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TITLE: Molecular and clinical correlates with prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy

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CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy (TRT) builds upon the radiosensitivity of prostate cancer with the specific expression of PSMA. We hypothesize that there are patient (germline) and/or tumor molecular characteristics such as DNA repair defects and active AR signaling as well as clinical characteristics that are associated with response (or lack thereof) to PSMA-TRT. We hypothesize that quantitative molecular imaging assessment of PSMA expression will be associated with response to PSMA-TRT. We also hypothesize that PSMA-TRT generates an immune response that may be identified and associated with patient outcome. In this proposal, we will utilize our retrospective and prospective data and sample sets to: (i) assess genomic biomarkers and gene expression changes associated with outcome from anti-PSMA targeted radionuclide therapy; (ii) assess clinical parameters associated with outcome from anti-PSMA- TRT; (iii) assess PSMA expression as determined by PSMA molecular imaging associated with response to anti-PSMA –TRT; and (iv) evaluate generation of an immune response following anti-PSMA-TRT in association with clinical outcome.

This project addresses the overarching challenge to develop effective new treatments and address mechanisms of resistance and particularly addresses the Focus Areas of Imaging and Targeted Radionuclide Therapy and Therapy and Mechanisms of Resistance and Response. As it is clear that prostate cancer is a radiosensitive disease, and PSMA is highly and selectively expressed, but not all patients respond to PSMA TRT, this proposal will rapidly translate into clinical progress for men afflicted with advanced prostate cancer in the near term. Furthermore, such targeted therapy may lead to future cures for men with micrometastatic disease in the high-risk clinically localized or biochemically recurrent settings.

15. SUBJECT TERMS: NONE LISTED

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1. INTRODUCTION:

Prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy (TRT) is a promising new class of drugs for men with metastatic prostate cancer. PSMA is an ideal target because its expression is highly specific for prostate cancer, it is expressed by the vast majority of hormone naïve and castration resistant tumors, and its cell surface expression lends opportunities for both imaging and therapy. Based on promising data led by our team and others looking at PSMA radionuclide therapy, ^{177}Lu--PSMA-617 is now completing a phase 3 trial for men with castration resistant prostate cancer. While the field is excited and encouraged by anti-tumor activity, there is still much to learn about patient and/or tumor molecular characteristics associated with response (or lack thereof) to PSMA-TRT. In this study, we are evaluating genomic biomarkers, clinical features, and PSMA molecular imaging of prospective cohorts of men treated with PSMA radionuclide therapy on our clinical trials.

2. KEYWORDS:

Prostate cancer specific membrane antigen (PSMA), metastatic prostate cancer, radionuclide therapy, biomarkers, genomics

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Our goal is to define molecular biomarkers and clinical features associated with outcomes from anti-PSMA targeted radionuclide therapy. We are performing prospective and retrospective genomic analyses of archival tissues, plasma samples, and metastatic biopsies from patients treated on prospective anti-PSMA targeted radionuclide clinical trials, with correlation of genomics with outcomes including PSA and radiographic response, progression free survival (PFS), and overall survival. We are also correlating genomics with PSMA PET/CT imaging to evaluate genomic characteristics associated with PSMA low or PSMA-heterogeneous disease and resistance to PSMA radionuclide therapy. PSMA expression and other imaging parameters are being quantified and correlated with response and outcomes. Immune responses following anti-PSMA targeted radionuclide therapy are also being measured, including an assay to measure serologic immunoreactivity to a targeted panel of antigens before/after PSMA-TRT. Overall this project will provide unprecedented insights into the molecular mediators of response and resistance to PSMA targeted radionuclide therapies with broad implications for the field.

What was accomplished under these goals?

We have made the following progress on our Aims according to our SOW:

Aim 1: To prospectively and retrospectively assess genomic biomarkers associated with outcome from anti-PSMA targeted radionuclide therapy

Major Task 1-1: Characterize genomic landscape of prior patients with mCRPC treated with PSMA-TRT [Tagawa and Beltran]

In the initial portion of this Aim, we analyzed clinically available genomic alterations in our retrospective dataset. The subset analyzed with whole exome sequencing (WES) and the larger dataset analyzed across CLIA-approved next generation sequencing platforms were reported in presentations at major scientific conferences in 2019 as described in section 1b (Reportable Outcomes) above.

