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.