OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier
CA199947

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT11971824

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 ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Oxygen impact on tumor metastasis dormancy and therapy

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2016 03/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
PA-014

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

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: Email*: cbeckwitt@gmail.com

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$443,294.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$443,294.00
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. SFLLL 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:
Street1*: 123 University Place
Street2:
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*: ornih@offres.pitt.edu

Signature of Authorized Representative*
Brian Balich

Date Signed*
08/05/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1234-Coverletter_Beckwitt_Resubmission_Final.pdf

424 R&R and PHS-398 Specific Table Of Contents

Appendix

Number of Attachments in Appendix: 3

OMB Number: 4040-0010
Expiration Date: 06/30/2016

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)

Funding Opportunity Number: PA-14-150. Received Date: 2015-08-05T09:50:00.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ● Yes ❍ No

If YES, check appropriate exemption number: ☐ 1 ☐ 2 ☐ 3 ☑ 4 ☐ 5 ☐ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number 00006790

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **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? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1235-Project_Summary_Beckwitt_Resubmission_Final.pdf
8. Project Narrative*	1236-Project_Narrative_Beckwitt_Resubmission_Final.pdf
9. Bibliography & References Cited	1237-Bibliography_Beckwitt_Resubmission_Final.pdf
10.Facilities & Other Resources	1238-Facilities_Beckwitt_Resubmission_Final.pdf
11.Equipment	1239-Equipment_Beckwitt_Resubmission_Final.pdf
12. Other Attachments	1240-Additional_Educational_Information_Beckwitt_Resubmission_Final.pdf

Funding Opportunity Number: PA-14-150. Received Date: 2015-08-05T09:50:00.000-04:00

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 to elucidate the impacts of oxygen tension on tumor dormancy phenotype and cell metabolism. Finally, select anti-tumor agents, including the chemo-therapeutics doxorubicin and cisplatin, will be incorporated into the liver MPS to determine whether therapeutic efficacy is dependent on cancer cell metabolic state and concurrently 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 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.

9. BIBLIOGRAPHY AND REFERENCES CITED

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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^{O}C and -20^{O}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 CO_2 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 Venkatarmanan. 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 facilties 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 Educational Information

Description of Dual-Degree Program
Richard Steinman, MD, PhD
Director Pitt-CMU MSTP

Structure of the University of Pittsburgh-Carnegie Mellon University 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. The PD2 course focuses on scientific design and career development strategies, and has developed a core curriculum on scientific reproducibility and data handling and biostatistics. The PD3 course 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 and 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, Kenneth Hallows 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 generally in four 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).
- 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.

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 below

Table 1. Outcomes of MSTP Postdoctoral Fellowship

Student (year)	Paper published during fellowship **1st au*** 2nd au	Paper submitted during fellowship **1st au*** 2nd au	Writing completed during fellowship **1st au*** 2nd au	Additional publications 1 year follow-up	Presentations
1 (2013)	1	**3*** 1	**2*** 1	1 first author 1 co-authored	-
2 (2013)	0	**2***	**1***	1 first authored	-
3 (2013)	0	0	3	0	AHA
4 (2013)	0	0	**1***	0	-
5 (2013)	0	0	**1***	1 first-author	-

6 (2014)	0	0	**3*** 1	N/A	Multiple
7 (2014)	**1***	0	0	N/A	-
8 (2014)	0	0	**1*** 2	N/A	-

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 also 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 1 and **Figure 2** summarize the program and display the general timeline, including all MSTP-specific coursework, and the corresponding competencies MSTP-specific activities instill respectively.

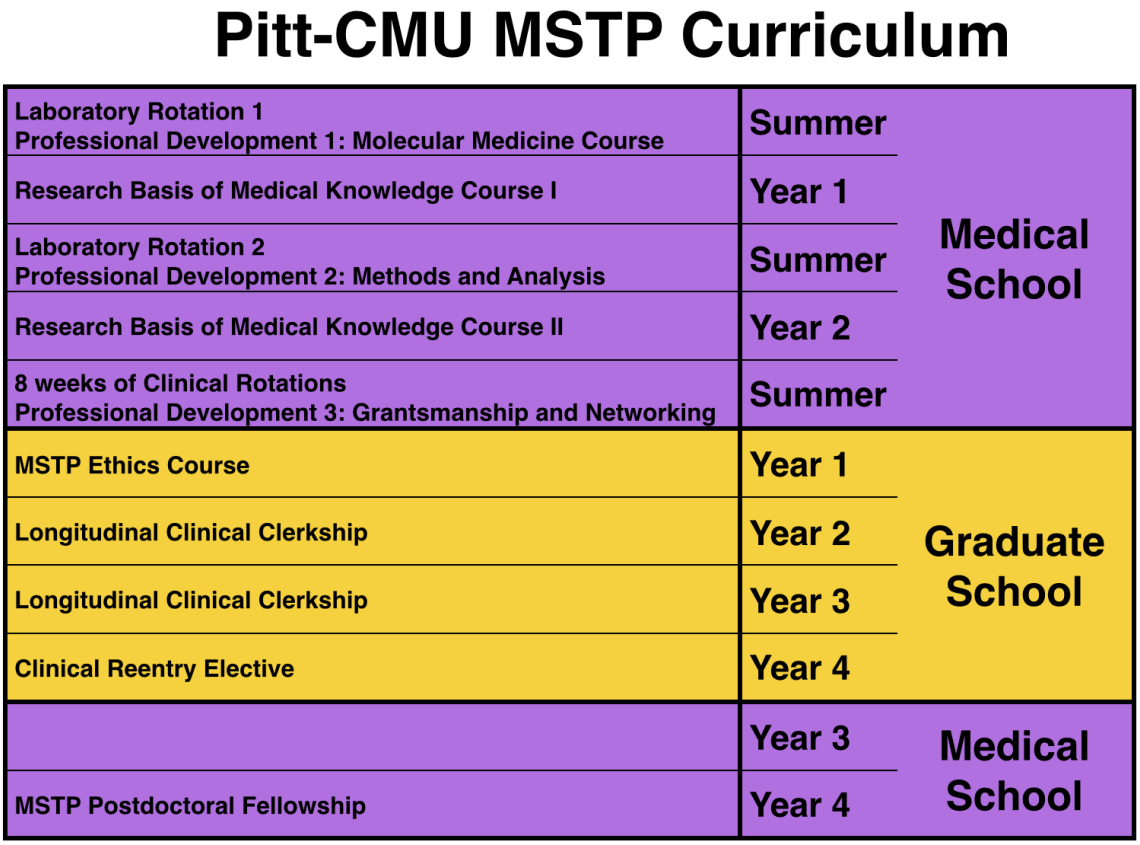


Figure 1. Curriculum structure of the MSTP. Only MSTP enrichment courses are shown. Medical school courses and graduate school courses are not shown. A 4-year graduate cycle is represented, although duration varies by program and student. **Average duration of our MSTP program is 7.8 years**

Competencies	Rotation	PD 1	PD 2	PD 3	Ethics	Workshops	Reentry	Committee	CA
Recognize innovative and significant science		X	X						
Articulate strong and weak components of research plans/ papers		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-of-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						
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					
Integrating clinical and research roles						X	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 lab work and build troubleshooting skills	X		X						
Strategically identify rotation laboratories		X							X
Achieving deliverables including publications, grants, national presentations	X								

Figure 2. Core Competencies instilled by MSTP-specific activities. Key competencies engendered by MSTP-specific activities. The coherent and comprehensive nature of our curriculum introduces and reinforces critical skills necessary for bourgeoning physician scientists. Blue represents cognitive skills, Red designates professional development skills and Green highlights deliverables. PD = Professional Development Courses, RBMK = Research Basis of Medical Knowledge Course, LCC = Longitudinal Clinical Clerkship, MSTP workshops, Student Committee Work and CA = Career Advisors.

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 Advisers as sounding boards for laboratory issues or for emerging interests.

In order to customize advice 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 166 alumnae. Since its founding 32 years ago, the program has had a 78% retention rate, with a rate of 88% of students matriculating since 2004. 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. Of 86 recent graduates still in training or just beginning faculty positions, 39 had received F- awards. We look forward to them leveraging that early success and skills gained from our MSTP curriculum to garner extramural funding to support them in academic positions.

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 and MS2 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, Clinical Experiences 1, 2, 3,* and Introduction to Medical Decision Making. He has also completed his first 8 week clinical clerkship in Combined Ambulatory Medicine and Pediatrics.

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, published in *Scientific Reports* in December, 2014. This level of early publication is not the norm, but not surprising since Dr. Wells is known to emphasize publication and presentation, and career transition planning, among his trainees.

