Original Investigation

Statin Use at the Time of Initiation of Androgen Deprivation Therapy and Time to Progression in Patients With Hormone-Sensitive Prostate Cancer

Lauren C. Harshman, MD; Xiaodong Wang, PhD; Mari Nakabayashi, MD; Wanling Xie, MS; Loana Valenca, MD; Lillian Werner, MS; Yongjiang Yu, PhD; Aaron M. Kantoff, BS; Christopher J. Sweeney, MBBS; Lorelei A. Mucci, ScD; Mark Pomerantz, MD; Gwo-Shu Mary Lee, PhD; Philip W. Kantoff, MD

IMPORTANCE Statin use has been associated with improved prostate cancer outcomes. Dehydroepiandrosterone sulfate (DHEAS) is a precursor of testosterone and a substrate for SLCO2B1, an organic anionic transporter. We previously demonstrated that genetic variants of *SLCO2B1* correlated with time to progression (TTP) during receipt of androgen deprivation therapy (ADT). Statins also use SLCO2B1 to enter cells, and thus we hypothesized that they may compete with DHEAS uptake by the tumor cells.

OBJECTIVE To evaluate whether statin use prolongs TTP during ADT for hormone-sensitive prostate cancer.

DESIGN, SETTING, AND PARTICIPANTS In vitro studies were performed using prostate cancer cell lines at an academic, comprehensive cancer center. Statin use was retrospectively analyzed in 926 patients who had received ADT for biochemical or metastatic recurrence or de novo metastatic prostate cancer between January 1996 and November 2013.

MAIN OUTCOMES AND MEASURES To determine whether statins interfere with DHEAS uptake, we performed in vitro studies using prostate cancer cell lines. Next, we queried our institutional clinical database to assess for an association between statin use and TTP during ADT using multivariable Cox regression analysis and adjusted for known prognostic factors.

RESULTS In vitro, we demonstrated that statins block DHEAS uptake by competitively binding to SLCO2B1. In our ADT cohort of 926 patients, 283 (31%) were taking a statin at ADT initiation. After a median follow-up of 5.8 years, 644 patients (70%) had experienced disease progression while receiving ADT. Median TTP during ADT was 20.3 months (95% CI, 18-24 months). Men taking statins had a longer median TTP during ADT compared with nonusers (27.5 [95% CI, 21.1-37.7] vs 17.4 [95% CI, 14.9-21.1] months; $P < .001$). The association remained statistically significant after adjusting for predefined prognostic factors (adjusted hazard ratio, 0.83 [95% CI, 0.69-0.99]; $P = .04$). The positive statin effect was observed for both patients with and without metastases (adjusted hazard ratio, 0.79 [95% CI, 0.58-1.07] for M0 disease and 0.84 [95% CI, 0.67-1.06] for M1 disease; P for interaction = .72).

CONCLUSIONS AND RELEVANCE Statin use at the time of ADT initiation was associated with a significantly longer TTP during ADT even after adjustment for known prognostic factors. Our in vitro finding that statins competitively reduce DHEAS uptake, thus effectively decreasing the available intratumoral androgen pool, affords a plausible mechanism to support the clinical observation of prolonged TTP in statin users.

Editorial page 428

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Philip W. Kantoff, MD, Division of Solid Tumor Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave, Dana 1230, Boston, MA 02215 (philip_kantoff@dfci.harvard.edu).

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The organic anionic transporter SLCO2B1 enables a variety of anticancer compounds and hormones to enter cells.[1] Among its substrates is the abundant adrenal androgen dehydroepiandrosterone sulfate (DHEAS), which is a precursor to more potent androgens, such as dihydroxytestosterone (DHT), which binds to the androgen receptor in normal and cancer cells. In prostate cancer (PC), expression of *SLCO2B1* increases on progression from hormone-sensitive to castration-resistant disease.[2] Our group and others have previously demonstrated that genetic variants in *SLCO2B1* are associated with the durability of response to androgen deprivation therapy (ADT), due to varied efficiency of androgen influx into cells.[3,4]

Interestingly, statins are also substrates of SLCO2B1. Past work has generally shown an inverse association between statin use and incidence of PC, as well as improved clinical outcomes.[5-8] Little is known about the impact of statin use and the durability of response to ADT, which is the cornerstone of treatment for metastatic hormone-sensitive PC.[9] Given the fact that both DHEAS and statins are substrates for SLCO2B1, we first sought to determine whether there was any interaction between statins and DHEAS influx by SLCO2B1 in PC cell lines. Then, using our institutional clinical database, we evaluated the association between statin use and time to progression (TTP) among patients with PC who were receiving ADT.

At a Glance

- Statins compete with dehydroepiandrosterone sulfate for influx by SLCO2B1, which may decrease the tumor's available androgen pool.
- We observed a median 10-month prolongation in time to progression during androgen deprivation therapy (ADT) in statin users compared with nonusers (27.5 vs 17.4 months; $P < .001$) in a cohort of 926 men receiving primary ADT.
- There was a 17% reduction in the hazard of progression during ADT after adjusting for predefined prognostic factors (adjusted hazard ratio, 0.83; 95% CI, 0.69-0.99; $P = .04$).
- The positive effect of statin use was observed for both patients with and without metastases.
- The widespread use of statins and their established safety profile make them attractive potential anticancer therapeutics as adjuvants to cytotoxic or androgen-ablating therapies.

