Androgen Receptor Upregulation Mediates Radioresistance after Ionizing Radiation

Daniel E. Spratt\textsuperscript{1,2,3}, Michael J. Evans\textsuperscript{2}, Brian J. Davis\textsuperscript{4}, Michael G. Doran\textsuperscript{3}, Man Xia Lee\textsuperscript{2}, Neel Shah\textsuperscript{2}, John Wongvipat\textsuperscript{2}, Kathryn E. Carnazza\textsuperscript{3}, George G. Klee\textsuperscript{5}, William Polkinghorn\textsuperscript{1,2}, Donald J. Tindall\textsuperscript{5}, Jason S. Lewis\textsuperscript{3}, and Charles L. Sawyers\textsuperscript{2}

Abstract

Clinical trials have established the benefit of androgen deprivation therapy (ADT) combined with radiotherapy in prostate cancer. ADT sensitizes prostate cancer to radiotherapy-induced death at least in part through inhibition of DNA repair machinery, but for unknown reasons, adjuvant ADT provides further survival benefits. Here, we show that androgen receptor (AR) expression and activity are durably upregulated following radiotherapy in multiple human prostate cancer models in vitro and in vivo. Moreover, the degree of AR upregulation correlates with survival in vitro and time to tumor progression in animal models. We also provide evidence of AR pathway upregulation, measured by a rise in serum levels of AR-regulated hK2 protein, in nearly 20% of patients after radiotherapy. Furthermore, these men were three-fold more likely to experience subsequent biochemical failure. Collectively, these data demonstrate that radiotherapy can upregulate AR signaling after therapy to an extent that negatively affects disease progression and/or survival. Cancer Res; 75(22); 4688–96. ©2015 AACR.

Introduction

Several phase III clinical trials have demonstrated a clear survival benefit when long-term adjuvant androgen deprivation therapy (LTADT) is added to concurrent ADT and external beam radiotherapy (1, 2). The addition of adjuvant ADT after radiotherapy is commonly referred to as LTADT; however, recent evidence suggests that simply increasing duration of ADT without the focus on when it is given in relation to radiotherapy does not improve outcomes, suggesting the timing and duration of ADT are critical (3). However, chronic androgen suppression can impact quality of life, motivating ongoing clinical studies to optimize the duration of androgen deprivation without compromising efficacy (3, 4).

We previously have shown that primary prostate tumors display heterogeneity in androgen receptor (AR) transcriptional output, which could result in differential sensitivity to ADT and to the relative clinical benefit of ADT when combined with radiotherapy (5). Furthermore, we and others have also shown that AR activates DNA repair pathways, providing further rationale for concurrent ADT/radiotherapy (5–7). Despite this radiosensitizing mechanistic action of ADT, clinical trials have demonstrated that adjuvant ADT has similar efficacy to that of concurrent ADT with radiotherapy (8), begging the question why adjuvant ADT sufficiently compensates for radiosensitizing concurrent therapy, and further improves survival even beyond concurrent use of ADT with radiotherapy (1).

In addition to variability in baseline AR signaling, select observations may suggest that AR signaling is upregulated by radiotherapy. For instance, in small patient series, a subset of patients have increases in secreted levels of AR target genes (e.g., PSA, hK2) during external beam radiotherapy (EBRT; refs. 9, 10). In addition, the AR target gene TMPRSS2 is upregulated in a human prostate cancer cell line exposed to therapeutic doses of radiotherapy (6). These considerations led us to more broadly study the impact of radiotherapy on AR signaling, and the association between high AR signaling after radiotherapy and measures of outcome, as this may have implications for the use and duration of adjuvant ADT after radiotherapy.

