AWARD NUMBER: W81XWH-14-1-0287

TITLE: The HGF/c-MET axis as a critical driver of resistance to androgen suppression in metastatic castrate-resistant prostate cancer

PRINCIPAL INVESTIGATOR: Todd M. Morgan

CONTRACTING ORGANIZATION: University of Michigan
Ann Arbor, MI

REPORT DATE: October 2016

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
The HGF/c-MET axis as a critical driver of resistance to androgen suppression in metastatic castrate-resistant prostate cancer

In this Physician Research Training Award, the primary goal is to elucidate the role of the HGF/c-MET axis in metastatic castration-resistant prostate cancer. During the current funding period, we have confirmed an inverse relationship between MET expression and AR signaling in prostate cancer cell lines. Our data supports negative regulation of MET by AR signaling, and we found that AR signaling inhibition in AR-positive CRPC models increased MET expression and resulted in susceptibility to ligand (HGF) activation. Likewise, our work over the past year showed that MET inhibition was only effective in blocking cancer phenotypes in cells with MET overexpression. Using multiple AR-positive CRPC models, as detailed in the initial proposal, we found that combined MET inhibition and enzalutamide (AR antagonist) treatment was more efficacious than either inhibitor alone. These data support the concept that the MET pathway may be an key mechanism of resistance in men with CRPC who undergo potent androgen signaling inhibition with abiraterone or enzalutamide.

Prostate cancer, metastasis, androgen deprivation therapy, human growth factor, MET, enzalutamide, abiraterone, cabozantinib, circulating tumor cells, disseminated tumor cells
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>5</td>
</tr>
<tr>
<td>3. Accomplishments</td>
<td>6</td>
</tr>
<tr>
<td>4. Impact</td>
<td>14</td>
</tr>
<tr>
<td>5. Changes/Problems</td>
<td>15</td>
</tr>
<tr>
<td>6. Products</td>
<td>16</td>
</tr>
<tr>
<td>7. Participants &amp; Other Collaborating Organizations</td>
<td>19</td>
</tr>
</tbody>
</table>
Introduction

In this Physician Research Training Award, the primary goals are both to further my training as a surgeon scientist and to elucidate the role of the HGF/c-MET axis in metastatic castration-resistant prostate cancer. In so doing, the overarching goal is to develop a durable cure of prostate cancer through a deeper understanding of prostate cancer metastasis and mechanisms of therapeutic resistance. The career development component of the award involves training in molecular biology techniques and biomarker discovery, with close mentorship from Drs. Arul Chinnaian and Russell Taichman. The primary scientific aims of this grant are to: 1) Define the mechanisms by which the HGF/c-MET axis facilitates proliferation and viability of CRPC cells in vitro, 2) Define the role of the HGF/c-MET axis in supporting resistance to androgen suppression in mCRPC patients with and without next generation anti-androgen therapy, and 3) Identify the mechanisms by which the HGF/c-MET axis emerges as a mediator of acquired resistance to abiraterone therapy.
Keywords

Prostate cancer, metastasis, androgen deprivation therapy, human growth factor, MET, enzalutamide, abiraterone, cabozantinib, circulating tumor cells, disseminated tumor cells
Accomplishments

**Major Task 1:** Establish the response to enzalutamide in distinct PCa cell lines and determine the extent to which c-MET inhibition recovers androgen suppression. (months 1-18)

We neared completion of Major Task 1 during the second year of this grant. This task did result in a manuscript which was published earlier this year:


In this manuscript, we provided mechanistic evidence establishing the inverse relationship between MET expression and AR signaling and demonstrated that MET inhibition was only effective in blocking cancer phenotypes in cells with MET overexpression. The manuscript indicates a possible explanation for the failure of cabozantinib in the recently published COMET-1 trial and supports the potential for combined MET and AR blockade in mCRPC.

**Subtask 3:** Evaluate impact of c-MET/HGF axis inhibition using c-MET siRNA, anti-HGF neutralizing antibody, and exogenous HGF in PCa cell lines

We previously showed that MET is upregulated when AR signaling is inhibited. In order to see whether the reverse holds true (ie downregulation of AR when MET is overexpressed, we measured protein levels of AR and MET in stable MET overexpressing LNCaP cells. These results showed that overexpression of MET in AR+ LNCaP cells does not affect endogenous AR protein levels. Thus, regulation of AR and MET appears to be one-directional in AR+ CRPC, with suppression of MET by active AR signaling through possible posttranslational modification.

