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Journal

European Urology, 73(5)

Authors

Luo, Jun
Attard, Gerhardt
Balk, Steven
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Publication Date

2018-05-01

DOI

10.1016/j.eururo.2017.11.038

Peer reviewed

Published in final edited form as:

Eur Urol. 2018 May ; 73(5): 715–723. doi:10.1016/j.eururo.2017.11.038.

Role of Androgen Receptor Variants in Prostate Cancer: Report from the 2017 Mission Androgen Receptor Variants Meeting

Jun Luo[a,*], **Gerhardt Attard**[b], **Steven P. Balk**[c], **Charlotte Bevan**[d], **Kerry Burnstein**[e], **Laura Cato**[f], **Artem Cherkasov**[g], **Johann S. De Bono**[h], **Yan Dong**[i], **Allen C. Gao**[j], **Martin Gleave**[g], **Hannelore Heemers**[k,l,m], **Mayuko Kanayama**[n], **Ralf Kittler**[o], **Joshua M. Lang**[p], **Richard J. Lee**[q], **Christopher J. Logothetis**[r], **Robert Matusik**[s], **Stephen Plymate**[t], **Charles L. Sawyers**[u], **Luke A. Selth**[v], **Howard Soule**[w], **Wayne Tilley**[v], **Nancy L. Weigel**[x], **Amina Zoubeidi**[g], **Scott M. Dehm**[y,z,aa,*], and **Ganesh V. Raj**[bb,cc,*]

[a]Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, MD, USA

[b]The Institute of Cancer Research, London, UK

[c]Hematology-Oncology Division, Beth Israel Deaconess Medical Center, Boston, MA, USA

[d]Department of Surgery & Cancer, Imperial College London, Imperial Centre for Translational & Experimental Medicine (ICTEM), Hammersmith Hospital Campus, London, UK

[e]Department of Molecular & Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL, USA

[f]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

[*]Corresponding authors. Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University, 600 N Wolfe St., Baltimore, MD 21287, USA (J. Luo); Masonic Cancer Center, University of Minnesota, 570 MCRB, 425 East River Parkway Minneapolis, MN 55455, USA (S.M. Dehm); Departments of Urology and Pharmacology, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., J8.130C Dallas, TX 75390, USA (G.V. Raj). jluo1@jhmi.edu (J. Luo); dehm@umn.edu (S.M. Dehm); ganesh.raj@utsouthwestern.edu (G.V. Raj).

Author contributions: Jun Luo had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Luo, Dehm, Raj.
Acquisition of data: Luo, Attard, Balk, Bevan, Burnstein, Cato, Cherkasov, De Bono, Dong, Gao, Gleave, Heemers, Kanayama, Kittler, Lang, Lee, Logothetis, Matusik, Plymate, Sawyers, Selth, Soule, Tilley, Weigel, Zoubeidi, Dehm, Raj.
Analysis and interpretation of data: Luo, Attard, Balk, Bevan, Burnstein, Cato, Cherkasov, De Bono, Dong, Gao, Gleave, Heemers, Kanayama, Kittler, Lang, Lee, Logothetis, Matusik, Plymate, Sawyers, Selth, Soule, Tilley, Weigel, Zoubeidi, Dehm, Raj.
Drafting of the manuscript: Luo, Dehm, Raj.
Critical revision of the manuscript for important intellectual content: Luo, Attard, Balk, Bevan, Burnstein, Cato, Cherkasov, De Bono, Dong, Gao, Gleave, Heemers, Kanayama, Kittler, Lang, Lee, Logothetis, Matusik, Plymate, Sawyers, Selth, Soule, Tilley, Weigel, Zoubeidi, Dehm, Raj.
Statistical analysis: None.
Obtaining funding: Luo, Dehm, Raj.
Administrative, technical, or material support: Luo, Dehm, Raj.
Supervision: None.
Other: None.

Financial disclosures: Other authors have no disclosure relevant to the subject matter.

[g]Department of Urologic Sciences, University of British Columbia, The Vancouver Prostate Centre, Vancouver, BC, Canada

[h]Division of Clinical Studies, The Institute of Cancer Research, London, UK

[i]Department of Structural and Cellular Biology, Tulane University School of Medicine, New Orleans, LA, USA

[j]Department of Urology, University of California Davis, Sacramento, CA, USA

[k]Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

[l]Department of Urology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

[m]Department of Hematology/Medical Oncology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

[n]Department of Urology, Juntendo University Graduate School of Medicine, Tokyo, Japan

[o]McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX, USA

[p]Department of Medicine, Carbone Cancer Center, University of Wisconsin, Madison, WI, USA

[q]Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[r]Division of Cancer Medicine, Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

[s]Department of Urologic Surgery, Vanderbilt Prostate Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

[t]Department of Medicine, University of Washington and VAPSHCS GRECC, Seattle, WA, USA

[u]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[v]Dame Roma Mitchell Cancer Research Laboratories and Freemasons Foundation Centre for Men's Health, Adelaide Medical School, The University of Adelaide, SA, Australia

[w]Prostate Cancer Foundation, Santa Monica, CA, USA

[x]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

[y]Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

[z]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

[aa]Department of Urology, University of Minnesota, Minneapolis, MN, USA

[bb]Department of Urology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA

[cc]Department of Urology and Pharmacology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA

Abstract

Context—Although a number of studies have demonstrated the importance of constitutively active androgen receptor variants (AR-Vs) in prostate cancer, questions still remain about the precise role of AR-Vs in the progression of castration-resistant prostate cancer (CRPC).

