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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 strategy that has the potential to dramatically improve outcomes is eradicating persistent senescent cancer cells that remain after ADT and likely play a key role in castration-resistant PC. We have demonstrated that ADT induces senescence in androgen-dependent PC cells and acts synergistically with the diabetes medication metformin to induce apoptosis in PC cells. ADT activates Akt, AMPK, mTORC1 and XIAP at various time points and silencing XIAP augments apoptosis induced by ADT, pointing to XIAP as a promising drug target to increase the activity of ADT in advanced prostate cancer. We have also shown that metformin enhances the antitumor activity of ADT in two PDX PC models. Analysis of the VA database revealed that metformin use in patients with advanced prostate cancer receiving ADT is associated with improved OS and cancer-specific survival. Our results point to metformin as a novel therapeutic strategy for castration-resistant PC.

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

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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

-COMPLETED

We have optimized the dosing and time to achieve a maximal coefficient index for synthetic lethality for 2 cell lines, LNCaP and LAPC4. VCaP was not utilized because of variable androgen responsiveness, and CWR22RV1 was removed from the study due to the inconsistent response to androgen deprivation. LAPC4 was used as an alternate cell line. In addition, we adopted another approach for inducing senescence: charcoal-stripped serum (CSS) instead of FBS.

We have demonstrated that the combination of androgen deprivation therapy (ADT, bicalutamide or CSS) and metformin inhibit prostate cancer cell growth more robustly than either agent alone (**Figure 1**).

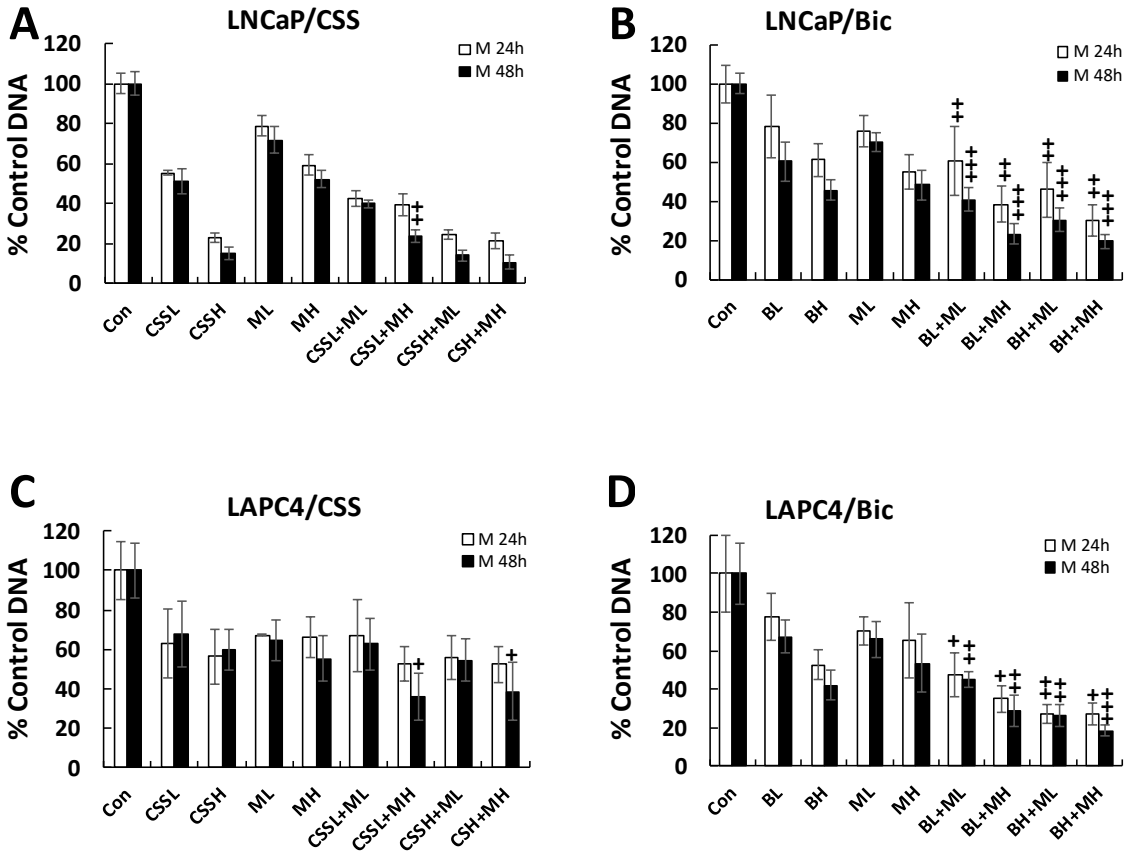


Figure 1. Combination of ADT with metformin decreases the growth of androgen-dependent prostate cancer cells compared to single-agent use. A&C, Cells were cultured in medium containing different percentages of FBS/CSS for 6 days and then followed by addition of metformin for 24 or 48 hours. Cell proliferation was measured by DNA assay. Con: 10% FBS; CSSL: 8% CSS + 2% FBS; CSSH:10% CSS; ML, metformin low dose (mM): LNCaP, 0.1; LAPC4, 2.5. MH, metformin high dose (mM): LNCaP, 1; LAPC4, 5. **B&D,** Cells were treated with bicalutamide for 6 days, followed by addition of metformin for 24 or 48 hours. Con: 10% FBS treated with vehicles. BL, bicalutamide low dose (μM): LNCaP, 1; LAPC4, 20. BH, bicalutamide high dose (μM): LNCaP, 5; LAPC 30. Metformin doses are the same as A&C. Data are shown as percentage of the control group, Mean ± SD. Synergistic effect was calculated by Calcusyn, moderate synergy (combination index CI: 0.7-0.85) +; synergy (CI: 0.3-0.7) ++, and strong synergy (CI: 0.1-0.3) +++.

Senescence was quantitated using SA-β-gal activity, flow cytometry and western blotting for p16 and p27. We have demonstrated that ADT induces senescence in androgen-dependent prostate cancer cells (**Figure 2**).

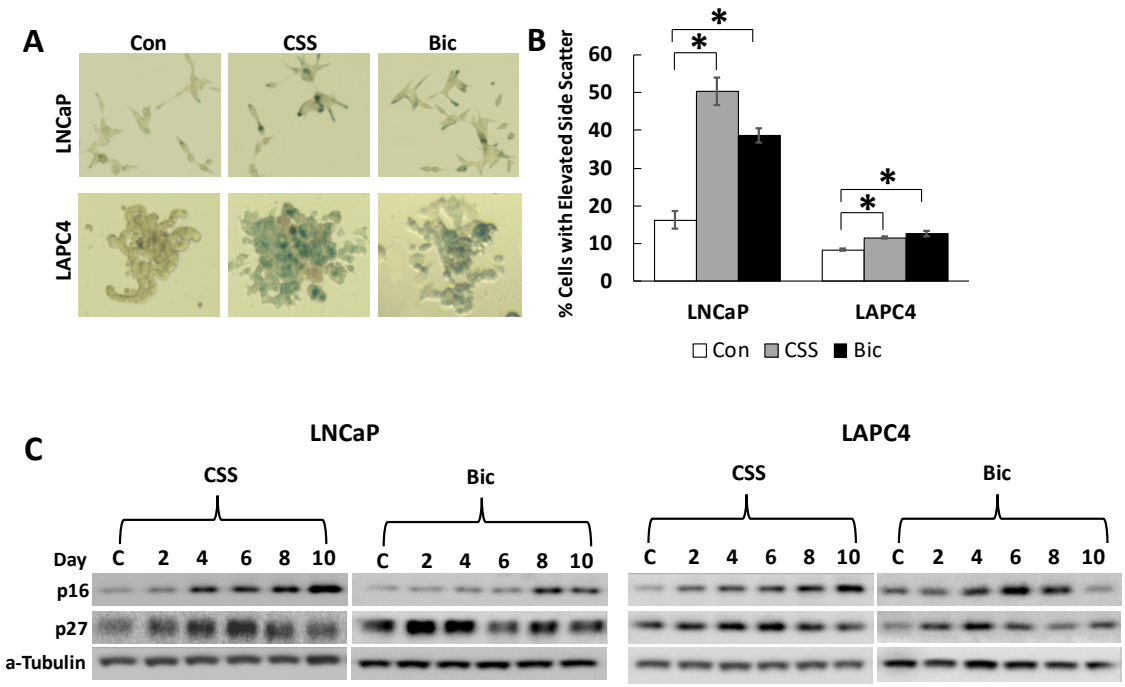


Figure 2. ADT induces phenotypic characteristics of cellular senescence in androgen-dependent prostate cancer cells. A, LNCaP or LAPC4 cells were cultured in CSS medium or treated with bicalutamide for 8 days, and then stained for SA-β-gal activity (magnification × 100). Con: 10% FBS in RPMI media; CSS: 10% Charcoal-stripped serum in RPMI media ; Bic, bicalutamide in complete RPMI media, LNCaP, 5μM; LAPC 30μM. **B,** Flow cytometry was used to examine the cellular complexity side scatter. The percentage of cells with side-scatter is shown in graph (Mean ± SD) * t-test, $p < 0.05$. ADT increases the fraction of cells with elevated cellular complexity and size. **C,** LNCaP and LAPC4 cells were cultured in CSS medium or treated with bicalutamide for days indicated, then whole cell lysates were harvested and analyzed for p16 and p27 expression by western blot. 30μg of protein was loaded, and α-tubulin was used as a loading control.

Apoptosis occurs maximally 48 hr after exposure to ADT in LNCaP and LAPC4 prostate cancer cells (**Table 1**). Apoptosis increases with the addition of metformin to the senescent cells.

Table 1. Addition of metformin increases apoptosis in senescence-induced cancer cells

	LNCaP	LAPC4
CSS	0.3	1.29
Bic	0.87	0.95
Met	1.21	1.13
CSS + Met	2.51	1.72
Bic + Met	2.00	2.97

Table 1: Apoptosis was assayed using Caspase-Glo and shown as fold change relative to control, mean value. CSS: 10% CSS; Bic, bicalutamide (μM), LNCaP, 5; LAPC4 30. Met: metformin (mM): LNCaP, 1; LAPC4, 5.

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

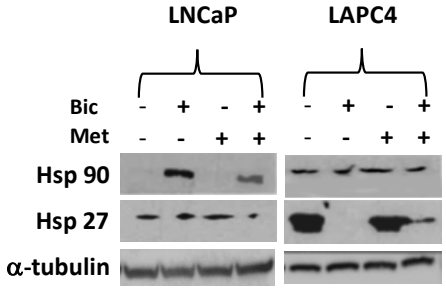
- PC cells will be treated with ADT and/or metformin as in subtask 1
- Collect GLB1-fluorescent and non-fluorescent cells by FACs and determine Hsf1, Hsp27, Hsp70 and Hsp90 mRNA and protein levels.
- Perform co-immunofluorescence with GLB1 and Hsf1, Hsp27, Hsp70, Hsp90, p-AMPK and AMPK
- Determine whether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

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

-COMPLETED

We have examined the proteotoxic stress response (PSR) 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 the prostate cancer cell line and individual heat shock proteins with regard to the effects of both ADT and metformin treatment. For example, we observed increased HSP90 in LNCaP cells, but not LAPC4 cells, with exposure to ADT, and decreased HSP27 in LAPC4 cells in response to ADT (**Figure 3**). Other HSPs were also not consistently altered.

Figure 3: Expression of Hsps in PC cells exposed to ADT. LNCaP and LAPC4 Cells were treated with bicalutamide for 6 days, and then followed by treatment of metformin for 1 or 2 days. Bic, bicalutamide (μM), LNCaP, 5; LAPC4 30. Met: metformin (mM): LNCaP, 1; LAPC4, 5.



Since we did not observe consistent PSR induction in prostate cancer cells following ADT, we have explored alternative signaling pathway(s) that may be associated with cell survival after ADT. AKT plays a critical role in cell survival, senescence and apoptosis-resistance (1). We observed that activated p-AKT (T308) increased in prostate cancer cells in response to ADT (**Figure 4**). AMPK is a cellular sensor that plays a key role in regulating energy homeostasis (2). We also observed that p-AMPK increased in prostate cancer cells 2-4 days after ADT treatment when senescence markers were minimally induced, consistent with AMPK activation. Subsequently, p-AMPK levels decreased in late terminal senescence (8-10 days).

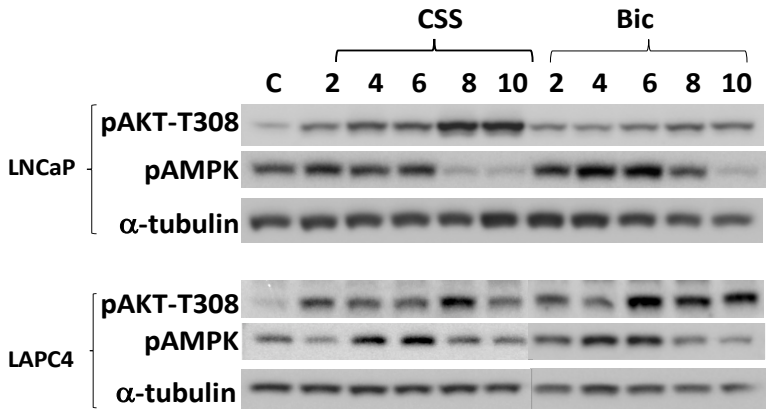


Figure 4: Time-dependent AKT and AMPK signaling alterations induced by ADT. LNCaP and LAPC cells were cultured with media containing 10% CSS or treated with complete culture media containing bicalutamide (LNCaP 5μM, LAPC4 30μM) as for the indicated number of days. 30μg of protein was used for each

AMPK is a known suppressor of mTORC1 signaling through phosphorylation of TSC2 (3). Persistent activation of AKT and inhibition of AMPK after long-term exposure to ADT was associated with mTORC1 activation as determined by increased p-p70S6K, a downstream target of mTORC1 (**Figure 5**). Activation of mTORC1 may serve as one of the pathways by which senescent prostate cancer cells persist. Consistent with this idea, addition of metformin, an activator of AMPK, to ADT, resulted in decreased p-p70S6K and a modest reduction in the senescence marker p16 (**Figure 5**), suggesting clearance of some senescent cells through metformin's effect on AMPK activation and subsequent inhibition of mTORC1.

