AWARD NUMBER: W81XWH-16-1-0278

TITLE: Synthetic Lethal Metabolic Targeting of Senescent Cells after Androgen Deprivation Therapy

PRINCIPAL INVESTIGATOR: , David F. Jarrard

CONTRACTING ORGANIZATION: University of Wisconsin System
Madison, WI 53715

REPORT DATE: July 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel 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 2018	Annual	1 July 2017 – 30 June 2018

4. TITLE AND SUBTITLE

Synthetic Lethal Metabolic Targeting of Senescent Cells after Androgen Deprivation Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0278

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David Jarrard

E-Mail: jarrard@urology.wisc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Wisconsin System
21 N Park St., Ste. 6401
Madison, WI 53715-1218

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel 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: Progression to castrate resistant prostate cancer for men with advanced prostate cancer (PC) results after the initiation of androgen deprivation therapy (ADT). One underutilized therapeutic strategy that has the potential to dramatically improve outcomes is eradicating the persistent cancer cells that remain after ADT and likely play a key role in the development of castration-resistant PC. The initiation of ADT induces susceptibilities in PC cells that make them amenable to synergistic treatment and improved cell killing.
See attached.

15. SUBJECT TERMS
prostate cancer, androgen deprivation therapy, senescence, proteotoxic stress, xenograft models, metformin, synthetic lethality

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	69	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Title: **Synthetic lethal metabolic targeting of senescent cells after androgen deprivation therapy**

1. INTRODUCTION

Progression to castrate resistant prostate cancer for men with advanced prostate cancer (PC) results after the initiation of androgen deprivation therapy (ADT). One underutilized therapeutic strategy that has the potential to dramatically improve outcomes is eradicating the persistent cancer cells that remain after ADT and likely play a key role in the development of castration-resistant PC. The initiation of ADT induces susceptibilities in PC cells that make them amenable to synergistic treatment and improved cell killing.

Androgen withdrawal in murine xenografts and human PC tissues is associated with a decrease in the proliferative index, but surprisingly low levels of apoptosis. We and others have demonstrated that a substantial portion of these persistent cells express markers of cellular senescence, a terminal growth arrest characterized by exit from the cell cycle and senescence-associated β-galactosidase expression. These senescent cells, although not proliferating, generate a protumor response, the senescent secretory phenotype, that may be detrimental to the patient and must be removed. However, the unique metabolic phenotype expressed by these persistent senescent cells is characterized by increased protein synthesis and notably an amplified proteotoxic stress response (PSR), a conserved survival pathway characterized by induction of multiple heat shock protein (Hsp) families coordinated by the master transcriptional regulator Hsf1. It is our overall hypothesis that the new senescent phenotype induced in prostate cancer cells by ADT may result in unique vulnerabilities to drugs targeting pathways such as the PSR that are critical for survival in the senescent state.

In preliminary data activation of the PSR in these residual cancer cells may represent a pathway critical for the survival of senescent PC cells. Further experiments have identified one agent, metformin, a widely-used, nontoxic oral antidiabetic drug that we propose to repurpose as synthetic lethal therapy in combination with ADT. We postulate that that metformin is synthetic lethal with ADT because it disables the principal PSR pathway mediated by Hsf1 in senescent PC cells that are already experience high levels of proteotoxic stress.

In Aim 1 we will examine the activity of metformin in eradicating senescent PCs following ADT in cellular models. In addition, we will determine whether metformin's actions are specifically mediated by inactivation of Hsf1 and resultant disruption of the PSR cell survival pathway mediated by Hsp27, Hsp70 and Hsp 90. In vitro and xenograft PC models utilizing overexpression of a phosphorylation-resistant Hsf1 mutant will be used to interrogate the specific role of the HSf1-mediated PSR in the synthetic lethal response. PC has a variable response to ADT. The ability of metformin to clear senescent cancer cells after ADT will be examined in Aim 2 in a series of human PC xenografts that exhibit variable responses to ADT. We will utilize a xenograft system consisting of human prostate cancer tumors that can be exposed to drug combinations in a physiologically relevant setting, the growth easily tracked, and the tumor readily harvested for detailed examination. Experiments will test whether synchronous ADT-metformin or their stepwise use leads to better tumor regression and longer survival. In addition, markers of response

will be investigated in the tumors focusing on the Hsps examined in Aim 1. Finally, in Aim 3 we will employ a health sciences research approach using the National Department of Veterans Affairs Corporate Data Warehouse to investigate a retrospective cohort of patients on ADT (~260,000 men), 8% of whom are on metformin (~21,000), to determine PC-specific mortality, biochemical recurrence-free survival and skeletal related events. This will provide further evidence for the implementation of this novel synthetic lethal therapeutic strategy.

Our studies have the potential to lead to a new treatment paradigm for PC by specifically targeting a unique vulnerability of senescent PCs (the PSR) that persist following ADT and likely contribute to androgen-resistance. The proposed study directly addresses mechanisms of resistance for men with high-risk cancer and furthermore, since metformin may mitigate the metabolic side effects of ADT, may improve the physical health of men with PC. When completed our new synthetic lethal approach to PC can be readily translated into the clinic since both ADT and metformin are safe and currently in use.

2. KEYWORDS

prostate cancer, androgen deprivation therapy, senescence, proteotoxic stress, xenograft models, metformin, synthetic lethality

3. ACCOMPLISHMENTS

SPECIFIC AIM 1:

Major Task 1: Determine whether the ADT-metformin synergistic response is mediated via disruption of the Hsf1-mediated proteolytic stress response (PSR).

Subtask 1: Characterize the effects of metformin on the viability of senescent PC cells following ADT (Jarrard/Cryns)

- PC cells will be treated with vehicle or biculatamide for 4 days followed by metformin or vehicle for 2-4 days
- Score senescent PC cells using SA-β-gal activity, GLB1 immunostaining and flow cytometry
- Evaluate apoptosis using co-immunofluorescence with GLB1 and active caspase-3 Ab and by annexin V labeling of GLB1-flourescent PC cells
- Cell lines: LNCaP, CWR22Rv1, VCaP in subtasks 1-3

Ongoing. We have optimized dosing to achieve a maximal coefficient index for synthetic lethality for 3 cell lines, LNCaP, CWR22Rv1 and LAPC4 (Figure 1). VCaP was not utilized because of variable androgen responsiveness. LAPC4 was used as a more consistent alternate.

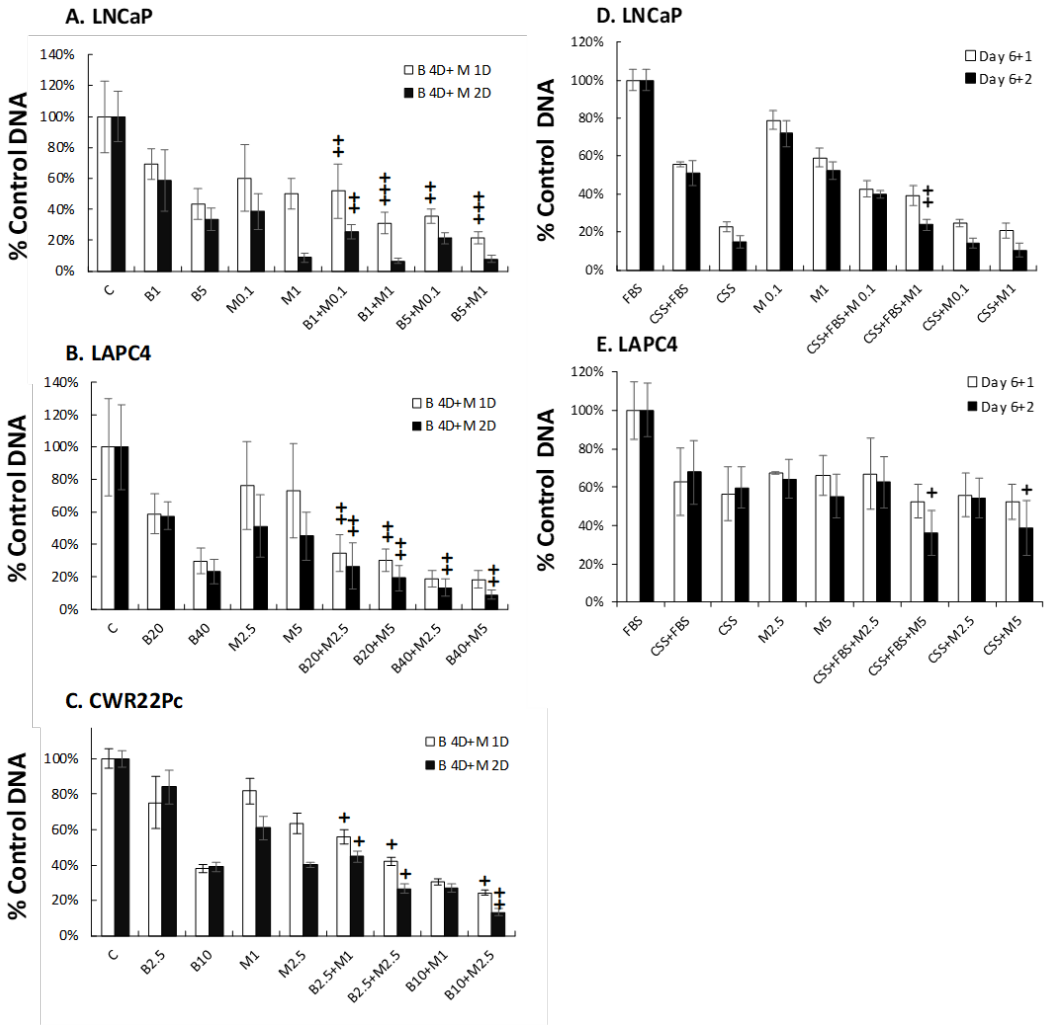


Figure 1. Combination of ADT with Metformin decrease the growth of androgen-dependent prostate cancer cells compared to single-agent use. A-C: Cells were treated with Bicalutamide for 4 days (B 4D), then followed by addition of Metformin for 1 day (M1D) or 2 days (M2D). C: control; B: Bicalutamide (uM); M: Metformin (mM). Number after B or M is the concentration of each drug. Synergistic effect was calculated by Calcusyn. +, moderate synergy (CI: 0.7-0.85); ++, synergy (CI: 0.3-0.7); +++, strong synergy (CI: 0.1-0.3). D-E: Cells cultured in medium containing 10% FBS (FBS), 8% CSS+2% FBS (CSS+FBS) or 10% CSS (CSS) for 6 days and then followed by addition of metformin as indicated does for each cell line.

Senescence has been quantitated using SA-β-gal activity, flow cytometry (cell size) and western blotting for p16 and p27 expression (Figure 2). Apoptosis occurs maximally at 48 hr after exposure to ADT. This increases with the addition of metformin to the senescent cells (Table 1).

Senescence occurs in a subpopulation and we are currently investigating options for increasing its response. Charcoal-stripped serum to remove androgens delivers a more robust senescence response is being used as an approach to generate senescence.

		Side scatter (%)
	Control	16.3 ± 2.3
LNCaP	CSS	50.3 ± 3.7
	Bic 5uM	38.7 ± 1.9
LAPC4	Control	8.4 ± 0.4
	CSS	11.6 ± 0.4
	Bic 40uM	12.6 ± 0.7
	Control	22.8 ± 0.3
CWR22PC	CSS	pending
	Bic 10uM	32.8 ± 1.2

Figure 2. ADT induces phenotypic characteristics of cellular senescence in androgen-dependent prostate cancer cells. A. LNCaP cells were cultured in CSS medium for up to 6 days or 1uM of bicalutamide for 4 days, stained for SA-β-gal activity, magnification × 100. Table 1. Flow cytometry was used to examine the degree of cellular complexity side scatter. ADT increases the fraction of cells with elevated cellular complexity and size, a characteristic of senescent cells.

LNCaP	**Fold change**
CSS	0.3
B1(mM)	0.87
M0.1(mM)	0.89
CSS+M (0.1mM)	2.31
B1(uM)+M0.1(mM)	2
LAPC4	
CSS	1.29
B20(uM)	0.95
M2.5 (mM)	1.13
CSS+M2.5(mM)	1.72
B20(uM)+M2.5(mM)	2.97
CWR22Pc	
CSS	
B10(uM)	1.45
M2.5(mM)	1.28
CSS+M 2.5mM	pending
B10(uM)+M2.5(mM)	4.13

Table 1. Addition of Metformin induced apoptosis in senescent cells. LNCaP, LAPC4 and CWR22PC cells were treated with Bicalutamide for 4 days or cultured in medium containing 10% CSS for 6 days followed by addition of Metformin as the doses shown above. Apoptosis was measured with caspase-Glo, normalized with cell number. The fold change relative to control.

In addition, we have done several studies to select cells. Sorting for SA-B-gal expression is too laborious and not optimal. We also attempted to flow sort based on size but this approach is too slow. We have assessed using senescence surface marker to isolate senescent cells. The expression of plasma membrane-associate proteins VSP26A, DCR2 and B2M increased in senescent cells. We are using biotinylated antibodies against these proteins, capturing with streptavidin magnetic beads, and isolating with a microchamber. These studies are ongoing.

Subtask 2: Characterize the effects of metformin on the PSR of senescent PC cells following ADT (Cryns/Jarrard).

- PC cells will be treated with ADT and/or metformin as in subtask 1
- Collect GLB1-fluorescent and non-fluorescent cells by FACs and determine Hsf1, Hsp27, Hsp70 and Hsp90 mRNA and protein levels.
- Perform co-immunofluorescence with GLB1 and Hsf1, Hsp27, Hsp70, Hsp90, p-AMPK and AMPK
- Determine whether AMPK binds directly to Hsf1 by co-immunoprecipitation.

Milestones: We predict that metformin will increase binding of AMPK to Hsf1 and inhibit the PSR.

Ongoing. We have examined the proteotoxic stress response in multiple prostate cancer cell lines in response to androgen deprivation therapy with and without metformin treatment. Analyses of the entire population of treated prostate cancer cells has yielded results that depend on prostate cancer cell line and individual heat shock proteins with regard to the effects of both androgen deprivation and metformin treatment.

Consistent observations include the induction of HSP 90 with exposure to ADT and metformin. In addition, HSP 27 decreases with ADT. Other HSPs are not consistently altered. We plan to

repeat these studies on senescent cells isolated from the entire population of cells by one of the strategies indicated in Subtask 1. In addition, the induction of Hsp90 suggests that Hsp90 inhibitors may be useful in combination with ADT to enhance cell death, a hypothesis we plan to test in future studies.

Subtask 3: Determine the functional role of site-specific phosphorylation of Hsf1 on the effects of metformin on senescent PCs following ADT (Cryns/Jarrard).

- Stably transduce PC cells with vector, WT Hsf1 or mutant S121A Hsf1 by lentiviral transduction.
- Treat PC cells stably expressing vector, WT or S121A mutant Hsf1 with ADT and/or metformin as in subtask 1
- Perform cell viability (subtask 1) and molecular characterization (subtask 2) assays on senescent PCs and the entire population of PCs.