![Figure 1](image-url)

**Figure 1:** AR and MET expression assessed by Western blot analysis in LNCaP cells overexpressing MET compared to vector controls. No change in AR expression is observed with MET overexpression.
To further understand the impact of HGF/MET signaling on cell invasion in MET+ and MET- CRPC cell lines, LNCaP and PC3 cells were cultured in 10% CSS-RPMI medium with or without DHT (1 ng/mL) for 24 hrs. Invasion assays were performed and exogenous HGF was used to activate HGF/c-MET signaling. Anti-HGF neutralizing antibody was used to block HGF function. Cells (100000 cells per well for LNCaP, 20000 per well for PC3) were added and incubated in for 48 hrs and calcein AM (4 uM) were used to stain live cells. LNCaP cells (AR+/MET-) responded to HGF with increased invasiveness in the absence of DHT, and this impact was no longer observed in the setting of anti-HGF neutralizing antibody. In contrast, when AR signaling was maintained with DHT, so significant effect of HGF or anti-HGF was observed. In contrast, PC3 cells responded to HGF regardless of DHT administration, and anti-HGF had an inhibitory effect in both settings. Interestingly, anti-HGF resulted in lower than baseline invasion levels for PC3 cells.

**Figure 2:** Invasion assays were performed in MET- (LNCaP) and MET+ (PC3) prostate cancer cell lines using DHT, HGF, and anti-HGF neutralizing antibody to elucidate the interaction between AR and MET for driving the invasive phenotype in these cell lines.

**Subtask 4:** Assess whether c-MET siRNA, anti-HGF neutralizing antibody, and/or absence of HGF in the setting of enzalutamide administration recovers the effects of androgen suppression in vitro.

For this subtask, we sought to better understand the impact of HGF and anti-HGF neutralizing antibody on c-MET expression and AR signaling. VCaP cells were cultured with with 10% DMEM media for 24 hrs. DHT (1 ng/mL) was then added with or without MDV3100 (10 μM) for another 24 hrs, and conditioned media from HS5 bone marrow stromal cells were then used to treat samples in the presence or absence of anti-HGF neutralized antibody (100 ng/mL). Expression of phosphor-
ERK and c-MET were evaluated. As shown in the figure, p-ERK and c-MET expression increase with enzalutamide, which also suppresses PSA expression. In the presence of anti-HGF neutralizing antibody, c-MET and p-ERK expression are substantially suppressed with persistent suppression of PSA expression.

<table>
<thead>
<tr>
<th></th>
<th>DHT (1 ng/ml)</th>
<th>ENZA</th>
<th>CM (TME)</th>
<th>Anti-HGF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-ERK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c-MET</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Actin</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 3:** Exposure of VCaP cells to DHT, enzalutamide, conditioned media from HS5 cells, and anti-HGF neutralizing antibody to assess relationship of HGF/MET pathway and AR signaling activity in response to suppression of these pathways.

In order to further assess the interplay between enzalutamide and HGF, we cultured LNCaP cells with 10% CSS-RPMI plus DHT 1 ng/mL for 24 hrs and then treated with or without MDV-1000 (10 ug/mL) for 24 hrs. Invasion assays were performed with and without conditioned medium (CM) from the human bone marrow stromal cell line HS-5 and with and without enzalutamide. Additionally, anti-HGF neutralizing antibody was used to block HGF in the conditioned medium. Increased cell invasion was observed with CM, as expected, regardless of enzalutamide exposure. However, anti-HGF neutralizing antibody was only effective in the setting of enzalutamide administration, further confirming the central role of the HGF/MET axis in the setting of potent AR signaling inhibition.
**Figure 4:** Invasion assays were performed using LNCaP cells. Conditioned medium (CM) from the human bone marrow stromal cell line HS-5 increases cell invasion both in the presence and absence of enzalutamide. Anti-HGF neutralizing antibody inhibits the effect of CM only in the setting of enzalutamide, supporting the role of the HGF/MET axis as a mechanism of resistance to enzalutamide.