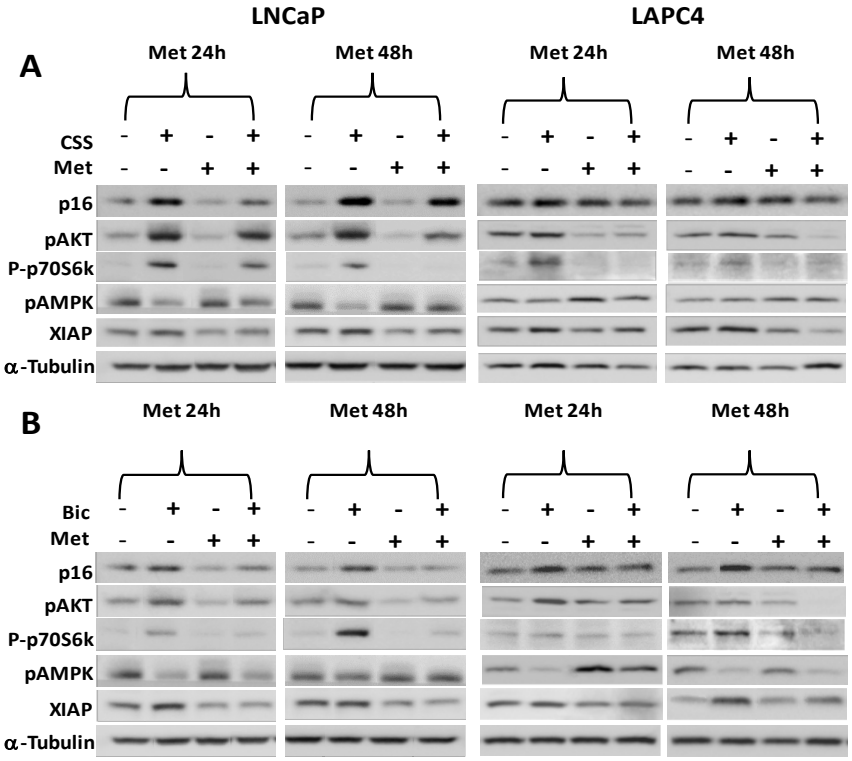
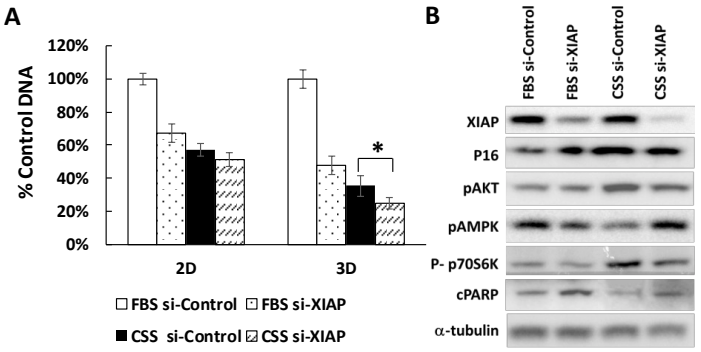


Figure 5. Late ADT induces mTORC1 signaling through activation of AKT and inhibition of AMPK. Metformin addition reactivates AMPK and inhibits mTORC1 signaling. PC cells were cultured in 10% CSS medium (**A**) or treated with bicalutamide (**B**) for 6 days followed by addition of metformin. Whole cell lysates were harvested and analyzed by western blotting. 30 μg of protein was used for each sample. Bic: bicalutamide (mM), LNCaP, 5; LAPC4, 30. Met: metformin (mM): LNCaP, 1; LAPC4, 5.

In addition, we found that the antiapoptotic protein XIAP was induced to varying degrees at different time points in prostate cancer cells by ADT and this induction was partly blocked by metformin (**Figure 5**). We next investigated the role of XIAP in the cell response to ADT and metformin by silencing XIAP in LNCaP cells. The combination of ADT and XIAP knockdown reduced LNCaP growth more robustly at day 3 than ADT plus the non-silencing control siRNAs (si-Control, **Figure 6A-B**). Moreover, XIAP knockdown resulted in enhanced apoptosis in ADT-treated cells compared to non-silencing controls plus ADT, indicating that a significant part of the survival response that ADT induces is XIAP-dependent (**Figure 6C**).



C

XIAP knockdown induces apoptosis in senescent cells using Caspase-Glo

	2D	3D
FBS si-Control	1.00 ± 0.15	1.00±0.17
FBS si-XIAP	1.62± 0.18	1.92±0.13
CSS si-Control	1.14± 0.06	1.70±0.19
***CSS si-XIAP**	2.84±0.21	2.51±0.14

Figure 6: Silencing XIAP enhances apoptosis in prostate cells treated with ADT. LNCaP cells were transfected with non-silencing control siRNA (si-Control) or siRNA targeting XIAP (si-XIAP), 24hr later cells were incubated with fresh media containing either 10% FBS or CSS. **A**. Cell proliferation was measured with a DNA assay at 2 and 3 days (2D and 3D) (Mean ± SD). *$p < 0.05$. **B.** Whole cell lysates were harvested and analyzed by western blotting. 30μg of protein was used for each sample. **C**. Apoptosis was measured by Caspase-Glo assay, and results were normalized to cell number. The fold change relative to cells transfected with si-Control and cultured in media containing 10% FBS is shown (Mean ± SD). *$p < 0.05$, comparison between CSS si-Control vs CSS si-XIAP.

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.

Due to the inconsistent alterations of HSPs by ADT in the investigated androgen-dependent prostate cancer cell lines, we altered our focus to AKT, AMPK, mTORC1 and XIAP as described in the previous section.

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

We did not investigate the function of site-specific phosphorylation of Hsf1 on the antitumor effects of ADT and metformin *in vivo* due to the inconsistent data regarding the PSR (Aim 1, Subtask 3).

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

- 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).
- Four groups were randomized using 2 xenografts (77 and 147) when flank tumors are 100mm3 to examine the i) castrated and treated with metformin (CM), ii) castrated and treated with vehicle control (CC), iii) sham surgery and treated with metformin (SM), and iv) sham surgery and treated with vehicle control (SC). Metformin was administered one week after surgery in drinking water at a dose of 2g / liter. Post-ADT Tumor growth and body weight were assessed weekly.

The growth of both PC PDXs was initially inhibited by castration; however, tumors began regrowing after 4-6 weeks (**Figure 7**). Metformin also modestly inhibited tumor growth compared to controls in both models. With the combination treatment, LuCaP 77 tumors at 7 weeks were significantly smaller than those in the castration ($p < 0.01$) or metformin ($p < 0.01$) alone groups. In the LuCaP 147 PDX model, tumors were significantly smaller at 6-8 weeks post-surgery in the combination therapy compared to those in the castration ($p < 0.01$) or metformin groups ($p < 0.01$).

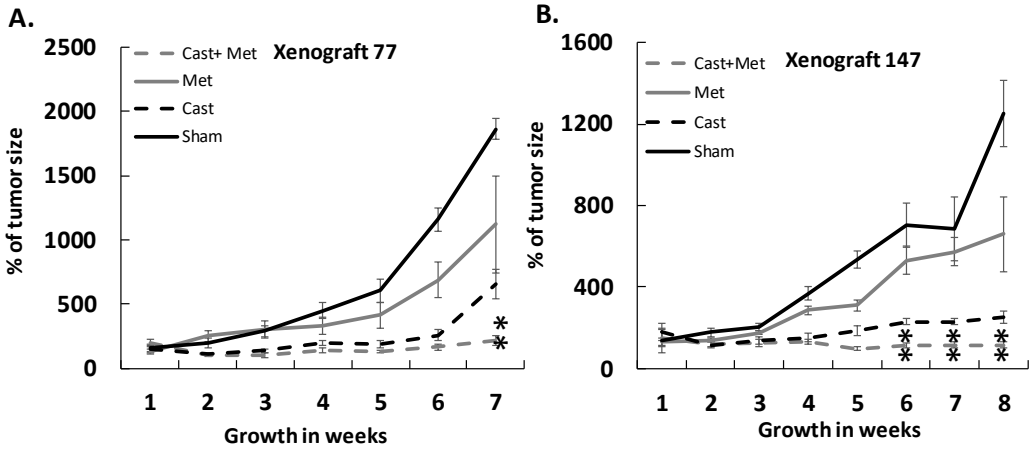


Figure 7: Metformin and ADT suppress tumor growth in patient-derived xenografts (PDXs) more robustly than ADT alone. A. LuCaP 77, **B.** LuCaP 147. The growth rates are shown as percentage of tumor size compared to the time point of sham surgery/castration (median ± SE). One-way ANOVA was used to analyze the differences between any 2 groups. $^{**}p < 0.01$ is considered as statistically significant between castration plus metformin treated group compared to castration alone (N=8 mice per group) Sham: sham surgery control, Cast: castration, Met: sham surgery with metformin treated, Cast+Met: castration and metformin treated.

We conclude from these animal studies that combined treatment with ADT and simultaneous metformin leads to an improved tumor response in prostate cancer PDX models.

A pilot study we performed using a smaller number of animals (8 vs 8) that underwent ADT and metformin treatment simultaneously versus delayed metformin treatment (10 days) suggested that simultaneous use of these 2 agents versus 10 d delay led to no appreciable differences in outcomes. 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 (PSR) 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).

-COMPLETED

Tissue Microarrays (TMA) were constructed from 58 tissue specimens (29 in duplicate) from the Xenograft 77 model and 54 tissue specimens (27 in duplicate) from the Xenograft 147 model. Antibodies for Ki67 and active cleaved caspase-3 (CC3) were used to quantify proliferation and apoptosis. For image analysis and quantification of the staining intensity, the VECTRA system was used. 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. Expression of Ki67 (proliferation) and cleaved caspase 3 (apoptosis) were evaluated by immunohistochemistry. H&E staining was used to differentiate nuclear and cytoplasmic tissue compartments. Two cores from the same tumor were averaged to give a more precise estimate.

Ki67 expression decreased in both the castration and castration plus metformin groups (**Figure 8**). A difference in CC3-positivity in tumors from different treatment groups was not detected (p=0.1905) in contrast to our *in vitro* data showing induction of apoptosis (**Figure 6**). We did not further pursue the staining of HSPs in this TMA due to the inconsistent activation of HSPs *in vitro*.

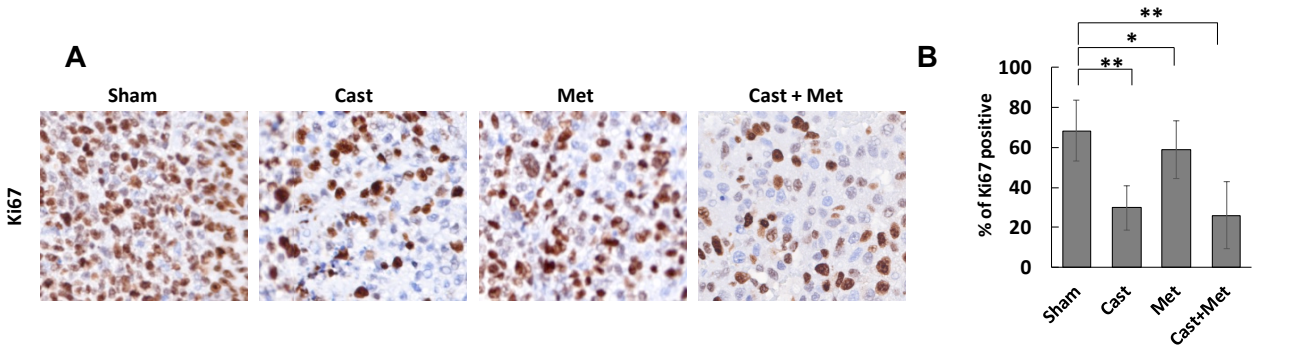


Figure 8. Castration, metformin and the combination reduce proliferation in a prostate cancer PDX model. Ki67 expression in LuCaP 147 tumors. Expression of Ki67 protein was detected in this TMA constructed from LuCaP 17 xenograft tumors, with duplicate cores per tissue. The VECTRA system was used for image capture and quantification of the staining intensity. **A**. Images of Ki67 staining. The brown color is positive staining. H&E was used as counter-staining. **B**. Data is shown as the percentage of Ki67-positive tumor cells (mean± SE). A one-way ANOVA was used to analyze the differences between any 2 groups. *p < 0.05, **p < 0.01.

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).

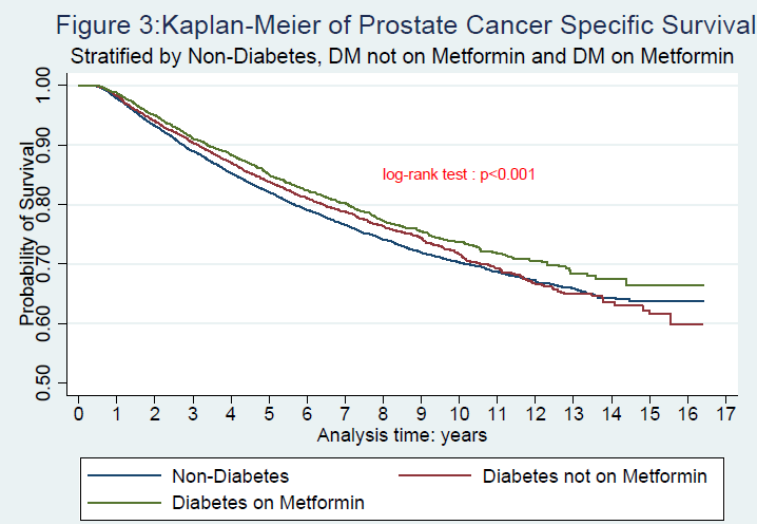
-COMPLETED

Milestone(s) Achieved:

Using national Veterans Affairs databases, we identified all men diagnosed with prostate cancer 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 not treated with 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.

