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PRINCIPAL INVESTIGATOR: Jean Jiang

RECIPIENT: University of Texas Health Science Center at San Antonio
San Antonio, TX 78229-3900

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Jean Jiang

E-Mail: jiangj@uthscsa.edu

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14. ABSTRACT

The long-term goal of our research is to develop a first-in-class therapy in the treatment of breast cancer bone metastasis. Our central hypothesis is the activation of Cx43 hemichannels in osteocytes by antibody lead to the suppression of metastatic breast cancer in bone. We propose to develop a new antibody immunotherapy for breast cancer bone metastasis, reduce the disease-associated symptoms and improve overall survival rate of the patients. We developed a monoclonal antibody (mAb2) against Cx43 that activates Cx43 hemichannels in osteocytes and inhibits breast cancer bone invasion and growth in mouse models. During our first year of this award, we conducted comprehensive examination of the therapeutic potency of mAb2 as proposed in the Specific Aim 1 in subtypes of breast cancer using intracardiac and syngeneic bone metastatic models. Data obtained suggests mAb2 not only reduces the chance of tumor metastasizing to bone tissue, but greatly improves life span. Additionally, the reduction of tumor growth in bone was also observed not only in triple negative breast cancer, but also in an ER-positive breast cancer model. In some cases, long term treatment of mAb2 leads to total tumor regression. Moreover, administration of mAb2 enhanced immunity by increasing populations of T lymphocytes known to suppress tumor. Taken together, these data establish the efficacy of mAb2 when administrated systematically.

15. SUBJECT TERMS

Breast cancer bone metastasis, Cx43 hemichannel, monoclonal antibody, drug efficacy, survival rate, affinity maturation

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1. INTRODUCTION

There is an urgent need to identify novel targets and develop new, specific therapies with improved therapeutic efficacies but less toxicity. Our team recently established connexin (Cx) 43 hemichannel as a de novo drug target for breast cancer bone metastasis. More importantly, we have developed a monoclonal, humanized antibody against Cx43 and have shown that activation of the hemichannels by this antibody inhibits breast cancer cell migration/invasion in vitro and bone metastasis in vivo with minimal toxicity. The objective of the proposed studies is to assess and optimize the therapeutic value of this novel, humanized antibody that represents a first-in-class therapy for breast cancer bone metastases which remains an unmet medical need. We will conduct preclinical studies on the optimized antibody therapy in the treatment of breast cancer bone metastasis. Below, we present data obtained during from experiments performed in this grant during the past year (September 30, 2017 through September 29, 2018) that support the efficacies of this antibody in suppression of breast cancer bone metastasis and improvement of animal survival rate. Moreover, we observed the effect of antibody on improvement of cancer immunity.

2. KEYWORDS:

Breast cancer bone metastasis, Cx43 hemichannel, monoclonal antibody, drug efficacy, survival rate, affinity maturation

3. ACCOMPLISHMENTS:

- **What were the major goals of the project? (**from grant)

The long-term goal of our research is to develop a first-in-class therapy in the treatment of breast cancer bone metastasis. Our central hypothesis is the activation of Cx43 hemichannels in osteocytes by antibody lead to the suppression of metastatic breast cancer in bone. We propose to develop a new antibody immunotherapy for breast cancer bone metastasis, reduce the disease-associated symptoms and improve overall survival rate of the patients. We developed a monoclonal antibody (HC2) against Cx43 that activates Cx43 hemichannels in osteocytes and inhibits breast cancer breast cancer bone metastasis in mouse models. Three specific aims are proposed; 1) To comprehensively evaluate the therapeutic potency of HC2 antibody in bone metastasis of breast cancer by determining the efficacy of HC2 in subtypes of breast cancer using intracardiac and syngeneic-mammary fat pad bone metastatic mouse models; 2) To produce the antibody drug under Good Laboratory Practice (GLP) guidelines for preclinical studies. We will develop a process for scaled-up production of the humanized antibody and characterize the antibody under GMP guidelines; and 3) To conduct preclinical studies of the lead humanized antibody. We will then conduct preclinical studies by determining the antibody's pharmacokinetic/pharmadynamic (PK/PD) profile and performing toxicity assays in mice and in rhesus monkeys.

Major goals as stated approved SOW:

Specific Aim 1 – To evaluate the therapeutic potency of HC2 in bone metastasis of breast cancer.	**Timeline**	**% completion**
Major Task 1: Determine the efficacy of HC2 in subtypes of breast cancer bone metastasis	Months	
Subtask 1: Use intracardiac injection model		
a) Inject WT mice with Py8119 with various dosages	1-3	70%
b) Inject WT mice with Py8119 with various durations	4-5	100%
Milestone # 1: Efficacy on triple negative breast cancer in WT mouse models	5	85%
c) Inject nude mice with MD-MB231 at various dosages and durations	6-9	85%
d) Inject nude mice with BC cells with various dosages and duration	9-12	100%
Milestone # 2: Efficacy of HC2 on triple negative breast cancer	12	92%
e) Inject nude mice with ER+ T47D cells with various dosages and durations	13-15	0%
Milestone # 3: Efficacy of HC2 on ER+ breast cancer		0%
f) Inject nude mice with ER+/Her2+ ZR-75-1 cells with various dosages and durations. (note: we used ER+/Her2+ BT474 cells instead, detailed justification is provided in the next section)	16-18	50%
Milestone #4: Efficacy of HC2 on ER+/Her2+ breast cancer	18	50%
Subtask 2: Use syngeneic-mammary fat metastasis model		
g) Inject Balb/c mice with 4T1.2 cells. with various dosages and durations	19-24	30%
Milestone # 5: Efficacy on bone metastasis from mammary fat pad	24	30%
Specific Aim 2 – Produce HC2 antibody drug at GLP standard and determine various properties of HC2		
Major Task 2: Develop a process of scale-up production of HC2 antibody	1-24	
Subtask 1: Develop production cell line using CHO cells.	1-4	100%
Subtask 2: Develop fed-batch cell culture process for eventual large scale production.	4-9	100%

Subtask 3: Develop downstream purification process for eventual large scale production.	3-10	100%
Subtask 4: Preformulation studies.	3-11	100%
Milestone #6: Generate a large quantify of HC2 at GLP standard.	11-17	100%
Major Task 3: Determine important characteristics of HC2 generated.		
Subtask 1: Determine antigen binding affinity, glycosylation, and biophysical properties (stability by heat denaturation, aggregation and deamidation).	13-18	0%
Milestone #7: Obtain results of characteristics of HC2.	13-24	0%

Specific Aim 3 – Conduct preclinical studies of HC2 antibody	**Timeline**	
Major Task 4: Conduct PK/PD studies	Months	
Subtask 1: Conduct bioanalysis of the antibody drug material	25-27	0%
Subtask 2: Conduct immunogenicity	28-30	0%
Subtask 3: Conduct formulation analysis	31-33	0%
Subtask 4: Conduct formulation analysis	31-33	0%
Subtask 5: Conduct single dose PK study in female rates and monkey	33-36	0%
Milestone #8: Obtain and analyze PK/PD data		0%
Major Task 5: Conduct general toxicity studies	Months	
Subtask 1: Determine general toxicity in mice	37-42	0%
Subtask 2: Determine general toxicity in Cynomolgus monkey	39-48	0%
Subtask 3: Tissue Cross Reactivity (TCR) of Test Article with human, monkey and rat tissues	42-48	0%
Subtask 4: Conduct formulation analysis	34-36	0%
Milestone #9: Obtain and analyze toxicity data		0%

Backup strategy	**Timeline**	
Major Task 6: Affinity maturation of HC2	Months	
Subtask 1: Construction of a HCDR3 phage library for affinity maturation of HC2	7-9	30%
Subtask 2: Panning of the HDR3 library for affinity improved antibodies	10-12	0%
Subtask 3: Test the affinity improved antibodies for in vitro functions	13-15	0%
Milestone #10: Obtain one affinity matured antibody named HC2-1		
Subtask 4: Construction of 2 HCDR3 and 2 LCDR3 phage libraries for affinity maturation of HC2-1	16-18	0%
Subtask 5: Panning of the four libraries for affinity improved antibodies	19-21	0%
Subtask 6: Test the affinity improved antibodies for in vitro functions	22-24	
Milestone #11: Obtain one affinity matured antibody named HC2-2		0%
Subtask 7: Construction of 2 HCDR3 and 2 LCDR3 phage libraries for affinity maturation of HC-2	25-27	0%
Subtask 8: Panning of the four libraries for affinity improved antibodies	28-30	0%
Subtask 9: Test the affinity improved antibodies for in vitro functions.	31-33	0%
Milestone #12: Obtain one affinity matured antibody named HC2-3.		
Major Task 7: Determine the efficacy of HC2-3 in subtypes of breast cancer bone metastasis	Months	
Subtask 1: Use intracardiac injection model		
a) Inject WT mice with Py8119 with various dosages	34-36	0%
b) Inject WT mice with Py8119 with determined optimized dosage and duration	37-38	0%

Milestone # 13: Efficacy on triple negative breast cancer in WT mouse models	39	
c) Inject nude mice with MD-MB231 with various dosages (5, 15 and 25 mg/kg) and durations	40-43	0%
Milestone # 14: Efficacy of HC2 on triple negative breast cancer	44	0%

What was accomplished under these goals?

