 first authored	
3	2013	0	0	3	none	AHA
4	2013	1*	0	1*	none	
5	2013	0	0	1*	1 first-authored	
6	2014	0	0	3*	N/A	multiple
7	2014	1*	0	0	N/A	
8	2014	0	0	1*	N/A	

5/17 graduates in 2013: 3/10 in 2014 undertook the mini-postdoc

Comments on the postdoc include "The experience helped me expand into a new area of research that I hope to continue. Moreover, it has already helped me to network [in residency at Hopkins] and will certainly play a role in my cardiology fellowship application."

"I had time to write several manuscripts during the postdoc time...it gave me more time to work on my ongoing projects so that it would make my research efforts during residency more productive."

Most students have reported that the MSTP has positively affected their clinical performance, e.g. "The analytical skills, critical thinking, goal oriented teamwork and emphasis on practical solutions in lab has translated to my improved clinical performance more than any amount of book studying and knowledge of diseases."

Figure 3 summarizes our course framework by year:

MSTP Curriculum

Laboratory Rotation (1) Professional Development 1 (molecular medicine course)	SUMMER	MEDICAL SCHOOL	MSTP Ethics Course	YEAR 1	GRADUATE SCHOOL
Research Basis of Medical Knowledge Course (1)	YEAR 1		Longitudinal Clinical Clerkship	YEAR 2	
Laboratory Rotation (2) Professional Development 2 (methods and analysis course)	SUMMER		Longitudinal Clinical Clerkship	YEAR 3	
Research Basis of Medical Knowledge Course (2)	YEAR 2		Clinical Reentry Elective	YEAR 4	
Clinical rotations; Laboratory Rotation(3) Professional Development 3 (grantwriting course)	SUMMER		MSTP Postdoctoral Fellowship	YEAR 3	MEDICAL SCHOOL
				YEAR 4	MEDICAL SCHOOL

Figure 3. Curriculum structure of the MSTP. Only MSTP enrichment courses are shown. The medical school courses and graduate school courses are not shown. A 4-year graduate cycle is shown, although the duration varies by program and student. Average duration of our MSTP program is 7.8 years.

Throughout: MSTP Workshop
(monthly, all students); **Career Advisor meetings** (twice yearly)

The following chart (Figure 4) highlights the coherent and comprehensive nature of our curriculum as it introduces and reinforces critical skills:

(blue—cognitive skills; red—professional development skills; black—deliverables)

PD (professional development courses); RBMK (research basis of medical knowledge course); LCC (longitudinal clerkship); MSTP workshops; Student committee work; CA (career advisors).

Competencies	Rotations	PD 1	PD 2	PD 3	RBMK	Ethics	Wkshps	Reentry	Committee	CA
Recognize innovative and significant science		X	X		X					
Articulate strong and weak components of research plans/papers		X	X	X	X					
Develop compelling and well supported hypotheses and research strategies	X		X	X						
Identify whether given experiment/plan is Type A, B, or C		X	X							
Framing scientific design grounded in state-or-the-art literature and techniques	X			X						
Coherent approach to working through ethical/integrity challenges						X	X			
Enhance work quality by accepting/responding to criticism			X		X					
Identifying strengths/weaknesses of mentor/mentee match and strategies to optimize interactions and outcomes			X							X
Frame and adapt detailed career development plan with milestones and evaluation criteria			X							X
Develop and execute networking strategy			X				X			X
Strategy to fill gaps in scientific knowledge	X	X		X						
Strategy to fill gaps in professional skills						X				
Collaborative and communication skills (peer and others)		X		X			X			
Integrating clinical and research roles								X		
Writing of clear, coherent abstracts and papers	X	X								
Writing of clear, thoughtful and compelling grant proposals				X						
Presenting own research eloquently (formal and informal)	X		X							
Collaborate to answer scientific/ethical questions						X	X		X	
Collaborate in support of social learning and curriculum							X		X	
Apply literature analysis to technical and intellectual labwork and build troubleshooting skills.	X		X							
Strategically identify rotation laboratories.		X								X
Achieving deliverables including publications, grants, national presentations	X									

Monitoring and evaluating student progress

Prior to matriculation, each new student is assigned a Career Advisor. The Program Director makes this assignment by matching student and faculty research interests. These advisors help orient and guide the students throughout their careers. Thesis advisors may be chosen from among any of the pre-approved training faculty, or the students in consultation with their Career Advisor may propose new faculty that are then reviewed by the Director and Co-Directors. The great majority of a trainee's time in the graduate program is spent in research training under the guidance of their research mentors, program leadership, and eventually their doctoral dissertation committee. In all of the programs, pre-doctoral students establish an advisory committee early in their training, and later an enlarged, doctoral dissertation committee is formed. For the most part the Universities require that the committees meet with the trainee twice a year and the MSTP tracks these meetings to be sure they occur and that feedback is available on line to the Career Advisors. The Career Advisor monitors the trainee's progress, including attending oral examinations or presentations made during the first year. All trainees are required to give an annual research presentation beginning with the end of the first year of graduate school.

The Pittsburgh MSTP stands out in its logistic integration of this wide net of career oversight. Through a secure on-line web database, each student advisor has access to all information necessary for them to appropriately advise the student. Depending on who is viewing it, this information COULD include any of the following: student demographics (e.g. undergraduate and objective test performance), attendance and performance on all MSTP activities (e.g. grades and evaluations in all courses), attendance of workshops and retreats, evaluations of laboratory rotations, dates and composition of thesis committee meeting, publications, performance on USMLE and clinical rotations, approved clinical training plan, biannual feedback from thesis and career advisors. But, NOT every advisor has access to all information. For example Thesis Advisors do not have access to Career Advisors notes. This enables students to use Career Advisors as sounding boards for laboratory issues or for emerging interests.

•In order to customize advise and resource allocation and to optimally position our students to succeed, we initiated a Self-Assessment study to be undertaken in conjunction with Career Advisor meetings by all MSTP students. The students share this Individual Development Plan and their updated biosketch with the Director and with their Career Advisor. The form is both competency-focused and goal-focused. Students identify specific skills that they want to develop in the next six months, set technical, intellectual and professional goals, identify how goals will be achieved and measured and the deliverables expected relative to the goals. Resources to reach goals and obstacles that could compromise success are enumerated and discussed. At six month intervals, progress toward short and long term goals enumerated through this process is discussed with the Career Advisor, and new goals are set.

Career counseling beyond the end of graduate and medical school. To better reflect the student's educational experience to prospective residency programs, the MSTP creates an executive summary that is either added to the Dean's Letter or submitted as a separate letter in the residency application. In this summary student evaluations, honors, presentations and participation in the combined degree training is described, rewarding students who altruistically give of their time and demonstrate prowess in working in groups.

A minimum of a year before graduating, students need to position themselves for postgraduate training in order to attain successful biomedical research positions. Six months to one year before trainees are expected to complete their doctoral program, they meet with the Program Director to discuss their future plans. Re-entry options are reviewed, advice given regarding applications, letters of recommendations, preparing a curriculum vitae, and so forth. The Program Director, as well as other members of the Career Advisory committee and faculty are prepared to provide all possible assistance in making the transition to postdoctoral training including residencies, fellowships and faculty positions and non-academic based positions. The MSTP office retains a file of information regarding postgraduate training opportunities, especially "research residencies" wherein MSTP graduates can continue to gain expertise in their dual responsibilities of research and clinical practice. Many of the current faculty are MD/PhDs and are very capable of participating in career planning for third and fourth year medical students who have completed their PhDs. Students interested in academic faculty positions need to successfully garner K- or RO-1- type support, and thus need to carefully plan their residencies and/or post- doctoral fellowships.

Program Outcomes

The Pittsburgh MSTP has 156 alumnae. The past 10 graduating classes have had a 85% completion rate. The large majority (82%) of Pittsburgh MSTP graduates are in academic residencies and fellowships or hold faculty positions in academia. Only 18 of the alumnae entered private practice instead of academia, NIH or industry. Most of those choosing private practice graduated the program in its early years, before the development of the carefully constructed enrichment curriculum described above. 9 graduates are Instructors, 30 are Assistant Professors, 9 are Associate Professors and 4 are Professors. We have 6 graduates in Industry and 3 at NIH. A query in 2011 of those who had completed training indicated that a majority of respondents spent over half of their time in research as PI's. Of 75 recent graduates still in training in residency or fellowship this past June, 36 had received F- awards. We look forward to them leveraging that early success and skills gained from our MSTP curriculum to earn academic faculty positions supported by extramural funding.

COLIN BECKWITT

Colin is an outstanding member of our MSTP who matriculated into the MSTP program in June, 2013 (beginning a summer rotation pre-MS1 year) and is in the midst of his MS2 year with entry into the Cellular and Molecular Pathology graduate program planned for September of 2015. As noted elsewhere in my LOR, Colin is an exceptionally productive and proactive student who is an intellectual leader in the program.

Colin has completed the following courses in the MSTP enrichment curriculum: Research Basis of Medical Knowledge (MS1); Professional Development Course-2 (Methods and analysis); MSTP Workshops Course. Also completed all required MS1 courses in the SOM curriculum, with the exception of one course (Methods in Logic and Medicine) that is waived for MSTP students. He is in good standing with both the MSTP and medical school, having completed the following SOM courses: (*Introduction to Being a Physician, Ethics, Law & Professionalism, Behavioral Medicine*); Fundamentals of Basic Science Block (FBS) (*Medical Anatomy, Fuel Metabolism, Human Genetics, Cellular and Pathologic Basis of Disease, Immunology in Health & Disease, Medical Microbiology*); Organ Systems Pathophysiology Block (OSP) (*Neuroscience, Introduction to Psychiatry*); Body Fluid Homeostasis (*Cardiovascular, Renal, Pulmonary*); Introduction to Patient Care Block (IPC) (*HIPAA Training for Medical Students, Medical Interviewing, Introduction to Physical Examination, Advanced Physical Examination 1, 2, and 3 (ongoing), Clinical Experiences 1, 3 (ongoing)*) and Introduction to Medical Decision Making.

His planned mentor for his CMP thesis work is Alan Wells, MD, Professor of Pathology and of Bioengineering, University of Pittsburgh. Colin has completed his 2nd of 3 summer lab rotations with Dr. Wells in a project investigating E-cadherin as a chemoresistance marker, and also began the work described in the appended application on oxygenation in micrometastasis. Notably, Colin's work in the first project earned him co-authorship on a manuscript, currently in review with *Scientific Reports*.

Colin will do his third summer rotation in Dr. Wells laboratory in July and August 2015 prior to starting graduate school. He will continue his work studying hypoxia in micrometastasis, building on his success incorporating and validating oxygen sensors in a continuous-perfusion primary liver culture device modeling micrometastases. Just prior to that rotation, Colin will complete 8 weeks of clinical rotations as per the MSTP curriculum structure; he will also take our Professional Development III course that summer.

The Cellular and Molecular Pathology program combines both basic science and clinical research to explore fundamental questions related to the biology and normal tissue differentiation and growth, as well as the cellular and molecular pathways leading to pathobiology of disease in human and animal models. Required courses cover topics from basic mechanisms to normal and abnormal tissue function. M.D. /Ph.D. students entering the CMP Program receive 19 credit hours for their M.D. coursework and MSTP rotations towards the 32 credit hours of coursework required by the University. In addition to the required research seminars each term, Colin could fulfill his requirements by taking the following courses of particular relevance to his project: *Molecular Mechanisms of Tissue Growth and Differentiation; Cancer Biology and Therapeutic; and Extracellular Matrix in Tissue Biology and Bioengineering* and could (besides the seminar) complete all coursework in his first (or the first half of his second) graduate year.

Colin's current career plan is to pursue basic science cancer research with a clinical specialty of surgical oncology. Specifically, he is interested in studying the mechanisms underlying tumor dormancy and identifying novel or repurposed treatments for combatting treatment-resistant cancers. In addition to working with Dr. Wells (a clinically active physician scientist) and other research mentors, Colin has already established relationships with two clinical mentors. Dr. Wallis Marsh is a nationally known hepatobiliary surgeon who trained under Thomas Starzl, and spent a large part of his career doing liver transplantation. Dr. Gary Gruen is an orthopedic trauma surgeon who focuses mostly on fracture repair and bone healing. Colin has spent several hundred hours in the OR and clinic with them.

MSTP students complete 2 longitudinal clinical clerkships (LCC's) during their time in graduate school. For each of these, students spend a collective 2 weeks time in a clinical setting of their choosing, often completed through ½ day per week of clinic time for twenty weeks during a given semester. These will afford Colin additional opportunities to identify and work with clinician scientist mentors in surgical oncology.

Although it is early in Colin's MSTP course, he has been very strategic in mapping out milestones for his combined degree trajectory. His goal is to complete his PhD training in the fall of 2018, returning to medical school in September of 2018 and graduating either in December 2019 or May of 2020. For the terms of the fellowship proposed, Colin seeks support for 38 months of research and 16-20 months of clinical work. Should Colin elect the route in which he graduates medical school in December 2019, he will then undertake the 5 month MSTP Postdoctoral Fellowship, described above. The post-doctoral fellowship is not included in the time of covered support requested in the current application.

Richard Steinman MD PhD
Associate Dean and Director, Pittsburgh MSTP
University of Pittsburgh School of Medicine
Steinman@pitt.edu
412 6233237

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Colin	Middle Name Henry	Last Name*: Beckwitt	Suffix:
Position/Title*:	MD/PHD Candidate			
Organization Name*:	University of Pittsburgh			
Department:	Pathology			
Division:	Medicine			
Street1*:	3550 Terrace Street			
Street2:	S723 Scaife Hall			
City*:	Pittsburgh			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	15213-2500			
Phone Number*:	412-647-7813	Fax Number:	E-Mail*: cbeckwitt@gmail.com	
Credential, e.g., agency login: CBECKWITT				
Project Role*: PD/PI		Other Project Role Category:		
Degree Type:		Degree Year:		
Attach Biographical Sketch*:		File Name		
Attach Current & Pending Support:		1251-Biosketch_Beckwitt_Final.pdf		

PROFILE - Senior/Key Person				
Prefix: Dr.	First Name*: Alan	Middle Name	Last Name*: Wells	Suffix: MD, DMSc
Position/Title*:	Professor			
Organization Name*:	University of Pittsburgh			
Department:	Pathology			
Division:	Medicine			
Street1*:	3550 Scaife Hall			
Street2:	S723 Scaife Hall			
City*:	Pittsburgh			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	15213-2500			
Phone Number*: 412-647-7813	Fax Number:	E-Mail*: wellsa@upmc.edu		
Credential, e.g., agency login: AWells				
Project Role*: Other (Specify)	Other Project Role Category: Mentor/Sponsor			
Degree Type: MD, DMSc	Degree Year: 1988, 1982			
Attach Biographical Sketch*:	File Name			
	1252- Wells_Biosketch_Beckwitt_Final.pdf			
Attach Current & Pending Support:	1253- Wells_Other_Support_Beckwitt_Final.pdf			

FELLOWSHIP APPLICANT BIOGRAPHICAL SKETCH**USE ONLY FOR INDIVIDUAL PREDOCTORAL and POSTDOCTORAL FELLOWSHIPS. DO NOT EXCEED FOUR PAGES.**

NAME OF FELLOWSHIP APPLICANT Colin Beckwitt	POSITION TITLE Pre-doctoral MD/PhD Candidate
eRA COMMONS USER NAME (credential, e.g., agency login) CBECKWITT	

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
Massachusetts Institute of Technology, Cambridge, MA	BS	06/2013	Bioengineering
University of Pittsburgh School of Medicine, Pittsburgh, PA	M.D./Ph.D.	In progress	Medicine / Cellular and Molecular Pathology

