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TITLE: Deploying a Novel Model of Merkel Cell Carcinoma for Development of Nove

PRINCIPAL INVESTIGATOR: Kenneth Y. Tsai, MD, PhD

CONTRACTING ORGANIZATION: H. Lee Moffitt Cancer Center

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Fort Detrick, Maryland 21702-5012

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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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E-Mail: kenneth.tsai@moffitt.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

H. Lee Moffitt Cancer Center & Research Institute, Inc.
12902 Magnolia Drive
Tampa, FL 33612-9492

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

There is no reported molecularly faithful model of UV-Merkel cell carcinoma. Such a model enables the exploration of cells of origin, cooperating genetic events, the role of the immune system, and serves as a preclinical platform for testing new therapies. Successful completion of this project will produce a quantum leap in our collective capability to study this disease from etiology on through to therapeutic intervention. The validation of these hypotheses and credentialing of a novel, first in kind, mouse model of non-viral MCC will make available to the community an extremely valuable resource. The vast majority of activities are related to ensuring that we are in a position to produce all the intermediate strains needed on an inbred C57BL/6 background. We are still in a position to complete the SOW as listed. We have made significant progress in Major Subtasks 2, 3, 6, 7. Our main objective is to credential a novel, first in kind, mouse model of virus-negative MCC and to test the relevance of key pathways implicated in virus-negative MCC and responses to immunotherapy. Along these lines, we have generated key data related to initial characterization of how UV exposure accelerates tumorigenesis, linkage to human MCC, and initial FACS analysis of the tumor microenvironment.

First, we have clearly shown that UV-exposure using a 3 month course of low dose UV irradiation (Newport Solar Simulator) dosed at 12.5 kJ/m2 weekly (broad band UVB) accelerates tumor formation in RPM mice.Next, we have performed some immunohistochemistry on our samples to show evidence of neuroendocrine differentiation (INSM expression) in mouse MCC tumors from RPM mice. This is accompanied by KRT8 expression, focal ATOH1 expression, consistent with MCC tumors.
Next, with our investigators at Baylor, we continue to show evidence of clear relatedness to human MCC through gene signature analysis showing clear similarity of mouse MCC tumors from the RPM mice to multiple sets of human MCC as well as small cell lung cancer (SCLC). Importantly we also show that neuroendocrine genes are significantly though heterogeneously upregulated in mouse MCC cell lines and tumors as compared to normal mouse keratinocytes. These show upregulation of NOTCH signaling, SOX2, ATOH1, ASCL1 as well as occasional upregulation of YAP / TAZ components. Finally, we have had the opportunity to probe the tumor microenvironment with FACS showing that the effect of anti PD-1 therapy in resistant mouse MCC tumors from RPM mice is upregulation of exhaustion markers on CD8+ T cells including LAG3, TIGIT, but also upregulation of similar markers in DC1 cells.

15. SUBJECT TERMS

Mouse model, Merkel cell carcinoma, immunocompetent, ultraviolet radiation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

There is no reported molecularly faithful model of UV-Merkel cell carcinoma. Such a model enables the exploration of cells of origin, cooperating genetic events, the role of the immune system, and serves as a preclinical platform for testing new therapies. Successful completion of this project will produce a quantum leap in our collective capability to study this disease from etiology on through to therapeutic intervention. The validation of these hypotheses and credentialing of a novel, first in kind, mouse model of non-viral MCC will make available to the community an extremely valuable resource.