Specific Aim 1 - To comprehensively evaluate the therapeutic potency of HC2 antibody in bone metastasis of breast cancer by determining the efficacy of HC2 in subtypes of breast cancer using intracardiac and syngeneic-mammary fat pad bone metastatic mouse models. These models use ER+, ER+/Her2+, and triple negative breast cancer cells in immuno-compromised nude mice and 4T1.2 breast cancer cells in immuno-competent mice.

Major Task 1: Determine the efficacy of HC2 in subtypes of breast cancer bone metastasis

Subtask 1: Use intracardiac injection model

a) and b) Inject WT mice with Py8119 with various dosages and durations.
Milestone #1. Efficacy on triple negative breast cancer in WT mouse models

Progress: As described in the statement of work, the purpose of the first sets of experiments is to determine breast cancer metastasis using intracardiac injection model in WT with various dosages and durations. To support the animal efficacy studies, more than 450 mg of the Cx43 targeting antibody was produced in 9 production lots.

The antibody used in the animal efficacy studies is a human-mouse chimeric antibody in which the variable regions are humanized and the Fc regions are of mouse origin. This design is to minimize the anti-drug immunogenicity in mouse studies. For MH-mab-2 production, heavy and light chain expression vectors of MH-mab-2 are co-transfected to $2.5x10^6$ Expi293 cells/ml by mixing with PEI-Max at a ratio of 1:1:3 (heavy chain: light chain: PEI-max in micro gram). The transfected Expi293 cells are cultured at 37^{o}C, 95% humidity, 8% CO_2 and on shaking platform at 125 rpm. Enhancer-1 and Enhancer-2 are added into the culture 24 hours post transfection. The culture is fed on day-1 and day-3. After 7 days cultivation, supernatant is collected by centrifugation at 12,000 rpm for 30 min. The 0.45 µm-filtered supernatant goes to protein-A column equilibrated with 20 mM sodium phosphate pH 7.2. After twice washing steps, the MH-mab-2 is eluted from the column with 0.1 M glycine pH2.6 and neutralized with 1/10 volume of 1M Tris•Cl pH 9.0. The eluted MH-mab-2 is buffer-exchanged to PBS using Amicon ultra centrifuge filter, sterile with 0.22 µm filter and stored at -80^{o}C. Table 1 summaries the production of MH-mab-2.

To explore the mechanistic aspect of antibody interaction with its Cx43 antigen, we have initiated a project to obtain crystal structure of the antibody/Cx43 complex. Toward this objective, we have prepared 2.1 mg of the MH-mab-2 Fab fragment. Briefly, digestion of MH-mab-2 with immobilized Papain is performed in the presence of cysteine at 37^{o}C in a shaker overnight. The immobilized enzyme was separated from the digest by centrifugation at 1,000 g for 10 min. The Fab fragments were separated from undigested IgG and Fc fragments

using an Immobilized Protein-A column. Figure 1 shows the purified intact MH-mab-2 IgG and Fab fragment in reduced and non-reduced SDS-PAGE gel.

Table 1. Production of MH-mab-2.

#	Name	Total mg	Summary of delivery							
			Lot #	Tubes	Conc. mg/ml	Amount mg	ml/tube	Buffer	Sterile	Shipping date
1	MH-mab-2	108.2	Lot#180425	25.0	4.33	108.00	1.0	PBS	Yes	180425
2	MH-mab-2	149.1	Lot#170426	1.0	2.57	2.57	1.0	PBS	Yes	180511
			Lot#170503	31.9	2.51	79.91	1.0	PBS	Yes	180511
			Lot#180425	15.4	4.33	66.70	1.0	PBS	Yes	180511
3	MH-Mab2-Fab	2.1	Lot#180430	1.0	2.12	2.12	1.0	PBS	No	180511
4	MH-mab-2	89.3	Lot#171130	15.0	3.01	45.20	15	PBS	Yes	180731
			Lot#171206	12.0	3.68	44.10	12	PBS	Yes	180731
5	MH-mab-2	101.5	Lot#180821	20.6	4.04	83.15	1.0	PBS	Yes	180912
			Lot#180829	4.00	4.58	18.31	1.0	PBS	Yes	180912
	Total	450.2								

With sufficient supply of the MH-mab-2 antibody, we made major efforts in developing intracardiac injection mouse model in WT using Py8119 cells in first few months of the current funding year. We injected various amounts of Py8119 cells, 20,000, 50,000, 100,000, and 200,000 and found 50,000 cell injection was an optimal cell amount for injection. We have successfully established this model system with reliable reproducibility. We then implanted Py8119 breast carcinoma cells into the circulation via intracardiac route. We administrated via i.p. route of HC2 antibody at 25 mg/kg either 30 min after or 2 days before tumor implantation and continued

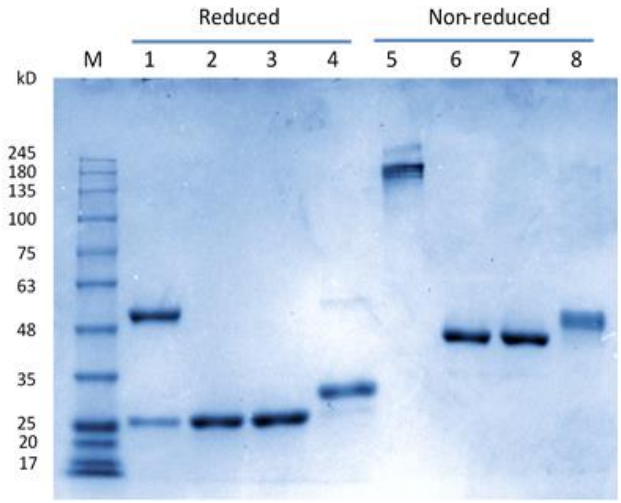


Figure 1. Purified intact MH-mab-2 IgG and Fab fragment in reduced and non-reduced SDS-PAGE gel. M, molecular marker standard. Lanes 1-4, reducing condition. Lanes 5-8, non-reducing condition. Lane 1, under reducing condition the intact IgG1 molecules is dissociated into two identical heavy chains with a molecule weight of ~50KD, and two identical light chains with a molecule weight of ~25KD. Lanes 2&3 under reducing condition the Fab fragment is dissociated into one heavy chain consisting of the variable region (VH) and constant region 1 (CH1) and one light chains with a molecule weight of ~25KD each. Lane 4, under reducing condition the Fc region of the antibody which consists of the hinge region, constant regions 2 & 3 (CH2-CH3) is dissociated into two identical fragments with a molecular weight of 30KD. Lane 5, under nonreducing condition the intact IgG1 molecule has a molecule weight of ~160KD. Lanes 6&377 under nonreducing condition the Fab fragment has a molecule weight of ~50KD. Lane 8, under reducing condition the Fc region of the antibody has a molecular weight of 60KD.

injection weekly for up to 8 weeks. The tumor progression and metastasis of Py8119-Luc cells were monitored weekly by bioluminescence imaging. The life span and survival rate were determined (Fig. 2). The metastasis of Py8119 cells via intracardiac injection model was primarily observed in lung, bone and brain (Fig. 2A). There is no significant difference if we administrated HC2 concurrently with the injection of Py8119 cells and all mice died after 20 days. However, if we administrated the antibody 2 days before tumor implantation, the life span of HC2 treated mice was significantly increased from up to 20 days to over 40 days (Fig. 2B). Interestingly, compared to saline treated control, treatment of HC2 antibody restricted the extent of tumor metastasis to other tissues. About 10% mice survived much longer. In this model, we did not observe incidence of bone metastasis. These data demonstrated the efficiency of HC2 in suppressing Py8119 breast carcinoma cancer metastasis and improve life span in WT mice.