**Subtask 5:** Determine the interplay between cancer cell c-MET activity and the bone marrow microenvironment for facilitating resistance to enzalutamide using bone marrow stromal cells

In order to further evaluate the role of the microenvironment in potentially facilitating resistance to enzalutamide, we again used CM from HS-5 bone marrow stromal cells and evaluated MET pathway activity under varying conditions. PC-3 cells only expressed p-
MET in the setting of HGF, and this was reversed by anti-HGF antibody. p-ERK was also much more strongly expressed in the setting of HGF, indicating downstream pathway activity. While p-MET is generally not detectable in LNCaP cells, we saw increased p-ERK and p_AKT when enzalutamide was added in the presence of CM, and this was inhibited by anti-HGF antibody. These data suggest that BMSCs can promote MET pathway activity in MET-PCa cells in the setting of enzalutamide.

**Figure 5:** MET pathway activity was assessed via Western blot in PC-3 and LNCaP cells using CM from the human bone marrow stromal cell line HS-5 and in the presence/absence of enzalutamide, HGF, and anti-HGF antibody.

**Major Task 2:** Isolation and characterization of CTCs and DTCs in patients with mCRPC on conventional ADT or enzalutamide

**Major Task 3:** Assess interplay between tumor HGF/c-MET pathway activity and response to abiraterone, and identify potential mechanisms of abiraterone resistance

As mentioned in the 2015 progress report, the key analyses on these two tasks will take place over the final 2 years of this grant. We have obtained a total of 14 bone marrow aspirates from 11 patients. This puts us on a lower pace than we would like to reach our final accrual goals, however this is an area we are devoting substantial attention to in order to recruit more patients to this study. We have recently simplified the logistics for obtaining bone marrow aspirates, allowing us to perform these aspirates on the same day as their clinic visit rather than necessitating a separate return visit for patients to undergo this procedure. We remain optimistic that we will be able to reach our targets. That said, we have been successful at recruiting patients for blood draws and have obtained 33 blood samples for CTC analyses from 24 patients on abiraterone and 18 blood samples from 17 patients on enzalutamide. This is in addition to 20 patients with mCRPC progressing on conventional ADT.

To assess CTCs and DTCs, we have refined our ability to measure gene expression across a panel of 96 genes. For platform validation, 180 GFP labeled prostate cancer cells (PC3,
LNCaP, VCaP) were sorted into 5 mL whole blood from a normal donor. Cells were captured using an immunomagnetic bead enrichment and visualized by immunofluorescence. Cells were then counted to assess for recovery rate. Additionally, 300 PC3 & LNCaP cells were spiked into 5 mL of whole blood from healthy controls and captured by immunomagnetic bead enrichment, followed by staining with DAPI, CD45, and PCa-CT (antibody cocktail, PSMA, EGFR, and pan-cytokeratin). To validate gene expression after CTC isolation, 10 prostate cancer cells (PC3, LNCaP, VCaP) were spiked into 5 mL whole blood from a normal control. Gene expression was then expressed by qPCR to assess whether the expression pattern matched that of what was expected for PC3, LNCaP, and VCaP cells.

**Figure 6:** GFP-labeled LNCaP, VCaP, and PC3 cells were spiked into whole blood and retrieved using immunomagnetic bead enrichment.

**Figure 7:** 10 cells at a time from each cell line were spiked into 5mL of whole blood. Gene expression was measured after immunomagnetic bead selection (left panel) and compared with known baseline gene expression data (right panel) from each of these lines.

While we do not yet have enough patients to look at the subsets of patients on each treatment (eg. abiraterone vs enzalutamide), we have assessed gene expression from CTCs across an initial combined set of mCRPC patients. These results further confirm our ability to determine gene expression from CTCs and will facilitate further assessment.
of mechanisms to resistance to therapy as patient accrual increases and as the outcomes in
the cohort mature.

Figure 8: Heatmap illustrating gene expression of prostate cancer-related genes
determined from CTCs in mCRPC patients. Expression was normalized to internal
housekeeping genes for each patient and to average expression in a set of normal controls
in order to control for background contamination from lymphocytes.