Objective—Key stakeholders and opinion leaders in prostate cancer convened on May 11, 2017 in Boston to establish the current state of the field of AR-Vs.

Evidence acquisition—The meeting "Mission Androgen Receptor Variants" was the second of its kind sponsored by the Prostate Cancer Foundation (PCF). This invitation-only event was attended by international leaders in the field and representatives from sponsoring organizations (PCF and industry sponsors). Eighteen faculty members gave short presentations, which were followed by in-depth discussions. Discussions focused on three thematic topics: (1) potential of AR-Vs as biomarkers of therapeutic resistance; (2) role of AR-Vs as functionally active CRPC progression drivers; and (3) utility of AR-Vs as therapeutic targets in CRPC.

Evidence synthesis—The three meeting organizers synthesized this meeting report, which is intended to summarize major data discussed at the meeting and identify key questions as well as strategies for addressing these questions. There was a critical consensus that further study of the AR-Vs is an important research focus in CRPC. Contrasting views and emphasis, each supported by data, were presented at the meeting, discussed among the participants, and synthesized in this report.

Conclusions—This article highlights the state of knowledge and outlines the most pressing questions that need to be addressed to advance the AR-V field.

Patient summary—Although further investigation is needed to delineate the role of androgen receptor (AR) variants in metastatic castration-resistant prostate cancer, advances in measurement science have enabled development of blood-based tests for treatment selection. Detection of AR variants (eg, AR-V7) identified a patient population with poor outcomes to existing AR-targeting therapies, highlighting the need for novel therapeutic agents currently under development.

Keywords

Androgen receptor variants; Androgen receptor variant 7; Castration-resistant prostate cancer; Abiraterone; Enzalutamide

1. Introduction

As prostate cancer is an androgen-dependent disease, the androgen receptor (AR) is the primary molecular target for systemic prostate cancer therapy. Despite initial robust responses to first-line androgen deprivation therapies (ADTs), nearly all patients with advanced prostate cancer progress to lethal castration-resistant prostate cancer (CRPC). Importantly, in CRPC, the AR continues to be the primary molecular driver, as evidenced by efficacy of novel hormonal therapies, abiraterone and enzalutamide, in CRPC patients [1–4]. While effective, therapies targeting AR are not curative, due to intrinsic and acquired resistance to first-line ADTs and novel hormonal therapies. Molecular mechanisms of

resistance are largely driven by AR aberrations including AR protein overexpression, AR gene amplification, AR gene mutations, and AR variants (AR-Vs) [5].

AR-Vs are truncated AR proteins lacking the AR ligand-binding domain (AR-LBD) [6]. While AR-Vs have frequently been detected in CRPC, their expression and functional role in benign prostate tissues and primary prostate cancers is not readily apparent. Structural rearrangements in the AR gene and alternative AR mRNA splicing are at least two mechanisms for expression of AR-Vs in CRPC [6]. Multiple AR-Vs arising from AR gene rearrangements and/or alternative splicing have been characterized. To date, AR splice variant-7 (AR-V7) has been studied in greatest detail owing to its relative abundance and frequency of detection in CRPC [7,8], as well as its potential clinical utility as a marker for treatment selection in men with metastatic CRPC (mCRPC) [9]. However, in-depth studies have also been conducted on other AR-Vs, including AR-V1, AR-V3, AR-V7, AR-V9, and ARv567es [10–12]. Structural differences of these AR-Vs are illustrated in Figure 1. Since AR-Vs contain the AR DNA-binding domain (DBD) and the AR transcriptional activation domain, they are capable of transcriptional regulation, in spite of the loss of the AR-LBD. Further, since the AR-Vs lack the AR-LBD, they are not regulated by either first-line or novel hormonal therapies currently used in the clinic. At the Mission Androgen Receptor Variants (MARS) 2 meeting, our efforts were streamlined to evaluate the role of AR-Vs as biomarkers, molecular drivers, and therapeutic targets. The authors identified key consensus, discussion points, and critical future work needed to advance the field.

2. Evidence acquisition

The MARS meeting was the second of its kind sponsored by the Prostate Cancer Foundation (PCF). This invitation-only event was attended by international leaders in the field and representatives from sponsoring organizations (PCF and industry sponsors). Eighteen faculty members gave short presentations, which were followed by in-depth discussions. Discussions focused on three thematic topics: (1) potential of AR-Vs as biomarkers of therapeutic resistance; (2) role of AR-Vs as functionally active CRPC progression drivers; and (3) utility of AR-Vs as therapeutic targets in CRPC.