In the next funding period, we plan to treat these mice with HC2 at 5 and 15 mg/kg in Py8119 intracardiac injection model and completed this subtask.

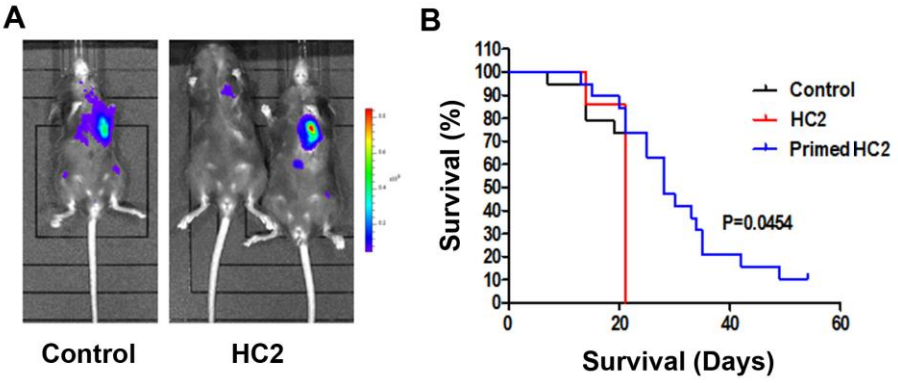


Figure 2. HC2 antibody administration reduced extent of Py8119 metastasis and improve life span. Py8119 (~50,000) cells were injected via intracardiac route and HC2 antibody was administrated either 30 min after or 2 days ahead of cancer implantation. The representative images of tumor cell distribution in the body are shown P=0.0454. (A). Animal survival rate was determined (B). n= 20 for control, n=7 for HC2 30 min after and n=19 for 2 days before cell implantation.

<u>c) Inject nude mice with MD-MB231with various dosages and durations. d) Inject nude mice with BC cells with various dosages and durations.</u>

Milestone # 2: Efficacy of HC2 on triple negative breast cancer

Progress: The purpose of this set of experiments was to determine human triple negative breast cancer cells using intracardiac injection model and two human breast cancer cell lines.

c) Inject nude mice with MD-MB231 cells. We first made major efforts in developing intracardiac injection mouse model in immune-compromised BalbC nude mice using a human triple-negative breast cancer line, MDA-MB-231 by injecting various amount of cells, 50,000, 100,000, 200,000 and 500,000 cells and identified 200,000 cells were the optimal cell amount for the proposed research. After we established this model system with reliable reproducibility, we implanted MDA-MB-231 human breast cancer cells into the circulation via intracardiac route. We administrated via i.p. route of HC2 antibody at 25 mg/kg 30 min after tumor implantation and followed by weekly injection for up to 6 weeks. The tumor progression and metastasis of MDA-MB-231-Luc cells were monitored weekly by bioluminescence imaging. The life span and survival rate were determined (Fig. 3). The metastasis of MDA-MB231 cells via intracardiac injection model was observed in lung, bone and brain (Fig. 3A). Similar to our observation for Py8119 cells, HC2 antibody treated groups tend to have restricted metastasis to other tissues. Even with concurrent injection of HC2 antibody with cancer cell implantation, the life span of HC2 treated mice was significantly extended from up to 50 days to more than 70 days (Fig. 3B). About 20% mice survived and lived with complete regression of tumor even after the stop of administration of HC2. However, there was no significant difference regarding the incidences of MDA-MB231 cells

metastasized to the bone between HC2 and control groups. These data demonstrated the efficiency of HC2 in suppressing extent of metastasis of triple negative MDA-MB231 human breast cancer and improve life span in nude mice.

In the next funding period, we plan to treat these mice with various dosages of HC2 at 5 and 15 mg/kg in MDA-MB231 intracardiac injection model and completed this subtask.

d) Inject nude mice with BC cells. During this funding period, we have successfully established triple negative BC metastasis model via intracardiac injection by injecting various amount of cells, 50,000, 100,000, 200,000 and 500,000 cells and identified 200,000 cells were optimal cell amount for the proposed research. We implanted triple negative BC human breast cancer cells into the circulation via intracardiac route. We administrated via IP route of HC2 antibody at 25 mg/kg 30 min after tumor implantation and followed by weekly injection for up to 6 weeks. The tumor progression and metastasis of BC-Luc cells were monitored weekly by bioluminescence imaging. The life span and survival rate were determined (Fig. 4).

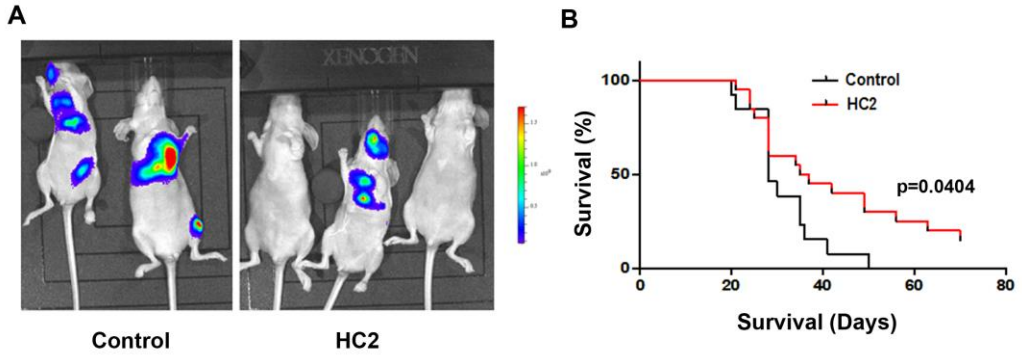


Figure 3. HC2 antibody administration reduced extent of MDA-MB213 metastasis and improve life span. MDA-MB231 (~200,000) cells were injected intracardiacally and HC2 antibody was administrated either 30 min after cancer implantation. The representative images of tumor cell distribution in the body are shown (A). Animal survival rate was determined (B). P=0.0404. n= 13 for control and n=16 for HC2 treated groups.

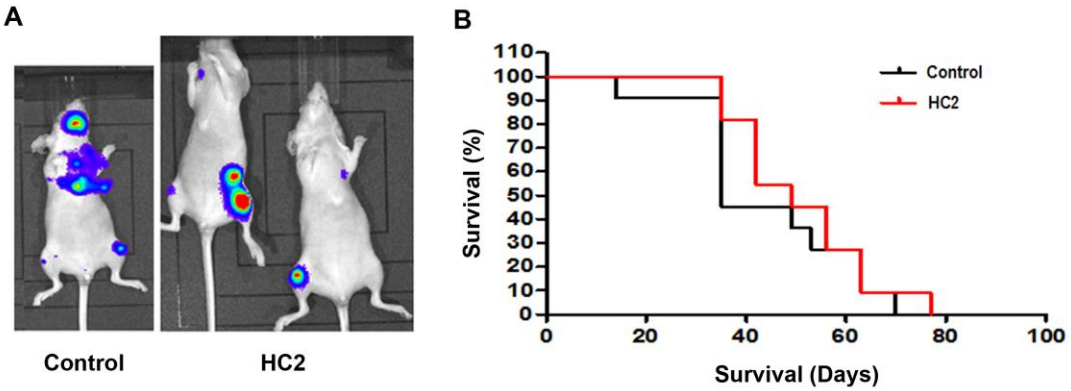


Figure 4. HC2 antibody administration reduced extent of BC metastasis, but has minimal effect during concurrent treatment. BC (MDA-MB1833) (~100,000) cells were injected intracardiacally and HC2 antibody was administrated 30 min after cancer implantation. The representative images of tumor cell distribution in the body are shown (A). Animal survival rate was determined (B). n= 15 for control and n=16 for HC2 treated groups.

