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Award Number: W81XWH-18-1-0492

TITLE: Targeting CDK4/6 in HER2-Positive Breast Cancer Brain Metastasis

PRINCIPAL INVESTIGATOR: Nancy U. Lin, M.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute

Boston, MA 02215-5450

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Nancy U. Lin
Jean Zhao

5d. PROJECT NUMBER

5e. TASK NUMBER

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Boston, MA 02215-5450

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this project is to use patient-derived xenograft (PDX) models and genetically-engineered mouse models (GEMMs) of HER2+ breast cancer brain metastases (BCBM) to test novel CDK4/6-based combination therapies for activity against HER2+ BCBM. By using anti-CDK4/6 drugs with other targeted therapies we will be able test the potential synergy of drug combinations against HER2+ BCBM. Due to pending approval of involving human subjects, we do not have any activities to report on using patient derived xenograft (PDX) models. However, we have made a considerable progress in mouse tumor cells and syngeneic mouse models of BCBM. Our positive preliminary results from mouse models laid the foundation for further developing effective therapeutic strategies against specific subtypes of breast cancer brain metastases, which is important for deliver pre-clinical information to guide the rational design of promising clinical trials.

15. SUBJECT TERMS
Breast cancer,

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ANNUAL TECHNICAL REPORT
Award Number: W81XWH-18-1-0492
Title: Targeting CDK4/6 in HER2-positive breast cancer brain metastases
Principal Investigator: Nancy U. Lin
Reporting Period: Sep 1, 2018 to Aug 31, 2019

For Cover Page and SF298 form

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2. Project Summary
Provide an unclassified summary of research findings to date. In approximately 200 words, state the purpose, scope, and major findings of the research. This must be an up-to-date report of the progress in terms of results and significance.

The purpose of this project is to use patient-derived xenograft (PDX) models and genetically-engineered mouse models (GEMMs) of HER2+ breast cancer brain metastases (BCBM) to test novel CDK4/6-based combination therapies for activity against HER2+ BCBM. By using anti-CDK4/6 drugs with other targeted therapies we will be able test the potential synergy of drug combinations against HER2+ BCBM.

Due to pending approval of involving human subjects, we do not have any activities to report on using patient derived xenograft (PDX) models. However, we have made a considerable progress in mouse tumor cells and syngeneic mouse models of BCBM. Our positive preliminary results from mouse models laid the foundation for further developing effective therapeutic strategies against specific subtypes of breast cancer brain metastases, which is important for deliver pre-clinical information to guide the rational design of promising clinical trials.

Main Body of Report

1. INTRODUCTION:

Breast cancer brain metastases (BCBM) remain a significant clinical challenge. This is especially true for patients with the HER2+ subtype of breast cancer where up to half of patients with metastatic disease eventually develop brain metastases. To date, there are no FDA-approved systemic therapies for the specific treatment of breast cancer brain metastases. Through careful molecular and genetic analysis of HER2+ BCBM patient samples we identified a high frequency *CDKN2A/2B* copy number loss in HER2+ BCBMs, suggesting hyperactivation of the CDK4/6-Rb pathway. We hypothesize that hyperactivation of the CDK4/6-Rb pathway in HER2+ BCBMs, via loss of *CDKN2A/2B*, would be vulnerable to treatment with CDK4/6 inhibitors whose activity maybe synergized in combination with immune therapies. This proposal is designed to investigate the efficacy of targeting CDK4/6 in HER2+ BCBM and develop CDK4/6-inhibitor based combinations with immune checkpoint blockade to treat HER2+ BCBM. In this study, we propose to use patient derived xenograft (PDX) models and syngeneic genetically-engineered mouse models (GEMMs) of HER2+ BCBM to test novel CDK4/6-based combination therapies for activity against HER2+ BCBM.

2. KEYWORDS:

HER2+ breast cancer, brain metastases, targeted therapy, immunotherapy, combination therapy, CDKN2A/2B, CDK4/6-Rb pathway, therapeutic resistance, immune checkpoint blockade, syngeneic GEMM, PDX, HER2 inhibitor, CDX4/6 inhibitor

3. ACCOMPLISHMENTS

3a. What were the major goals of the project?