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

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3. Hindupur, S. K., et al. (2015). The opposing actions of target of rapamycin and AMP-activated protein kinase in cell growth control. Cold Spring Harb Perspect Biol **7**(8): a019141.

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 in Boston MA), GU ASCO Meeting (Feb 2017 in Orlando, FL), the Prostate Cancer Foundation meeting (10/2017 in Washington DC) and multiple publications (see Products).

• 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, and submit the current manuscript in preparation for publication.

4. Impact

A growing body of evidence indicates that ADT induces a susceptible therapeutic niche that may be exploited to enhance therapeutic response. In the current study, we demonstrate for the first time a synergistic inhibitory effect of metformin combined with ADT on growth in human PC cell lines *in vitro* and PDX tumors *in vivo*. We find a novel role for XIAP, an anti-apoptotic protein driven by increased AKT signaling in PC cells after ADT. These observations mechanistically support our recent study demonstrating improved survival in patients taking metformin at the time of initiating ADT for advanced PC. The fate of PC cells after ADT includes apoptosis, quiescence and other phenotypes. In the current work, we demonstrate that subpopulations of androgen-sensitive PC cells after ADT exhibit molecular markers characteristic of senescence, such as increased SA-beta-gal-staining, cellular complexity and expression of cyclin dependent kinase inhibitor 2A p16INK4a. Apoptosis occurs early after ADT and is limited. In a series of xenograft tumors *in vivo*, surgical castration shows a similar induction of senescence with decreased proliferation as determined by Ki-67 expression. We also demonstrate that persistent activation of AKT and inhibition of AMPK following long-term ADT activates mTORC1 signaling, which may serve as one of the pathways by which senescent cells persist. A synergistic

reduction in cell number was noted with the addition of metformin to ADT *in vitro,* as well as a reduction in xenograft tumor growth with the combination *in vivo.* These studies provide a molecular rationale for the clinical observation that metformin improves both overall and cancer-specific survival in patients with advanced PCa receiving ADT. We would anticipate that other agents that activate AMPK efficiently may also act synergistically with ADT.

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 observed prostate cell line-specific and Hsp-specific alterations in response to ADT (Major Task 1, Subtask 2) Given inconsistent activation of the proteotoxic stress response, we did not investigate the functional role of Hsf1 (the major transcription factor for HSPs), on the effects of metformin on senescent PCs following ADT under Major Task 1, Subtasks 3 & 4.
- We have investigated alternative signaling pathway(s) that may be associated with cell survival after ADT. We found that Akt, AMPK, mTORC1 and the antiapoptotic protein XIAP are induced at various time points during ADT and that silencing XIAP augmented apoptosis by ADT.

6. Products

Manuscript in preparation:

Bing Yang, Shivashankar Damodaran, Tariq A. Khemees, Mikolaji Filon, Adam Schultz, Joseph Gawdzik, Tyler Etheridge, Dmitry Malin, **Kyle Richards, Vincent L. Cryns and David Jarrard**. Synthetic Lethal Metabolic Targeting of Androgen Deprived Prostate Cancer Cells with Metformin. In preparation.

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 Dec;200(6):1256-1263.
2. Etheridge T, Damodaran S, Schultz A, Richards KA, Gawdzik J, Yang B, **Cryns V**, **Jarrard DF**. Combination therapy with androgen deprivation for hormone sensitive prostate cancer: A new frontier. Asian J Urol. 2019 Jan;6(1):57-64.
3. Damodaran S, Lang JM, **Jarrard DF**. Targeting Metastatic Hormone Sensitive Prostate Cancer: Chemohormonal Therapy and New Combinatorial Approaches. J Urol. 2019 May;201(5):876-885.

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): 0000-0001-8773-6413

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: Bing Yang, MD, PhD

Project Role: Researcher

Researcher Identifier (e.g., ORCID ID): 0000-0001-8621-6838

Nearest person month worked: 5.7

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: Jinn-ing Liou

Project Role: Researcher

Researcher Identifier (e.g., ORCID ID):

Nearest person month worked: 0.36

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: 7.5

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: Elena Strekalova, PhD

Project Role: Assistant Scientist

Researcher Identifier (e.g., ORCID ID): 0000-0001-9271-5465

Nearest person month worked: 2.5

Contribution to Project: Elena Strekalova has worked together with Dr. Malin to characterize the therapeutic response of PCa cell lines to androgen deprivation therapy and metformin.

8. Special Reporting Requirements

NA

9. APPENDICES

Manuscripts:

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 Dec;200(6):1256-1263.
2. Etheridge T, Damodaran S, Schultz A, Richards KA, Gawdzik J, Yang B, **Cryns V**, **Jarrard DF**. Combination therapy with androgen deprivation for hormone sensitive prostate cancer: A new frontier. Asian J Urol. 2019 Jan;6(1):57-64.
3. Damodaran S, Lang JM, **Jarrard DF**. Targeting Metastatic Hormone Sensitive Prostate Cancer: Chemohormonal Therapy and New Combinatorial Approaches. J Urol. 2019 May;201(5):876-885.

Metformin Use is Associated with Improved Survival for Patients with Advanced Prostate Cancer on Androgen Deprivation Therapy

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

From the Section of Urology, Department of Surgery, William S. Middleton Memorial Veterans Hospital (KAR) and Departments of Urology (KAR, TMD, EJA, DFJ) and Medicine (JL, VLC), University of Wisconsin-Madison, Madison, Wisconsin

Abbreviations and Acronyms

ADT = androgen deprivation therapy
CDW = Corporate Data Warehouse
CSS = cancer specific survival
DM = diabetes mellitus
IPSW = inverse propensity score weighted
OS = overall survival
PCa = prostate cancer
PSA = prostate specific antigen
SRE = skeletal related event
VA = Veterans Administration

Purpose: Metformin is commonly prescribed for patients with type 2 diabetes mellitus. We hypothesized that metformin plus androgen deprivation therapy may be beneficial in combination. Our objective was to assess this combination in a retrospective cohort of patients with advanced prostate cancer.

Materials and Methods: Using national Veterans Affairs databases we identified all men diagnosed with prostate cancer between 2000 and 2008 who were treated with androgen deprivation therapy with followup through May 2016. Study exclusions included treatment with androgen deprivation therapy for 6 months or longer, or receipt of androgen deprivation therapy concurrently with localized radiation. Three patient cohorts were developed, including no diabetes mellitus, diabetes mellitus with no metformin and diabetes mellitus with metformin. Cox proportional HRs were calculated for overall survival, skeletal related events and cancer specific survival.

Results: After exclusions the cohort consisted of 87,344 patients, including 61% with no diabetes mellitus, 22% with diabetes mellitus and no metformin, and 17% with diabetes mellitus on metformin. Cox proportional hazard analysis of overall survival showed improved survival in men with diabetes mellitus on metformin (HR 0.82, 95% CI 0.78–0.86) compared to those with diabetes mellitus who were not on metformin (HR 1.03, 95% CI 0.99–1.08). The reference group was men with no diabetes mellitus. Cox proportional hazard analysis of predictors of skeletal related events revealed a HR of 0.82 (95% CI 0.72–0.93) in men with diabetes mellitus on metformin. Cox proportional hazard analysis of cancer specific survival showed improved survival in men with diabetes mellitus

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The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

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Editor's Note: This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1382 and 1383.

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on metformin (HR 0.70, 95% CI 0.64−0.77) vs those with diabetes mellitus without metformin (HR 0.93, 95% CI 0.85− 1.00). The reference group was men with no diabetes mellitus.

Conclusions: Metformin use in veterans with prostate cancer who receive androgen deprivation therapy is associated with improved oncologic outcomes. This association should be evaluated in a prospective clinical trial.

Key Words: prostatic neoplasms, metformin, gonadotropin-releasing hormone, analogs, derivatives, and diabetes mellitus

THE past decade has witnessed remarkable advances with 6 new therapies approved by the United States FDA (Food and Drug Administration) for the treatment of men with advanced PCa.[1] Despite these advances nearly 27,000 men died of PCa in 2017, highlighting the ongoing need for additional therapeutic options in men in whom conventional treatments fail.[2]

ADT remains the standard first line approach for metastatic PCa. It leads to regression but rarely to cure as hormone insensitive disease invariably develops from resistant clones. These cells that remain after the initiation of ADT represent an underexplored therapeutic niche which may improve therapy. In support a recent randomized clinical trial demonstrated that up-front chemotherapy with ADT improved survival by 10.5 months vs ADT alone in hormone naïve patients, suggesting that initiating ADT induces susceptibilities in PCa cells that make them amenable to synergistic treatments.[3]

Metformin, a commonly used insulin sensitizer, is a first line agent for patients with type 2 DM. There is scientific evidence for the antineoplastic effects that metformin may have for various cancers but its impact in men with advanced PCa and its usefulness in combination with other treatments remain poorly studied.[4,5] Metformin activates AMPK (AMP-activated protein kinase), which inhibits mTOR (mammalian target of rapamycin), 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 performed a large observational study evaluating the impact of metformin use on cancer outcomes in men with PCa who were being treated with ADT.

MATERIALS AND METHODS

Data Source

The study was approved by local institutional review boards. The VA provides care to more than 20 million veterans at a total of more than 1,400 centers. All care processes are captured via the VistA (Veterans Information System Technology Architecture) electronic health record, which provides a longitudinal view of patients receiving care nationwide, including diagnoses, procedures, medications, laboratory findings, physiological measurements, text notes and reports.[11] Data are aggregated from individual VistA systems to the VA CDW, where the data are prepared for use.

Study Population

To develop a cohort of men with PCa on ADT we identified all 558,252 men diagnosed with PCa (ICD-9 code 185) in the VA CDW from 2000 to 2008. In this cohort we included only the 129,672 men receiving ADT 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 from study 33,312 patients with no information on the ADT medication supply days, quantity or dose, those on ADT for 6 months or less and/or 10,960 receiving ADT concurrent with primary radiation therapy of the prostate, leaving a final cohort of 87,344 patients for our analytical file. ADT was entered as a time dependent variable in the models. Longitudinal data on patients were compiled until death or until the study end of May 31, 2016, at which point they were censored.

We divided the study population into 3 cohorts and defined DM in the VA using a previously published algorithm with ICD-9 codes 250.00 or 250.02.[12] Comparator groups included 1) no DM, 2) DM and no prescription of metformin for 180 days or longer during the study period and 3) DM with a prescription of metformin for 180 days or longer during the study period.

Outcomes of Interest

The primary outcome of interest in this study was OS. Secondary outcomes of interest included SRE and death from PCa (CSS). The dependent variable used in our analyses was the interval from the ADT starting date to death from any cause, SRE and/or death from PCa. SRE served 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 for whom metformin was prescribed for 180 days or longer. We did not exclude patients with exposure to insulin or other glucose lowering medications because the impact on

cancer outcomes is conflicting.[14,15] Prior clinical trials on metformin consisted of at least 24 weeks of exposure. Therefore, we chose to define drug use as 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 for patients to move from a period of exposure to a period of nonexposure.

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

Statistical Analysis

Medians were compared by the Mann-Whitney U test. The Fisher exact and chi-square tests were used to compare categorical variables. We performed multivariable Cox proportional hazard analyses to assess for independent predictors of OS, SRE and CSS. We then calculated a propensity score by multinomial logistic regression and used it to adjust the IPSW in the final models.[18] We constructed IPSW Kaplan-Meier curves of OS, SRE and CSS, and performed the log rank test. We also performed sensitivity analysis of CSS to account for competing risks as a result of death from other causes using a subdistribution hazard model adapted for time dependent covariates.[19,20] Finally, we performed subset IPSW multivariable Cox proportional hazard analyses to assess for independent predictors of OS, SRE and CSS in patients with PSA greater than 20 ng/ml at ADT initiation. Statistical significance was considered at 2-sided $p < 0.05$ and statistical analysis was performed with Stata® 14.

RESULTS

The total cohort available for analysis after exclusions consisted of 87,344 patients, including 53,893 (61%) in the no DM group, 18,934 (22%) in the DM without metformin group and 14,517 (17%) in the DM plus metformin group. The metformin group was younger with a median age of 71.0 years (IQR 64–76) compared to the no DM group (75.0, IQR 69–80) and the DM without metformin group (75.0, IQR 69–79, $p < 0.001$, see table).

The OS was longest in the metformin group as represented by the IPSW Kaplan-Meier curve ($p = 0.005$, fig. 1). The adjusted Cox proportional hazard multivariable analysis identified that the metformin group was associated with improved OS (HR 0.82, 95% CI 0.78–0.86, $p < 0.001$) vs the DM without metformin group (HR 1.03, 95% CI 0.99–1.08, $p = 0.18$) with the no DM group as the reference group. A dose-response relationship was observed in the cumulative duration of metformin use before and after IPSW with 36 months or more found to be most protective (HR 0.69, 95% CI 0.65–0.74, $p < 0.001$, supplementary table 1, http://jurology.com/).

