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Irene Lee, PhD

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Irene Lee, PhD

E-Mail: irenei_lee@dfci.harvard.edu

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Dana-Farber Cancer Institute
450 Brookline Ave
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Androgen Deprivation Therapy (ADT) remains the first-line treatment option for patients with advanced metastatic prostate cancer. However, despite initial responses to ADT, the majority of patients develop resistance to ADT and progress to castration resistant prostate cancer (CRPC). The mechanisms of resistance to ADT are poorly understood and remains an urgent unmet need in prostate cancer research. It has been hypothesized that AR splice variants play a causal role in conferring endocrine resistance in prostate cancer. ARv7 is the most frequently expressed AR splice variant in CRPC and is associated with poor prognosis and resistance to ADT. The overarching goal of this study is to further delineate the role and mechanisms governing ARv7 activity in CRPC. We hypothesize that the use of AR N-terminal disrupting inhibitors may target the ARv7 and demonstrate increased effectiveness in the treatment of CRPC.

15. SUBJECT TERMS
Prostate Cancer, Androgen Receptor, Androgen Receptor variant 7, castration resistant prostate cancer, transcription

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1. Introduction

Androgen Deprivation Therapy (ADT) remains the first-line treatment option for patients with advanced metastatic prostate cancer. However, despite initial responses to ADT, the majority of patients develop resistance to ADT and progress to castration resistant prostate cancer (CRPC). The mechanisms of resistance to ADT are poorly understood and remains an urgent unmet need in prostate cancer research. It has been hypothesized that AR splice variants play a causal role in conferring endocrine resistance in prostate cancer. ARv7 is the most frequently expressed AR splice variant in CRPC and is associated with poor prognosis and resistance to ADT. The ARv7 variant retains the same N-terminal domain and DNA-binding domain (DBD) as the full-length AR, but importantly, lacks the LBD, the intended target for enzalutamide and other AR-targeting inhibitors. The overarching goal of this study is to further delineate the role and mechanisms governing ARv7 activity in CRPC. The biology of ARv7 activity remains poorly understood, and a better understanding of ARv7 activity could be critical in improving therapeutic options for patients with castration resistant prostate cancer.

2. Keywords

Prostate Cancer, Androgen Receptor, Androgen Receptor variant 7, castration resistant prostate cancer, transcription

3. Accomplishments

Major Goals:

Aim 1: Determine the ARv7-dependent essential genes responsible for driving CRPC growth through CRISPR/Cas9 genetic screening.
Aim 2: Determine if EZH2 is required for ARv7-dependent transcriptional repression in CRPC.
Aim 3: Determine if novel AR N-terminal disrupting inhibitors target ARv7 and decrease CRPC tumor growth.

Results

CRISPR Screen Results in shGFP, shARv7, shARfl LN95

In order to identify the key genes downstream of ARv7 that mediate cell proliferation, we performed a genome-wide CRISPR screen in shGFP, shARv7, shARfl LN95 cells. The LN95 model is a CRPC cell line model that expresses ARv7. These cells have been engineered to inducibly express short hairpins directed at either GFP (control), ARv7, or ARfl in the presence of Doxycycline (Figure 1). We then performed the genome-wide CRISPR screen in the presence and absence of Doxycycline (Figure 2). We then compared each +DOX condition to its corresponding -DOX condition to eliminate those genes that are pan essential. From this analysis, we were able to determine those genes that are specifically essential in the shARv7 +DOX and the shARfl +DOX conditions. We then

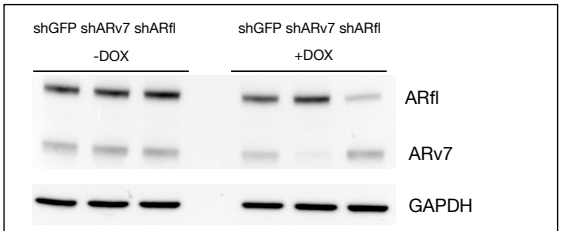

Figure 1. Expression of ARv7, ARfl, and GAPDH in shGFP, shARv7, shARfl LN95 cells.