2. KEYWORDS:

Mouse model, Merkel cell carcinoma, immunocompetent, ultraviolet radiation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Characterize differences in tumor behavior in RPM model variants
50% complete

Major Task 2: Identify the impact of specific UV exposure regimens on MCC development
75% complete

Major Task 3: Characterize the diversity of neuroendocrine differentiation in UV-exposed and RP, RM, PM models
25% complete

Major Task 4: Test vulnerability to TGFβ and BMP signaling suppression in mouse and human MCC cell lines.
0% complete

Major Task 5: Test relevance of EMT markers in mouse and human MCC lines.
0% complete

Major Task 6: Assess the tumor microenvironment in a transplantable model of MCC.
10% complete

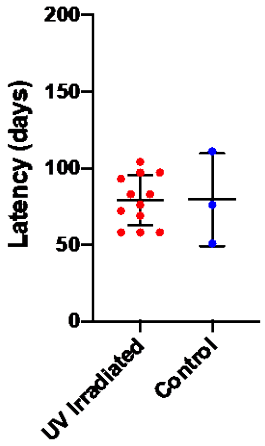
Major Task 7: Validate and provide rationales for novel combinations of immunotherapy
25% complete

What was accomplished under these goals?

Major Activities: The vast majority of activities are related to ensuring that we are in a position to produce all the intermediate strains needed on an inbred C57BL/6 background. We are still in a position to complete the SOW as listed. We have made significant progress in Major Subtasks 2, 3, 6, 7.

Specific Objectives: Our main objective is to credential a novel, first in kind, mouse model of virus-negative MCC and to test the relevance of key pathways implicated in virus-negative MCC and responses to immunotherapy. Along these lines, we have generated key data related to initial characterization of how UV exposure accelerates tumorigenesis, linkage to human MCC, and initial FACS analysis of the tumor microenvironment.

Significant Results: First, we have clearly shown that UV-exposure using a 3 month course of low dose UV irradiation (Newport Solar Simulator) dosed at 12.5 kJ/m2 weekly (broad band UVB) accelerates tumor formation in RPM mice.



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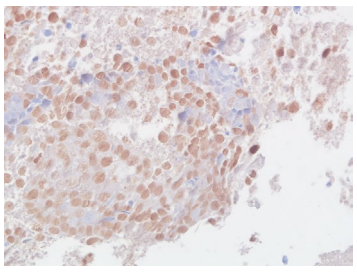

