World Journal of Urology
https://doi.org/10.1007/s00345-018-2382-8

ORIGINAL ARTICLE

The BET-inhibitor PFI-1 diminishes AR/AR-V7 signaling in prostate cancer cells

Marie C. Hupe[1] · M. Raschid Hoda[1] · Friedemann Zengerling[2] · Sven Perner[3] · Axel S. Merseburger[1] · Marcus V. Cronauer[1]

Received: 19 April 2018 / Accepted: 16 June 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Objective The bromodomain and extra-terminal (BET) family of proteins provides a scaffolding platform for the recruitment and tethering of transcription factors to acetylated chromatin, thereby modulating gene expression. In this study, we evaluated the efficacy of the BET-inhibitor PFI-1 to diminish AR/AR-V7 signaling and proliferation in castration-resistant prostate cancer cells.

Methods Prostate-specific antigen and androgen receptor (AR) protein were quantified by means of two commercial ELISAs. Transactivation of the AR, AR-V7 and Q641X was determined by reporter gene assays. Cell proliferation was measured using a colorimetric MTT-assay.

Results PFI-1 dose-dependently inhibited transactivation of full-length AR (non- mutated, i.e., wild-type or point-mutated/promiscuous forms) without affecting their cellular protein levels. Moreover, PFI-1 was active against C-terminally truncated constitutively active ARs like AR-V7 and Q641X. Prostate cancer cells exhibiting a transcriptionally active AR-signaling complex (LNCaP, 22Rv1) were more susceptible to the growth-inhibitory effects than the AR-negative PC-3 cells.

Conclusion The quinazolinone PFI-1 is a highly efficient inhibitor of AR-signaling-competent prostate cancer cells in vitro. PFI-1 could serve as a lead compound for the development of new therapeutics able to block AR/AR-V7 signaling in advanced prostate cancer.

Keywords BET inhibitor · BRD4 · Androgen receptor · AR-V7

Marie C. Hupe and M. Raschid Hoda equally contributed to this paper.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00345-018-2382-8) contains supplementary material, which is available to authorized users.

✉ Marcus V. Cronauer
marcus.cronauer@uksh.de

1 Department of Urology, University Hospital Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

2 Department of Urology, University of Ulm, 89075 Ulm, Germany

3 Pathology of the University Hospital Schleswig-Holstein, Campus Lübeck and Research Center Borstel, Leibniz Center for Medicine and Biosciences, Borstel, Germany

Introduction

Treatment of castration-resistant prostate cancer (CRPC) has dramatically changed over the past decade with the approval of new endocrine treatments such as abiraterone, enzalutamide or apalutamide (ARN-509, approval US only). Unfortunately, not all patients respond to second-generation therapies and neither abiraterone nor enzalutamide/apalutamide is curative. One mechanism enabling CRPC cells to escape the effects of endocrine treatments is the synthesis of constitutively active C-terminally truncated androgen receptor (AR) molecules lacking the entire or vast parts of their ligand/hormone binding domain (LBD) (Fig. 1). These AR forms, termed hereafter ARΔLBDs, are almost exclusively products of alternative splicing (AR-Vs); however, ARΔLBDs originating from some rare nonsense mutations have been described (Q641X, Q784*) [1–3]. Devoid of a LBD, all ARΔLBDs are unable to bind and respond to androgens or antiandrogens. With the emergence of ARΔLBDs in late