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

I am a second year MD-PhD student at the University of Pittsburgh with the goal to be a translational surgeon-scientist. I am pursuing a Ph.D. in pathology with a focus on cancer biology and metabolism, which I plan to supplement with clinical training in surgical oncology. My ultimate career goals are to run an NIH-funded lab, practice surgery, and teach at an academic institution. My dissertation research will investigate dormant micrometastasis phenotype and metabolism using a liver microphysiological system (MPS) which I will use to measure cancer cell metabolic state and the impact of oxygen availability (this latter aspect incorporating a novel hemoglobin-based oxygen carrier (HBOC)) on cell dormancy phenotype, metabolism, and treatment potentiation. I will be working with Alan Wells, MD, DMSc, who in addition to running a well-regarded research lab in cancer biology also practices clinical pathology. Performing my research under his mentorship will enhance my mastery of experimental techniques, intellectual experiment design, clinical integration, and networking and presentation opportunities. Moreover, the MSTP program at the University of Pittsburgh grants multiple opportunities for professional career development through numerous workshops, journal clubs, retreats, and courses dedicated to furthering my development as a surgeon-scientist. My undergraduate coursework at MIT provided me with a strong foundation in the biological sciences and instilled in me the mindset of an experimental scientist. My undergraduate research experiences allowed me to intellectually and experimentally approach a variety of difficult research questions, and cumulatively resulted in a second author paper and the submission of a white paper to Thorlabs, Inc. I am currently funded by the University of Pittsburgh MSTP T32 grant (see below) which has enabled me to pursue medical school coursework and summer research experiences. My past research experiences and ultimate career goal to integrate clinical surgical oncology and cancer biology investigation make me an ideal candidate for this fellowship. Dr. Wells's lab will help develop my skills as a researcher, which will be complimented by my clinical involvement throughout my graduate training. Moreover, the University of Pittsburgh MSTP and surgery departments strongly promote the integrative training of physician- and surgeon-scientists respectively, which will both facilitate the formation of strong mentoring relationships and my professional career development.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/ EMPLOYER
Experimental Instructor (July)	07/08	07/11	Bio-optics	Women's Technology Program, MIT	Steve Wasserman, PhD
Summer Undergraduate Researcher	06/09 06/10	09/09 09/10	Bioinstrumentation Bio-optics	Massachusetts Institute of Technology	Ian Hunter, PhD Steve Wasserman, PhD
Undergraduate Researcher	06/11	04/12	Biomaterials	Massachusetts Institute of Technology	Katharina Ribbeck, PhD

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/EMPLOYER
Summer Graduate Researcher	06/13	08/13	Tissue Engineering	University of Pittsburgh	Steven Badylak, DVM, PhD, MD
	06/14	08/14	Cancer Biology	University of Pittsburgh	Alan Wells, MD, DMS

Academic and Professional Honors

Honors and Awards

2013 Outstanding Contribution to the MIT Biological Engineering Community
 2013-2014 Graduate Student Researcher, Bioengineering, University of Pittsburgh
 2013-2015 Medical Scientist Training Program, University of Pittsburgh (T32, 5T32GM008208-25, -26)
 2013- Phi Beta Kappa, Honor Society

Memberships in Professional and Academic Societies

2013- Phi Beta Kappa, Honor Society
 2013- Medical Student Member, American Medical Association

C. Publications

Research Papers

Crouzier T, **Beckwitt CH**, Ribbeck K. Mucin multilayers assembled through sugar-lectin interactions. *Biomacromolecules*. 2012 Oct 8;13(10): 3401-8.
 Warita K, Warita T, **Beckwitt CH**, Schurdak ME, Vazquez A, Wells A, Oltvai ZN. Statin-induced mevalonate pathway inhibition attenuates the growth of mesenchymal-like cancer cells that lack functional E-cadherin mediated cell cohesion. 2014 in revision

D. Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY			MASSACHUSETTS INSTITUTE OF TECHNOLOGY		
2009	Introductory Biology	P	2009	Writing & Experience	P
2009	Physics I	P	2010	Reading Fiction	A-
2009	Calculus I (AP credit)	S	2010	Principles of Microeconomics	B+
2009	Calculus II	P	2011	Principles of Macroeconomics	A
2009	Differential Equations (taken at BU during high school, grade = A)	S	2011	Harmony and Counterpoint I	A+
2009	Linear Algebra (taken at BU during high school, grade = A)	S	2011	Chamber Music Society (Spr)	A-
2010	Principles of Chemical Science	A+	2011	Studio Accompanying: Pianists (Spr)	A
2010	Introduction to Computer Science and Programming	A	2011	Chamber Music Society (Fall)	A
2010	Physics II	A	2011	Studio Accompanying: Pianists (Fall)	A
2010	Organic Chemistry I	A	2012	Jazz Harmony and Arranging	A
2010	Genetics	A	2012	Problems of Philosophy	A
2010	Earth, Energy, & the Environment	A	2012	Jazz Composition	A
2010	Laboratory Chemistry	A			
2011	Physical Chemistry of Biomolec Sys	A+		UNIVERSITY OF PITTSBURGH	
2011	Biological Chemistry	A		SCHOOL OF MEDICINE	
2011	Organic Chemistry II	A		MSTP COURSEWORK	
2011	Lab Fundamentals in Biological Eng	A-			
2011	Biomolecular and Cellular Systems	A	2013	Molecular Medicine	S

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2012	Cell Biology	A	2013	Laboratory Research Rotation 1	S
2012	Molecular, Cellular, & Tissue Biomechanics	A	2013	MSTP Workshops	S
2012	Fields, Forces, and Flows	A	2013	Research Basis of Medical Knowledge (Fall)	S
2012	Undergraduate Independent Study in Chemistry	A	2014	Research Basis of Medical Knowledge (Spring)	S
2012	Mechanisms of Drug Action	A	2014	Methods and Analysis	S
2012	Instrument and Measurement for Biological Systems	A+	2014	Laboratory Research Rotation 2	S
2013	Molecular and Engineering Aspects of Biotechnology	A+	2014	Research Basis of Medical Knowledge 2 (Fall)	IP
2013	Biological Engineering Design	A+			
	UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE M.D. COURSEWORK (Science)			UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE M.D. COURSEWORK (Non-science)	
2013	Basic Science Fundamentals I	P			
2013	Basic Science Fundamentals 2	P	2013	Patient/Physician & Society (PPS1)	P
2013	Scientific Reasoning 1	P	2013	Introduction to Patient Care 1	P
2014	Basic Science Fundamentals 3	P	2014	Introduction to Patient Care 2	P
2014	Neuroscience/Psychiatry	P	2014	PPS2 Behavioral Medicine	P
2014	Body Fluid Homeostasis	P	2014	PPS3 Population Health	IP
2014	GI/Endo/Heme/Skin/Musk/Repro Sciences	IP	2014	Introduction to Patient Care 3	IP

MCAT: 330

Grade Legend:

“P” - Pass

1. Massachusetts Institute of Technology: All grades first semester were graded on a pass/no record system. A passed class was assigned a “P” whereas a failed class was not listed on the transcript (doesn't apply for any classes I took).

2. University of Pittsburgh School of Medicine M.D. Coursework: All classes are graded on a pass (P) or fail (F) basis

“S” - Satisfactory

1. Massachusetts Institute of Technology: An “S” indicates credit obtained from courses taken outside the university

2. University of Pittsburgh School of Medicine MSTP Coursework: An “S” indicates a passing grade.

“IP” – In Progress

For all coursework, “IP” indicates the course is currently being taken.

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 Alan Wells	POSITION TITLE Thomas J Gill III Professor of Pathology Executive Vice-Chair for Laboratory Medicine		
eRA COMMONS USER NAME AWells			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Brown University, Providence, RI	AB	1979	Biochemistry
Karolinska Institute, Stockholm, Sweden	DMSc	1982	Tumor Biology
Brown University, Providence, RI	MD	1988	Medicine
University of California, San Francisco, CA	PostDocFlw	1984-87	Tumor Biology
University of California, San Diego, CA	Resident/PDF	1988-91	Laboratory Med/Cell Biology

A. Personal Statement. Briefly describe why your experience and qualifications make you particularly well-suited for your role (e.g., PD/PI, mentor) in the project that is the subject of the application.

I am in excellent position to mentor Colin Beckwitt based on both my training and research aspects.

I have been actively involved in mentoring and training my entire professional career. This includes at the faculty level. As Vice-Chair for Laboratory Medicine, a major part of departmental responsibilities consists of advising and directing faculty, particularly junior ones, towards success. Formally, I have been the primary mentor on two K08, one K12 and five VA Medical Research Entry Program awards. I have also been on the mentoring team for another three such awards. All of these former trainees still currently have active research programs.

I have been involved in training pre-doctoral and post-doctoral students for almost two decades. At the doctoral level, I have been the major advisor for 15 graduated students (5 of which were MD-PhD, and 2 under-represented minorities), with five more currently in the laboratory (1 minority). All of the graduates had at least one first author publication at time of graduation and all but one had won regional and national recognitions; these students' subsequent careers have been successful including independent NIH funding as faculty members. In addition, I have been on 75 thesis committees (6 as Opponent, and at multiple outside institutions in six different countries in addition to my home institutions). I was also Director of the graduate Program in Integrative Molecular Biology at University of Pittsburgh.

I have been involved with the training and direction of under-represented minorities for almost two decades now. In the 1990's I was involved with Talladega College (Alabama) as an advisor to their biology program. For the past decade, I have been intimately involved with Tuskegee University (Alabama) on their MARC and MBRS programs as an external reviewer, advisor, and faculty mentor; in 2007 I was appointed as Adjunct Professor. I currently am program director of a training grant to introduce students from Tuskegee to prostate cancer research during the summer months funded by the DoD CDMRP in Prostate Cancer.

I am currently involved on the Executive Committees on two other training grants at UPitt. Cellular Approaches to Tissue Engineering and Regeneration (CATER) is a joint effort of the Department of Pathology in the School of Medicine and the Department of Bioengineering in the School of Engineering; this aims to educate investigators that bridge these two disciplines. The Angiogenesis Training Program, housed in the Department of Pathology is campus wide, attempting to spur interest in, and educate about pathophysiology of the vascular system.

My laboratory has been investigating tumor progression (focusing mainly on the hormone-responsive breast and prostate carcinomas) since its inception at the Birmingham VA and UAB twenty years ago, and supported by the VA Merit program and NIH grants continuously since. Initially we investigated invasion from the primary tumor nodule but over the last half decade have focused strongly on the whole of tumor progression including metastatic competency and phenotypic plasticity, being at the forefront of investigating cancer-associated Mesenchymal-to-Epithelial Transition (MET) or as we designate it Mesenchymal-to-Epithelial Reverting Transition (MERt) to imply both the incompleteness and plasticity of either the mesenchymal or epithelial phenotype in carcinoma cells. In studying this transition, we delved into the controlling aspect of the tumor micro-environment. Thus, based on our other studies in wound healing, which represents a self-correcting dysplasia plasticity, the critical role of 'stop signals' rose to the fore. Thus we queried if similar molecular events were critical in breast cancer progression,

emphasizing metastatic dissemination. This project was initiated four years ago with successful fruition in the recent publications on breast cancer phenotypic plasticity, that forms the basis of this proposal.

This phenotypic plasticity/switching behavior was made evident by the collaboration with Dr. Linda Griffith (MIT) using her liver organotypic bioreactor. The modification of this enabling technology to allow us to examine the early stages of metastatic seeding is the technical basis for this proposal. Additionally assistance for these projects has come from Dr. Raman Venkataramanan who is an expert in drug metabolism and is a leader in how sex hormones affect such metabolism. These studies are now supported as part of the NCATS-led Tissue Chip program, of which the UH3 continuation is supported in part by NCI. Colin's proposed project is part of this multi-disciplinary program involving the biological engineers at MIT (his alma mater), the imagers (Dr. Donna Stolz of Cell Biology) and pharmacologists (Dr. Venkataramanan) at Pitt, and a new collaborator, a transplant surgeon who is spearheading the hemoglobin-based oxygen carrier for organ preservation. Thus, we have the financial and intellectual resources to enable Colin to complete these studies.

B. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Employment:

- 1983-84 Visiting Scientist, Okayama University Medical School, Okayama, Japan
- 1991-97 Assistant Professor, Department of Pathology, University of Alabama at Birmingham
- 1992- Staff Pathologist, Veterans Administration Medical Center, Birmingham (-99), Pittsburgh (99-)
- 1997-99 Associate Professor, Department of Pathology and Cell Biology, UAB
- 1999- Professor and Vice-Chair, Department of Pathology, University of Pittsburgh, PA
- 1999- Medical Director, UPMC Clinical Laboratories
- 1999- Senior Member, University of Pittsburgh Cancer Institute
- 2001- Member & Head - Cell Biology, McGowan Institute for Regenerative Medicine, UPitt
- 2002-10 Chief Scientific Officer (acting), Precision Therapeutics, Pittsburgh, PA
- 2003- Professor (secondary appointment), Bioengineering, University of Pittsburgh
- 2007- Adjunct Professor, Tuskegee University, Alabama

Academic Honors:

- 1978 Phi Beta Kappa 1979 Sigma Xi
- 1979 Brown University, AB Biochemistry, *magna cum laude*
- 1979-80 ITT International Fellowship to Sweden
- 1982 Karolinska Institutet, DMS Tumor Biology, with Honors
- 1984-86 American Cancer Society (National) Postdoctoral Fellowship
- 1990 David J Epstein Award, University of California at San Diego
- 1989 & 90 Junior Investigator Award, Academy of Clinical Laboratory Physicians and Scientists
- 1992-94 Junior Faculty Development Awardee, Comprehensive Cancer Center, UAB
- 1999 Wellcome Travel Research Grant, for sabbatical at University College London
- 2001 American Society for Clinical Investigation, elected (ASCI)
- 2002 American Association of University Pathologists, elected (Pluto Club)
- 2002-03 President, Academy of Clinical Laboratory Physicians and Scientists
- 2006 Univ South Alabama School of Medicine, Medical Student Retreat, Keynote,
- 2007 Tsou King Lectureship, Graduate Student Research Day, Oregon State University
- 2008 Keynote Speaker, CLRI Jubilee Anniversary Symposium, Chennai, India
- 2008 Keynote Speaker, ETRS Annual Meeting, Malta
- 2009 President, American Association of University Pathologists (Pluto Club)
- 2011 William E Brown Outstanding MSTP Mentor Award, UPitt
- 2011 Distinguished Mentor, Biomedical Graduate Students Association, UPitt
- 2013 Provost Award for Excellence in Mentoring, UPitt

Government Advisory Service:

- 1993- Ad hoc Reviewer for NSF and VA grants
- 1995-97 DoD/US Army, Study Section for Breast Cancer Initiative - Pathobiology
- 1998 & 2001 NIH, Member (temporary), Physiological Chemistry (PC) Study Section
- 2000 - 2003 NIH, Member, SBIR/STTR Study Section
- 2000, 04-11 NIH, Member (temporary), SAT Study Section (permanent member, 2006 -; chair, 2010 -)
- 2001- occ NIH, NCRR/NIGMS, Member COBRE Grant Special Study Section
- 2002 & 2003 NIH, Chair, NIAMS Special Study Section
- 2007-pr Shriners Hospitals, SAB for Burns Research
- 1999 - 2007 California Breast Cancer Program, Pathogenesis and Biology of the Normal Breast sections

2011 – pr VA Merit Review, Oncology A Study Section (chair)

C. Selected recent peer-reviewed publications (in chronological order) germane to this proposal.

The listed papers are numbered chronologically. (of >220 total publications)(H factor = 49 SCOPUS, 61 Google)

Original Articles:

149. YL Chao, Q Wu, C Shepard, **A Wells** (2012). Hepatocyte-induced re-expression of E-cadherin in breast and prostate cancer cells increases chemoresistance. Clinical and Experimental Metastasis **29**, 39-50. PMID: 21964676.
151. YL Chao, Q Wu, M Acquafondata, R Dhir, **A Wells** (2012). Partial mesenchymal to epithelial reverting transition in breast and prostate cancer metastases. Cancer Microenvironment **5**, 19-28. PMID: 21892699. PMC3343195
153. Q Wu, R Dhir, **A Wells** (2012). Altered CXCR3 isoform expression regulates prostate cancer cell migration and invasion. Molecular Cancer **11**, 3e. PMID: 22236567. PMC3320557.
154. AS Meyer, SK Hughes-Alford, JE Kay, A Castillo, **A Wells**, FB Gertler, DA Lauffenburger (2012). 2D Protrusion but not motility predicts growth factor-induced cancer cell migration in 3D collagen. Journal of Cell Biology **197**, 721-729. PMID: 22665521. PMC3373410
156. J Jones, H Wang, J Zhou, S Hardy, T Turner, D Austin, Q He, **A Wells**, W Grizzle, C Yates (2012). Nuclear Kaiso indicates aggressive prostate cancer and promotes migration and invasiveness of prostate cancer cells. American Journal of Pathology **181**, 1836-1846. PMID: 22974583
157. J Grahovac, D Becker, **A Wells** (2013). Melanoma cell invasiveness is regulated at least in part by the epidermal growth factor-like repeats of tenascin-C. Journal of Investigative Dermatology **133**, 210-220. PMID: 22951722
160. D Taylor, JZ Wells, A Savol, C Chennubhotla, **A Wells** (2013). Modeling boundary conditions for balanced proliferation in metastatic latency. Clinical Cancer Research **19**, 1063-1070. PMID: 23329811. PMC3594128
164. J Jamison, D Lauffenburger, JH-C Wang, **A Wells** (2013). PKC ζ localization at the membrane increases traction force dependent on PLC β 1/EGFR signaling. PLoS One **8**, e77434. PMID: 24155954. PMC3796482
163. Z Ding, M Joy, R Bhargava, M Gunsaulus, N Lakshman, M Miron-Mendoza, M Petroll, J Condeelis, **A Wells**, P Roy (2014) Profilin-1 downregulation has contrasting effects on early vs late steps of breast cancer metastasis. Oncogene **33**, 2065-2074. PMID: 23686314. PMC3834125
168. DP Taylor, A Clark, S Wheeler, **A Wells** (2014). Hepatic nonparenchymal cells drive metastatic breast cancer outgrowth and partial epithelial to mesenchymal transition. Breast Cancer Research and Treatment **144**, 551-560. PMID: 24610032
169. B Ma, **A Wells** (2014). The MAP kinases p38 and ERK are involved in hepatocyte-mediated phenotypic switching in prostate cancer cells. Journal of Biological Chemistry **289**, 11153-11161. PMID: 24619413
172. SE Wheeler, AM Clark, DP Taylor, CL Young, V Pillai, DB Stolz, **R Venkataramanan**, D Lauffenburger, L Griffith, **A Wells** (2014). Spontaneous dormancy of metastatic breast cancer cells in an all human liver microphysiologic system. British Journal of Cancer, in press.
173. H Shao, S Li, SC Watkins, **A Wells** (2014). Alpha-actinin-4 is required for amoeboid-type invasiveness of melanoma cells. Journal of Biological Chemistry, in press.