- Specific Aim 1: to investigate the effects of combined CDK4/6 and HER2 inhibition in HER2-positive BCBM.
    - Major Task 1. To determine whether CDKN2A/2B status in HER2+ BCBM dictates response to CDK4/6 inhibition, and to evaluate the efficacy of CDK4/6 inhibition in combination with HER2-targeted therapy. (No activity)
    - Major Task 2. To evaluate the therapeutic response of HER2+ ER- BCBM to CDK4/6 inhibition in combination with HER2-targeted therapy. (No activity)

- Specific Aim 2: To investigate the effects of CDK4/6 inhibition on anti-tumor immunity in HER2-positive BCBM
    - Major Task 1: To determine the effects of CDK4/6 inhibition on HER2+ BCBM tumors autonomous immune response. (No activity)
    - Major Task 2. To evaluate whether combinations of CDK4/6 inhibition with immunomodulatory agents have CNS efficacy in syngeneic GEMMs of HER2+ BCBM tumors.
        - Subtask 1. Generate MMTV-Neu-IRES-Cre / PtenL/L (NIC/Pten) Cdkn2a-/- or Cdkn2b-/- (NIC/Pten/Cdkn) syngeneic tumors using in vitro CRISPR gene editing of NIC/Pten tumor cells (1-12 months, completed)
        - Subtask 2. Establish NIC/Pten/Cdkn and NIC/Pten orthotopic brain tumors by intracranial injection into syngeneic FVB female mice (12-24 months, completed)
        - Subtask 3. Treat NIC/Pten intracranial tumor bearing mice with 4 arms: abemaciclib or PD1 inhibitor or abemaciclib + PD1 inhibitor or vehicle measure response by change in tumor volume using bioluminescence and animal survival (18-24 months, ~50% completion)

3b. What was accomplished under these goals?

Aim 2 - Subtask 1. Generate MMTV-Neu-IRES-Cre / PtenL/L (NIC/Pten) and NIC/Pten/Cdkn syngeneic tumors using *in vitro* CRISPR gene editing of NIC/Pten tumor cells.

We have carried out successful CRISPR-mediated gene ablation of Cdkn2a in NIC/Pten^{null} brain mets tumor cells as shown in Figure 1 panel A. Interestingly, while NIC/Pten^{null} tumor cells have Cdkn2a mRNA expression, the p16 protein level was not detectable by WB analysis (Figure 1 B), suggesting a post transcription loss of p16 in NIC/Pten^{null} tumor cells. Hereafter, NIC/Pten^{null} tumor cells are labeled as NIC/Pten^{null}/p16^{null} tumor cells.

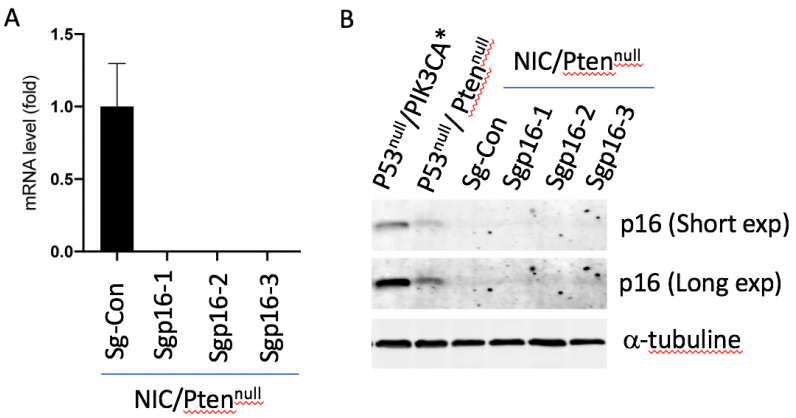

Figure 1. CRISPR-mediated deletion of Cdkn2a in NIC/Pten^{null} BCBM tumor cells. (A). Expression levels of mRNA in sg-control and 3 sgRNA against p16Ink4a (Cdkn2a). (B) Western blotting analysis of Cdkn2a-KO NIC/Pten^{null} KO cells. P53^{null}/PIK3CA* and P53^{null}/Pten^{null} mouse mammary tumor cells used as controls.