Published online: 22 June 2018

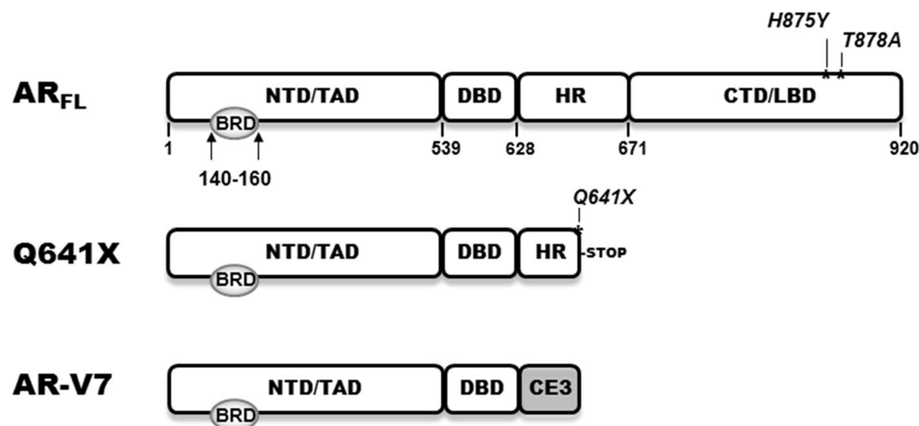

Fig. 1 *Androgen receptor variants in prostate cancer*: AR_{FL}, full-length AR; Q641X, truncated AR nonsense mutant; AR-V7, most prevalent androgen receptor splice variant. *Functional regions of the AR*: NTD/TAD, N-terminal domain/transactivation domain; DBD, DNA binding domain; HR, hinge region; CTD/LBD, C-terminal domain/ligand binding domain; CE3, AR-V7 specific C-terminus encoded by cryptic exon 3; BRD, BET protein binding to the AR-N-terminus between amino acids (aa) 140–160. *H875Y, *T878A, point mutations in the AR-LBD of 22Rv1 and LNCaP, respectively; *Q641X, nonsense mutation leading to stop a codon in the HR

stage prostate cancer (PCa), conventional endocrine therapies targeting androgen synthesis and/or androgen binding are prone to fail. To overcome the limitations of current endocrine therapies, there is an urgent need to identify new additional targets within the AR/AR-V-signaling network. There is experimental evidence that members of the bromodomain and extra-terminal (BET) family of proteins, the bromodomain-containing proteins (BRD), might be such targets [4, 5]. All members of the mammalian BRD-family, namely BRD2, BRD3, BRD4 and the testis-specific BRDT, share a common functional architecture comprising evolutionarily highly conserved protein modules such as two tandem bromodomains (BD1 and BD2) and an extra-terminal (ET) protein–protein interaction domain [6]. BRD-proteins recognize acetylated lysine residues in histones and other proteins by means of their BDs; the ET domain fulfills a regulatory function by recruiting specific effector proteins [7]. In summary, BRD-proteins are chromatin-binding adaptors that provide a tissue-specific scaffolding platform for the recruitment and tethering of active transcriptional complexes to acetylated histones and chromatin, and thus they control the expression of lineage-specific genes that are linked to cell cycle progression [6, 8]. Consistently, BRD-proteins were shown to modulate AR and c-MYC transcriptional activity in PCa cells [4, 9]. Although BRD2, 3 and 4 can physically interact with the AR, there is evidence that BRD4 is the preferred AR-interaction partner in PCa cells [4]. In line with these findings, a BRD-binding site was recently mapped to amino acids 140–160 of the amino-terminal/N-terminal domain of the AR (NTD, Fig. 1) [4]. As the NTD is essential for AR- and ARΔLBD transcriptional activity, inhibition of BRD-proteins presents a formidable opportunity to inhibit signaling of full-length AR (wild-type or point-mutated promiscuous forms) and ARΔLBDs (splice variants and nonsense mutants) (Fig. 1). In consequence, we analyzed the effects of the BRD2/BRD4-inhibitor PFI-1, a novel, highly selective acetyl-lysine mimetic [10, 11] on proliferation and transcriptional activity of AR, AR-V7 and Q641X in human PCa cell lines.

Materials and methods

Chemicals and plasmids

The BET-inhibitor PFI-1 targeting BRD4/BRD2 was purchased from Selleck Chemicals, Biozol, Eching, Germany. Expression constructs for wild-type AR, the AR splice variant AR-V7 and the AR nonsense mutant Q641X (formerly Q640X [1, 2]) were generous gifts from Dr. Jocelyn Céraline, Université de Strasbourg, Strasbourg, France. The reporter gene plasmid pARE2x-luc for the detection of AR/ARΔLBD-activity was provided by Prof. Zoran Culig, Innsbruck, Austria. All other chemicals/compounds, if not specified, were products of Sigma-Aldrich GmbH, Taufkirchen, Germany.

Cell culture

PC-3, 22Rv1 and LNCaP cells were purchased from the American Type Culture Collection, Manassas, VA, USA. Cells were routinely grown in RPMI-1640, 10% fetal bovine serum (FBS) and antibiotics (Gibco, Thermo Fischer Scientific, Dreieich, Germany) [12]. Steroid-free FBS used in reporter gene assays was provided by BioWest, Nuaillé, France.

Enzyme-linked immunosorbent assays for AR and PSA

Expression of full-length AR proteins was measured using the PathScan® Total Androgen Receptor Sandwich ELISA (Cell Signaling Technology, New England Biolabs GmbH, Frankfurt a.M., Germany). Prostate-specific antigen (PSA) levels in the cell culture supernatant of LNCaP cells were determined by means of a human kallikrein 3/PSA Quantikine® ELISA Kit, (Bio-Techne GmbH, Wiesbaden, Germany). Both assays were performed according to the manufacturer's instructions.

