AACR American Association for Cancer Research

FINDING CURES TOGETHER℠

INTERIM PROGRESS REPORT **Rev. 10/2014**	☒ **6 Month Report** ☐ **18 Month Report** ☐ **30 Month Report** ☐ **42 Month Report**
Grant Name: AACR-Triple Negative Breast Cancer Foundation-Carol's Crusade for a Cure Foundation Career Development Award for Metastatic Triple Negative Breast Cancer Research	
AACR Grant No.: 16-20-43-CURT	**Grant Term:** 07/01/2016-06/30/2019
Title of Project: Delineating the dynamics of triple negative breast cancer metastasis	
Grantee Percent Effort on Research Project: 10%	
Grantee Name: Christina Curtis	**Institution Name: Stanford University**
Human Subjects: ☒Yes ☐No Human Assurance Number: FWA00000935 Research Exempt? ☐Yes ☒No IRB status: approved IRB Date: 06/08/2016	Animal Subjects: ☐Yes ☒No Animal Consent: ☐Yes ☒No IACUC approval date: Click here to enter text. Animal Welfare Assurance No.: Click here to enter text.
Are biospecimens being used? ☒ Yes ☐No If yes, please check the type(s) used in this project: ☒ Tissue ☐ Cell Lines ☐ Plasma ☐ Serum ☐ Other, please specify Click here to enter text.	
GRANTEE CERTIFICATION: I hereby certify that the statements herein are true and accurate to the best of my knowledge. GRANTEE SIGNATURE: Christina Curtis DATE: 1/31/2017	

I. Description of Report Period's Activities: Briefly describe any progress toward project milestones during this reporting period. Do not repeat any progress from previous reports. Figures and/or tables are required and should be referenced herein. Information presented in this section will be considered confidential.

During this reporting period we have made steady progress towards our overarching goal of delineating the evolutionary dynamics of triple negative breast cancer (TNBC) metastasis within the three year grant period. To date, we have focused on Aim 1 which seeks to characterize spatio-temporal genomic heterogeneity in primary TNBCs and their paired metastases towards quantification of the underlying evolutionary dynamics. While it is appreciated that clonal diversification and selection underlie tumor progression, the dynamics of this process remain poorly understood and require a quantitative evolutionary and population genetic framework. To address this outstanding question, we build on our previous description of a spatial computational model of primary tumor growth and Bayesian statistical inference framework (*Nature Genetics*, 2015), which enables the inference of patient-specific tumor parameters such as the mutation rate and subclone fitness differences from multi-region sequencing (MRS). In particular, we have now extended our framework to incorporate additional parameters such as deme (subpopulation) size and different levels of selection, while simulating realistically sized '*virtual tumors*' composed of 10s of billions of cells. By exploiting summary statistics derived from MRS, we have

developed an approach to classify tumors according to different modes of evolution, namely effectively neutral evolution versus selection (**Figure 1**) (Sun et al, in revision). We have applied this new tool to diverse solid tumors for which MRS data was available, demonstrating its broad utility for diverse tumor types. Hence, this approach is now readily applicable to the TNBC MRS data being generated within this proposal and will provide novel insights into the mode of primary TNBC evolution with fundamental implications for the drivers of this process and metastatic progression.

Second, we have pioneered a novel computational and theoretical framework to infer the timing of metastatic divergence from MRS of paired primaries and metastases. Importantly, we have made this approach more generalizable by accounting for the mode of primary tumor growth based on the above classification and have demonstrated the power of this approach to quantify the timing of metastasis by applying this to an existing cohort of paired primaries and metastases. Thus, we anticipate being able to readily apply this framework to to the TNBC genomic data that are being generated.

Third, to enable the sensitive detection of subclonal variants from MRS data, as directly relevant to this project, we have developed a custom bioinformatics toolkit (Sun et al, in revision). This new method significantly improves over current approaches (designed for single sample data) such as *Mutect* and will aid the detection of low variant allele frequency (VAF) private and shared somatic single nucleotide variants (SSNVs), which in turn, inform the mode of primary tumor growth and metastatic divergence.