The metastasis of BC cells via intracardiac injection model was observed in lung, bone and brain (Fig. 4). With concurrent injection of HC2 antibody with cancer cell implantation, the life span of HC2 treated mice was not significantly different from saline control (Fig. 4).

This subtask is completed.

f) Inject nude mice with ER+/Her2+ ZR75 cells. (We changed to ER+/Her2+ BT473 cells)
Milestone # 3: Efficacy of HC2 on ER+ breast cancer

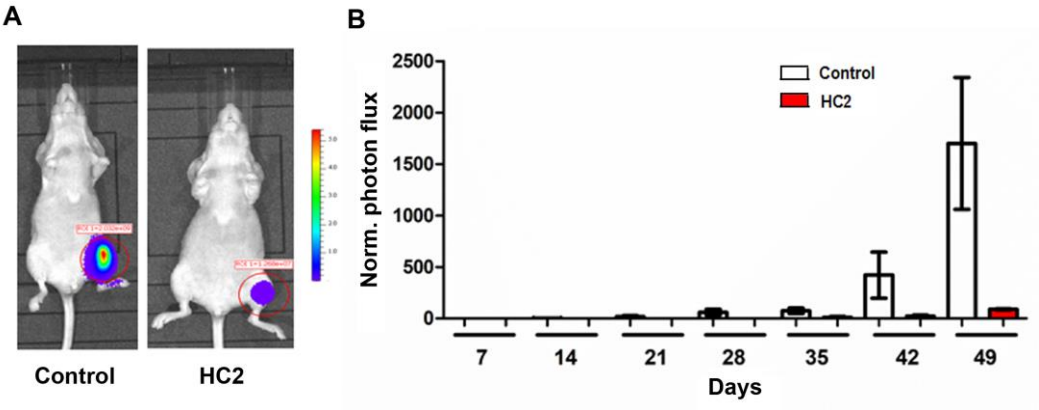


Figure 5. HC2 antibody administration suppressed BT474 tumor growth in bone. BT474 cells (~100,000) cells were injected intratibially and HC2 antibody was administrated either 30 min after cancer implantation. The representative images of tumor cells are shown (A). Tumor growth was imaged weekly and the size was quantified (B). n= 15 for control and n=16 for HC2 treated groups.

Progress: We could not establish stable ZR75-luc line stably expressing luciferase. We decided to use another ER+/Her2+ human breast cancer cell line, BT474 cells, which has been used to study breast cancer metastasis. Due to less aggressive feature of ER+/Her2 characteristics in breast cancer metastatic model, we decided to use intratibial injection to study tumor growth and metastasis in the bone. In this study, we first established the model by injecting various amount of BT474 cells, 20,000, 50,000, 100,000 and 200,000 cells and identified 100,000 as an optimal cell amount. We injected 100,000 BT474 cells into right tibia and at the same time, injected via i.p. route with 25 mg/kg HC2 once a week. The tumor progression and metastasis of BT474-Luc cells were monitored weekly by bioluminescence imaging. Our preliminary data showed that HC2 greatly suppressed ER+ BT474 human cancer cell growth in the bone tissue (Fig. 5B). At the meantime, it appears to protect bone tissue against bone resorption (Fig. 5C).

In the next funding period, we plan to increase animal numbers and determine the dosages of HC2, 5 and 15 mg/kg on BT474 cell in intratibial metastasis model and completed this subtask.

Subtask 2: Use syngeneic-mammary fat metastasis model

g) Inject Balb/c mice with 4T1.2 cells.
Milestone # 5: Efficacy on bone metastasis from mammary fat pad

Progress: We tried very hard to establish this synergic metastasis model originated from mammary fat pad. We implanted 4T1.2 cells into mammary fat pad with various amounts of cells, 50,000, 100,000, 200,000 and 500,000 cells. We observed that cell growth was concentrated around at mammary fat pad without any evidence of metastasis. When we removed the primary tumor from mammary fat pad, with original implantation at 500,000 cells, only lung metastasis was shown and animal died soon after. With lower amount, tumor appeared to spread to other tissues at the first week including bone, but at second week and beyond, cancer appeared to return to lung and animal died soon after.

In the next funding period, we plan to conduct caudal artery injection of this cell in the tail artery, an alternative model that has been used to study cancer bone metastasis and completed this subtask.

Other data – HC2 increases immunity against cancer

Progress: We observed that HC2 greatly improved survival rate and life span. We hypothesize that activation of Cx43 hemichannels in osteocytes by HC2 enhanced whole body immunity, leading to suppression of cancer and extension of life span. We isolated lymph nodes close to tibia where cancer cells were implanted. We showed that MDA-MB231 implantation significantly decreased T-helper CD4 and cytotoxic CD8+ lymphocytes, which are known lymphocytes direct attaching tumor cells. This reduction was mitigated by the treatment of HC2 antibody (Fig. 6). This data suggest that HC2 treatment improve immunity by increasing lymphocytes directly against cancer cell invasion.

In the next funding period, we plan to finish determining levels of lymphocytes in lymph nodes, tumor tissue and serum with implantation of Py8119 cells and HC2 administration in WT mice.

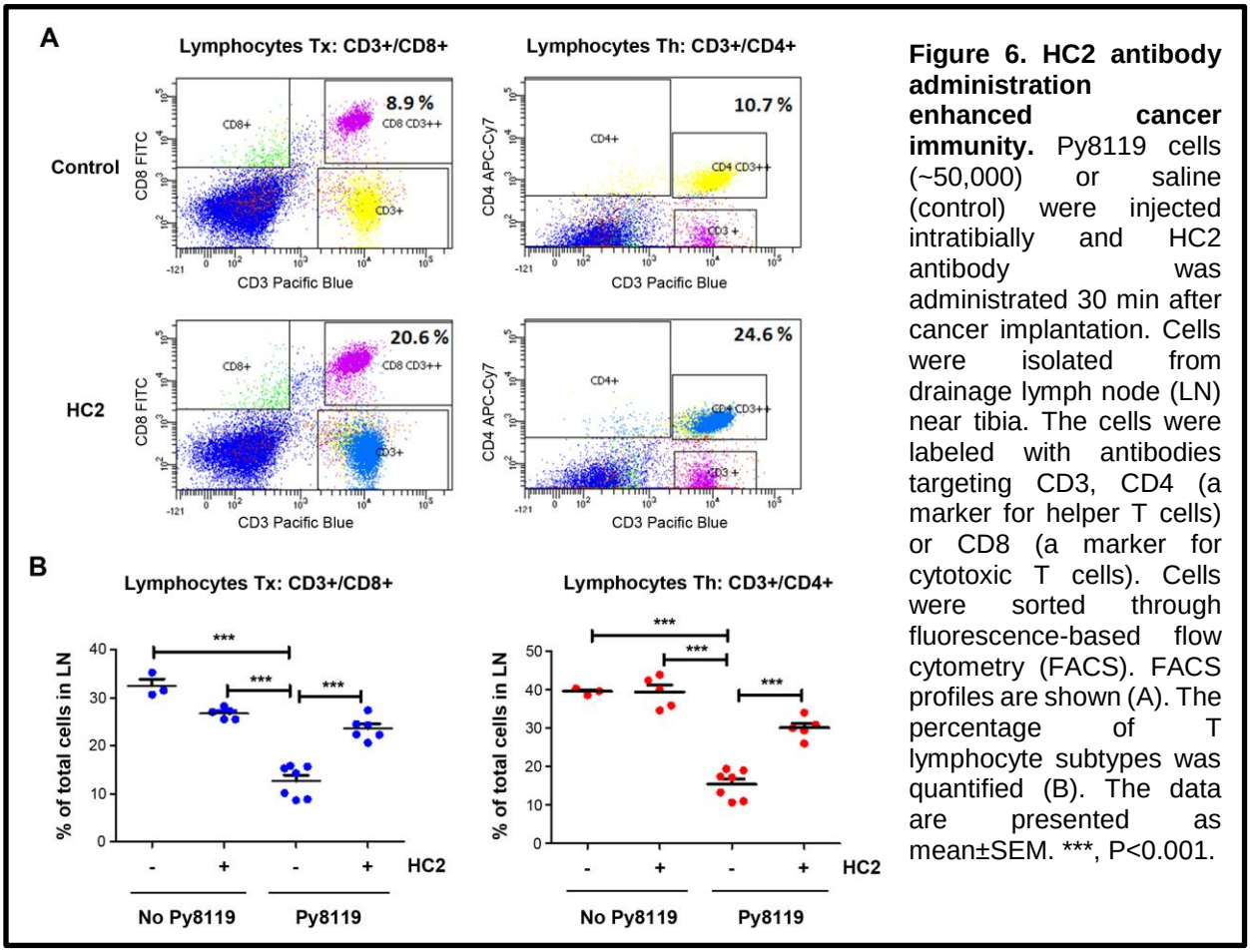


Figure 6. HC2 antibody administration enhanced cancer immunity. Py8119 cells (~50,000) or saline (control) were injected intratibially and HC2 antibody was administrated 30 min after cancer implantation. Cells were isolated from drainage lymph node (LN) near tibia. The cells were labeled with antibodies targeting CD3, CD4 (a marker for helper T cells) or CD8 (a marker for cytotoxic T cells). Cells were sorted through fluorescence-based flow cytometry (FACS). FACS profiles are shown (A). The percentage of T lymphocyte subtypes was quantified (B). The data are presented as mean±SEM. ***, P<0.001.

