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14. ABSTRACT

More women die from the estrogen receptor positive (ER+) breast cancer subtype than from any other. The proportion of early ER+ recurrences (=5 years since diagnosis) approaches that for all triple-negative breast cancers alone. Late recurrences (>5 years after diagnosis), the result of dormancy, are most common in ER+ disease and can arise decades after the initial diagnosis. Since recurrent breast cancers have escaped the effects of endocrine therapies, and are lethal, we will study endocrine resistance (Tamoxifen; Fulvestrant). Our primary objective is to identify what drives breast cancer growth and determine how to stop it. We will learn about why some breast cancers are aggressive and others are indolent, and why/how some breast cancers lay dormant for years and then re-emerge.

15. SUBJECT TERMS
Breast cancer, drug resistance, admixing, ecology, multiscale modeling

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1. Introduction

~70% of newly diagnosed breast cancers are ER+ [1]. Many of these women die because metastatic ER+ disease becomes treatment resistant. Resistance is multiscale, i.e., evident at many levels, with genetic, cellular, and phenotypic features (including intratumor heterogeneity; ITH), all are molecularly manifested, and functionally realized, as networked changes in the transcriptome and proteome. We will take a systems biology approach to portray the proteome and transcriptome topology of treatment-induced adaptive remodeling of cell admixtures in vitro and in vivo. Overarching goals are to understand the principles of this remodeling and uncover the mechanisms that confer endocrine resistance in breast cancer, leading to new treatment strategies.

2. Keywords

Drug resistance, admixing, ecology, multiscale modeling

3. Accomplishments

A) Major goals (and related subtasks) of the project from approved SOW:

SPECIFIC AIM 1 (specified in proposal)	Timeline	Site 1	Site 2	Percent complete	Date completed (if 100%)
Major Task 1 (Aim 1a)	Months				
Subtask 1: Determine prevalence of **R** and/or **P** cells in **S** populations	1-6	Dr. Clarke Dr. Sengupta			
Subtask 2: Determine the effects of different **S**:**R** ratios on response to TAM and ICI in MCF-7, LCC, T47D, and ZR-75-1 **S** and **R** matched cell models *in vitro*	1-18	Dr. Clarke Dr. Sengupta			
Milestone(s) Achieved: Identified effects of **S**:**R** ratio on responsiveness to TAM and ICI *in vitro* in multiple breast cancer cell models and identified optimal admix ratios for *in vivo* studies					
Local IACUC approval (annual renewal required only – approval for studies already in place)	1	Dr. Clarke			
Local IRB approval (add this award as an exemption for use of existing data – no new clinical data will be generated in this BT#2)	1	Dr. Clarke			
Subtask 3: Determine the effects of different **S**:**R** ratios on response to TAM and ICI in **S**:**R** matched cell models *in vivo* (models and admixes guided by the optimal* results in Major Task 1/Subtask 2) It is difficult to provide direct numbers until the *in vitro* work is completed. A standard design for a single would include the following (n=15/group as in application): R cells alone ± ICI (15+15=30) S cells alone ± ICI (15+15=30) R+S cells at a single ration ± ICI (15+15=30) Total = 90/experiment We may do 3 such experiments over the 18-month period for 270 mice.	6-24	Dr. Clarke Dr. Sengupta			
Milestone(s) Achieved: Identified effects of **S**:**R** ratio on responsiveness to TAM and ICI *in vivo*					
Major Task 2 (Aim 1b)					
Subtask 1: Determine the role of GJIC in the ability of **R** to make **S** cells resistant to TAM and ICI in MCF-7, LCC, T47D and ZR-75-1 matched cell models *in vitro* (guided by the optimal experimental conditions from Aim 1a)	6-18	Dr. Clarke Dr. Sengupta			
Subtask 2: Determine the role of microvesicles and protein secretion	6-18	Dr. Clarke Dr. Sengupta			