Review and Perspective Articles:

43. L Leloup, **A Wells** (2011). Calpains as potential anti-cancer targets. Expert Opinion on Therapeutic Targets **15**, 309-323. PMID: 21244345. PMC3076675.
51. **A Wells**, J Grahovac, S Wheeler, DP Taylor, B Ma, DA Lauffenburger (2013). Targeting tumor cell motility as a strategy against invasion and metastasis. Trends in Pharmaceutical Sciences **34**, 283-289. PMID: 23571046. PMC3640670
52. **A Wells**, LG Griffith, JZ Wells, DP Taylor (2013). The dormancy dilemma: Balanced proliferation versus quiescence. Cancer Research **73**, 3811-3816. PMID: 23794703. PMC3702639
53. J Grahovac, DA Lauffenburger, **A Wells** (2014). Matricellular proteins as regulators of cancer cell invasion. Lab Investigation **94**, 31-40. PMID: 24247562.
54. SE Wheeler, (alphabetically): JT Borenstein, AM Clark, M Ebrahimkhani, IJ Fox, L Griffith, W Inman, D Lauffenburger, T Nguyen, VC Pillai, R Prantil-Baun, DB Stolz, D Taylor, T Ulrich, **R Venkataramanan**, **A Wells**, C Young (2014). All-Human Model of Metastasis Therapy. Stem Cell Research and Therapy **4S1**, S11. PMID: 24565274. PMC in process

55. AM Clark, SE Wheeler, DP Taylor, VC Pillai, CL Young, R Prantil-Baun, T Nguyen, DB Stolz, JT Borenstein, DA Lauffenburger, **R Venkataramanan**, LG Griffith, **A Wells** (2014). A microphysiological system model of therapy for liver micrometastases. Experimental Biology and Medicine, in press. PMID: 24821820. PMC in process after embargo
56. LG Griffith, **A Wells**, DB Stolz (2014). Engineering liver. Hepatology, in press.
55. AM Clark, SE Wheeler, DP Taylor, VC Pillai, CL Young, R Prantil-Baun, T Nguyen, DB Stolz, JT Borenstein, DA Lauffenburger, R Venkataramanan, LG Griffith, **A Wells** (2014). A microphysiological system model of therapy for liver micrometastases. Experimental Biology and Medicine, in press.
56. LG Griffith, **A Wells**, DB Stolz (2014). Engineering liver. Hepatology, in press.

12. FY15 Year Support - Alan Wells

ACTIVE

- 1) DoD CDMRP (PC080566) (PI: Wells) 3/15/09 – 3/14/15
0.6 months
\$10,000 directs to Wells Lab
DoD CDMRP PCRP, HBCU Summer Training Program
“Pittsburgh-Tuskegee Prostate Training Program (PTPTP)”
The goal of this training program is to train in prostate cancer research 4 undergraduate students each year. These Tuskegee University students will undertake a summer project at the Univ Pittsburgh and then continue during the school year on the same project with a mentor at Tuskegee. This is a **training grant with no overlap. This project is not being renewed and will end prior to the start of this renewal.**
- 2) NIGMS (GM63569) (PI: Wells) 7/1/03 – **8/30/13** – 8/30/14 NCE
2.4 months
\$0 directs to Wells Lab during
NIH/NIGMS
NCE
“Dermal-epidermal communication during wound healing”
The major goal of this project is to determine whether IP-9 and IP-10 are key paracrine signaling molecules that communicate the degree of healing in the wounded skin. There is **no overlap in Aims, target molecules, molecular processes or endpoints** with the current proposal. The renewal has been submitted 2/2014 and is likely to be renewed as of 1/1/15.
- 3) NIGMS (GM69668) (PI: Wells) 1/15/04 – 11/30/15
2.0 months
\$119,000 directs to Wells Lab
NIH/NIGMS
“Spatial segregation of cell functioning during motility”
The major goal of this renewed project that started 1/1/08, is to determine how a cell segregates biochemical and biophysical pathways in a front-rear asymmetry during growth factor-induced motility. The working hypothesis centers on phospho-inositide generation behind the leading lamellipod actuates transcellular contractility in front of the nucleus. The cells of focus are keratinocytes, fibroblasts and endothelial cells. There is **no overlap in Aims, target molecules, molecular processes or endpoints with the current proposal.**
- 4) VA Merit Award (PI: Wells) 10/1/06 - 9/30/15
1.50 months
\$149,000 directs to Wells Lab
Veterans Administration
"Molecular Regulation of Cancer Progression"
The major goals of this project are to determine whether (a) E-cadherin expression protects from induced death and (b) whether E-cadherin expression improves metastatic seeding in animal models. **This is the grant currently being proposed for competitive renewal.**
- 5) NIH (1UH3TR000496) (PI: Griffith, MIT) 8/1/12 – 6/30/17
2.00 months
\$200,000 directs to Wells Lab
NIH/Clinical Translational Sciences directorate
“All Human Microphysical Model of Metastasis Therapy”
The major goals of this project are to (a) develop a microfluidic, flow- and perfusate-controlled organotypic liver bioreactor that mimics the human situation (MIT and Draper Labs), and (b) test that for tumor micrometastases behavior and growth/survival in the face of hormonal and chemotherapeutic challenges (UPitt). **There is no overlap with the current grant, but is complementary in that it is to establish an improved version of the MPS that is being used in this Merit Renewal proposal.**

6) NIH (UH3TR000503) (PI: Taylor, UPitt)

7/1/14 – 6/30/17

0.50 months

NIH/Clinical Translational Sciences directorate

\$25,000 directs to Wells Lab

“A 3D biomimetic liver sinusoid construct for predicting physiology and toxicity”

The goal of this UH3 proposal is to integrate these three organ models as a drug clearance and organ toxicity model, as well as a starting point for assessing distribution of orally administered drugs. The Wells lab role is to enable High Content Screening of tumor cells in the liver bilayer module so as to rapidly assess changes in actively growing tumor cells. **There is no overlap with the current grant.**

7) NCI (1R21CA188799-01) (PI: Yates, Tuskegee)

9/30/14 – 9/29/16

0.6 months

NIH/NCI Cancer Health Disparities Program

\$39,000 directs to Wells Lab

“Transcriptional Regulation of Breast Cancer Metastasis within the Tumor Microenvironment”

The goal of this proposal, directed by Clayton Yates, PhD at Tuskegee, who is collaborating in the current UH3 extension to provide prostate and breast cancer specimens from African-Americans, is to determine whether the shuttling between E-cadherin and the nucleus of this DNA methylation regulator dictates tumor cell phenotype. While this proposal is complementary, **there is no overlap as this is directed as the control of E-cadherin expression.**

PENDING

1) NIGMS (GM63569) (PI: Wells)

12/1/14 – 11/30/19

2.4 months

NIH/NIGMS

\$160,000 directs to Wells Lab

“Dermal-epidermal communication during wound healing”

This is a competing renewal of the above grant in NCE. The major goal of this project is to determine the role of key matrix proteins in educating the wound to avoid scarring and promote quiescence after healing. There is **no overlap in Aims, target molecules, molecular processes or endpoints** with the current proposal.

PHS Fellowship Supplemental Form

A. Application Type:

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference, as you attach the sections that are appropriate for this Career Development Award.

New Resubmission Renewal Continuation Revision

B. Research Training Plan

1. Introduction to Application

(for RESUBMISSION applications only)

2. Specific Aims*

1241-Specific_Aims_Beckwitt_Final.pdf

3. Research Strategy*

1242-Research_Strategy_Beckwitt_Final.pdf

4. Progress Report Publication List

(for RENEWAL applications only)

Human Subjects

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? Yes No

5. Human Subjects Involvement Indefinite?

Yes No

6. Clinical Trial?

Yes No

7. Agency-Defined Phase III Clinical Trial?

8. Protection of Human Subjects

1243-Corrected_HumanSubjects_Beckwitt_Final.pdf

9. Inclusion of Women and Minorities

10. Inclusion of Children

Other Research Training Plan Sections

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the use of vertebrate animals, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Vertebrate Animals Used? Yes No

11. Vertebrate Animals Use Indefinite?

12. Vertebrate Animals

13. Select Agent Research

14. Resource Sharing Plan

17. Respective Contributions*

1244-Corrected_Respective_Contributions_Beckwitt_Final.pdf

16. Selection of Sponsor and Institution*

1245-Selection_Sponsor_Institution_Beckwitt_Final.pdf

17. Responsible Conduct of Research*

1246-Responsible_Conduct_Research_Beckwitt_Final.pdf

PHS Fellowship Supplemental Form

C. Additional Information

Human Embryonic Stem Cells

1. Does the proposed project involve human embryonic stem cells?* Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

Fellowship Applicant

2. Alternate Phone Number: 339-987-9046

3. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):
DOTh: Other Doctorate	MD/PhD	2021-05

4. Field of Training for Current Proposal*: 1140 Metabolism

5. Current Or Prior Kirschstein-NRSA Support?* Yes No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)
Predocctoral	Institutional	07/01/2013	06/30/2014	5T32GM008208-25
Predocctoral	Institutional	07/01/2014	06/30/2015	5T32GM008208-26

6. Applications for Concurrent Support?* Yes No

If yes, please describe in an attached file:

7. Goals for Fellowship Training and Career* 1247-Goals_For_Fellowship_Beckwitt_Final.pdf

8. Activities Planned Under This Award* 1248-Activities_Planned_Beckwitt_Final.pdf

9. Doctoral Dissertation and Other Research Experience 1249-Doctoral_Dissertation_Research_Exp_Beckwitt_Final.pdf

10. Citizenship* U.S. Citizen or noncitizen national Permanent Resident of U.S. Pending
 Permanent Resident of U.S. Non-U.S. Citizen with temporary U.S. visa
(If a permanent resident of the U.S., a notarized statement must be provided by the time of award)

Institution

11. Change of Sponsoring Institution Name of Former Institution:*

PHS Fellowship Supplemental Form

D. Sponsor(s) and Co-Sponsor(s)

Sponsor(s) and Co-Sponsor(s) Information* 1250-Corrected_SponsorInformationWells_Beckwitt_Final.pdf

E. Budget

All Fellowship Applicants:

1. Tuition and Fees*:

None Requested Funds Requested

Year 1	\$56,694.00
Year 2	\$59,529.00
Year 3	\$62,505.00
Year 4	\$65,630.00
Year 5	\$61,692.00

Year 6 (when applicable)

Total Funds Requested: \$306,050.00

Senior Fellowship Applicants Only:

	Amount	Academic Period	Number of Months
2. Present Institutional Base Salary:			
3. Stipends/Salary During First Year of Proposed Fellowship:			
a. Federal Stipend Requested:	Amount		Number of Months
b. Supplementation from other sources:	Amount		Number of Months
	Type (sabbatical leave, salary, etc.)		
	Source		

F. Appendix

B.2. SPECIFIC AIMS

Tumor metastasis to the liver is one of the most common manifestations of advanced cancers and heralds mortality from the disease. The metastatic cascade is initiated by an epithelial to mesenchymal transition (EMT) of the primary tumor that allows for migration from the primary mass. Tumor cells next intravasate into the systemic circulation, through which they are transported to other organs. Extravasation and seeding of surviving tumor cells at these distant sites establishes micrometastases, most commonly only a few cells in size. In addition to immediately outgrowing to form clinically evident metastases, these micrometastases often enter a dormant state, a situation especially daunting in breast cancer. The quiescence of these cells not only prevents their detection by traditional imaging modalities, but it also imparts chemoresistance. Years to decades can pass before these cells convert to a more proliferative phenotype. Signals from the tumor microenvironment are crucial in both maintaining cell dormancy and eventual tumor outgrowth. We previously showed that cell signaling and extracellular growth factors influence metastatic outgrowth in a microphysiological model for liver metastasis, a “LiverChip.”

Rapidly proliferating cancer cells resort to glycolytic metabolism in order to accumulate the massive amounts of metabolic intermediates required for cell division, the so-called “Warburg Effect.” However, the phenotypically dormant cancer cells may exhibit a different metabolic profile, which may be crucial to both early diagnosis and treatment. **Our foundational model is that dormant micrometastases adapt a low metabolic and proliferative state concordant with chemoresistance and reduced glycolytic flux. We hypothesize that oxygen tension levels will dictate the switch between oxidative phosphorylation and glycolytic metabolism, and impact dormancy and chemoresponsiveness.** This will be tested in the following Aims:

Aim 1. Determine whether dormant cancer cells display decreased levels of glycolytic flux. Tumor dormancy is characterized by a quiescent state, in which cells undergo a G0/G1 arrest, similar in phenotype to the quiescence observed in normal differentiated cells. Interactions between resident and tumor cells are known to play a role in this arrest. Examining the metabolic profile of micrometastases, including measuring intracellular ATP, protein synthesis, and metabolic enzymatic levels, in the context of the LiverChip system described herein will elucidate the impacts of native tissue on tumor cell dormancy.

Aim 2. Determine whether different oxygen levels affect tumor cell dormancy. The supply of oxygen and nutrients is essential for rapidly proliferating tumors and is the main reason for the research into antiangiogenic molecules as cancer therapies. However, the effects of oxygenation on dormant tumor cell phenotype have not been described. We hypothesize that high oxygen tensions will disrupt tumor dormancy, causing cell proliferation and outgrowth. Controlled measurement and modification of oxygen tension may reveal whether dormant cells require a specific oxygen environment in order to maintain dormancy or outgrow to form clinically evident metastases.

Aim 3. Define whether select anti-tumor agents are dependent on tumor cellular metabolic state/tissue oxygen capacity. Chemoresistance of dormant micrometastases is responsible for the widespread very limited success of adjuvant chemotherapy. The responsiveness of dormant tumor cells to chemotherapeutic agents, such as cisplatin, and anti-metabolic drugs, such as statins, will be monitored in the LiverChip system. In doing so, a relationship between drug resistance and cellular metabolic state may be extrapolated and novel biomarkers for therapy efficacy may be deduced. Additionally, the local effects of these treatments on liver tissue, a common site for drug toxicity, will be examined.

The successful completion of these Aims will provide novel insights into basic tumor biology of metastases. The consideration that oxygen tension alters micrometastatic state of dormancy or outgrowth is an innovative concept that has not been amenable to study previously. The use of the cutting edge all human liver microphysiologic system, coupled with novel oxygen carriers will now allow us to alter oxygen delivery to address this key question. The establishment of this system will also promote translational studies into improving the efficacy of existing agents (drug rescue) and in the development of new adjuvant therapies for this mortal stage of cancer development.

