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14. ABSTRACT

Prostate cancer (PCa) remains the most common malignancy and the second leading cause of cancer-related death for men in the United States. Every year tens of thousands of men in the US undergo treatment with of radiation therapy as a primary treatment for localized PCa. While results are often excellent, approximately one third will develop biochemical recurrence in 10 years and up to a third of men with biochemical recurrence will die of their disease, particularly men with higher stage and higher grade disease. In addition, thousands of men receive adjuvant radiation therapy following radical prostatectomy to try and prevent recurrence or for palliation of metastatic disease. Thus, improving outcomes of radiation therapy will have an immediate impact in the lives of men with PCa. In this proposal, we will determine the extent to which inhibition of two key prosurvival signaling pathways (RET and FGF receptors), alone and/or in combination, can enhance the effectiveness of radiation therapy.

15. SUBJECT TERMS

NONE LISTED

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1. INTRODUCTION: Prostate cancer (PCa) remains the most common malignancy and the second leading cause of cancer-related death for men in the United States[1]. Every year tens of thousands of men in the US undergo treatment with of radiation therapy as a primary treatment for localized PCa. While results are often excellent, approximately one third will develop biochemical recurrence in 10 years and up to a third of men with biochemical recurrence will die of their disease [2], particularly men with higher stage and higher grade disease. In addition, thousands of men receive adjuvant radiation therapy following radical prostatectomy to try and prevent recurrence or for palliation of metastatic disease. Thus, improving outcomes of radiation therapy will have an immediate impact in the lives of men with PCa. In this proposal, we will determine the extent to which inhibition of two key prosurvival signaling pathways (RET and FGF receptors), alone and/or in combination, can enhance the effectiveness of radiation therapy.

COVID-19 has had a major impact on our on our research. Like most research institutions, BCM shut down completely for many months. We proceeded with reopening but in stages with reduced staff hours. Not only was time lost for experimentation but we also had to freeze all cell lines before we left the lab such that they had to be regrown (which takes considerable time) and thus we actually went backwards. Animal experiments were also shut down and mouse numbers were severely restrained. While Houston was emerging from a very bad COVID-19 pandemic last year, the delta variant has now emerged so hospitalizations are climbing steeply. We have requested no cost extension to try and finish experiments for this project.

2. KEYWORDS: prostate cancer; nerve, RET, GDNF, GFRα1, FGF, lenvatinib

3. ACCOMPLISHMENTS:

A. <u>Major Goals</u>

Major Task 1: Obtain regulatory approvals (Months 1-4)

All regulatory approvals have been obtained and maintained

Major Task 2: Determine role of GDNF/ GFRα1/RET signaling in resistance to radiation therapy (Months 4-36)

Subtask 1: In vitro studies of impact of RET signaling on radiation resistance (Months 4-24)

We have carried out an extensive set of in vitro experiments to assess the impact of RET signaling on the response PCa cells to radiation. A subset of this data is presented below.

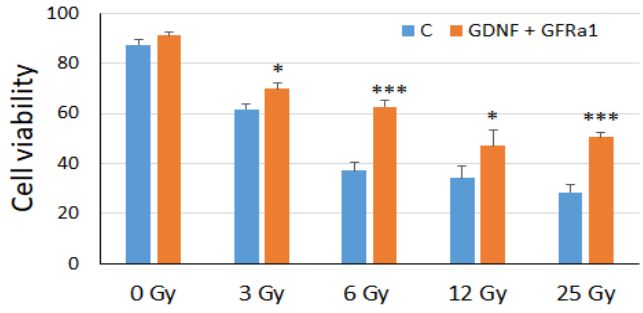


Figure 1. RET signaling enhances viability of LNCaP cells after radiation treatment. LNCaP cells received 3, 6, 12 or 25 Gy of ϒ-ray (Gammacell 1000) with or without the presence of GFRα1+ GDNF and cultured for 96 hr. Cell viability was evaluated with Trypan blue exclusion method. The cell viability is presented as mean percent ± SEM. *p<0.05; ***p<0.001 vs. vehicle control.