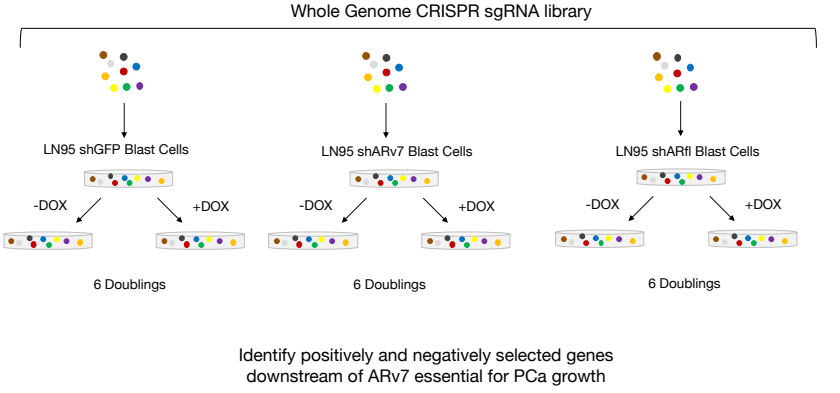

Figure 2. Overview of CRISPR screen.

overlapped these essential genes between the shARv7 and shARfl conditions to identify genes that are specifically essential only in the shARv7 condition, the shARfl condition, and essential in both (Figure 3A). We then performed gene ontology analysis to identify which pathways were enriched (Figure 3B). We identified that oxidative phosphorylation was an essential pathway in the shARv7 only, shARfl only, and overlapping genes. In the shARv7 only essential genes, we identified the Hallmark reactive oxygen species was specifically enriched and in the shARfl only genes we identified the Hallmark androgen response. These pathways may be critical downstream effectors of ARv7 and ARfl. Further validation of target genes and pathways is ongoing. Additionally, we examined known AR cofactors to determine their essentiality in the context of ARv7 and ARfl knockdown. We examined the essentialities of EZH2, FOXA1, GRHL2, and HOXB13, known well-established cofactors of AR. FOXA1 and HOXB13 become more essential in the shARv7 condition, while GRHL2 and EZH2 become less essential in the shARv7 condition (Figure 4). Loss of FOXA1 and HOXB13 could further decrease cell proliferation when ARv7 is knocked down. Concordantly, GRHL2 and EZH2 loss could further increase cell proliferation when ARv7 is knocked down and may suggest that these factors play a role in the ARv7 pathway.

