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# REPORT DOCUMENTATION PAGE

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## 1. INTRODUCTION:

Our **main objective** is to credential a novel, first in kind, mouse model of virus-negative MCC and to identify and test the relevance of key pathways implicated in virus-negative MCC. Our **central hypotheses** are that keratinocytes can be a cell of origin for Merkel cell carcinoma, that the resulting tumors recapitulate the human disease, and the Sleeping Beauty transposon system identifies genetic enhancers and suppressors.

## 2. KEYWORDS:

Merkel cell carcinoma, Retinoblastoma gene, Rb1, Tumor related protein 53, Trp53, keratinocyte, neuroendocrine, Myc

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

### **Major Task 1 Validate genomic profile of mouse model of MCC – 75%**

Subtask 1 – Perform RNASeq sequencing analysis of MCC tumors

Subtask 2 – Perform whole exome sequencing analysis of MCC tumors

### **Major Task 2 Validate marker profile of mouse model of MCC – 50%**

Subtask 1 - Generate comprehensive tissue marker profiles of MCC

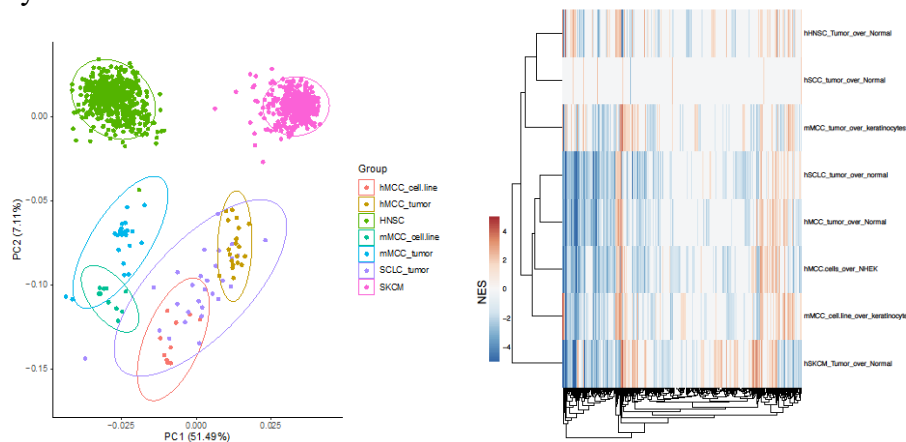
### **Major Task 3 Characterize tumor cohort (n=50) mice with Onc3 Sleeping Beauty system – 10%**

Subtask 1. Perform detailed phenotypic analysis from MCC model with Sleeping Beauty platform

Subtask 2. Perform initial characterization of genetic enhancers and suppressors of MCC

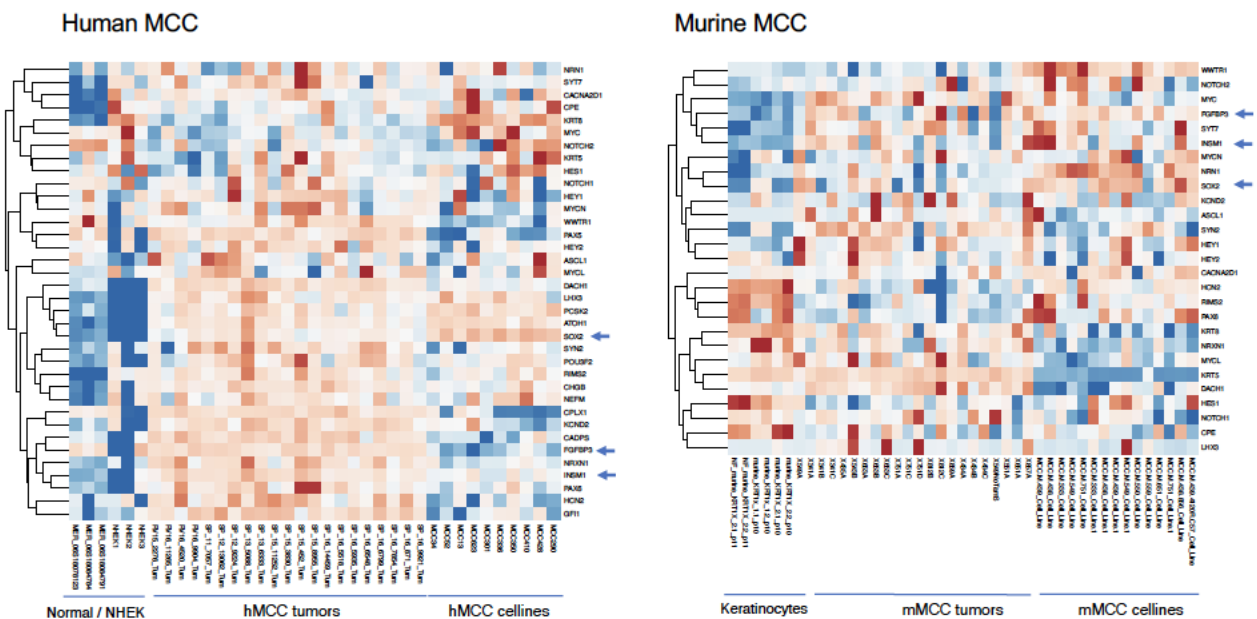
## What was accomplished under these goals?