We have also progressed in terms of the review and processing of the TNBC cohort. In particular, we have performed chart review of the TNBC cohort to ensure the comprehensive annotation of clinical and treatment information for these cases. We have focused on a subset of 11 TNBC cases, 9 of whom had involved nodes at diagnosis that can be sampled in addition to paired distant metastases. The metastases derive from the following sites: lung (n=5), skin (n=3), distant mediastinal lymph node (n=1), bone (n=1), pleura (n=1). For all cases, treatment information is known for 7 cases a treatment naïve primary tumor specimen is available, several of which include a paired pre and post-treatment primary tumor biopsy. Hence, we have assembled the necessary clinical information to enable robust analysis of the evolutionary dynamics of metastasis. We have also been able to identify additional TNBC cases with metastatic disease through the Oncoshare database and will evaluate the suitability of these tissue specimens as a validation set. We continue to work on microdissection, nucleic acid extraction, library generation and sequencing of these cases.

Given the importance of properly classifying the mode of primary TNBC growth for our inferences, we sought to evaluate whether this classification is equivalent irrespective of whether WGS or WES data is employed as we observed for other tumors (**Figure 1**). To this end, we performed multi-region WGS of a four regions of a primary TNBC with matched lymph node metastasis. Although analysis is still underway, it is evident from the MRS data that different tumor regions exhibit intra-tumor heterogeneity (ITH) at the copy number level (**Figure 2**), highlighting the crucial need for multiple sampling in TNBC. Ongoing analyses will evaluate the extent of ITH between different tumor regions at the mutational and copy number levels and the impact of greater numbers of samples on the inference of patient-specific parameters.

Finally, through our continued analyses of the METABRIC cohort of 2,000 breast tumors (*Nature*, 2012) for which long-term clinical outcomes are available, we will define molecular features associated with distant metastasis in TNBC. This orthogonal approach will complement our detailed analysis of the paired primary and metastatic archival cohort.

In ongoing work we will continue to generate and analyze the genomic data to define the landscape of somatic alterations in metastatic TNBC. The systematic comparison of primary/metastasis pairs will delineate both the functional drivers of this process and the underlying dynamics.