B.3. RESEARCH STRATEGY

A. Background and Significance

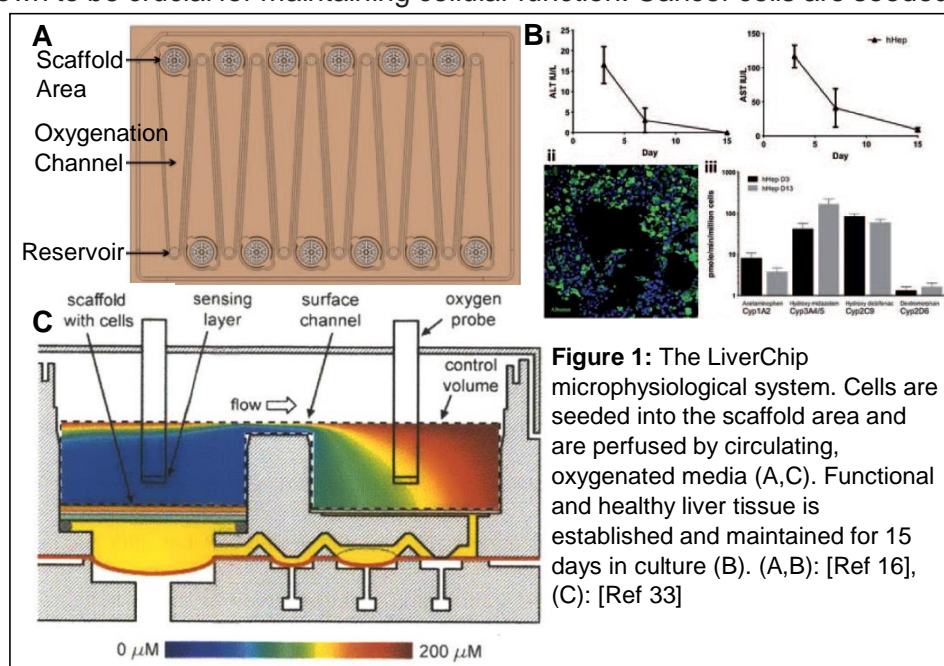
A1. Background

Relatively little is known about the role of oxygen in controlling micrometastatic states of dormancy or outgrowth, despite this being a critical step in cancer progression. Two main strategies have been proposed in the literature for the establishment of dormancy in micrometastases: “balanced proliferation” and “quiescence.” The balanced proliferation hypothesis attributes dormancy to an equilibrium between cell proliferation and cell death, allowing for Darwinian selection of tumor cells best fit to survive in their microenvironment¹, often resulting in an “angiogenic switch” characterized by the recruitment of blood vessels. In contrast, the quiescence hypothesis attributes dormancy to a cessation of cell proliferation, caused by a G0/G1 cell cycle arrest². Cellular senescence is unlikely, as it is an irreversible state which would prevent dormant cell outgrowth². Recent *in silico* models have shown quiescence most likely explains micrometastasis dormancy^{3,4}. This is supported by molecular analyses of circulating tumor cells (CTCs) from patients with small cell lung cancer⁵ and breast cancer⁶ demonstrating a lack of proliferative markers. Moreover, patients exhibiting CTCs void of proliferative markers are often refractory to chemotherapy⁷. These results suggest the large role cellular quiescence plays in cancer dormancy and chemotherapy resistance and motivates the investigation of mechanistic determinants of quiescence in an effort to divulge novel therapies.

Since Warburg’s discovery that tumor cells primarily utilize glycolysis for energy accumulation, despite abundant levels of oxygen, manipulating cancer metabolism has been sought as an avenue of therapy. Rapidly proliferating tumors depend on angiogenesis to accommodate their growing need for nutrient and oxygen delivery. Large, proliferative tumors upregulate micro-RNAs⁸ to augment their vascularity, and hence nutrient and oxygen supply. Moreover, hypoxic induction of transcription factors such as HIF-1 α has been associated with dysregulation of glycolytic enzymes^{9,10}, drug efflux pumps¹¹, and anti-apoptotic proteins^{11,12}. These results suggest that oxygen supply dictates the metabolic capacity and pharmacologic susceptibility of clinically significant cancers. Unfortunately, highly vascularized tumors frequently bode poor prognosis for cancer patients^{10,13,14}. While increasing oxygen availability has been shown to contribute to the progression of macro-scale cancers, **the impact of oxygen tension has not yet been investigated in tumors of the micro-scale. This study will examine for the first time the link between oxygen tension and the switch between tumor dormancy and progression in a novel microphysiological model for liver micrometastases.**

The all human liver microphysiological system (MPS) we employ (LiverChip, Zyoxel Ltd.) has been shown to be a physiologically relevant model for the investigation of dormant micrometastases (**Figure 1**)^{15,16,17,33}. In short, primary hepatocytes and non-parenchymal cells (NPCs) are seeded into a flexible, hydrogel scaffold. After a couple days of adaptation, these primary cells establish a micro-hepatic architecture capable of endogenous liver functions, such as albumin production. This tissue exists in a continuous perfusion system, since both nutrients and physical stimulus are known to be crucial for maintaining cellular function. Cancer cells are seeded

on this formed tissue on the third day of culture. After fifteen days of culture, the scaffolds are harvested and analyzed by immunohistochemistry to determine tissue microstructure. Analysis of scaffolds treated with chemotherapeutics or EdU/BrdU describes tumor drug susceptibility and proliferation respectively. Clinical chemistry is used to probe the functionality of the established liver tissue every two days by measuring concentrations of cytochrome P450 isoforms (CYP450), α 1-antitrypsin (A1AT), fibrinogen, and urea in collected supernatant samples during media changes. Moreover, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are measured as a marker for hepatocyte health. The addition of engineered ruthenium-based oxygen sensors and novel oxygen carriers will **enable this system to test the hypothesis that oxygen tension levels dictate the switch between oxidative phosphorylation and glycolytic metabolism, and thereby impact dormancy and chemoresponsiveness (Figure 2).**



A2. Significance

i. Clinical:

The dormancy of micrometastases presents several clinical problems. While the primary tumor can be effectively eliminated by surgery, radiotherapy, or chemotherapy, dormant micrometastases are commonly resistant to these treatments. The outgrowth of these micrometastases is the main source of morbidity and mortality in cancer patients. Additionally, these small groups of dormant cells are clinically undetectable using current diagnostic modalities, hindering the development of strategies to circumvent treatment insensitivity. It is clear that more effective therapies are needed to deal with micrometastatic cancer cells. *Understanding the influence of oxygen tension on dormancy maintenance and the potentiation of existing therapies may suggest effective ways to prevent clinically emergent disease from this early stage of dissemination.*

Understanding the influence of oxygen tension on micrometastasis metabolism, dormancy, and treatment susceptibility may 1) promote translational studies into improving the efficacy of existing therapies, 2) promote the development of new therapies specific for this stage in tumor development, 3) inspire new diagnostic modalities, and/or 4) suggest prophylactic methods to prevent emergence from dormancy in scenarios of anticipated, but not clinically evident, metastasis.

ii. Basic Tumor Biology:

The mechanisms for metastatic dormancy remain largely undetermined, due to the absence of physiologically relevant systems with the potential to establish and maintain dormant cancer cells. The liver MPS, which this research will employ, consistently establishes quiescent micrometastases, facilitating investigation into the mechanisms of dormancy maintenance and outgrowth. Investigation of the impact of oxygen tension on dormant cellular metabolism and treatment potentiation may suggest signaling and metabolic pathways responsible for the establishment and maintenance of dormancy. Additionally, instituting a more physiological robust oxygen delivery system, through the use of novel oxygen carriers, will facilitate investigation of factors contributing to the observed resistance to chemotherapeutic and anti-metabolic agents. Finally, measuring the health and functionality of the liver tissue, in which these micrometastases are established, will elucidate both microenvironmental alterations and potentially limiting toxicities of the therapies.

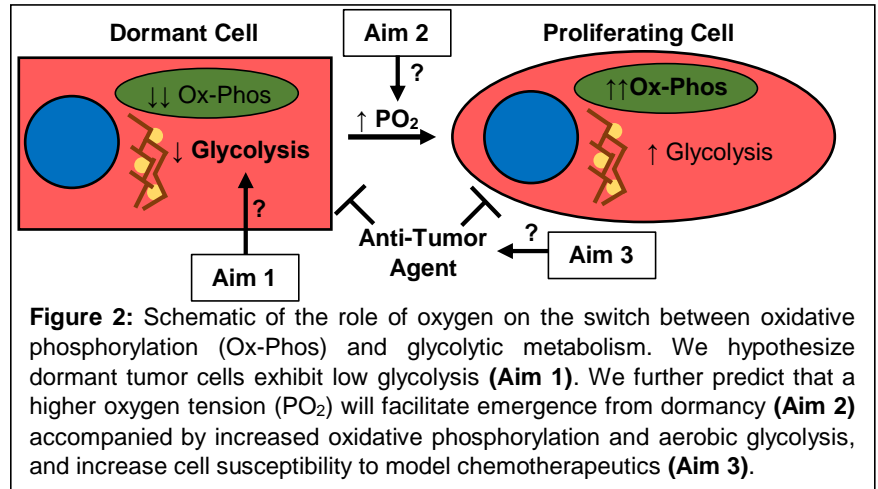
A3. Innovation

This work is highly innovative in its conception and technical approaches as the question of oxygen impact on tumor dormancy was previously inaccessible. Our foundational model is that micrometastases establish a low metabolic and proliferative state concordant with chemoresistance and low glycolytic flux, with this premortal phenotype modulated by oxygen tension. We previously established a liver MPS capable of replicating cancer cell dormancy in the absence of molecular engineering or pharmacologic intervention. This system relies on dissolved oxygen, replenished at an air-liquid interface, for metabolic needs. This work aims to establish a more physiological oxygen delivery system utilizing novel oxygen carriers. Hemoglobin-bound oxygen comprises 98% of total body oxygen³⁴ while the rest is dissolved in the plasma. The introduction of an oxygen carrier analogous to endogenous erythrocyte hemoglobin will facilitate robust modulation of the system's oxygen supply. Incorporation and characterization of this oxygen carrier can be extrapolated to other organotypic culture systems requiring physiological oxygen delivery. Moreover, this tunable variable will allow the impacts of the oxygen environment on dormancy maintenance (**Aim 2**) and treatment susceptibility (**Aim 3**) to be extrapolated.

B. Experimental Design

B1. Systems Used

This work will make use of a previously described all human three-dimensional liver culture system/MPS^{15,16,17}, which provides a physiologically relevant environment in which micrometastasis biology can be studied. This MPS allows for the continuous perfusion of an all human primary cell liver tissue and establishment of micrometastatic niches. Liver tissue production, metabolism, and health can be determined through measuring synthetic function (albumin, α 1-antitrypsin, urea), metabolic activity (CYP450 activities), and damage markers (AST, ALT) respectively. Cancer dormancy can be demonstrated through lack of proliferative



markers (**Figure 3**) and the chemoresistance commonly seen both clinically and experimentally. Previously developed oxygen sensors will give a sensitive, real-time readout of oxygen tension levels on both sides of the perfused tissue layer³³. Continuous perfusion and characterizations of both the liver tissue and micrometastases allow for the impact of introducing a novel oxygen delivery system to be measured.

The non-metastatic MCF-7 and highly aggressive MDA-MB-231 breast cancer cell lines will be used in all experiments to reflect epithelioid and a mesenchymal cancer phenotypes, respectively. MCF7 cells are hormone responsive and express E-cadherin on the cell surface to form cell-cell contacts even in culture. MDA-MB-231 cells are triple-negative breast cancer cells that suppress E-cadherin expression by extensive DNA promoter methylation, but also present high levels of EGF receptors.

B2. Aim 1. Determine whether dormant cancer cells display decreased levels of glycolytic flux.

Rationale: As previously stated, the all human liver MPS we employ is a relevant model for examining metastatic dormancy (**Figure 3**). Rapidly proliferating tumors have been characterized by high degrees of glucose uptake and consumption¹⁸. This trait has been exploited in standard imaging modalities such as positron emission tomography (PET) for diagnostic and prognostic purposes^{18,19}. High fluorodeoxyglucose (FDG) uptake correlates with poor outcome in head and neck²⁰ and neuroendocrine²¹ tumors. The Glut-1 glucose transporter has been found to be upregulated in cancers in response to hypoxia⁴². Moreover, inhibition of glucose uptake in cancer cells by flavanoids^{22,23} or transfected miRNA²⁴ decreases their proliferation. Inhibition of enzymes relevant to the glycolytic pathway has also been shown to decrease cancer proliferation. For example, inhibiting the PFKFB3^{25,26} and PFKFB4²⁷ isozymes of 6-phosphofructo-2-kinase, a constitutively active enzyme whose product (fructose-2,6-bisphosphate) is a key activator of glycolysis, has been found to reduce the proliferation and survival of cancer cells respectively by reducing glycolytic flux. These data suggest that glycolytic metabolism highly correlates with the proliferation of cancer cells and poor clinical prognosis. Aggressive cancers additionally exhibit reduced oxidative phosphorylation flux due to upregulation glycolytic and biosynthetic pathways. For example, PDK1 expression has been shown to shunt pyruvate away from oxidative phosphorylation in cancer cells due to inactivation of pyruvate dehydrogenase, contributing to overactive glycolysis and cancer aggression⁴³. ATP-citrate lyase, an enzyme that generates acetyl-CoA from cytoplasmic citrate, is upregulated in many cancers to increase lipid and cholesterol biosynthesis and protein isoprenylation⁴⁴. While the importance of glycolysis and oxidative phosphorylation have been studied in monolayer culture and clinical diagnostics, the relevance of glycolytic metabolism in cancer dormancy has not. We hypothesize cancer cells exhibiting a dormant, more epithelioid phenotype seen in micrometastases concomitantly show decreased glycolytic flux.

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Experimental Design: To determine alterations in glycolytic flux, several proteins typically upregulated in cancers will be quantified through imaging modalities. Glut-1 and PFKFB3 immunofluorescence (levels and localization to membrane) will detect changes in glucose uptake and glycolytic regulation, respectively, by dormant cells. To directly measure intracellular glucose levels, a glucose analog (2-NBDG) will be added on day 13 of culture. Upon scaffold harvesting and sectioning, fluorescent imaging will determine intracellular glucose accumulation over the previous two days. To determine the relative amounts of glucose undergoing glycolytic or oxidative phosphorylation metabolism, PDK1 immunofluorescence will be quantified and normalized to the glucose uptake measured previously. Oxidative phosphorylation flux will also be inferred by cytoplasmic ATP-citrate lyase levels. Total metabolic energy generation will be determined by measuring intracellular ATP levels indirectly with magnesium green staining. Cell dormancy will be assessed by the lack of EdU incorporation and lack of KI67 staining in RFP-positive cancer cells. Membrane E-cadherin immunofluorescence will determine whether cancer cells exhibit an epithelioid phenotype typically seen in dormancy. Since the goal of these experiments is to determine the metabolism of dormant cells, the KI67- cells (dormant) will be compared to the KI67+ cells (actively proliferating) for data analysis.