3. Evidence synthesis

The three meeting organizers (J.L., S.M.D., G.V.R.) conceived the idea of an invitation-only meeting focusing on AR-Vs. Three thematic topics were predefined prior to the meeting: (1) potential of AR-Vs as biomarkers of therapeutic resistance; (2) role of AR-Vs as functionally active CRPC progression drivers; and (3) utility of AR-Vs as therapeutic targets in CRPC. The meeting was sponsored by the PCF, Sanofi, Astellas, Janssen Research and Development LLC, and Sun Pharma, and held in Boston, MA, prior to the American Urological Association annual meeting. Academic physicians and scientists from the USA, the UK, Canada, Australia, and Japan, as well as representatives from four sponsoring pharmaceutical companies attended this meeting. Eighteen faculty members gave short presentations, which were followed by in-depth discussions. The three meeting organizers summarized major data discussed at the meeting, identified 26 key questions in the field, and synthesized this meeting report. The 26 key questions were included in an online survey sent

to all nonindustry participants after the meeting. Detailed voting results (percent and number of approval votes) are included in the three boxes of the Supplementary material, summarizing general consensus reached at the meeting.

3.1. Session 1: Measurement science pertaining to AR-Vs and other AR aberrations

3.1.1. Tissue-based testing of AR-Vs—Prior studies had established that AR-Vs can be detected at the RNA and protein levels in CRPC samples. Expression of several AR-Vs including AR-V7 has been reported in benign prostate tissue and primary prostate cancers [9]. Although AR-Vs were detected in untreated prostate tumors and benign prostate tissues; their levels were substantially lower and likely represented background splice events detectable by reverse transcription polymerase chain reaction and RNA-seq that may not lead to robust detection by RNA in situ hybridization (RISH) and immunohistochemistry (IHC). However, expression of AR-V7 is higher in CRPC, potentially due to AR gene amplification and/or induction of AR-V7 by ADTs [13–15], which occurs in CRPC cell lines and xenografts. Therefore, amplification of the AR gene, a frequent event in CRPC, is likely to underlie the increased expression of AR-Vs at this disease stage. Whether the selective advantage for AR gene amplification is overexpression of AR or AR-Vs (or both) remains to be established.

Dr. Richard Lee reported the development of a branched chain RNA in situ histochemistry (RNA ISH) assay for the detection of AR-V7 mRNA within archival prostate cancer tissue. This branched chain RNA ISH assays and other similar assays reported in the literature utilized 20-mer probes tiled across 500–1000 base pairs of the unique cryptic exon 3 (CE3) at the 3′ terminus of AR-V7 mRNA [16–18]. Using an automated ISH system, AR-V7 was detected in formalin-fixed paraffin-embedded (FFPE) samples from radical prostatectomy (n = 30, detection rate 1/30), metastatic, castration-sensitive prostate cancer (n = 22, detection rate 10/22), and mCRPC (n = 12, detection rate 12/12), suggesting that AR-V7 expression dramatically increases with disease progression [18]. In this pilot study, AR-V7 mRNA detection was also associated with the duration of response to first-line ADTs. Dr. Lee presented an individual case with serial tissues collected before, during, and after ADT showing progressively higher AR-V7 expression. Thus, automated RNA ISH assay is feasible for AR-V7 evaluation in archival FFPE prostate cancer tissue, and this small-cohort study suggests that baseline AR-V7 by this method is a negative predictive marker for treatment with ADTs.

Dr. Johann de Bono reported on advances in tissue-based measurements of AR-V7 mRNA and protein. The primary drawback of RNA ISH– and IHC-based detection of AR-V7 mRNA and protein has been the lack of specificity [19,20]. Dr. de Bono presented data on a novel RNA ISH method, as well as a new AR-V7 antibody developed by RevMab shown to be analytically valid with a single band on Western blot. The novel RNA ISH method detected a single splice junction specific to AR-V7 [20], and the new AR-V7 antibody did not detect false-positive signals reported in a previous study with the Abcam antibody [19]. These validated in situ detection methods enabled precision measurement of AR-V7 in morphologically intact clinical tissue specimens. A recent study reported a novel RISH detection method and compared the RISH results with the IHC results by the RevMab

antibody [20]. The findings further confirmed the improved specificity of the new antibody. Detection of AR-V7 by the novel RNA ISH method in a cohort of CRPC biopsies was generally associated with poorer response to abiraterone and enzalutamide [20]. Dr. de Bono suggested that AR-V7 mRNA detection alone may not correlate with AR-V7 protein levels, due to altered kinetics of AR-V7 protein degradation [21].

3.1.1.1 Synthesis: AR-V7 mRNA and protein can be detected in morphologically intact tissues. Tissue-based studies support the feasibility of measuring AR-V7 in metastatic tissue biopsies. As AR-V7 is infrequently detected in untreated patients by RISH or IHC, elevated AR-V7 expression may be an acquired event after hormonal therapies, although detection in untreated cases may theoretically have prognostic or predictive value. Both RISH and IHC detection values are continuous variables. Thus, differences in detection rate between studies could reflect the different cutoff values. In addition, difference in techniques, reliance on RNA versus protein, and sampling criteria may result in different detection rates.