Luciferase reporter gene assay

Reporter gene assays with plasmids coding for AR, AR-V7 and Q641X were performed as recently described [13, 14]. Luciferase reporter gene activities were measured on a TriStar² S LB 942 luminometer (Berthold Technologies) using a Dual-Luciferase Reporter Assay (Promega GmbH, Mannheim, Germany).

Proliferation assay

Cell proliferation of PCa cells was determined by means of a colorimetric MTT-assay as originally described by Mosmann et al. [15].

Results

The BET-inhibitor PFI-1 diminishes ligand-activated signaling of full-length AR in prostate cancer cells

As compared to untreated controls, PFI-1 diminished the levels of PSA secreted into the supernatant of LNCaP cells already after 24 h. The decrease of PSA was significant at a PFI-1 concentration of 10 µM (PSA decrease 69%, $p < 0.003$). PFI-1 concentrations > 20 µM did not further decrease the PSA-levels (data not shown). The ability of PFI-1 to impair AR transcriptional activity was furthermore confirmed in androgen-stimulated 22Rv1 cells transfected with a reporter gene construct (Fig. 1S). Downregulation of PSA following PFI-1 treatment was not paralleled by a reduction of AR protein in LNCaP cells (Fig. 2). Interestingly, 5 µM PFI-1 induced a statistically significant increase of AR levels as compared to PFI-1 untreated controls (increase 43%, $p < 0.05$). By contrast, PFI-1 concentrations ≥ 10 µM were unable to significantly reduce/increase AR levels (expressed in % untreated controls ± sdev (SD): 10 µM PFI-1, 93 ± 7%, $p > 0.05$; 20 µM PFI-1, 91 ± 10%, $p > 0.05$).

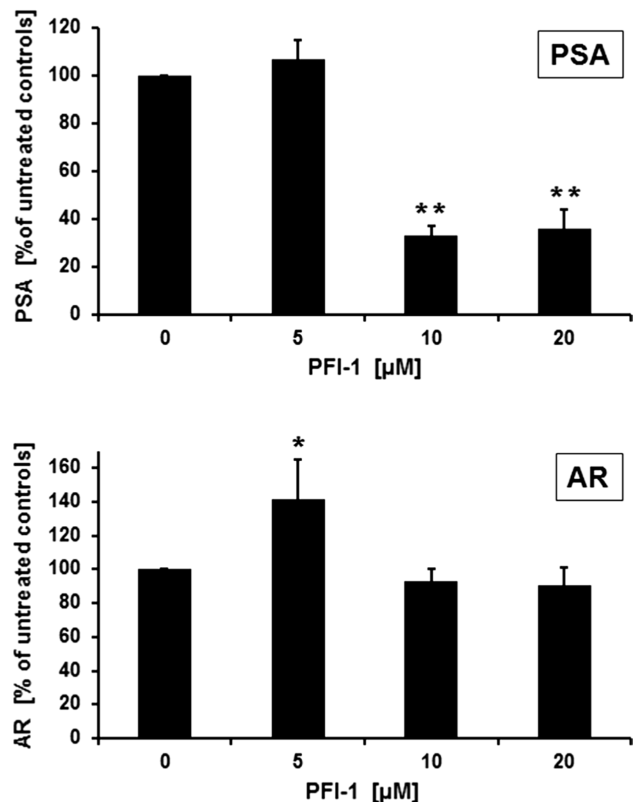

Fig. 2 *Effects of PFI-1 on PSA-protein expression (top)*: prostate-specific antigen secreted into the supernatant of LNCaP cells stimulated with 10 nM DHT for 24 h. Data represent one representative experiment of four run in triplicates. Results are expressed in % of PFI-1 untreated controls (100%) ± standard deviation (SD); $^{**}p < 0.05$. *Effects of PFI-1 on AR-protein levels (bottom)*: AR measured in LNCaP cell lysates grown in the presence of physiological levels of androgens. Data represent the mean of six independent experiments run in triplicate. Results are expressed as % of PFI-1 untreated controls (100%) ± SD, $^{*}p < 0.03$

PFI-1 impairs signaling of wild-type AR, AR-V7 and Q641X in prostate cancer cells