Colin is currently undertaking 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. He is taking our Professional Development III course this summer and begins graduate training in September 2015.

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 <u>not</u> 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
412 623 3237

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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:

	File Name
Attach Biographical Sketch*:	1256-Biosketch_Beckwitt_Resubmission_Final.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11971830 Funding Opportunity Number: PA-14-150 . Received Date: 2015-08-05T09:50:00.000-04:00

PROFILE - Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix
Dr.	Alan		Wells	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*	Fax Number:	E-Mail*
412-647-7813		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

	File Name
Attach Biographical Sketch*:	1257-Wells_Biosketch_Beckwitt_Resubmission_Final.pdf
Attach Current & Pending Support:	1258-Wells_Other_Support_Beckwitt_Resubmission_Final.pdf

OMB No. 0925-0001 (Rev. 08/12 Approved Through 8/31/2015)

APPLICANT BIOGRAPHICAL SKETCH

Use only for individual predoctoral and postdoctoral fellowships, dissertation research grants (R36),and Research Supplements to Promote Diversity in Health-Related Research (Admin Suppl). DO NOT EXCEED FIVE PAGES.

NAME OF APPLICANT: Colin Beckwitt

eRA COMMONS USER NAME (credential, e.g., agency login): CBECKWITT

POSITION TITLE: Pre-doctoral MD/PhD candidate

EDUCATION/TRAINING *(Most applicants will begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training and residency training if applicable. High school students should list their current institution and associated information. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge MA	BS	08/2009	06/2013	Bioengineering
University of Pittsburgh School of Medicine, Pittsburgh PA	M.D./Ph.D.	06/2013	05/2020	Medicine / Cellular and Molecular Pathology

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). This unique system will be used to probe the metabolic state of dormant versus proliferating cells and the impact of oxygen availability (through use of a novel hemoglobin-based oxygen carrier (HBOC)) on cell dormancy phenotype, metabolism, and treatment potentiation. My thesis mentor, Alan Wells, MD, DMSc, has expertise in this field of study and copious experience balancing both clinical and research responsibilities, through his continued involvement in 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 imparted 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. 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	START DATE (mm/yy)	END 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	06/09	09/09	Bioinstrumentation	Massachusetts Institute	Ian Hunter, PhD
Undergraduate Researcher	06/10	09/10	Bio-optics	of Technology	Steve Wasserman, PhD
Undergraduate Researcher	06/11	04/12	Biomaterials	Massachusetts Institute of Technology	Katharina Ribbeck, PhD
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
Graduate student researcher	07/15	Current	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
2015 Autopsy Discovery Program – 3rd place poster

Memberships in Professional and Academic Societies

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

C. Contributions to Science:

My three contributions to science have derived from undergraduate, medical school, and graduate research projects. These three experiences are organized chronologically below.

1. Undergraduate research: 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. These films demonstrated assembly independent of salt concentration (a unique trait when compared to polyelectrolyte films, e.g. poly-L-lysine) and a lack of cytotoxicity, permitting future clinical translation. I was involved with a patent submission for this technology.

Poster presentations:

Mucin multilayers assembled through sugar-lectin interactions
Beckwitt CH, Crouzier T, Ribbeck K: March, 2013

Peer-reviewed manuscripts:

Crouzier T, **Beckwitt CH**, Ribbeck K. Mucin multilayers assembled through sugar-lectin interactions. *Biomacromolecules*. 2012 Oct 8;13(10): 3401-8.

2. Medical school research: The anatomic pathology department runs an annual "Autopsy Discovery Program." The goal of this program is to introduce medical students to the diagnostic power of autopsies and promote research of a discovered pathology. I participated in this program in the spring of 2015 and conducted

research on a neuroendocrine tumor, pheochromocytoma. I analyzed slides of the pathologic specimen I dissected and won 3rd place at the poster session at the end of the program.

Poster presentations:
Pheochromocytoma: A Catecholamine-secreting Neuroendocrine Tumor of the Adrenal Medulla (3rd place)
Beckwitt CH, Trejo Bittar HE, Nine J: March, 2015

3. Graduate school research: My ongoing pre-doctoral research began as a 10-week summer laboratory rotation in 2014 with my sponsor, Dr. Alan Wells. My research focused on two topics: first, collecting preliminary data for my thesis work using the LiverChip system and second, working on a manuscript investigating statins as potential anti-cancer drugs. The later resulted in the publication of a manuscript (a rare feat during a rotation) and a poster. Since starting my PhD in July, 2015, I have started to prepare a review on human microchips technology to further my contribution to my field of study.

Poster presentations:
Statin inhibition of cancer cell growth tracks with mesenchymal phenotype and lack of functional E-cadherin mediated cell cohesion
Beckwitt CH*, Warita K*, Warita T, Schurdak ME, Clark A, Wheeler S, Vazquez A, Wells A, Oltvai ZN: August, 2014

Peer-reviewed manuscripts:
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. *Sci Rep*, 2014 Dec 23;4:7593
Beckwitt CH, Clark A, Wheeler S, Wells A. Artificial Bioreactors – Human Microchips for Drug Research. In *Machine Perfusion*, Paulo Fontes, ed. *In preparation* invited.

D. Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE
	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	
2009	Introductory Biology	P
2009	Physics I	P
2009	Calculus I (AP credit)	S
2009	Calculus II	P
2009	Differential Equations (taken at BU during high school, grade = A)	S
2009	Linear Algebra (taken at BU during high school, grade = A)	S
2010	Principles of Chemical Science	A+
2010	Introduction to Computer Science and Programming	A
2010	Physics II	A
2010	Organic Chemistry I	A
2010	Genetics	A
2010	Earth, Energy, & the Environment	A
2010	Laboratory Chemistry	A
2011	Physical Chemistry of Biomolec Sys	A+
2011	Biological Chemistry	A
2011	Organic Chemistry II	A
2011	Lab Fundamentals in Biological Eng	A-
2011	Biomolecular and Cellular Systems	A
2012	Cell Biology	A

YEAR	OTHER COURSE TITLE	GRADE
	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	
2009	Writing & Experience	P
2010	Reading Fiction	A-
2010	Principles of Microeconomics	B+
2011	Principles of Macroeconomics	A
2011	Harmony and Counterpoint I	A+
2011	Chamber Music Society (Spr)	A-
2011	Studio Accompanying: Pianists (Spr)	A
2011	Chamber Music Society (Fall)	A
2011	Studio Accompanying: Pianists (Fall)	A
2012	Jazz Harmony and Arranging	A
2012	Problems of Philosophy	A
2012	Jazz Composition	A
	UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE MSTP COURSEWORK	
2013	Molecular Medicine	S
2013	Laboratory Research Rotation 1	S

YEAR	SCIENCE COURSE TITLE	GRADE
2012	Molecular, Cellular, & Tissue Biomechanics	A
2012	Fields, Forces, and Flows	A
2012	Undergraduate Independent Study in Chemistry	A
2012	Mechanisms of Drug Action	A
2012	Instrument and Measurement for Biological Systems	A+
2013	Molecular and Engineering Aspects of Biotechnology	A+
2013	Biological Engineering Design	A+
	UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE M.D. COURSEWORK (Science)	
2013	Basic Science Fundamentals 1	P
2013	Basic Science Fundamentals 2	P
2013	Scientific Reasoning 1	P
2014	Basic Science Fundamentals 3	P
2014	Neuroscience/Psychiatry	P
2014	Body Fluid Homeostasis	P
2014	GI/Endo/Heme/Skin/Musk/Repro Sciences	P

YEAR	OTHER COURSE TITLE	GRADE
2013	MSTP Workshops	S
2013	Research Basis of Medical Knowledge (Fall)	S
2014	Research Basis of Medical Knowledge (Spring)	S
2014	Methods and Analysis	S
2014	Laboratory Research Rotation 2	S
2014	Research Basis of Medical Knowledge 2 (Fall)	S
	UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE M.D. COURSEWORK (Non-science)	
2013	Patient/Physician & Society (PPS1)	P
2013	Introduction to Patient Care 1	P
2014	Introduction to Patient Care 2	P
2014	PPS2 Behavioral Medicine	P
2014	PPS3 Population Health	P
2014	Introduction to Patient Care 3	P

MCAT: 33O
Step 1 of USMLE: 254
GRE: 162/166/5.5 (Verbal/Quantitative/Writing)

Grade Legend:
“P” – Pass (>70%)
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 dropped (N/A for me).
2. University of Pittsburgh School of Medicine M.D. Coursework: All classes are graded pass (P) or fail (F)

“S” - Satisfactory
1. Massachusetts Institute of Technology: An “S” indicates credit obtained from courses taken outside MIT
2. University of Pittsburgh School of Medicine MSTP Coursework: An “S” indicates a passing grade.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Alan Wells**

eRA COMMONS USER NAME (credential, e.g., agency login): **AWells**

POSITION TITLE: **Thomas J Gill III Professor of Pathology**
Executive Vice-Chair for Laboratory Medicine, University of Pittsburgh
Staff Pathologist Pittsburgh VA Health System

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	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	PostDocFl	1984-87	Tumor Biology
University of California, San Diego, CA	Resident	1988-91	Laboratory Med

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. We have the interest, collaborations, time, resources and monies to support his thesis work.