Materials and Methods

Cell lines

LNCaP and MDA-PCa2b cell lines used were purchased directly from the American Type Culture Collection and cultured according to recommended specifications. LNCaP-AR is an AR-overexpressing (wild-type) cell line originally derived from parental LNCaP with a luciferase probasin reporter. LNCaP-AR cell line was authenticated via AR overexpression by PCR, immunoblot, and luciferase assay. CWR22Rv1 were obtained from Marja Nevalianan, Thomas Jefferson University.
Cell culture
Cell lines were not kept in culture longer than 6 months. Growth conditions for each cell line are described in Supplementary Methods.

Cell irradiation
All described doses of radiation were delivered using Cesium-137 irradiator (Shepherd Mark, Model 68). Correction factors for decay were implemented, and the estimated dose rate of delivered was 184 cGy/min. All plates were continuously rotated with a turntable speed of 6 revolutions/minute to improve dose homogeneity.

Real-time PCR
Cells were plated and 24 hours later irradiated. At the specified time after radiotherapy, cells were collected for RNA extraction using the Qiagen Kit and RNA-easy Kit (QIAGEN, QIA Shredder, #79656, QIAGEN, RNeasy Mini Kit, #74106). cDNA was generated using the Applied Biosystems High capacity cDNA Reverse Transcription Kit (#4368814). Per manufacturer’s recommendations, Quantifast (QIAGEN, Quantifast SYBR Green PCR Kit, #204057) was used for PCR. All assays were performed in quadruplicate and normalized to actin. PCR primers can be found in Supplementary Methods.

Western blot analysis
Whole-cell lysates were prepared using 10% M-PER lysis buffer and clarified by centrifugation. Proteins were separated by 4% to 12% SDS-PAGE 15-well gel prepared as previously described. Primary antibodies for the following proteins were used: AR [Santa Cruz; AR (N-20), sc-816, 1:500-1,000 dilution], γ-H2AX [Millipore; Anti-phospho-Histone H2Ax (ser139), clone JBW301, 05-636, 1:500-1,000 dilution], pChk2 [Cell Signaling Technology; p-Chk2 (T68) (C13C1), 2197s, 1:1,000 dilution], and GAPDH [Abcam; GAPDH, ab9485 (1:10,000)]. Secondary antibodies used included Jackson Immuno Research, Goat anti-mouse HRP (115-035-003, 1:10,000 dilution), and Goat anti-rabbit HRP (111-035-003, 1-10,000 dilution).

18F-FDHT internalization assay
Internalization of 18F-DHT was investigated on LNCaP cells. Approximately 1 x 10^5 cells were plated in 12-well plates and incubated overnight, and the next day, the plates were irradiated with the specified increasing doses of radiotherapy. Twenty-four hours after radiotherapy, 2 mL of radiolabeled DHT (37 kBq/mL) was added to each well. The plates were incubated at 37°C for 1 hour. The medium was then collected and the cells were rinsed with 1 mL of PBS twice. Adherent cells were lysed with 1 mL of 1 mol/L NaOH. Each wash was collected, isolated, and counted for activity. For each plate, 2 wells were reserved for cell counting in order to normalize uptake per cell. This experiment was conducted in both charcoal-stripped media and full serum.

Neutral comet assay
LNCaP and LNCaP-AR cells were grown in described conditions above for 2 days, and the neutral comet assay was performed using CometAssay Electrophoresis System (CometAssay 2 Well ES Unit w/ Starter Kit and Power Supply: #4250-050-ESK-PS1) per assay protocol.

Immunofluorescence assays
LNCaP and LNCaP-AR cells were grown as described above in parallel for 24 hours on 4-chamber slides (Thermo Scientific, Lab-Tek II Chamber Slide w/cover CC2 Glass slide sterile, 154917), approximately 125,000 cells/well in 500 μL total volume. Following radiotherapy using the cell irradiator, cells were washed twice with PBS and fixed with 4% PFA and 0.2% Triton X-100. The primary antibody for AR [Santa Cruz; AR (N-20), sc-816, 1:200 dilution] or gamma-H2Ax (Millipore; Anti-phospho-Histone H2Ax (ser139), clone JBW301, 05-636, 1:200 dilution) incubated overnight at 4 degrees, and then washed followed by incubation with the secondary antibody for AR (Vector Laboratories; Dylight 594, D1-1594, 1:100 dilution) or gamma-H2Ax (Invitrogen; Alexa Fluor 488, A11001, 1:500 dilution) for 1 hour at room temperature, and stained for DAPI. Confocal microscopy (LSM 5 LIVE) with a 20 x/0.8 NA objective and focus was counted using Metamorph image analysis software (Molecular Devices). An average of 1,000 distinct nuclei was counted per time point.

