AWARD NUMBER:
W81XWH-22-1-0523

TITLE:
Biomarkers of Response to Combination Cabozantinib + Nivolumab in Advanced Renal Cell Carcinoma

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REPORT DATE:
July 2023

TYPE OF REPORT:
Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2023	Annual	01Jul2022-30Jun2023

4. TITLE AND SUBTITLE

Biomarkers of Response to Combination Cabozantinib + Nivolumab in Advanced Renal Cell Carcinoma

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-22-1-0523

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Brigham and Women's Hospital, Boston, MD

8. PERFORMING ORGANIZATION REPORT NUMBER

9, SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Immune checkpoint inhibitors (ICI) have changed the treatment management of metastatic clear cell renal cell carcinoma (mccRCC). However, many patients do not experience durable benefit. Thus, we aim to explore the clinical utility of previously identified biomarkers selecting ICI+VEGF-TKI therapy vs. VEGF-TKI therapy alone using specimen collection from phase III trial CheckMate 9ER. For markers that we successfully replicate the associative effect, we plan on developing a predictive model for patients treated with ICI+VEGF-TKI that can help clinicians estimate the probability that an event will occur. Model replication will be performed using specimens and clinical data from clinical trial JAVELIN Renal 101. In addition, we will also explore the potential associations that were previously uncovered between RNA-based or protein-based biomarkers and resistance to ICI monotherapy that may be overcome when combined with an VEGF-TKI using trials HCRN and OMNIVORE.

15. SUBJECT TERMS
Clinical biomarkers
Immunotherapies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	10	USAMRDC

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1. INTRODUCTION:

Immune checkpoint inhibitors (ICI) have changed the treatment management of metastatic clear cell renal cell carcinoma (mccRCC). However, many patients do not experience durable benefit. Thus, we aim to explore the clinical utility of previously identified biomarkers selecting ICI+VEGF-TKI therapy vs. VEGF-TKI therapy alone using specimen collection from phase III trial CheckMate 9ER. For markers that we successfully replicate the associative effect, we plan on developing a predictive model for patients treated with ICI+VEGF-TKI that can help clinicians estimate the probability that an event will occur. Model replication will be performed using specimens and clinical data from clinical trial JAVELIN Renal 101. In addition, we will also explore the potential associations that were previously uncovered between RNA-based or protein-based biomarkers and resistance to ICI monotherapy that may be overcome when combined with an VEGF-TKI using trials HCRN and OMNIVORE.

2. KEYWORDS:

Clinical biomarkers; Immunotherapies; Systemic therapies – discovery and development; Translational Research

3. ACCOMPLISHMENTS:

What were the major goals of the project?
Below is a list of major tasks and subtasks according to Specific Aims outlined in the grant proposal's SOW. Accomplishments and updates at this time are detailed in **bold** for this first annual report.

Tasks according to Aims
Subtask 1: Submit documents for institutional IRB review.
Milestone #1: IRB approval received. **This has been completed.**
Subtask 2: Submit IRB approval and necessary documents for HRPO review.
Milestone #2: HRPO approval received. **This has been completed.**
Subtask 3: • To obtain individual level clinical data CheckMate 9ER (CM9ER), JAVELIN Renal 101, HCRN and OMNIVORE. **This has been completed.**
Specific Aim 1: To identify immune cell populations in the ccRCC microenvironment that predict response or resistance to ICI+VEGF combination therapy.
Major Task 1 (Aim 1a): To demonstrate that mildly exhausted $CD8^+$ tumor infiltrating lymphocytes predict response to ICI+VEGF therapy.
Subtask 4: To score PD-L1 immunohistochemistry (IHC) and to validate IF image analysis results; To extract RNA in tumor areas; To assess immunophenotype of $CD8^+$ $PD\text{-}1^+$ $TIM\text{-}3^-$ $LAG\text{-}3^-$ cells via multiplex IF and image analysis. Human Anatomical Substances (HAS) used: de-identified tissue tumor samples from CM9ER. **Prior to shipping the specimens, BMS took measures to ensure that the subset of patients who consented to FFPE collection during the trial were not different compared to the overall, intent-to-treat population, with respect to progression-free survival endpoint. For this purpose, survival analyses were produced and compared between subsets. Upon successfully completion of the analyses which showed that both cohorts shared similar event rates, BMS sent the unstained slides to the Signoretti Lab in May 2023.**
Subtask 5: To correlate IF biomarker with clinical outcome