The proportion of patients with SREs was highest in the metformin group at 11.1% but time to SRE was also longest in the metformin group as represented by the IPSW Kaplan-Meier curve ($p = 0.005$, fig. 2). The adjusted Cox proportional hazard multivariable analysis identified that the metformin group was associated with a decreased risk of SRE (HR 0.84, 95% CI 0.74–0.96, $p = 0.009$) vs the DM without metformin group (HR 1.08, 95% CI 0.96–1.23, $p = 0.20$) with the no DM group as the reference group. A dose-response relationship was observed in the cumulative duration of metformin use before and after IPSW with 36 months or more found to be most protective (HR 0.70, 95% CI 0.59–0.83, $p < 0.001$, supplementary table 2, http://jurology.com/).

The proportion of patients documented to have died of PCa was lowest in the metformin group at 9.3% as shown by the IPSW Kaplan-Meier curve ($p < 0.001$, fig. 3). The adjusted Cox proportional hazard multivariable analysis identified that the metformin group was associated with improved CSS (HR 0.70, 95% CI 0.64–0.77, $p < 0.001$) vs the DM without metformin group (HR 0.93, 95% CI 0.85–1.00, $p = 0.0.054$) with the no DM group as the reference group. A dose-response relationship was observed in the cumulative duration of metformin use before and after IPSW with 36 months or more found to be most protective (HR 0.58, 95% CI 0.51–0.66, $p < 0.001$, supplementary table 3, http://jurology.com/). After accounting for competing risks as a result of death from other causes the decreased risk observed between metformin for 36 months or greater and prostate cancer mortality remained statistically significant (HR 0.66, 95% CI 0.58–0.75, $p < 0.001$).

The subset Cox proportional hazard multivariable analyses to assess for independent predictors of OS, SRE and CSS in patients with PSA greater than 20 ng/ml at the time of ADT initiation revealed no change in the noted associations (supplementary tables 1, 3 and 4, http://jurology.com/). However, the association with SRE was no longer statistically significant (supplementary tables 2, 5 and 6, http://jurology.com/).

DISCUSSION

This large observational study revealed that metformin use was associated with improved oncologic outcomes in men with PCa on ADT. Prior studies evaluating the impact of metformin in men with PCa focused on disease at diagnosis or early treatment. To our knowledge the current study is unique in evaluating the impact of metformin in men on ADT as these drugs may have an additive effect.

Characteristics of 87,344 patients with prostate cancer on androgen deprivation therapy

	No Diabetes	Diabetes		p Value
		No Metformin	Metformin	
No. pts	53,893	18,934	14,517	
Median age (IQR)	75.0 (69–80)	75.0 (69–79)	71.0 (64–76)	<0.001
No. race (%):				<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)	
No. Charlson comorbidity score (%):				<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)	
Greater than 3	926 (1.7)	932 (4.9)	621 (4.3)	
No. Agent Orange exposure (%)	1,804 (3.4)	696 (3.7)	975 (6.7)	<0.001
No. mos ADT (%):				<0.001
Less than 12	16,744 (31.1)	5,360 (28.3)	3,865 (26.6)	
12—Less than 24	14,509 (26.9)	4,903 (25.9)	3,629 (25.0)	
24—Less than 36	7,925 (14.7)	2,894 (15.3)	2,230 (15.4)	
36 or Greater	14,715 (27.3)	5,777 (30.5)	4,793 (33.0)	
Median ng/dl PSA IQR):*				<0.001
Less than 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)	
Greater than 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)	
No. diagnosis yr (%):				<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)	
No. Gleason score (%):				<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)	
No. local therapy (%)	3,964 (7.4)	1,387 (7.3)	1,788 (12.3)	<0.001
No. docetaxel (%)	1,803 (3.4)	508 (2.7)	584 (4.0)	<0.001
No. insulin (%)		8,755 (46.2)	9,297 (64.0)	<0.001
No. vital status (% deceased)	42,133 (78.2)	15,215 (80.4)	9,512 (65.5)	<0.001
Median yrs overall survival (IQR)	5.1 (2.5–8.8)	5.4 (2.7–9.0)	6.8 (3.5–10.1)	<0.001
No. prostate cancer death (%)	5,522 (10.3)	1,959 (10.4)	1,337 (9.2)	<0.001
Skeletal related event:				
No. pts (%)	4,863 (9.0)	1,833 (9.7)	1,609 (11.1)	<0.001
Median yrs to event (IQR)	4.7 (2.2–8.3)	4.9 (2.3–8.5)	6.1 (2.8–9.5)	<0.001

* At androgen deprivation therapy initiation.

Residual cancer cells after ADT are characterized by metabolic abnormalities which may be targeted preferentially by metformin.[10] Capturing prescription medication use is vital for this type of analysis and VA databases provided an ideal platform to perform this study since approximately 83% of VA enrollees who use 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 as monitored through 1 health care record, making outcomes easier to determine.

Our analysis, which controlled for multiple variables, identified that metformin use was associated with improved OS (HR 0.82) in dose dependent fashion. CSS also improved (HR 0.70), specifically in men with DM receiving metformin compared to the other groups. It was difficult to clearly define the patients in whom ADT was initiated for metastatic hormone sensitive PCa in this data set. However, controlling for PSA and performing subset analysis of patients with PSA greater than 20 ng/ml at ADT initiation confirmed the overall and cancer specific survival advantage to being on metformin. In this higher PSA subset there were improved outcomes in patients at higher risk for metastatic disease, the group in which ADT is typically initiated for modern, hormone sensitive PCa. The recognition of increased cardiac, bone density and other side effects has led to delaying ADT in many patients with micrometastatic disease.[22]

To our knowledge studies to date 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 receipt was associated with decreased PCa risk (OR 0.91) and biochemical recurrence following treatment (HR 0.81) but not with improved OS in patients with PCa (HR 0.86, 95% CI 0.64–1.14).[23] Our data do not discount a role for metformin in improving disease in the castrate resistant state. In a phase 2 clinical trial of metformin in 44 men with progressive castrate

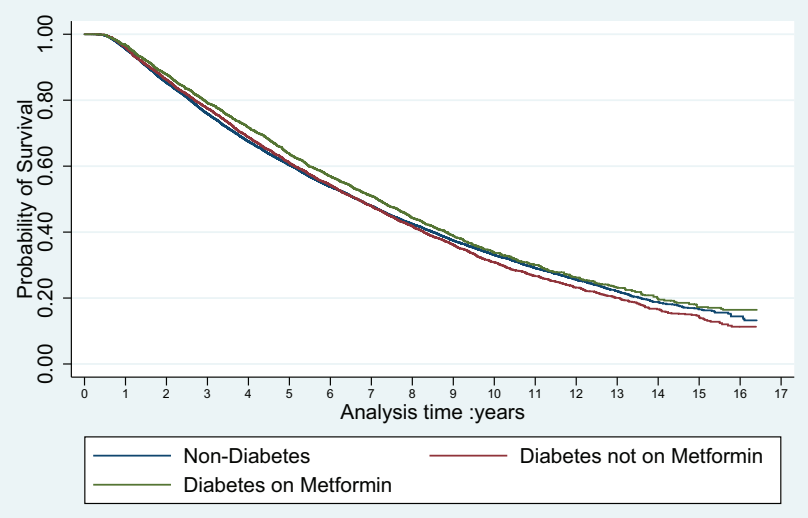

Figure 1. Kaplan-Meier curve of overall survival stratified by nondiabetes, DM plus metformin and DM without metformin after IPSW adjustment (log rank test p = 0.005).

resistant PCa, Metformin Hydrochloride as First-Line Therapy in Treating Patients With Locally Advanced or Metastatic Prostate Cancer (ClinicalTrials.gov NCT01243385), 36% of the men were free of progression at the 12-week followup with no grade 3 or 4 toxicity, suggesting some activity in this space.[24] The multi-arm, multistage, randomized STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy, ClinicalTrials.gov NCT00268476) clinical trial is currently recruiting patients in a metformin plus ADT arm to assess the safety and efficacy of this approach. In addition, the randomized, prospective, phase 3 PRIME (Metformin in Patients Initiating ADT as Prevention and Intervention of Metabolic Syndrome, ClinicalTrials.gov NCT03031821) clinical trial is under way to assess the proportion of patients in whom metabolic syndrome develops.

The duration of metformin receipt may influence outcomes as suggested by our data and those of others. Margel et al performed a retrospective cohort study to evaluate associations of the cumulative duration of antidiabetic drug use after PCa

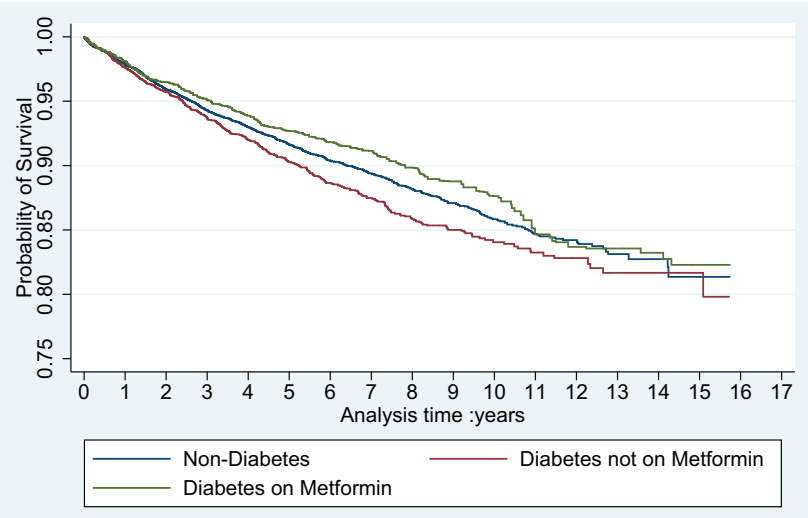

Figure 2. Kaplan-Meier curve of skeletal related events stratified by nondiabetes, DM plus metformin and DM without metformin after IPSW adjustment (log rank test p = 0.005).

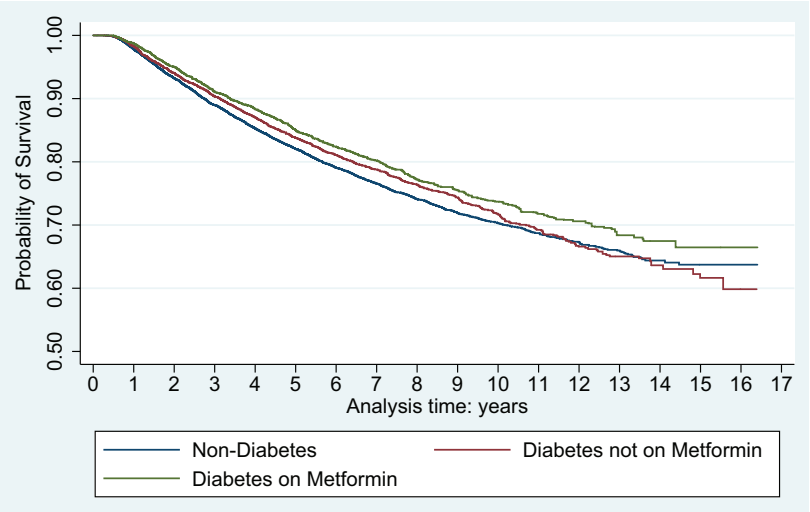

Figure 3. Kaplan-Meier curve of prostate cancer specific mortality stratified by nondiabetes, DM plus metformin and DM without metformin after IPSW adjustment (log rank test p = 0.001).

diagnosis with CSS and OS in patients with type 2 DM.[25] Each additional 6 months of metformin resulted in an adjusted CSS HR of 0.76 (95% CI 0.64–0.89). However, no relationship was seen between the cumulative use of other antidiabetic drugs and CSS or OS. Furthermore, we found similar adjusted HRs of OS and CSS in our cohort, noting that our study included patients without DM as a functional control group and all study patients were on ADT. This highlighted the difference in our study design.

In addition, we found that metformin was associated with a reduced risk of SRE, which we used as a measure of progression. Notably progression was not assessed in the study by Margel et al.[25] There was an increased incidence of SRE in the metformin group but 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 of progression since we thought that it was a more sensitive measure in this patient population, given the low rate of chemotherapy or novel antiandrogen therapies.

In our study we aimed to specifically assess the effects of metformin in patients on ADT based on the potential for an additive benefit of these 2 agents in preclinical studies.[6–10] In vitro and in vivo studies suggested that combining metformin with bicalutamide would result in reduced proliferation of androgen receptor positive cells and apoptosis of androgen receptor negative cells.[26] ADT induces senescence in a population of PCa cells,[27] which generates inherent susceptibilities that may be used. 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 showed that long-term ADT use may also induce metabolic syndrome and in turn increase the risk of cardiovascular morbidity.[28] Metformin may have benefits in reducing these effects, in addition to the direct antineoplastic activity.

There are several limitations to our study. 1) This was a retrospective observational study with potentially unmeasured confounding variables and/or missing variables. 2) Because national VA data are developed as an administrative data set via the CDW, we could not account for drug discontinuation reasons, key variable miscoding, complete laboratory data on the entire cohort, socioeconomic status, body mass index, exercise, smoking, local therapies received outside the VA or stage. In addition, we could not account for other potential health benefits of metformin which may have impacted our results, including an improvement in DM and cardiovascular health. However, our large sample size and our propensity score matching enabled us to control for other important confounding factors. 3) Finally, our population of aging veterans may lack external validity. Additional studies are warranted in other populations.

CONCLUSIONS

Metformin use was associated with improved OS, SRE and CSS in men with PCa who were also receiving ADT. We believe that 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.