Expected results, possible pitfalls, and alternative approaches: We expect dormant cancer cells to exhibit a lower glycolytic flux as compared to proliferating cancer cells. The LiverChip system we employ is ideal for examining

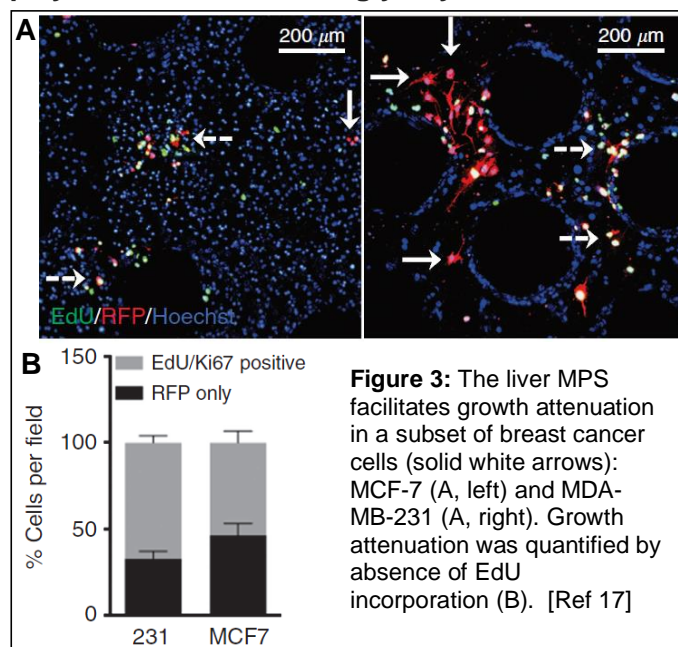


Figure 3: The liver MPS facilitates growth attenuation in a subset of breast cancer cells (solid white arrows): MCF-7 (A, left) and MDA-MB-231 (A, right). Growth attenuation was quantified by absence of EdU incorporation (B). [Ref 17]

the two phenotypic populations – proliferative and quiescent – exposed to the same microenvironment. Compared to proliferating cancer cells, dormant cancer cells will likely exhibit less Glut-1 and PFKFB3 immunostaining. If true, we expect to see dormant cells uptake less 2-NBDG over the two day period prior to scaffold harvesting. The reduced energy requirements of the dormant cells will likely decrease PDK1 levels. Chronic hypoxia-induced dormancy in cancer cells has previously shown decreased expression of Glut-1 and PDK1 as compared to acute hypoxic exposure³². Furthermore, ATP-citrate lyase levels will likely be decreased due to down-regulated PI3K-Akt signaling in quiescent cells⁴⁵. Finally, we expect to see lower levels of intracellular ATP in dormant cells.

It is possible that the expression of the glycolytic markers being measured will not be significantly different between the quiescent and proliferating cancer cells in our system. While unlikely, given the regulatory signaling factors previously discussed which link glycolytic upregulation and proliferation, the reduction in metabolic flux could be due to alternative pathways. For example, rapidly proliferating tumors often have elevated glutamine metabolism, whose high flux through the TCA cycle provides building blocks for lipid synthesis^{46,47}. Some cancers, in particular hepatocellular carcinoma, upregulate glutamine metabolism as a means for energy accumulation⁵⁴. So, if we find glycolytic flux is not reduced in dormant cells, glutamine flux will be investigated by probing for glutaminase and aspartate aminotransferase, two enzymes which facilitate glutamine utilization by the TCA cycle⁴⁸. The flexibility of the LiverChip system allows for investigation of multiple metabolic intermediates and extrapolation of potential mechanisms for metabolic suppression in dormant tumor cells.

B3. Aim 2. Determine whether different oxygen levels affect tumor cell dormancy.

Rationale: Oxygen supply plays a critical role in the proliferation and metastasis of human cancers. Previous work has shown that transcription factors, such as HIF-1 α , are stabilized during periods of hypoxia, and induce pro-survival, pro-angiogenic, and pro-glycolytic genes *in vivo* and *in vitro*^{10,28}. In the presence of oxygen, HIF-1 α is hydroxylated at two key proline residues in its oxygen degradation domain, targeting it for ubiquitination²⁹. Hypoxia also contributes to cancer chemotherapeutic resistance by slowing the cell cycle, diminishing the efficacy of alkylating agents, and upregulating p-glycoprotein (P-gp), an efflux pump associated with resistance to chemotherapeutic drugs such as adriamycin³⁰. Given the importance of oxygen supply to a proliferating tumor, anti-angiogenic agents have been explored as therapies, most commonly in combination with standard chemotherapy³¹. It is apparent that the efficacy of these antiangiogenic drugs depends greatly on the microenvironment of the tumor and the molecular pathways involved^{29,30,31}. While much work has been done investigating the role of oxygen on tumor progression, very little investigation has been conducted on dormant cancer cells. In monolayer culture during periods of prolonged, extreme hypoxia (1% O₂), a pancreatic cancer cell line was shown to induce cell dormancy through suppression of Akt, a constitutively active kinase in many human cancers³². Additionally, PPAR γ , a transcriptional mediator commonly expressed in many cancers^{35,36}, has found to be activated under hypoxic conditions^{37,38}. This result suggests oxygen tension may play some role in initiating or maintaining cancer dormancy. Through use of our all human liver MPS, we aim to investigate the relationship between oxygen and tumor cell dormancy. We hypothesize that dormant cells require a specific oxygen environment which, when perturbed, will facilitate outgrowth (**Figure 4**).

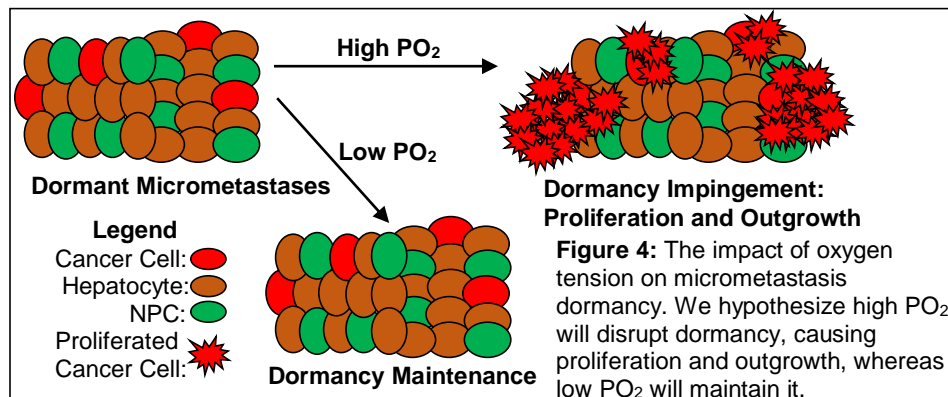


Figure 4: The impact of oxygen tension on micrometastasis dormancy. We hypothesize high PO₂ will disrupt dormancy, causing proliferation and outgrowth, whereas low PO₂ will maintain it.

Hypoxia also contributes to cancer chemotherapeutic resistance by slowing the cell cycle, diminishing the efficacy of alkylating agents, and upregulating p-glycoprotein (P-gp), an efflux pump associated with resistance to chemotherapeutic drugs such as adriamycin³⁰. Given the importance of oxygen supply to a proliferating tumor, anti-angiogenic agents have been explored as therapies, most commonly in combination with standard chemotherapy³¹. It is apparent that the efficacy of these antiangiogenic drugs depends greatly on the microenvironment of the tumor and the molecular pathways involved^{29,30,31}. While much work has been done investigating the role of oxygen on tumor progression, very little investigation has been conducted on dormant cancer cells. In monolayer culture during periods of prolonged, extreme hypoxia (1% O₂), a pancreatic cancer cell line was shown to induce cell dormancy through suppression of Akt, a constitutively active kinase in many human cancers³². Additionally, PPAR γ , a transcriptional mediator commonly expressed in many cancers^{35,36}, has found to be activated under hypoxic conditions^{37,38}. This result suggests oxygen tension may play some role in initiating or maintaining cancer dormancy. Through use of our all human liver MPS, we aim to investigate the relationship between oxygen and tumor cell dormancy. We hypothesize that dormant cells require a specific oxygen environment which, when perturbed, will facilitate outgrowth (**Figure 4**).

Experimental Design: To determine the influence of oxygen supply on tumor dormancy, the oxygen tension will be modulated by adding varied concentrations of a hemoglobin-based oxygen carrier (HBOC) to the culture medium. In order to calculate tissue oxygen supply and consumption, oxygen tension and the trans-tissue oxygen gradient will be measured using ruthenium-based oxygen sensors previously described³³. To determine the effects of adding an oxygen carrier and oxygen tension on the tumor microenvironment, cytokine, chemokine, and growth factor panels will be measured as previously described¹⁷. To assess the adequacy of oxygen delivery to cancer cells in various locations in the liver tissue, the degree of hypoxia will be assessed by HIF-1 α immunofluorescence. Finally, to determine if an increased oxygen supply alters metabolism or dormancy of dormant cancer cells, the glycolytic and oxidative phosphorylation fluxes and cell dormancy will be measured as described in **Aim 1**.

Expected results, possible pitfalls, and alternative approaches: We expect the introduction of an HBOC will modulate the tumor dormancy. Previous work has shown that factors such as PPAR γ , which inhibit cancer proliferation and invasion through upregulation of cyclin dependent kinase inhibitors p21 and p27³⁶, are activated under hypoxic conditions. Short-term exposure to PPAR γ ligands in nude mice has been found to reduce colorectal cancer metastasis and longer term exposure demonstrated the formation of quiescent, dormant groups of cells³⁹. These findings suggest increasing the oxygen supply to dormant cancer cells may cause a shift out of dormancy. An increased oxygen supply would be noted by a decrease in the trans-tissue oxygen gradient (more effective oxygen buffering by the HBOC system). Additionally, a higher population of KI67+, EdU-incorporating cells is expected due to emergence from dormancy. HIF-1 α expression is expected to decrease under an adequate oxygen supply. Increased oxygen tension will likely upregulate oxidative phosphorylation flux. The higher energy requirements of the emerged, proliferating cells will likely have higher glycolytic flux, despite oxygen-catalyzed degradation of hypoxia-response proteins (HIF-1 α) and the resulting decrease in transcription of the glycolytic enzymes and regulatory factors previously described.

Altered oxygen tension may also impact the hepatocytes and NPCs, the latter of which are known to play an important role in the tumor microenvironment¹⁷. Examining signaling molecules present in the microenvironment, such as proinflammatory cytokines (IL-6, IL-8) and growth factors (EGF), will determine the impact of oxygen tension on the LiverChip tissue.

It is possible that the incorporation of the HBOC will not cause dormant cells to outgrow. This could be due to intracellular signaling which maintains quiescence even in the presence of altered metabolism. Various signaling modalities such as kinases (Akt³², Erk⁴⁰) and checkpoint proteins (p16², p21³⁶, p27³⁶, p38⁴⁰) have been shown to be down-regulated and up-regulated respectively in dormant cells. Alternatively, signals from the hepatic microenvironment may play a role in dormancy maintenance. Hepatocyte co-culture has been shown to promote re-expression of E-cadherin in breast and prostate cancer cells, playing a role in the conversion of these micrometastases to a more epithelioid, quiescent phenotype⁴¹. Moreover, compromised β 1-integrin and EGF signaling from the environment may contribute to persistent dormancy even in the context of intracellular metabolic changes.

If increasing oxygen tension is not found to have a measurable effect on cell proliferation or quiescence, the factors mentioned above will be investigated to find the potential upregulated pathways that contribute to dormancy maintenance. Tumor cell membrane E-cadherin expression will be examined as a possible factor for dormancy maintenance. Additionally, growth factors and cytokines will be quantified to determine if the microenvironment is the cause for preserved quiescence. The LiverChip system is amenable to these investigations, all of which will provide more insight into the relationship between oxygen tension and tumor cell dormancy should our initial hypothesis prove incomplete.

B4. Aim 3. Define whether select anti-tumor agents are dependent on tumor cellular metabolic state.

Rationale: The previous two aims addressed examination of glycolytic metabolism in dormant cancer cells (**Aim 1**) and the influence of increasing oxygen tension on cancer cell dormancy and metabolic pathway fluxes (**Aim 2**). The current aim will investigate the influence of tumor metabolic state on select cancer therapies, namely chemotherapeutics (cisplatin and doxorubicin) and statins (atorvastatin; atorva, simvastatin; simva, fluvastatin; fluva). Previous work in the LiverChip system has shown a high degree of chemoresistance to cisplatin and doxorubicin among dormant cancer cells (**Figure 5A,B**) and preliminary in vitro data I collected showed exogenous E-cadherin expression increases the resistance of MDA-MB-231 cells to atorvastatin (**Figure 5C**). The increased resistance to

statin treatment can potentially be due to the quiescence induced by membrane E-cadherin expression, which converts the traditionally mesenchymal MDA-MB-231 cells to a more epithelioid phenotype⁴⁹ commonly seen in dormant cells. The fact that I found similar statin resistance is observed in the epithelioid DU-145 and MCF-7

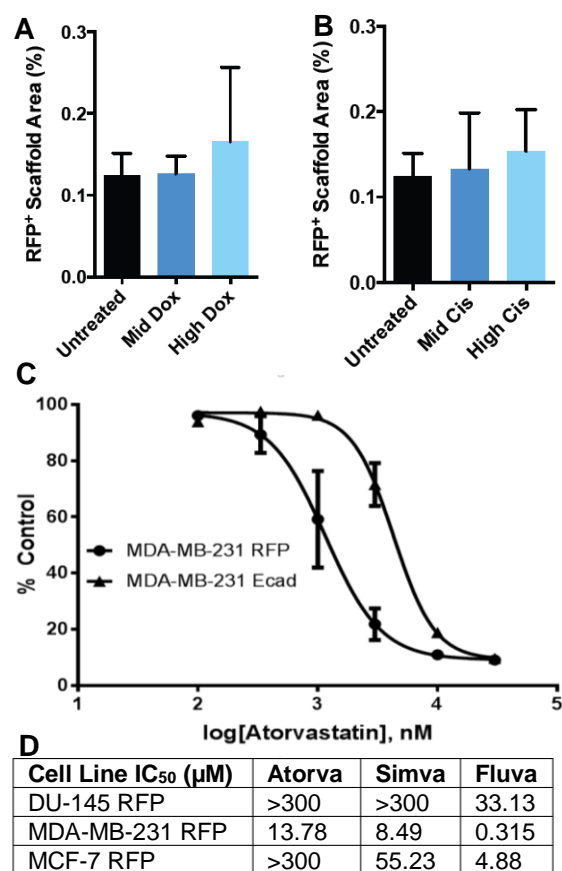


Figure 5: Resistance to doxorubicin (A) and cisplatin (B) in dormant MDA-MB-231 RFP+ cells. MDA-MB-231 RFP+ cells exogenously expressing E-cadherin are more resistant to atorvastatin-induced growth inhibition (C). Statin IC₅₀ values collected in RFP labeled cell lines (D). (A,B,D): Unpublished data from Wells lab. (C): Data in review (Warita et al, Scientific Reports).

cell lines supports this hypothesis. (**Figure 5D**). Chemotherapeutics and statins are widely used clinical drugs making them prime candidates to investigate the ability of oxygen to potentiate cancer therapy. Investigation of therapy resistance in this system may reveal new biomarkers that can be used to guide clinical treatment choices. Finally, local effects on hepatic tissue, a common site for drug toxicity, can be measured in this system.

Experimental Design: During each treatment condition, cellular metabolic state and dormancy phenotype will be monitored as described in **Aim 1**. Doses of doxorubicin and cisplatin will be used as previously described (**Figure 5A,B**). Doses of the three statins previously mentioned will be investigated around the IC_{50} measured in cell culture (**Figure 5C,D**). Treatments will be conducted using HBOC concentrations that provide significant metabolic changes, as assessed in **Aim 2**. Cancer cell viability will be determined by measuring the %RFP Scaffold Area (quantification of alive cancer cells). Hepatocyte toxicity and functionality will be measured using tests previously described in the **Background** section.

Expected results, possible pitfalls, and alternative approach: Assuming increased oxygen tension results in emergence from dormancy (as investigated in **Aim 2**), we expect increasing oxygen supply will potentiate both chemotherapeutic and statin efficacy (drug rescue). Since chemotherapeutic drugs like cisplatin rely on cellular proliferation for cytotoxicity, enhanced proliferation upon emergence from quiescence is expected to reduce cancer cell viability. Statins have been shown to be efficacious anti-tumor agents through inhibition of Akt signaling⁵⁰ and cell cycle proteins⁵¹. Our preliminary work has shown different cancer cell lines have varied susceptibility to statin treatment, more epithelioid, E-cadherin-expressing cell lines exhibiting a higher degree of statin resistance (**Figure 5C,D**). This finding makes sense given E-cadherin signaling results in inhibition of tumor cell growth^{49,52}. The two breast cancer cell lines were chosen to examine drug potentiation in both mesenchymal (MDA-MB-231) and epithelioid (MCF-7) cell lines to suggest for which cancer phenotype statin treatment may be beneficial. A higher degree of susceptibility to these anti-tumor agents will reduce the therapeutic concentration needed, which will reduce the measured hepatocyte toxicity and increase hepatocyte functionality.