Specific Aim 2 - Produce HC2 antibody drug at GLP standard and determine various properties of HC2.

Before the antibody is tested for its efficacy in humans, a lengthy and costly preclinical development process needs to be completed. A significant portion of this DoD project is to achieve several major milestones in preclinical efficacy, biological, and biophysical studies. However, the funds are not able to complete chemistry, manufacturing and control (CMC) development IND enabling studies and further clinical trials. As such, we have engaged in a commercial partnership with AlaMab Therapeutics, a US registered company to co-develop this antibody-based drug for breast cancer patients. Under the joint development arrangement, we have the funding required for an industrial scale CMC program to bring the drug candidate to human trials and ultimately FDA approval. Toward this goal, our two institutions led by UT Health San Antonio have jointly established a partnership with AlaMab Therapeutics to co-develop the antibody therapy. This arrangement has been reviewed and approved by the UT Health San Antonio's Office of Sponsored Program and Office of Technology Commercialization Office. We have also reported this arrangement to Dr. Henry J. Nothnagel who is the project manager for this DoD project. Dr. Nothnagel is very supportive of this arrangement as it helps to achieve our ultimate goal to bring this new therapeutic to market to treat breast cancer patients with bone metastasis, an unmet medical need.

Milestone # 6: Generate a large quantify of HC2 at GLP standard. To supply the large quantity of the antibody drug needed for IND-enabling preclinical studies, a CHO cell line that stably produces the monoclonal antibody in high titer was generated. A fed-batch cell culture process was established for a CHO cell line that stably produces the monoclonal antibody at a titer of >2g/L. A downstream purification process for eventual large scale production was established using material from a fed-batch cell culture process for a CHO cell line that stably produces the monoclonal antibody at a titer of >2g/L. Prefrmauation studies have completed with a concentration of 25mg/ml.

Major Task 2: Develop a process of scale-up production of HC2 antibody

Subtask 1: Develop production cell line using CHO cells

Progress: In collaboration with AlaMab, WuXi Biologics which is the CRO for development of the CMC package has generated pools of CHO cells that stably express the antibody. Through a series of cloning selection, multiple clones were identified which are capable of expressing >2g/L. Purified MH-mab-2 IgG1 expressed by this stable CHO cell line is shown in reduced and non-reduced SDS-PAGE gel (Fig. 7). Briefly, through molecular biology cloning techniques, vectors carrying either the heavy or light chains of the antibody was introduced into CHO cells by transfection followed by clonal selection, adaptation in serum-free production media and screening for high-titer producing clones. Multiple transfections were carried out and antibody expression titer was determined by POROS/HPLC. Best cell pools were proceeded to further product analysis and cloning. Limiting dilution method were used for cloning. Cells were plated at~0.4-0.6 cells/well. Photos were taken on Day 0, Day 1, Day 2 and Day 7 to demonstrate monoclonality. Based on early expression data, top clones/pool were chosen for expansion and fed-batch cell culture analysis in shake flasks. The top clones were selected for product quality analysis and after product quality analysis, the top clones were selected and tested to ensure the cell clones are free of

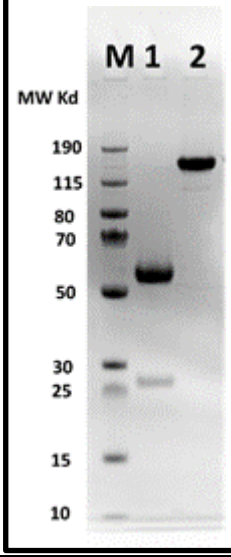


Figure 7. Purified MH-mab-2 IgG1 expressed by a stable CHO cell line in reduced and non-reduced SDS-PAGE gel. M, molecular marker standard. Lane 1, under reducing condition the intact IgG1 molecules is dissociated into two identical heavy chains with a molecule weight of ~50KD, and two identical light chains with a molecule weight of ~25KD. Lane 2 Lane 5, under nonreducing condition the intact IgG1 molecule has a molecule weight of ~160KD.

mycoplasma and other microbial contaminants (via Sterility assay). These final clones were subjected to cell culture expansion and bioreactor studies.

Subtask 2: Develop fed-batch cell culture process for eventual large scale production

Progress: A Pre-MCB (Master Cell Bank) (PCB) for top clones at 2 x 10^7 cells/vial. Product expression stability of the cell banks was evaluated. Growth kinetics, protein expression and product analytical characteristics were used to evaluate cell line stability. Small scale bioreactors were used to evaluate growth profiles of the top clones using a platform process in duplicate for each clone. Product quality assessment of the small scale bioreactor material and data from above were used to select the top and back-up clones. Upon clone finalization based on expression level, product quality, and cell line stability, an MCB under cGMP was prepared. Cells were filled in cryovials at 2.0 x 10^7 cells/vial and stored in liquid nitrogen (vapor phase). Cell culture, purification, and formulation development studies include media assessment and optimization, cell culture studies in bench-top culture system, purification resin assessment optimization, and purification optimization.

Subtask 3: Develop downstream purification process for eventual large scale production

Progress: Downstream purification process consists of two major tasks. The first is Resin Assessment Studies. Studies for yield, product purity, and to provide purification process using technologies and buffer formulations that are suitable for cost-effective operation at manufacturing scale was conducted. Material derived from the upstream cell culture studies were used for the downstream process development activities. Platform chromatography resins for capture and polishing steps to evaluate dynamic binding capacity and resolution was assessed. The resins that provide the best overall yield were chosen for future process development activities. The second task is Purification Optimization Studies. For further purification process development, experiments at small scale were conducted. Factors to address in the purification scheme include filtration/clarification process conditions (filter media, load, and flow rates), column load and elution conditions (pH, conductivity, buffer formulations, pooling criteria, pool volume, elution scheme), column packing, column size, and TFF/nanofiltration conditions (filter media, load, flow rate, TMP, concentration volume).

Subtask 4: Preformulation studies

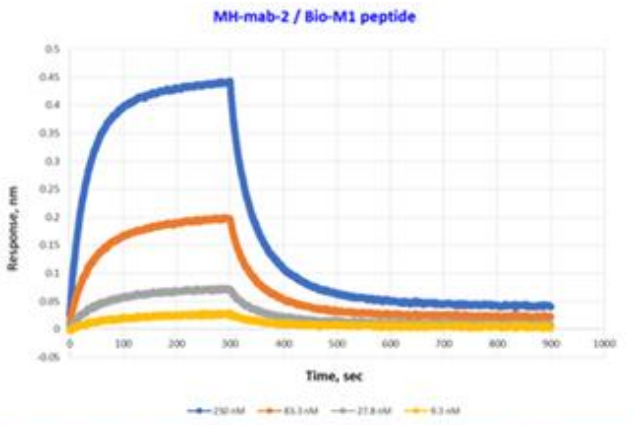
Progress: Using materials generated from small scale-up runs, WuXi developed preformulations using product as reference and evaluate solubility and stability of the formulation using biophysical tools including MicroCal DSC, DLS, etc, and stressed studies. Preformulation studies included buffer and pH assessment.
The formulations were stressed tested under the following stress conditions: 1. Heat (at 40° C or appropriately), 2. Agitation by vigorous stirring/mixing – comparison between static and motion at 25° C, 3. Freeze – Thaw (up to 5 cycles). Stressed materials were assessed using pH & Appearance (observation for changes in clarity, color or appearance), SEC-HPLC, CE-SDS, cIEF, UV Absorbance (A280)

Backup strategy
In this DoD project, we aim to develop humanized HC2 antibody into a drug therapy for the treatment of breast cancer bone metastasis. In general practice for drug development, the top drug candidate, in our case, HC2 will move forward to preclinical and subsequently, clinical studies. At the meantime, the backup drug candidate will also be developed. Here we plan to conduct antibody maturation and optimization process to generate a backup drug candidate. In this aim, we will optimize binding affinity and potency of the humanized antibody to further develop this antibody into a potential therapeutic drug.