Milestones: We predict that the S121A mutant Hsf1 will abrogate the effects of metformin on cell death and PSR following ADT.

Pending. We are awaiting the results of experiments on the isolated senescent cells for evidence of proteotoxic stress (*i.e*., activation of multiple Hsps). If we observe evidence of proteotoxic stress, we will perform these experiments as planned. As an alternate strategy, we plan to examine metabolic changes in PC cell lines treated with ADT, metformin or the combination by unbiased mass spectrometry-based metabolite profiling through an established metabolomics core facility. Validated metabolites altered by these treatments may lead to the identification of additional metabolic pathways that could be targeted to enhanced cell death by ADT.

Subtask 4: Determine the function of site-specific phosphorylation of Hsf1 on the antitumor effects of ADT and metformin in vivo (Jarrard/Cryns).

- Male nude mice with LnCaP and CWR22rv1 flank tumors stably expressing WT or S121A mutant Hsf1 will be randomized to 4 groups (8 mice per group): (1) vehicle + sham operation; (2) vehicle + castration; (3) metformin + sham operation; and (4) metformin + castration. Metformin will be tried simultaneously (groups 3 and 4) or sequentially (groups 5 and 6). Tumor size will be assessed weekly and serum PSA recorded. To assess senescent cell clearing, a parallel experiment will be performed using the same 4 treatment groups (8 mice per group) except that mice will be euthanized 4 weeks after castration and tumors harvested for analysis. (16X(6X3)) for 2 experiments (total 576)
- Mouse tissues will be analyzed for SA-β-gal activity, HP1γ, GLB1, p27, Hsp27, Hsp70, Hsp90, Ki67, active caspase-3 and TUNEL staining

PENDING.

Major Task 2: Examine the synthetic lethal response involving ADT-metformin in vivo in cancers of variable androgen sensitivity and test markers of response.

Subtask 1: To determine the optimal schedule for combining ADT and metformin and assess whether metformin eradicates senescent PC cells following ADT (Jarrard).

-COMPLETED

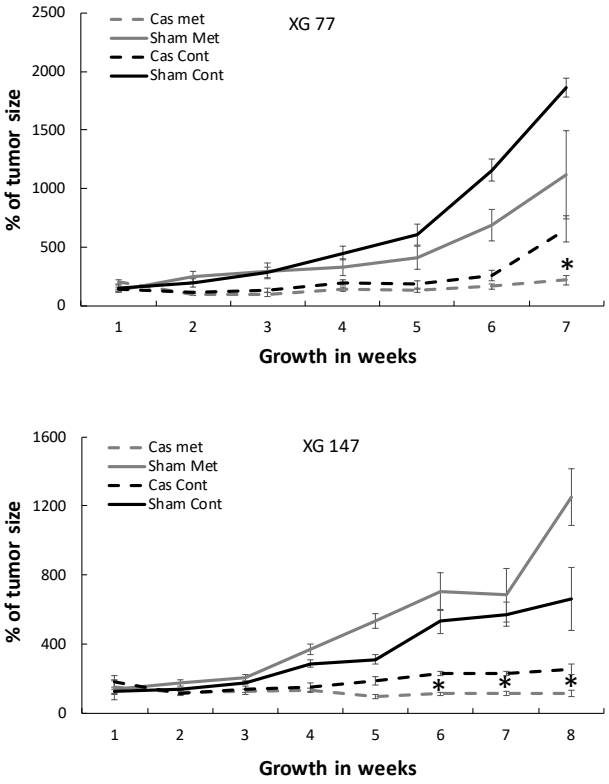


Figure 3 . LuCaP Tumor growth chart. Xenograft 77 growth chart till 7 weeks. The growth rate (percentage of original tumor size) of castrated mice was significantly lower than sham control and sham metformin treated mice (5-7 weeks), and the growth rate of mice treated with both castration and metformin at 7 weeks was further significantly lower compared with sham metformin ($p<0.01$) and castration control ($p<0.01$) mice. Xenograft 147 growth chart till 8 weeks. The growth rate (percentage of original tumor size) of castrated mice was significantly lower than sham control and sham metformin treated mice (4-8 weeks), and the growth rate of mice treated with both castration and metformin at 6-8 weeks was further significantly lower compared with sham metformin ($p<0.01$) and castration control ($p<0.01$) mice. N=8 mice per group, Median± SE

- We obtained approval by the USAMRMC ORP Animal Care and Use Review Office (ACURO), in addition to the local Institutional Animal Care and Use Committee (IACUC).
- Three groups were randomized using 2 xenografts (77 and 147) when flank tumors are 100mm^3 to examine the i) Castrated mice, ii) Mice were placed on Metformin (mg/kg) given orally daily in intact sham operated mice, iii) Mice were castrated and placed on Metformin (50mg/kg-1) given orally daily immediately post-castration. Post-ADT tumor size was assessed weekly.

Mice were divided into 4 treatment groups: sham control (SC), castration control (CC), sham metformin (SM) and castration metformin (CM). Both patient derived xenografts (PDXs) respond to castration (See figures). The individual tumor growth rates and median tumor growth rates, measured as percentage of original tumor size were significantly reduced in metformin treated mice in both Xg 77 and Xg 147. In Xg 77, the tumor size in CM mice at 6 weeks was significantly smaller compared with SM ($p=0.01$) and SC ($p= 0.01$) mice. The tumor size in CC mice trended towards significance at 6 weeks ($p=0.07$) and was significantly larger than CM mice at 13 weeks ($p=0.005$). In Xg 147, the tumor size in CM mice at 6 weeks was significantly smaller compared with CC ($p<0.01$), SM ($p<0.01$) and SC ($p<0.01$) mice. This trend continued at 13 weeks in both groups, with the smallest tumor sizes recorded for CM groups in both PDXs (Figure 3).

We conclude from these animal studies that treatment with ADT and simultaneous metformin leads to an improved tumor response in human PDX samples.

A pilot study we performed using a smaller number of animals (8 vs 8) that underwent ADT and metformin simultaneously versus delayed treatment with metformin (10 days) suggested that simultaneous use of these 2 agents versus a 10 d delay led to no appreciable differences in antitumor activity. This experiment was not further pursued.

Subtask 2: To determine whether PSR markers predict improved response to ADT-Metformin (Jarrard/Cryns). Xenograft tumors from the 2A will be sectioned and immunofluorescence will be used and quantitated using the automated Vectra™ system for Hsp27, Hsp70 and Hsp90. The proteolytic stress response(PSA) represented by these 3 genes in castrated animals harvested at 4wk (group i) will be statistically compared to tumor response, survival, PSA, and other markers including GLB1 in ADT-Metformin groups (iii and iv).

ONGOING
Tissue Microarrays (TMA) were constructed from 58 tissue specimens (29 in duplicate) for Xg 77 and 54 tissue specimens (27 in duplicate) for Xg 147 and antibodies for Ki67 and active cleaved caspase-3 (CC3) are used to quantify proliferation and apoptosis, respectively. For image analysis and quantification of the staining intensity, VECTRA system was used [18]. Cores with <5% epithelial component or loss of tissue were excluded from the analysis. Nuance system and inform 1.2™ software (Caliper Life Sciences, Hopkinton, MA) were used to for building spectral libraries on the basis of target signals of the two stained parameters.
Staining and analysis is currently ongoing.

Major Task 3: Determine whether metformin combined with ADT results in improved cancer-specific survival and longer time to secondary interventions in patients on these agents.

Subtask 1: We propose to utilize a robust observational cohort from the national Veterans Affairs (VA) database to specifically evaluate our hypothesis that metformin improves PC response to ADT, thereby directly examining the patient relevance of our preclinical data in validated patient population. Approvals (Jarrard/Richards).

-COMPLETED

Subtask 2: Data collection, organization with exclusion and inclusion from 2000-2008 (Jarrard/Richards).

-COMPLETED

Subtask 3: Analysis of primary and secondary predictive variables (Jarrard/Richards). Evaluate and control for other covariates including other diabetes medication administration history, age, race, Charlson-comorbidity score, agent orange exposure, family history of prostate cancer, tobacco use, blood type, local therapy (surgery or radiation), date of prostate cancer diagnosis, stage at diagnosis, Gleason score, and other medication administration history (finasteride, aspirin, and docetaxol).

-COMPLETED

Milestone(s) Achieved:
Using national Veterans Affairs databases, we identified all men diagnosed with PCa between 2000-2008 that were treated with ADT with follow-up through October of 2015. We excluded patients that were treated with ADT for ≤6 months or were receiving ADT concurrently with localized radiation therapy. We split these patients into three cohorts: 1. Patients without diabetes 2. Diabetics on metformin 3. Diabetics not treated with metformin. Our primary outcome was overall survival (OS) and secondary outcomes included skeletal related events (SRE) and prostate-cancer specific survival. Cox proportional hazards ratios were calculated for overall and disease specific survival.

The total cohort after exclusions consisted of 87,344 patients of which 53,893 (61%) were non-diabetics, 14,517 (17%) were diabetics on metformin, and 18,934 (22%) were diabetics not receiving metformin. The mean age was 75 ±11 years in the non-diabetics, 71 ±12 in the diabetics on metformin, and 75 ±10 in the diabetics no metformin (p<0.001). The median OS was 7.1 years in the non-diabetics, 9.1 years in the diabetics on metformin, and 7.4 years in the diabetics not treated with metformin.

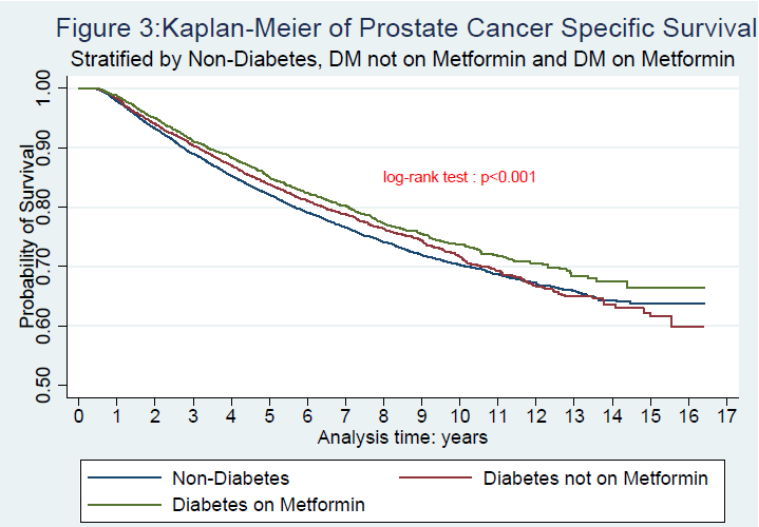


Figure 4. Kapan-Meier analysis of prostate cancer specific survival improves for men on metformin at the time of ADT initiation. Performed through the VA system.

Multivariable Cox proportional hazards analysis assessing for predictors of overall survival showed improved survival in diabetics on metformin (HR 0.77, 95% CI 0.74-0.81) vs. diabetics not treated with metformin (HR 0.99, 95% CI 0.95-1.03) with non-diabetics as referent group. Multivariable Cox proportional hazards analysis assessing for predictors of SRE revealed no association between metformin use (HR 0.99, 95% CI 0.92-1.07) and SRE. Lastly, multivariable Cox proportional hazards analysis assessing for predictors of prostate-cancer specific survival showed improved survival in diabetics on metformin (HR 0.72, 95% CI 0.67-0.78) and to a lesser effect

diabetics not treated with metformin (HR 0.87, 95% CI 0.81- 0.93) with non-diabetics as referent group.

We conclude that metformin use in Veterans with advanced prostate cancer receiving ADT is associated with improved OS and cancer-specific survival. The impact of metformin in prostate cancer patients should be evaluated in a prospective clinical trial.

Completed and abstract presented at the American Urological Association Meeting May 2017 and the GU ASCO meeting Feb 2017. Paper published J Urol 2018 (included in the appendix).

Opportunities for training and professional development?

These include an oncology fellow Dr Shiva Damoradan who has recently taken a clinical position at the University of Toledo. Additional trainees include Nathan Damaschke a graduate student who performed work with the tumor analysis. He is now doing a postdoctoral training at Northwestern University.

How were the results disseminated to communities of interest?
Abstract presentations at the American Urological Association meeting 5/2017 Boston MA and the GU ASCO meeting 10/2017.

• What do you plan to do during the next reporting period to accomplish the goals and objectives?
We plan to adhere to the proposed SOW with the exceptions noted under accomplishments.

4. IMPACT

Androgen deprivation therapy (ADT) induced by surgical or chemical castration limits the growth of androgen-responsive tissues, but is not curative and ultimately castration-resistant PC results. An innovative therapeutic niche that has not been successfully exploited to date is the persistent population of PC cells after ADT treatment that likely contributes to castration-resistant PC. We and others have demonstrated that a substantial portion of these persistent cells express markers of cellular senescence, a terminal growth arrest characterized by exit from the cell cycle. Persistent senescent cells exhibit a senescent-associated secretory phenotype, which results in enhanced protein translation and the accumulation of misfolded proteins, thereby activating a conserved proteotoxic stress response (PSR) characterized by induction of multiple heat shock protein (Hsp) families coordinated by the master transcriptional regulator Hsf1 that enables cell survival. We propose that this intrinsic PSR may represent a potential ‘Achilles heel’ that may be exploited to drive persistent PC cells into apoptosis using a synthetic lethal approach. Intriguingly, the safe and widely used diabetes oral agent metformin was recently reported to inhibit the Hsf1-mediated PSR. **The induction of cell senescence using ADT, followed by a synthetic lethal therapy**

approach using metformin to drive these senescent cells into apoptosis by disrupting the PSR, is a transformative concept has not been addressed to date. Although components of the PSR such as Hsp27 have been targeted previously, we postulate that our approach will be more effective because it targets the global PSR network, not just one of its many downstream mediators. Notably, metformin is an inexpensive drug with documented safety, even in nondiabetic patients that ***we propose to repurpose*** as synthetic lethal cancer therapy that can be readily translated into the clinic. This proposal addresses the PCRP overarching challenge of mechanisms of resistance in men with high-risk PC.

5. CHANGES PROBLEMS

We are using a modified approach to identify senescent prostate cancer cells after treatment as noted under Major Task 1, Subtask 1. We have also utilized a different approach employing charcoal-stripped serum to remove androgens. We also plan to perform metabolomics studies to identify metabolites altered by ADT, metformin or the combination. These studies may lead to new therapeutic targets.