It is possible that anti-tumor agent efficacy will not be altered by cancer cell metabolic state. While unlikely, especially if a strong effect of oxygen tension on tumor dormancy is observed, the stagnant efficacy could be due to modulation of signaling pathways previously suggested in **Aim 2** or alterations in tumor microenvironment signaling. Measuring potential mechanisms for continued treatment resistance, such as upregulated efflux proteins (P-gp), microenvironment growth factors and cytokines, and E-cadherin/EGF signaling, may suggest mechanisms for continued treatment resistance despite altered cellular metabolic state. If single drug regimens prove ineffective, the LiverChip system can be used to probe multidrug therapy efficacy and hepatotoxicity. Previous studies have shown simvastatin potentiates doxorubicin through increased activation of nuclear factor- κ B (NF- κ B)⁵³. Investigation of combination therapy in this system may suggest additional mechanisms for drug rescue which minimize hepatotoxicity and maximize anti-tumor efficacy.

C. Timeline: The Aims will be tackled in a staggered manner with the **Aim 1** encompassing the first two years, **Aim 2** being started near the end of the first year, and **Aim 3** being started at month 18, and extending to the end of the research period.

D. Summary and Future Directions

The completion of these investigations will provide novel insights into the basic tumor biology of micrometastases. Specifically, these aims will determine the influence of oxygen tension on micrometastatic dormancy and therapy potentiation (drug rescue). This increase in oxygen supply will be instilled by using a novel hemoglobin-based oxygen carrier (HBOC) that will mirror physiological oxygen delivery. We hypothesize that increased oxygen tension will impinge on micrometastatic dormancy and potentiate therapies targeting both cellular proliferation and metabolism.

These studies will suggest future investigations into the metabolic perturbations that accompany cancer dormancy. The incorporation of the HBOC advances the physiological relevance of the LiverChip system, facilitating further studies involving the interaction between the liver microenvironment and dormant micrometastases. Moreover, the impacts of altered oxygen supply can be extrapolated to other physiological systems (gut, kidney). Incorporating other tissue systems in series with the LiverChip, under continuous perfusion with media enhanced by the HBOC, may enable a more comprehensive and clinically translatable understanding of the factors which contribute to cancer dormancy and outgrowth.

6. HUMAN SUBJECTS

Scenario C, Exemption 4

The human cell acquisition and use will be at the University of Pittsburgh.

All studies have been deemed exempted by the University of Pittsburgh IRB and thus do not qualify as human subjects by NIH definition. However, the University of Pittsburgh considers the cells, cell lines and excess pathological specimens, that qualify under exemption 4e, as human subjects.

1. Involvement. Primary human cells are obtained from ATCC, Lonza, and Cascade Biologicals and from discarded tissue removed as part of clinical care and blood donated for transfusion. These are not covered by Human Studies, as there is no access to patient data or identifiers.

Human primary liver cells, mainly hepatocytes but also non-parenchymal cells, will be obtained for coculture and to seed the all human bioreactor. These derive from an federally-funded program to provide human hepatocytes for cellular transplantation and, if derived from normal tissue removed during therapeutic colorectal metastatic nodule resection, for qualified researcher uses; this program is directed by Dr. Stephen Strom (UPitt). These tissues are consented for research use, de-identified at time of surgery, and provided without PHI or other trackable information. These cells are not considered "human subjects" by NIH or DHHS.

The human cells obtained through the University of Pittsburgh sources (liver resections) will be from adults due to the nature of these diseases rarely occurring in children, though children will not be excluded. The de-identified specimens will be tagged to age, gender and race, and will generally conform to the composition of the population served in the Pittsburgh region. The commercially available cells will not always be tagged with demographic information. As no persons are recruited for this present study, we will not be able to balance gender, race or age compositions. Importantly, such issues are not considered major confounders for the cellular and tissue studies herein after accounting for cell viability and functioning.

2. Source. The cells will be obtained from public or commercial sources that retain donor anonymity and confidentiality. The primary cells that are derived from discarded liver and tumor resections are provided from the pathology service as de-identified and without linkages to trace back to patients.

3-6. Recruitment and risks. The human cell lines were obtained after therapeutic intervention or donation. These manipulations have been performed as part of routine clinical care and the obtaining of such tissue in excess to any needed for diagnostic purposes or are not suitable for human transplantation has been judged to present minimal risks to the patients. Patient/donor/volunteer confidentiality and data are maintained by the respective providing services. Specific disease and patient demographic data are not relevant to the investigations planned and thus will not be sought during this study. These study parameters have resulted in an exempt status as determined by the University of Pittsburgh IRB for the liver work.

All other sections of human studies (Inclusion and Enrollment information sheets) are not relevant as there are no Human Subjects per NIH definitions.

B.15. RESPECTIVE CONTRIBUTIONS

The proposal was conceived by the applicant, Colin Beckwitt, after a summer rotation in the Wells lab and discussions with the sponsor, Dr. Alan Wells, collaborators, Dr. Linda Griffith (MIT) and Dr. Paolo Fontes (UPitt), and laboratory members. The specific aims and research training plan were written entirely by the applicant, Colin Beckwitt, with advice and revisions from Wells, medical school faculty Dr. Don DeFranco, career advisor Dr. Kenneth Hallows, and referees Drs. Zoltan N. Oltvai, Forest White, Linda Griffith, and Richard Steinman.

The applicant will plan the experiments with collaborating laboratory colleagues, and present such plans to the mentor and in laboratory meetings for feedback. The applicant will then execute the experiments and collect the data proposed in the training plan. The results of these experiments will be shared with Dr. Wells during weekly one-on-one meetings. As the experiments are compiled, these will be presented at the microphysiological systems investigators meetings. Dr. Wells will be the primary overseer of the applicant's progress regarding the training plan described herein. The applicant will draft all manuscripts corresponding to his collected data, which will be discussed with and reviewed by Dr. Wells and all co-authors prior to journal submission. The applicant will receive assistance from members in the Wells lab who also work with the liver microphysiological system in the system's maintenance and his data analysis. He will write annual reports updating the funding agency on his progress with the proposed training plan, which will be discussed and reviewed by Dr. Wells prior to submission. Dr. Wells will aid in the research, professional, and clinical development of the applicant as outlined in the Research Training Plan.

B.16. SELECTION OF SPONSOR AND INSTITUTION

My ultimate career objective is to become a surgeon-scientist who runs an NIH-funded research laboratory, practices clinical surgery, and teaches. I believe that the mentorship of my sponsor, Dr. Alan Wells, in the context of both the University of Pittsburgh Medical Scientist Training Program (MSTP) and University of Pittsburgh Medical Center (UPMC) will provide me with a supportive and resourceful training environment through which I can both fulfill my current scientific and clinical goals and build a strong foundation for my future career.

I selected Dr. Alan Wells as my thesis mentor because he has an exceptional track record for training pre-doctoral students, five of whom were MD-PhD, who have subsequently launched successful careers including the establishment of independently NIH-funded laboratories. Additionally, Dr. Wells has an extraordinary enthusiasm for student mentorship in regards to both career and intellectual development. He has taken a special interest in providing me with opportunities for collaboration and networking. Impressively, within the first two weeks of my summer laboratory rotation, he had already introduced me to several clinical collaborators and facilitated my involvement with the experimentation and writing for another lab's manuscript. My experiences with him thus far have demonstrated that as a mentor he effectively balances guidance with student independence. I have already had the opportunity to independently design and formulate experiments, protocols, figures, and written work, with the knowledge that he will provide feedback whenever needed. He expresses appropriate skepticism regarding all collected data, questioning desired results as much as unexpected or undesired ones. He responds to email quicker than the majority of individuals I have met, despite having many time-consuming commitments. All of these traits give me extreme confidence in his abilities as both a mentor and role model. His active involvement in clinical pathology while managing an NIH-funded research lab will help me learn how to blend clinical practice and scientific research to achieve my career goals.

UPMC and the School of Medicine (SOM) have been frequently ranked among the best hospital systems and medical schools in the country, respectively. The proximity of UPMC, the University of Pittsburgh, and Carnegie Mellon University (CMU) facilitates easy and active scientific and clinical collaboration. In fact, the University of Pittsburgh is the most common institution cited on PubMed due to its high degree of collaboration with other universities. The SOM places a high emphasis on all medical students being involved in scientific or clinical research during their training in the form of a mandatory "Scholarly Project." The department of general surgery at UPMC is one of the most academic surgical departments in the country. The chair, Dr. Tim Billiar, is an active surgeon-scientist himself, boasting involvement in clinical trauma and management of a basic science lab. The sheer size of UPMC promotes a clinically diverse patient population which is optimal for enhancing my medical school training. The extent of research opportunities and integration with clinical practice are optimal for my ultimate goal of becoming a physician-scientist.

When I first interviewed for the University of Pittsburgh MSTP, I was impressed with the fact that MSTP students are required to do three summer laboratory rotations, three semesters of journal clubs, and three summer professional development courses, all within the first two years of the program. These classes build analytic and professional skills, such as grant writing and networking, that are crucial to successful career as a physician scientist. The required eight-week clinical clerkship prior to starting thesis work helps prepare students for the two longitudinal clinical clerkships during graduate school. MSTP students are also given the opportunity to do a Postdoctoral Fellowship during their fourth year of medical school. The many forms of clinical and research integration of this program will aid my development as a surgeon-scientist.

On a more personal note, I chose the University of Pittsburgh MSTP due to the kindness and helpfulness of the students and administration in the program. The director of the program, Dr. Richard Steinman, actively advocates for students, especially during times of difficulty. Biannual meetings with my career advisor allow me to discuss my current short term goals to further my long term career aspirations by use of an individualized development plan. The monthly workshops and annual retreats integrate students in all years of the program, facilitating opportunities for inter-student mentorship. I find the warm family of the MSTP to be an ideal training environment for me while I pursue my rigorous career aspirations to become a surgeon-scientist.

Finally, my graduate program, Cellular and Molecular Pathology (CMP), provides a diverse set of courses that I can use to supplement my thesis work. The CMP program also has extensive core facilities to aid in my research, including one of the most advanced imaging facilities in the country. The CMP program houses both scientific and clinical faculty, which will allow for easy collaboration and integration during graduate training.

In summary, I am confident that I chose a sponsor and training program whose mentorship and environment will allow me to complete the proposed research project and develop into an independently funded surgeon-scientist.

B.17. RESPONSIBLE CONDUCT OF RESEARCH

The University of Pittsburgh strongly emphasizes the responsible conduct of research. During my first year of medical school, I took a course called “Ethics, Law, and Professionalism” as part of the school’s “Patient, Physician, and Society” curriculum. The course met for two hours each week for 15 weeks, both in lecture and small group format, and discussed many important ethical issues such as informed consent, research and industry relations, genetic selection, and confidentiality. The lectures were taught by faculty from the schools of medicine, law, and bioethics and required the completion of several online training modules related to the responsible conduct of research. These lectures and small group sessions taught me the importance of many considerations when doing human subjects research through example case studies. The foundational knowledge built through the course was tested in both a midterm essay and a final exam. Independent of this course, the School of Medicine also required completion of several training modules related to the responsible conduct of research, including bloodborne pathogens, informed consent, and HIPAA.

The University of Pittsburgh Medical Scientist Training Program (MSTP) also heavily emphasizes research ethics. All MSTP students are required to take course called “Ethics for Medical Students” during one of their graduate school years. This class meets a total of four times for two hours each. The first session teaches students how to systematically apply analytical methods to the evaluation of ethical dilemmas. These skills are then applied to specific biomedical ethics cases in the following three sessions. Course topics include authorship, biomedical technologies and clinical trials, data falsification, and human subject research. Additionally, two of the monthly MSTP workshops each year feature faculty presentations on ethical dilemmas in the lab, clinic, or collaboration with industry. This past year, one such talk involved the design of pharmacologic clinical trials, collaboration with industry, and approaches to successful interactions with industry when pursuing resources for clinical translation of a lab-discovered compound. I have so far attended three of these ethics-based workshops.

As a student entering the department of Cellular and Molecular Pathology (CMP), I am required to take many online modules on the proper conduct of research to enhance my graduate training. So far, I have completed online modules through the Collaborative Institutional Training Initiative (CITI), sponsored by the University of Miami, on topics relating conflicts of interest in biomedical research. I also took an extensive module called “Responsible Conduct of Research” (RCR), which taught me important ethical considerations in a number of areas such as human subject research, plagiarism, authorship, collaborative research, managing conflicts of interest, data management, mentoring, peer review, and research misconduct. The Clinical and Translational Science Institute (CTSI) at the University of Pittsburgh hosts an RCR center that offers four to five workshops each month. This center is an excellent resource for obtaining in-person ethics training in a variety of topics. The topics covered by these workshops include research resource management, informed consent, authorship conflict, proper lab notebook keeping, and maximizing mentoring relationships. I plan to attend as many of these workshops as my schedule allows.

During my MD-PhD training, I will stay up-to-date with all required online research modules and participate in at least eight hours of classroom-based RCR training. During my first year of graduate school, I will take the “Ethics for Medical Students” course mentioned above. For the remaining three years, my classroom-based RCR training will consist of two MSTP workshops each year (2 hours) and several CSTI workshops (at least 6 hours). I believe research ethics is a crucial part of successful scientific and clinical investigation and expect these courses to supplement my technical scientific training and aid in my development to becoming an independently NIH-funded researcher.

C.7. GOALS FOR FELLOWSHIP TRAINING AND CAREER:

My goals for this **fellowship** are (1) to learn the scientific method of asking a worthwhile question that can be answered experimentally, (2) to develop experimental and intellectual aptitude and independence in the lab, (3) to develop expertise with data analysis, manuscript formulation, and presentation execution, (4) to further develop my professional skills, collaborative networks, and personal mentorships (clinical and scientific), and (5) to be exposed to clinical and research experiences that further my development as a budding surgeon-scientist. My ultimate **career** goals are to head an NIH-funded research lab, practice clinical surgery, and teach at an academic institution. I aim to continue research in the area of cancer biology, with a particular focus on the clinical application of tumor biology and therapeutic insights garnered from laboratory research. I want to pursue a residency in General Surgery, supplemented by a fellowship in Surgical Oncology. This clinical specialty will compliment my laboratory interests in investigative and translational cancer research. My dissertation work with the liver microphysiological system (MPS), focusing on dormant cell metabolism and the impact of oxygen tension on tumor dormancy, metabolism, and therapeutic potentiation will provide a strong foundation for future studies investigating basic and translatable tumor biology.

I am confident my training under the Kirschstein-NRSA fellowship will be vital to achieving my stated training and career goals. I am highly confident that my thesis mentor, Dr. Alan Wells, will provide both intellectual support and furthering my career as an aspiring surgeon-scientist. Dr. Wells has trained multiple MSTP students who have all been highly successful both in his lab and in their subsequent careers. In addition, Dr. Wells has many years of experience balancing both clinical and research responsibilities. These impressive traits and our shared interest in clinically translatable cancer biology make him the ideal mentor for my graduate and professional training.

Under Dr. Wells's guidance, I will learn the necessary skills to design and execute future research projects independently. These skills will aid in my future establishment of an NIH-funded laboratory. Through the research proposed by the training plan herein, I will become proficient with cell culture (both traditional and as a part of the liver microphysiological system), cell and tissue engineering, and a variety of imaging techniques, biochemical analyses, and molecular biology techniques. I will also be intimately exposed to biomaterial sciences and systems biology. More importantly for the future, I will develop my proficiency as a scientific thinker and writer through the formulation of proposal (for my comprehensives and thesis milestones), manuscripts, abstracts, posters, and presentations during my fellowship. I will develop expertise in data analysis, facilitated by the high throughput of the liver MPS system the Wells lab employs.

To develop my career, I will begin to network in my field of study at various conferences. It is the practice of the Wells lab to attend two meetings a year, always as a presenter, alternating between larger comprehensive meetings (such as AACR annual meeting) and smaller targeted meetings (such as Gordon/FASEB conferences or San Antonio Breast Cancer meeting). These gatherings will allow me to apply the lessons learned in the Cellular and Molecular Pathology Graduate Program and MSTP workshops, retreats and career guidance sessions. Parenthetically, the collaborative nature of the larger project in which my proposal fits, already has established networks at MIT and at a biotech company in the UK, CNBioInnovations, and research laboratories in the Boston area.

My clinical involvement during all four years of graduate school will provide me with experience balancing the career of a surgeon-scientist, insights into avenues of translation between these two integrated fields, and opportunities to strengthen clinical mentorships I have formed during my first two years of medical school. My research involvement during my last two years of medical school will provide me with opportunities to finalize research related to my dissertation and start a clinical research project of my own in my field of interest. My medical school curriculum thus far has provided me with both molecular and clinical knowledge relating to the study and treatment of disease. My graduate courses will supplement this foundation and provide further knowledge that will aid in my research investigations.