Clonogenic assay
LNCaP, LNCaP-AR, and CWR22Pc cells were grown in 6-well, tissue-culture–treated polystyrene plates (BD Falcon) in a series of serial dilutions (24,000, 12,000, 3,000, 1,000, 333, and 111 cells per well) at each dose. Cells received either 0, 2, 4, or 6 Gy of radiotherapy. Plates were incubated for 2 weeks, then washed and fixed with methanol, and stained with 0.2% crystal violet (Sigma) in 10% formalin (Sigma). Plates were scanned and counted by GelCount (Oxford Optronix) and its accompanying software (5).

Xenografts
All animal studies were conducted in compliance with the Research Animal Resource Center guidelines at our institution. Approximately 5-week-old male CB-17 SCID mice were obtained from Taconic Farms. A total of 2 x 10^6 LNCaP-AR cells were injected subcutaneously into the one (or both) flanks of intact male mice in a 1:1 mixture by volume of Matrigel and media. Experiments were initiated once tumors were palpable, and tumor volume measurements were estimated by hand caliper measurements in three dimensions. Tumors were harvested and analyzed for protein, mRNA, immunofluorescence, or immunohistochemistry.

Bioluminescence in vivo assay
AR function was determined in vivo by measuring luciferase activity of human LNCaP-AR xenografts grown in male mice. These tumors coexpressed exogenous AR and the AR-dependent reporter construct ARR2-Ph-Lac. d-Luciferin (Perkin Elmer) was dissolved in PBS to 15 mg/mL. Mice were injected with 200 μL (3 mg) via intraperitoneal injection. Following injection, mice were placed under anesthesia with a mixture of 2.5% isoflurane and oxygen for 5 minutes. The mice were images using the IVIS Spectrum for the duration of 30 seconds. Images were taken at specified time points before and after radiotherapy.

Patient serum analysis
Patient serum was collected at the Mayo clinic on an Institutional Review Board–approved study of prospective biomarker collection. Patients enrolled were almost exclusively low and intermediate risk by NCCN criteria. Most patients were treated with radiotherapy as monotherapy. No patients underwent a
radical prostatectomy. Serum specimens were collected, analyzed, and stored at baseline before the initiation of radiotherapy and at the first follow-up visit after treatment (3–7 months after radiotherapy). Both serum PSA and free PSA were measured utilizing the Hybritech assays on an Access analyzer (Beckman Coulter, Inc). Free-hK2 levels were measured utilizing a selective pair of monoclonal antibodies. The assay was implemented on the Access analyzer, and the cross-reactivity with PSA is negligible as previously described (11). The hK2 limit of detection was 1.5 pg/mL, and the day-to-day coefficient of variation set at <15% was <4 pg/mL.

Statistical analyses
All qPCR analyses, IHC comparisons, and comet assay comparisons were performed with a two-sided t test. Correlation statistics were performed using R² statistics. In vivo tumor growth comparisons were estimated by actuarial likelihood estimates using the inverse Kaplan–Meier method with log-rank statistics to obtain cumulative incidence rates. Statistical analysis was performed using SPSS version 21 (SPSS, Inc.). Patient data statistics were conducted using logistical regression to compare patients with and without a rise in hK2 post-radiotherapy, reported as an OR and 95% confidence interval (CI). Kaplan–Meier time point comparisons (72 months) were performed with a c² test. Baseline patient group comparison between those with and without hK2 gain was performed using the Fisher exact test or Mantel–Haenszel c² test for ordinal and categorical variables and the Wilcoxon–Mann–Whitney test for continuous variables. For all analyses, two-sided P values of ≤ 0.05 were considered statistically significant.