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EDITORIAL COMMENT

Metformin has been proposed to have efficacy in prostate cancer through several putative mechanisms. They include effects on insulin responsive prostate cancers via attenuation of hyperinsulinemia, inhibition of oxidative phosphorylation causing energetic stress in cancer cells and potentially delaying the development of castrate resistant prostate cancer, which hyperinsulinemia can potentiate.[1] Retrospective studies have supported the hypothesis that metformin can improve outcomes in patients with prostate cancer (reference 25 in article).[2] However, this report is by far the largest observational study to demonstrate a strong

association between metformin use and improved oncologic outcomes.

The time has come to confirm these findings as well as any benefit of decreasing the metabolic morbidities of androgen deprivation in prospective clinical trials. Two phase III studies are presently under way in this arena. The ongoing multi-arm comparative STAMPEDE study recently added a metformin arm to evaluate overall survival in patients with advancing or metastatic prostate cancer. The PRIME study compares metformin to placebo in patients in whom intermittent androgen deprivation therapy is initiated for metabolic morbidity and efficacy outcomes. These studies could confirm the benefit of one of the simplest and cost-effective therapies for prostate cancer in a long time.

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Review

Combination therapy with androgen deprivation for hormone sensitive prostate cancer: A new frontier

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Abstract Androgen deprivation therapy (ADT) has been the standard of care for the last 75 years in metastatic hormone sensitive prostate cancer (PCa). However, this approach is rarely curative. Recent clinical trials have demonstrated that ADT combined with other agents, notably docetaxel and abiraterone, lead to improved survival. The mechanisms surrounding this improved cancer outcomes are incompletely defined. The response of cancer cells to ADT includes apoptosis and cell death, but a significant fraction remains viable. Our laboratory has demonstrated both *in vitro* and *in vivo* that cellular senescence occurs in a subset of these cells. Cellular senescence is a phenotype characterized by cell cycle arrest, senescence-associated β-galactosidase (SA-β-gal), and a hypermetabolic state. Positive features of cellular senescence include growth arrest and immune stimulation, although persistence may release cytokines and growth factors that are detrimental. Senescent tumor cells generate a catabolic state with increased glycolysis, protein turnover and other metabolic changes that represent targets for drugs, like metformin, to be applied in a synthetic lethal approach. This review examines the response to ADT and the putative role of cellular senescence as a biomarker and therapeutic target in this context.

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1. Introduction

Prostate cancer (PCa) is the most common male malignancy in the western world and is a leading cause of cancer death [1]. For the last 75 years, advanced disease has been managed with androgen deprivation therapy (ADT), an approach that leads to disease regression, but rarely cure. Castration-resistant PCa generally results in death over 30–40 months depending on disease extent at diagnosis [2]. Multiple phase III clinical trials (ChemoHormonal Therapy Versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer [CHAARTED], A Randomized, Double-blind, Comparative Study of Abiraterone Acetate Plus Low-Dose Prednisone Plus Androgen Deprivation Therapy [ADT] Versus ADT Alone in Newly Diagnosed Subjects with High-Risk, Metastatic Hormone-naïve Prostate Cancer [mHNPC] [LATITUDE], Randomized Phase III Trial Comparing an Association of Hormonal Treatment and Docetaxel Versus the Hormonal Treatment Alone in Metastatic Prostate Cancers [GETUG-AFU 15], and Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy [STAMPEDE]) have recently demonstrated that in metastatic hormone-sensitive PCa, combining either docetaxel chemotherapy or abiraterone (an androgen signaling inhibitor) at the time of initiating ADT markedly improves overall survival in men [3–6]. These clinical observations suggest that ADT induces susceptibilities in PCa cells that make them amenable to synergistic treatment and improve cell killing. This underexplored area has the potential to have a major impact on PCa survival by improving the cancer response to ADT.

One phenotype that arises with extended replication or after cell stress is cellular senescence, in which cells are arrested yet viable [7]. Cellular senescence can also be induced after exposure to oxidative stress, DNA damaging agents, and ADT [8]. In breast cancer, another hormonally dependent cancer, senescence results after tamoxifen exposure in estrogen receptor positive cells [9]. Beneficial aspects of cellular senescence include a therapeutic arrest and immune stimulation. However, negative features include reentry of a subset of these persistent cells into the cell cycle and a secretory phenotype that may induce the growth of surrounding tumor cells. Thus, removal of these cells may lead to improved cancer outcomes.

The late Dr. Donald Coffey was always a strong supporter of "thinking outside the box". His work led to remarkable advances in their own right, but placed in the context of his mentees he had an even greater impact of the field. Dr. Coffey often marveled at nature and would often state "You are going to be surprised at the simplicity and beauty of the real answer". This review, dedicated to him, focuses on a surprising and major shift in our approach to advanced hormone sensitive PCa, that of the role of ADT and combined therapy. It is an area that requires significant research effort to understand and refine recent clinical discoveries. We will review cancer cell responses to ADT, seek to understand the clinical responses and mechanisms underlying these new combinatorial therapies with ADT, and discuss putative targeting of senescent cells after ADT as a therapeutic niche to improve outcomes.

2. Cancer cell responses to ADT

One of the best studied responses to ADT is apoptosis or programmed cell death. Kyprianou and colleague [10,11] performed some of the early work that androgen deprivation leads to apoptosis in pre-clinical PCa models. Androgen withdrawal in murine xenografts and human PCa tissues is associated with a decrease in the proliferative index, but surprisingly low levels of apoptosis encompassing only 2%–3% of cells [12–14]. Apoptosis typically occurs early after ADT within the first 72 h [15]. In addition to apoptosis, other cell death mechanisms induced with ADT include autophagy, necrosis, and necroptosis [16].

Autophagy is an evolutionarily conserved catabolic pathway that targets cellular organelles and cytoplasmic constituents to the lysosomes for degradation. Autophagy, although a cell death pathway, can also function as a survival mechanism exploited by cancer cells under various physiological stresses [17,18]. Androgen deprivation and tissue hypoxia, conditions which occur in prostate tissue after surgical or medical castration, lead to an increased adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity in a threshold dependent manner in murine xenografts [19]. Increased AMPK activity is associated with greater cell survival and the induction of autophagy. *In vitro* in hormone-sensitive PCa cells, autophagy has been reported to occur after bicalutamide, but this occurs at much higher toxic doses than those seen with the induction of cellular senescence [20].

Some tumor cells become quiescent after ADT and have the potential to reactivate. PCa stem-like cells are typically androgen receptor (AR) negative and may represent part of the normally quiescent cancer stem cell population that emerges and expands after ADT [21]. These cells are rare, but express a specific surface antigen profile ($CD44^{+}/\alpha_2\beta_1^{hi}/CD133^{+}$) when isolated from primary PCa tissues and show high levels of clonogenic ability [22].

3. Cellular senescence as a response to ADT in PCa

The phenotype of these residual cells after ADT is complex, but cellular senescence represents an intriguing response that has potential for therapeutic exploitation. Replicative senescence was first described as a phenotype in primary cells after extensive culture and replicative exhaustion *in vitro*, which was linked to telomere shortening [23]. More recently, DNA damage, increased oncogenic signaling, and oxidative stress have been found to result in induced or accelerated senescence [24]. The propensity of tumor cells to undergo senescence induction in response to multiple anti-cancer therapies has been demonstrated [25]. Notably, multiple solid tumor cell lines have shown dose-dependent cellular senescence induction in response to DNA damaging agents such as doxorubicin, diaziquone, cisplatin, and to a lesser degree ionizing radiation. Recently reported data suggest enzalutamide, an antiandrogen, potentiates DNA damage caused by radiation and significantly increases radiation-induced cellular senescence in LNCaP cells [26].

Senescent cells remain viable and metabolically active, but are permanently growth-arrested (Table 1) [24]. They are persistent, in contrast to cells undergoing programmed responses including apoptosis, autophagy, and/or mitotic catastrophe using conventional cytotoxic agents. Growth arrest is achieved and maintained in either G_1 or G_2/M phage, in part, by the increased expression of specific cyclin-dependent kinase inhibitors (CDKIs), including $p16^{Ink4a}$, $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ [27]. Interestingly, transformed neoplastic cells that lack cellular senescence-associated tumor suppressor genes present in non-transformed cells (*e.g.*, p53 and Rb) retain the capacity to become senescent with exposure to doxorubicin, docetaxel, and other chemotherapy agents [28,29].

Cultured *in vitro*, senescent cells develop a distinct and recognizable flattened and enlarged morphology with a prominent nucleus and increased cytoplasmic granularity. Most notably, these cells can be visualized using a staining technique based on senescence-associated β-galactosidase (SA-β-gal) activity [30]. This technique, which stains lysosomes in the perinuclear compartment blue at pH 6.0, is a widely accepted and utilized marker of cellular senescence, but has limitations *in vivo*, as it is not applicable to formalin-fixed archival tissues.

To resolve this issue, we validated one of the first antibodies to SA-β-gal protein (GLB1) *in vitro*, subsequently allowing the biology of cellular senescence in PCa *in vivo* to be interrogated [31]. In hormonally intact prostate tissues, quantitative imaging detects increased GLB1 expression in high-grade prostatic intraepithelial neoplasia (HGPIN) known to contain senescent cells compared to benign prostate tissues [31]. This work also demonstrated that in intermediate grade PCa increased GLB1 predicts prostate-specific antigen (PSA)-free survival. Furthermore, senescent cells are found less commonly in high grade (Gleason score 8—10) versus intermediate grade (Gleason score 6—7) cancers. These findings support a tumor suppression aspect of cellular senescence seen in skin and many other aging organs.

These studies in our laboratory, and subsequently others, have demonstrated that cellular senescence is induced in androgen sensitive cells after ADT [32,33]. Increased expression of the senescence-related proteins GLB1, the CDKI $p27^{Kip1}$, and chromatin-regulating heterochromatin protein 1γ (HP1γ) are detected in 50%—80% of androgen sensitive LNCaP cells after being cultured in androgen-free media [8]. In mice bearing LuCaP xenograft tumors *in vivo*, surgical castration similarly increases senescent markers [8]. In another study, immunohistochemistry of human prostate tumors removed after ADT induced with goserelin acetate (Zoladex) showed a similar induction of GLB1, HP1γ, and decreased Ki-67 [34]. ADT induces a cellular growth arrest consistent with cellular senescence, including hypophosphorylation of Rb, reduction of cyclin-dependent kinase activity, and a G_1/S block [27]. More recently, we have found that in patients undergoing ADT prior to prostate removal for cancer, elevated cellular GLB1 levels measured by VECTRA automated immunoquantitation are noted in as many as 50% of cancer cells [35]. GLB1 levels increase in tumors with longer ADT duration.

Despite permanent growth arrest, senescent cells are metabolically active and have increased energy demand, protein turnover, and glycolysis [36]. The increased energy demand is marked by activation of AMPK, a cellular energy sensor [37]. Increased glucose transport and amplified glycolytic enzyme and pyruvate kinase expression promote the utilization of glucose through non-oxidative glycolysis, similar to the Warburg phenomenon described in tumor cells [36]. Additionally, higher amino acid transport and increased protein synthesis generate cytokines that characterize the proinflammatory secretory-associated senescent phenotype (SASP). These metabolic changes present an opportunity for improved therapeutic approaches when combined with ADT.

Interestingly, AR signaling may play an important role in the cellular senescence response. Blocking the AR, or paradoxically applying supraphysiologic levels of androgens, such as those used in bipolar androgen therapy, may induce cellular senescence [38]. This may represent part of the biphasic growth response seen in AR expressing PCa cells, and induction of senescence in this context may function through the Src-P13K-Akt signaling pathway. Pharmacologic induction of senescence has also been demonstrated in murine xenografts and human PCa tissues using the naturally occurring AR antagonist atraric acid [39].

Table 1 Summary of clinical trial results using ADT in combination with other therapies for the treatment of metastatic hormone sensitive PCa.

Clinical trial	ADT+	Number of patients	Follow-up (month)	HR 95% CI	Reference
GETUG-AFU 15[a]	Docetaxel	385	50	1.01 (0.75—1.36)	Gravis et al., 2013 [40]
CHAARTED[b]	Docetaxel	790	28.9	0.61 (0.47—0.80)	Sweeney et al., 2015 [3]
LATITUDE[c]	Abiraterone	1199	30.4	0.62 (0.51—0.76)	Fizazi et al., 2017 [4]
STAMPEDE-Doc[d]	Docetaxel	2962	43	0.78 (0.66—0.93)	James et al., 2016 [5]
STAMPEDE-Abi[e]	Abiraterone	1917	40	0.63 (0.52—0.76)	James et al., 2017 [6]

PCa, prostate cancer; HR, hazard ratio; CI, confidential interval.
[a] Randomized Phase III Trial Comparing an Association of Hormonal Treatment and Docetaxel Versus the Hormonal Treatment Alone in Metastatic Prostate Cancers (GETUC-AFU 15).
[b] ChemoHormonal Therapy Versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer (CHAARTED).
[c] A Randomized, Double-blind, Comparative Study of Abiraterone Acetate Plus Low-Dose Prednisone Plus Androgen Deprivation Therapy (ADT) Versus ADT Alone in Newly Diagnosed Subjects with High-Risk, Metastatic Hormone-naïve Prostate Cancer (mHNPC) (LATITUDE).
[d] Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE) with Docetaxel (Doc).
[e] Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE) with Abi (Abiraterone).