The ability of PFI-1 to inhibit constitutive transcriptional activity of ARΔLBDs was tested in PC-3 cells transfected with AR-V7 and Q641X using a reporter gene assay. PC-3 cells transfected with a wild-type AR were subsequently treated with PFI-1 and DHT (10 nM) and served as controls. As seen in Fig. 3, PFI-1 dose-dependently diminished the transcriptional activities of AR, AR-V7 and Q641X. The constitutive activity of AR-V7 and Q641X was more affected by PFI-1 than the androgen-induced activity of the wild-type AR. Transactivation of AR-V7 and Q641X was already significantly inhibited by 23 and 58% at PFI-1 concentrations of 2.5 and 5 µM, respectively

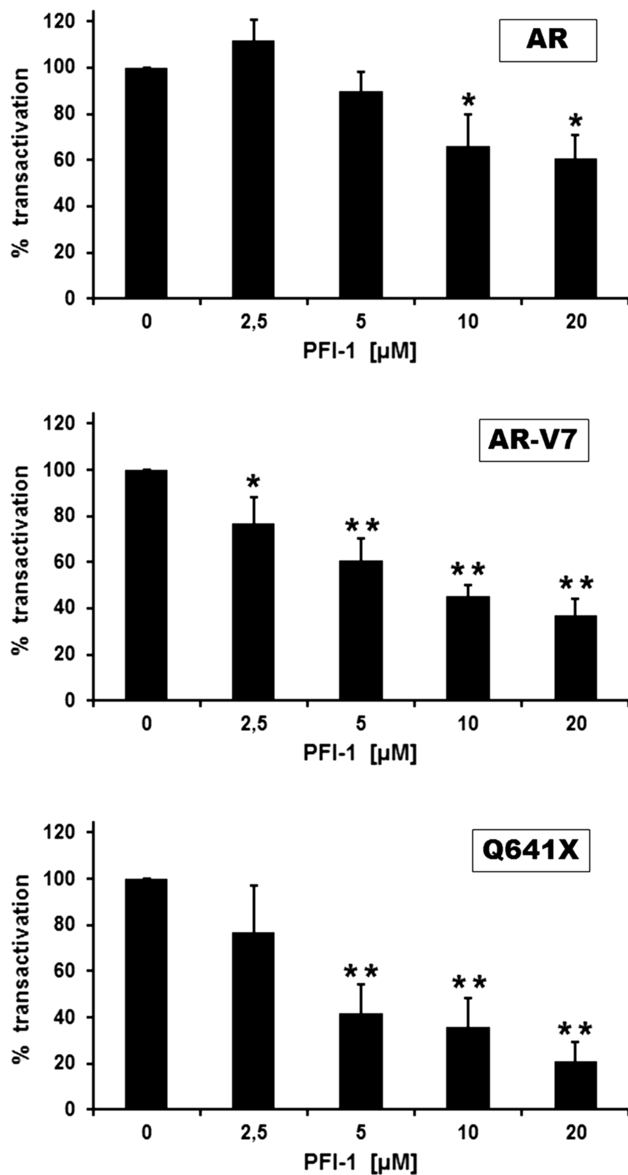

Fig. 3 *Effects of PFI-1 on AR and ARΔLBD signaling*: Plasmids coding for wild-type AR (AR), AR-V7 or Q641X (nonsense mutant) were co-transfected with the reporter gene constructs pARE2X-luc (AR activity) and pRL-TK (transfection efficiency) in PC-3 cells. Cells transfected with AR-V7 or Q641X were grown in the absence of androgens with increasing concentrations of PFI-1, and cells harboring the wild-type AR were additionally stimulated with 10 nM DHT for 24 h. Data represent one representative experiment of four run in triplicates. Results are expressed in % of PFI-1 untreated controls (100%) ± SD. AR (top): $*p<0.02$; AR-V7 (middle): $*p<0.02$, $**p<0.004$; Q641X (bottom): $**p<0.004$

($p<0.004$, and $p<0.02$). PFI-1 concentrations ≥ 10 µM reduced the transactivation of all AR types (reduction of transcriptional activities: ARwt: 34% at 10 µM, 39% at 20 µM, $p<0.02$; Q641X: 64% at 10 µM, 79% at 20 µM, $p<0004$ and AR-V7: 55 and 63% at 10 and 20 µM, $p<0.004$).