We have completed subtask 1 for this Aim.

Subtask 2 is underway.

Subtask 3 will occur following completion of subtask 2.

Major Task 1-2: Characterize genomic landscape of prospective patients with mCRPC treated with PSMA-TRT [Tagawa and Beltran]

Subtask 1 is partially complete and will be completed once accrual to the associated therapeutic clinical trials is completed.

Subtask 2 is partially complete and will be completed once accrual to the associated therapeutic clinical trials is completed.

Subtask 3 is planned following completion of subtasks 1 and 2.

Subtask 4 will occur following completion of subtask 3.

Progress to date: Because of the DNA damaging effects of ionizing radiation and of the relationship between the AR pathway and PSMA expression, we hypothesized that patients with germline or somatic gene alterations in DNA damage repair (DDR) pathways or DDR crosstalk pathways (AR, MYC) treated with PSMA-TRT may demonstrate differential treatment responses and outcomes. **Methods:** We examined a cohort of advanced PC patients with available germline (targeted) or/and somatic (targeted or whole exome) DNA testing, and clinical data (Halabi CALGB prognostic factors) and outcome. The Kaplan-Meier method and Cox regression analysis were used to evaluate the associations between mutations/copy number alterations (CNA) with PSA response (≥50%, ≥30%, any) and radiographic response, progression-free survival (PFS) and overall survival (OS). Stepwise forward-selection method was used in the multivariable regression model and p value for entry was set at 0.1. For final analyses, a $p \leq 0.05$ was used for statistical significance. We analyzed 53 patients treated with PSMA-TRT. 16 (30.2%) received ^{177}Lu-J591, 28 (56.6%) ^{177}Lu-PSMA-617, 4 (7.5%) both concurrently, 2 (3.8%) received ^{225}Ac-J591 (3 additional received more than 1 agent sequentially and are analyzed based upon 1st drug). 6 (11.3%) had pathogenic germline DDR mutations while 31 (58.5%) had ≥1 mutation/CNA in DDR genes. The most frequently affected DDR genes were: TP53 (n=21, 39.6%), BRCA2 (n=14, 26.4%), CHEK2

(n=10, 18.9%), FANCA (n=10, 18.9%), RB1 (n=9, 16.9%), ATM (n=5, 9.4%), ERCC5 (n=5, 9.4%), ERCC3 (n=3, 5.7%), ERCC2 (n=2, 3.8%), BRCA1(n=2, 3.8%), MSH6 (n=2, 3.8%), FANCD2 (n=2, 3.8%), FANCF (n=2, 3.8%). AR amplifications or resistance-mutations were found in 22 patients (41.5%), and MYC amplifications in 9 patients (16.9%). 19 (35.8%) patients had ≥50% PSA decline, 24 (45.3%) experienced ≥30% decline and 39 (73.6%) had any PSA decline following PSMA-TRT. 4 patients experienced a partial response while 18 had stable disease. Presence of BRCA2 inactivating mutations, deletions or losses was associated with any PSA decline (p=0.011). PFS was significantly longer in patients with RB1 deletion or loss (5 vs 3 mos, p=0.003). The presence of BRCA2 alterations was predictive of longer OS compared to wild-type patients (49 vs 17 mos, p=0.09). AR amplifications or resistance-mutations and MYC amplifications were both predictive of shorter OS (AR: 13 vs 63 mos, p=0.02; MYC: 8 vs 24 mos, p=0.06). On multivariate analysis, after adjusting for Halabi prognostic groups (low vs high risk), BRCA2 and AR alterations retained their significance as independent prognosticators of OS (BRCA2 HR 0.1 [0.02-0.42], p=0.002; AR HR 7.2 [2.09-25.14], p=0.002). **Conclusions:** Knowledge of molecular alterations in BRCA2, AR and RB1 genes may have potential utility for prediction of clinical outcomes in patients being considered for anti-PSMA targeted radionuclide therapies. We are expanding on these finding in larger cohorts.

<u>Aim 2: To prospectively and retrospectively assess clinical parameters associated with outcome from anti-PSMA targeted radionuclide therapy</u>.

Major Task 2-1: Associate clinical characteristics of patients with mCRPC with outcome from PSMA-TRT [Tagawa and Beltran]

Subtask 1 is complete.