Through this combination of didactic and heuristic education, immersion in a hands-on project, intellectual development, and continued clinical contact, I will be ready for the next steps of reaching my career goals. The teaching aspect will be provided by the experience of the Wells lab where there are many undergraduate trainees and graduate rotation students, each of whom work under the tutelage of a lab member. As the graduated responsibilities and assignments accrue, I will become more independent, while in a supportive environment that will provide continued feedback for improvement.

C.8. ACTIVITIES PLANNED UNDER THIS FELLOWSHIP

Year	Research	Clinical	Coursework	Other
1	80%	9%	10%	1%
2	80%	9%	10%	1%
3	88%	10%	1%	1%
4	88%	10%	1%	1%
5	10%	85%	0%	5%

Graduate School (Years 1 through 4):

Research: In allocating the majority of my time in the lab during the first four years, I will have the ability to master new techniques such as using an ex vivo liver microphysiological system (MPS), tissue sectioning, immunofluorescence (IF), image analysis, and metabolic and functional analyses of dormant micrometastases and liver tissue respectively. I will also design and write new protocols describing the incorporation of a hemoglobin-based oxygen carrier (HBOC) into the liver MPS, drug efficacy studies, and oxygen tension determination. I will collect and analyze my own data and organize my findings into written form amenable to journal submission. I will meet with my thesis mentor and sponsor, Dr. Wells, at least weekly individually and biweekly in the MPS group to discuss my research progress. Every two weeks, the MPS grant group (including NIH and DARPA funded investigators at MIT, CNBio Innovations, and at UPitt) has a webex, with NIH (NCATS and NCI) attending every other session; I participated in these last summer. I also will have the opportunity to present at lab meetings to my colleagues. I will have the opportunity to collaborate with Dr. Linda Griffith at MIT in application of my findings to additional organotypic systems currently under study. I will update my dissertation committee on my thesis progress bi-annually and plan to defend my thesis in the fall of 2018 or spring of 2019.

The Cellular and Molecular Pathology and Medical Scientist Training Program (MSTP) programs at the University of Pittsburgh also provide multiple opportunities for networking, mentorship, and presentation of my work. I will have the ability to give oral and poster presentations at many annual Pathology and MSTP retreats. I will attend at least two international conferences each year to present my work in poster and oral form as well as network in the field. These meetings will include AACR annual meeting and section meetings, the Metastasis Research Society biannual meeting, the San Antonio Breast Cancer meeting, and various FASEB or Gordon Conferences.

I will meet with my MSTP career advisor, Dr. Ken Hallows, biannually to discuss my research and future career trajectory and goals. My training will be enhanced by monthly MSTP seminars discussing issues pertinent to the career development of physician-scientists.

Clinical: To further my career as a budding surgeon-scientist, I will dedicate a half-day each week to seeing surgical patients in clinical and OR settings. Additionally, I will attend every General Surgery Grand Rounds, as long as it doesn't conflict with important graduate school commitments. These experiences will also allow me to strengthen clinical mentorships I have established during my first two years of medical school. I will complete two longitudinal clinical clerkships in my 3rd and 4th years of graduate school, one in oncology and one in surgical oncology to facilitate my transition into my last two years of medical school and supplement my oncologic research with primary patient exposures. Each longitudinal clinical clerkship is 20 weeks long, with one half-day each week dedicated to seeing patients in clinic with an experienced physician. I will continue to attend clinical tumor conferences, which discuss challenging surgical oncology cases, and health policy lunch talks to incorporate clinical and practical knowledge that will be useful as a practicing surgeon-scientist.

Coursework: I will participate in the Pathology Research Seminar class each semester until my thesis defense, which will give me an opportunity to present my work to peers and the faculty instructors. I will take the "Cancer Biology and Therapeutics" and "Extracellular Matrix in Tissue Biology and Bioengineering" during the fall and spring of my 1st graduate year respectively. Additional courses will occur in year two and three. I already took the "Multiparametric Microscopic Imaging" course last summer (2014) and will complete my final requirement for that class (a project aimed at utilizing multiple imaging technologies to answer a hypothesis) during the summer in which I enter graduate school.

Other: I will dedicate time to committee involvement in the pathology and MSTP programs.

Third Year of Medical School (Year 5):

Research: I will take one research month during my 3rd year of medical school to finish any work and manuscripts related to my dissertation in the Wells lab. If I have time during this month, I will seek a small clinical research project which I will complete during before finishing the program. Finalizing any manuscripts or revisions should not be an issue as I already fit this in this fall for the paper with Dr. Oltvai's group.

Clinical: I will take the clerkships required (40 weeks in total) so that I can focus on taking electives aimed at improving my exposure to surgical specialties so I can better aim my clinical focus in surgical oncology.

Other: I will continue to attend required monthly MSTP workshops and stay involved with MSTP or graduate program committees.

C.9. DOCTORAL DISSERTATION AND OTHER RESEARCH EXPERIENCES

I am very early in my MSTP program, being in the second year of predominantly clinical coursework. In the MSTP at University of Pittsburgh/CMU, trainees do not formally enter the graduate research until after the beginning of the third year. While it is unusual to apply for support at this time, it is possible due to the progress made during the past summer including the selection of a project that will become my dissertation topic. That is provided in this proposal. Here I will relate other research experiences. Prior to joining the University of Pittsburgh Medical Scientist Training Program (MSTP), I had undergraduate research experiences at the Massachusetts Institute of Technology (MIT) in three separate labs.

The summer prior to starting at MIT, I worked for Dr. Ian Hunter's BioInstrumentation Lab on a project called MICA (Measurement, Instrumentation, Control, and Analysis). The goal of the project is to create a line of sensors and generators, link them together wirelessly, connect them to a laptop, and use them for both education and research applications. My specific role involved designing physics experiments for the applications of these sensors and developing the sensor box to house them. I designed the housing in SolidWorks and then used stereolithography to print and mechanically test my designs.

The summer following my freshman year at MIT, I worked for Dr. Steven Wasserman on the construction, software development, and characterization of an optical trapping kit (OTK) that has been commercialized by Thorlabs, inc. An optical trap is a device which uses a collimated laser to exert force on small dielectric particles, most commonly bacteria or silica beads. My goals with this project were to improve the design of the instrument, provide helpful assembly instructions, and supply calibration data that could be used to assist other researchers who purchase the kit. In order to obtain repeatable trap operation, the laser beam must be both collimated and centered. I developed and documented reliable methods for collimation and alignment of the optical trap that are still in use today. In addition to optimizing the physical trap, I designed and coded a graphical user interface (GUI) in Matlab for trap operation. After performing multiple calibrations using the GUI I developed, I conducted two relevant assays to demonstrate the suitability of the trap for biological research. The first measured the stopping torque and rotational speed of spinning *E. coli* bacteria stuck to a microscope slide. The second stretched anchored DNA oligos to determine their contour length and compare to the expected value. This research experience culminated in the presentation of a white paper to Thorlabs. This trap that I assembled is still used in the MIT Bioengineering teaching curriculum (Course 20.309) as an experimental module.

My most significant undergraduate research experience was with Dr. Katharina Ribbeck on a project examining the formation of multilayered biomaterial films using mucin proteins and lectin crosslinkers. The ultimate goal of this project was to construct films to serve as antimicrobial lubricating agents and drug delivery devices. My primary responsibility was to characterize film growth and particle incorporation using fluorescence assays, quartz crystal microbalance (QCM), and confocal microscopy techniques. I determined the growth kinetics of films under conditions of varying substrate and salt concentrations and substrate pairs. I characterized the 3D morphology of these films using confocal microscopy and performed fluorescence recovery after Photobleaching (FRAP) experiments to determine substrate diffusivity. I utilized biotin-avidin linkages to load model drugs (ex. Taxol) into preassembled films or during assembly. To investigate the possibility for clinical use, I assayed the cytotoxicity of films in cell culture using HeLa cells. We found that film assembly was independent of salt concentration, in contrast to previously described polyelectrolyte films using substrates such as poly-L-lysine. Film thickness and water content grew exponentially with the number of multilayers incorporated. HeLa cell viability was found not significantly different, indicating mucin multilayer films have possible clinical utility. Although my research was supervised by Dr. Thomas Crouzier, a postdoctoral associate in the Ribbeck lab, I performed the majority of the experiments independently and designed new experiments in collaboration with him to further my research. My research project culminated with a presentation to the lab group, a second author paper in *Biomacromolecules*, and a patent disclosure.

These experiences not only satisfied my desire for discovery and invention, but also convinced me that my future must include investigative research in addition to the clinical contacts I craved. To enable this longer term, I realized I needed formalized training in a doctoral program and chose to pursue the MSTP route as the next step.

At the University of Pittsburgh MSTP at least two rotations are required before selecting an advisor and project. For these, I opted to wed my biological engineering skills and training with biological exploration. I chose to spend my first 10-week summer rotation working for Dr. Steven Badylak at the McGowan Institute for Regenerative Medicine. The lab pursues many areas related to tissue engineering and wound healing. The lab's focus on translational science and biomaterials-related projects drew my interest. In particular, my

summer work involved two projects: 1) Establishing a 3D-recellularized liver tissue and 2) quantifying polarized macrophage phagocytosis as a function of cell phenotype (classical versus alternative activation). The majority of my time was spent on the first project. The goal of the project was to decellularize whole rat livers and reseed primary rat hepatocytes, non-parenchymal cells, and sinusoidal endothelial cells to establish a functioning, healthy liver tissue. My responsibilities were to assist with harvesting whole rat livers, perfusing rat livers to obtain fresh hepatocytes and non-parenchymal cells, and characterize primary rat hepatocyte growth on two extracellular matrix (ECM) digests the lab common uses for regenerative applications. Additionally, I characterized cell membrane expression of CD47, a possible signaling factor to potentiate cell proliferation prior to reseeding, on primary rat hepatocytes using FACS. I found that primary rat hepatocytes express cell membrane CD47. My second project quantified and compared the phagocytosis of fluorescent *S. aureus* beads by unpolarized (M0), classically activated (M1), or alternatively activated (M2) macrophages. Macrophages were polarized using IFN γ and LPS (M1), IL-4 (M2), or ECM digests of bladder and small intestine. I found that M1-type macrophages exhibited lower phagocytosis than M2- or M0-type macrophages. Macrophages polarized by either form of ECM digest exhibited even lower rates of phagocytosis. My summer experience culminated with an oral presentation to the Badylak lab and a poster presentation at the annual MSTP retreat in the middle of August. This first summer rotation proved to be significantly helpful for my career development. The formalized structure of Dr. Badylak's lab improved my writing and communication. I learned many new techniques including FACS, hepatocyte cell culture, macrophage polarization, and hepatocyte isolation procedures that will be useful in my future research. Additionally, collaboration with a hepatic transplant surgeon, Dr. Paulo Fontes, led to my introduction to one of my most inspiring clinical mentors, Dr. Wallis Marsh, and increased my interest in translational surgical oncology.

Realizing that cancer had a special calling for me, I chose to do my second 10-week summer rotation with Dr. Alan Wells, who will serve as my sponsor for this fellowship, my thesis advisor, and a career mentor. I was initially introduced to Dr. Wells through two of my mentors at MIT, Dr. Linda Griffith and Dr. Doug Lauffenburger, who share an extensive history of collaboration with him. Dr. Wells's lab was an ideal choice for my second rotation given my MIT mentors' strong recommendations, his extensive history of training both graduate and dual-degree students, his abundant research funding, and the lab's scientific focuses. More than his qualifications, I felt a true connection with Dr. Wells both personally and intellectually at our first meeting. My summer work with Dr. Wells involved two projects: 1) Incorporation of oxygen measurements into his liver microphysiological system (MPS) and 2) Investigating statin efficacy as cancer therapeutics. For this first project, I developed several protocols including the calibration of and data collection with ruthenium oxygen sensors (will be used in the proposed work) and MPS supernatant analysis using a clinical blood gas machine (ABL5). I ran preliminary oxygen content tests incorporating a hemoglobin-based oxygen carrier (HBOC; will be used in the proposed work). For the second project, I collaborated with Dr. Zoltan Oltvai in the Department of Pathology to finish a manuscript investigating membrane E-cadherin as a marker for cancer cell resistance to statin therapy. My responsibilities were to finish experiments for the manuscript, and write parts of the discussion and methods, and edit the draft before submission. My experiments involved immunofluorescence, IC-50 determination, extensive cell culture, western blotting, and cholesterol uptake determination. The manuscript was submitted to *Scientific Reports* in August, 2014 and is currently in revision. I presented a poster on the manuscript, with particular emphasis on the data I collected, at the annual MSTP retreat at the end of the summer. My summer experience made me feel comfortable with the lab environment, experiments, and members, begin to explore my own research independence, and allowed me to gather preliminary data for starting my thesis work in the Wells lab in July, 2015.

All of these research experiences have been invaluable in my training as a scientific investigator. I have learned how to formulate hypotheses, execute experiments, and formulate my findings into manuscripts and presentations. Additionally, I have expanded my scientific and clinical networks through collaborations associated with my research projects. These skills I've developed, guided by professional and individualized mentorship, will allow me to be successful during my graduate education and in future research endeavors.

SECTION II – SPONSOR AND CO-SPONSOR INFORMATION**Alan Wells, MD DMSc, Primary Sponsor/Mentor**

Dr. Wells is the Thomas Gill III Professor of Pathology in the School of Medicine, with a secondary appointment in BioEngineering in the School of Engineering at the University of Pittsburgh. He is also the Medical Director for all the UPMC (University of Pittsburgh Medical Center) Clinical Laboratories, through their network of 20 hospitals across western Pennsylvania. On the research side he has expertise in tumor cell biology and cell signaling. Over the past decade, along with Dr. Linda Griffith, he has pioneered the concept of ex vivo all human bioreactors to study the microenvironment of the metastatic niche. This is the system that Colin will use to explore the role of oxygen utilization on this organ site. Wells has graduated over 25 students at the sole or co-advisor, including 5 MD-PhD trainees, and will be the primary mentor for Colin throughout his training. He will serve to guide him in his experimental designs and approaches and to help to foster his career development as a physician-scientist.

(A) RESEARCH SUPPORT AVAILABLE

This table summarizes all funding sources in the Wells lab for work relevant to Colin's training plan. The Directs are funds available to the Wells lab specifically; for instance the NIGMS R01 also has subcontracts to MIT and other investigators at UPitt, thus leaving \$~120,000/year for the Wells lab. (Other funding from NIH to explore wound healing is not noted due to different focus of study, and funding to other members of the Wells lab are also not included.)

Source	Grant #	Title	PI	Dates	Annual Direct
Other	N/A	Thomas Gill Endowed Chair	Wells, Alan	ongoing	\$130,000
NIH	UH3TR000496	"All Human Microphysical Model of Metastasis Therapy"	Griffith, Linda (MIT), Wells is UPitt PI	07/01/14-06/30/17	\$187,000
NIH	UH3TR000503	"A 3D biomimetic liver sinusoid construct for predicting physiology and toxicity"	Taylor, Lans (UPitt)	07/01/12-06/30/17	\$25,000
VA	Merit Award	"Molecular Regulation of Cancer Progression"	Wells, Alan	10/01/11-09/30/15	\$149,000
NIH	R21CA188799 NCI Health Disparities	"Transcriptional Regulation of Breast Cancer Metastasis within the Tumor Microenvironment"	Yates, Clayton (Tuskegee)	09/30/14-09/29/16	\$39,000
NIH	R01GM69668	"Spatial segregation of cell functioning during motility"	Wells, Alan	01/15/04-11/30/15	\$119,000

(B) SPONSORS' PREVIOUS FELLOWS/TRAINEES

Dr. Wells has graduated 22 pre-doctoral as major advisor, 7 predoctoral as co-advisor, and 16 post-doctoral students. Of these, 5 were trained as part of an MD-PhD program, two more were FMG who are now in academic positions in the US, and 5 post-doctoral fellows were physician-scientists, who are still functioning in that capacity. Five of these trainees were African-American. He currently trains two graduate students and four post-doctoral fellows. Colin will be the only current MSTP student when he joins the laboratory officially in the summer of 2015. Dr. Wells has been recognized for his training of graduate students by the UPitt MSTP students and the UPitt Biomedical Graduate Programs students, as well as the Provost's office for training of post-doctoral fellows.