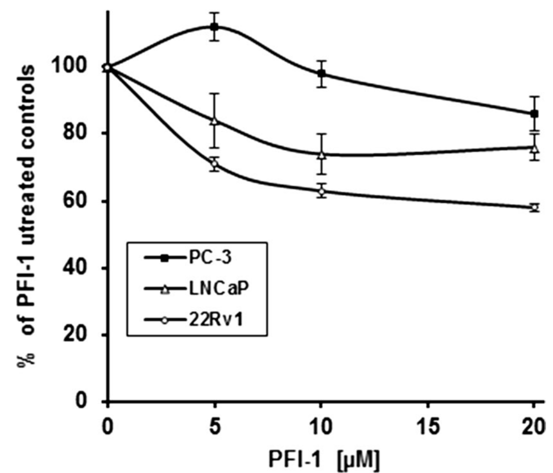

Fig. 4 LNCaP (AR+), 22Rv1 (AR+, AR-V7+) and PC-3 (AR−) were grown for 24 h in the presence or absence of increasing concentrations of PFI-1 (0–20 µM). Cell proliferation was measured by means of an MTT-assay. The results are expressed in % of untreated controls set at 100%

Anti-proliferative effects of PF-1 are more pronounced in AR-positive prostate cancer cells

The fact that PFI-1 suppressed the transactivation of all AR forms prompted us to analyze its effects on the proliferation of LNCaP (AR+), 22Rv1 (AR+ , AR-V7+) and PC-3 (AR−) grown in the presence of physiological levels of androgens. Cell proliferation was determined by means of a colorimetric MTT-assay after 24 h. As shown in Fig. 4, LNCaP and 22Rv1 cells were more susceptible to the inhibitory effects of PFI-1 than PC-3 cells, suggesting that the anti-proliferative properties of PFI-1 are linked to its inhibitory effects on the AR transcriptional machinery. A statistically significant increase of cell death was furthermore confirmed in LNCaP and 22Rv1 cells using trypan blue dye exclusion assay (Fig. 2S).

Discussion

As prostate cancer depends on androgens for growth and survival, androgen depletion and blockade of androgen binding to its cognate receptor have been the mainstay of hormonal treatment for over six decades [16]. Unfortunately, the benefits of endocrine therapies are only transitory and the disease almost invariably recurs as CRPC, characterized by an intra-tumorous reactivation of AR signaling despite castrate levels of circulating testosterone. The molecular mechanisms leading to aberrant AR activity in CRPC cells include AR amplification/overexpression, gain of function mutants broadening AR ligand specificity, activation or enhancement of AR signaling by growth factors and

cytokines, intra-tumorous androgen synthesis as well as synthesis of C-terminally truncated, constitutively active ARs [17, 18]. Devoid of a functional LBD, these ARΔLBDs (AR-Vs, Q641X, Q784*) are unable to bind androgens/antiandrogens and pose a major threat to LBD-directed therapies [1–3]. To inhibit aberrant AR or ARΔLBD signaling, small molecule inhibitors targeting functional domains involved in transactivation (NTD/TAD) or DNA binding (DBD) of the AR (Fig. 1) are currently being developed [19, 20]. All of these agents hold promise, but are still in the early stages of development [21, 22].

Steroid receptors like the AR but also ARΔLBDs rely on a huge network of cooperating molecules to enact their signaling effects on the cell. In consequence, AR co-factor-directed inhibitors could lead to an inhibition or disruption of the AR/ARΔLBD-signaling network [23, 24]. Recently, members of the BET family of proteins, namely BRD2, 3 and 4, have been identified as AR-interacting proteins [4]. BET proteins provide a scaffolding platform for the recruitment and tethering of active transcriptional complexes to acetylated histones and chromatin. BET inhibitors such as JQ1 or I-BET762 act through competitive binding to the bromodomain (BD) pocket, thereby blocking the acetyl-lysine recognition motif of BET proteins. These inhibitors are highly effective modulators of the c-MYC-signaling complex in a broad spectrum of different neoplasms [25]. The identification of BRD4, as part of the AR-transcriptional complex opens a new alternative to disable AR and/or ARΔLBD signaling in CRPC using specific BET inhibitors [4, 5, 26, 27].