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 (-94), 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	Tsoo 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 Award, Biomedical Graduate Students Association, UPitt		
2013	Provost Award for Excellence in Graduate 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 – 14 VA Merit Review, Oncology Study Section (chair, 2012 - 14)

C. Contributions to Science

For a full list of the papers generated by this Merit Award please see the Progress Report. The listed papers are numbered chronologically. (of 230+ total publications, >30 since 2012 inclusive)(H factor = 55 by Scopus, 65 by Google)

For a full list of my publications please see Google Scholar:
https://scholar.google.com/citations?user=LZ6DltgAAAAJ&hl=en

This cancer program has focused on carcinoma progression from a localized cancerous growth invading through barrier matrices through ectopic seeding, and now emergence as lethal metastases. The foundational work has involved (1) deciphering the intracellular signals that link the microenvironmental cues to migration and invasiveness, (2) determining involvement of these pathways in tumor invasion and dissemination, (3) phenotypic plasticity during ectopic seeding, (4) cues that keep the ectopic tumor cells alive in a hostile, foreign microenvironment, and (5) development of ex vivo all human organotypic model systems with which to study these early events in metastatic seeding and tumor emergence from dormancy.

1. Cell Signaling of Migration – We have been at the forefront of deciphering the key signaling nexi that control growth factor-induced cell motility for over two decades. As carcinoma cells establish autocrine signaling loops that are critical for tumor progression, most commonly through the EGF receptor, we focused on the distinction between this chemokinetic motility and the underlying basal haptotactic cell movement. This involved both biochemical dissections and systems modeling to determine intervention points. These findings provided for the background for early cycles of this Merit Award program.

53. A Glading, P Chang, DA Lauffenburger, **A Wells** (2000). EGF receptor activation of calpain is required for fibroblast motility and occurs via an ERK/MAP kinase signaling pathway. Journal of Biological Chemistry 275, 2390-2398. PMID: 10644690
86. A Iwabu, K Smith, FD Allen, DA Lauffenburger, **A Wells** (2004). EGF induces fibroblast contractility and motility via a PKC□-dependent pathway. Journal of Biological Chemistry 279, 14551-14560.
93. S Hauteniemi, S Kharait, A Iwabu, **A Wells,** DA Lauffenburger (2005). Modeling and prediction of signal transduction cascades using decision trees. Bioinformatics 21, 2027-2035.
161. H Shao, T Travers, C Camacho, **A Wells** (2013). The carboxyl tail of alpha-actinin-4 regulates its susceptibility to m-calpain and thus functions in cell migration and spreading. International Journal of Biochemistry and Cell Biology 45, 1051-1063. PMC3633689.
177. T Travers, H Shao, BA Joughin, DA Lauffenburger, **A Wells**, C Camacho (2015). Novel regulatory mechanism by tandem phosphorylation sites in the intrinsically disordered N-terminal region of ACTN4. Science Signaling 8, ra51.

2. Tumor Invasion driven by Cell Motility – It is critical to establish that the intracellular signals established for growth factor mediated motility were involved in cancer invasion. This was the original impetus for this Merit Award program. We initially established this in prostate cancer in animal models and more recently moved this to ex vivo models so as to study the cellular events during such invasion. These findings were powered by the earlier and current Merit Awards. The more recent papers using complex 3D models demonstrate the value of such in discerning targetable molecular events even in simplified models.

36. T Turner, M Van Epps-Fung, J Kassis, **A Wells** (1997). Molecular inhibition of PLC□□signaling abrogates DU-145 prostate tumor cell invasion. Clinical Cancer Research 3, 2275-2282.
76. A Mamoune, JH Luo, DA Lauffenburger, **A Wells** (2003). m-Calpain as a target for limiting prostate cancer invasion. Cancer Research 63, 4632-4640.
107. MH Zaman, LM Trapani, A Siemeski, D McKeller, H Gong, RD Kamm, **A Wells**, DA Lauffenburger, P Matsudaira (2006). Migration of tumor cells in three-dimensional matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proceedings of the National Academy of Sciences (USA) 103, 10889-10894.
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
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 289, 32717–32728.

3. Epithelial-Mesenchymal Plasticity in Tumor Progression – The major issue in carcinoma treatment today is metastatic disease. Advances in imaging, detection and local treatment have shown that tumors often seed ectopic sites very early in their development. However, our approaches to metastatic disease have not advance apace, mainly due to a gap in our understanding of what occurs on a molecular level during the very inefficient step of micrometastatic seeding. Our lab, supported in part by the past two cycles of this Merit Award, provided the first evidence of adaptive phenotypic plasticity of de-differentiated mesenchymal carcinoma cells undergoing a reversion to a more epithelial phenotype both ex vivo and in animal models, this includes not just responsiveness to the microenvironment but also reprogramming of the epigenetic imprinting in the carcinoma cells. This provides the scientific basis for the current proposal that examines the implications of this plasticity for tumor metastasis and dormancy (with the last paper addressing the question of emergence from dormancy).

113. CC Yates, CR Shepard, D Stolz, **A Wells** (2007). Co-culturing human prostate carcinoma cells with hepatocytes leads to increased expression of E-cadherin. British Journal of Cancer 96, 1246-1252. PMID: 1740636

R37. A Wells, C Yates, CR Shepard (2008). E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas. Clinical and Experimental Metastasis 25, 621-628. PMID: 18600305. PMC2929356

141. YL Chao, CR Shepard, **A Wells** (2010). Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. Molecular Cancer 9, 179. PMID: 20609236. PMC2907333.

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

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

4. Survival and Chemoresistance Signaling – Limiting the efficacy of our chemotherapy, tumor cells in the metastatic environment appear resistant to chemotherapy in a generalized sense (as opposed to agent-specific adaptation/mutation). With our collaborators we discovered that signaling from surface-restricted EGF receptor promotes survival even in the face of death cytokines and chemotherapeutics (for both normal and transformed cells). This occurs physiologically in that the cryptic matrikine tenascin-C was found to have this then novel property as we discovered. We have shown that this matricellular component also promotes the survival of stem cells, as well as tumors in preliminary studies. Furthermore, we have found that tumor cells quiesce during dormancy, rather than in a cycling state with nodule size constraints. These findings motivate our focus on emergence, and targeting the emergent cells as potentially sensitive to new agents.

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.

162. M Rodrigues, CC Yates, A Nuschke, L Griffith, **A Wells** (2013). The matrikine tenascin-C protects multipotential stromal cells/mesenchymal stem cells from death cytokines such as FasL. Tissue Engineering 19, 1972-1983. PMID: 23541003; PMC: 3725854

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

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

175. K Warita, T Warita, C Beckwitt, M Schurdak A Vazquez, **A Wells**, ZN Oltvai (2015). Statin-induced mevalonate pathway inhibition attenuates the growth of mesenchymal-like cancer cells that lack functional E-cadherin mediated cell cohesion. Scientific Reports 4, e7593.

5. Ex vivo Organotypic Models for Tumor Progression and Dormancy – In our investigations we found that the current model systems, while informative, were limiting as to probing the early events in dissemination, in particular the cell biological and molecular events. Over the past two Merit Award cycles, we collaborated with Linda Griffith at MIT to adapt a liver organotypic bioreactor to metastasis research. To further improve the relevance, we migrated this system to an all human tissue so as to be able to recreate human-specific cyto-/chemo-kine signaling and drug metabolism. This system allowed our team for the first time to achieve predictable and reproducible quiescent dormancy (without any molecular engineering), with an ability to re-awaken these cells as emergent outgrowths.