Results
We first tested whether radiotherapy had direct biologic effects on AR regulation and expression. Four human prostate cancer cell lines (LNCaP, LNCaP-AR, MDA-PCa2b, and CWR22Pc) showed a dose-dependent increase in AR mRNA after exposure to 1, 6, and 12 Gy at 24 hours after radiotherapy (Fig. 1A). AR protein levels were also upregulated in three (LNCaP, LNCaP-AR, and CWR22Pc) of the four cell lines at the same time points (Fig. 1B). AR nuclear protein translocation was also enhanced by radiotherapy, as immunofluorescence showed that the overall percentage of nuclear AR, measured 24 hours after radiotherapy, was 25%–45% fold higher compared with vehicle control (Supplementary Fig. S1A). To provide further evidence of AR protein upregulation, we used a ligand binding assay with 18F-labeled dihydrotestosterone (18F-FDHT; Supplementary Fig. S1B). When 18F-FDHT was added to LNCaP cells plated in androgen-free media and irradiated with increasing doses of radiotherapy, even at 1 Gy, there was a significant increase in uptake of 18F-FDHT. This increase is consistent with increased AR protein expression and/or available ligand binding sites, and was abrogated when the experiment was performed in androgen-replete serum (due to competition of unlabeled ligand with 18F-FDHT).

To test if radiotherapy-induced AR upregulation occurs in vivo, subcutaneous LNCaP-AR tumors were established in mice and treated with 10 Gy of conformal external beam radiotherapy, and

Figure 1.
Radiotherapy induces increased expression of the AR. A and B, LNCaP, LNCaP-AR, MDA-PCa2b, and CWR22Pc cell lines were treated with either 0, 1, 6, or 12 Gy or EBRT and the cells harvested for mRNA measured by qPCR (A) and protein by Western blot (B). C and D, LNCaP-AR-derived xenografts were treated with 10 Gy of conformal EBRT and compared with nonirradiated controls, and mRNA for AR was measured by qPCR (C) and protein by Western blot (D). E, LNCaP-AR xenografts were treated with 10 Gy of EBRT and harvested 5 days after treatment, fixed and formalin and paraffin embedded, and stained by IHC and immunofluorescence for AR. Significance level indicated by *, P < 0.05, or P value listed in figure. RT, radiotherapy.
mRNA after EBRT (Fig. 3A). Of note, the magnitude of AR line panel, LNCaP and LNCaP-AR, and with CWR22Pc, which has fl
whether these differences correlated with tumor cell survival. We performed serial imaging 1, 3, 5, and 7 days after EBRT. This approach demonstrated increases in AR output over baseline at any time (Dmax of 65 Gy), thereby serving as a control. Five days after radiotherapy, the tumors were harvested, paraffin-embedded, sectioned, and assessed for AR expression analysis by immunohistochemistry and immunofluorescence (Fig. 1E). To quantify the change in AR intensity, the immunofluorescence slides were digitized, random areas of tumor were captured, and approximately 600 cells in the control and radiotherapy group were quantified via staining intensity by standard software computation. Mean AR expression was increased compared with nonirradiated tumors (P < 0.0001), but with considerable heterogeneity, suggesting that a subgroup of cells may be "primed" to respond to radiotherapy in this manner (Fig. 1E). Collectively, these results show that AR mRNA and protein are acutely upregulated after EBRT within 24 hours and are maintained in a heterogeneous manner even after a single fraction of EBRT.