Alternative approach: We plan to introduce Cdkn2a into NIC/Pten^{null}/p16^{null} cells to make NIC/Pten^{null}/p16+ tumor cells to evaluate the effect of the presence and absence of p16 on the therapeutic response to CDK4/6 inhibition.

Aim 2 - Subtask 2. Establish NIC/Pten/Cdkn and NIC/Pten orthotopic brain tumors by intracranial injection into syngeneic FVB female mice.

We have established NIC/Pten^{null}/p16^{null} orthotopic brain tumor models via both intracranial injection and intracarotid injection methods (Figures 2 and 3 and not shown). We have proceeded to Subtask 3 on evaluation of therapeutic responses to CDK4/6 inhibitor, HER2 inhibitor and PD-1 blockade and their combinations.

Aim 3 - Subtask 3. Treat NIC/Pten^{null}/p16^{null} intracranial tumor bearing mice with various inhibitors and combinations, and measure responses by change in tumor volume using bioluminescence and animal survival.

We started with treatment of a cohort of NIC/Pten^{null}/p16^{null} intracranial tumor allograft-bearing mice with CDK4/6 inhibitor (abemaciclib) and HER2 inhibitor (ONT380, tucatinib). Both of these drugs have potent brain penetration. As shown in Figure 2, while tucatinib had some activity as a single agent, the combination of tucatinib and abemaciclib had a greater effect on survival benefit. We have also tested the combination of abemaciclib and PD1 blockade as shown in Figure 3. These results show that these combination therapeutic approaches are able to extent the survival of brain tumor bearing animals, more combinations of targeted therapy and immunotherapy are needed to identify most effective therapy to improve the therapeutic outcome, e.g. stable remission or eradication of tumor cells.