R34. C Yates, CR Shepard, G Papworth, A Dash, DB Stolz, S Tannenbaum, L Griffith, **A Wells** (2007). Novel three-dimensional organotypic liver bioreactor to directly visualize early events in metastatic progression. Advances in Cancer Research 97, 225-246. PMID: 17419948

139. BL Hood, J Grahovac, MS Flint, M Sun, N Charro, D Becker, **A Wells**, TP Conrads (2010). Proteomic analysis of laser microdissected melanoma cells from skin organ cultures. Journal of Proteome Research 9, 3656-3663. PMID: 20459140. PMC3733114
R55. 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 239, 1170-1179. PMID: 24821820. PMC in process after embargo
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 111, 2342-2350.

D. FY15 Year Support - Alan Wells
ACTIVE
1) VA Merit Award (PI: Wells) 10/1/06 - 9/30/15
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 the grant being competitively renewed with a percentile of 11.2.** There is **no overlap in Aims, target molecules, molecular processes or endpoints** with the current proposal.
2) NIGMS (GM63569) (PI: Wells) 12/1/14 – 11/30/18
NIH/NIGMS
“Dermal-epidermal communication during wound healing”
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.
3) NIGMS (GM69668) (PI: Wells) 1/15/04 – 11/30/15
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. **This the grant being competitively renewed with a percentile of 16.** There is **no overlap in Aims, target molecules, molecular processes or endpoints** with the current proposal.
4) NIH (1UH3TR000496) (PI: Griffith, MIT) 8/1/12 – 6/30/17
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.**
5) NIH (UH3TR000503) (PI: Taylor, UPitt) 7/1/14 – 6/30/17
NIH/Clinical Translational Sciences directorate
“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.**
6) NCI (1R21CA188799) (PI: Yates, Tuskegee) 9/30/14 – 9/29/16
NIH/NCI Cancer Health Disparities Program
“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 is complementary, **there is no overlap as this is directed as the control of E-cadherin expression.**

12. FY16 Year Support - Alan Wells

ACTIVE

1) NIGMS (GM63569) (PI: Wells) 1/1/15 – 11/30/18
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.

2) NIGMS (GM69668) (PI: Wells) 1/15/04 – **11/30/15**
2.0 months
NIH/NIGMS $119,000 directs to Wells Lab
"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. This grant is under consideration for renewal with a percentile of 16%.**

3) VA Merit Award (PI: Wells) 10/1/06 - **9/30/15**
1.50 months
Veterans Administration $149,000 directs to Wells Lab
"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. **There is no overlap with the current grant. This grant is under consideration for renewal with a percentile of 11%.**

4) NIH (1UH3TR000496) (PI: Griffith, MIT) 8/1/12 – 6/30/17
2.00 months
NIH/Clinical Translational Sciences directorate $200,000 directs to Wells Lab
"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.**

5) 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.**

6) NCI (1R21CA188799-01) (PI: Yates, Tuskegee) 9/30/14 – 9/29/16
0.6 months
NIH/NCI Cancer Health Disparities Program $35,000 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.**

PHS Fellowship Supplemental Form

OMB Number: 0925-0002

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)	1244-Introduction_Beckwitt_Resubmission_Final.pdf
2. Specific Aims*	1245-Specific_Aims_Beckwitt_Resubmission_Final.pdf
3. Research Strategy*	1246-Research_Strategy_Beckwitt_Resubmission_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	1247-HumanSubjects_Beckwitt_Resubmission_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	1248-Resource_Sharing_Plan_Beckwitt_Resubmission_Final.pdf
17. Respective Contributions*	1249-Respective_Contributions_Beckwitt_Resubmission_Final.pdf
16. Selection of Sponsor and Institution*	1250-Selection_Sponsor_Institution_Beckwitt_Resubmission_Final.pdf
17. Responsible Conduct of Research*	1251-Responsible_Conduct_Research_Beckwitt_Resubmission_Final.pdf

PHS Fellowship Supplemental Form

OMB Number: 0925-0002

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)
Predoctoral	Institutional	07/01/2013	06/30/2014	5T32GM008208-25
Predoctoral	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* 1252-Goals_For_Fellowship_Beckwitt_Resubmission_Final.pdf

8. Activities Planned Under This Award* 1253-Activities_Planned_Beckwitt_Resubmission_Final.pdf

9. Doctoral Dissertation and Other Research Experience 1254-Doctoral_Dissertation_Research_Exp_Beckwitt_Resubmission_Final.pdf

10. Citizenship*

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

Institution

11. ☐ Change of Sponsoring Institution

Name of Former Institution:*

Funding Opportunity Number: PA-14-150. Received Date: 2015-08-05T09:50:00.000-04:00

PHS Fellowship Supplemental Form

OMB Number: 0925-0002

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

Sponsor(s) and Co-Sponsor(s) Information*	1255-SponsorInformationWells_Beckwitt_Resubmission_Final.pdf

E. Budget

All Fellowship Applicants:

1. Tuition and Fees*:

❍ None Requested ● Funds Requested

Year 1	$58,305.00
Year 2	$61,220.00
Year 3	$64,281.00
Year 4	$60,433.00
Year 5	$63,455.00
Year 6 (when applicable)	
Total Funds Requested:	$307,694.00

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary:	Amount	Academic Period	Number of Months
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

1241-Normole_Biosketch_Resubmission_Final.pdf1242-IRB 0506140 Approval 2015-2016.pdf1243-IRB Exempt Tumor Studies.pdf

Funding Opportunity Number: PA-14-150. Received Date: 2015-08-05T09:50:00.000-04:00

B.1 INTRODUCTION

Thank you to the reviewers for such comprehensive and thoughtful feedback and for the encouraging impact score of 36. This introduction addresses the helpful and important concerns brought up in my Summary Statement. These and other important aspects of my resubmission will be addressed in list format below. Space does not permit us to address here all the issues; changes addressing these concerns are in the text.

1. *Data analysis and statistics:* Reviewer 1 stated the importance of proper analysis of the data, given the MPS incorporates two systems: the liver system and the tumor cells. I agree that this point is essential to the success of my project. To help with this, I have recruited a statistician to my mentorship team (Daniel Normolle, PhD). Dr. Normolle is the director of the University of Pittsburgh Cancer Institute Biostatistics facility and has been involved with the design, analysis and interpretation of clinical, in vivo, ex vivo and in vitro studies of gastrointestinal cancers for over 20 years. His Biosketch (including funding support) is included in my resubmitted application. In addition to his massive technical expertise, his mentoring potential is remarkable - he has mentored both PhD and MD-PhD students for the past 22 years. As such, he is the perfect mentor to help me develop skills in statistical analysis and in developing models for the complex MPS I will use for my thesis work. To additionally address this issue, I have added a statistical analysis section to my Research Strategy and have colored it in blue. In brief, a Mann-Whitney U-test will be used for comparing two populations (e.g., dormant and proliferating tumor cells) and a one-way or two-way ANOVA will be used for comparing three or more populations (e.g., varying oxygen tensions or therapeutic dosages) for one or two independent variables respectively. The significance level for these statistical tests will be $P<0.05$.

I further thank Reviewer 1 for astutely noting that some of the agents being used in this project (the statins) impact both the liver system and the tumor cells, with the impact on the liver system possibly further impacting the tumor cells. This is both an expected and desired result for the MPS, as its goal is to determine the effects of these interventions as would occur clinically. The effects of these interventions on the tumor microenvironment, and the resulting implications on tumor cell functioning combined with tumor-specific effects, will govern clinical efficacy. As such, distinction of the effects of statins (or chemotherapeutic agents) on the liver system or tumor cells individually is not the goal of this work. Rather, this work aims to encompass both effects to reflect the clinical situation as accurately as is possible.

2. *Preliminary data:* During the summer between first and second year of medical school, I tackled the background work enabling this study, as shown by secondary authorship on the statin paper. I have now returned to research full time in July 2015 after my second year of medical school. Given the pre-clinical and clinical education effort, the preliminary data with the oxygen carrier is minimal.

3. *Resource Sharing Plan:* I have now included a resource sharing plan as part of my application.

4. *Budget and period of support:* All three reviewers recommended reducing the time of my fellowship and reviewer 2 recommended reducing the budget amount. My SRO, Dr. Mark Damico, kindly explained over the phone to ask for the amount of time and money I need to complete my training for both graduate and medical school. After review of my proposal, he will determine the budget and period of support that will fall within NRSA guidelines if I am funded.

5. *Human Subjects exemption:* The University of Pittsburgh IRB has deemed this study NOT to involve human subjects; this documentation has been sent to the NIH. All of the human cells are obtained commercially or via the NIH funded LCTDS Program that de-identifies the source and thus distributes as exempted.