Methods

Cell Lines and Reagents

The hormone-sensitive PC cell lines LNCaP and 22RV1 were used for the in vitro studies. LNCaP and 22RV1 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 culture medium and supplemented with 10% fetal bovine serum (FBS) and antibiotics. For the cell proliferation studies, all PC cells were cultured in Phenol-Red free RPMI 1640/10% charcoal-stripped FBS. 293T cells were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium with 10% FBS and antibiotics. All cell lines were regularly screened for mycoplasma (Sigma Venor GeM Mycoplasma Detection Kit). Dehydroepiandrosterone sulfate was obtained from BioVender R&D Products. Atorvastatin calcium was obtained from Santa Cruz Biotechnology, and fluvastatin sodium, pravastatin sodium, and simvastatin were purchased from Selleckchem.

shRNA

We constructed an inducible short hairpin SLCO2B1 (shSLCO2B1) RNA-expressing plasmid using the "all-in-one" pLKO-Tet-On lentiviral vector (see eMethods in the Supplement). Lentiviruses were packaged using 293T cells. The stable inducible shSLCO2B1-expressing cell lines were established by selection in puromycin hydrochloride. The efficiency of knocking down *SLCO2B1* expression was assayed after induction with 1 µg/mL of doxycycline hyclate for 48 hours. The LNCaP and 22RV1 cell lines were transfected with scrambled short hairpin RNA (shRNA), and these were used as negative controls.

Quantitative Reverse-Transcription Polymerase Chain Reaction

A quantity of 100 ng total RNA was extracted from each PC cell line and analyzed by means of reverse-transcription polymerase chain reaction (RT-PCR) (see eMethods in the Supplement). All RT-PCR experiments were performed in triplicate.

DHEAS Uptake Assay

Cells were harvested in the PBS buffer and triple-washed with incubation buffer (140mM NaCl, 5mM KCl, 1mM KH_2PO_4, 1.2mM $MgSO_4$, 1.5mM $CaCl_2$, 5mM D-glucose, and 12.5mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], pH 7.7). Aliquoted cells were incubated with each statin or dimethyl sulfoxide (control) in incubation buffer at 37°C for 10 minutes and then treated with different DHEAS concentrations. At defined time points, the cells were triple-washed with cold incubation buffer to stop DHEAS uptake. The cells were then lysed using 1% Triton X-100 solution in 1 × PBS on ice for 30 minutes. The total protein concentration was measured in the cleared cell lysate supernatant by protein (bicinchoninic acid) assay (Thermo Scientific). The quantity of intracellular DHEAS was determined using the DHEAS enzyme-linked immunosorbent assay kit (BioVendor) and adjusted to the protein concentration of the cell lysate.

Cell Proliferation Assay

Cell proliferation was determined using the WST-1 assay (Roche). Briefly, control and shSLCO2B1-lentivirus–infected cells were cultured in 96-well plates in the presence of doxycycline at a confluence of approximately 10% in androgen-depleted medium for 2 days followed by treatment with atorvastatin and/or DHEAS. Cell proliferation assays were carried out on different days after treatment. Each experiment was performed in triplicate.

Clinical Cohort Study

This study was approved by the Dana-Farber Cancer Institute institutional review board. All patients provided written consent to 01-045, a protocol that collects clinical, treatment, and outcomes data on our patients with PC. Using this database,[10] we identified 1265 patients with hormone-sensitive PC who had

been treated with ADT (with or without an antiandrogen) between January 1996 and November 2013. Patients were excluded if they had insufficient follow-up data on prostate-specific antigen (PSA) level after ADT administration (n = 131) or if statin use status was unknown (n = 208), which left 926 patients for this analysis.

Clinicodemographic data were captured from the database. The electronic medical record was reviewed for dates of initiation and progression during ADT. Patients were defined as statin users if they were using statins at the time of ADT initiation. Progression was defined as a minimum of 2 increases in PSA level. Date of progression was defined as date of first increase in PSA level (nadir plus ≥0.02 ng/mL) (see additional details in the eMethods in the Supplement).

Statistical Analysis

Patient and disease characteristics were summarized as frequencies or the median and range of values. Characteristics were compared between statin users and nonusers using χ^2 and Wilcoxon rank-sum tests. The primary outcome variable was TTP during ADT, defined as the duration of time from ADT initiation to the date of disease progression or censorship at the date of last follow-up visit in patients who were progression-free. The association between statin use and TTP during ADT was analyzed by means of multivariable Cox regression to estimate hazard ratios (HRs) and 95% CIs, adjusting for predefined prognostic factors: biopsy Gleason score, primary therapy type, use of prior ADT in conjunction with local therapy, metastatic status, and PSA at ADT initiation (see additional details in the eMethods in the Supplement). We chose to evaluate these factors a priori given our group's past work showing their prognostic value.[11]

For in vitro studies, data were represented as the mean (SD) of at least 3 biological repeats. Comparison between 2 independent groups (or cell lines) was performed by a 2-tailed *t* test. $P < .05$ was considered statistically significant for all analyses.