4. Clinical trials suggest unique PCa susceptibilities after ADT

The application of ADT has always been approached as the sole systemic therapy for metastatic hormone sensitive PCa, traditionally using agents that target the substrate (testosterone) or block the AR. In a major paradigm shift, several recent trials using either docetaxel or androgen signaling inhibitors in combination with ADT for metastatic hormone sensitive PCa have demonstrated remarkable improvements in survival. This suggests ADT induces susceptibilities in cancer cells that may be exploited. The phase III CHAARTED trial (ChemoHormonal Therapy Versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer), demonstrated upfront chemotherapy with concurrent docetaxel and ADT improves survival by 13.6 months versus ADT alone in hormone naïve patients (Table 1) [3]. The docetaxel group also experienced a significant delay in biochemical, symptomatic, or radiographic progression compared to controls (20.1 *vs.* 11.7 months; hazard ratio [HR] = 0.61; 95% confidence interval [CI] = 0.51 to 0.72; $p < 0.001$). These results were confirmed in the STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy) [5] and GETUG-AFU 15 trials (Randomized Phase III Trial Comparing an Association of Hormonal Treatment and Docetaxel Versus the Hormonal Treatment Alone in Metastatic Prostate Cancers) [40]. Docetaxel functions to inhibit the transport of the AR, potentiating ADT action [41]. It is also a microtubule inhibitor having direct toxicity to cells. We anticipate this combination results in fewer persistent senescent cells than ADT alone post-treatment, but further mechanistic study is required regarding the synergistic activity of this combination.

The phase III LATITUDE trial (A Randomized, Double-blind, Comparative Study of Abiraterone Acetate Plus Low-Dose Prednisone Plus Androgen Deprivation Therapy [ADT] Versus ADT Alone in Newly Diagnosed Subjects with High-Risk, Metastatic Hormone-naïve Prostate Cancer [mHNPC]) demonstrated upfront therapy with concurrent abiraterone and luteinizing hormone-releasing hormone agonists improves overall survival versus ADT alone (median not reached versus 34.7 months, HR = 0.62; 95% CI: 0.51—0.76; $p < 0.001$) in hormone naïve patients [4]. The abiraterone group also experienced a significant delay in radiographic progression-free survival compared to control (33.0 months *vs.* 14.8 months; HR = 0.47; 95% CI: 0.39—0.55; $p < 0.001$). The abiraterone and ADT arm of the STAMPEDE trial also showed improved outcomes [6]. Abiraterone irreversibly inhibits the Cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17) enzyme expressed in adrenal tissues, reducing androgen synthesis from all sources [42]. Although the combination of CYP17 inhibition and ADT demonstrates more effective androgen depletion than either agent alone [43], resistance to abiraterone develops, likely through increase CYP17 expression [44], enzymatic alterations [45], and/or gain of function 3β-hydroxysteroid dehydrogenase type 1 mutation [46]. These clinical observations suggest that the initiation of ADT induces susceptibilities in PCa cells that make them amenable to synergistic treatment and improved cell killing. Further study is required to delineate the synergistic activity of abiraterone and ADT, along with clearly defining the mechanisms of resistance in castration-resistant PCa.

5. Exploiting cellular senescence as synthetic therapy

Cellular senescence may have detrimental features. Oncogene-induced cellular senescence imposes selective pressures that promote the outgrowth of senescence-resistant aggressive tumor cell subpopulations [47]. Although cellular senescence is cytostatic and offers a potential survival advantage in some models, long-term exposure of surrounding cells to the SASP, and resulting proinflammatory cytokines and growth factors, may have deleterious effects on surrounding cells [7]. In a recent *Nature* paper, it was reported that lymphoma cells released from chemotherapy-induced senescence results in a population of cells exhibiting a stem cell phenotype that exhibits highly aggressive growth potential upon escape from cell-cycle blockade [48]. This population is enriched in relapsing hematologic tumors. It has been proposed that ADT-induced cellular senescence might play a role in the chemoresistance that arises with intermittent ADT [32].

The cellular senescence phenotype presents unique opportunities that have the potential to be exploited for therapeutic cure (Fig. 1) [49]. Dörr and colleagues [36] have induced senescence in lymphoma cells and used compounds that target the inhibition of glucose transporters, glycolytic enzymes, and adenosine triphosphate (ATP) depletion to generate synthetic lethality in cancer cells (Table 2). These combinations lead to improved survival and elimination of cancer cells through caspase-12- and caspase-3-mediated endoplasmic-reticulum-related apoptosis [36]. Additionally, higher amino acid transport and increased protein synthesis generate cytokines that characterize the proinflammatory SASP. These findings highlight the hypercatabolic nature of senescent cells after induction with ADT and other agents that is therapeutically exploitable by synthetic lethal metabolic targeting. These approaches have been largely unexplored to date in PCa, but as outlined below, interesting supportive data exist.

The therapeutic induction of cellular senescence from chemotherapeutics, such as cyclophosphamide, results in augmented protein translation with the resultant accumulation of misfolded proteins, which activates the conserved proteotoxic stress response (PSR) [36]. The substantial proteotoxic stress in cellular senescence evokes energy-consuming countermeasures for cell survival. The PSR is orchestrated by the transcription factor heat shock factor 1 (Hsf1), which regulates the gene expression of several families of molecular chaperones (heat shock protein (Hsp) 27, Hsp70, Hsp90 and others) that attenuate proteotoxic stress and promote cell survival by preventing protein aggregation, refolding non-native proteins, or delivering them to the proteasome [54]. Anabolic tumor cells are more dependent on the Hsf1-mediated PSR than normal cells for cell survival [55]. The PSR may represent an additional novel targetable vulnerability of senescent transformed cells that can be exploited therapeutically, a hypothesis that has yet to be tested.

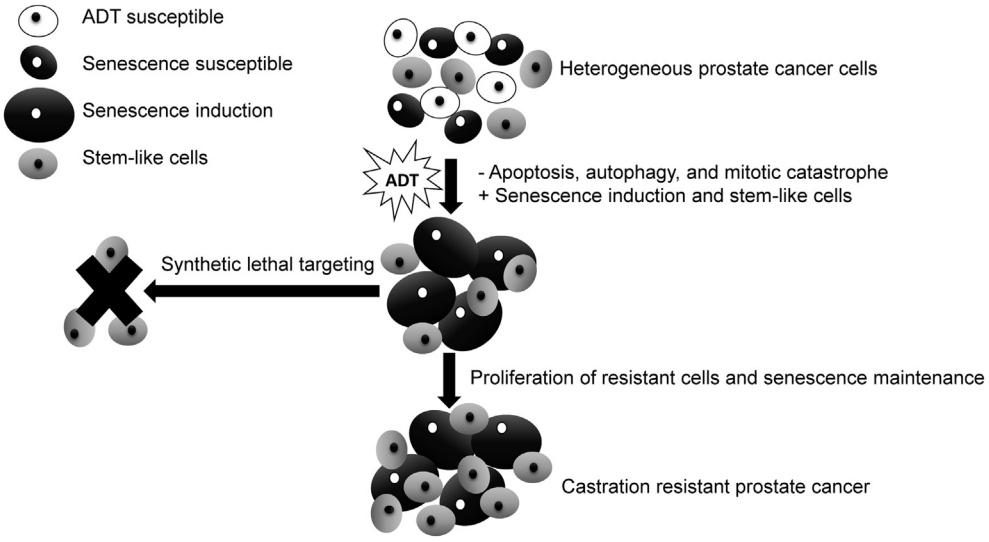

Figure 1 Synthetic lethal targeting of ADT induced cellular senescence for improved prostate cancer cell killing. ADT, androgen deprivation therapy.

Table 2 Presence of the cellular senescence phenotype offers therapeutic opportunities.

Characteristic	Synthetic lethal targeting of senescent cells
Cell morphology	
SA-β-gal positive	
Enlarged cell with prominent nuclei and cytoplasmic granularity	
Metabolic alterations	
Hypermetabolic	Glucose transport inhibitors phloretin, cytochalasin B, 2-deoxy-D-glucose [36]
Glycolysis	AMPK inhibitor compound C [36]/AMPK activator and mTOR inhibitor metformin [50,51]
High protein turnover	Lysosomal V-ATPase inhibitors bafilomycin A1 and concanamycin A [36]
Secretory phenotype	
Pro-inflammatory cytokines	Tumor microenvironment cancer-based immunotherapy [52]
Growth factors	Growth factor and growth factor receptor inhibitors (*e.g.* VEGF, IGF-1 inhibitors) [53]

AMPK, adenosine monophosphate-activated protein kinase; ATPase, adenosine triphosphatase; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; SA-β-gal, senescence-associated β-galactosidas; VEGF, vascular endothelial growth factor.

Metformin is an oral anti-diabetic agent in the biguanide class that has generated interest as a cancer therapy. Metformin is an intriguing drug that may not only enhance chemotherapy response for established tumors, but also demonstrates anticancer activity as a single agent [56]. Metformin inhibits the mammalian target of rapamycin (mTOR), a central regulator of cell growth and survival, in part by activating AMPK [51]. Additionally, metformin inhibits Hsf1 by activating AMPK, which in turns phosphorylates Hsf1 on Ser 121 to inhibit its activity [57]. This disables the protective effects of Hsf1 against proteotoxic stress. Synergistic activity after neoadjuvant chemotherapy was suggested in an analysis showing improved clinical response in diabetic patients with breast cancer receiving metformin and neoadjuvant chemotherapy [58]. These patients received doxorubicin, which is well known for its senescence-inducing properties in prostate and breast cancer [28,29].

Given the metabolic susceptibilities that ADT induces, metformin leads to increased cell kill when combined with ADT. Metformin has been combined with the antiandrogen bicalutamide *in vitro* and in animal models [59]. This combination significantly reduced clonogenicity ($p < 0.005$) and tumor growth with greater effects in AR-positive cells. In unpublished data, we have found LNCaP, LaPC4, and CWR22 PCa lines all demonstrate synthetic lethal responses as calculated by Calcusyn (Biosoft, Cambridge, UK) to low dose bicalutamide (1—5 umol/L) to induce senescence initially, followed by metformin at low dose (0.1—1 mmol/L) [60]. Increased apoptosis peaks 2 days after metformin application.

In a large observational study of 87 344 veterans, we demonstrated that metformin combined with ADT improved overall survival (HR = 0.82, 95% CI: 0.78—0.86), reduced the risk of skeletal related events (HR = 0.84, 95% CI: 0.74—0.96), and improved cancer specific survival

(HR = 0.70, 95% CI: 0.64—0.77) [61]. Skeletal related events, defined as pathologic fracture, spinal cord compression, and/or necessity for bone radiation or surgery due to pain or impending fracture, was used as one surrogate for disease progression. Although retrospective observational studies do not prove causality, these data merit further clinical trial investigation. Notably, metformin is an inexpensive, widely used drug associated with minimal side effects even when used in the non-diabetic population. Therefore, few barriers exist to prevent its clinical translation in PCa.

Statins are another widely used and inexpensive drug that may provide synergistic lethality with ADT given the metabolic profile of senescent cells. Statins are a class of oral anti-hypercholesterolemia agents that inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase. Although they are associated with myopathy and elevated transaminases, the side effect profile is favorable [62]. The use of statins reduces PSA *in vivo* [63], through downregulation of AR expression and activity due to enhanced proteolysis of the AR protein [64]. In LNCaP cells, the combination of simvastatin and ADT demonstrates increased growth inhibition in AR positive lines [65]. Statins also compete with the dihydrotestosterone precursor for Solute Carrier Organic Anion Transporter Family Member 2B1 mediated transport, thus decreasing androgen availability in PCa cells [66]. Men with hormone sensitive PCa undergoing ADT and taking statin drugs experienced a delayed time to disease progression compared to men not taking statins (median 27.5 months; 95% CI: 21.1—37.7 *vs.* 17.4 months; 95% CI: 14.9—21.1). Simvastatin has also been shown to decrease the secretory phenotype of senescent human fibroblasts, including interleukin-6, by inhibiting protein prenylation [67]. Whether statins directly induce cell death in senescent cells is unknown as of yet.

Other characteristics of senescent cells include a hypermetabolic phenotype comprised of enhanced glycolysis and protein turnover, providing a critical cellular senescence associated metabolic liability that may be targeted therapeutically. Senescent cells are selectively susceptible to inhibition of glucose transporters (*e.g.* cytochalasin B), or to the pharmacological competitor (2-deoxy-D-glucose) [36]. Senescent cells also rely on an intact lysosomal protein degradation machinery to buffer proteotoxic stress. Exposure of senescent cells to bafilomycin A1 or concanamycin A, specific inhibitors of lysosomal V-ATPases, or to a cocktail of lysosomal protease inhibitors all generate increased death in therapy induced senescent cells [36]. With use of these inhibitor in cell culture, senescent cell death occurred at significantly higher rates. They provide an avenue for therapeutic exploitation that should be examined further.

6. Conclusion and future directions

ADT has been used as the primary approach to advanced hormone sensitive PCa for over 75 years. Recent clinical trials have demonstrated combining ADT with other agents improves survival. ADT and docetaxel (or abiraterone) should be considered standard of care for patients with metastatic hormone sensitive PCa given recent trial results. Research is needed to define and understand these observations to further improve our progress in this area. Cellular senescence is a distinctive phenotype characterized by metabolic alterations and growth arrest. ADT induced cellular senescence in PCa may be deleterious; however, it may offer a unique opportunity for synthetic lethal targeting of residual PCa cells. Several recent retrospective hypothesis-generating studies suggest combining ADT with agents that target metabolism, including metformin, may improve patient survival. Future work should focus on further delineating the response of tumors to ADT and cellular senescence biology in this context.

Conflicts of interest

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Author contributions

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Review Article

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

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Abbreviations and Acronyms

ADT = androgen deprivation therapy

AR = androgen receptor

CHAARTED = Chemohormonal Therapy versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer

CRPC = castration resistant PC

GETUG—AFU = Groupe d'Étude des Tumeurs Uro-Genital and Association Française d'Urologie trial

HSPC = hormone sensitive PC

mHSPC = metastatic HSPC

PC = prostate cancer

PSA = prostate specific antigen

QOL = quality of life

STAMPEDE = Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy

Purpose: Androgen deprivation therapy alone has been the standard of care for metastatic hormone sensitive prostate cancer for the last 75 years. This review focuses on recent trials and mechanisms which highlight the new paradigm of combining androgen deprivation therapy with other agents, changing the treatment of patients with prostate cancer who have advanced disease.