6. Oxygen impact on liver parenchymal organization and lipid as a proliferation fuel source: To address reviewer 2's comments regarding these two topics, I have added sections to my Research Strategy document and have colored them blue. In brief, oxygen tension guides physiologic liver zonation, which causes metabolic sub-specialization of the parenchyma. This trait allows the liver to be responsive to alterations in oxygen supply. Also, lipids are an important fuel for cancer cells, especially during hypoxia, but are typically derived from upregulation of *de novo* synthesis pathways rather than extracellular uptake.

7. Cell line heterogeneity: The two cancer lines used become phenotypically heterogenous when introduced into the liver microenvironment, this appears to be reflective of the human situation. We will score outgrowth and dormancy along with phenotype of the individual cells to account for these changes.

8. Primary cell heterogeneity: Primary liver function is monitored using clinical function tests (e.g., albumin/urea) and damage markers (e.g. AST/ALT). Incorporation of primary cells allows this system to be used to screen for potential biomarkers that affect the tumor microenvironment. In future years, the primary breast cancer cells will be monitored as to phenotype. Additionally, we will use the inoculated cells as controls for phenotypic markers.

9. *Publication strategy/Clinical translation:* To further emphasize these crucial aspects of my training plan, I have added sections to my Research Strategy document and have colored them blue. In short, my mentor and I am acutely aware of publication needs, as shown by inclusion on the Warita et al. paper and my current work on a review article on oxygen carriers in microphysiological systems. Clinical translatability is the reason for using an all-human liver MPS, in that this captures both drug metabolism and toxicity, and tumor cell efficacy. Thank you for taking the time to read and consider my resubmitted proposal.

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. While some micrometastases immediately outgrow to form clinically evident metastases, many often enter a dormant state that not only prevents their detection by traditional imaging modalities but 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 resuming proliferation. We previously showed that cell signaling and extracellular growth factors influence metastatic outgrowth in our LiverChip microphysiological model for liver metastasis. Given the reliance of rapidly proliferating tumor cells on glycolytic metabolism, the so-called “Warburg Effect,” oxygen abundance may play a role in the transition from the dormant to the proliferative state.

Our hypothesis is that dormant micrometastases adapt a low metabolic/low proliferative state concordant with chemoresistance and reduced glycolytic flux. We hypothesize that rising oxygen tension levels initiate the switch from oxidative phosphorylation to glycolytic metabolism and thus moderate dormancy and chemoresponsiveness. The controlled examination of metabolic inputs and outputs in a co-culture environment is made possible through our 3D microfluidic liver-on-a-chip using human hepatocytes, non-parenchymal cells, and stable RFP-expressing MCF-7 and MDA-MB-231 breast cancer cell lines. Specifically, we will:

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 host and tumor cells are known to play a role in this arrest. Examining the metabolic profile of micrometastases, including measuring intracellular ATP, glucose uptake, and metabolic enzymatic levels, using the LiverChip system will elucidate the impact of host 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, but the effects of oxygenation on the dormant tumor cell phenotype have not been described. We hypothesize that high oxygen tension 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 to maintain dormancy and whether there is a threshold beyond which they outgrow to form clinically evident metastases.

Aim 3. Define whether select anti-tumor agents are dependent on tumor cell metabolic state/tissue oxygen capacity. Chemoresistance of dormant micrometastases is responsible for the limited success of adjuvant chemotherapy in many types of cancer. We will use the LiverChip to measure the responsiveness of dormant tumor cells to chemotherapeutic agents (e.g., cisplatin) and anti-metabolic drugs (e.g., statins) for increasing oxygen tension. These data may suggest a relationship between drug resistance and tumor cell metabolic state and point to novel biomarkers of therapy efficacy.

Overall Impact: The successful completion of our Aims will provide novel insight into an understudied aspect of basic tumor biology, the role of oxygen tension in micrometastatic dormancy. While our human LiverChip microphysiologic system, coupled with novel oxygen carriers and biosensors, will allow us to address this specific question, our approach can be applied to other organotypic culture systems requiring physiological oxygen delivery. Our findings will inform the design of translational studies to improve the efficacy of existing agents (drug rescue) and to develop new therapies that exploit our findings related to tumor cell dormancy.

Contribution to Training: I will learn non-parametric statistical techniques (e.g., Mann Whitney and Kruskal-Wallis) to analyze the physiologic data and develop a model to control for confounding variables such as cell type heterogeneity and the influence of changes in oxygen, exogenous agents, cell ratios, and lipolysis on liver tissue function. Finally, the skills acquired and data obtained will advance my career development as a physician-scientist in surgical oncology.

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 microenvironment[1], often resulting in an “angiogenic switch” prompting 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 arrest[2]. Cellular senescence is unlikely, as it is an irreversible state which would prevent dormant cell outgrowth[2]. Recent i*n silico* models have shown quiescence most likely explains micrometastasis dormancy[3,4]. Similarly, molecular analyses of circulating tumor cells (CTCs) from patients with small cell lung cancer[5] and breast cancer[6] show a lack of proliferative markers. Patients with CTCs void of proliferative markers are often refractory to chemotherapy[7]. These results suggest the large role cellular quiescence plays in cancer dormancy and chemoresistance 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, despite abundant levels of oxygen, manipulating cancer metabolism has been sought as an avenue of therapy. Large, proliferative tumors upregulate micro-RNAs[8] to augment their vascularity in order to accommodate their growing need for nutrient and oxygen delivery. Moreover, hypoxic induction of transcription factors such as HIF-1α has been associated with dysregulation of glycolytic enzymes[9,10], drug efflux pumps[11], 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 micro-scale tumors**. **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 human hepatocytes and non-parenchymal cells (NPCs) are seeded into a flexible, hydrogel scaffold. After 2 days of adaptation, these primary cells form a micro-hepatic architecture capable of endogenous liver functions (e.g. albumin production). This tissue exists in a continuous perfusion system, since both nutrients and physical stimuli are known to be crucial for maintaining cellular function. Cancer cells are seeded on this formed tissue on the third day of culture. Scaffolds are harvested after 15 days in the MPS. Immmuno-fluoresence is used to resolve tissue microstructure. Analysis of scaffolds treated with chemotherapeutics or EdU/BrdU depicts tumor drug efficacy and proliferation respectively. Clinical chemistry probes the functionality of the liver tissue every two days by measuring cytochrome P450 isoform (CYP450) concentrations, α1-antitrypsin (A1AT), fibrinogen, and urea collected from supernatant samples during media changes. Hepatocyte health is determined by alanine amino-transferase (ALT) and aspartate aminotransferase (AST) levels. 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).**

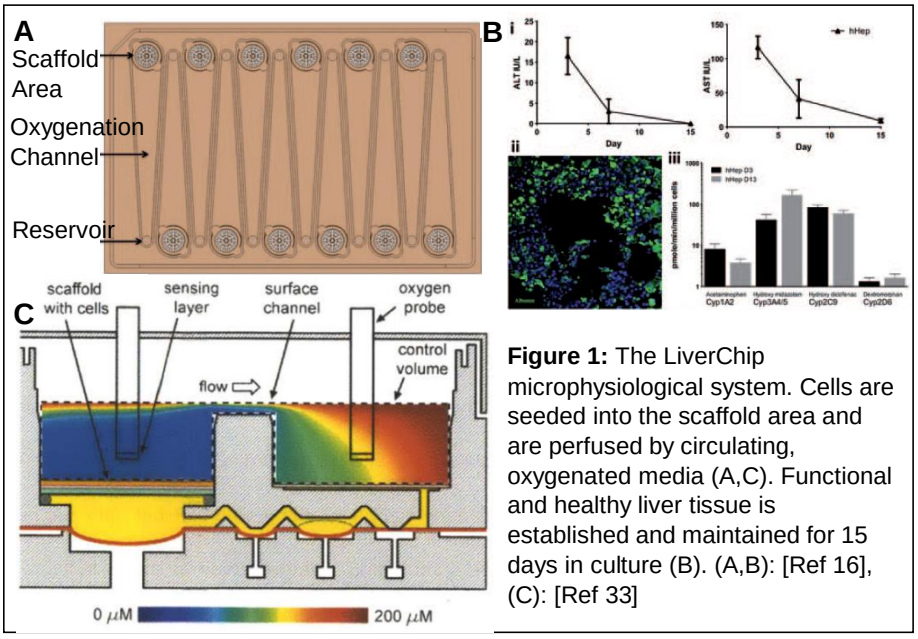


Figure 1: The LiverChip microphysiological system. Cells are seeded into the scaffold area and are perfused by circulating, oxygenated media (A,C). Functional and healthy liver tissue is established and maintained for 15 days in culture (B). (A,B): [Ref 16], (C): [Ref 33]

A2. Significance

i. Clinical: Micrometastatic dormancy presents several clinical problems. While the primary tumor can be effectively eliminated by surgery, radiotherapy, or chemotherapy, dormant micrometastases are commonly resistant to these therapies. 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.