Results

Dependence of Atorvastatin's Inhibition of DHEAS Uptake on SLCO2B1

We examined the effect of 4 different statins—atorvastatin, fluvastatin, pravastatin, and simvastatin—on DHEAS uptake in the androgen-dependent LNCaP and the partially androgen-dependent 22RV1 PC cell lines (**Figure 1**A). Dehydroepiandrosterone sulfate uptake in PC cell lines was concentration and time dependent (Figure 1B and C and eFigure 1 in the Supplement). When incubated with DHEAS at a physiological concentration (2.5 µM) for 60 minutes, the 22RV1 cell line, which has a relatively high level of *SLCO2B1* expression, displayed the most active DHEAS uptake of more than 300 pmol/mg compared with approximately 60 pmol/mg protein for LNCaP (Figure 1B). A quantity of 100 µM atorvastatin significantly decreased DHEAS influx by approximately 50% in both cell lines when cells were incubated with 2.5 µM DHEAS. Among the 4 statins that we studied, pravastatin had the most substantial inhibitory effect on DHEAS uptake in both cell lines, whereas a more prominent effect of simvastatin was shown for 22RV1 cells than that for LNCaP cells (Figure 1B). However, 10 or even 100 µM atorvastatin or simvastatin was insufficient to inhibit DHEAS uptake in LNCaP, which has a relatively low level of *SLCO2B1* expression when the concentration of 100 µM DHEAS was used (eFigure 1 in the Supplement). These results suggest, not surprisingly, that different statins compete with DHEAS for the same transporter but with varying efficiency and that this effect is cell line dependent.

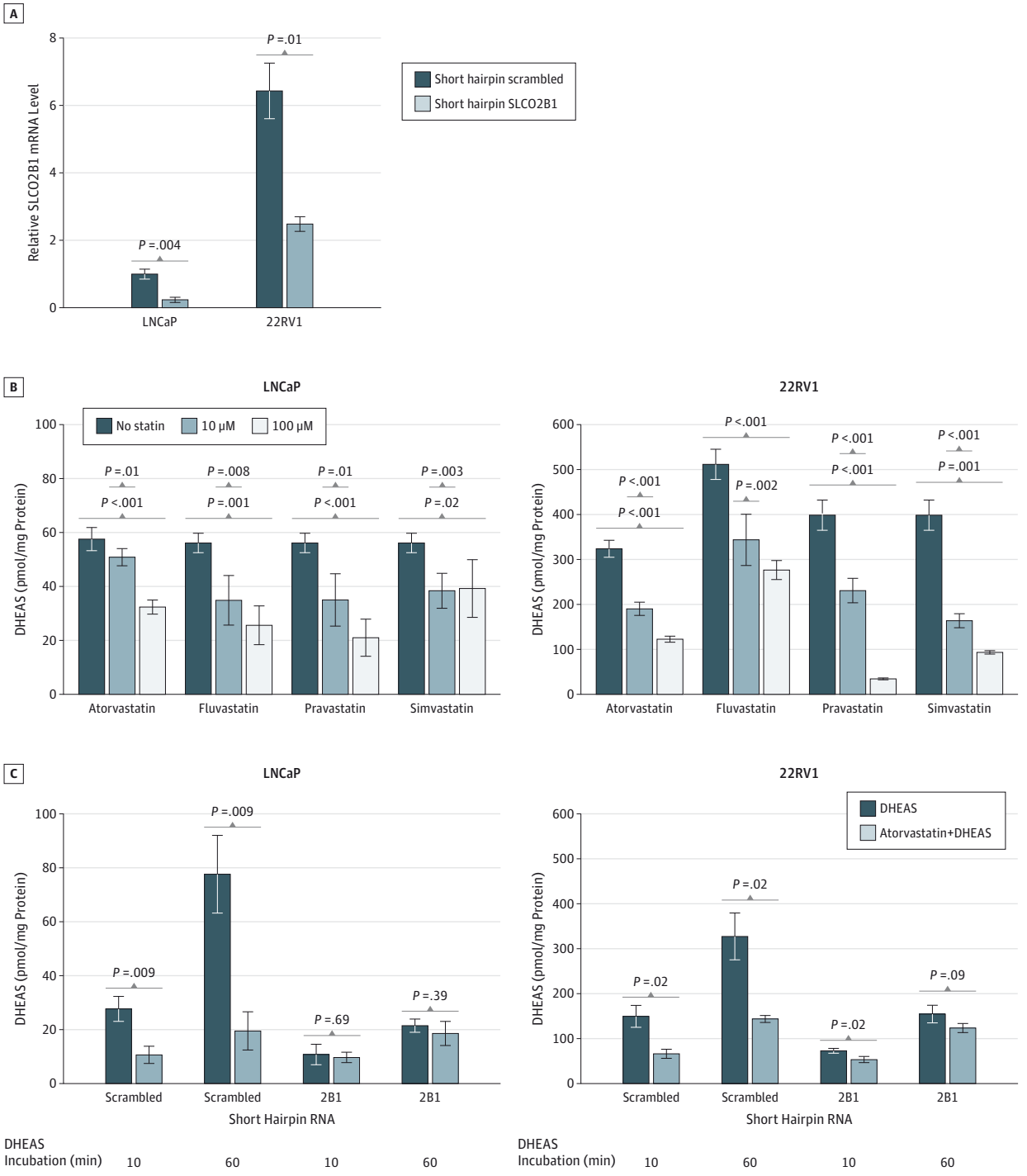
To determine the dependence of DHEAS uptake on SLCO2B1, we constructed inducible *SLCO2B1*-deficient stable 22RV1 cell lines using the lentiviral-derived tetracycline inducible shRNA knock-down system. We were unable to establish an inducible *SLCO2B1*-deficient stable cell line in LNCaP; thus, we used a transient-inducible knock-down of *SLCO2B1* LNCaP cells in this study (shRNA-*SLCO2B1*). After successfully knocking down *SLCO2B1* (Figure 1A), we found that DHEAS uptake was substantially decreased to approximately 50% in 22RV1 and approximately 70% in LNCaP from that observed in control cells (cells transfected with scrambled shRNA) (Figure 1C). These results indicate that SLCO2B1 plays an essential role in DHEAS import into PC cells (Figure 1C). More importantly, knocking down *SLCO2B1* abolished atorvastatin's inhibition of DHEAS uptake, further suggesting that atorvastatin competes with DHEAS for binding to their transporter, SLCO2B1.