The BET-inhibitor PFI-1 is a highly selective cell-permeable acetyl-mimetic targeting the bromodomains of BRD2/BRD4. Unlike the most common BET inhibitors, the triazolodiazepines JQ1, I-BET762 and OTX015, the quinazolinone PFI-1 was designed to serve as a lead compound for a new group of BET inhibitors [10, 28]. The present study demonstrates that PFI-1 is able to suppress AR/ARΔLBD signaling and proliferation in human PCa cell lines at concentrations previously shown to inhibit leukemia cells in vitro [11]. In LNCaP cells, PFI-1 diminished the amount of PSA secreted into the cell culture supernatant in a dose-dependent manner. The ability of PFI-1 to impair DHT-stimulated AR activity was furthermore confirmed in 22Rv1 cells using a reporter gene assay. The observation that PFI-1 suppresses AR-dependent reporter gene activity suggests that the decrease of secreted PSA into the supernatant of LNCaP cells is due to an inhibition of the AR transcriptional machinery rather than a direct effect of PFI-1 on PSA secretion. Downregulation of PSA following PFI-1 treatment was not paralleled by a reduction of AR-protein levels in LNCaP cells. To our surprise 5 μM PFI-1 increased the amount of AR protein in LNCaP, whereas concentrations ≥ 10 μM were unable to significantly reduce/increase cellular AR-protein levels. Although various BET inhibitors attenuate AR transcriptional activity, their effect on AR-protein levels remains controversial [4, 5, 9]. To date, there is no clear explanation for the differential modulation of endogenous AR-protein levels by different BET inhibitors in PCa cells. The conflicting experimental data could be due to different cellular models (LNCaP, 22Rv1, VCaP) or incubation times used in the studies [4, 5]. Although LNCaP and 22Rv1 are widely used for the study of AR signaling, both cell lines harbor androgen-inducible but point-mutated full-length ARs (AR^{T878A}, AR^{H875Y}) that can be activated by a variety of steroid hormones including antiandrogens (Fig. 1). Only VCaP express a wild-type AR but co-express (like 22Rv1) AR-V7. Due to the diversity of AR forms expressed in the experimental models, it is tempting to speculate that PFI-1-mediated regulation of full-length ARs could depend on the cellular context. Moreover, the chemical structures of the BET inhibitors as well as their mechanisms of action (acetyl-lysine mimetics: JQ1, I-BET762, ABBY-074 or BET degrader: AR-V771) may be responsible for off-target effects being, at least in part, responsible for the conflicting results [4, 5, 9, 26, 27]. The selective blockade of AR-V7, Q641X and wild-type AR by PFI-1 was analyzed in a reporter gene assay. PFI-1 dose-dependently inhibited the transactivation of all receptor variants in a dose-dependent manner. Interestingly, in reporter gene assays, the constitutive activity of AR-V7 and Q641X was more affected by PFI-1 than the androgen-stimulated transactivation of AR wild type. The reason for this discrepancy remains unknown. The acetyl-lysine mimetic PFI-1 primarily blocks the recognition of acetylated histones by BET proteins like BRD4. One might object that reporter gene assays are not suitable for the study of BET inhibitors, as plasmid DNA including that of the reporter constructs is not bound to histones. However, there is experimental evidence from HEK 293 cells that plasmid DNA is packed into nucleosomes within 24 h after transfection [29]. Although BD1 and BD2 interact with acetylated chromatin, they also interact with non-histone proteins like the AR [4]. BRD4 is involved in the recruitment of transcriptional regulatory complexes to chromatin through various protein recognition modules such as BD1, BD2 and the ET domain. Inhibition of the acetyl-lysine binding pocket in the BD1 (and to a lesser extend of BD2) by the BET inhibitor JQ1 was recently shown to disrupt AR–BRD4 interaction. Interestingly, this leads to an inhibition of AR signaling independent of BRD4's ability to bind acetylated histones [4].

In addition to its anti-AR effects, the anti-proliferative effects of PFI-1 were more pronounced in AR-positive PCa cells (LNCaP, 22Rv1) as compared to AR-negative PCa cells (PC-3). This finding is in agreement with a previous study, analyzing the effects of the BET inhibitor JQ1 on the proliferation of various PCa cells [4]. Interestingly, the robust

inhibition of PSA in LNCaP cells (64–67%) did not translate into a comparable reduction of cell proliferation (24–26%). Although the reason for this discrepancy remains unknown, the fact that PSA expression does not necessarily correlate with PCa cell growth has also been described for bicalutamide, cyproterone acetate or goserelin [30].

The ability of PFI-1 to inhibit transactivation of full-length ARs (AR, AR^{T878A}, AR^{H875Y}), AR-V7, and Q641X, as well as its distinct anti-proliferative properties in AR/AR-V7-positive cells, highlights the importance of BET proteins in normal and aberrant AR signaling in PCa cells. Besides their anti-AR activity, BET inhibitors were also shown to enhance or restore enzalutamide sensitivity in CRPC cells [31, 32]. Given the fact that most BET inhibitors have been proven to be safe with manageable reversible toxicity in clinical trials, BET inhibitors are promising candidates for future therapies of advanced PCa [33]. To maximize the potential of BET inhibition, future studies should focus on the development of new compounds and/or synergistic combination therapies that may ultimately be able to address CRPC for which currently only few therapeutic options exist.