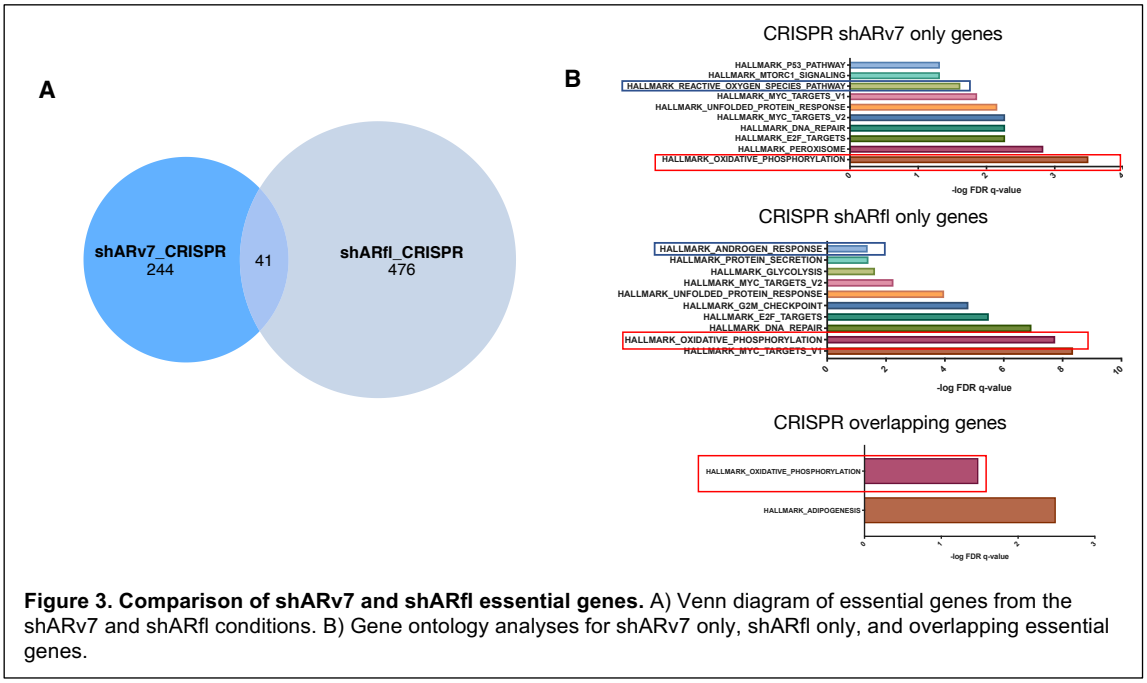

Figure 3. Comparison of shARv7 and shARfl essential genes. A) Venn diagram of essential genes from the shARv7 and shARfl conditions. B) Gene ontology analyses for shARv7 only, shARfl only, and overlapping essential genes.

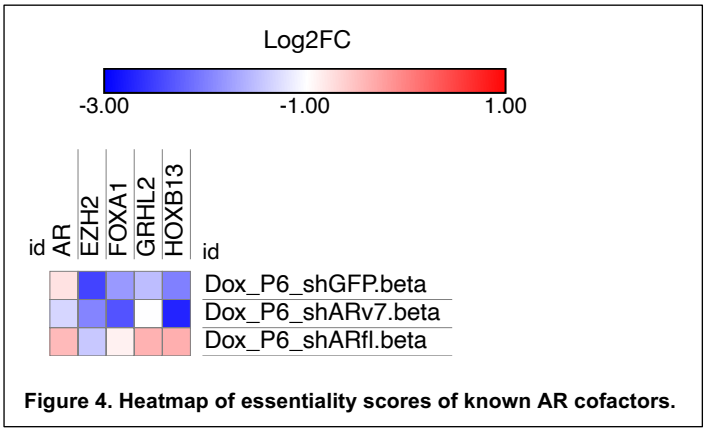

Figure 4. Heatmap of essentiality scores of known AR cofactors.

Defining the ARv7 cistrome

In an effort to better understand the mechanism of action of ARv7, we have defined the ARv7 cistromes in a number of CRPC cell lines: 22RV1, LN95, and VCaP-16. VCaP-16 cells are CRPC cells developed by our collaborator, Dr. Steve Balk, that are resistant to the AR antagonist, enzalutamide. We have performed ChIP-seq of both the ARfl and ARv7 in VCaP-16 cells to better understand the dynamics of AR recruitment to its regulatory regions. Similar to what we have previously observed in LN95, the majority of ARv7 binding sites in VCaP-16 overlap with ARfl binding sites (Figure 5). We then overlapped the VCaP-16 ARv7 binding sites with the cistromes of ARv7 in 22RV1 and LN95 cells, we found that ARv7 binding is quite heterogenous (Figure 6). In the Vehicle treatment group, only 255 binding sites were common among all three cell lines. In each cell line, the majority of ARv7 binding sites were unique to each cell line and not shared. We then performed Cistrome Toolkit GIGGLE similarity analysis which queries publicly available ChIP-seq data and returns datasets that are most similar to the input dataset. We input the unique ARv7 binding sites for each cell line and queried the datasets that are most similar to identify potential members of the ARv7 transcriptional complex (Figure 7). We found that each cell line had unique datasets that were most similar to the ARv7 dataset, potentially indicating that ARv7 is regulated distinctly in each cell line. For each cell line, the AR dataset was either the top or among

the topmost similar datasets. For LN95, CHD1, NKX3.1, and HOXB13 were among the most similar datasets. For 22RV1, SRC1 and SOX2 were among the top-ranking datasets; SOX2 is not a well-known cofactor of AR and it has been implicated in promoting castration resistant prostate cancer and stemness. Finally, for VCaP-16, EZH2 and ERG factors rank highly amongst the most similar datasets; EZH2 and AR is an active area of interest and will be further explored in the future.