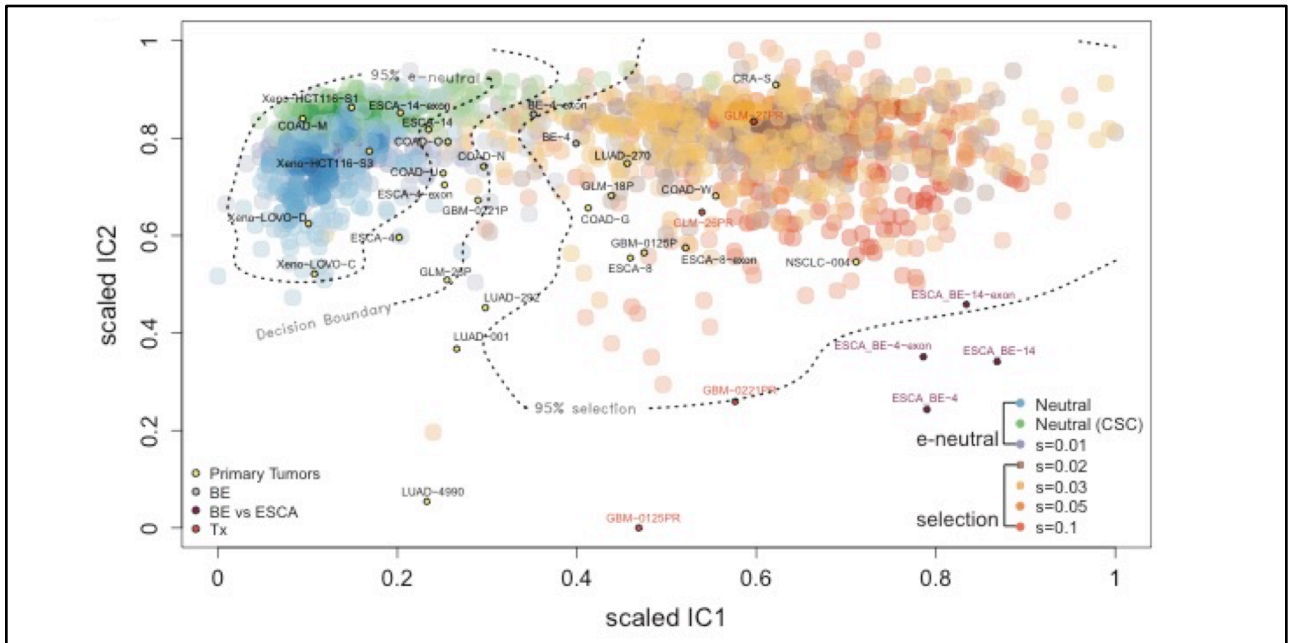

Figure 1. **Classification of distinct modes of tumor evolution from multi-region sequencing.** Independent component analysis (ICA) of simulated and real tumors based on the five aforementioned features. The independent components clearly separate the simulated tumors (large transparent colored circles) under *effectively (e) neutral growth* (neutral, neutral (CSC) and weak selection, s=0.01) versus *positive selection* (moderate-s=0.02-0.1). Here 200 tumors were simulated under each model. The decision boundary for an SVM trained on the two IC components based on the simulated tumors is indicated by dashed line, where countours corresponding to the 95% probability of being classified as effectively neutral (*e-neutral*) or under selection are shown. Small circles corresponding to patient samples are labeled by their corresponding sample ID, and color-coded according to the nature of the sample, as indicated in the legend.

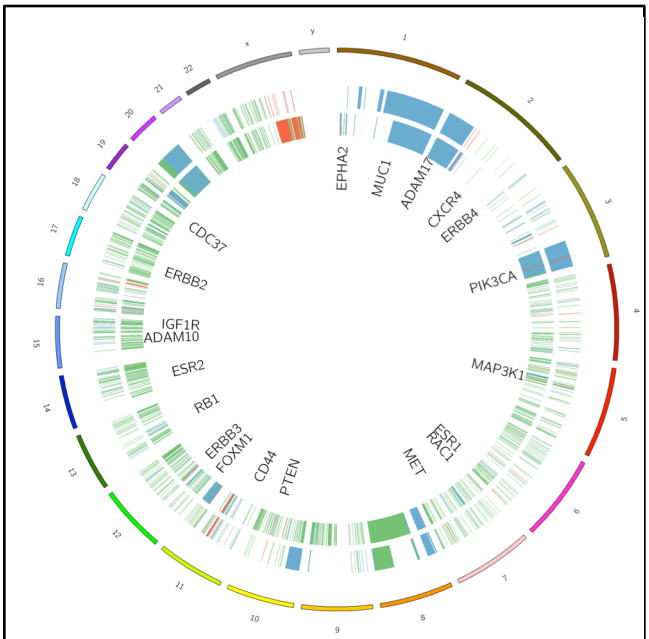

Figure 2. **Multi-region sequencing of two sectors of a primary TNBC reveals intra-tumor copy number heterogeneity.** Circos plot illustrating somatic copy number alterations (CNAs) in Region 1 (outer circle) and Region 2 (inner circle) of a primary TNBC, where blue indicates gain (CN=3), green indicates amplification (CN>=4), and red indicates deletion.

II. **<u>Modifications:</u> Are you proposing any modifications to the original Research Project plan or anticipating changes to the research design or specific aims?**

☐Yes ☑No

If yes, discuss these changes and the factors contributing to the need for adjustments to the project plan. Detailed justification and approval from AACR are necessary for any change in specific aims.

III. **<u>Additional Funding:</u> Have you received any additional funding during this reporting period to support this Research Project?**

☐Yes ☑No

If yes, please complete the table below and list any additional funding support.