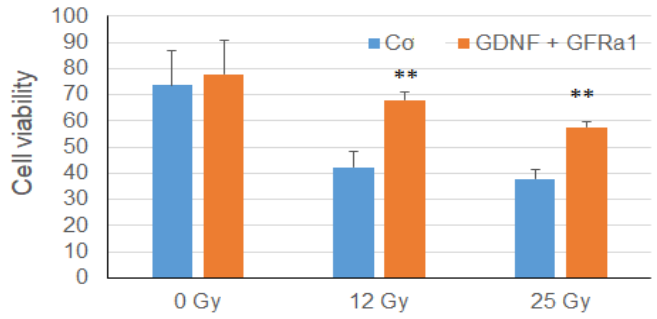


Figure 2. RET signaling enhances viability of 22RV1 cells after radiation treatment. 22RV1 cells received 12 or 25 Gy of ϒ-ray (Gammacell) with or without the presence of GFRα1+ GDNF and cultured for 96 hr. Cell viability was evaluated with the Trypan blue exclusion method. The cell viability is presented as mean percent ± SEM. **p<0.01 vs. vehicle control.

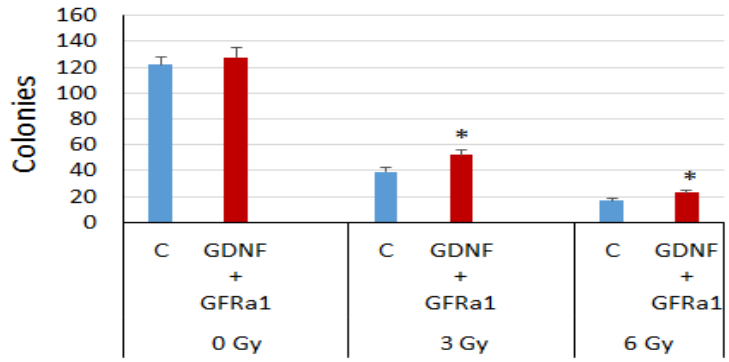


Figure 3. RET signaling enhances clonogenic survival of LNCaP cells after radiation treatment. 1000 LNCaP cells were seeded in 35 mm culture dishes for 16 hours and treated with or without GDNF and GFRα1 for 6 hours. Cells then received 0 Gy, 3 Gy or 6 Gy of ϒ-ray and cultured for 21 days. The colonies containing at least 50 cells were counted. The number of surviving colonies is presented as mean ± SEM. *p<0.05, vs. vehicle control.

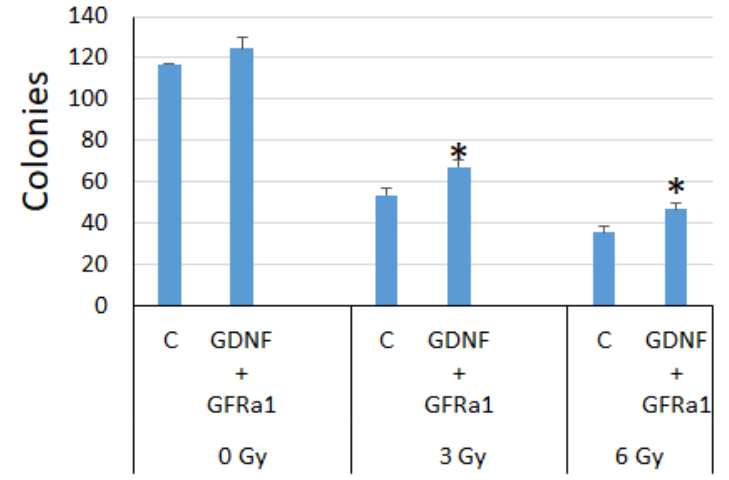


Figure 4. RET signaling enhances clonogenic survival of 22RV1 cells after radiation treatment. 1000 22RV1 cells were seeded in 35 mm culture dishes for 16 hours and treated with or without GDNF and GFRα1 for 6 hours. Cells then received 0 Gy, 3 Gy or 6 Gy of ϒ-ray and cultured for 21 days. The colonies containing at least 50 cells were counted. The number of surviving colonies was presented as means ± SEM. *p<0.05, vs. vehicle control.