Major Task 6: Affinity maturation of HC2

Subtask 1: Construction of a HCDR3 phage library for affinity maturation of HC2

Progress: Before the phage-displayed antibody libraries for affinity maturation are constructed, we first investigated the binding affinity of the antibody to its Cx43 antigen. Briefly, MH-mab-2 binding affinity is determined by Octet RED96. Biotinylated Bio-M1 peptide is applied to SA-biosensor (Streptavidin). The Bio-M1 peptide/SA-biosensors are incubated with different concentration of MH-mab-2 for association and then placed in kinetics buffer for dissociation. The binding affinity of MH-mab-2 to Bio-M1 peptide is calculated with fitting binding curve by a software provided by Octet



KD (M)	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2
1.15E-07	9.51E+04	1.09E-02	1.4217	0.9673

Figure 8. Binding affinity of the antibody to Cx43 M1 peptide.

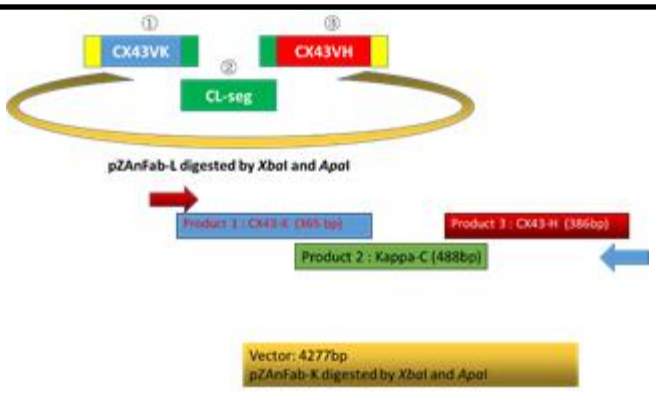
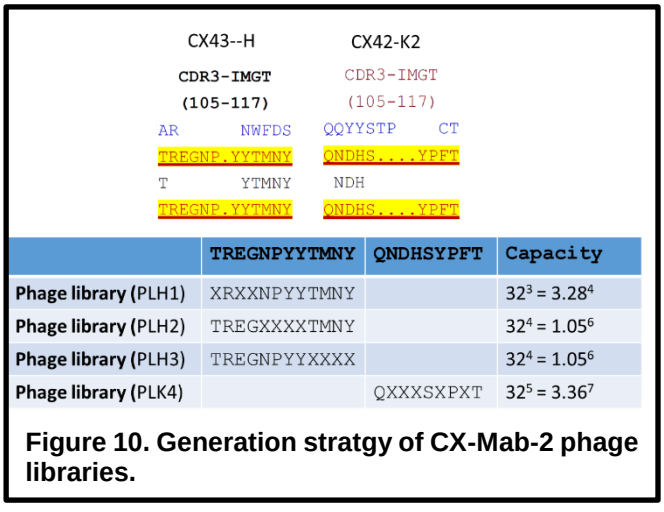


Figure 9. Construction of the plasmid of pZAnFab-CX43 for affnity maturation of the antibody by phage-display.

(Fig. 8). Our goal is to improve the Kd by >10 fold over the current Kd value of 115nM.

We then began to generate humanized HC2 by grafting the antigen binding sequences or complementarity determining regions (CDRs) of the antibody to the framework of a human IgG (humanization). We have successfully constructed a vector that is capable of displaying the antibody as a Fab fragment on phage surface or expressing soluble Fab proteins in the periplasmic space of *E. coli* (Fig. 9**).** We will improve affinity of the humanized antibody by a phage displayed antibody affinity maturation protocol. Briefly, amino acid residues of CDR regions of the antibody will be mutated and a large number of mutants in the range of > 1 x 10^9 will be generated in a phage library. Affinity improved antibody cloned will be identified by panning the phage library. The concept and methods of antibody affinity maturation are well established. The peptide that was used to generate the parental monoclonal antibody is identical for mouse and human. The binding affinity of the antibodies for the peptide will be determined and clones with high antigen binding affinity will be chosen for further characterization. We have designed three libraries which focus on the mutations in the heavy chain CDR3



	TREGNPYYTMNY	QNDHSYPFT	Capacity
Phage library (PLH1)	XRXXNPYYTMNY		$32^3 = 3.28^4$
Phage library (PLH2)	TREGXXXXTMNY		$32^4 = 1.05^6$
Phage library (PLH3)	TREGNPYYXXXX		$32^4 = 1.05^6$
Phage library (PLK4)		QXXXSXPXT	$32^5 = 3.36^7$

Figure 10. Generation stratgy of CX-Mab-2 phage libraries.

domain, and one library which focuses on the mutations in the light chain CDR3 domain (Figure 10).
In the original plan, we proposed to construct a pilot library to validate the protocol during the first year, and construct four libraries during the 2nd funding year. After careful planning, we focused the protocol development without the need of a pilot library.

Subtask 2: Panning of the HDR3 library for affinity improved antibodies

Progress: As described above, a pilot library was not constructed for the panning protocol validation. Four libraries are currently under construction, and we anticipate the completion of the library construction and the panning steps during the 2nd funding year as scheduled.

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

Dr. Jiang assisted and trained postdoctoral fellow Dr. Manuel Riquleme. Dr. Riquelme received his Ph.D. from Pontificia Universidad Católica de Chile, Chile. Dr. Riquelme had extensive experience in studying connexin channels in his graduate studies. Since joining the laboratory, he has gained extensive technical skills and experiences in cancer bone metastasis mouse models, cancer biology and research in developing antibody therapeutics. Dr. Riquelme presented his work at Metastasis Research Society (MRS) Meeting in Princeton in August, 2018 and won a poster award for young investigators. He has also presented his research at American Society of Bone and Mineral Research (ASBMR) Annual Meeting in October, Montreal, Canada and won a Minority Poster Competition Award, one of only 5 winners. He attends weekly lab meetings and seminars, and journal clubs in every two weeks. Dr. Riquelme interacts with various faculty members in our May's and MD Anderson Cancer including Professors LuZhe Sun (Cancer metastasis and stem cells), Zhiqiang An (antibody development and cancer therapeutics), Virginia Kaklamani (clinical research in breast cancer and breast cancer bone metastasis), Joel Michalek (statistical analysis in cancer research), Andrew Brenner (Breast cancer metastasis) and Taylor Curiel (Cancer Immunology).

Dr. Jiang assisted and trained research assistant Hongyun Cheng. Ms. Cheng has helped maintaining and breeding mouse colonies, preparing reagents and supplies. She also helped preparing cancer cells for injection into animals, imaging analysis and data analysis. She attends weekly lab meetings and weekly seminar and journal clubs in every two weeks. Ms. Cheng interests with Dr. Jiang and Dr. Riquelme on a daily basis.

As Partnering PI of the project, Dr. An worked closely with Dr. Wei Xiong who is a scientist with expertise in antibody engineering and production. Dr. Xiong carried out antibody expression and production in mammalian expression system (HEK293) during the year. Dr. Xiong has initiated to develop stable CHO cell lines for large scale production of lead antibodies. Before joining UTHealth, Dr. Xiong spent six years as a Senior Scientist in Department of Biotherapeutics and Antibody Discovery, Lexicon Pharmaceuticals Inc., Woodlands, Texas. While at Lexicon, he mastered several key antibody technologies such as antibody production in mammalian expression systems and screening of phage display libraries, humanization of antibody sequences. Dr. Xiong presented his work at the joint project team meetings rotating between Houston and San Antonio.

