Supplementary Online Content


eMethods
eFigure 1. The Impact of Statins on DHEAS Uptake by PC Cells
eFigure 2. Decrease of DHEAS Induced PC Cell Proliferation Is Atorvastatin (ATO) Concentration Dependent
eFigure 3. Statin Use by Year of ADT Initiation

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

shRNA

We constructed the inducible shSLCO2B1 RNA expressing plasmid using the “all-in-one” pLKO-Tet-On lentiviral vector. The sequence of SLCO2B1 shRNA oligos: forward 5'-ccggGAGGAGGGTTTGCTAATCTtcgagAGATTAGCAAACCCTCTCCTCttttt-3' and reverse 5'-aattaaaaaGAGGAGAGGGTTTGCTAATCTtcgagAGATTAGCAAACCCTCTCCTC-3'.

Quantitative RT-PCR

The following primers were used to measure the targeted gene expression level: SLCO2B1, forward 5'-GTTTCGGCGAAAGGTCTTAGCAG-3', reverse 5'-CCATCCTGCTTTCTCGTGACT-3' and GAPDH, forward 5'-CAGCCTCAAGATCATCAGCA-3', reverse 5'-GTCTTCTGGGTGGCAGTGAT-3'. The primer pair efficiencies for SLCO2B1 and GAPDH were calculated according to the linear regression of the log serial (8 ×) dilution factor versus the correspondent threshold cycle (Ct) values. The slope of the regression line was used to calculate the primer pair efficiencies (E), i.e., $E = 10^{(-1/slope)}$. The primer pairs with efficiencies close to 2 were used.

Statistical Analysis

TTP on ADT was defined as the duration of time from ADT initiation to the date of ADT progression. To meet the endpoint of ADT progression, a patient needed to have two rises in PSA (at least 1 week apart) above a nadir value while receiving ADT, with the first rise greater than the nadir PSA plus 0.02 ng/mL and the second rise greater than the first
rise. The date of progression was defined as the date of the first rise. Radiologic progression or initiation of a second hormonal therapy for a rising PSA before fulfillment of the definition of progression was also considered a progression event. The date of starting the next therapy was the progression date in those cases. Among those who were progression-free, TTP was censored at the date of last follow-up visit or PSA value.

The association between statin use and TTP on ADT was estimated from multivariable Cox regression, adjusting for pre-defined prognostic factors (biopsy Gleason score, primary therapy type, use of prior ADT in conjunction with local therapy, metastatic status, and PSA at ADT initiation) based on our previous work using a cohort of 553 patients who were treated with ADT (Ross Cancer 2008). No formal model selection was used, but multiple sensitivity analyses were conducted by (1) using a stratified multivariable Cox regression, stratified by year of ADT initiation using 5-year increments; (2) using a “full” model adjusted for all available baseline clinical variables (i.e. age at ADT initiation, TMN stage at diagnosis, PSA at diagnosis, and time from diagnosis to ADT initiation) as well as stratified by years of ADT initiation. Consistent results were observed in these various sensitivity analyses (adjusted HR for statin use=0.83, 95% CI: 0.68, 1.01). Other baseline variables in Table 1 (age, TMN stage at diagnosis, PSA at diagnosis and time from diagnosis to ADT initiation) were not independent predictors on multivariable analysis (HRs ranging from 0.94 to 1.20, p>0.25). In addition, we observed underlying high correlation between these baseline covariates. For example, patients with a high PSA at ADT initiation generally also a more elevated PSA at diagnosis (Pearson correlation= 0.92). Among those who did not receive local therapy, the majority (63%)

© 2015 American Medical Association. All rights reserved.
had M1 or N1 disease at diagnosis, and the median duration from diagnose to ADT initiation was only <1 month compared to a median of 4.3 years in patients with a local therapy. Therefore, the final model presented in Table 2 is a parsimonious model adjusted for the pre-defined risk factors for TTP on ADT instead of a full model to avoid the multicolinearity issue due to inclusion of redundant, highly correlative covariates in regression analysis.
eFigure 1. The Impact of Statins on DHEAS Uptake by PC Cells

DHEAS uptake in PC cells with 100 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 (NS).
eFigure 2. Decrease of DHEAS Induced PC Cell Proliferation Is Atorvastatin (ATO) Concentration Dependent

LNCaP cells were maintained in androgen depleted medium for 2 days followed by the addition of 80 nM DHEAS and different concentrations of ATO to the culture medium for 6 days. NT = no DHEAS was added in the culture medium as a control group.

Relative cell numbers were calculated as percentages of the cell numbers at day 0 (100%).
Statin use tended to increase over time with significantly more users after 2006 compared to prior to 2006.