Having demonstrated that radiotherapy leads to increased AR expression and nuclear localization in vitro and in vivo, we asked if downstream AR transcriptional output was altered. Indeed, LNCaP-AR cells treated with increasing dose of radiotherapy (0, 1, 6, and 12 Gy) EBRT and harvested 24 hours after treatment showed increased expression of established canonical AR-target genes (KL2K, KL3K, TMPRSS2; Fig. 2A).

TMPRSS2, KL3K, and KL2K were also upregulated in LNCaP-AR xenografts during the first week after treatment with 10 Gy of conformal EBRT, but this induction was not observed in all mice and the magnitude of induction varied by the target gene analyzed. KL2K was the most consistently upregulated of the tested target genes (95% of mice experienced an upregulation over untreated controls), whereas PSA demonstrated an increase in less than half of the mice (47%) and TMPRSS2 was intermediate (73%; Fig. 2B). Because kinetics of AR induction in this model is variable during the first week after radiotherapy, we used the androgen responsive ARR-Pb-luciferase reporter system in the LNCaP-AR model to perform serial imaging 1, 3, 5, and 7 days after EBRT. This approach to measure AR pathway activation also revealed heterogeneity in AR induction across the cohort, with 19 of the 25 mice (76%) demonstrating increases in AR output over baseline at any time during the first week after radiotherapy (Fig. 2C). Minor stochastic mutational changes that arise during tumor growth may manifest as larger changes in radiosensitivity as well as AR output.

Given the variable range of AR upregulation after EBRT (either among cell lines in vitro or among tumors in vivo), we next asked whether these differences correlated with tumor cell survival. We first performed a clonogenic survival assay with the isogenic cell line panel, LNCaP and LNCaP-AR, and with CWR22Pc, which has relatively low baseline AR expression but large induction of AR mRNA after EBRT (Fig. 3A). Of note, the magnitude of AR upregulation was more highly correlated with the surviving fraction (P < 0.005, Fig. 3B) than the baseline AR level. This finding is consistent with more direct assessments of radiotherapy-induced DNA damage by neutral comet assay (Fig. 3C) and by gamma-H2AX induction by immunofluorescence (Fig. 3D) or Western blot (Supplementary Fig. S2A), all of which show more rapid resolution of DNA damage in LNCaP-AR compared with parental LNCaP.

To study the impact of AR induction on survival in vivo, we treated a cohort of mice bearing subcutaneous LNCaP-AR xenografts with 10 Gy of EBRT. We tracked AR induction by bioluminescence (as in Fig. 2C) and monitored tumor volume changes over 4 weeks (Fig. 3E). The maximum percent change in bioluminescence during the first week after radiotherapy was significantly correlated with time to tumor progression (R² 0.77, P < 0.0001). Furthermore, time to progression in tumors with the largest increase in AR output after EBRT (>50%) was significantly shorter than those with a modest increase (0%–50%) or a decline in AR output (P > 0.001, Fig. 3F). Of note, pretreatment tumor bioluminescence signal did not correlate with time to tumor progression (Supplementary Fig. S2B), consistent with the results from our in vitro data showing that post-radiotherapy AR induction was more highly correlated with radioreistance than baseline AR levels (Fig. 3D).