Grasping the influence of oxygen tension on micrometastasis metabolism, dormancy, and treatment susceptibility may lead to 1) improvement of existing therapies, 2) development of new therapies specific for clinically-silent micrometastases, 3) define new diagnostic modalities, and/or 4) suggest prophylactic methods to prevent emergence from dormancy in scenarios of anticipated, but not clinically evident, metastasis.

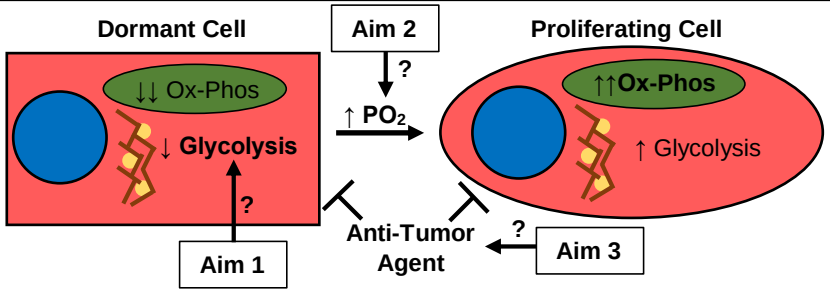


Figure 2: Schematic of the role of oxygen on the switch between oxidative phosphorylation (Ox-Phos) and glycolytic metabolism. We hypothesize dormant tumor cells exhibit low glycolysis **(Aim 1)**. We further predict that a higher oxygen tension (PO_2) will facilitate emergence from dormancy **(Aim 2)** accompanied by increased oxidative phosphorylation and aerobic glycolysis, and increase cell susceptibility to model chemotherapeutics **(Aim 3)**.

An effect of oxygen tension on the tumor cell growth or efficacy of chemotherapy would have direct clinical implications. If we find a relationship between oxygen and therapy responsiveness, then translational studies would determine if different oxygen levels (hyperbaric or altitude) improve chemo-responsiveness. Additionally, therapies could be developed to activate in these hypoxic environments (so as to minimize activity in normal tissue and limit toxicity), though this would take longer to move to the clinic. Finally, these studies may suggest different management in situations of altered oxygen delivery in patients with other confounding disease such as anemia, lung disease, or heart failure.

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's 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 oxygen[34] while the rest is dissolved. Use of an oxygen carrier analogous to endogenous erythrocyte hemoglobin will facilitate robust modulation of the system's oxygen supply and can be extrapolated to other organotypic culture systems. 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.

A4. Publication and Communication Strategy

Science is, at its base, communication. All findings must be disseminated and made available so as to impact the larger community of researchers. I will pursue the two-pronged strategy extant in the Wells Lab. Papers form the core as these are freely available to all and form a written record for all to critique and follow. I anticipate a first author paper from each Aim, with secondary authorships where work contributes to others in the lab and collaborating labs. The second part is to present at national and international meetings, as well as local retreats and symposia. I will be attending two meetings annually on average, with Dr. Wells insisting on presenting (posters or oral) at all meetings attended. Given the novelty of the project, I will be presenting unpublished data at these meetings, as is the norm for the Wells Lab.

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, α1-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

layer[33]. 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 (both stable RFP-expressing) 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 consumption[18]. 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 neck[20] and neuroendocrine[21] tumors. The Glut-1 glucose transporter has been found to be upregulated in cancers in response to hypoxia[42]. Moreover, inhibition of glucose uptake in cancer cells by flavanoids[22,23] or transfected miRNA[24] 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 PFKFB4[27] 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 glycolytic metabolism highly correlates with the proliferation of cancer cells and poor clinical prognosis. Aggressive cancers also exhibit reduced oxidative phosphorylation flux due to upregulation of 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 aggression[43]. 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 isoprenylation[44]. 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.

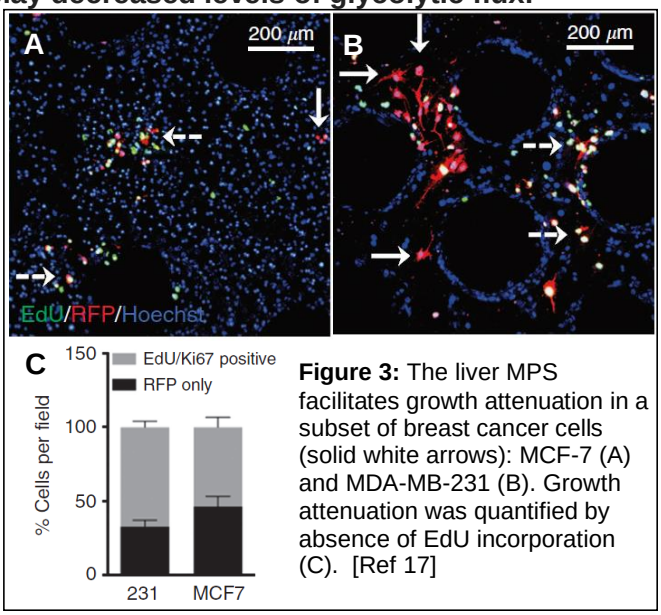


Figure 3: The liver MPS facilitates growth attenuation in a subset of breast cancer cells (solid white arrows): MCF-7 (A) and MDA-MB-231 (B). Growth attenuation was quantified by absence of EdU incorporation (C). [Ref 17]

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. Recently, we have developed the means to disintegrate the hydrogel scaffolds housing the liver tissue and cancer cells without damaging their cell membranes (data not shown). This facilitates the isolation of the cancer cells, following the culture period, for individual evaluation. As such, FACS (cancer cells are RFP-tagged) combined with RNA-seq will be used to confirm the quantitative immunofluorescence results. The KI67- cells (dormant) will be compared to the KI67+ cells (actively proliferating) for data analysis to uncover differences between the two populations.

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 is ideal for examining these two populations – proliferative and dormant – exposed to the same microenvironment. Compared to proliferating cancer cells, dormant cancer cells will likely exhibit less Glut-1 and PFKFB3 immunostaining (for Glut-1 we are more interested in surface staining as the transporter does shuttle between exposed and intracellular vesicles). 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 in 2D culture has previously shown lower expression of Glut-1 and PDK1 as compared

to an acute hypoxic exposure[32]. Furthermore, ATP-citrate lyase levels will likely be lower due to decreased PI3K-Akt signaling in quiescent cells[45]. Finally, we expect to see lower levels of intracellular ATP in dormant cells. We would interpret these findings as consistent with our model of low oxygen promoting dormancy.

It is possible that the expression of the measured glycolytic markers will not differ significantly between the quiescent and proliferating cancer cells. While unlikely, given the regulatory 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 amino acid and lipid synthesis[46,47]. Some cancers, in particular hepatocellular carcinoma, upregulate glutamine metabolism as a means for energy accumulation[54]. 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 cycle[48].

Lipids also have been shown to provide fuel for cancer cell proliferation. Hepatocytes and stellate cells possess a large capacity for lipid storage which, in this system, may provide an alternative fuel for the tumor cells right at the site of micrometastases. However, studies have shown rapidly proliferating cancer cells preferentially upregulate de novo synthesis pathways rather than obtain lipids from their microenvironment[55,56]. Other studies show cancer cells activate lipoprotein lipase to uptake fatty acids from their environment[57] and that the storage of lipids is important for cell functioning, especially in times of hypoxia[58]. If we see no difference in glycolytic flux, we will investigate fatty acid uptake or de novo synthesis pathways as alternative energy generators for proliferation. The LiverChip system is amenable to studying many metabolic intermediates and extrapolating 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-1α, 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-1α is hydroxylated at two key proline residues in its oxygen degradation domain, targeting it for ubiquitination[29]. Hypoxia also impacts chemoresistance by slowing the cell cycle, reducing the efficacy of alkylating agents, and upregulating p-glycoprotein (P-gp), an efflux pump associated with resistance to chemotherapeutic drugs such as adriamycin[30]. Given the importance of oxygen supply to a proliferating tumor, anti-angiogenic agents have been explored as therapies, most commonly in combination with standard chemotherapy[31]. 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% O_2), a pancreatic cancer cell line was shown to induce cell dormancy through suppression of Akt, a constitutively active kinase in many human cancers[32]. Additionally, PPARγ, 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)**.