The major activities have centered on genomic characterization of our model of Merkel cell carcinoma and proving that they represent human MCC adequately to function as valid preclinical platforms. The major objectives have been to assess these similarities using RNAseq and focusing on how background of the strains may affect the ability to generate the Sleeping Beauty transposon system.



The plot and heatmap above show using Principal Components Analysis (left) and Gene Set Enrichment analysis (GSEA, right) show deep relatedness between mouse Merkel cell carcinoma (MCC) to human MCC, human small cell lung carcinoma, but not melanoma and not squamous cell carcinoma. This is an important validation step of showing the model appropriately represents human MCC. We have decided to postpone whole exome sequencing based until the strains are more backcrossed into the C57BL/6 background – this minimizes background variation as an explanation for differences in tumor latency and aggressiveness.

Comprehensive marker profile has been predicated on some immunohistochemistry as well as looking for evidence of clear neuroendocrine differentiation across MCC tumors and cell lines from both humans and mouse.



The latter is more advanced. The above figure shows that both human (left) and mouse (right) MC tumors and cell lines reflect a spectrum of neuroendocrine markers and factors known to be expressed by MCC. These include INSM1, SOX2, POU2F3 and define a spectrum of neuroendocrine differentiation across MCC which can be modeled effectively.

**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?**

A No Cost Extension has been approved for 12 months. We plan to finish out the tasks as originally proposed.

#### **4. IMPACT:**

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

This base line model remains the only credible model of virus-negative Merkel cell carcinoma (MCC) we are aware of. We continue to believe that this work will enable an entire generation new findings by identifying unifying commonalities among neuroendocrine carcinomas and by providing a platform for preclinical studies in MCC.

**What was the impact on other disciplines?**

Thus far, the impact is to confirm that indeed keratinocytes can be cells of origin for Merkel cell carcinoma. We anticipate that we will be able to generate a unified approach for modeling and studying neuroendocrine carcinomas.

**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 had breeding problems moving the ONC3 / transposases alleles into Rb/p53/Myc mice with what we surmise is unanticipated neonatal lethality. Our solution is to backcross the model to the C57BL/6 background. The individual Rb1, Trp53, and Myc alleles are within one generation of being on the appropriate background.

**Changes that had a significant impact on expenditures**

None. We are committed to finishing the work with the NCE.

**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**

Nothing to Report. IRB 00000971 (MCC 21946) Approval: 5/2/2022

**Significant changes in use or care of vertebrate animals**

Nothing to Report. IACUC PROTOCOL #: R IS00010732. Approval: 5/27/2022

**Significant changes in use of biohazards and/or select agents**

Nothing to Report.

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **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 [unified Societies for Investigative Dermatology Meeting], Tokyo, Japan, 5/9-5/13/2023, “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?

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.*

Name: Kenneth Tsai  
Project Role: Principal Investigator  
Researcher Identifier (e.g. ORCID ID):  
Nearest person month worked: 1  
Contribution to Project: Principal Investigator

Name: Omar Chavez  
Project Role: Research Associate  
Researcher Identifier (e.g. ORCID ID):  
Nearest person month worked: 4  
Contribution to Project: Managing mouse colonies. Performing experiments

Name: Irmak Aksit  
Project Role: Research Associate  
Researcher Identifier (e.g. ORCID ID):  
Nearest person month worked: 2  
Contribution to Project: Performing experiments.

**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: Deploying a Novel Model of Merkel Cell Carcinoma for Development of Novel Therapeutic Approaches

Time Commitments: 0.96 calendar months

Supporting Agency: USArmy CDMRP RCRP Idea - W81XWH-22-1-0921 (Tsai, PI)

Performance Period: 09/01/2022 – 08/31/2024

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. Michael Mann**

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

**What other organizations were involved as partners?**

Nothing to Report.

**8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:**

**9. APPENDICES:**