6. PRODUCTS

Abstracts:
Metformin Use Associated with Improved Survival in Veterans with Advanced Prostate Cancer: A Large Observational Study. Kyle A. Richards, Jinn-ing Liou, Vincent Cryns, Tracy M. Downs, E. Jason Abel, and David F. Jarrard (American Urological Association Meeting. Boston MA 2017)

Publications:

1. Richards KA, Liou JI, Cryns VL, Downs TM, Abel EJ, **Jarrard DF**. Metformin Use Is Associated with Improved Survival in Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy. J Urol. 2018 Jun 22. pii: S0022-5347(18)43412-X. PubMed PMID: 29940252.
2. Damodaran S, Lang JM, **Jarrard DF**. Targeting Metastatic Hormone Sensitive Prostate Cancer: Chemohormonal Therapy and New Combinatorial Approaches. J Urol. (*In Press*)

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Senior key personnel have been working on the project since the initiation of the project with no changes.

The following individuals have worked on the project:

Name: David F. Jarrard, MD
Project Role: Principal Investigator
Researcher Identifier (e.g., ORCID ID): 0000-0001-8444-7165
Nearest person month worked: 1.2
Contribution to Project: David Jarrard has conceived and designed the study, reviewed all of the data and the analysis of all of the results on the project, wrote and revised the manuscript.

Name: Vince Cryns, MD
Project Role: Co-Principal Investigator
Researcher Identifier (e.g., ORCID ID): 0000-0003-0355-2268
Nearest person month worked: 2.4
Contribution to Project: Vince Cryns has conceived and designed the study, reviewed all of the data and the analysis of all of the results on the project, wrote and revised the manuscript.

Name: Kyle Richards, MD
Project Role: Co- Investigator
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 0.3
Contribution to Project: Dr Richards has reviewed the data and the analysis of all of Aim 3 on the project, co-wrote and revised the manuscript.

Name: Shiva DAMODARAN
Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 4
Contribution to Project: Shiva has optimized the animal xenografts and metformin treatment and is currently performing the animal studies.

Name: Bing Yang, MD, PhD
Project Role: Researcher
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 3.6
Contribution to Project: Bing Yang has prepared all the PCa cell lines used in this study and performed the analysis on the cell lines, organized the data and assisting with the mouse studies.

Name: Joe Gawdzik
Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 1
Contribution to Project: Joe has assisted with the cell culture experiments for Task 1 and data analysis and collection.

Name: Jinn-ing Liou
Project Role: Researcher
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 0.3
Contribution to Project: Jinn-ing has generated the data and the analysis of all of Aim 3 on the project, co-wrote and revised the manuscript.

Name: Dmitry Malin, PhD
Project Role: Associate Scientist
Researcher Identifier (e.g., ORCID ID): 0000-0002-5728-7511
Nearest person month worked: 6
Contribution to Project: Dmitry Malin has analyzed human PCa cell lines for markers of proteotoxic stress in response to androgen deprivation therapy with or without metformin treatment and assisted with the design of these experiments.

Name: Emmanuel Sampene, PhD
Project Role: Biostatistician
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 0.36
Contribution to Project: Dr. Sampene has provided biostatistical support for these studies.

Name: Zhanhai Li
Project Role: Biostatistician
Researcher Identifier (e.g., ORCID ID): 0000-0003-2687-2838
Nearest person month worked: 0.6
Contribution to Project: has provided comprehensive biostatistical support for these studies.

8. APPENDICES

Manuscripts.

Author's Accepted Manuscript

Metformin Use Is Associated with Improved Survival in Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy

Kyle A. Richards , Jinn-ing Liou , Vincent L. Cryns , Tracy M. Downs , E. Jason Abel , David F. Jarrard

PII: S0022-5347(18)43412-X
DOI: 10.1016/j.juro.2018.06.031
Reference: JURO 15685

To appear in: *The Journal of Urology*
Accepted Date: 13 June 2018

Please cite this article as: Richards KA, Liou Ji, Cryns VL, Downs TM, Abel EJ, Jarrard DF, Metformin Use Is Associated with Improved Survival in Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy, *The Journal of Urology*® (2018), doi: 10.1016/j.juro.2018.06.031.

Title: Metformin Use Is Associated with Improved Survival in Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy

Running Title: Metformin and prostate cancer

Authors: Kyle A. Richards MD[1, 2], Jinn-ing Liou MS[3], Vincent L. Cryns MD[3], Tracy M. Downs MD[2], E. Jason Abel MD[2], and David F. Jarrard MD[2]

Institution:

1. William S. Middleton Memorial Veterans Hospital
   Department of Surgery, Section of Urology
   2500 Overlook Terrace, Madison, WI 53705
2. The University of Wisconsin-Madison
   Department of Urology
   1685 Highland Avenue, Madison, WI 53705
3. The University of Wisconsin-Madison
   Department of Medicine
   1685 Highland Avenue, Madison, WI 53705

Corresponding authors and address:

Kyle A. Richards, MD

Assistant Professor, Department of Urology; Chief of Urology VA

The University of Wisconsin-Madison

1685 Highland Avenue Madison, WI 53705

Fax: 608-262-6453; Tel: 608-262-0759

Email: richardsk@urology.wisc.edu

David F. Jarrard

Professor, Department of Urology, Associate Director UW Carbone Cancer Center

The University of Wisconsin-Madison

1685 Highland Avenue Madison, WI 53705

Fax: 608-262-6453; Tel: 608-262-0759

Disclosures: The authors have nothing to disclose.

Precis for use in the Table of Contents: Metformin use is associated with improved oncologic outcomes in men with advanced prostate cancer on androgen deprivation therapy. This association should be evaluated in a prospective study.

Acknowledgements: This material is the result of work supported with resources and the use of facilities at the William S. Memorial Veterans Hospital. The contents do not represent the views of the U. S. Department of Veterans Affairs or the United States Government. This work was supported by a Department of Defense grant via the Prostate Cancer Research Program #PC150221. The authors would like to acknowledge and thank all the women and men that have served their country in the Unites States Armed Forces.

Text pages (including title page, references, figure legends): 18

Tables: 3 **Figures:** 3

Word count:

Abstract: 247

Manuscript text: 2500

Keywords: prostatic neoplasms; metformin; gonadotropin-releasing hormone/analogs and derivatives; diabetes mellitus; Hospitals, Veterans

Author contributions:

Conceptualization: Richards, Cryns, and Jarrard

Data curation: Richards and Liou

Formal analysis: Richards and Liou

Funding acquisition: Richards, Jarrard, Cryns

Investigation: Richards, Cryns, Downs, Abel, and Jarrard

Methodology: Richards, Cryns, Downs, Abel, and Jarrard

Project administration: Richards, Jarrard, Cryns

Resources: Richards, Jarrard, Cryns

Software: Richards and Liou

Supervision: Richards, Jarrard, Cryns

Validation: n/a

Visualization: n/a

Writing- original draft preparation: Richards and Jarrard

Writing- review and editing: Richards, Liou, Cryns, Downs, Abel, and Jarrard

Abstract:

Purpose: Metformin is commonly prescribed for patients with type 2 diabetes mellitus (DM). We hypothesize that metformin plus androgen deprivation therapy (ADT) may be beneficial in combination. The objective was to assess this combination in a retrospective cohort of patients with advanced PCa.

Methods: Using national Veterans Affairs databases, we identified all men diagnosed with PCa between 2000-2008 that were treated with ADT with follow-up through May of 2016. Exclusions included treatment with ADT for ≤6 months or ADT receipt concurrently with localized radiation. Three patient cohorts were developed: No DM, DM no metformin, and DM on metformin. Cox proportional hazards ratios were calculated for overall survival (OS), skeletal-related events (SRE) and cancer-specific survival (CSS).

Results: The cohort after exclusions consisted of 87,344 patients: 61% were no DM, 22% were DM no metformin, and 17% were DM on metformin. Cox proportional hazards analysis for OS showed improved survival in DM on metformin (HR 0.82, 95% CI 0.78-0.86) vs. DM no metformin (HR 1.03, 95% CI 0.99-1.08) with no DM as referent group. Cox proportional hazards analysis for predictors of SRE revealed HR 0.82 (95% CI 0.72-0.93) for DM on metformin. Cox proportional hazards analysis for CSS showed improved survival in DM on metformin (HR 0.70, 95% CI 0.64-0.77) vs. DM no metformin (HR 0.93, 95% CI 0.85- 1.00) with no DM as referent group.

Conclusion: Metformin use in Veterans with PCa receiving ADT is associated with improved oncologic outcomes. This association should be evaluated in a prospective clinical trial.

Introduction:

The past decade has witnessed remarkable advances with six new therapies approved by the United States Food and Drug Administration for treatment of men with advanced PCa[1]. Despite these advances, nearly 27,000 men died from PCa in 2017 highlighting the ongoing need for additional therapeutic options for men that fail conventional treatments[2]. Androgen deprivation therapy (ADT) remains the standard first-line approach for metastatic PCa and leads to regression, but rarely cure as hormone-insensitive disease invariably e develops from resistant clones. These cells that remain after the initiation of ADT represent an underexplored therapeutic niche that may improve therapy. In support, a recent randomized clinical trial demonstrated that upfront chemotherapy with ADT improves survival by 10.5 months versus ADT alone in hormone naïve patients suggesting that the initiation of ADT induces susceptibilities in PCa cells that make them amenable to synergistic treatments[3].

Metformin is a commonly used insulin sensitizer and is a 1st line agent for patients with type 2 diabetes mellitus (DM). There is scientific evidence for the antineoplastic effects metformin may have for various cancers, but its impact on men with advanced PCa and its utility in combination with other treatments remains poorly studied[4, 5]. Metformin activates AMP-activated protein kinase (AMPK) which inhibits the mammalian target of rapamycin (mTOR)- a central regulator of cell growth[6, 7]. ADT has been shown to induce senescence in androgen sensitive cells, a phenotype with high glycolysis and proteolytic turnover[8-10]. Given these data, we hypothesized that metformin may be beneficial in combination with ADT to target PCa cells that persist after ADT leading to improved survival. To test this approach, we conduct a large observational study evaluating the impact of metformin use on cancer outcomes in men with PCa being treated with ADT.

Materials and Methods:

Data Source:

The study was approved by local Institutional Review Boards (IRB). The VA provides care to over 20 million Veterans at over 1,400 centers. All care processes are captured via an electronic health record (EHR) known as the Veterans Information System Technology Architecture (VistA) that provides a longitudinal view for patients receiving care nationwide including diagnoses, procedures, medications, labs, physiologic measurements, text notes and reports[11]. Data are aggregated from individual VistA systems to the VA Corporate Data Warehouse (CDW) where it is prepared for use.

Study Population:

To develop a cohort of men with PCa on ADT, we identified all men diagnosed with PCa (ICD-9 code 185) in the VA CDW from 2000-2008 (n=558,252). Within this cohort, we included only those with ADT use (n=129,672) by querying the pharmacy domain for VA formulary approved ADT medications including leuprolide, goserelin, bicalutamide, flutamide, and nilutamide from 2000 through May 31, 2016. These were the only approved ADT medications on formulary during the study period. We excluded patients with no information for ADT medication supply days/quantity/dose, those taking ADT for ≤ 6 months (n=33,312), and/or those receiving ADT concurrently with primary radiation therapy of the prostate (n=10,960) leaving us a final cohort of 87,344 patients for our analytic file. ADT was entered as a time-dependent variable in the models. Longitudinal data on each patient was compiled until death or study end of May 31, 2016 at which point they were censored.

We divided the study population into three cohorts and defined DM within the VA using a previously published algorithm with ICD-9 codes 250.00 or 250.02[12]. Comparator groups included (i) no DM; (ii) DM and no prescription of metformin ≥ 180 days during study period; (iii) DM and having a prescription for metformin for ≥ 180 days during the study period.

Outcomes of Interest:

The primary outcome of interest in this study is overall survival (OS). Secondary outcomes of interest for this study include skeletal related events (SRE) and death from PCa (CSS). The dependent variable used in our analyses is the time interval between the starting date of ADT to death from any cause, SRE, and/or death from PCa. SRE was used as a surrogate for progression using a previously described claims-based model to identify SRE[13].

Predictors and Measures:

The metformin group consisted of patients who had metformin prescribed for ≥ 180 days. We did not exclude patients that had exposures to insulin or other glucose lowering medications because their impact on cancer outcomes is conflicting[14, 15]. Prior clinical trials on metformin consisted of at least 24 weeks exposure; therefore, we choose to define drug use of at least 180 days based on this and other studies[12, 16]. There were no metformin users in the no DM group. Metformin use was entered as a time-dependent variable in the models, allowing patients to move from a period of exposure to a period of non-exposure.

Covariates adjusted for in the analyses included demographic and clinical characteristics of each patient included age at ADT initiation, race, Charlson-comorbidity score (CCI), Agent Orange exposure, prostate specific antigen (PSA) at initiation of ADT, year of diagnosis, Gleason score, receipt of local therapy[17], receipt of docetaxel, and insulin use.

Statistical Analysis:

Statistical analysis was performed using Stata 14 (College Station, TX). Comparison of medians was performed using the Mann-Whitney U-test. Fisher's exact and chi-squared tests were used for comparison of categorical variables. We performed multivariable Cox proportional hazards analyses to assess for independent predictors of OS, SRE, and CSS. We then computed a propensity score by multinomial logistic regression and then utilized this for inverse propensity score weighted (IPSW) adjustment in the final models[18]. We constructed IPSW Kaplan-Meier curves for OS, SRE, and CSS and performed log rank tests. We conducted a sensitivity analysis for CSS to account for competing risks as a result of death from other causes using a subdistribution hazards model adapted for time-dependent covariates[19, 20]. Finally, we performed a subset IPSW multivariable Cox proportional hazards analyses to assess for independent predictors of OS, SRE, and CSS in the patients with PSA > 20 at time of ADT initiation. A two-sided p-value of < 0.05 was considered significant.

Results:

The total cohort available for analysis after exclusions consisted of 87,344 patients of which 53,893 (61%) were no DM, 18,934 (22%) were DM no metformin, and 14,517 (17%) were DM on metformin. The metformin group was younger with a median age 71.0 (IQR 64-76) compared to the no DM (75.0, IQR 69-80) and DM no metformin (75.0, IQR 69-79; $p<0.001$) groups (table 1).

The OS was longest in the metformin group as represented in the IPSW Kaplan Meier curve (Figure 1, $p=0.005$). The adjusted Cox proportional hazards multivariable analysis identified the metformin group to be associated with improved OS (HR 0.82, 95% CI 0.78-0.86; $p<0.001$) versus DM no metformin group (HR 1.03, 95% CI 0.99-1.08; $p=0.18$) with the no DM

as the referent group. A dose-response relationship was observed regarding cumulative duration of metformin use both before and after IPSW as metformin use ≥ 36 months being the most protective (HR 0.69, 95% CI 0.65-0.74; p<0.001) (table 2).

The proportion of patients with SRE was highest in the metformin group (11.1%) but the time to SRE was longest in the metformin group as represented in the IPSW Kaplan Meier curve (Figure 2, p=0.005). The adjusted Cox proportional hazards multivariable analysis identified the metformin group to be associated with decreased risk of SRE (HR 0.84, 95% CI 0.74-0.96; p=0.009) versus DM no metformin group (HR 1.08, 95% CI 0.96-1.23; p=0.20) with the no DM as the referent group. A dose-response relationship was observed regarding cumulative duration of metformin use both before and after IPSW as metformin use ≥ 36 months being the most protective (HR 0.70, 95% CI 0.59-0.83; p<0.001) (supplementary table 2).