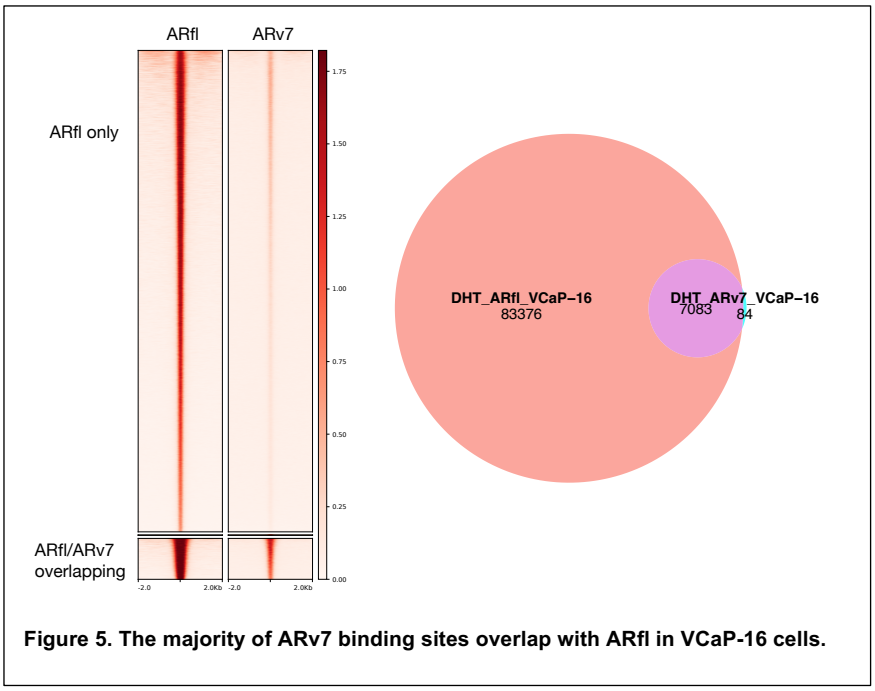

Figure 5. The majority of ARv7 binding sites overlap with ARfl in VCaP-16 cells.

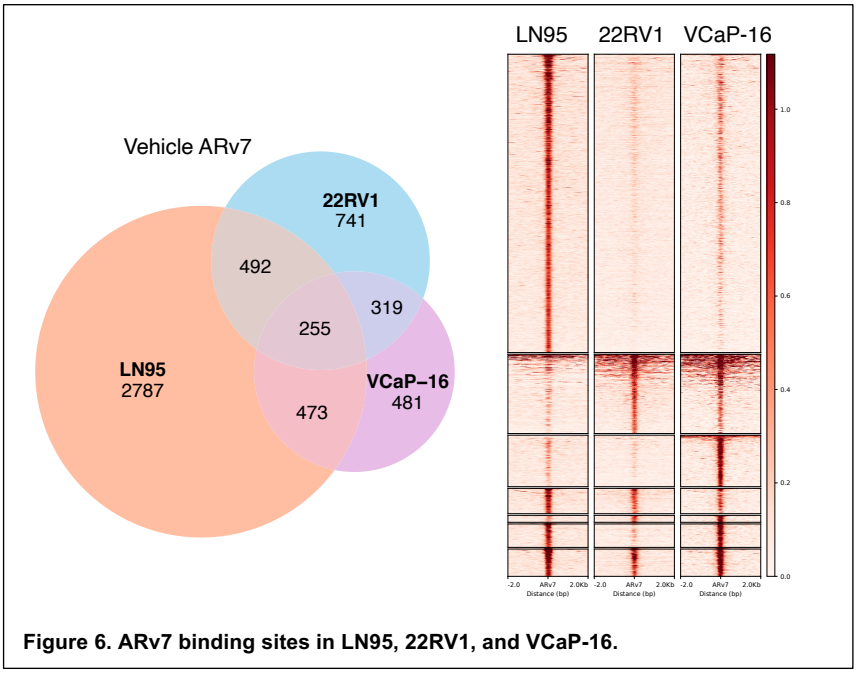

Figure 6. ARv7 binding sites in LN95, 22RV1, and VCaP-16.