Acknowledgements The authors wish to thank Beate Godau and Christine Marschke for expert technical assistance.

Author contributions MCH: data collection and protocol development; MHR: data collection; FZ: data collection; SP: data analysis; ASM: data analysis and manuscript writing/editing; MVC: project development, data analysis, and manuscript writing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Azoitei A, Merseburger AS, Godau B, Hoda MR, Schmid E, Cronauer MV (2017) C-terminally truncated constitutively active androgen receptor variants and their biologic and clinical significance in castration-resistant prostate cancer. J Steroid Biochem Mol Biol 166:38–44
2. Lapouge G, Erdmann E, Marcias G, Jagla M, Monge A, Kessler P, Serra S, Lang H, Jacqmin D, Bergerat JP, Céraline J (2007) Unexpected paracrine action of prostate cancer cells harboring a new class of androgen receptor mutation—a new paradigm for cooperation among prostate tumor cells. Int J Cancer 121:1238–1244
3. Han D, Gao S, Valencia K, Owiredu J, Han W, de Waal E, Macoska JA, Cai C (2017) A novel nonsense mutation in androgen receptor confers resistance to CYP17 inhibitor treatment in prostate cancer. Oncotarget 8:6796–6808
4. Asangani IA, Dommeti VL, Wang X, Malik R, Cieslik M, Yang R, Escara-Wilke J, Wilder-Romans K, Dhanireddy S, Engelke C, Iyer MK, Jing X, Wu YM, Cao X, Qin ZS, Wang S, Feng FY, Chinnaiyan AM (2014) Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 510:278–282
5. Chan SC, Selth LA, Li Y, Nyquist MD, Miao L, Bradner JE, Raj GV, Tilley WD, Dehm SM (2015) Targeting chromatin binding regulation of constitutively active AR variants to overcome prostate cancer resistance to endocrine-based therapies. Nucleic Acids Res 43:5880–5897
6. Fujisawa T, Filippakopoulos P (2017) Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. Nat Rev Mol Cell Biol 18:246–262
7. Taniguchi Y (2016) The bromodomain and extra-terminal domain (BET) family: functional anatomy of BET paralogous proteins. Int J Mol Sci 17:1849. https://doi.org/10.3390/ijms17111849
8. Wu SY, Chiang CM (2007) The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. J Biol Chem 282:13141–13145
9. Wyce A, Degenhardt Y, Bai Y, Le B, Korenchuk S, Crouthame MC, McHugh CF, Vessella R, Creasy CL, Tummino PJ, Barbash O (2013) Inhibition of BET bromodomain proteins as a therapeutic approach in prostate cancer. Oncotarget 4:2419–2429
10. Fish PV, Filippakopoulos P, Bish G, Brennan PE, Bunnage ME, Cook AS, Federov O, Gerstenberger BS, Jones H, Knapp S, Marsden B, Nocka K, Owen DR, Philpott M, Picaud S, Primiano MJ, Ralph MJ, Sciammetta N, Trzupek JD (2012) Identification of a chemical probe for bromo and extra C-terminal bromodomain inhibition through optimization of a fragment-derived hit. J Med Chem 55:9831–9837
11. Picaud S, Da Costa D, Thanasopoulou A, Filippakopoulos P, Fish PV, Philpott M, Fedorov O, Brennan P, Bunnage ME, Owen DR, Bradner JE, Taniere P, O'Sullivan B, Müller S, Schwaller J, Stankovic T, Knapp S (2013) PFI-1, a highly selective protein interaction inhibitor, targeting BET bromodomains. Cancer Res 73:3336–3346
12. Schütz SV, Schrader AJ, Zengerling F, Genze F, Cronauer MV, Schrader M (2011) Inhibition of glycogen synthase kinase-3β counteracts ligand-independent activity of the androgen receptor in castration resistant prostate cancer. PLoS One 6(9):e25341
13. Streicher W, Zengerling F, Laschak M, Weidemann W, Höpfner M, Schrader AJ, Jentzmik F, Schrader M, Cronauer MV (2012) AR-Q640X, a model to study the effects of constitutively active C-terminally truncated AR variants in prostate cancer cells. World J Urol 30:333–339
14. Zengerling F, Azoitei A, Herweg A, Jentzmik F, Cronauer MV (2016) Inhibition of IGF-1R diminishes transcriptional activity of the androgen receptor and its constitutively active, C-terminally truncated counterparts Q640X and AR-V7. World J Urol 34:633–639
15. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
16. Merseburger AS, Hammerer P, Rozet F, Roumeguère T, Caffo O, da Silva FC, Alcaraz A (2015) Androgen deprivation therapy in castrate-resistant prostate cancer: how important is GnRH agonist backbone therapy? World J Urol 33(8):1079–1085
17. Perner S, Cronauer MV, Schrader AJ, Klocker H, Culig Z, Baniahmad A (2015) Adaptive responses of androgen receptor signaling in castration-resistant prostate cancer. Oncotarget 6:35542–35555
18. Jentzmik F, Azoitei A, Zengerling F, Damjanoski I, Cronauer MV (2016) Androgen receptor aberrations in the era of abiraterone and enzalutamide. World J Urol 34:297–303
19. Sadar MD (2012) Advances in small molecule inhibitors of androgen receptor for the treatment of advanced prostate cancer. World J Urol 30:311–318
20. Dalal K, Roshan-Moniri M, Sharma A, Li H, Ban F, Hassona MD, Hsing M, Singh K, LeBlanc E, Dehm S, Tomlinson Guns ES,