Major Task 2 (Aim 1b): To discover novel immune cell types and/or pathways specific to immune cells that are associated with response or resistance to ICI+VEGF therapy using high-definition spatial proteomics (exploratory).
Subtask 6: To use MIBI to identify cell populations and interactions. Human Anatomical Substances (HAS) used: de-identified tissue tumor samples from CM9ER. **We are currently working with BMS to receive tissue samples for the high-plex proteomic studies.**
Subtask 7: To correlate novel immune cell types with clinical outcomes.
Milestone #3: Aim 1 completed; manuscript preparation and submission
Specific Aim 2: To identify gene signatures and genomic alterations that predict response to ICI+VEGF combination therapy in mccRCC
Major Task 3 (Aim 2a): To demonstrate that high levels of high T-effector and/or cell-cycle gene signatures predict response to ICI+VEGF combination therapy
Subtask 8: To perform RNA-seq/WES analysis of tumor samples to characterize molecular clusters of cell-cycles and T-eff signature. Human Anatomical Substances (HAS) used: de-identified WES/RNA samples used from CM9ER. **BMS sent WES/RNAseq data to the Choueiri/Braun Labs in April 2023.**
Subtask 9: To correlate levels of T-eff and/or cell-cycle gene signatures with clinical outcomes
Major Task 4 (Aim 2b): To demonstrate that high levels of myeloid cell-associated genes identify patients that are refractory to anti-PD1 monotherapy but can respond to ICI+VEGF combination therapy.
Subtask 10: To perform RNA-seq of tumor samples to assess myeloid cells. Human Anatomical Substances (HAS) used: de-identified WES/RNA samples from CM9ER and HCRN+OMNIVORE. **As noted in Subtask 8, WES/RNAseq data for the CM9ER trial has been sent to Choueiri/Braun Labs. With regards to data of the OMNIVORE trial, we observed that some samples were previously shipped to an off-site storage for logistical reasons. In a research meeting, it was determined that 96 frozen OCT-embedded samples were currently available for analyses. Additional steps are being taken to populate additional samples. Determination of the finalized inventory is currently ongoing and expected to be produced at the next meeting (end of July 2023). As for HCRN samples, WES and RNAseq data have already been generated by the Signoretti/Braun groups.**
Major Task 5 (Aim 2c): To demonstrate that loss of 9p21.3 in the context of immune cell infiltration identifies patients that are refractory to anti-PD1 monotherapy but can respond to ICI+VEGF combination therapy
Subtask 11: loss of 9p21.3 will be assessed via WES analysis of tumor and normal specimens. Human Anatomical Substances (HAS) used: de-identified WES/RNA samples from CM9ER and HCRN+OMNIVORE.
Milestone #4: Aim 2 completed; manuscript preparation and submission
Specific Aim 3: To develop and externally validate a model for prediction of PFS using biomarkers and conventional clinic-pathologic variables
Subtask 12: To develop a prognostic model for prediction of PFS at 6 month and at 12 month in patients treated with nivo+cabo from the CM9ER cohort, using clinic-pathologic variables and candidate biomarkers **(those that replicated in Aims 1 and 2a).** The most optimal model will be selected based on measures of predictive accuracy (discrimination, calibration, goodness of fit, reclassification). Exploratory endpoints will include OS. Model validation will be performed in the ave+axi arm from the JAVELIN cohort. Human Anatomical Substances (HAS) used: de-identified WES/RNA samples from CM9ER and JAVELIN. **WES/RNAseq data and clinical data for both the CM9ER and JAVELIN trials have been received and are available for the development and external validation of the predictive model.**
Milestone #5: Aim 3 completed; manuscript preparation and submission

What was accomplished under these goals?