Inhibition of SLCO2B1-Mediated DHEAS Uptake and PC Cell Proliferation

To further test our hypothesis, we investigated whether inhibition of cell growth was SLCO2B1 and DHEAS dependent. Thus, we used the WST-1 assay to examine the impact of atorvastatin, the most commonly used statin clinically, and DHEAS on tumor proliferation before and after *SLCO2B1* was knocked down.

A concentration of at least 2.5 µM atorvastatin is known to inhibit LNCaP cell proliferation and induces autophagy.[12] The concentration of atorvastatin ranges from 5 to 270 nM in patient serum,[13] and we have demonstrated that inhibition of DHEAS-induced cell growth is dependent on atorvastatin concentrations in this range (eFigure 2 in the Supplement). Thus, we chose 200 nM of atorvastatin for our cell proliferation assay. A quantity of 80 nM DHEAS significantly increased cell proliferation in LNCaP and 22RV1 lines that were maintained in androgen-depleted medium. Cell numbers nearly doubled by day 6 for LNCaP (**Figure 2**A). At day 6, DHEAS induced a roughly 6-fold increase in LNCaP cell number, compared with a roughly 3-fold increase in the absence of DHEAS ($P < .001$). However, treatment with atorvastatin (200 nM) inhibited this DHEAS-induced cell proliferation. Consistent with this finding, knocking down *SLCO2B1* abolished DHEAS-induced cell proliferation in LNCaP and 22RV1 (Figure 2B). Furthermore, treatment with atorvastatin did not significantly inhibit the growth of *SLCO2B1* knocked-down cells. In summary, our data demonstrate that atorvastatin can efficiently block SLCO2B1-mediated DHEAS uptake and DHEAS-induced cell growth in androgen-dependent PC cell lines.

Figure 1. Statin-Related Inhibition of Dehydroepiandrosterone Sulfate (DHEAS) Uptake by SLCO2B1 in Prostate Cancer (PC) Cells