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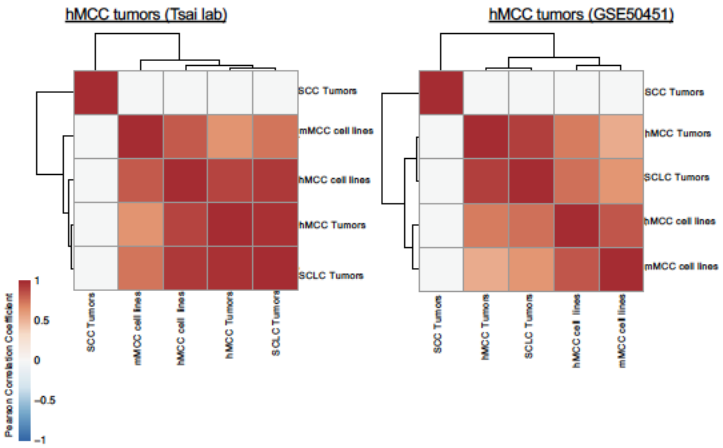
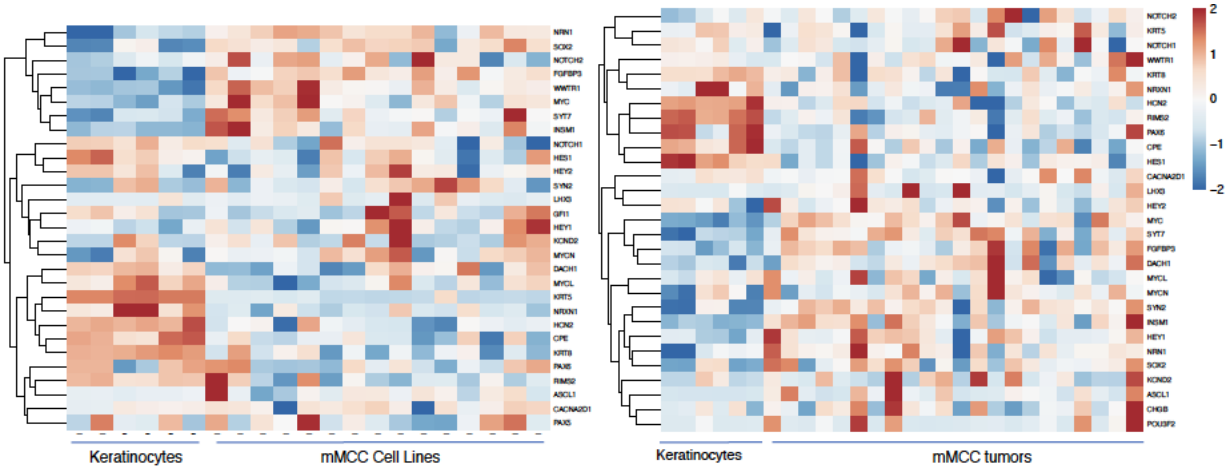
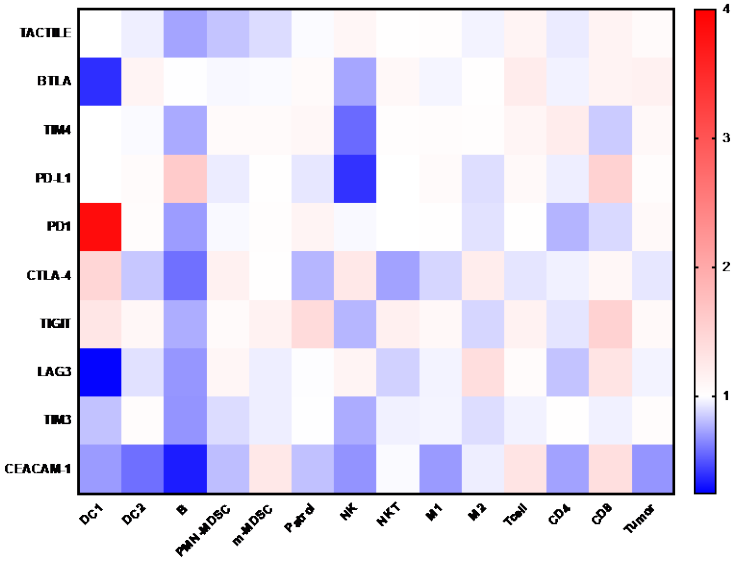


Importantly we also show that neuroendocrine genes are significantly though heterogeneously upregulated in mouse MCC cell lines and tumors as compared to normal mouse keratinocytes. These show upregulation of NOTCH signaling, SOX2, ATOH1, ASCL1 as well as occasional upregulation of YAP / TAZ components.



Finally, we have had the opportunity to probe the tumor microenvironment with FACS showing that the effect of anti PD-1 therapy in resistant mouse MCC tumors from RPM mice is upregulation of exhaustion markers on CD8+ T cells including LAG3, TIGIT, but also upregulation of similar markers in DC1 cells.



These results must be verified and expanded to RM, RP, PM mice as proposed.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

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

Complete the remainder of tasks especially with respect to the comparison of RM, PM, RP mice in terms of comparing molecular profiles and tumor latencies and frequencies. Testing the immunotherapies in mice will be the logical conclusion. The corresponding human data proposed for cross species comparison is well in hand as are the computational pipelines to accomplish that analysis.

4. IMPACT:

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

This impact thus far has really been informing what is possible in modeling and trying to compare it to a new virally driven model we are developing as well as a parallel model utilizing L-MYC rather than C-MYC. Our work is anticipated to have impact that stretches across MYC signaling as well as other neuroendocrine carcinomas driven by similar genetic alterations.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report.