Based on our initial analyses, CTC-based expression of AURKA (HR 3.40, 95% CI 1.47 – 7.85), WNT5a (HR 2.71, 95% CI 1.43 – 5.13), and BMP7 (HR 2.1, 95% CI 1.25 – 3.52) all appear to be strong predictors of mortality in this patient population. While MET and HGF expression were not prognostic in our initial cohort-wide analyses, we will be able to better test the study hypothesis with increased patient accrual.

Figure: Waterfall plots showing association between expression of AURKA, WNT5a,
and BMP7 in CTCs with early mortality among a cohort of patients with advanced
mCRPC.
Key Research Accomplishments

Research accomplishments:
- Publication of manuscript based on Aim 1 of this grant.
- Further confirmation of MET as a key mechanism of resistance in AR-negative prostate cancer
- Delineating impact of anti-HGF neutralizing antibody which diminishes invasion only in settings of AR-/MET+ prostate cancer in vitro.
- Demonstration of impact of conditioned media from bone marrow stromal cells in promoting cell invasion through the HGF/MET axis in the setting of potent AR suppression.
- Confirmation of the ability to reliably assess gene expression from small numbers of cancer cells present in whole blood.
- Assessment of CTCs and DTCs in increasing numbers of patients with mCRPC and continued active collection of patient data, facilitating our eventual goal of understanding mechanisms of resistance to therapy using a liquid biopsy approach.

Training accomplishments:
- Completion of R01 boot camp program at University of Michigan for training in grant writing
- Attended and presented at 2016 Biomarkers and Diagnostics World Conference
- Faculty at 2016 Future Directions in Urology conference
- Attended 2016 DOD IMPaCT meeting
- Attended 2016 SPORE prostate cancer workshop
- Continue as instructor/faculty of medical school/graduate courses

Conferences/journal clubs:
- Attend monthly prostate cancer seminars
- Meet with mentors (Drs. Taichman and Chinnaiyan) regularly to discuss research progress/career development
- Continue leadership/active roles in Urology Grand Rounds, GU tumor board, P01 collaborative conferences
- Presented at institution-wide Taubman Medical Research Institute Emerging Scholars Symposium (spring 2016)

Clinical responsibilities
- Continue Urology clinic
- Continue operative schedule

Professional accomplishments: Promotion to Associate Professor with tenure, awarded 2016 Prostate Cancer Foundation Challenge Award (co-PI)
Impact

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

We have continued to make significant progress to date honing in on a specific cancer promoting pathway, the HGF/MET axis, as a key mechanism of resistance to potent androgen signaling inhibition. We have shown that MET expression is only elevated and drives cancer phenotypes in cell line models of AR- prostate cancer. Our publication earlier this year provided a potential explanation for the failure of cabozantinib in the recently published COMET-1 trials, as most of the patients in the pivotal phase 3 trial likely had intact androgen signaling. Our data in Aim 1 provides a strong rationale for targeting MET only when the HGF/MET axis is driving disease progression, likely corresponding to the AR- setting. Our work in Aims 2 and 3 has led to the development of a CTC-based approach that appears to reliably assess gene expression in men with mCRPC. We have nominated AURKA, WNT5a, and BMP7 expression in CTCs as potential prognostic markers in mCRPC, and we expect further accrual to help shed additional light on the importance of the HGF/MET axis in patients progressing on abiraterone and enzalutamide.

2) What was the impact on other disciplines?

Nothing to report

3) What was the impact on technology transfer?

Nothing to report

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

Nothing to report
Changes/Problems

1) Changes in approach and reasons for change

Greater emphasis over the past year on CTC analyses over DTC analyses due to better accrual of blood samples compared to bone marrow aspirates. However, we anticipate a shift towards DTC analyses as bone marrow aspirate accrual increases.

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

Recruitment for bone marrow aspirates has been slower than anticipated. In addition to adding a $100 honorarium for patients who enroll in order to increase participation, we are now able to perform bone marrow aspiration on the same day as patients consent in clinic. Thus, a separate procedure visit will no longer be needed, making the logistics far simpler.

3) Changes that had a significant impact on expenditures

Nothing to report

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

Nothing to report

5) Significant changes in use or care of human subjects

Nothing to report

6) Significant changes in use or care of vertebrate animals.