3.1.2. Blood-based detection of AR-Vs and other AR aberrations—Previous studies have utilized circulating tumor cells (CTCs) [16,22–25], plasma exosomes [26], peripheral blood mononucleated cells [27], and even whole blood samples [28–31] for the detection of AR-Vs (mainly AR-V7) in men with mCRPC. Among these, the CTC-based AR-V7 test has been analytically validated and implemented in Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories on the basis of clinical correlative findings [25,32]. However, CTC-based AR-V7 tests may be limited. Critically, determination of the AR-V7 status and its quantification were not possible in a significant proportion of mCRPC patients who were CTC negative. In addition, many other relevant molecular targets are compatible with blood-based measurements, including AR amplification/mutation, AR-Vs other than AR-V7, as well as non-AR genomic alterations implicated in CRPC progression.

Novel CTC platforms may partially address this limitation. Dr. Joshua Lang demonstrated the feasibility of the VERSA system to detect mRNAs of multiple AR-Vs using CTCs captured using antibodies targeted to cell surface proteins EpCAM and TROP2. The overall AR-V7 detection rate was 6/26 (26%) in this study, in line with the literature. In addition to AR-V7, AR-V9 was also detected at a high frequency [11]. Using this platform, ARv567es was detected at a lower frequency (~1% of patients). The system allowed analysis of additional genes involved in epithelial–mesenchymal transition and neuroendocrine differentiation.

Dr. Mayuko Kanayama reported the Juntendo University pilot study, where they evaluated the detection of AR-V7–positive CTCs along with examination of prostate-specific membrane antigen (PSMA) in 19 Japanese mCRPC patients treated with different CRPC therapies. The overall AR-V7 detection rate was 26% (5/19). In addition to AR-V7, Dr. Kanayama discussed the potential negative predictive value of PSMA detection (positive rate 47%, 9/19) in this small cohort.

Drs. Martin Gleave and Gerhard Attard presented data on blood-based measurements through the isolation of circulating tumor DNA (CtDNA). Published data have established

the feasibility of detecting genomic alterations in CtDNA samples in patients with CRPC and evolution in these genomic alterations over time under selective pressures of treatment [33–35]. A wide spectrum of genomic alterations (AR, DNA repair genes, TP53, PIK3CA, and RB1) detected in CtDNA samples are also detected in matched metastatic tissues. In addition, CtDNA assays may reveal greater heterogeneity of alterations in some patients than is possible through biopsy of a single metastatic site, suggesting that metastatic tissue biopsy may not be required to determine the somatic status of clinically actionable prostate cancer driver genes [36]. CtDNA assays, therefore, may be utilized for the development of prognostic and predictive biomarkers. There is a growing need to optimize panels and develop CLIA-certified assay for prospective validation of CtDNA markers.

3.1.2.1. Synthesis: It is feasible to measure AR aberrations (AR-V7, AR-V9, AR amplification, and AR mutation) as well as other disease drivers using blood-based assays. However, no studies have integrated these measurements. The challenge ahead is to perform analytical as well as clinical validation of individual and integrated assays with concerted efforts.

3.2. Session 2: AR-V functional role and regulation

3.2.1. Regulation of gene expression by AR-Vs—At the functional level, ADTs cause increased expression of AR and AR-Vs due to relief of androgen/AR-mediated suppression of AR gene transcription [37]. Thus, AR-Vs (and full-length AW [AR-FL]) induced by ADTs may regulate the same genes regulated by androgen-activated AR-FL. An alternative model is that elevated AR-Vs may confer a distinct transcriptional program and cellular phenotype. Dr. Nancy L. Weigel used an inducible AR-V7 system to show that while AR-V7 induces canonical AR genes such as *FKBP5* and *KLK3*, RNA-seq analysis revealed expression of distinct genes that were associated with AR-V7 induction. For example, EDN2, ETS2, SRD5A1, ORM1, BIRC3, HSP27, and HES1 were specifically induced following induction of AR-V7 expression, while SGK1 was specifically induced by AR-FL activation [38]. Interestingly, the AR-V7–specific target genes, *EDN2*, and *ETS2* are genes that can be regulated by AR if the pioneer transcription factor FOXA1 is depleted. Conversely, AR can induce RASSF3 only when FOXA1 is present, but AR-V7 does not induce RASSF3 under either condition. When ARv567es was investigated, there also appeared to be significant overlap with AR-V7 and AR-FL targets. However, there may also be ARv567es-specific target genes owing to the retention of the AR hinge region in ARv567es as opposed to AR-V7 (Fig. 1).

Dr. Laura Cato showed LNCaP95 cells (LNCaP cells derived from long-term passage under castrate conditions) may depend on both AR-FL and AR-V7 for short-term proliferation, as evidenced by slowed LNCaP95 cell growth after dox-inducible knockdown of either AR-FL or AR-V7 [14]. Using the AR-V7 RevMab and an AR C-terminal antibody for ChIP-seq, they demonstrate that AR-V7 and AR-FL bind to the same genomic location in this dox-inducible model. AR-V7 chromatin binding was reduced in response to AR-FL knockdown and vice versa; knockdown of AR-V7 reduced AR-FL chromatin binding. Further work is ongoing to identify similarities and differences in the transcriptional activities of the two receptors.