Materials and Methods: We searched the peer reviewed literature on the PubMed® and Web of Science® databases through January 2018 using the key words, "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 using androgen deprivation therapy combinations for metastatic hormone sensitive prostate cancer can be broadly grouped into chemohormonal studies (docetaxel) or trials of androgen signaling inhibitors. The CHAARTED (Chemohormonal Therapy versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer) and STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy) studies showed a survival advantage when combining androgen deprivation therapy with chemotherapy, as well as increased time to progression to castration resistant status. The abiraterone arm of the STAMPEDE and LATITUDE trials, which analyzed combining androgen deprivation therapy with abiraterone, revealed improved overall and progression-free survival. Androgen deprivation therapy generates a number of phenotypes in resistant cancer cells, including quiescence, autophagy and cellular senescence. Senescent cells represent a metabolic target for synergistic lethality with drugs such as metformin. Ongoing trials are under way to examine the effect of combining newer antiandrogens and novel drugs with androgen deprivation therapy in patients with metastatic hormone sensitive prostate cancer.

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Conclusions: Combination therapy has evolved as the standard of care for metastatic hormone sensitive prostate cancer. The ideal combination is tailored to patients after individualized counseling taking into account general health and comorbid illness status.

Key Words: prostatic neoplasms, castration-resistant; neoplasm metastasis; drug therapy, combination; antineoplastic agents; cellular senescence

Prostate cancer is the most common cancer in males with an estimated new case incidence of 164,690 and an estimated mortality of 29,430 expected in 2018. Despite an overall 5-year survival rate of 98.2%, mHSPC has a dismal 30% 5-year survival rate.[1] Conventional treatment of mHSPC has been ADT since the landmark discovery by Huggins and Hodges in 1941 demonstrating the hormonal sensitivity of PC.[2] Metastatic HSPC treated with ADT transitions to the CRPC stage with a median survival of approximately 3 years.[2] Progression to CRPC is associated with a deterioration in QOL. In the recently concluded STAMPEDE trial patients were found to spend three-quarters of overall survival after the 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 disease management at earlier time points.

Approaches to improve the response rate 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% to 3% improvement in 5-year survival with a wide range of uncertainty.[4] For a number of years researchers had considered earlier application of cytotoxic therapy in an effort to delay progression to castrate disease. The addition of chemotherapy to ADT was tested in the last 30 years and this approach was used in a number of published randomized trials, as summarized by Millikan et al.[5] However, the lack of cytotoxic therapy, which improved survival in CRPC cases, led to minimal advances. In CRPC more recently docetaxel chemotherapy resulted in an incremental 2.5-month improvement in median survival, leading to its approval in 2004.[6] These findings lead to the initiation of trials earlier in the disease in patients with a larger disease burden at ADT initiation.

ADT induces a number of unique responses in prostate cancer cells, of which some 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 combined 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 in patients with mHSPC.

METHODS

We performed PubMed and Web of Science database searches of the peer reviewed mHSPC literature on the mechanisms of cellular persistence after ADT as well as combination therapies that use ADT with another therapeutic agent (fig. 1). 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 of combination therapy with ADT in the setting of mHSPC, the synergy mechanism, side effects and QOL. The mechanisms of cellular persistence after ADT are also discussed with special emphasis on senescence. Combination therapies ongoing and to be considered in the near future are examined.

RESULTS

Metastatic Hormone Sensitive Prostate Cancer

Chemohormonal Therapy Trials. Early studies of chemotherapy combined with ADT were less optimistic, in part due to toxicity and the lack of active agents. Millikan et al performed a phase III trial in 286 patients with mHSPC who received 3, 8-week cycles of ketoconazole and doxorubicin alternating with vinblastine and estramustine, given in addition to standard ADT vs 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 led to consideration of its use at the time of ADT initiation in patients with a higher tumor burden.

The CHAARTED study was the first trial to show that adding 6 cycles of docetaxel 75 mg/m^2 every 3 weeks to standard ADT significantly improved outcomes in men with mHSPC (table 1).[8] The 13.6-month improved overall survival in the chemohormonal group compared to the ADT group (median overall survival 57.6 vs 44 months, HR 0.61, 95% CI 0.47–0.80, p <0.001) represented one of the

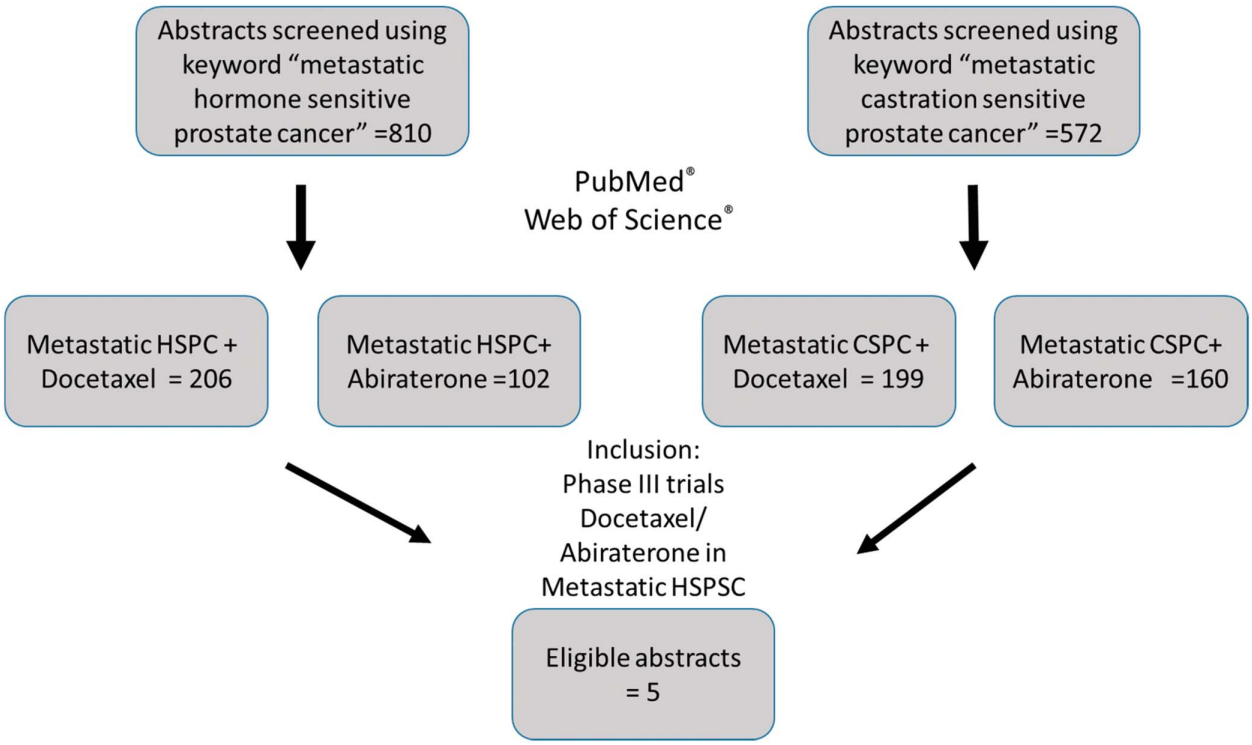

Figure 1. Review criteria used for study selection. *CSPC,* castration sensitive prostate cancer.

largest improvements in survival in patients with advanced PC in the modern era. Time to the CRPC transition was also prolonged in the chemo-hormonal therapy arm (20.2 vs 11.2 months, HR 0.61, 0.51–0.72). On stratified analysis of patients with high volume disease, defined as visceral metastases, or 4 or more bone lesions with at least 1 lesion beyond the vertebral bodies and pelvis, there was almost 17-month improved overall survival, which was not seen for low volume disease (51.2 vs 34.4 months, HR 0.63, 0.50–0.79, p <0.0001). This was attributable to the higher proportion of hormone resistant cells in the high volume disease subpopulation, which contributed to resistance to hormonal manipulation.

The STAMPEDE study 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 which received ADT alone, ADT plus docetaxel, ADT plus zoledronate and ADT plus docetaxel plus zoledronate, respectively. In addition to metastatic disease, the study also included lymph node involvement, high risk locally advanced disease (T3/4 plus Gleason score 8–10, T3/4 plus prostate specific antigen 40 ng/ml or greater or Gleason score 8–10 plus prostate specific antigen 40 ng/ml or greater) and recurrent disease previously treated with definitive local therapy. There was a 10-month improvement

Table 1. *Phase III combination trials with ADT in metastatic hormone sensitive prostate cancer*

	ADT + Docetaxel/ADT			ADT + Abiraterone/ADT	
	GETUG-AFU 15	CHAARTED	STAMPEDE-Docetaxel	LATITUDE	STAMPEDE-Abiraterone
No. pts	192/193	397/393	592/1,184	597/602	960/957
Median age	63/64	64/63	65/65	68/67	67/67
Median survival:					
Overall*	58.9/54.2 Mos	57.6/44 Mos	81/71 Mos	66%/49%	83%/76%
HR (95% CI)	0.88 (0.68–1.14)	0.61 (0.47–0.80)	0.78 (0.66–0.93)	0.62 (0.51–0.76)	0.63 (0.52–0.76)
Progression-free†	22.9/12.9 Mos	20.2/11.7 Mos	37/20 Mos	33/14.8 Mos	75%/45%
HR (95% CI)	0.72 (0.57–0.91)	0.61 (0.51–0.72)	0.61 (0.53–0.70)	0.47 (0.39–0.55)	0.29 (0.25–0.34)

* CHAARTED and STAMPEDE 5-year survival, and LATITUDE and STAMPEDE-Abiraterone 3-year survivor rate with survival in STAMPEDE including M0 (nonmetastatic) and M1 (metastatic) subsets at 60 vs 45 months for STAMPEDE-docetaxel in M1 subset combination and ADT arms, respectively.
† CHAARTED time to CRPC, STAMPEDE clinical, radiographic and biochemical failure-free survival and LATITUDE radiographic survival.

in overall survival in the chemohormonal therapy arm compared to the ADT arm (81 vs 71 months, HR 0.78, 95% CI 0.66–0.93, p = 0.006). The secondary end points of failure-free survival and time to the first skeletal related event were also significantly better in the chemohormonal therapy arm.

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

In GETUG-AFU and CHAARTED side effects were more common in the chemohormonal therapy arm. The most common grade 3 or greater adverse events in GETUG-AFU and CHAARTED were neutropenia at 32% and 12%, febrile neutropenia at 7% and 6%, and fatigue at 7% and 4%, respectively. In each study there were negligible grade 3 or greater adverse events in the ADT arm. Diarrhea, stomatitis, and motor and sensory neuropathy were the less common adverse effects, which developed in less than 1% of the population in the CHAARTED study. STAMPEDE reported additional toxicity in the chemohormonal therapy arm compared with that of ADT alone (grade 3 or greater adverse events in 52% vs 32% of patients). This was mostly due to toxicity during the first 6 months on trial, when grade 3 or greater adverse events were reported in 36% of the chemohormonal therapy arm vs 17% in the ADT arm. A 1-year analysis of 1,998 patients with available profiles revealed a balanced rate of grade 3 or greater adverse events of 10% in each arm. There were 2 deaths in the chemohormonal therapy arm and 72 patients (13%) discontinued treatment. QOL assessment was done 3, 6, 9 and 12 months after randomization in CHAARTED using the FACT (Functional Assessment of Cancer Therapy)-Prostate score. Although QOL scores with docetaxel decreased at 3 months, it was better at 12 months in patients who received docetaxel vs ADT alone.[13]

Chemohormonal therapy may improve survival because of the existence and emergence of hormonally resistant cellular clones during the CRPC transition. Interestingly, each trial that demonstrated a survival advantage had a time delay between the start of ADT and chemotherapy, including 120 days in CHAARTED and 90 days in STAMPEDE. In GETUG-AFU 15 the patients were required to enroll within 2 months of starting ADT and almost half of them enrolled within 15 days of starting ADT. Some have suggested that this might explain the decreased survival benefit with chemohormonal therapy in this trial. A timed sequence of chemohormonal therapy contributes to maximum synergy by targeting hormone resistant cells when they are most vulnerable.[14] Microtubule targeting chemotherapy inhibited nuclear translocation of androgen receptor in preclinical studies, which also potentially contributes to synergy with ADT.[15]

Combined Androgen Deprivation Therapy and Androgen Synthesis Inhibitors. 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] Approval of this drug in the prechemotherapy and post-chemotherapy era led to its application to earlier disease. Resistance to ADT is driven in part by up-regulation of androgen receptor signaling through adrenal androgen production, intratumor testosterone production and modification of androgen receptors.[17] The neoadjuvant combination of abiraterone plus prednisone and ADT markedly reduced the tumor burden in men with newly diagnosed, high risk, localized prostate cancer, suggesting a potential role for inhibiting extragonadal androgen biosynthesis before the emergence of resistant clones.[18] These findings led to 2 randomized, phase III trials testing the efficacy of abiraterone and ADT in mHSPC.