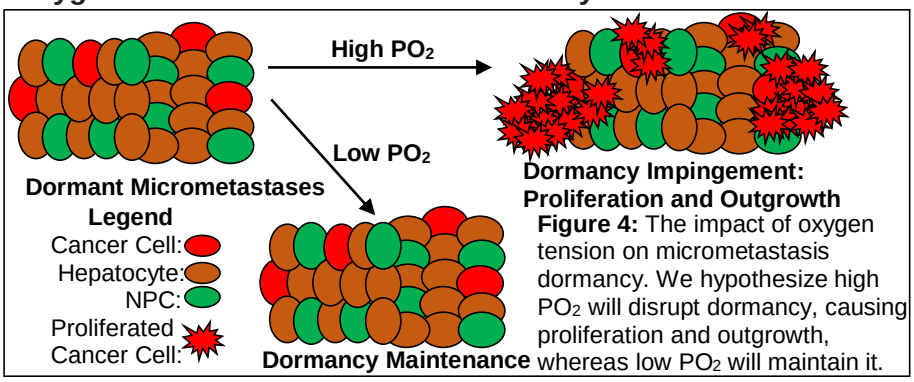


Figure 4: The impact of oxygen tension on micrometastasis dormancy. We hypothesize high PO_2 will disrupt dormancy, causing proliferation and outgrowth, whereas low PO_2 will maintain it.

Oxygen also plays a key role in the liver tissue context, since it is a highly vascularized organ whose oxygen tension varies depending on the proportional blood supply and subtissue architecture from the arterial and portal systems. The oxygenation status of the liver tissue governs parenchymal function. The relatively oxygen rich periportal area has oxidative metabolism and cholesterol synthesis whereas the relatively oxygen poor perivenous area exhibits glycolysis and xenobiotic metabolism by the CYP450 system[59]. Thus, altering oxygen supply may alter liver cell functioning. As the predilection for dormancy or emergence has not been discerned based on hepatic oxygen tension considerations, this ex vivo system offers a unique opportunity.

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 described[33]. 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 described[17]. To access the adequacy of oxygen delivery to cancer cells in various locations in the liver tissue, the degree of hypoxia will be assessed by HIF-1α immunofluorescence and measuring both input and effluent oxygen levels. 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**. Finally, liver metabolic functioning will be measured as described in the **Background** to detect any alterations in parenchymal remodeling. All of these parameters will be quantitatively modeled using mass transfer principles learned during my engineering undergraduate days (namely the combined convection-diffusion-consumption and Navier-Stokes equations).

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 PPARγ, which inhibit cancer proliferation and invasion through upregulation of cyclin dependent kinase inhibitors p21 and p27[36], are activated under hypoxic conditions. Short-term exposure to PPARγ ligands in nude mice has been found to reduce colorectal cancer metastasis and longer term exposure demonstrated the formation of quiescent, dormant groups of cells[39]. 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-1α 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-1α) and the resulting decrease in transcription of the glycolytic enzymes and regulatory factors previously described. This would be interpreted as increased oxygen leading to emergence. While this may be a harbinger of negative biological effects, it also may make the tumors more susceptible to chemotherapies and other agents, as we note that emergent tumor cells are more readily killed while the dormant cells are basically chemoresistant[17].

Altered oxygen tension may also impact the hepatocytes and NPCs, the latter of which play an important role in the tumor microenvironment[17]. 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. Similarly, increased oxygen supply may encourage liver functions akin to periportal hepatocytes (see rationale). Conversely, hypoxia may stress the stellate or parenchymal cells to express these same cytokines, negating the beneficial direct effects of hypoxia on keeping the cancer cells in dormancy.

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 (Akt[32], Erk[40]) and checkpoint proteins (p16[2], p21[36], p27[36], p38[40]) 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, aiding the conversion of these cells to a more epithelioid, quiescent phenotype[41]. Moreover, compromised β1-integrin and EGF signaling from the environment may contribute to persistent dormancy even in the context of intracellular metabolic changes.

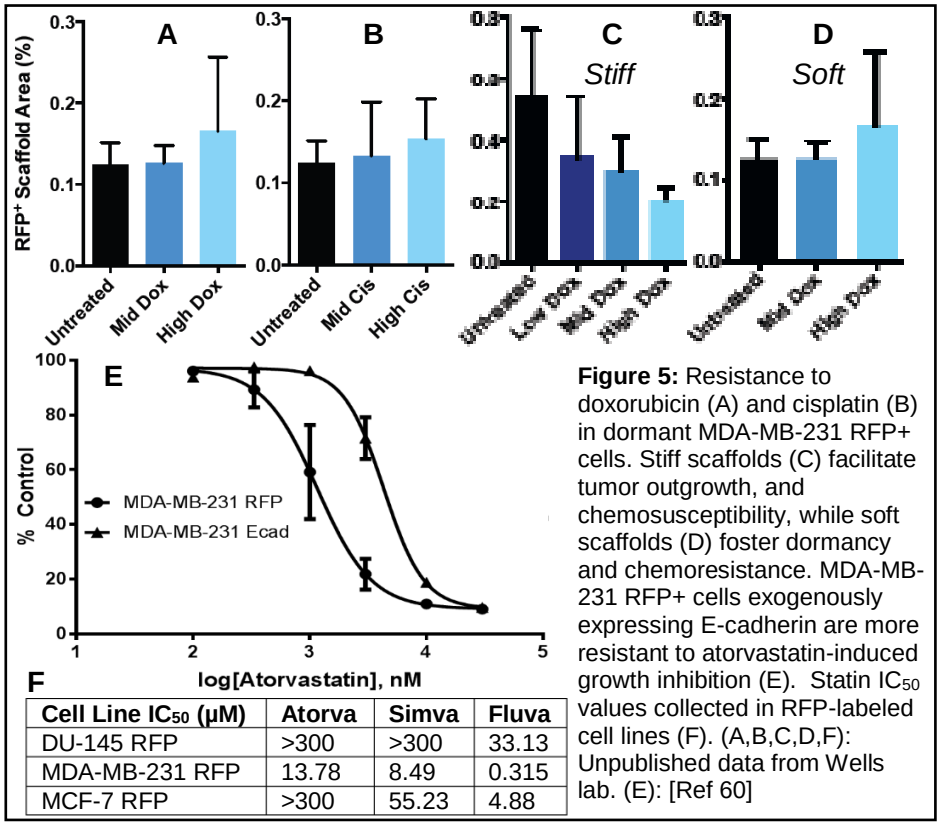
If increasing oxygen tension is not found to have a measurable effect on cell proliferation or quiescence, p-Akt and p–Erk levels will be determined to investigate pro-proliferation signals. Tumor cell membrane E-cadherin expression will be stained as high expression would be a possible cause of dormancy 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)**, dependent on the mechanical properties of the cell scaffold **(Figure 5C,D)**, with stiff scaffolds promoting outgrowth and chemoresponsiveness and soft scaffolds promoting dormancy and chemoresistance. In vitro data I collected showed exogenous E-cadherin expression increases the resistance of MDA-MB-231 cells to atorvastatin **(Figure 5E)**. 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 phenotype[49] commonly seen in dormant cells. The fact that I found similar statin resistance is observed in the epithelioid DU-145 and MCF-7 cell lines supports this hypothesis. **(Figure 5F)**. 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-D)**. Doses of the three statins previously mentioned will be used near the IC_{50} measured in cell culture **(Figure 5E,F)**. Treatments will include 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 determined as previously described in the **Background**.

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 signaling[50] and cell cycle proteins[51]. 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 5E,F)**. 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, impinging less on hepatocyte function or causing toxicity.



F

Cell Line IC_{50} (μM)	Atorva	Simva	Fluva
DU-145 RFP	>300	>300	33.13
MDA-MB-231 RFP	13.78	8.49	0.315
MCF-7 RFP	>300	55.23	4.88

Figure 5: Resistance to doxorubicin (A) and cisplatin (B) in dormant MDA-MB-231 RFP+ cells. Stiff scaffolds (C) facilitate tumor outgrowth, and chemosusceptibility, while soft scaffolds (D) foster dormancy and chemoresistance. MDA-MB-231 RFP+ cells exogenously expressing E-cadherin are more resistant to atorvastatin-induced growth inhibition (E). Statin IC_{50} values collected in RFP-labeled cell lines (F). (A,B,C,D,F): Unpublished data from Wells lab. (E): [Ref 60]

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 in pathways previously suggested in **Aim 2** or in the tumor microenvironment. Measuring efflux protein expression (P-gp), microenvironment growth factors and cytokines, and E-cadherin/EGF signaling, may be required to determine the cause of continued resistance. If single drug regimens prove ineffective, we will probe multidrug therapy efficacy and hepatotoxicity. Previous studies have shown simvastatin potentiates doxorubicin through increased activation of NF-κB[53]. Investigation of combination therapy may suggest additional mechanisms for drug rescue which minimize hepatotoxicity and maximize anti-tumor efficacy.