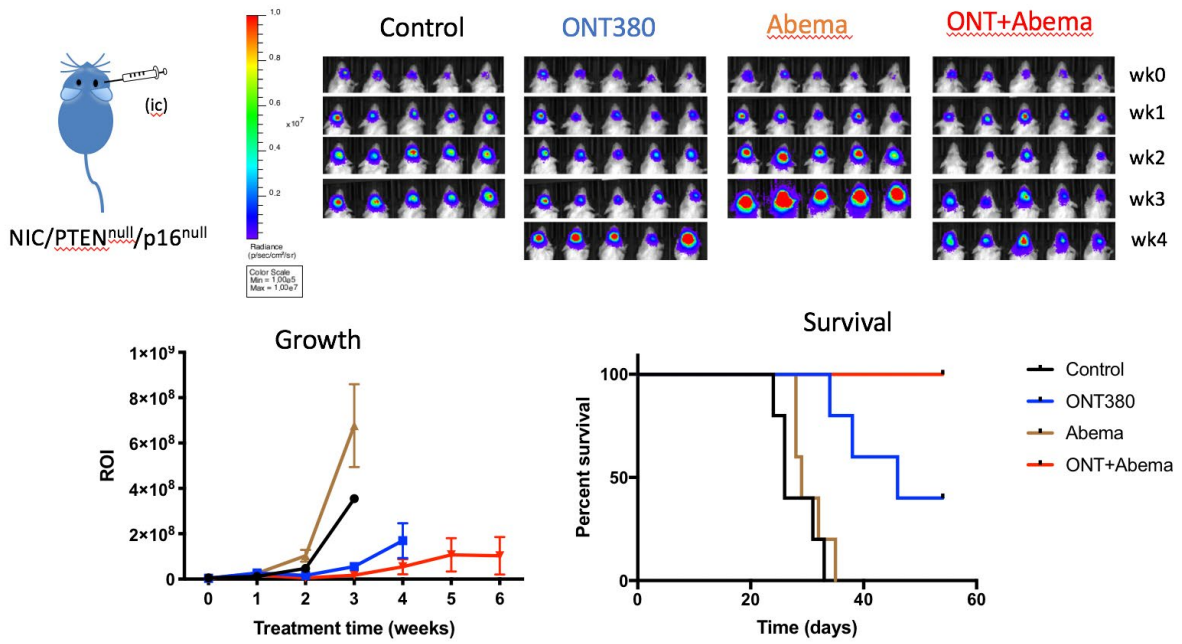

Figure 2. Combination of CDK4/6 inhibitor (abemaciclib) and HER2 inhibitor (ONT380, tucatinib) on NIC/Pten^{null}/p16^{null} intracranial tumor bearing mice.

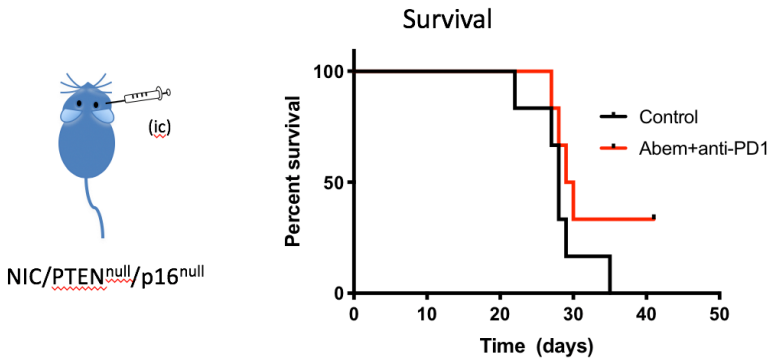

Figure 3. Combination of CDK4/6 inhibitor (abemaciclib) and anti-PD1 mouse antibody on NIC/Pten^{null}/p16^{null} intracranial tumor bearing mice.

3c. What opportunities for training and professional development has the project provided?

Nothing to Report.

3d. How were the results disseminated to communities of interest?

Nothing to Report

3e. What do you plan to do during the next reporting period to accomplish these goals?

We recently received the notice from USAMRDC Office of Research Protections (ORP) that ORP Human Research Protection Office (HRPO) concurs with the DFCI IRB Office's determination of research not involving human subjects (IRB Study Number 19-420, Proposal Number BC171657, Award Number W81XWH-18-1-0491, 9/17/2019). With this approval, we plan to proceed our project with a major effort on working with PDX models as described in the proposal. Specifically, we will move ahead immediately with these tasks outlined in the SOW under the Specific Aim 1 to investigate the effects of combined CDK4/6 and HER2 inhibition in HER2-positive BCBM using PDX models established at DFCI during the next reporting period to accomplish the goals and objectives.

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 the items below:*

4a. What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

4b. What was the impact on other disciplines?

Nothing to Report.

4c. What was the impact on technology transfer?

Nothing to Report.

4d. What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

5a. Changes in approach and reasons for change:

Nothing to Report.

5b. Actual or anticipated problems or delays and actions or plans to resolve them:

Work with PDX models was delayed as the necessary HRPO approval for use of human materials took significantly longer to complete than originally anticipated. However, the approval is now in place and we are moving ahead with the research plan as expediently as possible.

5c. Changes that had a significant impact on expenditures:

Expenditures in the initial year of this grant have been lower than originally budgeted due to the delays encountered in receiving HRPO approval for use of human materials. The approval is now in place and our rate of expenditure will increase accordingly.

5d-f. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

5d. Significant changes in use or care of human subjects: *Nothing to Report.*

5e. Significant changes in use or care of vertebrate animals: *Nothing to Report.*
5f. 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."*

Nothing to Report.

6a. Publications, conference papers, and presentations:

Nothing to Report.

6b. Website or other internet site:

Nothing to report.

6c. Technologies or techniques:

Nothing to report.

6d. Inventions, patent applications, and/or licenses:

Nothing to report.