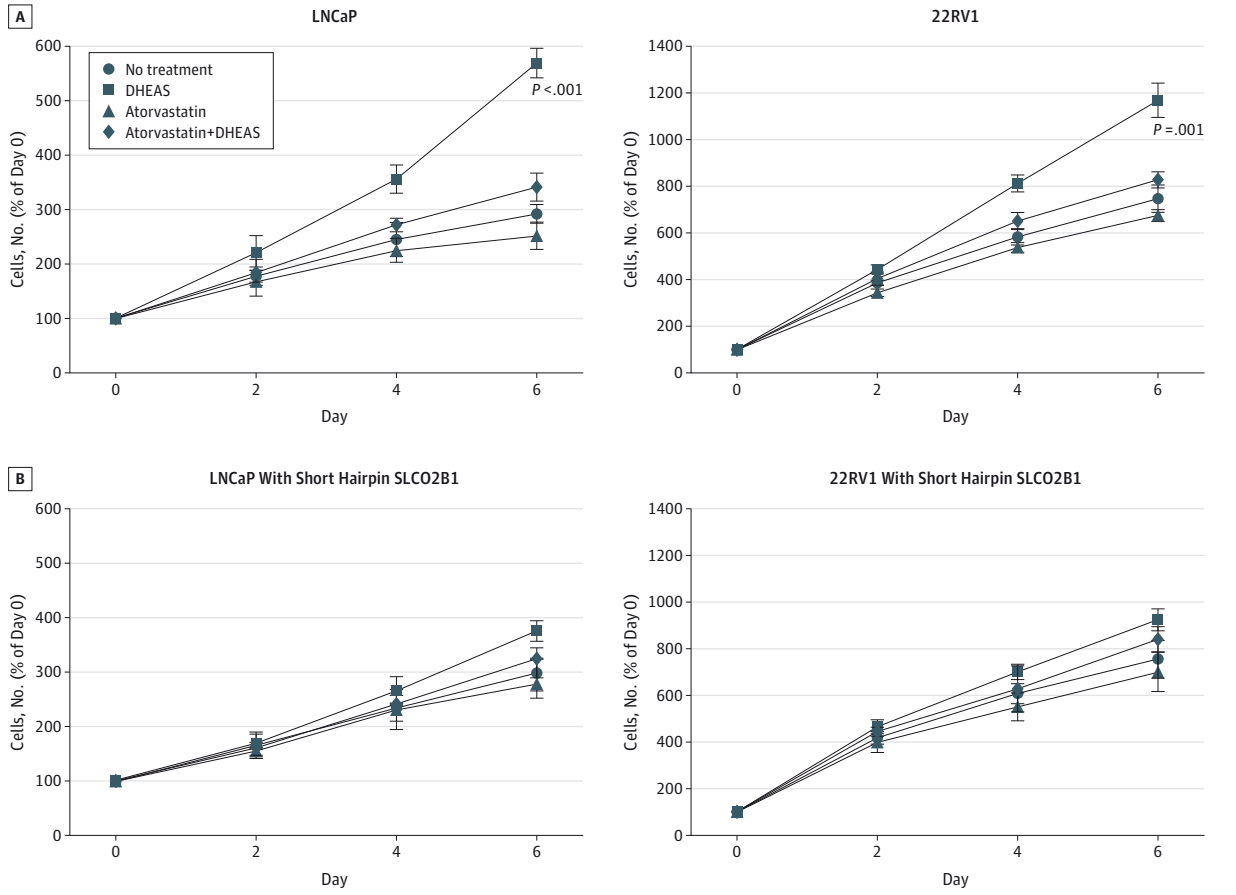


A, Relative mRNA levels in PC cells before and after *SLCO2B1* is knocked down. B, Uptake of DHEAS in PC cells with 2.5 μM DHEAS and different concentrations of statins when incubated for 60 minutes. Statistical analysis was performed by comparing each condition with the DHEAS 2.5 μM and no statin state except when indicated. C, Uptake of DHEAS in PC cells before (scrambled short hairpin RNA) and after (short hairpin RNA 2B1) *SLCO2B1* is knocked down when incubated with 2.5 μM DHEAS and 100 μM atorvastatin for 10 and 60 minutes. Statistical analysis was performed by comparing each condition with scrambled short hairpin RNA after 10 minutes with DHEAS except when indicated. *P* = .02 for the comparison between scrambled short hairpin RNA with 10 vs 60 minutes of DHEAS incubation for LNCaP and .01 for 22RV1. Other P values are indicated in the figure. Bars indicate means and error bars indicate standard deviation.

Clinical Cohort Study

Of the 926 patients included, 283 patients (31%) were taking a statin at ADT initiation. Most patients (93%) continued statin use at the time of progression or at last follow-up. Statin use generally increased over time from 1996 to 2013 (eFigure 3 in the Supplement). Of the 643 nonusers, 72

Figure 2. Effect of Atorvastatin on Dehydroepiandrosterone Sulfate (DHEAS)-Induced Prostate Cancer (PC) Cell Proliferation



Atorvastatin decreases DHEAS-induced PC cell proliferation by competing for influx through SLCO2B1. A, WST-1 assay of cell proliferation in PC cell lines. B, WST-1 assay of cell proliferation in PC cell lines after *SLCO2B1* is knocked down. For proliferation assays, cells were maintained in androgen-depleted medium with doxycycline for 2 days followed by the addition of 80 nM DHEAS and 200 nM atorvastatin to the culture medium for the indicated time. Relative cell numbers were calculated as percentages of the cell numbers at day 0 (100%).

started statin therapy while receiving ADT; in these patients, median (IQR) time from ADT initiation to initial statin use was 24 (12 to 48) months.

Patient and disease characteristics at diagnosis and at ADT initiation are detailed in **Table 1**. Statin users tended to have a lower median (IQR) PSA level both at diagnosis (9.1 [5.5-17.0] vs 11.8 [6.3-40.0] ng/mL) and at ADT initiation (10.3 [4.6-28.2] vs 12.5 [4.4-59.1] ng/mL). Median (IQR) duration from diagnosis to ADT initiation was longer in statin users (3.85 [1.2-7.8] vs 2.33 [0.1-5.6] years). Users were more likely to have lower stage disease (56% vs 44% T1 disease; $P = .005$) and less likely to have de novo metastases (11% vs 18%; $P = .01$) or nodal involvement (5% vs 10%; $P = .03$) at diagnosis. Statin users were more likely to have undergone local therapy (82% vs 68%; $P < .001$) or to have received ADT as part of local therapy (33% vs 26%; $P = .02$). Statin users were less likely to have metastases at ADT initiation (53% vs 63%; $P = .005$) (Table 1).

At the time of data capture, 70% (n = 644) of patients had disease progression during ADT therapy by PSA level. Median (range) follow-up was 5.8 (0.1-15.9) years. Median TTP during ADT for all patients irrespective of statin use was 20.3 (95% CI, 17.5-23.6) months. Statin users at ADT initiation had a significantly longer median TTP during ADT (27.5 [95% CI, 21.1-37.7] vs 17.4 [95% CI, 14.9-21.1] months; $P < .001$) (**Figure 3**A). When adjusting for predefined prognostic clinical factors including biopsy Gleason score, type of primary therapy, use of prior ADT in conjunction with localized therapy, metastatic status, and PSA level at initiation of ADT, the relative reduction for risk of progression was 17% (adjusted HR, 0.83 [95% CI, 0.69-0.99]) (**Table 2**).[11] The results were similar when the model was further stratified by year of ADT initiation using 5-year increments (adjusted HR, 0.83 [95% CI, 0.69-1.00]) or if we excluded the 72 patients who started using a statin while receiving ADT as nonusers (adjusted HR, 0.71 [95% CI, 0.59-0.85]). Moreover, the association between statin use and TTP was observed regardless of whether patients had radiographic evidence of metastatic disease compared with biochemical relapse only at ADT initiation (adjusted HR, 0.79 [95% CI, 0.58-1.07] for M0 disease; adjusted HR, 0.84 [95% CI, 0.67-1.06 for M1 disease; P for interaction = .72) (Figure 3B and C).