Lastly, we asked if there is any clinical evidence of post-radiotherapy changes in AR output and whether these changes correlate with clinical outcome in a cohort of 227 men with predominantly low- and intermediate-risk prostate cancer who were enrolled in a prospective trial to collect baseline and serial serum free-hK2 (KL2K) measurements after radiotherapy. All of these men had evaluable baseline and post-radiotherapy samples collected within 6 months of radiotherapy. No men in the trial received adjuvant ADT, and there was no difference in use of concurrent ADT between groups (P = 0.861). Of these 227 men, 40 had an increase in hK2 after treatment (defined as a rise above the pre-radiotherapy treatment value within 6 months of radiotherapy; Fig. 4A and Table 1) versus 187 who had no increase over baseline. These two groups were well balanced in regard to T-stage, Gleason score, and age. However, the 40 men with a rise in hK2 after EBRT showed trends of lower baseline total PSA, free PSA, and Free-hK2, which would be expected to portend lower recurrence rates. In spite of these baseline differences that are associated with a favorable prognosis, the men with increased free-hK2 levels after EBRT experienced a greater than 3-fold increase in biochemical failure (median time from radiotherapy to biochemical failure of 32.9 months IQR, 17.4–48.2). Specifically, patients with an hK2 gain versus those with no hK2 gain had biochemical failure rates of 17.5% versus 5.3%, respectively (OR, 3.39; 95%CI, 1.23–9.39; P = 0.019; Fig. 4B). Furthermore, at 72 months after treatment, the patients without an hK2 gain had significantly higher rates of freedom from PSA progression than those with an hK2 gain (94.2% vs. 83.5%, P = 0.027; Fig. 4C). Importantly, hK2 gain was defined very conservatively as it was not normalized to tumor shrinkage after treatment. Because a considerable fraction of prostate cancer cells die during radiotherapy, one would expect overall serum hK2 levels would decline after radiotherapy. Thus, an absolute rise in hK2 after therapy could represent a large increase in hK2 protein expression on a per cell basis.

Discussion
For decades the understanding of why the addition of ADT to radiotherapy improves survival has remained elusive (12–14).
This question was further complicated by the knowledge that the addtion of ADT to radical prostatectomy failed to improve survival (15). The recent discovery that ADT inhibits nonhomologous end joining, a critical DNA repair process, provides a compelling answer to years of observed clinical trial outcomes (5–7). However, the new found clarity on this topic has subsequently created new questions. For instance, multiple randomized trials have demonstrated that the use of adjuvant ADT after combined ADT/radiotherapy also improves survival, but by unknown mechanisms (1,14). In contrast, a recently reported phase III trial tested whether an increase in the duration of ADT prior to the start of radiotherapy would improve outcome, but failed to demonstrate a benefit for prolonged neoadjuvant ADT (3). These results suggest the timing of ADT in relationship to radiotherapy is perhaps more critical than simply the duration of use.

In the present report, we address the related issue of adjuvant ADT and provide mechanistic insight into why this clinical practice may be beneficial. Specifically, we show that radiotherapy induces upregulation of AR expression and activity across a panel of human prostate cancer cell lines, and the magnitude of this upregulation is more strongly correlated with increased viability in a clonogenic survival assay than baseline AR expression. This association was confirmed in xenograft experiments, where LNCaP-AR tumors with the greatest percent increase in AR signaling after radiotherapy showed more rapid time to progression. These preclinical findings appear to be relevant in patients,

Figure 2.
AR transcriptional output is increased following radiotherapy. A, LNCaP-AR in vitro qPCR assessment of AR target genes PSA, TMPRSS2, and KLK2 mRNA expression after EBRT (0, 1, 6, 12 Gy). B, LNCaP-AR xenografts irradiated with 10 Gy and harvested over the first week after radiotherapy and mRNA by qPCR was analyzed for the AR target genes TMPRSS2, PSA, and KLK2. C, bioluminescence assay of 25 SCID mice with LNCaP-AR xenografts imaged at baseline and then treated with 10 Gy of conformal EBRT. Subsequent imaging performed during the first week after radiotherapy, and the max increase in bioluminescence was recorded. The bottom plot demonstrates the acute and persistent increase in AR output measured by bioluminescence from mouse #1 in the companion graph. Significance level indicated by *, P < 0.05, or P value listed in figure.
because we found that men experiencing an increase in serum hK2 levels after radiotherapy were 3 times more likely to experience a biochemical failure than those with unchanged or declining hK2 after treatment. Finally, we demonstrate that baseline AR and AR output do not correlate with tumor response in vitro, in vivo, or in our patient serum data of hK2 levels.