The proportion of patients that were documented to have died from PCa was lowest in the metformin group (9.3%) as represented in the IPSW Kaplan Meier curve (Figure 3, p<0.001). The adjusted Cox proportional hazards multivariable analysis identified the metformin group to be associated with improved CSS (HR 0.70, 95% CI 0.64-0.77; p<0.001) versus the DM no metformin group (HR 0.93, 95% CI 0.85-1.00; p=~~0.~~0.054) with the no DM as the referent group. A dose-response relationship was observed regarding cumulative duration of metformin use both before and after IPSW as metformin use ≥ 36 months being the most protective (HR 0.58, 95% CI 0.51-0.66; p<0.001) (table 3). After accounting for competing risks as a result of death from other causes, the decreased risk observed between metformin use ≥ 36 months and prostate cancer mortality remained statistically significant (HR 0.66, 95% CI 0.58-0.75; p<0.001).

The subset Cox proportional hazards multivariable analyses assessing for independent predictors of OS, SRE, and CSS in patients with PSA > 20 at time of ADT initiation revealed no change in the associations noted in tables 2-3 or supplementary table 1 although the association with SRE is no longer statistically significant (supplementary tables 2-4).

Discussion:

This large observational study identifies metformin use to be associated with improved oncologic outcomes in men with PCa on ADT. Prior studies evaluating the impact of metformin on men with PCa have focused on disease at diagnosis or early treatment. The current study is unique in evaluating the impact of metformin in men on ADT as these drugs may have an additive effect. Residual cancer cells after ADT are characterized by metabolic abnormalities that may be targeted preferentially by metformin[10]. Capture of prescription medication utilization is vital for this type of analysis and VA databases provide an ideal platform for performing this study since approximately 83% of VA enrollees who use their VA pharmacy benefits fill prescriptions through a VA pharmacy[21]. Additionally, the VA provides continuous and equal access care for the majority of these Veterans, monitored through one healthcare record, making outcomes easier to determine.

Our analysis, controlling for multiple variables, identified metformin use to be associated with improved OS (HR 0.82) in a dose dependent fashion. CSS (HR 0.70) was also improved specifically for diabetics taking metformin compared to the other groups. It is difficult to clearly define those patients who had ADT initiated for metastatic hormone sensitive PCa in this dataset. However, controlling for PSA as well as performing a subset analysis of patients with PSA >20

at initiation of ADT, confirmed the overall and cancer-specific survival advantage to being on metformin. This higher PSA subset demonstrates improved outcomes in patients with a higher risk of metastatic disease, the group in which ADT is typically initiated in modern hormone sensitive PCa. The recognition of increased cardiac, bone density, and other side effects has led to delaying ADT for many patients with microscopic metastatic disease[22].

To date, studies have not focused on a potential additive role of metformin at the time of ADT initiation. In a meta-analysis of 21 eligible studies, metformin use was associated with decreased PCa risk (OR 0.91) and biochemical recurrence following treatment (HR 0.81), but not associated with improved OS in patients with PCa (HR 0.86, 95% CI 0.64-1.14)[23]. Our data does not discount a role for metformin in improving disease in the castrate resistant state. In a Phase 2 clinical trial (NCT01243385) studying metformin in 44 men with progressive castrate resistant PCa, 36% of patients were progression-free at 12-week follow-up, with no grade 3 or 4 toxicity, suggesting some activity in this space[24]. The Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE) multi-arm multi-stage randomized clinical trial is currently recruiting patients into a metformin plus ADT arm to assess the safety and efficacy of this approach (NCT00268476). In addition, the Metformin in Patients Initiating ADT as Prevention and Intervention of Metabolic Syndrome (PRIME) randomized prospective phase 3 clinical trial is underway to assess the proportion of patients that develop metabolic syndrome (NCT03031821).

The duration of metformin may influence outcomes as suggested by our data and others. Margel *et al* performed a retrospective cohort study to evaluate associations between cumulative duration of antidiabetic drug use after PCa diagnosis and CSS/OS among patients with T2DM[25]. Each additional 6 months of metformin use resulted in an adjusted CSS HR of 0.76 (95% CI

0.64-0.89), but there was no relationship seen between cumulative use of other antidiabetic drugs and CSS or OS. Furthermore, we note similar adjusted hazards ratios for OS and CSS in our cohort noting that our study included the non-diabetic patients as a functional control group and all patients in our study were on ADT highlighting the difference in study design in our study. In addition, we found metformin to be associated with reduced risk of SRE, which we used as a measure of progression (progression was not assessed in the Margel et al study). Although there was an increased proportion of SRE in the metformin group, when controlling for time and other covariates, the risk of SRE was attenuated in the metformin group. We chose the SRE algorithm as a measure for progression as we felt it was a more sensitive measure in this patient population given the low rates of chemotherapy or novel anti-androgen therapies.

Our study aimed to specifically assess the effects of metformin use in patients on ADT based on the potential for additive benefit between these agents in pre-clinical studies[6-10]. In vitro and in vivo studies suggest that a combination of metformin with bicalutamide results in reduced proliferation in androgen receptor-positive cells and apoptosis in androgen receptor-negative cells[26]. ADT induces senescence in a population of PCa cells[27], which generates inherent susceptibilities that may be utilized. These cells have high levels of protein turnover and gluconeogenesis rendering them susceptible to proteolytic inhibitors and agents that alter sugar metabolism[10]. Metformin activates AMPK a sensor of cellular energy change and switches on energy producing pathways as well as inhibiting mTOR[6, 7]. This leads to apoptosis of these residual cells providing a molecular rationale for this response. Other studies found that long-term ADT use may also induce metabolic syndrome and in turn increase the risk of cardiovascular morbidity[28]. Metformin may have added benefits in reducing these effects in addition to their direct antineoplastic activity.

There are several limitations in our study. First, this is a retrospective observational study with potential unmeasured confounding variables and/or missing variables. Second, as the national VA data is developed as an administrative dataset via the CDW, we are unable to account for reasons for drug discontinuation, miscoding of key variables, complete laboratory data for the entire cohort, socioeconomic status, body mass index, exercise, smoking, local therapies received outside the VA, and stage. In addition, we are unable to account for other potential health benefits of metformin that may have impacted our results including improvement in diabetes and cardiovascular health. However, our large sample size and our propensity score matching allows us to control for other important confounding factors. Finally, our population of aging Veterans may lack external validity and additional studies are warranted in other populations.

Conclusion:

Metformin use was associated with improved OS, SRE, and CSS in men with PCa also taking ADT. We believe these findings may be related to an additive antineoplastic effect between metformin and ADT. Additional studies are warranted to further validate these findings and establish causation via well designed clinical trials.

Figure Legends:

Figure 1: Kaplan-Meier curve for overall survival stratified by non-diabetes, diabetes on metformin, and diabetes not on metformin (Log rank test) after inverse propensity score weighted (IPSW) adjustment

Figure 2: Kaplan-Meier curve for skeletal related events stratified by non-diabetes, diabetes on metformin, and diabetes not on metformin (Log rank test) after inverse propensity score weighted (IPSW) adjustment

Figure 3: Kaplan-Meier curve for prostate cancer specific mortality stratified by non-diabetes, diabetes on metformin, and diabetes not on metformin (Log rank test) after inverse propensity score weighted (IPSW) adjustment

References:

1. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm; accessed on 8/15/17
2. Siegel RL, Miller KM, and Jemal A. Cancer statistics, 2017. Ca Cancer J Clin 2017; 67: 7-30.
3. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med 2015; 373(8): 737-46.
4. Jiralerspong S, Palla SL, Giordano SH, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 2009; 27: 3297-3302.
5. Pollak M. Metformin and other biguanides in oncology: Advancing the research agenda. Cancer Prev Res (Phila) 2010; 3: 1060-1065.
6. Rocha GZ, Dias MM, Rapelle ER, et al. Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. Clin Cancer Res 2011; 17(12): 3993-4005.
7. Dowling RJ, Zakikhani M, Fantus IG, et al. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res 2007; 67(22): 10804-12.
8. Ewald JA, Desotelle JA, Church DR, et al. Androgen deprivation induces senescence characteristics in prostate cancer cells in vitro and in vivo. Prostate 2013; 73(4): 337-45.
9. Pernicova Z, Slabakova E, Kharaishvili G, et al. Androgen depletion induces senescence in prostate cancer cells through down-regulation of Skp2. Neoplasia 2011; 13(6): 526-36.
10. Dorr JR, Yu Y, Milanovic M, et al. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature 2013; 501 (7467): 421-425.
11. https://www.hsrd.research.va.gov/for_researchers/vinci/
12. Chen-Pin W, Javier H, Lorenzo C, et al. Statins and finasteride use differentially modify the impact of metformin on prostate cancer incidence in men with type 2 diabetes. Ann Transl Med Epidemiol 2014; 1(1). Pii:1004.
13. Aly A, Onukwugha E, Woods C, et al. Measurement of skeletal related events in SEER-Medicare: a comparison of claims-based methods. BMC Medical Research Methodology 2015; 15: 65. doi: 10.1186/s12874-015-0047-5.
14. Hou G, Zhang S, Zhang X, et al. Clinical pathologic characteristics and prognostic analysis of 1013 breast cancer patients with diabetes. Breast Cancer Res Treat 2013; 137(3): 807-816.
15. Curie CJ, Poole CD, Jenkins-Jones S, et al. Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival. Diabetes Care 2012; 35(2): 299-304.
16. Curie CJ, Poole CD, and Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009; 52: 1766-1777.

17. Ritch CR, Graves AJ, Keegan KA, et al. Increasing use of observation among men at low risk for prostate cancer mortality. J Urol 2015; 193: 801-806.
18. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. Multivariate Behav Res 2011; 46: 399-424.
19. Fine JP and Gray RJ. A proportional hazards model for subdistribution of a competing risk. J Am Stat Assoc 1999; 94: 496-509.
20. Ruan PK and Gray RJ. Analyses of cumulative incidence functions via non-parametric multiple imputation. Stat Med 2008; 27: 5709-5724.
21. The VA PBM Drug Utilization Annual Report, Veterans Affairs Pharmacy Benefits Management Services, Washington, DC. 1st Edition, November 2016.
22. Chen DY, See LC, Liu JR et al. Risk of cardiovascular ischemic events after surgical castration and gonadotropin-releasing hormone agonist therapy for prostate cancer: A nationwide cohort study. J Clin Oncol 2017; 35 (32): 3697-3705.
23. Yu H, Yin L, Jiang X, et al. Effect of metformin on cancer risk and treatment outcome of prostate cancer: a meta-analysis of epidemiological observational studies. PLoS ONE 2014; 9(12): e116327.
24. Rothermundt C, Hayoz S, Templeton AJ, et al. Metformin in chemotherapy-naïve castration-resistant prostate cancer: a multicenter phase 2 trial (SAKK 08/09). European Urology 2014; 66: 468-474.
25. Margel D, Urbach DR, Lipscombe LL, et al. Metformin use and all-cause and prostate cancer-specific mortality among men with diabetes. J Clin Oncol 2013; 31: 3069-3075.
26. Colquhoun AJ, Venier NA, Vandersluis AD, et al. Metformin enhances the antiproliferative and apoptotic effect of bicalutamide in prostate cancer. Prostate Cancer and Prostatic Diseases 2012; 15: 346-252.
27. Blute ML Jr, Damaschke N, Wagner J, et al. Persistence of senescent prostate cancer cells following prolonged neoadjuvant androgen deprivation therapy. PLoS One 2017; 12(2): e0172048.
28. Bosco C, Crawley D, Adolfsson J, et al. Quantifying the evidence for the risk of metabolic syndrome and its components following androgen deprivation therapy for prostate cancer: a meta-analysis. PLoS One 2015; 10(3): e0117344.

Table 1: Cohort characteristics: 87,344 patients with prostate cancer on ADT

	Non-diabetic (N=53,893)	Diabetes no Metformin (N=18,934)	Diabetes with Metformin (N=14,517)	P-value
Age, median (IQR)	75.0 (69-80)	75.0 (69-79)	71.0 (64-76)	<0.001
Race, n (%)				<0.001
White	35,416 (65.7)	11,141 (58.8)	8,760 (60.3)	
Black	8,791 (16.3)	4,707 (24.9)	3,337 (23.0)	
Other	9,686 (18.0)	3,086 (16.3)	2,420 (16.7)	
Charlson-comorbidity score, n (%)				<0.001
0-1	42,490 (78.8)	14,065 (74.3)	10,960 (75.5)	
2-3	10,477 (19.4)	3,937 (20.8)	2,936 (20.2)	
> 3	926 (1.7)	932 (4.9)	621 (4.3)	
Agent Orange exposure, n (%)	1,804 (3.4)	696 (3.7)	975 (6.7)	<0.001
Duration ADT use				<0.001
< 12 months	16,744(31.1)	5,360(28.3)	3,865(26.6)	
12-24 months	14,509(26.9)	4,903(25.9)	3,629(25.0)	
24-36 months	7,925(14.7)	2,894(15.3)	2,230(15.4)	
≥ 36 months	14,715(27.3)	5,777(30.5)	4,793(33.0)	
Prostate specific antigen (PSA)*, median (IQR)				<0.001
<4	14,191(26.3)	5,332(28.2)	4,591(31.6)	
4-10	7,738(14.4)	2,970(15.7)	2,809(19.4)	
>10	16,768(31.1)	5,921(31.3)	4,253(29.3)	
Missing	15,196(28.2)	4,711(24.9)	2,864(19.7)	
Year of diagnosis, n (%)				<0.001
2000-2004	41,496 (77.0)	15,225 (80.4)	10,453 (72.0)	
2005-2008	12,397 (23.0)	3,709 (19.6)	4,064 (28.0)	
Gleason score, n (%)				<0.001
6	3,487 (6.5)	1,438 (7.6)	1,402 (9.7)	
7	4,542 (8.4)	1,630 (8.6)	1,719 (11.8)	
8-10	6,094 (11.3)	2,126 (11.2)	1,985 (13.7)	
Missing	39,770 (73.8)	13,740 (72.6)	9,411 (64.8)	
Local therapy, n (%)	3,964 (7.4)	1,387 (7.3)	1,788 (12.3)	<0.001
Docetaxel, n (%)	1,803 (3.4)	508 (2.7)	584 (4.0)	<0.001
Insulin use, n (%)		8,755 (46.2)	9,297 (64.0)	<0.001
Vital status, n (% deceased)	42,133 (78.2)	15,215 (80.4)	9,512 (65.5)	<0.001
Overall survival, median, years (IQR)	5.1 (2.5-8.8)	5.4 (2.7-9.0)	6.8 (3.5-10.1)	<0.001
Death from prostate cancer, n (%)	5,522 (10.3)	1,959 (10.4)	1,337 (9.2)	<0.001
Skeletal related event, n (%)	4,863 (9.0)	1,833 (9.7)	1,609 (11.1)	<0.001
Time to skeletal related event, years, median (IQR)	4.7 (2.2-8.3)	4.9 (2.3-8.5)	6.1 (2.8-9.5)	<0.001

*PSA at initiation of ADT (ng/dl)

Table 2: Cox proportional hazards multivariable analysis assessing predictors of overall survival before and after inverse propensity score weighted (IPSW) adjustment.