Table 1. Patient and Disease Characteristics at Diagnosis and at Androgen Deprivation Therapy (ADT) Initiation by Statin Use[a]

Characteristic	All (N = 926)	Statin Use		*P* Value
		No (n = 643)	Yes (n = 283)	
Age at diagnosis, No./median (IQR), y	881/61 (55-67)	616/60 (55-66)	265/62 (56-67)	.02
PSA level at diagnosis, No./median (IQR), ng/mL	813/10.7 (6-29)	570/11.8 (6-40)	243/9.1 (6-17)	<.001
Race, No. (%)				
White	858 (93)	596 (93)	262 (93)	.95
Black	41 (4)	30 (5)	11 (4)	
Hispanic	5 (0.5)	3 (0.5)	2 (0.7)	
Asian	4 (0.4)	2 (0.3)	2 (0.7)	
Other	7 (0.8)	5 (0.8)	2 (0.7)	
Unknown	11 (1)	7 (1)	4 (1)	
Clinical T stage, No. (%)				
T1	442 (48)	283 (44)	159 (56)	.005
T2	206 (22)	149 (23)	57 (20)	
T3-4	48 (5)	38 (6)	10 (4)	
Tx or unknown	230 (25)	173 (27)	57 (20)	
Clinical M stage, No. (%)				
M0	338 (36)	235 (36)	103 (36)	.01
M1	148 (16)	117 (18)	31 (11)	
Mx or unknown	440 (48)	291 (45)	149 (53)	
Clinical N stage, No. (%)				
N0	337 (36)	235 (36)	102 (36)	.03
N1	78 (8)	64 (10)	14 (5)	
Nx or unknown	511 (55)	344 (54)	167 (59)	
Biopsy Gleason score, No. (%)				
≤6	134 (14)	85 (13)	49 (17)	.11
7	310 (34)	207 (32)	103 (36)	
≥8	376 (41)	272 (42)	104 (37)	
Unknown	106 (11)	79 (12)	27 (10)	
Type of local therapy, No. (%)				
Radical prostatectomy with or without RT	388 (42)	268 (42)	120 (42)	<.001
RT only or other	285 (31)	172 (27)	113 (40)	
None	253 (27)	203 (32)	50 (18)	
ADT as part of local therapy, No. (%)				
No	668 (72)	478 (74)	190 (67)	.02
Yes	258 (28)	165 (26)	93 (33)	
PSA level at ADT initiation, No./median (IQR), ng/mL	849/11.8 (4.4-45)	580/12.5 (4.4-59.1)	269/10.3 (4.6-28.2)	.04
Time from diagnosis to ADT initiation, No./median (IQR), y	881/2.88 (0.2-6.1)	616/2.33 (0.1-5.6)	265/3.85 (1.2-7.8)	<.001
Metastases at ADT initiation, No. (%)				
No	372 (40)	239 (37)	133 (47)	.005
Yes	554 (60)	404 (63)	150 (53)	
Concomitant use, prior to progression, No. (%)				
5-α Reductase inhibitor	32 (4)	25 (4)	7 (2)	.28
Antiandrogen	658 (71)	459 (71)	199 (70)	.74
Chemotherapy	72 (8)	40 (6)	32 (11)	.008

Abbreviations: IQR, interquartile range; PSA, prostate-specific antigen; RT, radiotherapy.

[a] Percentages may not total 100% because of rounding.

Discussion

Continued reliance of the tumor on androgen receptor signaling and residual androgens contributes to progression to castration-resistant prostate cancer (CRPC). In our preclinical studies, we revealed that DHEAS and various statins compete for binding to the transporter SLCO2B1 and that treatment with statins likely competitively inhibits DHEAS uptake. We demonstrated that the adrenal androgen DHEAS, an important precursor to DHT, the active metabolite that PC uses, stimulates PC cell proliferation and that a statin drug, atorvastatin, can diminish DHEAS-stimulated proliferation. This mechanism may explain the observation that statin use may be associated with improved clinical outcomes in PC and drove us to query whether statin use might influence TTP during ADT in patients with hormone-sensitive PC. Given the vagaries of defining progression in PC, in our clinical study, we narrowly defined TTP by PSA level alone, allowing the use of PSA level as a pharmacodynamic end point of androgen action.

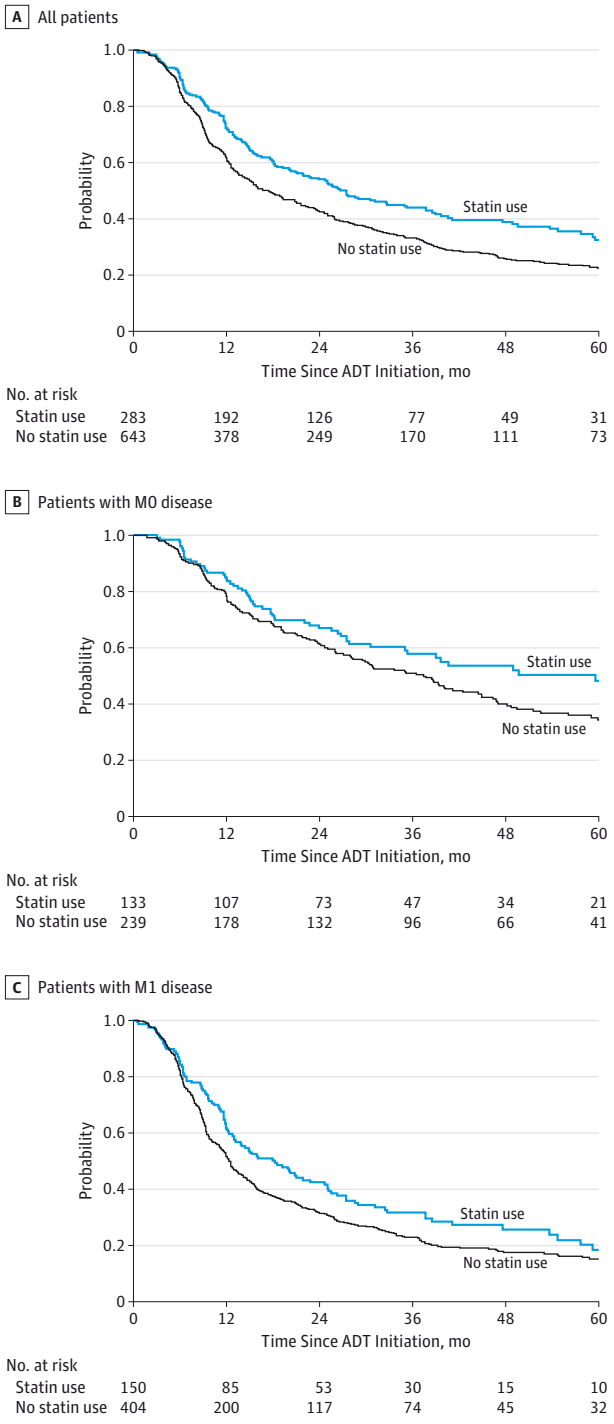
Statins are administered widely in the United States for their clinically meaningful lipid-lowering properties. Most epidemiological studies have shown significant associations between statin use and decreased incidence of advanced PC, risk of recurrence after local treatment, mortality, and PSA levels relative to nonusers.[5,6,14-16] Conversely, most cohort and case-controlled studies have shown no association between statin use and overall PC risk.[7,8,17-19] To our knowledge, none have reported on the impact of statin use on TTP during ADT.