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

We have decided that it is better to have a larger impact in the long run by ensuring that all the strains related to this project are fully backcrossed/ While our original thought at the time of grant submission was that this was largely in place, we did not explicitly commit to this goal but now realize it is significantly in the long run to do so.

Changes that had a significant impact on expenditures

Nothing to Report.

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

Significant changes in use or care of human subjects

None to report.

Significant changes in use or care of vertebrate animals

None to report.

Significant changes in use of biohazards and/or select agents

None to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report.

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

Nothing to Report.

Other publications, conference papers and presentations.

International Societies for Investigative Dermatology, Tokyo, Japan, 5/9-5/13/2023, Oral Presentation: Prieto et al. Establishment of a new immunocompetent mouse model of Merkel cell carcinoma

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Kenneth Tsai, MD, PhD
Project Role:	Principal Investigator
Nearest person month worked:	1
Contribution to project:	Responsible for overall project.
Funding Support:	N/A

Name:	Karol Prieto Sarmiento
Project Role:	Postdoctoral Fellow
Nearest person month worked:	4
Contribution to project:	Responsible for key experiments.
Funding Support:	N/A

Name:	Amrit Koirala
Project Role:	Bioinformatics Analyst
Nearest person month worked:	1
Contribution to Project:	Amrit worked closely with Dr. Coarfa on the analysis of the multi-omics analysis, including both data generated by the proposed grant and relevant publicly available datasets. Amrit had frequent meetings with Dr. Coarfa to discuss projects, pitfalls and alternative approaches.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Kenneth Tsai
NEW/ACTIVE:
Title: Credentialing a Mouse Model for Merkel Cell Carcinoma
Time Commitments: 0.60 calendar months
Supporting Agency: USArmy CDMRP RCRP Concept - W81XWH-22-11084 (Tsai, PI)
Performance Period: 09/20/2022-09/29/2024 (NCE)

Title: Analytical tools for studying the tumor microenvironment leveraging spatial transcriptomics
Time Commitments: 0.36 calendar months
Source of Support: NIH/NCI U01CA274489-01 (Fridley, PI)
Project/Proposal Start and End Date: 09/01/2022 – 08/31/2025

Title: Translational studies for targeted alpha-particle therapy for rare melanomas
Time Commitments: 0.24 calendar months
Supporting Agency: USArmy CDMRP HT9425-23-1-0909 (Morse, PI)
Performance Period: 09/01/2023-08/31/2027

Overlap: No overlap for NEW/ACTIVE awards above.

INACTIVE:
Title: Defining and modeling pediatric melanoma development
Time Commitments: 0.12 calendar months
Supporting Agency: Florida Biomedical Research Program - 9LA03 (SmalleyK, PI)
Performance Period: 04/01/2019-03/31/2022

Title: Exploiting the Immune Mapping of the Tumor Microenvironment to Improve Immunotherapy of Melanoma
Time Commitments: 1.14 calendar months
Supporting Agency: Dr. Miriam and Sheldon Adelson Medical Research Foundation (Mulé, PI)
Performance Period: 10/01/2019-09/30/2022

Title: Compartmentalized and System Interaction of the Skin Microbiome in Cancer Immunotherapy Response
Time Commitments: 0.006 calendar months
Supporting Agency: LEO Foundation Netherlands (PTE: The Jackson Laboratory- Oh, PI)
Performance Period: 11/01/2019-03/31/2023

Title: Genetic Drivers of Merkel Cell Carcinoma
Time Commitments: 0.06 calendar
Supporting Agency: Moffitt Melanoma & Skin Cancer Center of Excellence (Tsai, PI)
Performance Period: 08/01/2020-06/30/2022

Title: The role of afatinib therapy in immunotherapy refractory cutaneous SCC
Time Commitments: 0.06 calendar
Supporting Agency: Moffitt Melanoma & Skin Cancer Center of Excellence (Tsai, PI)
Performance Period: 08/01/2020-06/30/2022

INACTIVE (cont'd)
Title: Metabolic Reprogramming of the Tumor Microenvironment in Cutaneous SCC
Time Commitments: 0.06 calendar
Supporting Agency: Moffitt Melanoma & Skin Cancer Center of Excellence (Tsai, PI)
Performance Period: 08/01/2020-05/31/2022
Overlap: No scientific or budgetary overlap with the proposed CDMRP proposal.