(transwell) in the ability of R to make S cells resistant to TAM and ICI in MCF-7, LCC, T47D and ZR-75-1 matched cell models *in vitro* (informed by the optimal experimental conditions identified in Aim 1a)					
Milestone(s) Achieved: Identified role GJIC, microvesicles and protein secretion (transwell) in the ability of **R** to make **S** cells resistant to drug and how this is affected by different **S**:**R** ratios. Identified conditions to allow design and execution of *in vivo* studies with guggulsterone and/or GW4869 (experiments will be done if supported by data and if time permits)					
Subtask 3: Collect and store materials (*e.g.,* cell lysates) from optimal conditions for omics studies in Aim 2	1-24	Dr. Clarke Dr. Sengupta			
SPECIFIC AIM 2 (specified in proposal)	**Timeline**	**Site 1**	**Site 2**		
Major Task 3 (Aim 2a)					
Subtask 1: Collect RNA and protein from the materials stored from Aim 1a (this will be done as the optimal experiments are identified above)	1-24	Dr. Clarke Dr. Sengupta	Dr. Wang	50% Site 2	
Subtask 2: Perform array and proteome data collection, processing of raw data from Major Task 3/Subtask 1 (above), and *initial* analyses (*e.g.,* CAM, kDDN)	1-24	Dr. Clarke Dr. Sengupta	Dr. Wang	50% Site 2	
Milestone(s) Achieved: Create initial signaling maps of what is communicated by **R** to **S** to confer resistance and how this is affected by different **S**:**R** ratios					
Major Task 4 (Aim 2b)					
Subtask 1: Build initial mathematical models of cell population remodeling dynamics (*in vitro* and *in vivo* data)	4-24	Dr. Bansal			
Subtask 2: Build final mathematical models of cell population remodeling dynamics (*in vitro* and *in vivo* data)	24-36	Dr. Bansal			
Milestone(s) Achieved: Identified how endocrine therapies and the starting ratios of **S**:**R** cells affects population responses to treatment					
Major Task 5 (Aim 2c)					
Subtask 1: Use the data from Aims 1 and 2 to design and execute novel drug combination and scheduling studies *in silico* (mathematical modeling), *e.g.,* ICI+DNMTi	18-36	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	
Subtask 2: Design and execute novel drug combination and scheduling studies *in vitro* using the predictions in Major Task 5/Subtask 1	18-36	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	
Milestone(s) Achieved: Identified novel optimized (activity vs. toxicity) combination regimens *in vitro*.					
Subtask 3: A small number of predictions from the *in vitro* modeling in Major Task 5/Subtask 2 will be tested *in vivo* (we anticipate completing ~5 such animal studies)	18-36	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	