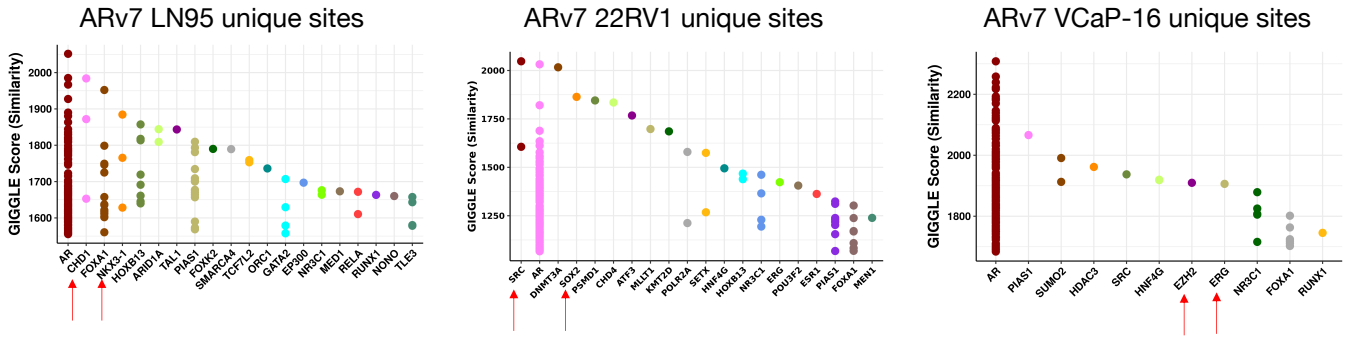

Figure 7. Cistrome Toolkit GIGGLE similarity analysis of LN95, 22RV1, VCaP-16 unique ARv7 binding sites.

Assessing next-generation Thio-2 derivatives in CRPC cell proliferation

In order to identify a potential therapeutic strategy for targeting the ARv7, we have tested next-generation Thio-2 derivatives. Thio-2 has been shown to disrupt the interaction between AR and its coactivator/cochaperone, Bag-1L. Depletion of Bag-1L has been previously demonstrated to impair AR transcriptional activity and decrease prostate cancer cell growth. Bag-1L binds to AR at the AR N-terminus and therefore, could theoretically also bind AR variants that lack the ligand binding domain, such as ARv7. In collaboration with Dr. Andrew Cato, we have assessed a number of next-generation Thio-2 derivatives for their ability to inhibit prostate cancer cell growth. We have identified one promising derivative, A4B17, which appears to decrease AR+ prostate cancer cell line growth but not AR- prostate cancer cell lines (Figure 8).

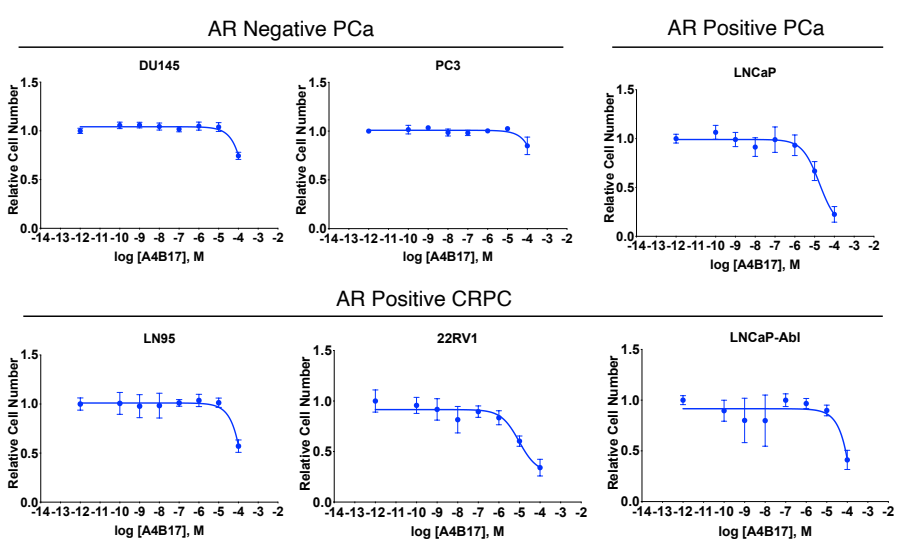

Figure 8. Prostate cancer cell line growth following treatment with Thio-2 derivative, A4B17.

Further interrogation into ARv7 targeting therapeutic strategies will be performed in the future. In addition to Thio-2 derivatives, we plan to assess the Arvinas AR PROTAC, ARV-110, to determine if it could be used to disrupt ARv7 transcriptional activity. Our previous data and current cistrome results have indicated that ARv7 binding is dependent upon ARfl, which may make an AR degrader approach feasible.