Title: Altered Tumor-Immune Synapse in Merkel Cell Carcinoma Drives Immune Evasion
Supporting Agency: Moffitt Melanoma & Skin Cancer Center of Excellence (Tsai, PI)
Time Commitments: 0.06 calendar
Performance Period: 08/01/2020-05/31/2022

Title: Exploring miR-29 in melanoma progression and prevention
Time Commitments: 0.36 calendar months
Supporting Agency: NIH/NCI – R21CA256141-01A1(Karreth,PI)
Performance Period: 08/01/2021-07/31/2023

Dr. Sungjune Kim
Dr. Kim departed Moffitt and is no longer active on this project.

Dr. Cristian Coarfa
NEW/ACTIVE:
Title: Novel-Metabolomics and Radiomics for Predicting HCC Risk
Time Commitment: 0.36 CM
Supporting Agency: Cancer Prevention & Research Institute of Texas (RP220119) (Dr. H. El-Serag PI)
Performance Period: 03/01/2022-02/28/2026

Title: Prevention of Hepatocellular Carcinoma Related to Metabolic
Time Commitment: 0.6 CM
Supporting Agency: National Cancer Institute (grant #5 P01 CA263025-02) (Dr. H. El-Serag, PI)
Performance Period: 07/01/2022-06/30/2027

Title: Understanding and Targeting the Pathophysiology of Youth-onset Type 2 Diabetes- Texas Children's Center
Time Commitment: 0 CM (0.6 CM in 2025-2029)
Supporting Agency: National Institute of Diabetes & Digestive and Kidney Diseases (#1U01DK134982-01) (Dr. F. Bacha, PI)
Performance Period: 03/05/2023-01/31/2029

Title: Impacts of Structural Racism on Racial and Ethnic Disparities in Perinatal Health
Time Commitment: 0.36 CM
Supporting Agency: National Institute of Child Health & Human Development (grant #1R01HD111500-01) (Dr. E. Symanski; Dr. K. Whitworth (Contact), PI)
Performance Period: 05/03/2023-02/29/2028

Title: Targeting Castration Resistant Prostate Cancer via Potent Inhibition of Signaling Lipids
Time Commitment: 0.36 CM
Supporting Agency: National Cancer Institute (grant #5 R01 CA251560-02) (Dr. S. Kaochar, PI)
Performance Period: 09/01/2022-08/31/2027

Title: Mechanisms of Cancer Drug Resistance and Sensitivity CORE B: Systems Bioinformatics Core
Time Commitment: 0.53 CM
Supporting Agency: National Cancer Institute / UT M.D. Anderson Cancer Center (#1 U54 CA274321-01) (Dr. V. Sandulache; Dr. J. Myers (Contact), MPI)
Performance Period: 09/20/2022-08/31/2027

Title: Differential changes in energy metabolism in response to mechanical tension give rise to human scaring heterogeneity
Time Commitment: 0.24 CM
Supporting Agency: National Institute of General Medical Sciences (grant #1 R01 GM141366-01A1) (Dr. S. Balaji, PI)
Performance Period: 07/01/2023-03/31/2028

Project Title: Regulation of atrophy-induced progenitor cells in the gastric corpus
Time Commitment: 0.6 CM
Supporting Agency: National Institute of Diabetes and Digestive and Kidney Diseases (#2 R01 DK094989-12) (Dr. J. Mills, PI)
Performance Period: 09/11/2012-04/30/2028