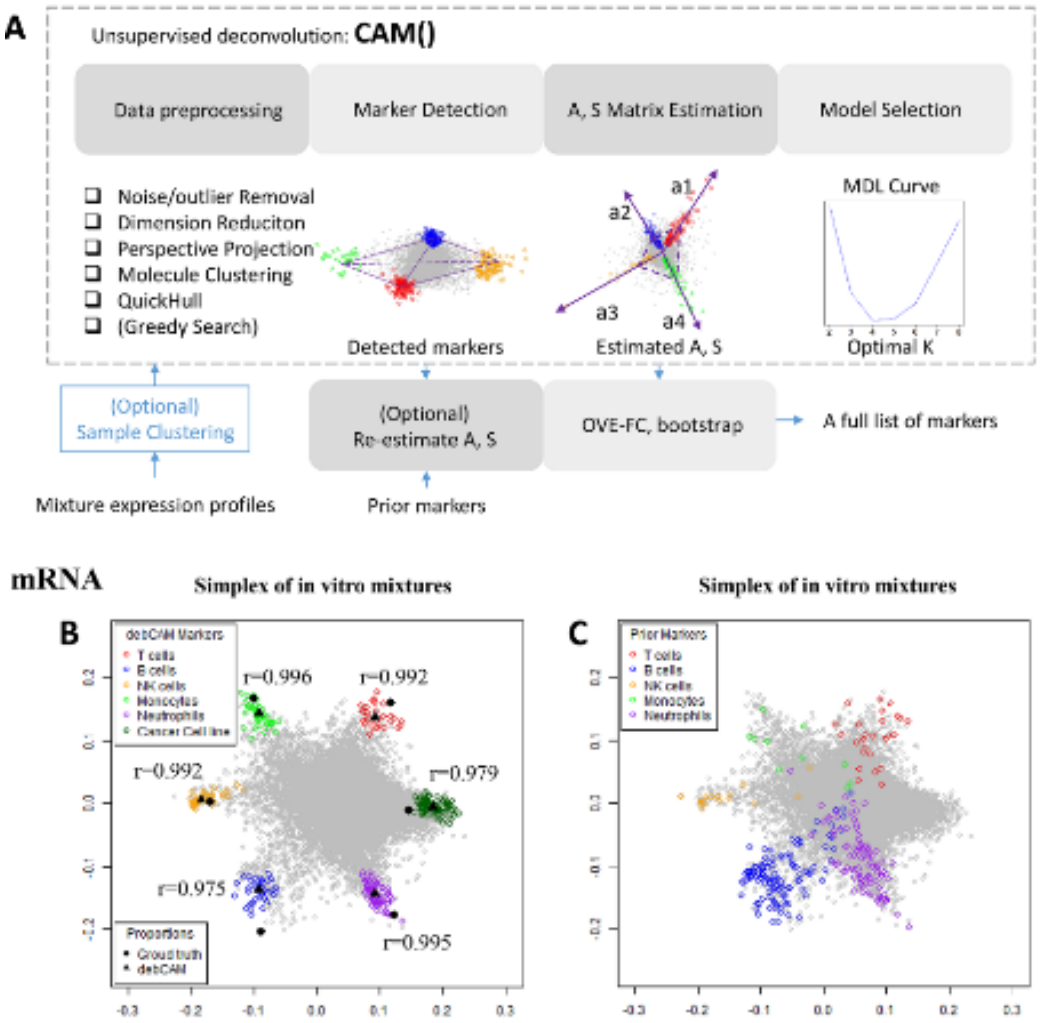
It is difficult to provide direct numbers until the *in vitro* work is completed. A standard design for a single would include the following (n=15/group as in application): R cells alone + Vehicle (15) S cells alone + Vehicle (15) R cells alone + Drug A and + Drug B (15+15=30) S cells alone + Drug A and + Drug B (15+15=30) R+S cells at a single ratio with Vehicle, + Drug A and + Drug B (15+15+15=45) Total = 135/experiment We may do 4 such experiments over the funding period (n=540 maximum number mice).					
Milestone(s) Achieved: Identified novel optimized (activity vs. toxicity) combination regimens *in vivo*.					
SPECIFIC AIM 3 (specified in proposal)	**Timeline**	**Site 1**	**Site 2**		
Major Task 6 (Aim 3a)					
Subtask 1: Initial CAM and kDDN modeling of microarray data from human tumors (public and in-house datasets); data will be fed back to Aim 2 to increase clinical relevance	1-12		Dr. Wang	100% Site 2	8/31/19
Subtask 2: Update models using outcomes from Aim 2 and study if candidate molecules from Aim 2 are associated with clinical outcome (univariate and multivariate)	12-36	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	
Milestone(s) Achieved: Identified clinically relevant molecules associated with ITH and endocrine resistance					
Subtask 4: A small number of predictions from the *in vitro* modeling in Major Task 5/Subtask 3 will be tested *in vivo* (~5 such experiments will be done) A small number of predictions from the in vitro modeling in Major Task 5/Subtask 2 will be tested in vivo (we anticipate completing ~5 such animal studies) It is difficult to provide direct numbers until the in vitro work is completed. A standard design for a single would include the following (n=15/group as in application): R cells alone ± Drug A (15+15=30) S cells alone ± Drug A (15+15=30) R cells alone ± Drug B (15+15=30) S cells alone ± Drug B (15+15=30)	18-36	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	