Training Opportunities

Professional Development Meetings

1. DFCI-BIDMC Monthly SPORE Meetings
2. DFCI-BIDMC Monthly PO1 Meetings
3. DF/HCC Prostate Program Retreat
4. FEBS 2019 Advanced Course: Epigenomics, Nuclear Receptors, and Disease

Results Disseminated

Nothing to Report

Future Studies
We plan to perform CRISPR screens in additional engineered shGFP, shARv7, shARfl CRPC cell lines to determine the essential genes that confer resistance and sensitivity to ARv7. Additionally, we will validate candidate target genes and determine their impact on cell proliferation, apoptosis, and ARv7 transcriptional activity. Furthermore, we will plan to expand on our understanding of ARv7 transcriptional activity. We have identified a number of potential cofactors of ARv7 from our ChIP-seq analysis, including EZH2, and will plan to interrogate their roles in ARv7 transcriptional activity. Finally, we plan to assess the efficacy of next-generation Thio-2 derivatives and additional strategies to potentially therapeutically target the ARv7 in CRPC.

4. Impact

Impact on the Development of Principle Discipline
This study begins to address the DOD's Overarching Challenges to "Develop treatments that improve outcomes for men with lethal prostate cancer" and "Define the biology of lethal prostate cancer to reduce death." Our study aims to further explore the requirement of Androgen Receptor in castration resistant prostate cancer (CRPC) and the role that the splice variant ARv7 plays in promoting growth and survival of CRPC cells. Resistance to Androgen Deprivation Therapy (ADT) remains a significant obstacle in the treatment of prostate cancer patients. The majority of patients treated with ADT will at some point, develop resistance to this therapy and progress to CRPC which is lethal. Understanding the mechanisms of ADT resistance will be imperative to determine how to overcome resistance to ADT therapies. We hypothesized that targeting the splice variant, ARv7, is the key to overcoming ADT resistance. Our results have indicated that ARv7 seems to regulate distinct essential gene pathways from ARfl and we plan to further examine these pathways in order to potentially exploit additional therapeutic vulnerabilities in CRPC. Additionally, we have shown that ARv7 binding to its regulatory regions is diverse and dependent upon its cellular context. Future studies will be focused on further understanding the mechanisms that underly ARv7-dependent transcription. Finally, we will continue to test therapeutics that can target and inhibit ARv7 activity. Our study has the potential to address the DOD's Overarching Challenges, by furthering our understanding of the biology of lethal prostate cancer and to perhaps suggest novel therapeutic approaches to treat men with lethal prostate cancer.

Impact on Other Disciplines
Nothing to Report

Impact on Technology Transfer
Nothing to Report

Impact on Society
Nothing to Report

5. Changes/Problems

Changes in Approach
Nothing to Report

Actual/anticipated Problems or Delays
We anticipate that the global COVID-19 pandemic will result in significant delays in data collection and new experiments being conducted. Our institute closed to all experimentation and wet lab research for approximately 3 months from March – June 2020. We are currently allowed to perform experiments again, however, the number of lab members at any one time working in the lab is limited. Therefore, we anticipate that progress on this project will be delayed because of the global pandemic.

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

6. Products

Journal Publications

Lee, II, Kuznik, NC, Rottenberg, J, Brown, M, and Cato, ACB. (2019). Bag-1L: a promising therapeutic target for androgen receptor-dependent prostate cancer. *J Mol Endocrinol, 62*(4), R289-R299.

Other Products
Nothing to Report

7. Participants and Other Collaborations

Individuals who have worked on project
Name: Irene Lee, PhD
Project Role: PI
Research Identifier (ORCID): 0000-0002-5749-9389
Nearest person month worked: 12
Contribution to project: Dr. Lee has conceived of the project, performed the experiments, data analysis, and data collection

Name: Myles Brown, MD
Project Role: Mentor
Nearest person month worked: 1
Contribution to project: Dr. Brown has provided guidance, support, and critical feedback of the project

Change in active support
Nothing to Report

Other organizations involved as partners
Organization Name: Beth Isreal Deaconness Medical Center
Location of Organization: Boston, MA, USA
Partner's contribution to project: collaboration

Organization Name: Karlsruhe Institute of Technology (KIT)
Location of Organization: Karlsruhe, Germany
Partner's contribution to project: collaboration

8. Special Reporting Requirements
Nothing to Report

9. Appendices
Nothing to Report