Role	Name of Grant/Funding Source	Title of Project	Funding Agency	Grant Term	Amount of Funding	Percent Effort	List of Specific Aims as Stated in Grant Proposal (summaries are NOT acceptable)

The Milestones Report and the Financial Report are also due at this time and should be uploaded to the proposalCENTRAL Award Management System as separate documents.

2016 AACR-Triple Negative Breast Cancer Foundation-Carol's Crusade for a Cure Foundation Career Development Award for Metastatic Triple Negative Breast Cancer Research **July 1, 2016 - June 30, 2019**

NAME: Curtis, Christina

Aim 1: Characterizes spatio-temporal genomic heterogeneity in primary TNBCs and their paired metastases and performs the unbiased discovery of metastasis drivers.

Aim 2: Delineates the evolutionary dynamics of TNBC metastasis.

Milestones

The Milestones Template is meant to list the various steps necessary to complete your research goals and the estimated time it will take to complete each step. Please list your name and the specific aims for the proposed project at the top of the template. Underneath each time period, identify the steps that will be needed to accomplish the aim(s) in that time period. For each step, note the corresponding aim in parentheses. Rows may be added/deleted to this template as needed. For the purposes of submitting this template with your application, only the information requested above inputted into Column A is needed. Reporting progress towards milestones further utilizing this template will be incorporated into the biannual reporting requirements for the project if funded. (To view an accurately completed Milestones Template please visit ftp://ftp.aacr.org, username: programs, password: aacr123.)

	M 6						M 12						M 18						M 24						M 30						M 36		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Step Ongoing? Y/N	Step Complete? Y/N
YEAR 1																																						
1 - 6 Months (briefly describe each milestone below)																																					Y	
Pathology review, tissue processing, and nucleic acid extraction from paired primary TNBCs and metastases (Aim 1)	X	X	X	X	X	X	X	X	X	X	X	X																									Y	
Library preparation for WES of TNBC cohort (Aim 1)	X	X	X	X	X	X	X	X	X	X	X	X																									Y	
7 - 12 Months (briefly describe each milestone below)																																						
Multi-region WES of paired primary TNBCs and metastases, batch 1 (Aim 1a)				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X																			Y	
Inference of somatic SNVs and CNAs from WES data using custome pipelines (Aim 1b)							X	X	X	X	X	X	X	X	X	X	X	X																			Y	
YEAR 2																																						
13 - 18 Months (briefly describe each milestone below)																																						
Multi-region WES of paired primary TNBCs and metastases, batch 2 (Aim 1a)																																						
Inference of somatic SNVs and CNAs from WES data using custome pipelines (Aim 1b)																																						
19 - 24 Months (briefly describe each milestone below)																																						
Bioinformatic prioritization of candidate metastasis drivers (Aim 1d)																																						
Reconstruction of TNBCs phylogenies from multi-region WES data (copy number and mutational) (Aim 2a)																																						
YEAR 3																																						
25 - 30 Months (briefly describe each milestone below)																																						
Deep targeted sequencing (TS) of patient-specific alterations in multiple specimens from paired primary TNBCs and metastases (Aim 1c)																																						
Refinement of TNBCs phylogenies from multi-region deep TS data (Aim 2a)																																						
Inference of the evolutionary dynamics of TNBC metastasis within our spatial computational framework (Aim 2b)																																						
31 - 36 Months (briefly describe each milestone below)																																						
Inference of patient-specific parameters (Aim 2b)																																						
Submit manuscript for publication																																						