R+S cells at a single ratio + Drug A + Drug B (15+15=30) Total = 150/experiment We may do 3-5 such experiments over the funding period (n=750 maximum number mice).					
Milestone(s) Achieved: Identified novel therapeutic strategies for ER+ breast cancer to prevent, delay or reverse resistance, and do so within minimized toxicity. These insights could be used to design clinical trials to be done outside this research program.					
Major Task 7 (Aim 3b)					
Subtask 1: Test candidate molecules from the model predictions in Aims 2 and 3a. For example, as described in the narrative section, genes upregulated in resistant cells relative to sensitive cells will be overexpressed (cDNA; regulable and/or constitutive promoters) in sensitive cells and knocked down in resistant (RNAi) if their mRNA or protein is still present in sensitive cells. The gene will be knocked out (CRISPR) in resistant cells if the gene is known to be lost or expression is undetectable in sensitive cells. The reverse experiments will be done where a gene is down regulated or lost in resistant cells relative to its expression/presence in sensitive cells.	12-36 months	Dr. Clarke Dr. Sengupta Dr. Bansal	Dr. Wang	10% Site 2	
Milestone(s) Achieved: Identified mechanistically relevant molecules associated with ITH and endocrine resistance					

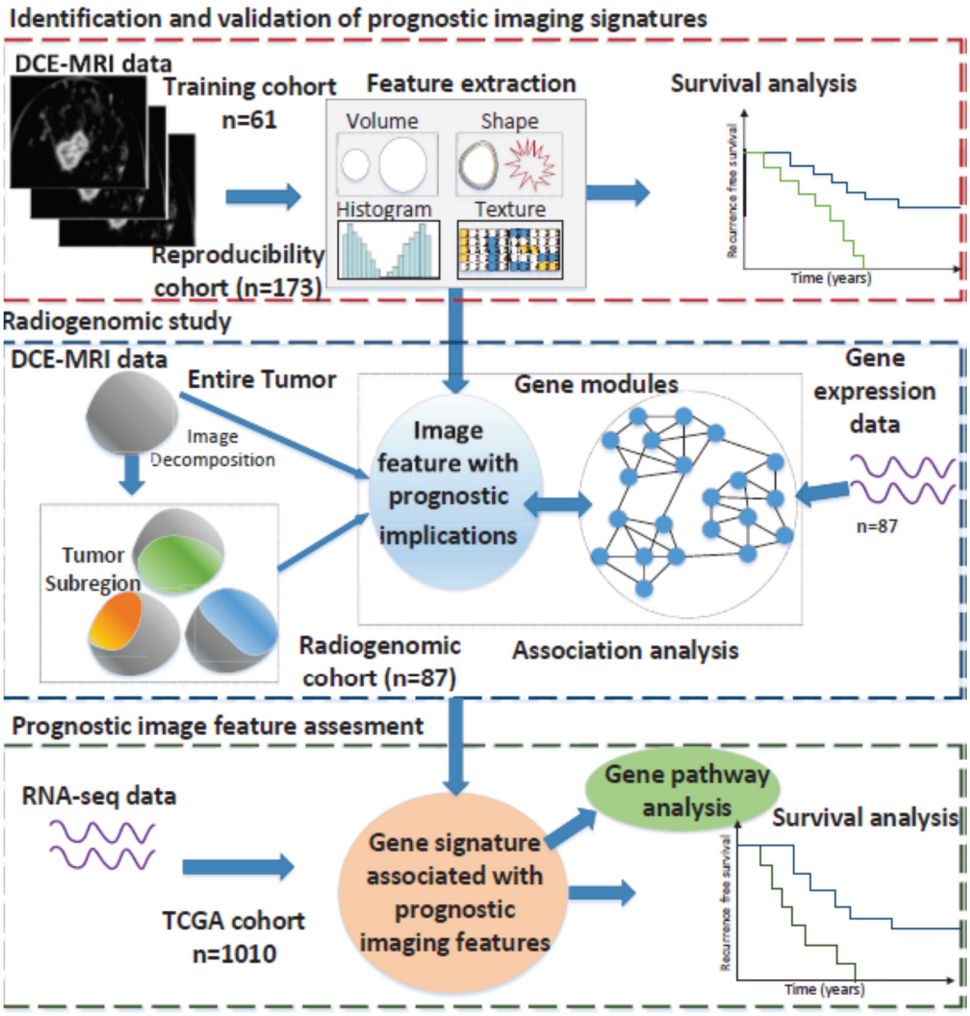
B) What was accomplished under these goals?