Given the biologic heterogeneity observed in PC, what becomes clear is that the ability of a therapy to thwart the development of *lethal* PC is more important than decreasing the overall incidence.[20] A recent meta-analysis collated data from 27 observational studies encompassing nearly 2 million patients and assessed the association of statin use with the risk of developing PC.[5] The pooled analysis revealed a 7% reduction in the risk of developing any PC (relative risk, 0.93 [95% CI, 0.87-0.99]; $P = .03$). However, 7 of the studies specifically assessed the association between statin use and the risk of clinically significant or advanced PC. All but 1 showed a relative risk reduction, which ranged from 7% to 49% with statin use (relative risk, 0.51-0.93). As reviewed by Mucci and Stampfer,[20] there have been at least 5 additional published studies since 2012 that demonstrate an inverse association between statin use and PC mortality.

Our study evaluated the impact of statin use in a patient population with more advanced disease, all of whom had either biochemical or metastatic recurrence after local therapy or de novo metastatic disease for which they commenced ADT. We found that statin use at the time of ADT initiation was associated with a significant increase in TTP during ADT even after adjusting for established prognostic factors such as Gleason score at biopsy, type of primary therapy be it radical prostatectomy or radiotherapy, use of ADT with the primary therapy, or presence of metastases and PSA level at ADT initiation.[11]

Multiple preclinical models have assessed the direct effects of statins on PC. Zheng and colleagues[21] evaluated ator-

Figure 3. Kaplan-Meier Analysis of Time to Progression (TTP) During Androgen Deprivation Therapy (ADT) According to Statin Use in All Patients



A, All patients. B, Patients with no visible radiographic metastasis but biochemical (prostate-specific antigen) failure (M0). C, Patients with metastasis (M1) at ADT initiation. Across the entire cohort, patients using statins at the time of ADT initiation had a significantly longer median TTP during ADT (27.5 vs 17.4 months; $P < .001$). The association between statin use and TTP was observed regardless of whether patients had evidence of metastatic disease (C) compared with biochemical relapse (B) only at ADT initiation (adjusted hazard ratio, 0.79 [95% CI, 0.58-1.07] for M0; adjusted hazard ratio, 0.84 [95% CI, 0.67-1.06] for M1; P for interaction = .72).

Table 2. Association of Statin Use With Time to Progression (TTP) During Androgen Deprivation Therapy (ADT) in Multivariable Cox Regression Analysis for 926 Patients

Variable	No.	Failure, No.	HR (95% CI)	*P* Value
Statin use at ADT initiation				
No	643	480	1 [Reference]	.04
Yes	283	164	0.83 (0.69-0.99)	
Biopsy Gleason score				
<7	134	70	1 [Reference]	<.001
7	310	196	1.17 (0.88-1.54)	
8	376	297	1.57 (1.19-2.08)	
Unknown	106	81	1.57 (1.13-2.17)	
Primary therapy				
Radical prostatectomy with or without radiotherapy	388	228	1 [Reference]	<.001
Radiotherapy only or other	285	193	1.33 (1.08-1.64)	
None	253	223	1.81 (1.43-2.30)	
ADT used as part of local therapy				
No	668	478	1 [Reference]	.72
Yes	258	166	1.04 (0.84-1.29)	
Metastasis at ADT initiation				
No	372	210	1 [Reference]	<.001
Yes	554	434	1.56 (1.29-1.89)	
Prostate-specific antigen level at ADT initiation, ng/mL				
<10	387	218	1 [Reference]	.03
10 to <20	131	95	1.27 (0.99-1.63)	
≥20	331	271	1.32 (1.07-1.64)	
Unknown	77	60	1.39 (1.04-1.87)	

Abbreviation: HR, hazard ratio.

vastatin and celecoxib in xenograft models. They observed a reduction in PC cell growth even after castration and a delay in progression to androgen independence. In vitro, Murtola et al[22] evaluated the effects of simvastatin using cell lines ranging from normal prostate epithelium to resected primary tumors to more advanced CRPC-like cell lines such as LNCaP and VCaP. They found that simvastatin inhibited early-stage cell lines but not the more advanced CRPC-like subtypes.[5,22] Although not directly assessing mechanism, meta-analysis of multiple randomized clinical trials and individual cohort studies in humans have shown that statin use lowers testosterone levels.[23,24]