3.2.1.1. Synthesis: Laboratory research using cell-line model systems may be used to dissect gene expression and chromatin-binding programs directed by AR-FL and AR-Vs. The majority of chromatin-binding events and transcriptional targets of AR-Vs and androgen-activated AR-FL display significant overlap. However, genome-wide analyses have suggested that an AR-V–specific transcriptional program may exist. The interplay between AR-FL, AR-V7, and other AR-Vs deserves further in-depth investigation, particularly in the setting of the native molecular context of clinical specimens.

3.2.2. Role of AR dimerization and AR coregulators—An important question in the AR-V field is whether AR-V functions as a dimer, and if so, how AR-V/AR-FL homo- and heterodimers influence therapeutic targeting [39]. Studies published from Dr. Yan Dong's laboratory [40–42] focused on interactions between AR-FL and various AR-Vs. Using tagged forms of AR-FL and AR-Vs, Dr. Dong suggested that AR-Vs form dimers through DBD/DBD interactions, and that this dimerization is required for function but not nuclear localization. AR-Vs can modulate the function and localization of AR-FL through formation of AR-FL/AR-V heterodimers, which are mediated by the DBD/DBD interactions, but also binding of the AR-V N terminus with the AR-FL C terminus. Importantly, this AR-V/AR-FL heterodimer was not inhibited by enzalutamide. The critical function of the dimer was supported by mutually dependent co-occupancy of genomic sites by AR-FL and AR-V7. AR coregulators are important for AR-FL transcriptional activity [43] and may drive context-specific AR functions. The AR-V cistrome consists of canonical androgen response elements (AREs) and overlaps with AR-FL, and AR-V–specific genes may reflect the biphasic nature of AR transcriptional activation of certain target genes [10,44], indicating that the same or a similar set of coregulators may be involved in AR-FL and AR-V function. However, AR-Vs such as ARv567es and AR-V7 have reduced affinity for AREs compared with AR-FL [10], and altered kinetics of DNA binding may require different sets of coregulators. Dr. Luke Selth presented his work on dissecting the sets of coregulators bound by AR and ARv567es using Rapid Immunoprecipitation Mass-spectrometry of Endogenous proteins (RIME) [45]. This work showed a high degree of overlap between the AR-FL and ARv567es interactomes, but also yielded differences that may arise from coregulators specific to the AR-LBD and/or unique binding surfaces on the variant protein. One new example of an AR-FL and ARv567es-shared coregulator recently identified by Dr. Selth's group is GRHL2 [45], which was shown to participate in a feed-forward transcriptional loop with active AR-FL and AR-Vs that likely drives their activity in CRPC. Dr. Selth also noted that there are likely to be differences in regulation of AR/AR-V by the ubiquitin–proteasome system [46], as AR-Vs lose interaction and dependency on HSP90 [47].

3.2.2.1. Synthesis: Current data indicate that chromatin binding and transcriptional activation mediated by AR-Vs require homodimerization. While evidence also exists to support a role for heterodimerization between AR-Vs and AR-FL, the relative contributions of AR-V homodimers and AR-FL/AR-V heterodimers to the activation of AR-FL/AR-V target genes remain to be characterized. RIME represents an effective approach to study complexes nucleated by these homo- and heterodimers, and will be useful for characterizing AR-FL and AR-V interactomes in clinical specimens.

3.2.3. AR-V as a potential disease driver—Whether AR-Vs drive therapeutic resistance in CRPC remains an unresolved topic. Studies in favor of AR-Vs functioning as drivers of resistance have come from models where AR-Vs are endogenously expressed at high levels, and their knockdown restores sensitivity to castration and/or antiandrogens [44,48]. In contrast, studies arguing against AR-Vs functioning as drivers of resistance have come from models where AR-Vs are introduced ectopically or are expressed at extremely low levels relative to AR-FL. For instance, Dr. Charles Sawyers discussed his early published work indicating that AR-Vs lack the key properties of drug resistance drivers. In these studies, overexpression of AR-V7 in LNCaP cells was able to confer gain of function in terms of ligand-independent growth but did not impart resistance to enzalutamide, suggesting that AR-Vs may require AR-FL for gain of function [49]. In this context, Dr. Sawyers indicated that rapid induction of AR-V7 by ADTs may be a by-product of the increased transcription of the AR gene and simply reflects a mechanism for rapid induction of AR-FL expression by ADTs [14,49]. Another argument against a driver role for AR-Vs came from his observations with a prostate cancer cell line (Myc-Cap) derived from the Hi-Myc mouse, which expressed AR-Vs but remained sensitive to castration and enzalutamide. However, he also noted the Myc transgene in this model was under the control of an androgen-responsive promoter, which clouds interpretation. The murine AR-V, although structurally different from the human AR-V, demonstrated in vitro functional activities similar to human AR-V. Additionally, the 3′ terminal exons in these murine AR-Vs were not located within the AR gene locus, as is the case for human AR-V7. Instead, these 3′ terminal exons were located hundreds of kb upstream or downstream of the AR locus, indicating that underlying AR gene rearrangements were responsible for their splicing into AR mRNA.