Figures 1 and 2 show that RET signaling induced by treatment with GDNF1 and GFRα1 enhances cell viability after radiation treatment of LNCaP and 22RV1 PCa cells. Apoptosis was decreased in a similar experiment (data not shown). A similar impact on clonogenic survival after radiation was also observed (Figures 3 and 4)

Subtask 2: In vivo orthotopic experiments to determine if RET knockdown enhances radiation sensitivity (Months 8-36)

After obtaining all regulatory approvals and required trainings, we have initiated dose finding external beam radiation studies using the mouse orthotopic models, given that detailed studies of tumor survival after radiation published to date have not used orthotopic models. We have

therefore tested different doses of external beam radiation in mice bearing orthotopic tumors of LNCaP or 22RV1 cells. For LNCaP cells, a single dose at 2.5 Gy was sufficient to reduce luciferase activity by 46%. This is within our desired response range for wild-type LNCaP orthotopic tumors. For the 22RV1 tumors, two doses of 5Gy were required to reduce luciferase activity 42%, which is within our target range. Unfortunately further mouse experiments had to be aborted after initiation due to COVID-19 closure of the animal facilities. We are awaiting word as to when we can resume activities.

Subtask 3: Correlative studies in human PCa to determine if expression of RET is associated with poor outcomes following radiation therapy (Months 4-28)

We have established a database of men treated for primary prostate cancer with external beam radiation at the MED VAMC from 1995 to 2009. A total of 1839 patients have been identified. We are actively entering data into the database. Of note, to date only a small fraction of the biopsies have very small tumors that would not be suitable for analysis. Immunohistochemical staining has been previously validated[3] and we will begin staining cases within the next few months and begin scoring.

Major Task 3: Evaluate role of FGFR signaling in promoting survival of radiated PCa cells (Months 4-36)

Subtask 1: In vitro studies of impact of FGFR signaling on radiation resistance (Months 4-18)

Our initial studies sought to test the hypothesis that nerves enhance survival of prostate cancer cells after radiation. In order to test this hypothesis, we incubated dorsal root ganglia (DRG) with either LNCaP or 22RV1 prostate cancer cell lines and subjected them to mock irradiation, 3 Gy radiation or 6 Gy radiation treatments. The cells were then used for clonogenic assays and number of surviving clones counted. As can be seen in Figure 5, both for LNCaP and 22RV 1 cells incubation with dorsal root ganglia significantly enhance survival after radiation. It should be noted that incubation with dorsal root ganglia also increased the number of clones and mock irradiated cells due to increased numbers of cells in response to growth factor secreted by the dorsal root ganglia. However the fold change in surviving cells was much higher in the irradiated cells, particularly in the cells treated with 6Gy (~1.4 versus 3-fold).

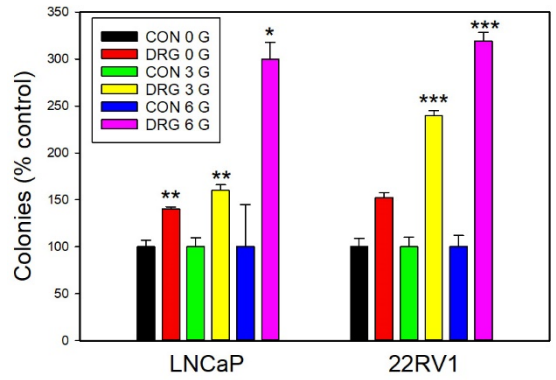
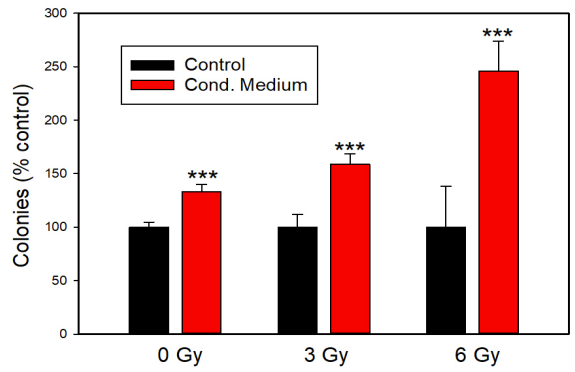


Figure 5. Dorsal root ganglia enhance survival after radiation. Dorsal root ganglia and incubated with matrigel and incubated with LNCaP or 22RV1 cells. Control plates contained matrigel only. Cells were then either mock irradiated or subjected to three or six Gy of external beam radiation. Cells were harvested used for clonogenic assays and number of clones with greater than 50 cells enumerated. The number of surviving colonies is presented as percent control mean at same radiation dose ± SEM. *p<0.05. **p<0.01; ***p<0.001, Vs. matrigel only control at same level of radiation, t-test