Prior studies have reported that approximately 20% of men have increases in PSA during EBRT treatment (without the use of ADT), whereas the remainder had negatively sloping PSA declines (9). These results have previously been ignored due to the absence of evidence for clinical significance of inferior treatment outcomes (16). This clinical heterogeneity
is also seen in our preclinical studies. One hypothesis that might explain the heterogeneity is variable amounts of hypoxia present in tumors, particularly since ADT has been shown to reduce prostate cancer hypoxia, suggesting an interplay with AR signaling (17, 18). Our results suggest that AR activity during and after ADT/EBRT should be more closely studied with serum and imaging biomarkers to determine their prognostic significance. One potential implication is that adjuvant ADT may only be necessary for those men whose tumors upregulate AR as a response to radiotherapy. Alternatively, more potent AR inhibition using second-generation ADT might prevent or mitigate the negative consequences of AR upregulation after radiotherapy.

The underlying biologic mechanism by which EBRT upregulates AR expression remains to be defined. Because AR mRNA levels are elevated in a dose-dependent manner by EBRT, it is possible that a transcription factor that is sensitive to genotoxic stress elevates AR transcription. Potential candidates include Ku70 and Ku80, NF-κB, and the STAT family (19–21). Ku is particularly promising given that the Ku70/Ku80 heterodimer recruits DNA-PKcs to double DNA strand breaks, and it has been demonstrated that both Ku70 and Ku80 directly interact with the ligand binding domain of the AR (21, 22). In addition, the heterogeneity in magnitude and kinetics of radiotherapy-induced AR upregulation seen across in vitro and in vivo experiments is consistent with a stochastic variable that affects radiotherapy response or a preexisting subset of tumor cells primed to respond to radiotherapy in this manner as an adaptive resistance mechanism. These critical points are areas for future investigation.

**Disclosure of Potential Conflicts of Interest**

B.J. Davis reports receiving commercial research grant from Takeda UK LLC; has received speakers bureau honoraria from ASTRO, DMCI Inc., and Prospect Medical Inc.; has ownership interest (including patents) in Pfizer; and is a consultant/advisory board member for EDAP Technomed Inc. J. Wongvipat has ownership interest (including patents) in patents for Enzalutamide/MDV3100/Xtandi. G.G. Klee reports receiving commercial research support from and has ownership interest (including patents) in Beckman Coulter. W. Polkinghorn is CEO at and has ownership interest (including patents) in Driver Group. D.J. Tindall reports receiving commercial research grant from Beckman Coulter. C.L. Sawyers has provided expert testimony for co-inventor of MDV3100 (enzalutamide) and is entitled to royalties. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Table 1. Baseline characteristics of patients by hK2 gain status