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	1.03	(0.98-1.07)	0.23	1.02	(0.98-1.07)	0.29
Metformin < 12 months	1.08	(0.98-1.20)	0.14	1.06	(0.95-1.19)	0.26
Metformin 12-24 months	1.15	(1.05-1.25)	<0.001	1.12	(1.02-1.24)	0.02
Metformin 24-36 months	0.97	(0.87-1.07)	0.51	0.94	(0.84-1.05)	0.28
Metformin ≥ 36 months	0.68	(0.64-0.72)	<0.001	0.69	(0.65-0.74)	<0.001
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.87	(0.83-0.90)	<0.001	0.90	(0.83-0.99)	0.03
24-36 months	0.67	(0.64-0.71)	<0.001	0.68	(0.61-0.75)	<0.001
≥ 36 months	0.40	(0.38-0.41)	<0.001	0.40	(0.36-0.43)	<0.001
Age (continuous)	1.04	(1.037-1.042)	<0.001	1.04	(0.035-1.044)	<0.001
Race						
White	Referent	Referent		Referent	Referent	
Black	0.94	(0.91-0.98)	0.01	0.86	(0.79-0.93)	<0.001
Other	0.97	(0.92-1.02)	0.22	0.99	(0.90-1.09)	0.84
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.16	(1.11-1.21)	<0.001	1.20	(1.11-1.31)	<0.001
> 3	1.77	(1.63-1.91)	<0.001	2.03	(1.79-2.29)	<0.001
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	0.98	(0.90-1.07)	0.65	1.00	(0.86-1.17)	0.98
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	0.83	(0.80-0.86)	<0.001	0.82	(0.77-0.88)	<0.001
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	0.97	(0.93-1.03)	0.31	0.98	(0.89-1.08)	0.65
>10	1.45	(1.39-1.52)	<0.001	1.43	(1.32-1.55)	<0.001
Gleason score						
6	Referent	Referent		Referent	Referent	
7	1.11	(1.06-1.16)	<0.001	1.14	(1.04-1.24)	0.01
8-10	1.60	(1.53-1.67)	<0.001	1.64	(1.51-1.79)	<0.001
Local therapy						
No	Referent	Referent		Referent	Referent	
Yes	0.90	(0.86-0.95)	<0.001	0.95	(0.86-1.05)	0.32

*PSA at initiation of ADT

Table 3: Cox proportional hazards multivariable analysis assessing predictors of prostate cancer specific survival before and after inverse propensity score weighted (IPSW) adjustment.

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	0.92	(0.86-0.99)	0.03	0.92	(0.85-0.99)	0.03
Metformin < 12 months	1.02	(0.86-1.22)	0.78	0.99	(0.82-1.19)	0.91
Metformin 12-24 months	1.03	(0.88-1.20)	0.72	0.98	(0.83-1.16)	0.82
Metformin 24-36 months	0.87	(0.72-1.03)	0.11	0.84	(0.69-1.03)	0.09
Metformin ≥ 36 months	0.56	(0.50-0.63)	<0.001	0.58	(0.51-0.66)	<0.001
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.79	(0.73-0.85)	<0.001	0.75	(0.65-0.86)	<0.001
24-36 months	0.59	(0.54-0.64)	<0.001	0.58	(0.49-0.68)	<0.001
≥36 Months	0.29	(0.27-0.32)	<0.001	0.29	(0.24-0.34)	<0.001
Age (continuous)	1.02	(1.02-1.03)	<0.001	1.02	(1.01-1.02)	<0.001
Race						
White	Referent	Referent		Referent	Referent	
Black	1.07	(1.00-1.15)	0.05	0.99	(0.87-1.12)	0.88
Other	1.01	(0.92-1.10)	0.88	1.02	(0.86-1.21)	0.80
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.13	(1.06-1.22)	<0.001	1.18	(1.03-1.35)	0.02
> 3	1.51	(1.30-1.74)	<0.001	1.76	(1.42-2.18)	<0.001
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	0.94	(0.82-1.08)	0.39	0.85	(0.66-1.11)	0.23
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	0.73	(0.68-0.77)	<0.001	0.72	(0.63-0.81)	<0.001
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	1.05	(0.96-1.16)	0.29	1.10	(0.91-1.32)	0.32
>10	1.99	(1.84-2.15)	<0.001	2.02	(1.74-2.35)	<0.001
Gleason score						
6	Referent	Referent		Referent	Referent	
7	1.20	(1.09-1.31)	<0.001	1.26	(1.06-1.50)	0.01
8-10	2.36	(2.18-2.55)	<0.001	2.51	(2.14-2.95)	<0.001
Local therapy						
No	Referent	Referent		Referent	Referent	
Yes	1.17	(1.08-1.27)	<0.001	1.34	(1.16-1.56)	<0.001

*PSA at initiation of ADT

Figure 1: Kaplan-Meier of Overall Survival

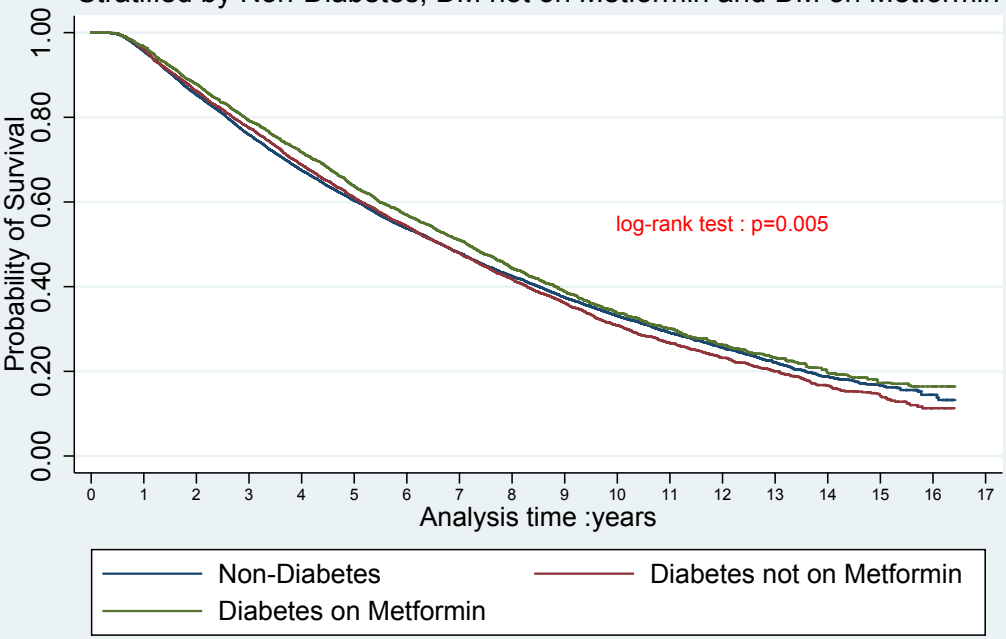


Figure 2: Kaplan-Meier of Skeletal Related Events

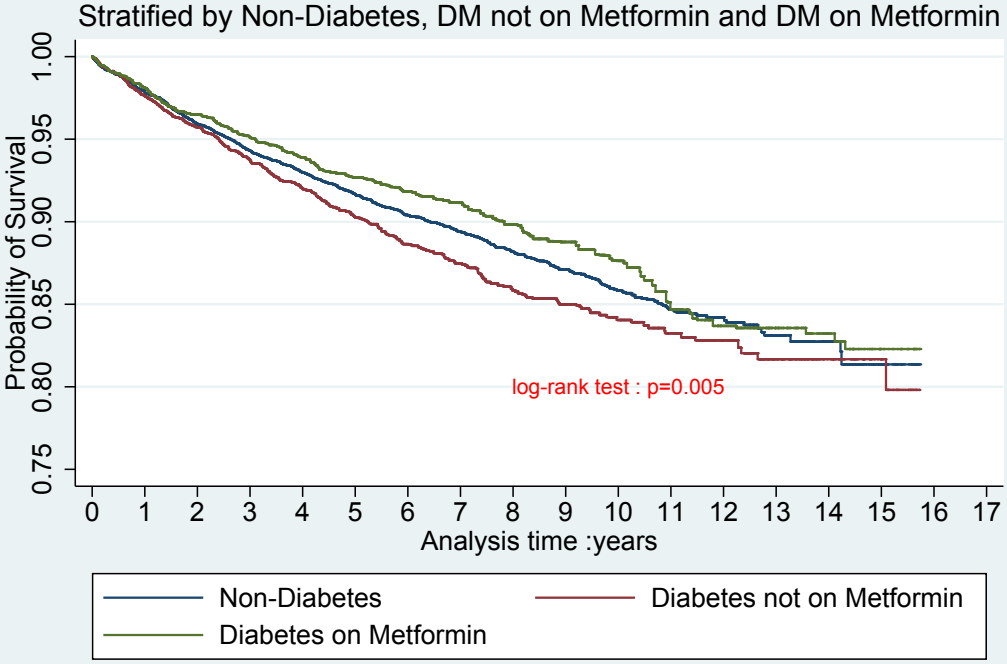
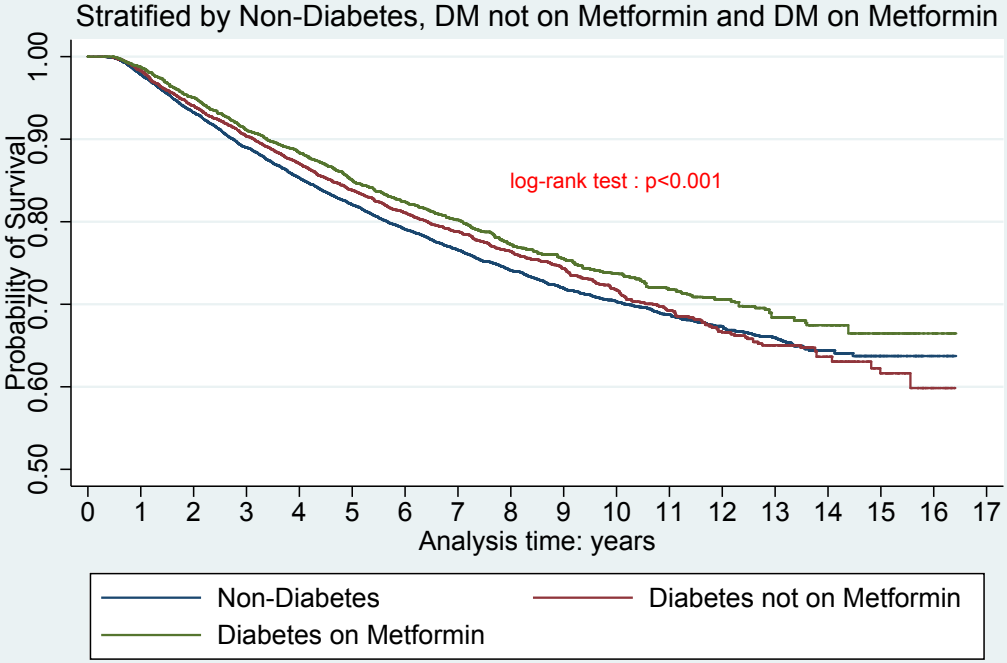


Figure 3:Kaplan-Meier of Prostate Cancer Specific Survival



Alphabetical List of Abbreviations:

CSS = Cancer specific survival

CCI = Charlson comorbidity index

OS = Overall survival

PSA = Prostate Specific Antigen

SRE = Skeletal related event

DM = Diabetes mellitus

VA = Veterans Affairs

Supplementary table 1: Cox proportional hazards multivariable analysis assessing predictors of skeletal related events before and after inverse propensity score weighted (IPSW) adjustment.

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	1.11	(0.99-1.24)	0.07	1.14	(1.01-1.29)	0.03
Metformin < 12 months	0.94	(0.70-1.25)	0.66	0.92	(0.69-1.24)	0.59
Metformin 12-24 months	0.96	(0.75-1.23)	0.77	0.95	(0.73-1.23)	0.70
Metformin 24-36 months	1.15	(0.90-1.46)	0.26	1.00	(0.77-1.30)	1.00
Metformin ≥ 36 months	0.71	(0.60-0.82)	<0.001	0.70	(0.59-0.83)	<0.001
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.91	(0.81-1.02)	0.12	0.79	(0.64-0.98)	0.03
24-36 months	0.81	(0.71-0.93)	0.00	0.81	(0.64-1.04)	0.09
≥ 36 months	0.58	(0.51-0.66)	<0.001	0.54	(0.43-0.68)	<0.001
Age (continuous)	1.01	(1.00-1.01)	0.08	1.00	(0.99-1.01)	0.96
Race						
White	Referent	Referent		Referent	Referent	
Black	1.01	(0.90-1.12)	0.92	1.02	(0.86-1.22)	0.78
Other	0.91	(0.79-1.06)	0.23	0.98	(0.75-1.27)	0.86
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.11	(0.99-1.24)	0.07	1.15	(0.94-1.40)	0.17
> 3	1.50	(1.19-1.89)	0.00	1.63	(1.14-2.33)	0.01
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	1.07	(0.91-1.27)	0.42	0.92	(0.69-1.25)	0.61
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	1.04	(0.94-1.14)	0.48	1.13	(0.96-1.34)	0.15
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	1.22	(1.06-1.40)	0.01	1.05	(0.82-1.35)	0.68
>10	1.96	(1.74-2.20)	<0.001	1.61	(1.31-1.99)	<0.001
Gleason score						
6	Referent	Referent		Referent	Referent	
7	1.01	(0.89-1.16)	0.85	0.93	(0.74-1.19)	0.58
8-10	1.64	(1.45-1.85)	<0.001	1.60	(1.28-1.99)	<0.001

*PSA at initiation of ADT

Supplementary Table 2: Cox proportional hazards multivariable analysis assessing predictors of overall survival in cohort of men with PSA > 20 at time of ADT initiation before and after inverse propensity score weighted (IPSW) adjustment