Nothing to report

7) Significant changes in use of biohazards and/or select agents

Nothing to report
Products

Publications:


Submitted publications:


Presentations/abstracts:

- Montgomery, J.S., Morgan, T.M., Thelen-Perry, S., He, F., Lee, C.T.: A Phase I/II Trial of Prehabilitation in Patients Undergoing Cystectomy for Bladder Cancer, Annual


- Salami, Simpa Samuel; Udager, Aaron; Miller, Brady Garland; Morgan, T.M.: Characterization of urothelial carcinoma with seminal vesicle involvement in locally


• “Is the HGF/MET axis still a relevant target in advanced prostate cancer?” Fred Hutchinson Cancer Research Center, October 2015, Seattle, Washington

• “Panel Discussion: Management of High Risk Prostate Cancer (moderator)” North Central Section AUA, November 2015, Amelia Island, Florida

• “Making sense of the current prostate cancer biomarker landscape” North Central Section AUA, November 2015, Amelia Island, Florida

• “AR and MET: Is there a link?” Ninth Annual SPORE Prostate Cancer Program Retreat, March 2016, Ft Lauderdale, Florida

• “From Resistance Pathways to Liquid Assays” A. Alfred Taubman Medical Research Institute Emerging Scholars Symposium, April 2016, AATMRI, University of Michigan
Participants and Other Collaborating Organizations

1) What individuals have worked on the project?

<table>
<thead>
<tr>
<th>Name</th>
<th>Yugang Wang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role</td>
<td>Research Scientist</td>
</tr>
<tr>
<td>Person month worked</td>
<td>2</td>
</tr>
<tr>
<td>Contribution</td>
<td>Work on cell line experiments and processing of clinical samples</td>
</tr>
<tr>
<td>Funding</td>
<td>Departmental start-up funding</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Amy Gursky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role</td>
<td>Research Coordinator</td>
</tr>
<tr>
<td>Person month worked</td>
<td>2</td>
</tr>
<tr>
<td>Contribution</td>
<td>Work on patient recruitment, sample acquisition, database tracking</td>
</tr>
<tr>
<td>Funding</td>
<td>Myriad Genetics, Inc</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Therese Roth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role</td>
<td>Research Technician</td>
</tr>
<tr>
<td>Person month worked</td>
<td>2</td>
</tr>
<tr>
<td>Contribution</td>
<td>Work on cell line experiments</td>
</tr>
<tr>
<td>Funding</td>
<td>Prostate Cancer Foundation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Yuanyuan Qiao</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role</td>
<td>Post-doc</td>
</tr>
<tr>
<td>Person month worked</td>
<td>1</td>
</tr>
<tr>
<td>Contribution</td>
<td>Work on cell line experiments</td>
</tr>
<tr>
<td>Funding</td>
<td>Chinnaiyan lab, NIH</td>
</tr>
</tbody>
</table>

2) Changes in active other support:

There has been no change in the PI’s effort on the current award. The PI has completed the funding period on the SPORE Career Development Award and Myriad Genetics funding is complete. New active other support is as follows:

15-PAF04482 (Morgan) 07/01/2015-06/30/2017
0.60 Calendar Months
NCCN
Title: Tissue-based genomics for risk stratification in localized renal cell carcinoma
Role: PI

14-PAF05733 (Keller) 10/01/2015-09/30/2020
0.72 Calendar Months
NIH
Title: The Biology of Prostate Cancer Skeletal Metastases
Role: Co-I

15-PAF05436 (Morgan) 12/01/2015-11/30/2020
0.36 Calendar Months

NIH
Title: Reducing the Effects of Active Surveillance Stress, Uncertainty and Rumination thru Engagement in Mindfulness Education (REASSURE ME)
Role: PI

16-PAF03431 (Morgan) 01/01/2016-12/31/2017
0.60 Calendar Months
GenomeDx Biosciences Inc.
Title: Prospective Randomized Trial of Genomic Classifier Impact on Adjuvant Treatment Decisions in High-Risk Post-RP Patients
Role: PI

16CHAL05 (Taichman) 08/22/2016-08/22/2018
0.60 Calendar Months
NIH
Title: Mechanisms of PCa Relapse in Marrow
Role: Co-Investigator