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FINANCIAL REPORT

				EXPENSES				
Grantee: Christina Curtis **Grantee Institution: Stanford University** **AACR Grant No.: 16-20-43-CURT** **Grant Term: 07/01/2016 - 06/30/2019** **Reporting Period: 07/01/2016 - 12/31/2016**		**(COLUMN A) Total Budget**	**(COLUMN B) Current Budget ***	**(COLUMN C) Actual Previously Reported**	**(COLUMN D) Current Six Month's Actual**	**(COLUMN E) Cumulative Expense (COL. C + D)**	**(COLUMN G) Current Year Balance (COL. B - D)**	**(COLUMN H) Cumulative Balance (COL. A - E)**
				From:7/1/16 **To**:12/31/16	**From**: **To**:			
A. Personnel	1	$116,424	$45,108	$22,131	$0	$22,131	$45,108	$94,293
B. Fringe Benefits	2	$36,936	$13,758	$6,746	$0	$6,746	$13,758	$30,190
Subtotal Personnel Costs**	**3**	**$153,360**	**$58,866**	**$28,877**	**$0**	**$28,877**	**$58,866**	**$124,483**
C. Consultant Costs	4	$0	$0	$0	$0	$0	$0	$0
D. Equipment	5	$0	$0	$0	$0	$0	$0	$0
E. Laboratory Supplies**	6	$6,000	$2,000	$12,765	$0	$12,765	$2,000	($6,765)
F. Animal Care	7	$0	$0	$0	$0	$0	$0	$0
G. Travel	8	$7,500	$2,500	$1,081	$0	$1,081	$2,500	$6,419
H. Patient Care (In-patient)	9	$0	$0	$0	$0	$0	$0	$0
I. Patient Care (Out-patient)	10	$0	$0	$0	$0	$0	$0	$0
J. Consortium/ Contractual: Direct Costs	11	$0	$0	$0	$0	$0	$0	$0
K. Other Expenses**	12	$83,140	$36,340	$0	$0	$0	$36,340	$83,140
Subtotal Direct Cost: Non-Personnel	**13**	**$96,640**	**$40,840**	**$13,846**	**$0**	**$13,846**	**$40,840**	**$82,794**
Subtotal Direct Cost	**14**	**$250,000**	**$99,706**	**$42,723**	**$0**	**$42,723**	**$99,706**	**$207,277**
L. Indirect Cost	15	$0	$0	$0	$0	$0	$0	$0
TOTALS: (Lines 3,14, and 15)	**16**	**$250,000**	**$99,706**	**$42,723**	**$0**	**$42,723**	**$99,706**	**$207,277**
Less: Payments Received from AACR to Date, Including Advances	**17**					$37,500		
Cash on Hand at the End of Period	**18**					**($5,223)**		

Not all budget categories are allowable for all grants. Please review V. Use of Grant Funds. in the fully executed Grant Agreement for details.

* Enter the line item budget amounts. In the second year and, if necessary, all proceeding years, the budget amounts in Column A should reflect the cumulative budget total of all years of the grant term to date. This information should be taken from Exhibit C of your Grant Agreement or the most current executed modification.

** Must be itemized in the indicated space below.

Per V. Use of Grant Funds., in the fully executed Grant Agreement, prior approval from the AACR is required to significantly rebudget. Significant rebudgeting is defined as when expenditures in a single direct cost budget category deviate (increase or decrease) from the categorical commitment level established by the approved budget by more than 20% of the annual grant amount. To obtain prior approval, the Grantee must submit a request in writing, along with a revised budget and budget justification utilizing the templates provided by the AACR.

Itemized List (can add or delete additional rows): Itemized amounts must total Column D amount.

Personnel: Provide name and title (note if individual is a postdoctoral or clinical research fellow) and amounts per individual.

Laboratory Supplies:

Sequencing library preparation	$	1,315.39
Sequencing	$	11,450.00

Other Expenses:

Name of Preparer:	Miguel De Los Santos	**Email: Miguel De Los Santos**	**Phone: 650-725-8141**

Only signed copies of this report will be accepted.

AUTHORIZED SIGNATURE: I hereby certify that the costs incurred (Columns C and D) are identified on the Project Budget, within the budgeted amount estimated for such costs, have been properly recorded in the accounting records of the Project, and are supported by written documentation.

		Research Accountant	**1/31/2017**
		Title	**Date**
/	**Miguel De Los Santos**	migueld@stanford.edu	**650-725-8141**
Authorized Signature	**Typed Name**	**Email**	**Phone**