Intratumor heterogeneity serves as both, an underexploited information source in characterizing complex tumor phenotypes, and a major confounding factor in studying individual subclones. Having analytic tools to define the molecular landscape of intratumor heterogeneity, and to determine how subclones are remodeled with phenotypic transitions, will be essential in the proposed research. We develop a fully unsupervised deconvolution method to dissect complex tumors into molecularly distinctive subclones based on bulk expression profiles. We implement an R package, deconvolution by Convex Analysis of Mixtures (debCAM) that can automatically detect subclone-specific markers, determine the number of constituent subclones, calculate subclone proportions in individual samples, and estimate subclone-specific expression profiles, **Fig. 1A**. We demonstrate the performance and biomedical utility of debCAM on gene expression, methylation, proteomics, and imaging data. With enhanced data preprocessing and prior knowledge incorporation, debCAM software tool will allow biologists to perform a more comprehensive and unbiased characterization of intratumor heterogeneity and remodeling in many biomedical contexts (e.g., cell-cell communications).

We have tested debCAM on biologically mixed gene expression profiles involving both cancer subclone and immune cell subtypes (GSE64385). The 12 samples represent the mixtures of five immune cell subtypes and one cancer cell line in various known proportions. The optimal scatter simplex blindly identified by debCAM is given in **Fig. 1B**, showing six distinctive vertices (**Fig. 1B**). To validate the accuracy of subtype-specific markers blindly detected by debCAM, we superimpose the color-coded known markers in the scatter simplex showing close proximity to the corresponding vertices (**Fig. 1C**, the cancer cell line specific markers are unavailable for comparison). More convincingly, the sample-wise subtype proportions estimated by debCAM match the ground truth almost perfectly, with r = 0.975~0.996.



We further performed radiogenomics feature based deconvolution of intratumor heterogeneity on breast cancer. The multiscale data are generated from TCGA cohort (n=87), covering DCE-MRI (TCGA-BRCA) and gene expressions (TCGA). The MRI tumour area was decomposed by debCAM-CM, resulting in three microvasculature compartments that represent plasma input, fast-flow kinetics and slow-flow kinetics. The prognostic MRI features were associated with the gene expression modules of cancer-related pathways. Furthermore, a multigene signature for each prognostic imaging feature was established (p=0.035), and the prognostic value for overall survival was confirmed in an additional cohort from TCGA (p=0.027).



Regulatory rewiring of molecular networks represents an important information source in characterizing complex diseases with phenotypic transitions. We have developed one of the first few data-driven and knowledge-fused differential network analysis methods, namely, Differential Dependency Network (DDN), to detect statistically significant coupling or uncoupling between pairs of molecular entities that are dependent on the specific phenotypes or experimental conditions. We have conducted some pilot DDN analyses on the multi-omics data on ovarian cancer. The DDN analysis identifies in a complex and often unknown overall molecular circuitry a network of differentially connected molecular entities. The subnetwork of hub molecular entities is used to assist in the inference of potential key pathways, and to generate novel hypotheses for further validation and investigations.

For selected pathways, DDN analysis on MAPK signaling pathway also highlighted AKT gene as one of the hub genes pivotal in the network rewiring events. AKT is modulated by PTEN mutation which is one of the key definers of HRD status. DDN method is used as an integrated analysis tool to detect rewiring events in KSR between HRD and non-HRD groups on the LC-MS/MS quantitated expression of kinases and HuProt array-based activity quantitation of substrates. Two kinases PTK2 and PTK2B linked with significant rewiring events were found dysregulated in ovarian cancer in cell line models.

C) What opportunities for training and professional development has the project provided?

Two doctoral graduate research assistants are supported in part by this project. In return, they have also contributed to developing data analytics tools and pipelines.

D) How were the results disseminated to communities of interest?

debCAM: a Bioconductor R package for fully unsupervised deconvolution of intratumor heterogeneity, has been disseminated. Availability and implementation: http://bioconductor.org/packages/debCAM

DDN availability and Implementation: Open-source DDN Java code with compiled package are freely available for download at http://apps.cytoscape.org/apps/kddn

E) What do you plan to do during the next reporting period to accomplish the goals?

We will apply both debCAM and DDN tools to analyze the data generated in-house at Georgetown University Medical Center site.

We will jointly interpret the results and develop sensible hypotheses.