Cherkasov A, Rennie PS (2014) Selectively targeting the DNA-binding domain of the androgen receptor as a prospective therapy for prostate cancer. J Biol Chem 289:26417–26429
21. Antonarakis ES, Chandhasin C, Osbourne E, Luo J, Sadar MD, Perabo F (2016) Targeting the N-terminal domain of the androgen receptor: a new approach for the treatment of advanced prostate cancer. Oncologist 21:1427–1435
22. Hupe MC, Offermann A, Perabo F, Chandhasin C, Perner S, Merseburger AS, Cronauer MV (2018) Inhibitors of the androgen receptor N-terminal domain: therapies targeting the Achilles' heel of various androgen receptor molecules in advanced prostate cancer. Urol A 57:148–154
23. Culig Z, Santer FR (2012) Androgen receptor co-activators in the regulation of cellular events in prostate cancer. World J Urol 30:297–302
24. Foley C, Mitsiades N (2016) Moving beyond the androgen receptor (AR): targeting AR-interacting proteins to treat prostate cancer. Horm Cancer 7:84–103
25. Fu LL, Tian M, Li X, Li JJ, Huang J, Ouyang L, Zhang Y, Liu B (2015) Inhibition of BET bromodomains as a therapeutic strategy for cancer drug discovery. Oncotarget 6:5501–5516
26. Raina K, Lu J, Qian Y, Altieri M, Gordon D, Rossi AM, Wang J, Chen X, Dong H, Siu K, Winkler JD, Crew AP, Crews CM, Coleman KG (2016) PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proc Natl Acad Sci USA 113:7124–7129
27. Faivre EJ, Wilcox D, Lin X, Hessler P, Torrent M, He W, Uziel T, Albert DH, McDaniel K, Kati W, Shen Y (2017) Exploitation of castration-resistant prostate cancer transcription factor dependencies by the novel BET inhibitor ABBV-075. Mol Cancer Res 15:35–44
28. Ferri E, Petosa C, McKenna CE (2016) Bromodomains: structure, function and pharmacology of inhibition. Biochem Pharmacol 106:1–18
29. Mladenova V, Mladenov E, Russev G (2014) Organization of plasmid DNA into nucleosome-like structures after transfection in eukaryotic cells. Biotechnol Biotechnol Equip 23(1):1044–1047. https://doi.org/10.1080/13102818.2009.10817609
30. Peternac D, Klima I, Cecchini MG, Studer UE, Thalmann GN (2006) Prostate specific antigen expression does not necessarily correlate with prostate cancer cell growth. J Urol 176:354–360
31. Asangani IA, Wilder-Romans K, Dommeti VL, Krishnamurthy PM, Apel IJ, Escara-Wilke J, Plymate SR, Navone NM, Wang S, Feng FY, Chinnaiyan AM (2016) BET bromodomain inhibitors enhance efficacy and disrupt resistance to AR antagonist in the treatment of prostate cancer. Mol Cancer Res 14:324–331
32. Shah N, Wang P, Wongvipat J, Karthaus WR, Abida W, Armenia J, Rockowitz S, Drier Y, Bernstein BE, Long HW, Freedman ML, Arora VK, Zheng D, Sawyers CL (2017) Regulation of the glucocorticoid receptor via a BET-dependent enhancer drives antiandrogen resistance in prostate cancer. Elife 6:e27861. https://doi.org/10.7554/elife.27861
33. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE (2010) Selective inhibition of BET bromodomains. Nature 468:1067–1073