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	1.03	(0.96-1.10)	0.44	1.02	(0.95-1.11)	0.59
Metformin < 12 months	1.07	(0.89-1.28)	0.49	1.04	(0.86-1.26)	0.65
Metformin 12-24 months	1.20	(1.03-1.40)	0.02	1.20	(1.00-1.45)	0.05
Metformin 24-36 months	0.95	(0.80-1.14)	0.59	0.92	(0.73-1.15)	0.44
Metformin ≥ 36 months	0.69	(0.62-0.78)	<0.001	0.72	(0.64-0.81)	<0.001
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.72	(0.67-0.77)	<0.001	0.71	(0.60-0.84)	<0.001
24-36 months	0.51	(0.47-0.56)	<0.001	0.52	(0.44-0.62)	<0.001
≥ 36 months	0.28	(0.26-0.31)	<0.001	0.30	(0.25-0.35)	<0.001
Age (continuous)	1.03	(1.03-1.04)	<0.001	1.03	(1.02-1.04)	<0.001
Race						
White	Referent	Referent		Referent	Referent	
Black	0.95	(0.89-1.01)	0.09	0.82	(0.72-0.95)	0.01
Other	1.04	(0.95-1.13)	0.40	1.11	(0.97-1.29)	0.14
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.15	(1.08-1.23)	<0.001	1.18	(1.00-1.38)	0.05
> 3	1.66	(1.47-1.88)	<0.001	2.02	(1.68-2.44)	<0.001
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	0.95	(0.82-1.11)	0.54	0.87	(0.60-1.26)	0.46
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	0.86	(0.82-0.92)	<0.001	0.89	(0.78-1.00)	0.05
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	1.31	(1.12-1.53)	0.001	1.10	(0.84-1.46)	0.49
>10	0.96	(0.85-1.08)	0.52	0.80	(0.68-0.96)	0.01
Gleason score						
6	Referent	Referent		Referent	Referent	
7	1.17	(1.07-1.27)	0.00	1.18	(0.99-1.41)	0.06
8-10	1.75	(1.62-1.90)	<0.001	1.78	(1.52-2.08)	<0.001
Local therapy						
No	Referent	Referent		Referent	Referent	
Yes	1.00	(0.91-1.10)	0.94	1.18	(0.99-1.40)	0.06

*PSA at diagnosis

Supplementary Table 3: Cox proportional hazards multivariable analysis assessing predictors of skeletal related events in cohort of men with PSA > 20 at time of ADT initiation before and after inverse propensity score weighted (IPSW) adjustment

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	1.08	(0.89-1.30)	0.45	1.08	(0.88-1.34)	0.46
Metformin < 12 months	0.66	(0.37-1.17)	0.16	0.64	(0.39-1.05)	0.08
Metformin 12-24 months	1.02	(0.68-1.54)	0.91	1.19	(0.77-1.83)	0.44
Metformin 24-36 months	1.16	(0.73-1.85)	0.52	1.04	(0.65-1.66)	0.87
Metformin ≥ 36 months	0.70	(0.52-0.93)	0.02	0.75	(0.54-1.04)	0.08
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.88	(0.73-1.07)	0.19	0.78	(0.55-1.10)	0.16
24-36 months	0.67	(0.52-0.85)	0.00	0.59	(0.38-0.93)	0.02
≥ 36 months	0.43	(0.34-0.54)	<0.001	0.36	(0.25-0.52)	<0.001
Age (continuous)	1.00	(0.99-1.01)	0.95	1.00	(0.99-1.02)	0.85
Race						
White	Referent	Referent		Referent	Referent	
Black	0.90	(0.76-1.08)	0.26	0.83	(0.62-1.11)	0.20
Other	1.05	(0.82-1.34)	0.69	0.87	(0.58-1.31)	0.52
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.08	(0.90-1.31)	0.40	0.82	(0.60-1.10)	0.19
> 3	1.47	(1.03-2.10)	0.03	1.08	(0.67-1.74)	0.76
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	1.18	(0.89-1.57)	0.25	0.95	(0.57-1.57)	0.84
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	1.19	(1.01-1.41)	0.04	1.15	(0.87-1.52)	0.34
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	0.89	(0.59-1.35)	0.58	0.77	(0.36-1.69)	0.52
>10	0.74	(0.53-1.03)	0.07	0.69	(0.40-1.20)	0.19
Gleason score						
6	Referent	Referent		Referent	Referent	
7	0.96	(0.74-1.25)	0.77	0.97	(0.60-1.55)	0.89
8-10	1.77	(1.40-2.22)	<0.001	1.71	(1.11-2.64)	0.01
Local therapy						
No	Referent	Referent		Referent	Referent	
Yes	10.24	(8.64-12.13)	<0.001	12.46	(9.30-16.70)	<0.001

*PSA at diagnosis

Supplementary Table 4: Cox proportional hazards multivariable analysis assessing predictors of prostate cancer-specific survival in cohort of men with PSA > 20 at time of ADT initiation before and after inverse propensity score weighted (IPSW) adjustment

				IPSW		
	HR	95% CI	P-value	HR	95% CI	P-Value
Diabetes						
Non-diabetic	Referent	Referent		Referent	Referent	
Diabetic no metformin	0.94	(0.84-1.06)	0.30	0.94	(0.83-1.07)	0.34
Metformin < 12 months	0.99	(0.75-1.32)	0.95	0.88	(0.65-1.20)	0.42
Metformin 12-24 months	1.22	(0.95-1.56)	0.12	1.20	(0.91-1.58)	0.21
Metformin 24-36 months	0.84	(0.62-1.14)	0.27	0.87	(0.63-1.22)	0.43
Metformin ≥ 36 months	0.61	(0.50-0.73)	<0.001	0.63	(0.51-0.78)	<0.001
Duration ADT use						
< 12 months	Referent	Referent		Referent	Referent	
12-24 months	0.65	(0.58-0.73)	<0.001	0.53	(0.42-0.67)	<0.001
24-36 months	0.45	(0.39-0.52)	<0.001	0.36	(0.27-0.46)	<0.001
≥ 36 months	0.21	(0.18-0.24)	<0.001	0.21	(0.16-0.26)	<0.001
Age (continuous)	1.01	(1.01-1.02)	<0.001	1.01	(1.00-1.02)	0.14
Race						
White	Referent	Referent		Referent	Referent	
Black	0.98	(0.88-1.08)	0.64	0.86	(0.70-1.05)	0.14
Other	1.14	(0.99-1.30)	0.06	1.20	(0.94-1.53)	0.14
Charlson-comorbidity score						
0-1	Referent	Referent		Referent	Referent	
2-3	1.11	(1.00-1.24)	0.06	1.17	(0.93-1.47)	0.18
> 3	1.40	(1.13-1.72)	0.00	1.62	(1.18-2.23)	0.00
Agent Orange exposure						
No	Referent	Referent		Referent	Referent	
Yes	1.07	(0.86-1.32)	0.56	0.89	(0.55-1.44)	0.63
Year of diagnosis						
2000-2004	Referent	Referent		Referent	Referent	
2005-2008	0.81	(0.74-0.89)	<0.001	0.81	(0.66-0.98)	0.03
Prostate specific antigen (category)*						
<4	Referent	Referent		Referent	Referent	
4-10	1.11	(0.87-1.43)	0.41	1.07	(0.70-1.64)	0.76
>10	0.95	(0.78-1.15)	0.57	0.80	(0.57-1.13)	0.21
Gleason score						
6	Referent	Referent		Referent	Referent	
7	1.14	(0.98-1.34)	0.10	1.32	(0.97-1.80)	0.08
8-10	2.34	(2.03-2.69)	<0.001	2.79	(2.12-3.67)	<0.001
Local therapy						
No	Referent	Referent		Referent	Referent	
Yes	1.29	(1.13-1.48)	<0.001	1.54	(1.22-1.96)	<0.001

*PSA at diagnosis

Targeting Metastatic Hormone Sensitive Prostate Cancer: Chemohormonal Therapy and New Combinatorial Approaches

Shivashankar Damodaran[1] damodaran@urology.wisc.edu , Joshua M. Lang[2] jmlang@medicine.wisc.edu and David F. Jarrard[1,2] jarrard@urology.wisc.edu

[1] Dept. of Urology, University of Wisconsin School of Medicine and Public Health,

[2] University of Wisconsin Carbone Cancer Center, University of Wisconsin–Madison

[2] Department of Medicine, University of Wisconsin School of Medicine and Public Health.

Corresponding author: David F. Jarrard

Professor Urologic Surgery, Dept of Urology

Associate Director for Translational Research, Carbone Cancer Center

7037, Wisconsin Institutes of Medical Research, 1111 Highland Avenue, Madison, WI 53792; Tel: (608) 263-9534; Email: jarrard@urology.wisc.edu

Abstract:

Purpose: Androgen deprivation therapy(ADT) alone has been the standard of care for metastatic hormone sensitive prostate cancer(HSPC) for the last 75 years. This review focuses on recent trials and mechanisms that highlight a new paradigm, combining ADT with other agents, changing the management of prostate cancer patients with advanced disease.

Methods: A PubMed® and Web of Science® database search on peer-reviewed literature was performed through January 2018 using the keywords “metastatic hormone sensitive prostate cancer”, “ metastatic castration sensitive prostate cancer”, “docetaxel”, “abiraterone” and “senescence in cancer”. ClinicalTrials.gov was queried for ongoing studies. Relevant data recently presented at major urology and medical oncology meetings were also evaluated.

Results: Recently published Phase III trials employing ADT combinations for metastatic hormone sensitive prostate cancer can be broadly grouped into chemohormonal(docetaxel) or androgen signaling inhibitors. Chemohormonal therapy versus androgen ablation randomized trial for extensive disease in prostate cancer(CHAARTED) and Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy(STAMPEDE) studies report a survival advantage in combining ADT with chemotherapy, and an increased time for progression to castration resistant status. The abiraterone arm of STAMPEDE and LATITUDE, which analyzed combining ADT with abiraterone; reports an improved overall and progression- free survival. ADT generates a number of phenotypes in resistant cancer cells, including those of quiescence, autophagy and cellular senescence. Senescent cells present a metabolic target for synergistic

lethality with drugs such as metformin. Ongoing trials are examining the effect of combining newer antiandrogens and novel drugs with ADT in patients with mHSPC.

Conclusions: Combination therapy has evolved as the standard of care for mHSPC. The ideal combination is tailored to patients after individualized counseling taking into account general health and co-morbid illness status.

Estimated word count: 4030

I. Introduction

II. Methods of data acquisition.

III. Chemohormonal therapy trials

- a. CHAARTED
  - i. Improvement in mOS and time to CRPC
  - ii. Cancer volume as a determinant of heterogeneity of outcomes
- b. STAMPEDE- docetaxel
  - i. Multiarm, multistage model
  - ii. High risk disease inclusion criteria
  - iii. Improved mOS and failure free survival
- c. GETUG trial updates
- d. Side effects and quality of life with chemohormonal therapy
- e. Mechanism of synergy in chemohormonal therapy
  - i. Targeting resistant cells
  - ii. Timing of chemohormonal therapy for maximum synergy

IV. ADT + androgen signaling inhibitor trials

- a. STAMPEDE- abiraterone
  - i. Multiarm, multistage model
  - ii. Relative risk reduction for OS and for failure free survival at 3 years
- b. LATITUDE
  - i. mHSPC with high risk features
  - ii. Improved radiographic progression free survival at 3 years
- c. Side effects and quality of life with abiraterone

d. Mechanism of synergy – abiraterone vs docetaxel
    i. CYP17 inhibitor; near total suppression of extra gonadal androgen synthesis
    ii. Simultaneous targeting vs sequential targeting

V. Mechanism of cellular persistence after ADT
a. Adaptation vs selection
    i. Docetaxel combination targets selection
    ii. Abiraterone combination targets adaptation
b. Senescence as a mechanism of cellular persistence
    i. Cell death mechanisms
    ii. Senescence – a underutilized mechanism
    iii. Marker of senescence
c. Potential of metabolic targeting
    i. Metformin – mechanism of action and potential synergy
    ii. Statins for combination therapy

VI. Optimizing treatment approaches for HSPC patients
a. Abiraterone vs docetaxel for mHSPC
    i. Toxicity profile of abiraterone and docetaxel
    ii. Duration of treatment as a determinant
    iii. Magnitude of treatment effect
    iv. Cost analysis

VII. Ongoing trials and future

VIII. Conclusions

Introduction:

Prostate cancer(PC) is the most common cancer of males with an estimated new case incidence of 164,690 and an estimated mortality of 29,430 expected for 2018. Despite overall five-year survival rates of 98.2%, metastatic hormone-sensitive prostate cancer (mHSPC) has a dismal five-year survival rate of 30%[1]. The conventional treatment of mHSPC has been Androgen Deprivation Therapy(ADT) since the landmark discovery by Huggins and Hodges in 1941 demonstrating the hormonal sensitivity of PC. Metastatic HSPC patients treated with ADT transitions to castrate resistant prostate cancer(CRPC) stage with a median survival of approximately 3 years[2]. Progression to CRPC is associated with a deterioration in quality of life. In the recently concluded STAMPEDE trial, patients were found to spend three-quarters of their overall survival time after mHSPC diagnosis in the CRPC state highlighting the significant alteration in the natural history of the disease with newer treatments [3]. Treatment continues to evolve with a focus on management of the disease at earlier timepoints.

Approaches to improve response rates to ADT or decrease side-effects have included intermittent hormone therapy, use of an antiandrogen with medical or surgical castration or antiandrogens alone [4]. An analysis of these trials detailed the minimal benefits of these approaches, including only 2-3% improvement in 5-year survival, with a wide range of uncertainty [4]. For a number of years researchers had considered an earlier application of cytotoxic therapy in an effort to delay progression to castrate disease. The addition of chemotherapy to ADT had been tested over the last 30 years and there were a number of published randomized trials using this approach as summarized by Logethetis and colleagues[5]. However, the lack of cytotoxic therapy that improved survival in CRPC lead to minimal advances. In CRPC more recently, docetaxel chemotherapy resulted in an incremental

improvement in median survival of 2.5 months leading to its approval in 2004 [6]. These findings lead to the initiation of trials earlier in the disease for patients with larger disease burdens at the time of ADT initiation.

ADT induces a number of unique responses in prostate cancer cells, some that lead to cellular persistence and the development of castration resistance. Recent Phase III trials have demonstrated striking improvements in patient survival with combined ADT and docetaxel as well as ADT and androgen synthesis inhibitors. The synergistic targeting of hormonally sensitive prostate cancer with ADT in combination with other novel agents is a new chapter in the evolution of prostate cancer treatment. These trials and new approaches are reviewed in this study, emphasizing that combination therapy is now the standard of care for patients with metastatic HSPC.

Methods:

We performed PubMed® and Web of Science® database searches of the peer-reviewed literature in mHSPC on the mechanisms of cellular persistence after ADT as well as combination therapies that utilize ADT with another therapeutic agent. Original studies of this subject as well as a small number of reviews were analyzed for strengths and weaknesses. We provide a comprehensive review of prospective Phase III trials that employ combination therapy with ADT in the setting of mHSPC, mechanism of synergy, side effects and quality of life. The mechanisms of cellular persistence after ADT, with special emphasis on senescence are also discussed. Combinatorial therapies ongoing and to be considered in the near future are examined.