Subtask 2 is partially complete and will be completed once accrual to the associated therapeutic clinical trials is completed.

Subtask 3 is underway in the retrospective cohort and will be completed in the prospective cohort once patient follow up on the associated therapeutic clinical trials is completed.

Progress: We evaluated 46 pts treated with PSMA targeted therapies between 2007-2018 after progression on at least two therapeutic lines, including abiraterone or enzalutamide (76.1%). 28 (60.9%) pts were Halabi high-risk group. PSA decline by at least 50% was observed in 34.8%, median PFS was 5.77 months (95% CI 4.33-7.28), and median OS was 19.15 months (95% CI 12.23-51.25). WES data (n=28) showed an incidence of *AR*, *BRCA1*, *BRCA2*, *ATM* alterations (copy number variations and point somatic mutations) in 71.4% (n=20), 11.1% (n=3), 29.6% (n=8), and 14.3% (n=4), respectively. Variables found with backward selection with AIC criterion for PFS and OS suggest significant clinical and molecular predictors of PFS/OS (Table 1). **Conclusion:** Knowledge of previous therapy with AR-directed drugs, baseline LDH, ALP, and *AR* and *BRCA1/BRCA2* alterations may have potential clinical utility in patients being considered for anti-PSMA

therapies.

Table 1. Predictors of PFS and OS in advanced prostate cancer patients treated with PSMA targeted therapy

Backward stepwise selection for PFS			**Backward stepwise selection for OS**		
Variable	**HR (95% CI)**	**P**	**Variable**	**HR (95% CI)**	**P**
Previous abi/enza	2.75 (0.93,8.08)	0.067	Previous abi/enza	6.78 (1.17,39.21)	0.032
Baseline LDH	1.01 (1.00,1.02)	0.003	Baseline ALP	1.02 (1.01,1.04)	<0.001
BRCA1 alteration	0.05 (0.01,0.53)	0.012	BRCA2 alteration	0.07 (0.01,0.53)	0.010
BRCA2 alteration	0.26 (0.09,0.76)	0.014	AR alteration	8.38 (1.26,55.84)	0.028

Aim 3: To prospectively and retrospectively assess PSMA expression as determined by PSMA molecular imaging associated with response to anti-PSMA targeted radionuclide therapy

Major Task 3-1: Associate clinical characteristics of prior patients with mCRPC with outcome from PSMA-TRT [Tagawa and Bander]

Subtask 1 is complete and has been reported in a presentation at a major scientific conference in 2019 as described in section 1b (Reportable Outcomes) above.

Subtask 2 is underway and will be completed once accrual and follow up on the associated therapeutic clinical trials is completed.

Aim 4: To evaluate generation of an immune response following anti-PSMA targeted radionuclide therapy in association with clinical outcome

Major Task 4-1: To assay serologic immunoreactivity to a targeted panel of antigens before/after PSMA-TRT and associate with outcome [Tagawa and Bander]

Collection of specimens for subtasks 1 and 2 is underway

Analysis will be completed once accrual to the associated therapeutic clinical trials is completed.

Major Task 4-2: To assay serologic immunoreactivity against a broad array of antigens before/after PSMA-TRT and associate with outcome. [Tagawa and Bander]

Subtask 1 was attempted, but unsuccessful due to loss/damage of old serum.

Collection of specimens for subtask 2 is underway
Analysis will be completed once accrual to the associated therapeutic clinical trials is completed.

Major Task 4-3: To assess immunogenic cell death following PSMA-TRT
Subtask 1 was attempted, but unsuccessful due to loss/damage of old serum. [Tagawa and Bander]
Collection of specimens for subtasks 2 and 3 is underway
Analysis will be completed once accrual to the associated therapeutic clinical trials is completed.

We previously obtained IRB approval to perform the work associated with the primary clinical trials. Subsequently it was determined that additional review that was separate from the clinical trial protocols was warranted in order to be reviewed by HRPO. The protocol entitled "Molecular and Clinical Correlates With Prostate-Specific Membrane Antigen (PSMA)–Targeted Radionuclide Therapy" was submitted to the WCM IRB and the study was determined to be IRB exempt. This protocol was subsequently submitted to HRPO for review. Initial parts of the study have been completed with initial data presented at 4 international scientific conferences as detailed below.