Dr. Steven Balk presented data on progressive increases of AR-V7 expression in VCaP cells treated with enzalutamide in vitro and combination of abiraterone/enzalutamide in vivo. Using these cells with high AR-V7 expression, he showed that AR activity was inhibited by knockdown with an siRNA targeted to AR exon 1 (which encodes the AR amino terminus [NTD]) or the 3′ terminal exon CE3 of AR-V7. Interestingly, AR activity in these cells was not inhibited by siRNA that selectively knocked down AR-FL. In contrast to this adaptive model, VCaP cells treated acutely with enzalutamide and displaying rapid induction of AR-V7 displayed very low AR activity, suggesting that additional cofactors may be important for AR-V function in settings of acute versus adapted AR-V7 expression.

3.2.3.1. Synthesis: Discrepancies have been noted in the contribution of AR-Vs to the phenotype of therapeutic resistance in CRPC. It is possible that these discrepancies may be due to whether knockdown or overexpression approaches were used to interrogate AR-V function. Additionally, several of the models used for AR-V knockdown experiments and displaying AR-V–driven resistance phenotypes also harbor structural rearrangements in the AR gene and/or were adapted to long-term treatment with enzalutamide. This suggests that alterations in AR gene structure or adaptive changes cofactor milieu may be important determinants of AR-V function. Additionally, it has recently been shown that AR-V9, AR-V1, and additional AR-Vs utilizing CEs in AR intron 3 are coordinately expressed in CRPC and susceptible to knockdown with siRNAs/shRNAs that had previously been thought to

target AR-V7 exclusively [11,50]. Thus, AR-V7 knockdown studies reported in the literature were actually inhibiting expression of multiple AR-Vs simultaneously. Further work is required to elucidate the impact of these parameters on AR-V function as drivers of resistance in CRPC.

3.3. Session 3: Therapeutic targets and strategies

3.3.1. Utility of targeting AR amino terminus—The AR-NTD (amino acids 1–538) is structurally unique among steroid receptors: it is much longer, has a stronger transactivation domain, and is critical for the transactivation and function of the AR. Experimental and bioinformatic analyses reveal that the AR-NTD is intrinsically disordered in solution and exists as an ensemble of interconverting conformations. In response to environmental stresses, the AR-NTD may rapidly and reversibly fluctuate between conformations. These alterations in domain structure may enable transient interactions between AR-NTD and protein coregulators that may allosterically regulate AR function. Analyses by circular dichroism, Fourier transform infrared spectroscopy, secondary structure prediction, and mutagenesis have revealed variations in the degree of intrinsic disorder of different regions of the AR-NTD: some domains may adopt more stable secondary structure than others. While the flexibility and intrinsic disorder of the AR-NTD in solution and the lack of a crystal structure hamper rational design of drugs using virtual docking approaches, the relative stability of some domains makes them potentially targetable. In addition, the lack of sequence homology between the NTDs of AR and other members of the steroid hormone receptor family suggests that drugs targeting the AR-NTD will be more selective for the AR, yielding fewer side effects.

The primary advantage of drugs targeting the AR-NTD is the potential to fundamentally target all forms of the AR, including those that drive resistance to AR-LBD–targeting therapies. Since the AR-NTD is retained in all forms of biologically active AR, including amplified AR-FL, AR-LBD mutations (eg, W741C for bicalutamide and F876L for enzalutamide), and AR-Vs, drugs targeting AR-NTD should be effective against all these AR forms. The addition of such drugs to the CRPC armamentarium is likely to have significant clinical utility to prevent and overcome drug resistance.

A bisphenol A derivative EPI-001 has been shown to bind the AF-1 region of the AR-NTD and inhibit AR function. EPI-001 has been shown to inhibit AR-NTD transactivation, inhibit proliferation of cell lines expressing various forms of AR, and selectively block AR–protein interactions and recruitment of the AR to DNA response elements [51]. A derivative of EPI-001 is currently in phase I clinical trials. Importantly, EPI-001 provided proof of principle of the translatability of drugs targeting the AR-NTD; however, its utility as a therapeutic agent remains to be proved.

3.3.2. Development of novel agents—Dr. Artem Cherkasov performed in silico screening of 150 million compounds to identify drugs targeting the AR DBD [52]. As the AR DBD is shared between AR-FL, AR-LBD mutations, and AR-Vs, such an approach is likely to target all forms of the AR. Indeed, their lead compound VPC-14449 inhibits binding of AR-FL as well as AR-V7 and ARv567es to chromatin, and consequently blocks

transcriptional activity driven by AR [53]. Dr. Cherkasov and colleagues also developed VPC-17005 to block AR dimerization, and this compound resulted in the abrogation of AR function. Importantly, VPC-17005 selectively inhibited AR DNA binding without affecting other steroid hormone receptors, including progesterone receptor, estrogen receptor, and glucocorticoid receptor. Further development of these drugs could pave the way forward for rational design of drugs targeting the undruggable transcription factors.