Results:

Chemohormonal therapy trials for metastatic HSPC

Early studies of chemotherapy combined with ADT were less optimistic in part due to a lack of active agents and toxicity. Logethetis and colleagues conducted a phase III trial in 286 patients with mHPSC receiving three 8-week cycles of ketoconazole and doxorubicin alternating with vinblastine and estramustine, given in addition to standard ADT versus ADT alone [5]. No difference in time to progression or overall survival was noted. Furthermore, 51% of patients experienced grade 3 or worse adverse events, including thromboembolic events. Another trial featured mitomycin, cyclophosphamide, epirubicin and fluorouracil with no improvement in survival[7]. In 2004, improved survival in CRPC with docetaxel chemotherapy subsequently lead to consideration of its use at the time of ADT initiation for patients with higher tumor burden.

Chemohormonal therapy versus androgen ablation randomized trial for extensive disease in prostate cancer(CHAARTED) was the first trial to show that the addition of 6 cycles of docetaxel 75 mg/m^2 every 3 weeks to standard ADT significantly improved outcomes in men with mHSPC [8]. The 13.6 months improved overall survival in the chemohormonal group compared to ADT group (mOS 57.6 months vs 44 months HR 0.61 [95% CI, 0.47 to 0.80; P<0.001]) represented one of the largest improvements in survival patients with advanced PC have seen in the modern era. The time to CRPC transition was also prolonged in the chemohormonal therapy arm (20.2 vs 11.2 months; 0.61 [0.51-0.72]). In a stratified analysis of high-volume disease patients(defined as presence of visceral metastases or ≥4 bone lesions with at least 1 lesion beyond the vertebral bodies and pelvis) there was an improved overall survival of almost 17 months (OS 51.2 months vs 34.4 months; HR 0.63 [0.50-0.79]; P<0.0001), which was not seen in low volume disease. This was attributed to the higher proportion of

hormone-resistant cells in high-volume disease subpopulation, contributing to resistance to hormonal manipulation.

Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy(STAMPEDE) uses a unique Phase II/III trial design to investigate new agents under the umbrella of a single trial. Additional arms are added to the study as new approaches are, designed[9]. Patients were initially stratified into 4 groups that received ADT alone, ADT + docetaxel, ADT + zoledronate and ADT + docetaxel + zoledronate. In addition to metastatic disease, lymph node involvement, high-risk locally advanced (with at least two features from T3/4, Gleason score of 8–10, and prostate-specific antigen ≥40 ng/mL) and recurrent disease previously treated with definitive local therapy were also included. There was a 10-month improvement in the overall survival in the chemohormonal therapy arm compared to the ADT arm (81 months vs. 71 months alone arm; HR, 0.78 [95% CI, 0.66–0.93]; P = 0.006). Secondary end points of failure-free survival and time to first skeletal related event were also significantly better in the chemohormonal therapy arm.

A third study using this approach, The Groupe d'Etude des Tumeurs Uro-Genital and Association Française d'Urologie trial(GETUG-AFU 15), which also compared chemohormonal therapy to ADT did not report improved outcomes. However, a post hoc analysis of data from high volume disease subset showed a non-significant 20% reduction of death[10,11]. A recent meta-analysis of aggregate data of high volume disease patients from CHAARTED and GETUG-AFU 15 trials showed a survival advantage with pooled HR of 0.68([95% CI 0.56; 0.82], p<0.00), but a significant heterogeneity of treatment effect in combination treatment arm between high volume and low volume subgroups, p=0.017 [12]. This suggests other approaches, notably biomarker based on tumor genotype, might better inform patient selection for treatment.

In both GETUG and CHAARTED side effects were more common in the chemohormonal therapy arm. The most common grade ≥3 adverse events were neutropenia, (32% in GETUG, 12% in CHAARTED), febrile neutropenia (7% in GETUG, 6% in CHAARTED) and fatigue (7% in GETUG, 4% in CHAARTED), while the ADT arm had negligible grade ≥3 adverse events. Diarrhea, stomatitis, motor and sensory neuropathy were the less common adverse effects occurring in <1 % of the population in CHAARTED study. STAMPEDE reported additional toxicity in the chemohormonal therapy arm compared with ADT alone, (grade ≥3 adverse events 52% compared to 32%). This was mostly due to toxicity during the first 6 months on trial, when grade ≥3 adverse events were reported in 36% of the chemohormonal therapy arm versus 17% in ADT. A one year analysis of 1998 patients with available profiles revealed a balanced rate of grade ≥3 adverse events of 10% in both arms. There were 2 deaths in the chemohormonal therapy arm and 72 patients(13%) discontinued treatment. Quality of Life(QOL) assessment was done at 3, 6, 9 and 12 months after randomization in CHAARTED using a Functional Assessment of Cancer Therapy (FACT)–Prostate score. Although QOL scores with docetaxel decreases at 3 months, it was better at 12 months for patients who had docetaxel versus ADT alone [13].

Chemohormonal therapy may improve treatment because of the existence and emergence of hormonally-resistant cellular clones during the CRPC transition. Interestingly, both trials that demonstrated a survival advantage, had a time delay between start of ADT and chemotherapy(120 days in CHAARTED and 90 days for STAMPEDE). In GETUG-AFU15, patients were required to enroll within 2 months of starting ADT and nearly half of patients enrolled within 15 days of starting ADT, which some have suggested might explain the decreased survival benefit with chemohormonal therapy in this trial. Timed sequence of chemohormonal therapy contributes to maximum synergy by targeting hormone resistant cells when they are most vulnerable[14]. Microtubule targeting chemotherapy inhibits nuclear

translocation of androgen receptor in preclinical studies, which also potentially contribute to synergy with ADT[15].

Combined ADT and androgen synthesis inhibitors for mHSPC

Abiraterone inhibits cytochrome P-450 CYP17, a critical enzyme in androgen biosynthesis in the testes, adrenal gland and prostate. Its active D4A metabolite contributes to its antitumor effects through blockade of multiple steroidogenic enzymes and antagonism of the androgen receptor[16]. The approval of this drug in the pre and post-chemotherapy space lead to its application to earlier disease. Resistance to ADT is driven in part by upregulation of androgen-receptor signaling through adrenal androgen production, intratumoral testosterone production, and modification of androgen receptors [17]. The neoadjuvant combination of abiraterone plus prednisone and ADT markedly reduced tumor burden in men with newly diagnosed, high-risk, localized prostate cancer suggesting a potential role for inhibiting extra gonadal androgen biosynthesis before the emergence of resistant clones [18]. These findings lead to two randomized Phase III trials testing the efficacy of abiraterone and ADT in mHSPC.

In STAMPEDE-abi investigators reported their outcomes with the combination of abiraterone and prednisone at the time of initiation of ADT [19]. They used the multistage, multiarm setting similar to previous trials enrolling patients with newly diagnosed metastatic, node-positive, or high-risk locally advanced disease. Patients relapsing with high-risk features after previous treatment with radical surgery or radiotherapy were also included. The group consisted of 1917 patients with 52% having metastatic disease, 20% node-positive or node-indeterminate nonmetastatic disease, and 28% had node-negative high risk nonmetastatic disease. Patients were randomized to receive ADT alone or a combination of ADT + abiraterone 1000mg + prednisolone 5 mg. This trial also mandated radiotherapy for patients with node-

negative, nonmetastatic disease and provided the option of radiotherapy for patients with node-positive, nonmetastatic disease. Treatment duration continued until PSA, radiologic, or clinical progression. For radiotherapy planned patients, treatment was for 2 years or until any type of progression, whichever came first. Results demonstrated improved overall survival at 3 years in the combination therapy group, with a 37% reduction in the relative risk (hazard ratio for death, 0.63; 95% confidence interval [CI], 0.52 to 0.76; P<0.001) compared to ADT. The failure-free survival found a 71% relative risk reduction (hazard ratio for treatment failure, 0.29; 95% CI, 0.25 to 0.34; P<0.001).

In LATITUDE, a multicenter phase III trial, 1199 patients with newly diagnosed mHSPC were enrolled within 3 months of diagnosis and randomized to receive a combination of ADT + abiraterone 1000mg + prednisolone 5 mg or ADT alone + placebo [20]. The trial was conducted at 235 sites in 34 countries in Europe, the Asia–Pacific region, Latin America, and Canada. Patients needed at least 2 out of 3 high risk features including Gleason score ≥ 8, visceral metastasis and ≥ 3 bone lesions on imaging. After 3 years, two thirds of patients in combination group survived compared to only half of patients in the placebo (ADT) group, with a relative risk reduction of 38% (hazard ratio, 0.62; 95% confidence interval [CI], 0.51 to 0.76; P<0.001). The risk reduction was similar to STAMPEDE at the end of 3 years of follow-up (38% vs 37%). The radiographic progression free survival was improved in combination treatment group with a 53% risk reduction (hazard ratio, 0.47; 95% CI, 0.39 to 0.55; P<0.001).

Abiraterone was well tolerated with adverse events primarily related to elevated mineralocorticoids. Grade 3 hypertension was reported in 20% of patients in LATITUDE compared to 10 % in placebo. Hypokalemia was also higher in abiraterone arm that required treatment discontinuation in 2 patients. STAMPEDE reported ≥ grade 3 events in 15% of combined therapy arm compared to 11% in ADT arm. Hypertension, elevated transaminases

and respiratory events were reported more often in combination therapy arm. Both studies mention these side effects were medically manageable and seldom caused life threatening adverse events.

Combination therapy with abiraterone is based on the hypothesis that incomplete blockade of androgen production by ADT leads to tumor cell adaptation. By blocking CYP17 a near total suppression of extra gonadal androgen production especially from within the tumor cell contributes to improved tumor clearance when used synergistically with ADT[21]. The median time to start abiraterone was 8 weeks after initiating ADT in the STAMPEDE trial and at the same time in the LATITUDE trial. Thus, synchronous targeting with combination therapy does not appear to alter outcomes, unlike chemohormonal therapy which requires sequential targeting. In this context, the accessory sources of androgen production are blocked leading to near total androgen suppression in contrast with chemohormonal therapy, where the hormone resistant population are targeted.

Mechanisms of cellular persistence after ADT:

Two distinct, but not mutually exclusive mechanisms have been proposed in the transition from hormone sensitive cells to CRPC. Tumor cells may either acquire new alterations that enable them to survive in the castrated state (adaptation) or pre-existing cells capable of surviving hormonal therapy may be selected after a course of ADT (selection)[22]. Increased levels of intratumoral androgens due to incomplete blockade by ADT, amplification of the androgen receptor(AR) gene, splice variations and gain of function mutations, changes in the expression of co-regulatory molecules to stimulate transcription after antiandrogen binding and bypass of the androgen receptor signaling pathway are examples of adaptive

mechanisms[23,24].The selection model is based on the hypothesis that clones of cells with inherent resistance to ADT are selected after ADT application. The presence of a heterogeneous population of androgen dependent and androgen independent cells therefore exist before initiation of ADT [25]. The finding of quiescent stem cells[26] and AR deficient neuroendocrine cells which are inherently resistant to ADT supports this hypothesis[27]. In this context, chemohormonal therapy targets selection and abiraterone targets adaptive mechanisms, respectively.

ADT causes cellular changes in prostate cancer tissues. Apoptosis, autophagy, necrosis and necroptosis are cell death mechanisms activated after initiating ADT[28]. Senescence is a less studied adaptive mechanism in androgen sensitive cells[29]. Replicative senescence was first described as a phenotype in primary cells after extensive culture and replicative exhaustion in vitro that was linked to telomere shortening[30]. Induced or accelerated senescence in cancer cells results from DNA damage, increased oncogenic signaling, and oxidative stress. Senescent cells remain viable and metabolically active, in contrast to apoptosis or autophagy, but are growth arrested[31]. Markers of cellular senescence include senescence-associated β-galactosidase(GLB1) expression. Tissues from patients undergoing neoadjuvant ADT before radical prostatectomy demonstrate viable cells that frequently express GLB1 and accumulate after ADT[32]. Although senescence is cytostatic, cells express a secretory-associated senescent phenotype(SASP), and resulting proinflammatory cytokines and growth factors, may have permissive effects on surrounding cancer cells. The unique metabolic phenotype expressed by these persistent senescent cells is characterized by a high metabolic rate, increased glycolysis, high rates of protein synthesis and downregulation of the 5' adenosine mono-phosphate-activated protein kinase(AMPK) pathway [33]. A strategy to remove these persistent senescent cells may maximize responses to ADT and improve long-term patient outcomes.

Given the unique phenotype of these residual cancer cells, it is interesting to speculate that other less toxic, agents that alter metabolism might impact outcomes with ADT. One agent is the well-known oral glyburide drug metformin. Metformin directly acts on the tumor cells by inhibiting the respiratory mitochondrial electron transport chain inhibiting gluconeogenesis, decreasing glucose uptake, as well as activation of AMPK [34]. It also inhibits fatty acid synthesis, lipid peroxidation and Kreb's cycle, which are crucial for PC cell survival [35]. Metformin represses AR-mediated signaling in hormone-sensitive cell lines [36] and enhances the antiproliferative and apoptotic effect of the antiandrogen bicalutamide [37]. A putative role for metformin acting synergistically with chemotherapy has been noted in therapy-resistant stem cells in breast and pancreatic tumors [38]. Interest has also arisen in examining concurrent use of statins and ADT given inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A(HMG-CoA) enzyme at the rate limiting step in the mevalonate pathway of cholesterol synthesis and potentially important in cancer.[2] Previous combination trials using celecoxib and zoledronic acid in combination with ADT for patients starting on long term hormonal therapy did not show a survival advantage [9,39].

In a recently published retrospective study of over 87,000 patients who were placed on ADT for advancing PC, metformin improved overall survival, cancer-specific survival and reduced skeletal metastases when compared to diabetics on insulin or nondiabetic patients[40]. These data suggest that the combination of metformin with ADT could synergistically eliminate persistent cancer cells after ADT and delay the onset of CRPC. This approach ultimately requires a prospective trial. STAMPEDE is currently evaluating the combination of metformin and ADT(arm J) against the standard of care ADT(ARM a) and this ongoing analysis is expected to shed light on this synergistic approach[41].

Selection of Patients for Combination Therapy in mHSPC:

Combined therapy now represents a standard of care for men with mHSPC. ADT plus docetaxel can be offered to patients with mHSPC who are eligible for chemotherapy, particularly those with a high metastatic burden or rapid pace of disease.[8] Barriers to the use of docetaxel include advanced patient age, poor performance status, coexisting illnesses, and patient preferences. Hematological side effects, neuropathy and fatigue are more common in chemohormonal therapy compared to ADT, and chemotherapy-related deaths, although rare (1-3%), were documented in all three randomized trials in which docetaxel was added. Both CHAARTED and STAMPEDE- docetaxel Phase III trials employed an 18-week course of therapy (6 cycles each consisting of 3 weeks) and about one fourth of patients in the chemohormonal therapy arm (26 and 23%, respectively) did not complete the full course of therapy. This becomes even more important in community practice where patients with mHSPC are commonly older than those enrolled in the above mentioned clinical trials [42].