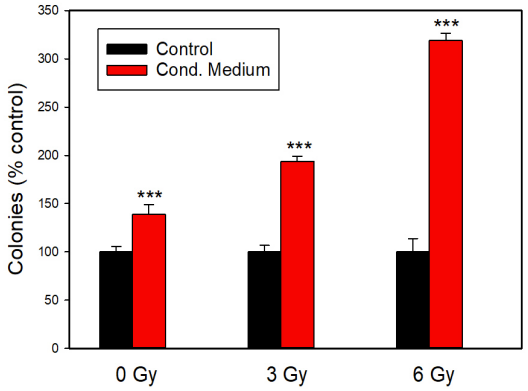


Figure 6. LNCaP (left) or 22RV1 cells (right) were incubated in serum free medium with conditioned medium from DRG with matrigel or matrigel alone (control). Cells were then either mock irradiated or subjected to three or six Gy of external beam radiation. Cells were harvested used for clonogenic assays and number of clones with greater than 50 cells enumerated. The number of surviving colonies is presented as percentage of control mean at same radiation dosage ± SEM. ***p<0.001, Vs. matrigel only control at same level of radiation, t-test

To determine if secreted factors are, at least in part, responsible for the observed increase in survival we prepared conditioned media (CM) from DRGs and incubated this CM with LNCaP and 22RV1. Cells were then mock irradiated (0 Gy) or radiated with 3 or 6 Gy and clonogenic survival assessed. Control was CM without DRG. As shown in Figure 6, CM substantially increased clonogenic survival.

To determine if FGFR signaling via plays an important role in promoting radiation resistance due to nerves we cultured LNCaP cells with conditioned medium from dorsal root ganglia (DRG) for 24 hours, treated the cells with 0, 3 or 6 G of radiation and clonogenic survival assessed. As can be seen in Figure 7, AZD4547 treatment at 300 nM significantly decreased clonogenic survival

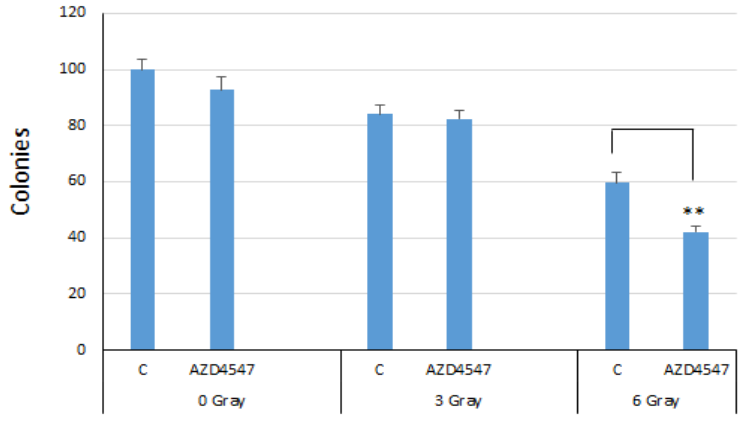


Figure 7. AZD4547 inhibits survival after radiation in dorsal root ganglion conditioned medium. Mouse dorsal root ganglia (DRG) were dissected and cultured in 20 ul matrigel for 5 days and conditioned medium (CM) prepared. The CM was used to culture the LNCaP cells with/without AZD4547 for 24 hours. Cells were then receiving different doses of γ-ray radiation treatment (0 G, 3G, and 6G) and continue to be cultured in growth medium for 21 days. Cell colonies were fixed, stained and colonies with at least 50 cells counted. The number of surviving colonies was presented as means ± SEM. **p<0.01; vs. vehicle control

Subtask 2: In vivo orthotopic experiments to determine if FGFR inhibition enhances radiation sensitivity (12-36)

Unfortunately, these experiments have been delayed by COVID-19.