5 Representative Trainees:

Mark Fung, MD PhD. (1994-1997) Dr. Fung completed his MD and PhD training through the MSTP at the University of Alabama at Birmingham. During this time, he won awards for both graduate student and medical student research days, was a Young Investigator of the Academy of Clinical Laboratory Physician and Scientists (ACLPS), and was named the Outstanding Graduate Student in Pathology in 1997. He went on to complete a residency in Laboratory Medicine/Clinical Pathology at Washington University and a fellowship in Transfusion Medicine at UPMC where he was funded as a College of American Pathologists (CAP) Foundation Scholar. He is currently an Associate Professor and Director of Transfusion Services at the University of Vermont. He has been involved nationally in setting both clinical policy and research directions through the American Association of Blood Banks and CAP, and has been a subaccount site for the NIH-funded Hemostasis Clinical Research Network. He had 3 first author (and one secondary author) publications as a graduate student.

Jeff Chou, MD PhD. (1998-2002) Dr. Chou completed his MSTP program in 2002 at the University of

Pittsburgh. During this time he won the best poster at the UPitt/CMU joint MD-PhD retreat and best graduate presentation in the Department of Pathology. He went on to a residency in Internal Medicine at University of Washington and an oncology fellowship at the Fred Hutchinson Cancer Center, where his research was funded by a Cancer Research Institute award. He is now an academic oncologist specializing in colorectal cancer. He had 2 first author (and two secondary author) publications as a graduate student.

Clayton Yates, PhD. (2000-2005) Dr. Yates finished his PhD training in 2005, during which time he won numerous awards for presentations, and funding as a Cellular Approaches to Tissue Engineering and Regeneration (CATER) fellow (NIH training grant funding) and an AACR Minority Scholar Award. After a post-doctoral fellowship at Georgia Tech, he joined the faculty at Tuskegee University where he remains as a tenured Associate Professor of Biology. He has been funded through the MBRS programs and now holds an R21 from the NCI program in health disparities research. Importantly to the present proposal, he was the first student to work on ex vivo all human bioreactors/microphysiologic systems (MPS) for human metastasis research. He had 3 first author (and 3 secondary author) publications as a graduate student.

Cecelia Yates, PhD. (2005-2009) Dr. Yates graduated with numerous recognitions locally and nationally. She was funded by a NIH Minority Supplement award during her thesis work. She was named the Young Investigator Awardee by the European Tissue Repair Society in 2007 and the Experimental Pathologist in Graduate Training Awardee by American Society for Investigative Pathology (ASIP) in 2008. During her post-doctoral fellowship at UPitt, she was garnered the ASIP Excellence in Research, recognizing the top post-doctoral fellow in the field. She is now an Assistant Professor of Nursing (tenure track) at Univ Pittsburgh, pursuing both basic mechanisms of skin fibrosis and how these conditions underlie health disparities. She had 4 first author (and two secondary author) publications as a graduate student.

Yvonne Chao, MD PhD. (2007-2011) Dr. Chao was recognized locally as the best graduate presentation at the University of Pittsburgh Science 2008, and nationally with a DoD CDMRP Breast Cancer predoctoral fellowship, AACR Scholar in Training award in 2009, Susan Komen Scholar in Training award in 2010, ACLPS Young Investigator in 2009, and American Society for Clinical Pathology Academic Excellence and Achievement awardee in 2010. She is now a resident in Internal Medicine at UPMC, in preparation for an oncology fellowship. She had 3 first author (and 2 secondary author) publications as a graduate student.

(C) TRAINING PLAN, ENVIRONMENT, RESEARCH FACILITIES

C.1. RESEARCH TRAINING PLAN (Primary Sponsor's Overview):

In order to become an independent physician-scientist, Colin's program will comprise laboratory training, career development and networking, as well as a research focus on metastasis. This approach is aimed at providing Colin the skills to pursue his career goals centered on cancer research. This will use a focused research proposal to teach the general skills of research – posing and answering a question in the scientific method.

The training program and environment is designed to provide for the future questions of “is this worth investigating” (significance) and “is this the person who should direct this study” (investigator). Thus, the proposed study, that will be central to this training and occupy most of the time, focuses on the emerging field of re-emerging dormant metastatic cells. As the microenvironment is strongly intertwined with regulating cancer cell dormancy and escape in the metastatic niche, this project has obvious implications in address the clinical conundrum of latent relapse in breast cancer. Further, Colin will be well positioned to be a leader in a new area of study as this work explores a novel hypothesis using innovative, cutting edge microphysiological systems (MPS) and technologies. The training program designed will enhance Colin's techniques and knowledge as well as his networks through with the collaborations outlined below and provide him with the specific skill set with which to accomplish the transition.

Formal courses will be as prescribed for his graduate work. Already Colin has completed ethics and statistics training, and the general graduate course. He will now enroll in the Cellular and Molecular Pathology Graduate Program, and take the key courses required, including Molecular Pathobiology, Cancer Biology and Therapeutics, and Extracellular Matrix in Tissue Biology and Bioengineering. This will be supplemented by courses in Computation and Systems Biology (Wells has a cross-appointment) in the new areas of biocomputation and modeling in systems biology.

LABORATORY TRAINING

Colin's time will predominantly involve investigating the proposed project. This will serve not only for specific technical and metastasis training, but also be the template to learn the scientific method. The first step is conceiving of a question both worth answering and be capable of investigation. The proposal herein is a major aspect of this training. Colin conceived of this after many discussions with myself and Drs. Griffith and Fontes. The hypothesis, aims, and approach were developed by Colin, with only superficial, iterative review by myself

and other faculty mentors and advisors. This proposal will also serve as a first draft of his comprehensive exam, which takes the form of an NIH proposal in the CMP program. Thus, hypothesis generation and experimental design will be a consistent process through all years of his graduate investigative work. Not only will Colin get feedback from faculty, but a major aspect of the weekly CMP graduate seminars is individual presentation of thesis work.

The technical aspects of the work will require him to master a number of both novel and established techniques that will enable him to transition to an independent laboratory and train the people therein in both standard approaches and innovative technologies that will mark his future laboratory with a distinctive approach. At the cutting edge of imaging, including live tissue and cell imaging, Colin will work with the University of Pittsburgh Center for Biologic Imaging under Drs. Donna Stolz and Catherin Baty. This facility can image LiverChip cultures using 2 photon imaging, in addition to routine high-level imaging. The CBI runs an imaging course that Colin will complete during his fellowship.

The innovative all-human LiverChip model will be fundamental to the experiments. For this Colin has already been trained by fellow trainees, Dr. Sarah Wheeler, Dr. Amanda Clark and Dr. Bo Ma. As technical aspects of the MPS are developed and improved, further training will be acquired by visiting trainees from the lab of the originator, and long-term collaborator, Dr. Linda Griffith at MIT.

Working with trainees of Dr. Lauffenburger at MIT, a pioneer in the field of systems biology, Colin will receive training on signaling profiling using multiplex assays and computational approaches for analyzing biological networks.

CANCER TRAINING

In order to facilitate enhancing the clinical aspects of Colin's cancer training, Colin will be a participant in the Breast Cancer Program at the University of Pittsburgh Cancer Institute (UPCI). As breast cancer is the major type of carcinoma studied in the system, this will serve as a model cancer.

UPCI is an NCI designated comprehensive cancer center and has formed a collaboration with the Magee Womens Research Institute (MWRI) to create a Women's Cancer Research Center (WCRC) with 168 researchers from a variety of Departments including: Pathology, Medicine, Pharmacology and Chemical Biology, Surgery, Ob/Gyn and Reproductive Services, Psychiatry, Biomedical Informatics, Radiology, Medical Education, Biostatistics, Epidemiology, Microbiology and Molecular Genetics, Hematology/oncology, Health Science, Cell Biology and Physiology, Bioengineering and Pharmaceutical Science; Wells is an active member of this group. The WCRC is geographically close to the Wells laboratory, the Magee Women's Hospital (for patient based research) and the UPCI; this geographic proximity and diverse departmental background fosters unique opportunities for collaboration and discovery in addition to a rich environment for education and training.

The WCRC provides a weekly work-in-progress seminar at which Colin will present annually. This work-in-progress seminar is a relaxed environment where trainees, faculty and physician-scientists present current, unfinished projects to get constructive feedback in the early stages of a project. In addition to this there is a monthly journal club specifically for the WCRC in which he will also be an active participant. Clinical breast cancer research also has a strong presence at the University of Pittsburgh thanks to the Magee-Womens Breast Cancer Program of UPMC Cancer Centers, another collaboration with the UPCI and co-directed by Dr. Adam Brufsky. This clinical research group specializes in translational research including dozens of clinical trials annually. The breast cancer clinicians and translational scientists hold a weekly breast cancer conference to discuss cases and treatments. Colin will attend this weekly to better understand the clinical needs that are currently unmet by research and to better translate his research into the clinical setting to help patients. As with the two prior trainees in the Wells laboratory supported by the DoD CDMRP in Breast Cancer, Colin will have one-on-one patient contact under the guidance of Dr. Brufsky to more fully understand the patient perspective. In addition to their meetings with survivors and advocates, and with support groups to expose Colin fully to the issues faced by patients.

The UPCI itself also offers many seminars and programs that Colin will attend as applicable.

TRAINING ENVIRONMENT

The Wells laboratory is comprised of a dozen trainees at all levels that come from a wide range of educational backgrounds. This has resulted in a diverse yet integrated variety of research projects primarily focused on how cells respond to and modify signals from their microenvironment. The diversity provides an excellent setting that will aid Colin in the proposed work by receiving feedback and assistance with from fellow trainees with an array of specialties. The physicians, PhD fellows, and trainees meet weekly for lab meeting and a journal club, which promote the occurrence of said feedback and assistance. At these presentations, Colin will be coached on delivery and communication. Additionally, all trainees are required to attend and present at one or more national meetings annually. Dr. Wells meets one-on-one each week at defined times with each member of the laboratory during which they review progress, plan for upcoming experiments, and discuss

career progression. Meetings are more frequent when working on grants and papers, etc. All trainees are required to write their own papers and grant applications, which are reviewed extensively as part of the training, with graded independence based on the progress of the individual trainee.

EXTERNAL CONNECTIONS

To continue developing Colin's scientific network and in order to present his findings he intends to attend at least one international conference per year (such as the International Biennial Congress of the Metastasis Research Society, currently scheduled for 2016), as well as at least one national conference (such as the AACR annual meeting or specialty meeting and later the San Antonio Breast Cancer meeting) that focus on cancer metastasis.

C.2. ENVIRONMENT AND RESEARCH FACILITIES:

My laboratory occupies 2,000 square feet in Scaife Hall at the University of Pittsburgh. The specifics of the laboratory, trainee office spaces, common and shared equipment are detailed elsewhere. Suffice to say that Colin will have full and unfettered access to all these laboratory, departmental, and common resources as a member of my laboratory and by extension of the University of Pittsburgh Cancer Institute (UPCI), the McGowan Institute for Regenerative Medicine (MIRM), and the School of Engineering. Additionally, he will have access to the resources provided by the UPMC Clinical Laboratories and Tissue Banks, as well as the Magee Women's Research Institute of Magee Hospital.

As a member of the MPS project team, Colin is expected to spend time back at MIT (his alma mater) in the laboratory of Dr. Linda Griffith (Dept Biological Engineering), learning the technical details of the MPS system and the nascent 3D printing of vascular conduits. All prior and current trainees involved in this project have experienced at least two visits of more than one week (up to 6 months) each, with MIT trainees also coming to Pitt on a regular basis. While there, and through Dr. Griffith's aegis, he will have full access to those facilities and cores. Lastly, Dr. Paolo Fontes, who is developing the hemoglobin-based oxygen carrier for organ preservation prior to transplantation, will provide his facilities and expertise at MIRM.

(D) NUMBER OF FELLOWS/TRAINEES SUPERVISED DURING FELLOWSHIP

At the start of Colin's graduate student tenure, I expect to have two other graduate students, 4 post-doctoral fellows, 2 technicians, and 2 junior faculty in my laboratory. Colin will be the sole MSTP student.

(E) APPLICANT'S QUALIFICATIONS AND POTENTIAL FOR A RESEARCH CAREER

Dr. Wells' Statement:

I am excited to have Colin join my laboratory to undertake this project. I met Colin due to the intercession of my colleagues at MIT who had rave reviews of this Biological Engineering student who was coming to UPitt for the MSTP program. They told me I had to grab him for our projects. He had an almost perfect record at MIT (the B+ in microeconomics being the only blemish). That he could grasp complex problems was demonstrated by being the top student in the P-Chem course they taught, and his creativity was evident as again the top student in the Engineering Design course. These all led to his co-authorship on a paper in *Biomacromolecules* that came from a one academic year stint as a UROP in Biological Engineering; this level of productivity exceeds the norm even for this talented group of undergraduates. For these accomplishments, not only was he invited into Phi Beta Kappa, but was designated for his contributions to the MIT Biological Engineering program.

Here at UPitt, Colin has continued his excellence in the classroom. Most importantly, his interest and involvement in research has only accelerated. His early submission of this proposal, even before officially transitioning into the graduate phase of the MSTP. After meeting shortly after he came to UPitt, we planned on his rotating in my laboratory the second summer. Despite this delay and the intense first year of medical school, he came to our lab meetings when possible and familiarized himself with the parameters of the metastatic microenvironment and TissueChip projects. This background work allowed this past summer to be highly productive. He had come into the summer having formulated with me and Dr. Fontes, the concept of looking at the role of oxygen in the micrometastatic niche, prior to any angiogenesis. This allowed him to work together with some of Dr. Fontes' interns and summer trainees to characterize the oxygen carrier, and work with two post-doctoral fellows in my laboratory adapt real time oxygen level measurements to the MPS system. With Colin's input, we now have the oxygen sensors working, obtaining data every 10 seconds for up to 30 days, with stable measurements. These aspects of the project utilized his engineering and computational skills.

Colin was not satisfied with missing out on wet lab work, so he became intimately involved in a collaborative project repurposing statins for anti-cancer work. Working with a collaborator and his trainee, Colin

probed the mechanistic reason behind the variable reporting of whether people on statins experience lower rates of cancer-related death; the epidemiologic literature on this supports both outcomes of fewer and no difference. Querying cells from the NCI 60 panel, we are now reporting in a manuscript in revision, that the tumor cells are induced into growth arrest and/or apoptosis only if they are essentially mesenchymal, whereas the carcinomas presenting a more epithelial phenotype with surface expression of E-cadherin are relatively resistant to statins as a single agent. While Colin came to this work late in its development, he contributed key interventional studies aimed at E-cadherin expression that were critical for the mechanistic insights, and warranted inclusion as an author. When the original submission was returned for revision, Colin was the first to address issues despite being in the midst of his organ-specific courses of the second year of medical school.

The project that Colin proposes herein is ambitious in its intellectual scope and challenging in its technical requirements. This is precisely the type of investigation that Colin wants to undertake. The role of oxygen in metastatic competency, emergence or response to agents has been largely unexplored. However, recent insights into glycolytic mitochondrial metabolism by cancer and stem cells, and tracking of extravasated tumor cells to the venous end of capillaries adds relevance to this question. These findings lead one to posit that low oxygen promotes metastatic seeding, and possibly dormancy. The logical consequence is that oxygen tension would then also affect emergence. Thus, the oxygen levels would affect responses to therapeutic agents, and may require metastasis-targeted interventions based on oxygen tensions. This key question is now approachable with the *ex vivo* MPS to study the micrometastatic niche, and the novel oxygen carrier proposed herein to modulate the oxygen carrying capacity of the media. Establishment of this system would place Colin at the forefront of metastasis research and provide a springboard for his investigative career.

An ancillary benefit of this project is the collaborative nature of the proposal. These investigations will require Colin to spend time at MIT learning the microphysiologic system and 3D printing of vascular conduits. In addition, he will work closely with the transplant surgeons, a positive for both his research and clinical goals. As Colin has already worked well with these two groups and that of Dr. Oltvai, I am confident that these interactions will enhance his education and grow his network.

In short, Colin is a special applicant who has as much promise as any of the more than two dozen graduate students that I have trained. He possesses all of the necessary qualities to achieve his goals. I believe that he will not only have a positive impact on the scientific community, but also on his future patients, colleagues, and students.