We will revise the analytics experimental designs toward more focused testing on the hypotheses.

We will further improve the data analytics tools by incorporating the feedback from GUMC team.

4. Impact

Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

The debCAM R package implements and tests the latest functionalities of the debCAM algorithm pipeline in the literature. We have demonstrated real biomedical utilities of debCAM tool on gene expression, proteomics, methylation, and imaging data. These applications have led to novel findings and hypotheses. The present software also enhances data preprocessing, accelerates robust scatter simplex identification, and integrates supervising information such as known markers.

What was the impact on other disciplines?

Both debCAM and DDN tools can have broad applications in data sciences, engineering, and geosciences.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

5. Changes/Problems

Nothing to Report.

Changes in approach and reasons for change

N/A.

Actual or anticipated problems or delays and actions or plans to resolve them

N/A.

Changes that had a significant impact on expenditures

N/A.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

N/A.

Significant changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

6. Products

Journal publications.

[1] Chia-Hsiang Lin, Ruiyuan Wu, Wing-Kin Ma, Chong-Yung Chi, and Yue Wang, "Maximum Volume Inscribed Ellipsoid: A New Simplex-Structured Matrix Factorization Framework via Facet Enumeration and Convex Optimization," ***SIAM Journal on Imaging Sciences***, vol. 11, no. 2, pp1651-1679, 2018.

[2] Jun Ruan, Zhen Liu, Ming Sun, Yue Wang, Junqiu Yue, and Guoqiang Yu, “DBS: a fast and informative segmentation algorithm for DNA copy number analysis,” ***BMC Bioinformatics***, 20:1, 2019. https://doi.org/10.1186/s12859-018-2565-8

[3] Ming Fan, Peng Zhang, Yue Wang, Weijun Peng, Shiwei Wang, Xin Gao, Maosheng Xu, Lihua Li, “Radiomic analysis of imaging heterogeneity in tumours and the surrounding parenchyma based on unsupervised decomposition of DCE-MRI for predicting molecular subtypes of breast cancer,” ***European Radiology***, https://doi.org/10.1007/s00330-018-5891-3.

[4] Ming Fan, Pingping Xia, Bin Liu, Lin Zhang, Yue Wng, Xin Gao, Lihua Li, “Tumour Heterogeneity Revealed by Unsupervised Decomposition of Dynamic Contrast-Enhanced Magnetic Resonance Imaging is Associated with Underlying Gene Expression Patterns and Poor Survival in Breast Cancer Patients,” ***Brest Cancer Research***, 2019. (accepted)

[5] Robert Clarke, John J Tyson, Ming Tan, William T Baumann, Lu Jin, Jianhua Xuan, and Yue Wang, “Systems biology: perspectives on multiscale modeling in research on endocrine-related cancers,” ***Endocrine-Related Cancer***, 2019. (accepted)

Books or other non-periodical, one-time publications.

[1] Niya Wang, Lulu Chen, and Yue Wang, "Mathematical modelling and deconvolution of molecular heterogeneity identifies novel subpopulations in complex tissues", Book Chapter, ***Transcriptome Data Analysis: Methods and Protocols***, Springer, 2018.

Other publications, conference papers, and presentations.

N/A.

Website(s) or other Internet site(s)

N/A.

Technologies or techniques

N/A.

Inventions, patent applications, and/or licenses

N/A.

Other Products

Software: debCAM, DDN.

7. Participants & Other Collaborating Organizations

Name: Yue Wang
Project Role: Principal Investigator (Partnering PI)
Research Identifier (ORCID ID):
Nearest person months worked: 1.0
Contribution to project: Designed data analytics methodology

Name: Lulu Chen
Project Role: Graduate Research Assistant
Nearest person months worked: 6.0
Contribution to project: Developed data deconvolution tool debCAM

Name: Minta Lu
Project Role: Graduate Research Assistant
Nearest person months worked: 6.0
Contribution to project: Developed integrated pathway prioritization tool IPP

Has there been a change in the active other support of the PI or senior personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
Nothing to report (aside from Partnering PI institution, Virginia Tech).

8. Special Reporting Requirements

The Initiating PI (Robert Clarke, PhD) has submitted an independent annual report for this period.

9. Appendices