B5. Statistical Analysis: All quantitative results obtained for each parameter for dormant cells and proliferative cells will be analyzed individually to note metabolic traits that differ between the two populations. These results will be statistically analyzed using a Mann-Whitney U-test with a significance level of $P<0.05$. For the oxygen and drug experiments, a one-way or two-way ANOVA with a significance level of $P<0.05$ will be used for comparisons involving one or two independent variables respectively.

C. Timeline: The Aims will be tackled in a staggered manner: **Aim 1** involving the first two years, **Aim 2** starting near the end of the first year, and **Aim 3** starting 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). The oxygen supply will be altered using a novel 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.

Assuming that we find a positive correlation between oxygen level and growth profiles of the tumor cells, we would push for clinical translation. Passively, we could pursue case-control or cohort look backs in the UPCI database for emergence and anemia or oxygen therapy for other conditions. Interventionally, one could envision trialing oxygen supplementation during chemotherapy to see if the masses show amplified responsive shrinkage.

6. HUMAN SUBJECTS

Scenario C, Exemption 4

The exemption reads: “Research involving the collection or study of existing data, documents, records, **pathological specimens**, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that **subjects cannot be identified**, directly or through identifiers linked to the subjects.”

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, despite qualifying under the NIH exemption 4e, the University of Pittsburgh considers the cells, cell lines and excess pathological specimens 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 co-culture and to seed the all human bioreactor. These derive from a federally-funded program called the Liver Tissue Cell Distribution System (LTCDS: http://www.peds.umn.edu/gi/ltcds/) with the purpose of providing human hepatocytes for cellular transplantation and, if derived from normal tissue removed during therapeutic resection, for qualified researcher uses; this program is directed by Dr. David Geller (UPitt). Under the umbrella of the LTCDS, 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, as per the exemption stated above.

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.

14. Resource Sharing Plan

As has been our ongoing custom, we will freely share any unique resources developed for all non-commercial purposes. We will fully adhere to the NIH Policy on Sharing of Unique Research Resources. If these inventions/resources become patentable, we will ensure the availability of such, and distribute such to the research community, as we have in the past.
Large data sets will be shared by publication as supplementary data or if not possible by posting on a university website (Pitt or MIT depending on the source of the data).

B.15. RESPECTIVE CONTRIBUTIONS

The proposal was conceived by the applicant, Colin Beckwitt, after a summer rotation in the Wells lab (with continued interactions during the second year of medical school) and discussions with the sponsor, Dr. Alan Wells, collaborators, Dr. Linda Griffith (MIT) and Dr. Paulo 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, previous career advisor Dr. Kenneth Hallows, Dr. Michelle Kienholz, Dr. Dan Normolle, 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 also work with Dr. Dan Normolle, director of the University of Pittsburgh Cancer Institute's biostatistics facility, on statistical analysis of the collected data. The applicant 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 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 students, 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, which resulted in a 3rd author publication in December, 2014. 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 Pitt 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 MSTP, I was impressed that 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. My experience with the Combined Ambulatory Medicine and Pediatrics Clerkship reinforced and built upon my previous two medical school years. 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. Clinically, during my 8-week clerkship in Combined Ambulatory Medicine and Pediatrics, I was continuously exposed to the importance of patient confidentiality during patient interactions and use of the Electronic Medical Record systems.

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 four of these ethics-based workshops.

As a student in 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 each year. 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 highly confident that my thesis mentor, Dr. Alan Wells, who has run a VA merit program and NIH grant supported laboratory investigating tumor progression for over twenty years, will provide both intellectual and professional support to further 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 training.

Under Dr. Wells's guidance, I will learn the necessary skills to design and execute future research projects independently with the goal of establishing my own NIH-funded lab. The research proposed by the training plan herein will develop proficiency with cell culture (both traditional and as a part of the liver MPS), cell and tissue engineering, and a variety of imaging, biochemical, 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 proposals (for my comprehensives and thesis milestones), manuscripts, abstracts, posters, and presentations. I will develop expertise in data analysis and statistical methods, facilitated by the high throughput of the liver MPS system and mentorship from Dr. Dan Normolle, director of the biostatistics program at the University of Pittsburgh Cancer Institute.

It is the practice of the Wells lab to attend two scientific meetings per year 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 and network with scientists in my field of study. 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. Specifically, I will spend time with Drs. Wallis Marsh and Gary Gruen. Dr. Wallis Marsh is a well renowned hepatobiliary surgeon who trained with Dr. Thomas Starzl while he was pioneering liver transplantation. Dr. Marsh's clinical team is responsible for harvesting the tissue from which the cells for my experiments are isolated. Dr. Gary Gruen is a leader in the field of orthopedic trauma surgery who has a longstanding record for mentoring medical students. From these two outstanding clinical mentors, I will foster surgical knowledge and skills essential for my future career goals as a surgeon-scientist. My research involvement during my last two years of medical school will focus on finalizing research related to my dissertation and beginning 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.

Building skills essential to teaching will also be provided during this fellowship with my experience in 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 gradated responsibilities and assignments accrue, I will become more independent, while in a supportive environment that will provide continued feedback for improvement. 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.

C.8. ACTIVITIES PLANNED UNDER THIS FELLOWSHIP

Funding Year	Program Year	Research	Clinical	Coursework	Other
1	G2	80%	9%	10%	1%
2	G3	85%	9%	5%	1%
3	G4	89%	9%	1%	1%
4	MS3	10%	85%	0%	5%
5	MS4	40%	55%	0%	5%

Second, Third, and Fourth Years of Graduate School (Fellowship Years 1 through 3):

Research: In allocating the majority of my time in the lab during the first three years of my fellowship, 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 meeting, 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 and to collaborate with Dr. Linda Griffith at MIT on the 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 with researchers 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. Saleem Khan, 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 Grand Rounds and surgical workshops, as my graduate school commitments allow. 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 to facilitate my transition into my last two years of medical school and supplement my 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 “Cancer Biology and Therapeutics” and “Extracellular Matrix in Tissue Biology and Bioengineering” during the fall and spring of my 1st graduate year respectively, with additional courses in years two and three. I took “Multiparametric Microscopic Imaging” in the summer of 2014 and will complete my final requirement for that class (a project utilizing multiple imaging technologies to answer a hypothesis) during the summer after my 1st graduate year.

Other: I will dedicate time to committee involvement in the pathology and MSTP programs. I am currently on the MSTP student interviewing committee.

Third and Fourth Years of Medical School (Fellowship Years 4 and 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 before finishing the program. Finalizing any manuscripts or revisions should not be an issue as demonstrated by my participation in finalizing a paper last fall with Dr. Oltvai’s group amidst medical school coursework. The MSTP offers a 5 month mini-post-doc during the second half of the 4th year of medical school, which I will pursue after finishing my medical school clinical training.

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, in particular surgical oncology, during my 4th year.

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, just starting graduate school in July, 2015 after finishing the first two years of predominantly clinical coursework. The progress made during my rotation with Dr. Wells last summer, including the selection of a project that will be my dissertation topic, allowed me to design and submit this proposal during December of my second year of medical school. My resubmitted proposal is enclosed. Here I will describe other research experiences. Before joining the 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 that 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 reliable 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 IFNγ 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, lead 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, on which I am third author, was published in *Scientific Reports* in December, 2014. 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 Colin 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 students as major advisor, 7 pre-doctoral 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 is only current MSTP student in the laboratory since joining officially in July, 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: The productive publication records during training evince a laboratory emphasis on communication and publication that Colin will follow.

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 a 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 Therapuetics, 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.

STATISTICS TRAINING

Most of the projects in the Wells lab are highly quantitative, collaborating with engineers and computational and systems biologists. Colin's project is no exception, as we are exploring a highly quantitative question of oxygen-carrying capacity and mass transfer of oxygen. In addition, the multitude of effects of altered oxygen levels, and drugs (on both the liver tissue and the cancer, with liver tissue effects then indirectly impacting the cancer cells) will require greater attention to the statistical handling of the data. To ensure proper analysis and training for Colin, we have recruited to the team Dr. Dan Normolle, director of the biostatistics facility at the University of Pittsburgh Cancer Institute, who has trained PhD and MD-PhD students since 1993 and has been involved with data analysis from *in vitro*, *ex vivo*, and *in vivo* studies of gastrointestinal cancers, including the liver, for over twenty years.

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 Medicince (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. This paper has been published in *Scientific Reports*.

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.