<table>
<thead>
<tr>
<th></th>
<th>Total (N=227)</th>
<th>No hK2 increase (N=187)</th>
<th>hK2 increase (N=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.0000</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (0.4%)</td>
<td>1 (0.5%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>225 (99.6%)</td>
<td>185 (99.5%)</td>
<td>40 (100.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age at baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>227</td>
<td>187</td>
<td>40</td>
<td>0.6933</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>68.5 (7.0)</td>
<td>68.6 (6.8)</td>
<td>67.9 (7.7)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>70.0</td>
<td>69.0</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>64.0, 74.0</td>
<td>64.0, 74.0</td>
<td>64.0, 75.0</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline serum PSA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>227</td>
<td>187</td>
<td>40</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>6.2 (6.6)</td>
<td>6.7 (6.8)</td>
<td>3.7 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.0</td>
<td>6.0</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>1.7, 8.8</td>
<td>2.7, 9.1</td>
<td>0.2, 4.8</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline serum-free PSA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>227</td>
<td>187</td>
<td>40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.9 (0.9)</td>
<td>0.9 (0.8)</td>
<td>0.6 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>0.2, 1.2</td>
<td>0.4, 1.3</td>
<td>0.3, 0.7</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline serum-free hK2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>227</td>
<td>187</td>
<td>40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>56.3 (76.7)</td>
<td>65.2 (81.5)</td>
<td>14.4 (16.4)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>49.0</td>
<td>49.0</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Q1, Q3</td>
<td>13.0, 74.0</td>
<td>22.0, 79.0</td>
<td>3.9, 19.0</td>
<td></td>
</tr>
<tr>
<td>Clinical T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.0000</td>
</tr>
<tr>
<td>T1</td>
<td>134 (59.3%)</td>
<td>110 (59.1%)</td>
<td>24 (60.0%)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>88 (38.9%)</td>
<td>73 (39.2%)</td>
<td>15 (37.5%)</td>
<td></td>
</tr>
<tr>
<td>T3/T4</td>
<td>4 (1.8%)</td>
<td>5 (1.6%)</td>
<td>1 (2.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Gleason group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>148 (65.2%)</td>
<td>122 (65.2%)</td>
<td>26 (65.0%)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>70 (30.8%)</td>
<td>58 (31.0%)</td>
<td>12 (30.0%)</td>
<td></td>
</tr>
<tr>
<td>≥8</td>
<td>9 (4.0%)</td>
<td>7 (3.7%)</td>
<td>2 (5.0%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Fisher exact test.  
<sup>b</sup>Wilcoxon–Mann–Whitney test.  
<sup>c</sup>Mantel–Haenszel $\chi^2$ test.

Grant Support

This study was supported by The Molecular Pharmacology & Chemistry Program Core (J.S. Lewis), The Radiological Society of North America 2013 Research Resident Grant #BR1150 (D.E. Spratt), the 2014 Rebecca and Nathan Milikowsky Prostate Cancer Foundation Young Investigator Award (D.E. Spratt), David H. Koch Young Investigator Award from the Prostate Cancer Foundation (M.J. Evans), the 2012 DARAS MSKCC institutional grant (D.E. Spratt and M.J. Evans), NIH P50CA091956 MAYO CLINIC SPORE (D.J. Tindall, G.G. Klee, and B.J. Davis), grant from the T.J. Martell Foundation (D.J. Tindall), NIH P50CA09262 MSKCC SPORE and the NIH P50CA091956 (C.L. Sawyers), and the Howard Hughes Medical Institute (C.L. Sawyers). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 6, 2015; revised July 22, 2015; accepted August 11, 2015; published OnlineFirst October 2, 2015.

www.aacjrournals.org

References


Acknowledgments

The authors thank the Radiochemistry & Molecular Imaging Probe Core of MSKCC (P30 CA008748) for supply of the 18F-FDHT and the MSKCC ICMIC. They also thank both the MSKCC Prostate SPORE NIH P50CA09262, the Mayo Prostate SPORE NIH P50CA091956, and the Howard Hughes Medical Institute. D.E. Spratt and M.J. Evans are funded by Rebecca and Nathan Milikowsky and David H. Koch Young Investigator Awards from the Prostate Cancer Foundation, respectively, and the authors thank them for their support.

Writing, review, and/or revision of the manuscript: D.E. Spratt, M.J. Evans, B.J. Davis, M.G. Doran, N. Shah, K.E. Camazza, G.G. Klee, D.J. Tindall, J.S. Lewis, C.L. Sawyers

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.E. Spratt, B.J. Davis, M.G. Doran, M.X. Lee, G.G. Klee

Study supervision: B.J. Davis, J.S. Lewis, C.L. Sawyers

AR Upregulation after Radiotherapy Mediates Radioresistance
Androgen Receptor Upregulation Mediates Radioresistance after Ionizing Radiation

Daniel E. Spratt, Michael J. Evans, Brian J. Davis, et al.

Cancer Res 2015;75:4688-4696. Published OnlineFirst October 2, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-0892

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2015/10/02/0008-5472.CAN-15-0892.DC1

Cited articles
This article cites 21 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/22/4688.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/75/22/4688.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.