In STAMPEDE-Abiraterone the investigators reported outcomes of the combination of abiraterone and prednisone at the time of ADT initiation.[19] They used a multistage, multi-arm setting similar to that of previous trials enrolling patients with newly diagnosed metastatic, node positive or high risk locally advanced disease. Patients who relapsed with high risk features after previous treatment with radical surgery or radiotherapy were also included. The group consisted of 1,917 patients, of whom 52% had metastatic disease, 20% had 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 plus abiraterone 1,000 mg plus prednisolone 5 mg. This trial also mandated radiotherapy in patients with node negative nonmetastatic disease and provided the option of radiotherapy for patients with node positive nonmetastatic disease. Treatment continued until PSA, radiological or clinical

progression. In patients in whom radiotherapy was planned treatment was administered 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 compared to ADT (death HR 0.63, 95% CI 0.52–0.76, p <0.001). Failure-free survival showed a 71% relative risk reduction (treatment failure HR 0.29, 95% CI 0.25–0.34, p <0.001).

In the multicenter, phase III LATITUDE trial 1,199 patients with newly diagnosed mHSPC were enrolled within 3 months of diagnosis and randomized to receive a combination of ADT plus abiraterone 1,000 mg plus prednisolone 5 mg or ADT alone plus placebo.[20] The trial was done at a total of 235 sites in 34 countries in Europe, the Asia-Pacific region, Latin America and Canada. Patients needed at least 2 of 3 high risk features, including Gleason score 8 or greater, visceral metastasis and 3 or more bone lesions on imaging. At 3 years two-thirds of the patients in the combination group survived compared to only half of those in the placebo (ADT) group with a relative risk reduction of 38% (HR 0.62, 95% CI 0.51–0.76, p <0.001). The risk reduction was similar to that in STAMPEDE at the end of 3 years of followup (38% vs 37%).[9] Radiographic progression-free survival was improved in the combination treatment group with a 53% risk reduction (HR 0.47, 95% CI 0.39–0.55, 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% on placebo. Hypokalemia was also higher in the abiraterone arm, requiring treatment discontinuation in 2 patients. STAMPEDE reported grade 3 or greater events in 15% of the combined therapy arm compared to 11% in the ADT arm.[9] Hypertension, elevated transaminases and respiratory events were reported more often in the combination therapy arm. Both studies mentioned that 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 almost total suppression of extragonadal androgen production, especially from within the tumor cell, contributes to improved tumor clearance when used synergistically with ADT.[21] 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 almost total androgen suppression, in contrast to chemohormonal therapy, in which the hormone resistant population is targeted.

Mechanisms of Cellular Persistence after Androgen Deprivation Therapy

Two distinct and not mutually exclusive mechanisms have been proposed in the transition from hormone sensitive cells to CRPC. Tumor cells may acquire new alterations which enable them to survive in the castrated state (adaptation) or preexisting cells capable of surviving hormonal therapy may be selected after a course of ADT (selection).[22] Increased levels of intratumor androgens due to incomplete blockade by ADT, amplification of the AR gene, splice variations and a gain of function mutations, changes in the expression of coregulatory molecules to stimulate transcription after antiandrogen binding and bypass of the AR 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. Therefore, a heterogeneous population of androgen dependent and androgen independent cells exists before ADT initiation.[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 mechanisms and abiraterone targets adaptive mechanisms.

ADT causes cellular changes in prostate cancer tissue. Apoptosis, autophagy, necrosis and necroptosis are the 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, in which growth is arrested.[31] Markers of cellular senescence include senescence associated GLB1 (β-galactosidase) expression. Tissues from patients who underwent neoadjuvant ADT before radical prostatectomy demonstrated viable cells that frequently expressed GLB1 and accumulated after ADT.[32] Although senescence is cytostatic, cells express a SASP (secretory associated senescent phenotype). The 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 down-regulation of the AMPK (5' adenosine monophosphate activated protein kinase) 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 ADT outcomes. One agent is the well-known oral glyburide drug metformin. Metformin directly acts on tumor cells by inhibiting the respiratory mitochondrial electron transport chain inhibiting gluconeogenesis and decreasing glucose uptake, as well as activating AMPK.[34] It also inhibits fatty acid synthesis, lipid peroxidation and the Krebs 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 inhibition of the HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) enzyme at the rate limiting step in the mevalonate pathway of cholesterol synthesis and the potential importance in cancer.[2] Previous combination trials using celecoxib and zoledronic acid combined with ADT in patients starting long-term hormonal therapy did not show a survival advantage.[9,39]

In a recently published retrospective study of more than 87,000 patients who were placed on ADT for advancing PC, metformin improved overall and cancer specific survival, and reduced skeletal metastases compared to diabetic men on insulin or nondiabetic men.[40] These data suggest that combining 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 K) against the standard of care ADT (arm A) and this ongoing analysis is expected to shed light on this synergistic approach.[41]

Patient Selection for Combination Therapy of Metastatic Hormone Sensitive Prostate Cancer

Combined therapy now represents a standard of care in 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 a rapid pace of disease (fig. 2).[8] Barriers to docetaxel use include advanced patient age, poor performance status, coexisting illness and patient preference. Hematological side effects, neuropathy and fatigue are more common for chemohormonal therapy than for ADT and chemotherapy related deaths, although rare at 1% to 3%, were documented in all 3 randomized trials in which docetaxel was added. The CHAARTED and STAMPEDE docetaxel phase III trials used an 18-week course of therapy (6 cycles, each consisting of 3 weeks), and 26% and 23% of patients, respectively, in the chemohormonal therapy arm 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 CHAARTED and STAMPEDE clinical trials.[42]

Abiraterone has a better side effect profile than docetaxel and, being an oral agent, it is easier to administer in the urologist office. Of patients 12% discontinued therapy due to toxic effects. In LATITUDE 88% of patients completed therapy without a dose modification.[20] In the abiraterone arm of STAMPEDE only a few patients discontinued therapy due to toxicity.[19] In LATITUDE 63% of patients reported grade 3 or 4 adverse events in the abiraterone group compared to 48% in the placebo group.[20] In STAMPEDE 47% of patients in the combination group reported grade 3 or greater adverse events compared to 33% in the placebo group.[19] Most of the latter adverse events were 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 revealed a threefold increase in grade 3 or greater cardiac and hepatic events, and a twofold increase in grade 3 or greater vascular events in the abiraterone combination group.[43]

Quality of life and side effects are important to consider in most men who were otherwise asymptomatic. In patients with low volume disease or significant comorbid conditions ADT alone remains an appropriate treatment option, which should be discussed in individualized counseling. The duration of treatment with abiraterone is longer at 2 years or more. This raises concerns about safety, especially in patients with preexisting risk factors for cardiovascular disorders and stroke. STAMPEDE excluded men with a significant cardiac history, limiting generalizations of benefit or toxicity in 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 the toxicity associated with abiraterone, including hyperglycemia, cardiovascular risks, osteopenia

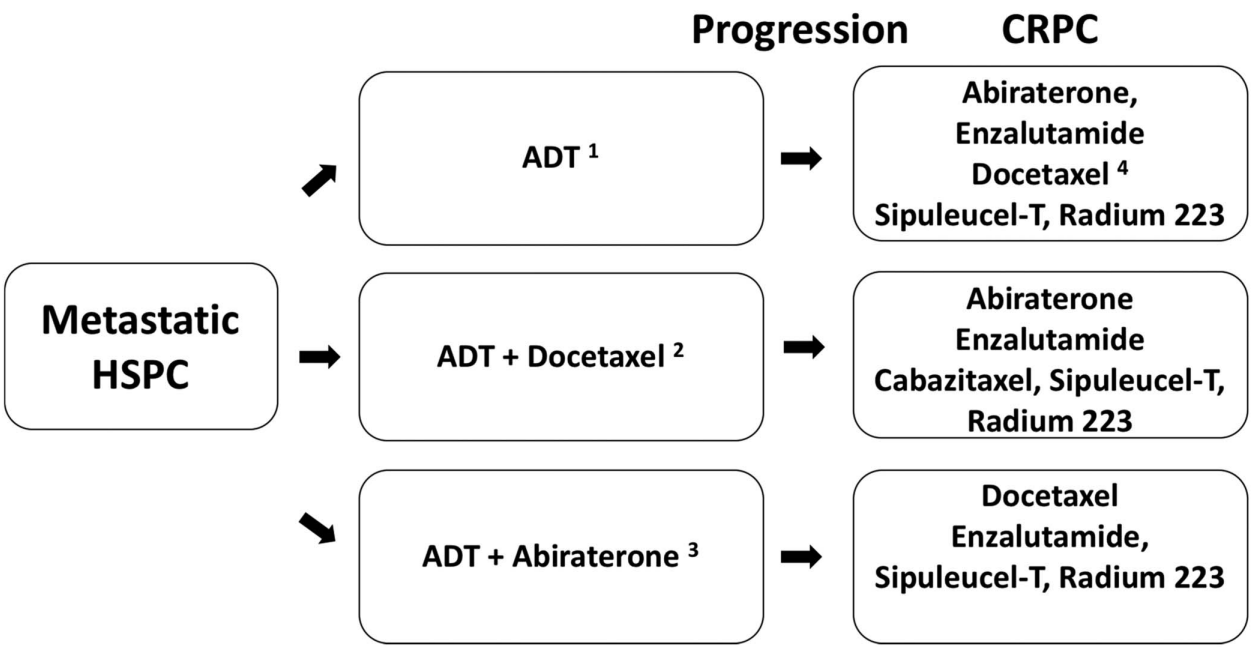

Figure 2. Optimizing treatment of newly diagnosed mHSPC. *1,* patients with poor performance status or desire to avoid therapy side effects may elect ADT alone. *2,* CHAARTED showed 37% relative risk reduction in high volume metastatic subgroup (more than 4 metastases) compared to 27% overall. *3,* LATITUDE included patients with de novo metastatic disease and 2 of 3 high risk features, namely Gleason score 8 or greater, visceral metastasis and 3 or more bone lesions on imaging. *4,* treatment depends on life expectancy and performance status.

and/or osteoporosis. The requirement for concurrent prednisone with abiraterone can limit its use in patients with brittle diabetes, patients with chronic gastric ulcers and patients with infection.

This raises the question of the ideal therapeutic agent in the setting of mHSPC. To our knowledge a direct head-to-head comparison of ADT plus abiraterone or docetaxel has not been performed, limiting conclusions. A recent analysis of STAMPEDE indicated that contemporaneously randomized patients showed no evidence of a difference in overall or PC specific survival, or in symptomatic skeletal events.[44] Interestingly, failure-free survival favored abiraterone, likely reflecting the PSA response and the mechanism of action. The docetaxel cohort had more durable survival after failure. Toxicity was similar between the arms with an 11% prevalence of grade 3 or 4 toxicity at 1 year. The question has been raised whether a combination of abiraterone plus docetaxel (and ADT) might lead to an additive benefit in survival. Data on this will emerge from the ongoing PEACE1 (Phase III Study for Patients with Metastatic Hormone-naïve Prostate Cancer) trial.

The cost of long-term abiraterone treatment is also a factor that physicians and patients should consider before starting therapy. While the cost of docetaxel for a 6-cycle course is estimated to be 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, to our knowledge they have not been reported in the HSPC setting. Given the extended treatment duration of abiraterone in HSPC cases, often exceeding 2 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 III trials have led to a wealth of new trials in the mHSPC space. Combining ADT with the androgen axis inhibitors enzalutamide, apalutamide and orteronel are ongoing (table 2). Enzalutamide is an androgen signaling inhibitor with multiple actions, including blocking AR translocation to the nucleus, AR binding to DNA and receptor mediated DNA transcription.[47] It has been approved in men with advancing CRPC before and after docetaxel chemotherapy, and recently for

Table 2. *Ongoing phase III trials of combination therapy with ADT in metastatic hormone sensitive prostate cancer*

Trial Type/Name	ClinicalTrials.gov Identifier	Phase/No. Enrolled	Intervention	Primary End Point	Estimated Completion
Hormonal:					
ENZAMET	NCT02446405	III/1,125	ADT + enzalutamide vs ADT + nonsteroidal anti-inflammatory drug	Overall survival	9/2020
ARCHES	NCT02677896	III/1,100	ADT + enzalutamide vs ADT + placebo	Radiographic progression-free survival	4/2020
STAMPEDE	NCT00268476	II/III/–	ADT vs ADT + abiraterone + enzalutamide (Arm J)	Overall survival	2020
TITAN	NCT02489318	III/1,000	ADT + apalutamide vs ADT + placebo	Overall + radiographic progression-free survival	11/2020
SWOG S1216	NCT01809691	III/1,304	ADT + orteronel vs ADT + bicalutamide	Overall survival	3/2022
Metabolic (STAMPEDE)	NCT00268476	II/III/–	ADT vs ADT + metformin (Arm K)	Overall survival	2020
Chemotherapy (PEACE1)	NCT01957436	III/1,168	ADT +/– docetaxel +/– abiraterone +/– radiation	Overall + radiographic progression-free survival	5/2019

nonmetastatic CRPC with rapidly rising PSA, as shown in PROSPER (Safety and Efficacy Study of Enzalutamide in Patients With Nonmetastatic Castration-Resistant Prostate Cancer). It is being evaluated as combination therapy with ADT for mHSPC separately as well as an adjunct with another androgen axis inhibitor, abiraterone. Apalutamide, which is similar to enzalutamide in action, was recently approved for nonmetastatic CRPC with rapidly rising PSA while on ADT (SPARTAN, Study of Apalutamide [ARN-509] in Men With Non-Metastatic Castration-Resistant Prostate Cancer).[48] Orteronel is a selective nonsteroidal 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 was 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 over expressing CRPC clones.[50] This approach represents an intriguing and potentially less toxic strategy for future trials.

CONCLUSIONS

It is remarkable that for 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 men with mHSPC have demonstrated significant improvements in survival and QOL. ADT and docetaxel or abiraterone should be considered the standard of care in 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 the onset of CRPC, as shown in several recent combination trials. Novel approaches targeting metabolic pathways have the potential to be synthetically lethal with ADT by targeting the multiple susceptibilities induced by ADT. This area needs to be explored in further clinical trials.

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