Major Task 4: Evaluate potential use of lenvatinib as a concurrent treatment to enhance radiation therapy (Months 4-36)

Subtask 1: In vitro studies of impact of lenvatinib on radiation resistance (Months 4-24)

To determine if lenvatinib can inhibit radiation resistance induced by nerves via secreted factors, we cultured LNCaP cells with conditioned medium from dorsal root ganglia (DRG) for 24 hours, treated the cells with 0, 3 or 6 G of radiation in the absence or presence of lenvatinib and clonogenic survival assessed. As can be seen in Figure 8, lenvatinib treatment significantly decreased clonogenic survival in cells treated with 6G of radiation

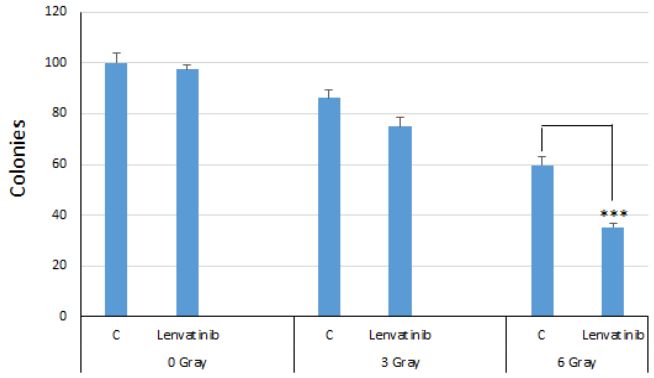


Figure 8. Lenvatinib inhibits survival after radiation in dorsal root ganglion conditioned medium Mouse dorsal root ganglia (DRG) were dissected and cultured in 20 ul matrigel for 5 days and comditioned medium (CM) prepared. The CM was used to culture the LNCaP cells with/without Lenvatinib for 24 hours. Cells were then receiving different doses of γ-ray radiation treatment (0 G, 3G, and 6G) and continue to be cultured in growth medium for 21 days. Cell colonies were fixed, stained and colonies with at least 50 cells counted. The number of surviving colonies was presented as means ± SEM. ***p<0.001; vs. vehicle control

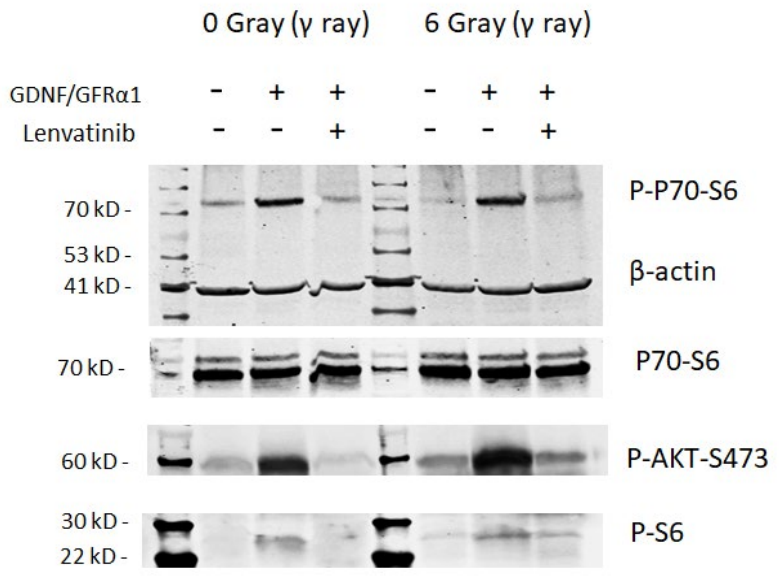


Figure 9. Inhibition of key signaling pathways by lenvatinib.

LNCaP cells were treated with GDNF/GFRα1 in the presence of lenvatinib after radiation with 6 Gy or mock irradiation. Levels of phosphorylated p70-S6, AKT and S6 were assessed by Western blotting. Total p70-S6 kinase was also measured. β-actin was used as a loading control.

Examination of key signaling pathways activated by GDNF/GFRα1 and inhibited by lenvatinib were carried out by Western blotting in LNCaP cells as shown in Figure 9. Phosphorylation of AKT, p70-S6 kinase and S6 was significantly inhibited by lenvatinib and this was not effected by radiation treatment.

Subtask 2: In vivo orthotopic experiments to determine if lenvatinib enhances radiation sensitivity (Months 14-36)

These experiments have been delayed due to COVID-19 closure of the animal facilities.

Subtask 3: Determination of signaling pathways impacted by lenvatinib in PCa (Months 4-14)

These experiments will be carried out with an expanded reverse phase protein array. The Core facility was closed due to COVID-19 but has now been reopened.

B. Training and Professional Development

Junior investigators Kazunari Nohara Ph.D and Yun Zhu Ph.D to Baylor College of Medicine to worked on this project.