Dr. Allen Gao showed that niclosamide, a Food and Drug Administration–approved drug to treat tapeworm infections, displayed activity against AR-V7 function [54–56]. A gene expression signature associated with treatment with niclosamide overlapped significantly with AR knockdown. This activity against AR-V7 may be driven by AR degradation as evidenced by MG132, a 26S proteasome inhibitor, inhibiting niclosamide-mediated AR-V7 protein degradation. Niclosamide inhibited growth of enzalutamide-resistant C4-2B cells, and synergized with treatment with abiraterone and enzalutamide [55]. Phase Ib/II clinical trials combining niclosamide and abiraterone/enzalutamide in mCRPC patients are currently ongoing.

Additional indirect strategies to interfere with AR-V function are to target their interactions with either protein coregulators or downstream transcriptional targets. Dr. Kerry Burnstein proposed indirectly targeting AR-V activity by disrupting interactions with key AR-V coactivators such as VAV3 and other AR amino terminal–interacting regulators [57]. She showed that a coactivator-enhanced AR-V transcriptional target could be exploited therapeutically in CRPC xenograft models. Similarly, another indirect approach to targeting AR-Vs rose out of an observation that certain kinase inhibitors inhibited the growth of AR-positive cells but not of AR-negative cells. Dr. Stephen Plymate presented data showing that bumped kinase inhibitors (BKIs) could inhibit prostate cancer cells that are driven by the constitutively active AR-V7. One proposed mechanism of action was inhibition of serine 81 phosphorylation on both AR-FL and AR-V7. Of note, in the absence of androgen, serine 81 is phosphorylated in cells expressing AR-FL and AR-V7. They also demonstrated that their candidate BKIs inhibited the growth of the AR-FL–driven LuCaP35 human PDX model in noncastrate mice as well as AR-V7–driven LNCaP95 xenografts in castrate mice. Their current BKI PK data demonstrate that they can reach effective EC50 levels in mice with a single daily oral dose, and there was no observable toxicity after 6 wk of treatment. Importantly, the BKIs have a narrow kinase target range. They will continue to modify the BKIs to achieve increased potency.

Dr. Robert Matusik showed that nuclear factor kB (NFkB) induces expression of AR-V7, and inhibition of NFkB using bortezomib reduced AR-V7 levels and restored CRPC responsiveness to antiandrogens in cell line and xenograft models using CRW22RV1 and C4-2B cells. Further, neuropeptides released by neuroendocrine prostate cancer activate the gastrin-releasing peptide receptor to induce NFkB and AR-V7 expression resulting in CRPC [58,59]. Other inhibitors of NFkB, such as methotrexate or LC-1 (dimethylaminoparthenolide), would reduce AR-V7 expression in CRPC cell lines and restore responsiveness to antiandrogens.

Dr. Amina Zoubeidi suggested that a significant fraction (9/35) of AR-positive enzalutamide-resistant xenografts are potentially AR indifferent, as indicated by a lack of prostate-specific antigen (PSA) expression [60], and have biologic similarity to pluripotent and neuroendocrine tumors. Stem-cell factors likely emerge early during therapeutic inhibition of the AR pathway, which can then be followed later by the expression of neuroendocrine markers. Inhibition of EZH2 can reverse the process back to an AR-driven state. Dr. Zoubeidi discussed BRN2, which was found to be overexpressed in CRPC tumors associated with low serum PSA. She further demonstrated that BRN2 was a transcription factor that likely functioned as a master regulator of enzalutamide-induced neuroendocrine transdifferentiation required for the expression of neuroendocrine markers. Mechanistically, AR suppressed BRN2, and this negative feedback can be relieved by enzalutamide. Thus, BRN2 is a potential target in advanced CRPC, and inhibition of BRN2 in combination with enzalutamide is being explored.

3.3.2.1. Synthesis: The unresolved question of whether AR-Vs function as the primary molecular drivers in CRPC has led to the question of whether AR-Vs are viable therapeutic targets for CRPC. Since AR-Vs lack the AR-LBD, novel drugs would be required to target AR-Vs. Since the amino terminus of AR-Vs is identical to the amino terminus of AR-FL, therapeutic strategies developed against AR-Vs are likely to have effects against all forms of the AR. This rationale has spawned significant interest from multiple investigators to target the AR-NTD or downregulate the expression of AR-Vs. However, approaches to target the N terminus of the AR are limited by the intrinsic disorder and lack of a crystal structure of this domain.

4. Conclusions

To realize the potential of translating laboratory discoveries to patient benefit, it is important to understand the biology, measurement science, and relevant experimental therapeutic approaches from multiple perspectives. In the 9 yr since the first report of AR-Vs, critical advances have been made. Importantly, testing platforms have been developed to facilitate AR-V measurements for biomarker-driven or biomarker-stratified clinical trials, and AR-Vs are being explored as a therapeutic target. In spite of these advances, there remains a need to conduct prospective trials to further assess the clinical utility of AR-Vs in mCRPC, and future efforts are also needed to improve blood-based testing platforms beyond AR-V7 by integrating multiple AR aberrations to enable robust treatment selection and patient selection. In addition, how AR-Vs mediate genomic function as a transcription factor, particularly in homo- versus heterodimer contexts, remains incompletely characterized. Additionally, an unresolved question that remains is whether AR-Vs drive therapeutic resistance in CRPC and, if so, under which specific contexts. Blood-based detection of AR-V7 identified a patient population with poor outcomes to existing therapeutic agents, highlighting the need to develop novel therapeutic approaches for mCRPC. Finally, there is a pressing need to develop markers and therapeutic approaches targeting AR-indifferent prostate cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Astellas Pharma, Sun Pharma, Janssen Oncology, and Sanofi Genzyme for their unrestricted financial sponsorship of the MARS2 meeting.