Dr. Xiong interacted frequently with Dr. Jiang who is the initiating PI of the project, and members of Dr. Jiang's team on project related technical, material, and scientific matters.

Dr. An recruited Dr. Yi Du into the project as an instructor. Dr. Yi obtained his Ph.D. and Postdoctoral Fellow training in Dr. Mien-Chie Hung's laboratory in the Department of Molecular and Cellular Oncology at UT-MDACC. During his Postdoctoral training, he investigated the mechanism of PARP inhibitor resistance in triple negative breast cancer. His findings demonstrated that receptor tyrosine kinase c-Met contributes to the resistance of cells to PARP inhibitor through direct phosphorylation of PARP1 at tyrosine 907. His work further demonstrated that combination of PARP and c-Met inhibitors is a potential therapeutic strategy to overcome PARP inhibitor resistance. These results were published in Nature Medicine in 2016. Dr. Du has developed strong knowledge and experience in cancer biology, biochemistry, molecular biology, and translational research through his extensive Ph.D. and postdoctoral training in Dr. Hung's laboratory. After joining the program, Dr. Du initiated the affinity maturation experiments for the lead antibody. Dr. Du presented his work at the joint project team meetings rotating between Houston and San Antonio. Dr. Xiong interacted frequently with Dr. Jiang who is the initiating PI of the project on technical and scientific matters.

- **How were the results disseminated to communities of interest?**

As detailed below, we have presented our preliminary results at three scientific meetings. Locally on Research Day for postdoctoral fellows here and Department of Biochemistry and Structural Biology Annual Retreat at UTHSCSA. Nationally, we attended the Metastasis Research Society (MRS) Meeting (Princeton, NJ), American Society of Bone and Mineral Research (ASBMR) Annual Meeting (Montreal, Canada), and the Keystone Symposia: Antibodies as Drugs: Translating Molecules into Treatments (Whistler Conference Center, Whistler, British Columbia, Canada). In addition, we have held two research progress meetings in December 2017 (San Antonio, TX) and June 2018 (Houston, TX). In the San Antonio meeting, two consumer advocates, Ms. Dale Eastman, a breast cancer survivor and current Chair of advocacy and Government Relations at Alamo Breast Cancer Foundation, and Ms. Denise Barlow, a breast cancer survivor, veteran and patient advocate attended the meeting and provided invaluable feedbacks of our research and their personal perspectives of community interests.

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

Our DoD project has two major objectives. The first one is to validate efficacy of the Cx43 targeting antibody in multiple clinically relevant animal models. We have made significant progress toward this goal by demonstrating efficacy of the antibody in several animal models. This is on-going effort and we will continue the animal studies in the 2nd year. As we have demonstrated efficacy during the 1st year, we will focus on our effort on dose response studies, which guide the dose selection in human clinical trial in the coming years. Another objective of the in vivo animal studies is to define potential biomarkers which will guide patient stratification during clinical testing of the antibody.

The second goal of the project is to develop an IND-enabling preclinical package for the experimental antibody therapy. By engaging a biotech company and collaborating with a CRO, we have made significant amount of the drug material for a series of downstream preclinical testing of the antibody including drug stability, formulation, PK/PD and safety assessment in animal species. These activities will continue into the 2nd, 3rd and 4th year of the project.

In addition to the above mentioned goals, we have also initiated a backup strategy by affinity maturing the leading antibody. An affinity improved antibody may be used to replace the current antibody, should the antibody encounters unforeseeable deficiencies during preclinical development which is an unlikely scenario. An affinity improved antibody will be used in both in vitro and in vivo studies as a comparator to the parent antibody in mechanistic, efficacy, and biomarker studies.

To ensure successful completion of the 2nd year goals, we will continue: 1. Regularly schedule team meetings rotating between Houston and San Antonio; 2. Attend local, national and international meeting by team members to keep up the rapidly developing knowledge in the field and to disseminate our findings to the larger scientific community; 3. Retain, recruit and train junior members of the team; and 4. Manage the complex collaborative arrangement among two universities, a biotech partner, and CROs.

4. IMPACT:

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

As stated in the original application, the results and understanding generated from this research project promise to have two major impacts, which could revolutionize the cure of patients with breast cancer. 1) The data gained will help generate an entirely new class of antibody therapy that targets to a novel mechanism with high efficacy and low toxicity. 2) The exciting collaboration of the researchers and experts in bone and cancer biology, antibody drug development and clinicians from academia, pharmaceutical industry offers a unique and practical advantage to move from the "bench to the bedside". More recently, we have developed a collaboration with a biotech company, which will help move this drug development from preclinical to clinical phases. The work completed thus far strongly suggest our drugs exhibit efficiencies in animal models; thereof, may ultimately be developed to treat patients with breast cancer bone metastasis. Our results also suggest that protocols we have developed are important models to study metastasis of various subtypes of breast cancer.

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

In the related study, we showed that the antibody treatment increased immunity that enhanced the ability of the whole body defensive system against cancer and increased life span. This research finding could have a significant impact on cancer treatment, just only in breast cancer bone metastasis but also in treating other cancer types.

- **What was the impact on technology transfer?**

Preclinical development of antibody-based drugs is very costly and the funds provided by the DoD grant helps to achieve several major milestones in efficacies and biological studies. However, the funds are not able to complete IND enabling studies and further clinical trials. We are engaging a commercial partnership with AlaMab Therapeutics to co-develop this antibody-based drug for breast cancer patients. Under the joint development arrangement, we believe that this collaboration between our two institutions, DoD and the commercial partner will achieve our ultimate goal to bring this new therapeutic to market to treat breast cancer patients with bone metastasis, an unmet medical need. This disclosure and request have been reviewed and approved by the UT Health and DoD.

- **What was the impact on society beyond science and technology?**

There are currently no FDA-approved treatments specifically targeting to breast cancer bone metastasis

5. CHANGES/PROBLEMS:

Nothing to Report

Changes in approach and reasons for change

There is a minor change in one of the animal studies. We decided to use human ER+/Her2+ BT474 cells instead of human ER+/Her2+ ZR75 cells. The technical reason is that we could not establish stable T47D-luc line stably expressing luciferase. BT474 cells, which has been established as a model to study breast cancer metastasis.

There is a minor change in the back-up strategy of developing an affinity improved antibody. In the original plan, we proposed to construct a pilot library to validate the protocol during the first year, and construct four libraries during the 2nd funding year. After careful planning, we focused the protocol development without the need of a pilot library during the first year. Instead, four libraries are currently under construction, and we anticipate the completion of the library construction and the panning steps during the 2nd funding year as scheduled. There will be no delay in the objective at the end of 2nd year.

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

We delayed lower dosage experiments primarily because of two reasons; first it took time to establish the reliable, systematic metastasis models; second, we need to obtain reliable efficiency

data under higher dosages before we pursue lower dosage assays. However, we already started working on some experiments planning for Year 2 as described in the research accomplishments. There will be no delay in the objective at the end of 2nd year.

Changes that had a significant impact on expenditures

None. All costs are been as expected.

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

Nothing to Report

6. PRODUCTS:

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

  Journal publications.

  *Graham, S.V., ***Jiang, J.X.,** and Mesnil, M. Connexins and pannexins: important players in tumorgenesis, metastasis and potential therapeutics. (2018) *International Journal of Molecular Sciences*. (*Co-first authors). 19, 1645

  Books or other non-periodical, one-time publications. Nothing to Report

  Other publications, conference papers, and presentations.

  August 4, 2018. Presented at Metastasis Research Society meeting, Princeton University, NJ

  Riquelme, MA, Gu, S., and Jiang, J.X. Osteocytic connexin hemichannels in suppression of breast cancer bone metastasis and potential therapeutic application.

  September 28, 2018. Presented at American Society for Bone and Mineral Research (ASBMR) meeting at Montreal, Canada

  Riquelme, M.A., Gu, S., An, Z., and Jiang, J.X. Suppression of breast cancer bone metastasis by osteocytic connexin hemichannels, a potential therapeutic target.

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

Nothing to Report

- **Technologies or techniques**

  Nothing to Report. The antibody used in this study was already protected intellectually prior to the grant award.