C. Dissemination to communities of interest

Nothing to report

D. Plans for coming year

We plan to proceed with the outlined Statement of Work. However, the rate of progress will in part be determined by the course of the COVID-19 pandemic in Houston.

4. IMPACT

Our data to date supports the concept that RET and FGF signaling from nerves impacts cancer cell survival after radiation and can potentially be targeted in patients

5. CHANGES/PROBLEMS

We have adapted the shielding used in the irradiator to make it suitable for irradiating orthotopic tumors while minimizing radiation of bone marrow and the gastrointestinal tract.

COVID-19 has significantly impacted our progress in the past year as described above but we are getting back to the laboratory.

6. PRODUCTS

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Participants

Name: Michael Ittmann MD PhD
Project Role: Principal investigator
Nearest person month worked: 1.2 calendar months
Contribution to Project: Overall coordination and data analysis. He has also carried out extensive work on the patient database.

Funding Support: The following changes in funding support have occurred since this proposal was activated:

Merit Review (Ittmann) 4/1/2019-3/31/2023 3.0 calendar
Dept of Veterans Affairs
A Novel Oncogenic Axis in African American Prostate Cancer
The goal of this project is to characterize the role of RGS12 in African American prostate cancer. No overlap.

DOD Prostate Cancer Research Program Idea (Mitsiades) 10/1/2018-9/30/2021 0.6 calendar
DOD Prostate Cancer Program
Sensitization of castration resistant prostate cancer to chemotherapy via BRCA-1/BRCA-2 induced DNA replication stress
The goal of this proposal is to enhance the efficacy of chemotherapy in advanced prostate cancer by inducing DNA replication stress. Dr. Ittmann is providing pathology support. No overlap.

RO1CA227559 (Sreekumar/Palapattu) 05/01/2019-04/31/2024 0.12 calendar
NIH
Metabolic Rewiring Promotes AA PCa by Regulating Stromal-Epithelial Interaction
The goal of this proposal is to examine metabolism in African American prostate cancer Dr. Ittmann is providing pathology support. No overlap.

PC181023 (Lotan) 9/1/2019-8/31/2022 0.24 calendar
DOD Prostate Cancer Research Program HDA
Epigenomic Landscape of Primary Prostate Cancer in African American Men
The goal of this proposal is to examine the epigenetic alterations in African American prostate cancer. We will provide samples for a validation cohort. No overlap.

2U54MD007597-31 (Kwabi-Addo) 08/01/19-07/31/24 0.6 calendar
NIH (BCM directs)
Epigenetic regulated genes in African American Prostate Cancer Patients
The goal is to understand the biological basis for the more aggressive clinical behavior of prostate cancer in African American men and to begin to develop predictive tools to help manage prostate cancer in African American men. No overlap.

W81XWH-19-1-0410 (Frigo) 7/15/2019-7/14/2022 0.6 calendar
DOD Prostate Cancer Program (salary support only)
Therapeutic Targeting of the Glutamine Transporter SLC1A5 in Advanced Prostate Cancer
To rigorously evaluate SLC1A5's role in prostate cancer metabolism and test whether SLC1A5 represents a viable therapeutic target. No overlap.

Name: Sean McGuire MD PhD
Project Role: Qualified Collaborator
Nearest person month worked: 2.4 calendar months
Contribution to Project: Assists with overall project coordination and data analysis
Funding Support: No changes in funding support

Name: Shu Feng, MD PhD
Project Role: Co-investigator
Nearest person month worked: 12 calendar months
Contribution to Project: Dr Feng has carried out much of the in vitro work described above
Funding Support: No changes in funding support

Name: Jianghua Wang MD
Project Role: Co-investigator
Nearest person month worked: 1.2 calendar months
Contribution to Project: Dr. Wang has assisted with in vitro and in vivo experiments.
Funding Support: No changes in funding support

Collaborating organizations

None. This is an internal collaboration within Baylor College of Medicine

8. REFERENCES

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2. Bruce JY, Lang JM, McNeel DG, Liu G: Current controversies in the management of biochemical failure in prostate cancer, Clin Adv Hematol Oncol 2012, 10:716-722
3. Ban K, Feng S, Shao L, Ittmann M: RET Signaling in Prostate Cancer, Clin Cancer Res 2017, 23:4885-4896

9. APPENDICES

None.