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

Trainees and fellows in our groups participate and lead analyses related to this study. Through meetings and interactions between scientific and clinical investigators, trainees and fellows are provided unique learning opportunities in translational research. Dr. Conteduca and Dr. Vlachostergios were first authors and presented abstracts on findings from this study at GU ASCO, AACR, and ASCO in 2019.

How were the results disseminated to communities of interest?

Results were presented as meeting abstracts at four national/international meetings in 2019 (GU ASCO, AACR, ASCO, and ESMO). We have also participated in meetings, seminars, and interviews to disseminate results including through the Prostate Cancer Foundation and UroToday.

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

The study tasks above are in progress and on track. We anticipate continued progress this year on optimizing assay performance and in our analysis of data. We plan to present new findings at. national /international meetings and to publish results.

4. IMPACT:

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

PSMA targeted radionuclide therapy is a promising drug approach for men with metastatic castration resistant prostate cancer. This study is providing new insights into molecular mediators of response and resistance to PSMA targeted radionuclide therapy, which may help in the future to select the patients most likely to benefit and to inform the development of effective combination strategies to prevent or target resistance mechanisms. The planned genomic analyses and correlation with imaging and immune markers will also provide new knowledge on tumor heterogeneity and how host responses impact therapy response and progression.

What was the impact on other disciplines?

Results from this project may provide insights into biomarker of response /resistance to radionuclide targeted therapies in other tumor types.

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

Due to multiple communication issues, HRPO approval was delayed (initial communication was that a separate protocol was not necessary). This has now been resolved.
Two subaims involved previously collected and frozen serum from subjects enrolled in prior clinical trials.
This serum was unfortunately lost and those subaims will not be able to be performed.

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

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

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents
Not applicable

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications. Nothing to report

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

Other publications, conference papers and presentations.

Vincenza Conteduca, Clara Oromendia, Panagiotis J. Vlachostergios, Amy Hackett, Charlene Thomas, Aidan Case, Jyothi Manohar, Kenneth Eng, Andrea Sboner, Karla V. Ballman, Olivier Elemento, David M. Nanus, Himisha Beltran, Scott T. Tagawa. Clinical and molecular analysis of patients treated with prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy. Presented at the 2019 Genitourinary Cancers Symposium, *J Clin Oncol* 2019

Panagiotis J. Vlachostergios, Vincenza Conteduca, Amy Hackett, Jyothi Manohar, Aileen Lee, Aidan Case, Michael Sun, Muhammad J. Niaz, Olivier Elemento, Ana M. Molina, David M. Nanus, Himisha Beltran, Neil H. Bander, Scott T. Tagawa. Prognostic value of BRCA2 and AR gene alterations in advanced prostate cancer patients treated with PSMA-targeted radionuclide therapies. Presented at the 2019 AACR Annual Meeting.

Panagiotis J. Vlachostergios, Muhammad Junaid Niaz, Seyed Ali Mosallaie, Paul J. Christos, Amy Hackett, Joseph R. Osborne, Yuliya Jhanwar, Lauren Gracey, Ana M. Molina, David M. Nanus, Neil Harrison Bander, Scott T. Tagawa. Association of noninvasive, radiographic measurement of prostate-specific membrane antigen (PSMA) expression with response to PSMA-targeted radionuclide therapy (TRT). Presented during poster discussion session of the 2019 ASCO Annual Meeting, *J Clin Oncol* 2019

S.T. Tagawa, J. Osborne, A. Hackett, M.J. Niaz, V. Cooley, P. Christos, P. Vlachostergios, C. Thomas, L. Gracey, H. Beltran, A. Molina, D.M. Nanus, J. Babich, S. Vallabhajosula, A. O. Sartor, K. Ballman, N.H. Bander. Preliminary results of a phase I/II study of fractionated dose 177Lu-PSMA-617 for progressive metastatic castration resistant prostate cancer (mCRPC). Presented in poster discussion session of 2019 ESMO annual meeting. *Annals of Oncology* 2019

Website(s) or other Internet site(s): Nothing to report

Technologies or techniques Nothing to report

Inventions, patent applications, and/or licenses Nothing to report

Other Products Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person

month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

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