Jun Luo certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Jun Luo has served as a paid consultant/advisor for Sun Pharma, Janssen, and Sanofi; has received research funding to his institution from Orion, Astellas, Sanofi, and Gilead; and is a coinventor of a technology that has been licensed to A&G, Tokai, and Qiagen. Gerhardt Attard received honoraria, speaker fees, and/or research support from and/or has conducted clinical trials for Astellas, Pfizer, Janssen, Sanofi, ESSA, and Arno that have an interest in targeting the androgen receptor in prostate cancer; and is included in the Institute of Cancer Research rewards to inventors list for abiraterone. Laura Cato is an employee at Sanofi-Genzyme. Johann S. De Bono served on advisory boards for multiple pharmaceutical and biotech partners including AstraZeneca, Astellas, Daiichi Sankyo, Genentech, Genmab, GSK, Merck Serono, MSD, Pfizer Oncology, Sanofi-Aventis, and Taiho; and is an employee of the Institute of Cancer Research, a not-for-profit research organization, which has a commercial interest in abiraterone acetate and PARP inhibitors for DNA repair defective cancers. Allen C. Gao has stock and other ownership interests in Pandomedx, Inc. Joshua M. Lang has Salus Discovery and LLC-ownership interest; and is a Sanofi consultant. Richard J. Lee is on the advisory board of Janssen. Christopher J. Logothetis is a coinventor of enzalutamide and entitled to royalties from the University of California. Scott M. Dehm has served as a paid consultant/advisor for Medivation/Astellas and Janssen Research and Development, LLC; and has received research funding from Janssen Research and Development, LLC. Ganesh V. Raj is the founder of C-diagnostics, GaudiumRx and EtiraRx, has received grants from Bayer and Janssen, and serves as a consultant/speaker for Janssen, Medivation/Pfizer, Astellas, Bayer, and Sanofi.

***Funding/Support and role of the sponsor*:** None.

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Precision detection methods for androgen receptor variants have been developed and will enable treatment selection in men with castration-resistant prostate cancer. Further investigation is needed, and there is a pressing need to develop novel therapeutic approaches to overcome this putative resistance mechanism.

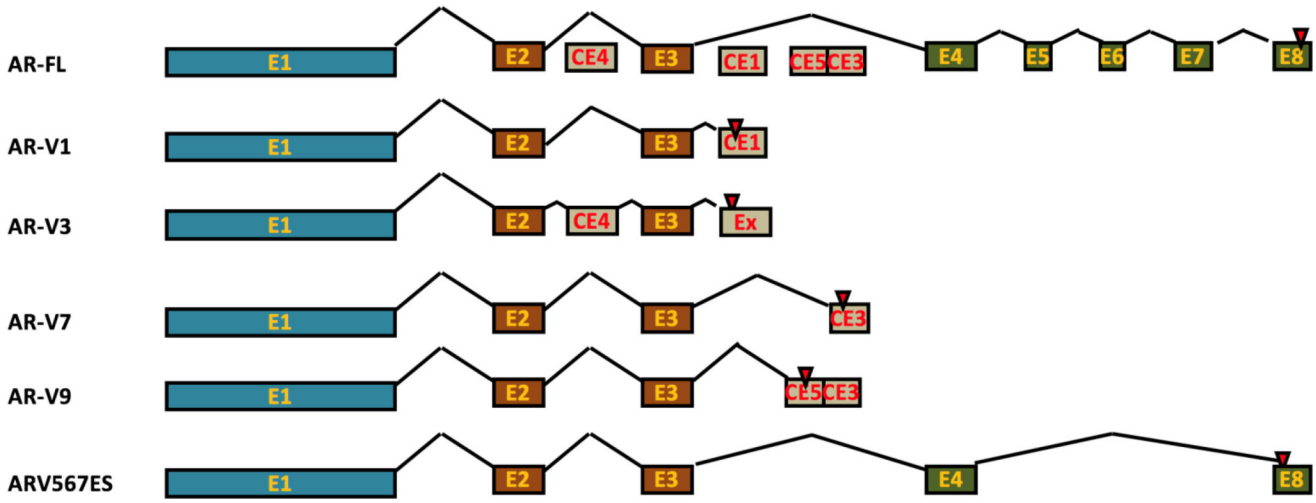

Fig. 1.

Transcript structure for representative AR-Vs. Stop codon positions are marked for each AR transcript. AR = androgen receptor; AR-FL = full length androgen receptor; AR-V = androgen receptor variant; E1–E8 = canonical androgen receptor exons 1–8; CE1–5 = cryptic exons 1–5; Ex = unknown exon in AR-V3.