6e. Other Products:

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. What individuals have worked on the project? *(1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period.*

Name: Nancy U. Lin
Project Role: PD/PI
Researcher Identifier (ORCID): https://orcid.org/0000-0003-2263-5413
Nearest person month worked: 1
Contribution to project:
Dr. Lin has worked closely with Dr. Zhao to review preclinical experiments to ensure they are clinically relevant, and to review results of the experiments to co-plan next steps. Dr. Lin has completed the IRB protocol which will allow for work on human-derived biospecimens. The IRB protocol is near activation. Dr. Kabraji has worked with both Dr. Lin and Dr. Zhao to carry out preclinical experiments.
Funding support: This award

7b. Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?
If the active support has changed for the PD/PIs or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission

of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously.

Yes. There have been several changes to the active support of Dr. Nancy U. Lin since it was last reported

NEW ACTIVE AWARDS:

BCRF-18-098 (Lin) 10/01/07 – 09/30/20 1.2 CM
Breast Cancer Research Foundation $208,333
Improving Therapeutic Options for Metastatic Breast Cancer
Specific Aims: 1) To test the safety and efficacy of a brain permeable dual PI3K/mTOR inhibitor (GDC-0084) in combination with trastuzumab in patients with HER2-positive breast cancer brain metastases. Specific Aim 2: To evaluate the clinical utility of a screening program for brain metastases in patients with metastatic breast cancer initiating first- or second-line chemotherapy. Specific Aim 3: To study mechanisms of resistance to HER2-directed therapy, specifically via analysis of circulating free DNA (cfDNA).
Role: Principal Investigator

P50CA168504-06A1 (Winer) 07/05/2019 – 05/31/2024 1.2 CM
NIH/NCI $106,699 (Lin Portion)
Dana-Farber/ Harvard Cancer Center SPORE in Breast Cancer – Project 3
Aim 1. To evaluate the role of PTEN loss, which is frequently seen in BCBM, in the growth and maintenance of HER2+ brain metastases, and to evaluate whether strategies to target the PI3K/PTEN/mTOR pathway have therapeutic effects against HER2+ BCBM. Aim 2. To evaluate the role of the cyclin D1/CDK4/Rb pathway, which is frequently activated in BCBM, in the growth and maintenance of BCBM, and to evaluate whether strategies to target the pathway have therapeutic effects in ER+/HER2- or HER2+ BCBM.
Role: Project 3 Co-Leader (Clinical)

No Num Available (Lin) 07/01/19-06/30/22 0.24 CM
NCCN/Pfizer $93,333
Enhancing Academic-Community-Patient Partnerships in Metastatic Breast Cancer Care
This award is a collaboration with DFCI and the Embrace Program designed to develop and assist in oversight of the Request of Proposals (RFP) led by NCCN/Pfizer. Funding will primarily be devoted to support the expansion of the EMBRACE program at DFCI through CRC support to assist with the following components: 1) implementation of return of results from tumor testing and trial matching in DFCI satellites, 2) development and distribution of patient surveys and 3) continued effort to more fully integrate Embrace into our breast oncology clinic.

No Num Available (Lin) 06/04/19-09/25/20 0.24 CM
Merck, Sharp & Dhome Corp. $172,127
MSI prevalence epidemiologic study at Dana-Farber Cancer Institute
The aims of this project are 1) to validate a bioinformatics algorithm to impute mismatch repair deficiency (MMR-D) status based upon somatic tumor testing using the Dana-Farber Cancer Institute (DFCI) Oncopanel assay, 2) to describe the prevalence of mismatch repair deficiency (MMR-D) among adult and pediatric patients diagnosed with solid tumors, as assessed by this algorithm, 3) to describe outcomes to immunotherapy in patients with MMR-D tumor status, and 4) to explore other associated genetic alterations that impact upon response to immunotherapy.

7c. What other organizations were involved as partners? *Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research,*

exchanged personnel, or otherwise contributed.If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS
Independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks must be clearly marked with the responsible PI and research site. A report must be submitted to https://ers.amedd.army.mil for each unique award.

Independent reports are being submitted by each PI.

9. APPENDICES *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

None.