Abiraterone has a better side effect profile than docetaxel and being an oral agent, is easier to administer in the urologists office. Few people discontinued therapy due to toxic effects (12%). In LATITUDE 88% of patients completed therapy without a dose modification and in the abiraterone arm of STAMPEDE, only few patients discontinued therapy due to toxicity. In LATITUDE 63% reported Grade 3 or 4 adverse events with abiraterone group compared to 48% with placebo, while in STAMPEDE 47% patients in combination group reported grade ≥ 3 adverse events compared to 33% in placebo group, the majority related to mineralocorticoid side effects including hypertension, fluid retention and hypokalemia. Altered liver transaminases also occur more frequently with abiraterone and must be monitored. A meta-analysis of these trials found a three-fold increase in grade ≥ 3 cardiac and hepatic events and a two-fold increase in grade ≥ 3 vascular events in abiraterone combination group [43].

Quality of life and side effects are important to consider in a majority of men who were otherwise asymptomatic. For patients with either low volume disease or significant comorbidities conditions, ADT alone remains an appropriate treatment option and should be discussed in individualized counseling. The duration of treatment with abiraterone is longer (2+ years) which raises concerns about safety especially in those patients with pre-existing risk factors for cardiovascular disorders and stroke. STAMPEDE excluded men with significant cardiac history limiting generalizations of either benefit or toxicity for those patients. A short course of docetaxel might be preferred in patients with good performance status to avoid the long-term effects of steroids and toxicities associated with abiraterone administration including hyperglycemia, cardiovascular risks and osteopenia/osteoporosis. The requirement for concurrent prednisone with abiraterone can limit its use for brittle diabetics, patients with chronic gastric ulcers, and infection.

This raises the question of the ideal therapeutic agent in the setting of mHSPC. A direct head to head comparison of ADT plus abiraterone or docetaxol has not been performed limiting conclusions. A recent analysis of STAMPEDE indicates that contemporaneously randomized patients showed no evidence of a difference in overall or PC specific survival, nor in symptomatic skeletal events [44]. Interestingly, failure-free survival favored abiraterone, likely reflecting PSA responses and mechanism of action, but the docetaxol cohort had more durable survival after failure. Toxicity was similar between arms with the prevalence of grade 3 or 4 toxicity at one year 11%. The question has been raised whether a combination of abiraterone plus decetaxel (and ADT) might lead to an additive benefit in survival. Data regarding this will emerge from the ongoing PEACE-1 trial.

Cost of long term abiraterone treatment is also a factor that physicians and patients should take into consideration before starting therapy. While the cost of docetaxel for a 6 cycle course is estimated to cost about $20,000, the cost of abiraterone for a 2-year course can exceed $120,000 per patient [45,46]. While cost analyses have been completed for abiraterone in men with CRPC, they have not been reported in the HSPC setting. Given the extended duration of treatment of abiraterone in HSPC, often exceeding two years, the potential costs can be significant. The fluid nature of prescription drug coverage across different insurers, especially for oral agents, has made it difficult to predict year-to-year costs of these agents. This represents a new world for many prescribers in which monitoring patient costs for these agents is a critical issue requiring close collaboration with oncology pharmacists. The emergence of new assistance programs for these expensive therapies requires dedicated staff to guide patients through these applications.

Ongoing and Future Trials

Recent positive phase 3 trials have led to a wealth of new trials in the mHSPC space. Combining ADT with androgen axis inhibitors enzalutamide, apalutamide and orteronel are ongoing (Table 2). Enzalutamide is an androgen signaling inhibitor with multiple actions including blocking translocation of the androgen receptor to the nucleus, androgen receptor binding to DNA, and receptor-mediated DNA transcription [47]. It has been approved for men with advancing CRPC before and after docetaxel chemotherapy, and recently for nonmetastatic CRPC with a rapidly rising PSA (PROSPER). It is being evaluated as combination therapy with ADT for mHSPC separately, as well as an adjunct with another androgen axis inhibitor, abiraterone. Apalutamide is similar to enzalutamide in action and was recently approved for nonmetastatic CRPC with rapidly rising PSA, while on ADT (SPARTAN) [48]. Orteronel is a

selective non-steroidal inhibitor of 17, 20 lyase, a key enzyme in androgen synthesis. It has shown significant activity in the setting of CRPC [49]. Combination therapy with these additional androgen axis inhibitors is expected to contribute to the evolving landscape of mHSPC management.

The synergistic combination of chemotherapy with androgen axis inhibition is also evaluated in combination with radiation therapy(Table 2). Metformin, is also being assessed in combination with ADT in arm K of STAMPEDE. The side effect profile and low cost would make this an attractive adjunct in the management of prostate cancer if the results show a synergistic benefit. Finally, ADT induces an AR-specific T cell response suggesting that ADT combined with AR-directed immunotherapy might be an alternate approach to prevent the development of AR overexpressing CRPC clones [50]. This approach represents an intriguing, potentially less toxic, strategy for future trials.

Conclusions:

It is remarkable that more than half a century after its introduction, androgen suppression remained the preferred front-line approach to the treatment of hormonally sensitive metastatic prostate cancer. With the development of cytotoxic regimens with clinically relevant activity in CRPC, earlier combination trials in mHSPC have demonstrated significant improvements in survival and QOL. ADT and docetaxel or abiraterone should be considered standard of care for these patients.

ADT induces adaptive changes in PC cells and selects resistant clones, leading to castration resistance. The susceptibilities generated by ADT can be synergistically targeted to improve survival outcomes and delay onset of CRPC as shown with several recent combination trials. Novel approaches targeting metabolic pathways have potential to be synthetic lethal with

ADT by targeting multiple susceptibilities induced by ADT, an area that needs to be explored in further clinical trials.

References:

1. institute Nc. Cancer Stat Facts: Prostate Cancer. https://seer.cancer.gov/statfacts/html/prost.html. Accessed 03/15/2018.
2. Tangen CM, Hussain MH, Higano CS, et al. Improved overall survival trends of men with newly diagnosed M1 prostate cancer: a SWOG phase III trial experience (S8494, S8894 and S9346). *The Journal of urology.* 2012;188(4):1164-1169.
3. James ND, Spears MR, Clarke NW, et al. Survival with Newly Diagnosed Metastatic Prostate Cancer in the "Docetaxel Era": Data from 917 Patients in the Control Arm of the STAMPEDE Trial (MRC PR08, CRUK/06/019). *European urology.* 2015;67(6):1028-1038.
4. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Prostate Cancer Trialists' Collaborative Group. *Lancet (London, England).* 2000;355(9214):1491-1498.
5. Millikan RE, Wen S, Pagliaro LC, et al. Phase III trial of androgen ablation with or without three cycles of systemic chemotherapy for advanced prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2008;26(36):5936-5942.
6. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *The New England journal of medicine.* 2004;351(15):1502-1512.
7. Schweizer MT, Antonarakis ES. Chemotherapy and its evolving role in the management of advanced prostate cancer. *Asian journal of andrology.* 2014;16(3):334-340.
8. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. *The New England journal of medicine.* 2015;373(8):737-746.
9. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. *Lancet (London, England).* 2016;387(10024):1163-1177.
10. Gravis G, Fizazi K, Joly F, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2013;14(2):149-158.
11. Gravis G, Boher JM, Joly F, et al. Androgen Deprivation Therapy (ADT) Plus Docetaxel Versus ADT Alone in Metastatic Non castrate Prostate Cancer: Impact of Metastatic Burden and Long-term Survival Analysis of the Randomized Phase 3 GETUG-AFU15 Trial. *European urology.* 2016;70(2):256-262.
12. Gravis G, Boher JM, Chen YH, et al. Burden of Metastatic Castrate Naive Prostate Cancer Patients, to Identify Men More Likely to Benefit from Early Docetaxel: Further Analyses of CHAARTED and GETUG-AFU15 Studies. *European urology.* 2018.
13. Morgans AK, Chen YH, Sweeney CJ, et al. Quality of Life During Treatment With Chemohormonal Therapy: Analysis of E3805 Chemohormonal Androgen Ablation

Randomized Trial in Prostate Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2018:JCO2017753335.
14. Eigl BJ, Eggener SE, Baybik J, et al. Timing is everything: preclinical evidence supporting simultaneous rather than sequential chemohormonal therapy for prostate cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2005;11(13):4905-4911.
15. Zhu ML, Horbinski CM, Garzotto M, et al. Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer. *Cancer research.* 2010;70(20):7992-8002.
16. Li Z, Bishop AC, Alyamani M, et al. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature.* 2015;523(7560):347-351.
17. Yamaoka M, Hara T, Kusaka M. Overcoming persistent dependency on androgen signaling after progression to castration-resistant prostate cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2010;16(17):4319-4324.
18. Taplin ME, Montgomery B, Logothetis CJ, et al. Intense androgen-deprivation therapy with abiraterone acetate plus leuprolide acetate in patients with localized high-risk prostate cancer: results of a randomized phase II neoadjuvant study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2014;32(33):3705-3715.
19. James ND, de Bono JS, Spears MR, et al. Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. *The New England journal of medicine.* 2017.
20. Fizazi K, Tran N, Fein L, et al. Abiraterone plus Prednisone in Metastatic, Castration-Sensitive Prostate Cancer. *The New England journal of medicine.* 2017.
21. Danila DC, Morris MJ, de Bono JS, et al. Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2010;28(9):1496-1501.
22. Zong Y, Goldstein AS. Adaptation or selection--mechanisms of castration-resistant prostate cancer. *Nature reviews Urology.* 2013;10(2):90-98.
23. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nature reviews Cancer.* 2001;1(1):34-45.
24. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2005;23(32):8253-8261.
25. Isaacs JT, Coffey DS. Adaptation versus selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation therapy as studied in the Dunning R-3327-H adenocarcinoma. *Cancer research.* 1981;41(12 Pt 1):5070-5075.
26. Clevers H. The cancer stem cell: premises, promises and challenges. *Nat Med.* 2011;17(3):313-319.
27. Bonkhoff H. Neuroendocrine cells in benign and malignant prostate tissue: morphogenesis, proliferation, and androgen receptor status. *Prostate Suppl.* 1998;8:18-22.

28. Zhang KX, Firus J, Prieur B. To die or to survive, a fatal question for the destiny of prostate cancer cells after androgen deprivation therapy. *Cancers (Basel).* 2011;3(2):1498-1512.
29. Ewald JA, Desotelle JA, Church DR, et al. Androgen deprivation induces senescence characteristics in prostate cancer cells in vitro and in vivo. *The Prostate.* 2013;73(4):337-345.
30. Hayflick L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. *Exp Cell Res.* 1965;37:614-636.
31. Campisi J. Cancer, aging and cellular senescence. *In Vivo.* 2000;14(1):183-188.
32. Blute ML, Jr., Damaschke N, Wagner J, et al. Persistence of senescent prostate cancer cells following prolonged neoadjuvant androgen deprivation therapy. *PLoS One.* 2017;12(2):e0172048.
33. Guo YN, Wang JC, Cai GY, et al. AMPK-mediated downregulation of connexin43 and premature senescence of mesangial cells under high-glucose conditions. *Exp Gerontol.* 2014;51:71-81.
34. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J.* 2000;348 Pt 3:607-614.
35. Zadra G, Photopoulos C, Loda M. The fat side of prostate cancer. *Biochim Biophys Acta.* 2013;1831(10):1518-1532.
36. Wang Y, Liu G, Tong D, et al. Metformin represses androgen-dependent and androgen-independent prostate cancers by targeting androgen receptor. *The Prostate.* 2015;75(11):1187-1196.
37. Colquhoun AJ, Venier NA, Vandersluis AD, et al. Metformin enhances the antiproliferative and apoptotic effect of bicalutamide in prostate cancer. *Prostate Cancer P D.* 2012;15(4):346-352.
38. Hirsch HA, Iliopoulos D, Tsichlis PN, et al. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer research.* 2009;69(19):7507-7511.
39. James ND, Sydes MR, Mason MD, et al. Celecoxib plus hormone therapy versus hormone therapy alone for hormone-sensitive prostate cancer: first results from the STAMPEDE multiarm, multistage, randomised controlled trial. *Lancet Oncol.* 2012;13(5):549-558.
40. Richards KA, Liou JI, Cryns VL, et al. Metformin Use Is Associated with Improved Survival in Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy. *The Journal of urology.* 2018.
41. Gillessen S, Gilson C, James N, et al. Repurposing Metformin as Therapy for Prostate Cancer within the STAMPEDE Trial Platform. *European urology.* 2016;70(6):906-908.
42. Gerritse FL, Meulenbeld HJ, Roodhart JM, et al. Analysis of docetaxel therapy in elderly (>/=70 years) castration resistant prostate cancer patients enrolled in the Netherlands Prostate Study. *European journal of cancer (Oxford, England : 1990).* 2013;49(15):3176-3183.
43. Rydzewska LHM, Burdett S, Vale CL, et al. Adding abiraterone to androgen deprivation therapy in men with metastatic hormone-sensitive prostate cancer: A systematic review

and meta-analysis. *European journal of cancer (Oxford, England : 1990).* 2017;84:88-101.
44. Sydes MR, Spears MR, Mason MD, et al. Adding abiraterone or docetaxel to long-term hormone therapy for prostate cancer: directly randomised data from the STAMPEDE multi-arm, multi-stage platform protocol. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2018;29(5):1235-1248.
45. Ramaekers BLT, Riemsma R, Tomini F, et al. Abiraterone Acetate for the Treatment of Chemotherapy-Naive Metastatic Castration-Resistant Prostate Cancer: An Evidence Review Group Perspective of an NICE Single Technology Appraisal. *Pharmacoeconomics.* 2017;35(2):191-202.
46. Pilon D, Queener M, Lefebvre P, et al. Cost per median overall survival month associated with abiraterone acetate and enzalutamide for treatment of patients with metastatic castration-resistant prostate cancer. *J Med Econ.* 2016;19(8):777-784.
47. Hoffman-Censits J, Kelly WK. Enzalutamide: a novel antiandrogen for patients with castrate-resistant prostate cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2013;19(6):1335-1339.
48. Smith MR, Saad F, Chowdhury S, et al. Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. *The New England journal of medicine.* 2018;378(15):1408-1418.
49. Fizazi K, Jones R, Oudard S, et al. Phase III, randomized, double-blind, multicenter trial comparing orteronel (TAK-700) plus prednisone with placebo plus prednisone in patients with metastatic castration-resistant prostate cancer that has progressed during or after docetaxel-based therapy: ELM-PC 5. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2015;33(7):723-731.
50. Olson BM, Gamat M, Seliski J, et al. Prostate Cancer Cells Express More Androgen Receptor (AR) Following Androgen Deprivation, Improving Recognition by AR-Specific T Cells. *Cancer Immunol Res.* 2017;5(12):1074-1085.