Title: Single Cell Analysis of Epigenetic Mechanisms That Regulate HIV-1 CNS Latency and Neuropathogenesis
Time Commitment: 1.8 CM
Supporting Agency: National Institute of Mental Health (grant #1 R01 MH 134392-01) (Dr. C. Coarfa; Dr. C. Walss-Bass; Dr. A. Rice (Contact), MPI)
Performance Period: 07/06/2023-04/30/2028

Project Title: Common Post-Infectious Premature Epigenetic-Aging
Time Commitment: 1.2 CM
Supporting Agency: National Institute on Aging (grant #1 R01 AG078268-01) (Dr. A. DiNardo, PI)
Performance Period: 08/15/2023-04/30/2028

Overlap: No overlap for NEW/ACTIVE awards above.

INACTIVE:
Title: REST-mediated epigenomic and transcriptomic signatures in neuropathic pain
Time Commitment: 0.48 CM
Supporting Agency: NCI Project Number: 5 R01 NS112280-02 (Majumder, S., PI)
Performance Period: 09/2019 – 07/2023

Title: Gene X Environment Interactions in the Pathogenesis of Uterine Fibroids

Time Commitment: 0.24 CM
Project Number: 7 R01 E028615-06
Supporting Agency: NIEHS PTE: University of Chicago (Al-Hendy/Walker, PI)
Performance Period: 11/2020 – 07/2022

Title: Minority PDX Development and Trial Center: Baylor College of Medicine and MD Anderson Cancer Center Collaboration on Mechanistic Studies to Dissect and Combat Health Disparities in Cancer
Time Commitment: 0.60 CM
Supporting Agency: NCI Project Number: 5 U54 CA233223-04 (Mitsiades, N., PI)
Performance Period: 09/2018 – 06/2023

Title: MYCN Reprograms Neuroblastoma Metabolism
Time Commitment: 0.24 CM
Supporting Agency: DOD, Project Number: W81XWH-19-1-0556 (Barbieri, E., PI)
Performance Period: 09/2020 – 08/2022

Title: MPI - Mechanisms of Prevention of Polycyclic Aromatic Hydrocarbon (PAH)- Mediated Lung Carcinogenesis by Omega-3 Fatty Acids
Time Commitment: 0.36CM
Supporting Agency: CPRIT Project Number: RP190279 (Moorthy, B., PI)
Performance Period: 03/2019 – 02/2022

Title: Use of Microbial Based Countermeasures to Mitigate Radiation Induced Intestinal Damage/RAD0101
Time Commitment: 0.24 CM
Supporting Agency: NASA Project Number: 1NNX16AO69A (RAD0101) (Blutt. S., PI)
Performance Period: 10/2020 – 09/2022

Title: Environmental Health Outcomes Research Among Hurricane Harvey Survivors
Time Commitment: 0.12 CM
Supporting Agency: NIEHS Project Number: 3 R21 ES029616-02S1 (Oluyomi, A., PI)
Performance Period: 08/2020 – 02/2022

Title: Replication Stress and DNA Damage Response Drives ESR1 Mutant Metastasis
Time Commitment: 0.00 CM
Supporting Agency: NCI Project Number: 2 R01 CA072038-21 (Fuqua, S., PI)
Performance Period: was not appointed to grant

What other organizations were involved as partners?

This award includes a subaward with Baylor College of Medicine, Houston, TX. Cristian Coarfa, PhD/Baylor is Co-Investigator. Dr. Coarfa is assisted by Bioinformatics Analyst, Amrit Koirala. Their contribution to the project is to perform the analyses of the transcriptomics and epigenomics data generated by this project. In addition, Dr. Coarfa will identify and supervise integration data generated by the application with relevant publicly available datasets, including transcriptomics, epigenomics, proteomics. Dr. Coarfa meets regularly via teleconference with Dr. Kenneth Tsai (PI), to discuss the progress and discuss potential pitfalls and alternative approaches.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable.

QUAD CHARTS:

Not applicable.

9. APPENDICES:

None.