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

  Nothing to Report

- **Other Products**

  In this study, we are testing efficiency of this antibody and develop this reagent into therapeutics in treating breast cancer bone metastasis. As described above, the data we have collected thus far are supportive of anti-cancer efficacy of the reagent. Additional experiments will be performed in years 2-4 to further characterize and develop this potential therapeutic drug. We plan to publish these results to disseminate the information to the public.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Dr. Jean X. Jiang's team members.

Name	*Jean X. Jiang*
Project Role	*Principal Investigator*
Research Identifier	*n/a*
Nearest person month worked	*3.6*
Contribution to project	*Overall management of the project. Supervise and coordinate all experiments, help analyze the data, and prepare publications and presentation*
Funding Support	*NIH RO1AR072020, NIH RO1EY012085, NIHRO1CA196214, NIHRO1AG045040, AlaMab Therapeutics, Welch Foundation AQ-1507.*
Name	*Joel Michalek*
Project Role	*Co-investigator*

Research Identifier	*n/a*
Nearest person month worked	*0.6*
Contribution to project	*Help with the design of animal treatment studies with respect to sample size calculation*
Funding Support	*NIH P30 CA054174, NIH R01 HL111718*
Name	*LuZhe Sun*
Project Role	*Co-investigator*
Research Identifier	*n/a*
Nearest person month worked	*0.2*
Contribution to project	*Provide technical consultation on experimental design for bone metastasis studies with various subtypes of breast cancer cells*
Funding Support	*NIH P30 CA-54174, NIH R01CA179120, NIH R01CA192564, NIH R01CA196214, NIH T32CA148724*
Name	*Virginia Kaklamani*
Project Role	*Co-investigator*
Research Identifier	*n/a*
Nearest person month worked	*0.2*
Contribution to project	*Provide consultation on experimental designs including dosage and duration of the antibodies applied and study outcomes*
Funding Support	*NIH RO1AR072020, NIH RO1EY012085, RO1CA196214, RO1AG045040, Welch Foundation AQ-1507.*
Name	*Manuel Riquelme*
Project Role	*Postdoctoral Associate*
Research Identifier	*n/a*
Nearest person month worked	*12*
Contribution to project	*Conducting the in vitro and in vivo biological experiments including the design of individual experiments and collection of data.*
Funding Support	*None*
Name	*Hongyun Cheng*
Project Role	*Research Assistant*
Research Identifier	*n/a*
Nearest person month worked	*12*
Contribution to project	*Provide technical assistance to the proposed experiments including maintaining and breeding mice, preparing reagents and supplies, cell and tissue culturing, etc*
Funding Support	*None*

Dr. Zhiqiang An's team members.

Name	*Zhiqiang An*
Project Role	*Partnering Principal Investigator*
Research Identifier	*n/a*
Nearest person month worked	*2.8*
Contribution to project	*Working with Dr. Jean Jiang, initiating PI, on overall management of the project. Supervise and coordinate all antibody related experiments, help analyze the data, and prepare publications and presentation*
Funding Support	*CPRIT* RP150551 (PI), *DoD BC170897 (Co-I), NIH/NIDDK 5R01DK109001 (Co-I), Immune-Onc (Co-I), Merck (PI)*
Name	*Ningyan Zhang*
Project Role	*Co-investigator*
Research Identifier	*n/a*
Nearest person month worked	*2.8*
Contribution to project	*Manage and design antibody engineering aspects of the project*
Funding Support	*CPRIT* RP150551 (Co-I), *Immune-Onc (PI), Merck (Co-I)*
Name	*Yi Du*
Project Role	*Postdoctoral Associate/Instructor*
Research Identifier	*n/a*
Nearest person month worked	*6.3*
Contribution to project	*Conducting experiments related to affinity maturation of the lead antibody including design of the phage-displayed antibody maturation libraries.*
Funding Support	*None*
Name	*Wei Xiong*
Project Role	*Research Scientist*
Research Identifier	*n/a*
Nearest person month worked	*6*
Contribution to project	*Conducting experiments related to antibody engineering and expression, and antibody-antigen interaction.*
Funding Support	*None*

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

The following grants have been completed since last reporting period:

Sun

R01ES022057 08/23/12 - 4/30/17 20%

NIEHS/NIH
Effect of bisphenol A exposure on mammary stem cell function and transformation
The major goal of the project is to determine the effect of bisphenol A alone or in combination with irradiation or obesity on the function of murine and primate mammary stem/progenitor cells and on mammary tumorigenesis.
Role: PI

Kaklamani

Intelligent Automation, Inc. (Holt) 10/13/2016-06/18/2017 2%
NIH NCI
Contract HHSN261201600025C MIND
CAP: Monitoring for Cognitive Impairment and Dysfunction in Cancer Patients, #16-0036
Goal of the study is to find ways to evaluate cognitive impairment from chemotherapy (chemobrain) using a mobile app.
Role: Co-investigator

Cancer Center Pilot Project (Kist) 01/01/2017-12/31/2017 1%
A study attempting to improve the comfort during screening mammography, #16-0045
The aim of the study is to evaluate whether the use of topical lidocaine or calming music improve anxiety and pain related to mammogram
Role: Co-investigator

Cancer Center Pilot Project (Kaklamani) 01/01/2017-12/31/2017 1%
Factors influencing decision on prophylactic surgery in Hispanic high risk breast cancer patients of South Texas and their health care providers, CTMS #16-0095
The major goal of the study is to evaluate reasons behind the decision to not undergo prophylactic in women at high risk for breast cancer
Role: Co-investigator

Cancer Center Pilot Project (Kaklamani) 01/01/2017-12/31/2017 1%
A Phase 0, Investigator Initiated Study, Evaluating the Impact of Omega 3 Free Fatty Acid Supplementation on Breast Cancer, CTMS #16-0119
The aim of the study is to evaluate whether omega 3 fatty acids can decrease the amount of inflammatory markers in women with early stage breast cancer
Role: Co-investigator

Cancer Center Pilot Project (Kaklamani) 01/01/2017-12/31/2017 1%
Evaluation of Cryotherapy and TRPA1 Receptors in Chemotherapy Induced Neuropathy, #17-0033
The goal of the study is to evaluate whether the use of cold gloves can decrease the incidence of neuropathy in women receiving taxane based chemotherapy for breast cancer
Role: co-investigator

An

CPRIT RP150230 3/1/2015-2/28/2018 15%
Counteracting tumor evasion of antibody immunity by a novel therapeutic strategy
In this proposal, we hypothesize that hinge cleavage of antitumor antibodies impairs host humoral immunity against cancer, and that anti-hinge antibodies can rescue the impaired Fc functions of antitumor antibodies.
Role: PI
Potential Overlap: none

The following grants have become active since last reporting:

Jiang

RO1 (AR072020) 08/01/2018-07/31/2023 20%
NIH/NIAMS
"Connexin channels in transducing mechanical signals in bone"
The major objective of this grant is to determine the specific mechanistic role of Cx43 hemichannels in mediating the anabolic effect of mechanical loading on the skeletal tissues
Role: PI
Potential Overlap: none

Research funding
AlaMab Therapeutics 12/01/2017-11/30/2020 15%
"Development of therapeutics for disease Indications"
The major objective of this grant funding is to determine antibody efficacies in neurological and bone disease indications.
Role: PI
Potential Overlap: none

Sun

R21CA198389-01A1 Ye (PI) 08/01/2017-07/30/2019
NCI/NIH
Noninvasive Detection of Prostate Cancer with a Label-Free Imaging System
The main objective is to use cell refractive indices (RI) as a native contrast parameter for label-free, noninvasive detection of prostate cancer. A novel imaging system based on a patented photonic crystal biosensor will be designed and constructed for label-free detection of malignant prostate cancer cells from urine with unparalleled sensitivity and specificity.
Role: Co-Investigator
Potential Overlap: none

1F32CA228435-01 Zeballos (PI) 05/01/2018-08/31/2019 1%
NCI/NIH
The major goal is for the training of the postdoctoral fellow, Carla Zeballos, M.D., to obtain her Ph.D.
Role: Mentor

Potential Overlap: none

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
A duplicative report has been submitted by the Partner PI, Dr. Zhiqiang An.

QUAD CHARTS: Not applicable.

9. APPENDICES: None