Statins may impart antitumor effects through various mechanisms. They inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an enzyme critical in cholesterol biosynthesis.[25] Cholesterol is an essential component in the synthesis of steroid hormones such as the androgens that drive PC. Thus, a reduced pool of cholesterol building blocks may stymie cancer growth because rapidly proliferating cells such as tumor cells require high levels of available cholesterol.[26] Other potential antitumor effects of statins include inhibition of cell proliferation, inflammation, angiogenesis, invasion, and metastasis, as well as induction of apoptosis and autophagy.[5,26-30]

We uncovered another potential mechanism by which statins may contribute to PC treatment. Using PC cell lines, we found that statins and DHEAS compete for the same transporter, SLCO2B1, and that statin administration competitively reduces uptake of DHEAS and, in the case of atorvastatin, subsequent tumor cell proliferation. Thus, statins effectively decrease the available intratumoral androgen pool, which may enhance cancer control. These cell line findings provide a plausible mechanism to support our clinical observations of increased TTP during ADT in statin users.

There are several limitations to our clinical study, many of which are inherent to retrospective analyses. Overall, statin users tended to have better cancer risk profiles at diagnosis and at ADT initiation. At ADT initiation, statin users tended to have less advanced PC with less nodal or metastatic disease. In contrast, statin users were more likely to have received prior ADT with local therapy, which is generally thought to shorten the durability of their subsequent use.[11] However, when adjusted for these imbalances in our multivariate analysis, the association with TTP remained. Other sociodemographic and behavioral differences (eg, income, education, health service use pattern, insurance status), medications (eg, metformin), and comorbidities were not captured by our institutional clinical database. Whereas these factors may affect the statin use pattern, timing of diagnosis, and the biological characteristics of PC, their impact on response duration during ADT as assessed by PSA level criteria is less clear. We did not capture toxicity data, but if statins plus ADT induced substantial or additive toxic effects (eg, fatigue, myalgias), one would have expected the opposite result of shorter TTP during ADT therapy. In addition, a "healthy user bias" could be at play in

that men taking statins likely see physicians more regularly and, thus, on average may either be healthier or have their comorbidities under better control. Increased access to and regular use of the health care system may lead to an earlier diagnosis of PC, which could affect duration of ADT therapy. However, our multivariate analyses controlled for PSA level at diagnosis and stage, and the relative reduction in risk of progression persisted.

Another confounding variable, which we did not specifically address, was the contribution of different statins to TTP. It is conceivable that differences in statin potency and/or their pharmacokinetic properties[27] could result in a dose-response relationship, which could have influenced the magnitude of the results. Finally, we do not know the impact of other androgens. After ADT therapy, serum testosterone is largely depleted whereas DHEAS, which is produced by the adrenal gland, remains abundant. Whereas the relative contributions of persistent testosterone, de novo synthesis of testosterone, and conversion of DHEAS to testosterone are unknown in the testosterone-depleted state induced by ADT, we believe that the latter mechanism is likely to be the dominant one. Future studies should directly evaluate the impact of statin use on testosterone and DHT levels.

Conclusions

We have shown that statins compete with DHEAS for influx by SLCO2B1, which may decrease the tumor's available androgen pool. Clinically, this may translate to improved cancer outcomes, which we observed in our institutional cohort of men receiving primary ADT. Even when controlled for known prognostic factors, men taking statins had significantly longer TTP while receiving ADT than nonusers. The mechanism through which statins exert their activity in PC is likely multifactorial, including antiproliferative and proapoptotic effects. However, the most plausible mechanism is the reduction in the tumor's androgen stores through a combination of decreased availability of the cholesterol precursor required for de novo synthesis and decreased transport of existing precursor androgens such as DHEAS via competitive binding of SLCO2B1. The widespread use of statins and their established safety profile make them attractive potential anticancer therapeutics as adjuvants to cytotoxic or androgen-ablating therapies. Ultimately, these results require prospective validation. More than 10 prospective trials are ongoing or maturing that will further characterize the role of statins as anticancer therapies in PC.[31]

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Author Affiliations: Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts (Harshman, Nakabayashi, Valenca, Sweeney, Pomerantz, P. W. Kantoff); Gelb Center for Translational Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts (Wang, Yu, Lee, P. W. Kantoff); Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, Massachusetts (Xie, Werner); Department of Urology, Shanghai Jiao Tong University School of Medicine, Xinhua Hospital, Shanghai, China (Yu); Apple Tree Partners, New York, New York (A. M. Kantoff); Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts (Mucci).

Author Contributions: Dr P. W. Kantoff had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Harshman and Wang served as co–first authors, each with equal contribution to the manuscript.
Study concept and design: Wang, P. W. Kantoff.
Acquisition, analysis, or interpretation of data: Harshman, Wang, Nakabayashi, Xie, Valenca, Werner, Yu, A. Kantoff, Sweeney, Mucci, Pomerantz, Lee, P. W. Kantoff.
Drafting of the manuscript: Harshman, Wang, Nakabayashi, Sweeney, Lee, P. W. Kantoff.
Critical revision of the manuscript for important intellectual content: Harshman, Wang, Xie, Valenca, Werner, Yu, A. Kantoff, Sweeney, Mucci, Pomerantz, Lee, P. W. Kantoff.
Statistical analysis: Wang, Xie, Mucci.
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Study supervision: Lee, P. W. Kantoff.

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