We have obtained tissue specimens as well as WES and RNAseq data for correlatives and linked clinical data from the primary phase III trial (CM9ER), as previously planned in the SOW. Similarly, WES and RNAseq data as well as clinical data were obtained for JAVELIN Renal 101, which will serve as our replication cohort when the time comes. While the final number of specimens is still being determined for HCRN+OMNIVORE, we are still on-schedule as the use of these specimens will only come at the end of year 2 (as previously planned). Of note, WES and RNAseq data have already been generated for the HCRN trial.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

Within the next 12 months, we will proceed with the Aims and sub-aims as proposed in the SOW.

Specific Aim 1: To identify immune cell populations in the ccRCC microenvironment that resistance to ICI+VEGF combination therapy.

Major Task 1 (Aim 1a): To demonstrate that mildly exhausted $CD8^+$ tumor infiltrating lymphocytes predict response to ICI+VEGF therapy.

For this purpose, the Signoretti Lab will first proceed to and perform multiplex immunofluorescence staining of the tissue samples. The immunophenotype of $CD8^+$ $PD\text{-}1^+$ $TIM\text{-}3^-$ $LAG\text{-}3^-$ cells will be assessed by image analysis and validation by a pathologist. The biomarker will then be correlated with clinical outcomes using CM9ER. Scoring PD-L1 immunohistochemistry will also be performed. Results pf IF and ICH analyses will be correlated with clinical outcomes. We do not anticipate any issues at this point.

Major Task 2 (Aim 1b): To discover novel immune cell types and/or pathways specific to immune cells that are associated with response or resistance to ICI+VEGF therapy using high-definition spatial proteomics (exploratory).

For this task, the Signoretti Lab will obtain the tissue samples through a collaboration with BMS. They will then perform high-plex proteomic analyses to identify cell type and states as well as spatial interactions correlated with clinical outcomes using tissue samples CM9ER.

Specific Aim 2: To identify gene signatures and genomic alterations that predict response to ICI+VEGF combination therapy in mccRCC.

Major Task 3 (Aim 2a): To demonstrate that high levels of high T-effector and/or cell-cycle gene signatures predict response to ICI+VEGF combination therapy.

The Choueiri/Braun Labs will perform analyses of the RNAseq data from the CM9ER trial to determine molecular clusters of cell-cycles and T-effector signatures and then test their association with clinical outcomes.

Major Task 4 (Aim 2b): To demonstrate that high levels of myeloid cell-associated genes identify patients that are refractory to anti-PD1 monotherapy but can respond to ICI+VEGF combination therapy.

The Choueiri/Braun Labs will obtain RNAseq data from tumor samples from OMNIVORE trial. RNAseq data from the CM9ER trial and the pooled cohort of the HCRN and OMNIVORE trials will then be analyzed to test the hypothesis that high levels of myeloid cell-associated genes are associated with poor outcomes in patients from the HCRN and OMNIVORE trials but not in patients from the CM9ER trial.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS:
Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

6. PRODUCTS:
Nothing to report.

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

Other publications, conference papers and presentations.
Nothing to report.

- **Website(s) or other Internet site(s)**
  - Nothing to report.

- **Technologies or techniques**
  - Nothing to report.

- **Inventions, patent applications, and/or licenses**
  - Nothing to report.

- **Other Products**
  - Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The following individuals are either Principal Investigators, or have worked at least one person month per year on the project for the Year 1 reporting period:

- BWH

- Sabina Signoretti, Principal Investigator (decreased effort from .84 to .72 calendar months). She is responsible for the overall project at BWH.

- Aseman Bagheri, Research assistant (previously not listed is at 10 calendar months). She helped organizing the samples from the CheckMate-9ER trial.

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

Signoretti:

Completed:

Tissue-based predictive biomarkers for Cabozantinib therapy in metastatic renal cell carcinoma
5R21CA238053

Predictive biomarkers for nivolumab treatment in metastatic renal cell carcinoma
W81XWH1810481

Developing a Translation Pipeline for VHL Mutant Malignancies
U01CA236489

New:

Biomarkers of response to combination cabozantinib+nivolumab in advanced Renal cell Carcinoma
W81XWH2210523

Tissue-based biomarkers of anti-PD-1-based therapy in metastatic renal cell carcinoma
1R01CA266424

Mechanisms by which LSD1 Promotes Neuroendocrine Differentiation and Small Cell Lung Cancer
1R37CA269990

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Not applicable.

QUAD CHARTS:
Not applicable.

9. APPENDICES:
Not applicable