METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF APALUTAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY UPLC

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ABSTRACT

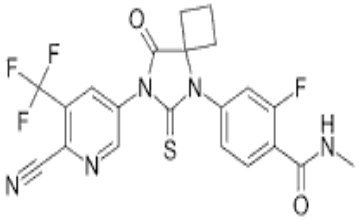
A simple, robust, economic method was developed for the estimation of Apalutamide by RP-UPLC technique. Chromatographic conditions used are stationary phase BEH C18 (100mm 2.1mm 1.8µm), Mobile phase 0.01N KH_2PO_4: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 0.3ml/min, detection wave length was 234nm, column temperature was set to 30°C and diluent was mobile phase conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.5 for intermediate precision. LOD and LOQ are 0.24µg/ml and 0.72µg/ml respectively. By using above method assay of marketed formulation was carried out 99.32% was present. The described method for simultaneous determination of Abiraterone and Enzalutamide was validated successfully and provides a useful tool for therapeutic drug monitoring in patients treated with these agents.

Key words: UPLC, Apalutamide, Method development, ICH Guidelines.

1. INTRODUCTION :

Apalutamide chemically known as 4-{7-[6-cyano-5-(trifluoromethyl)pyridin-3-yl]-8-oxo-6- sulfanylidene-5,7-diazaspiro[3.4]octan-5-yl}-2-fluoro-N-methylbenzamide**.**It is a potent androgen receptor antagonist that selectively binds to the ligand-binding domain and blocks nuclear translocation [1]. Indicated for the treatment of patients with non-metastatic, castration-resistant prostate cancer (NM-CRPC). Persistent androgen receptor (AR) signaling is a common feature of castration-resistant prostate cancer (CRPC), attributed to AR gene-amplification, AR gene mutation, increased AR expression or increased androgen biosynthesis in prostate tumors [2]. Its a main metabolite, N-desmethyl apalutamide, is a less potent inhibitor of AR, and Apalutamide primarily undergoes CYP2C8 and CYP3A4-mediated metabolism to its pharmacologically active metabolite, N-desmethyl apalutamide. The contribution of CYP2C8 and CYP3A4 in the total metabolism of apalutamide is approximately 58% and and 13% following single dose but changes to 40% and 37%, respectively at steady-state.

Structure:



Method validation was performed as per regulatory guideline. The assay had a good linearity over the range of 0.93-2000 ng/mL. The intra and inter-batch accuracy and precision (%RE & RSD) across quality controls met the acceptance criteria for all the analytes. Stability studies showed that all the analytes were stable on DBS cards for one month. This novel method has been applied to analyze the DBS samples of enzalutamide, N-desmethylenzalutamide, darolutamide and ORM-15341 obtained from a pharmacokinetic study in mice.

2. MATERIALS AND METHODS:

2.1 APPARATUS AND CHROMATOGRAPHIC PARAMETERS:

UPLC instrument used was of waters Uplc System with Auto Injector and Acquity TUV detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Apalutamide solutions. Sonicator (Ultrasonic sonicator) P^H meter (Thermo scientific) Micro balance (Sartorius) Vacuum filter pump.

2.2 Drug samples:

Apalutamide pure drugs (API), Combination Apalutamide tablets (ERLEADA), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from RANKEM.

2.3 Reagents and solutions:

Methanol HPLC Grade (RANKEM), Acetonitrile HPLC Grade (RANKEM), HPLC grade Water (RANKEM) Glacial Acetic acid.

2.4 Analytical methodology

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 30mg of Apalutamide transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (600µg/ml of Apalutamide).

Preparation of Standard working solutions (100% solution): 1ml of Apalutamide from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml of Apalutamide).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (600 µg/ml of Apalutamide).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Apalutamide)

Preparation of buffer:

Buffer: 0.01N Potassium dihyrogen ortho phosphate:

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution.

0.1%OPA Buffer: 1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Mobile phase:

Acetonitrile 50%,water 50%

Linearity sample Preparation: Accurately weighed 30mg of Apalutamide transferred 50ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (600µg/ml of Apalutamide). **25% Standard solution:** 0.25ml each **50% Standard solution:** 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml (30µg/ml of Apalutamide).

3.RESULTS AND DISCUSSION:

METHOD DEVOLOPMENT:

Based on drug solubility and p^{ka} value following conditions has been usd to develop the method estimation of Apalutamide.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

A Standard solution of Apalutamide working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 1.

Optimization Peak

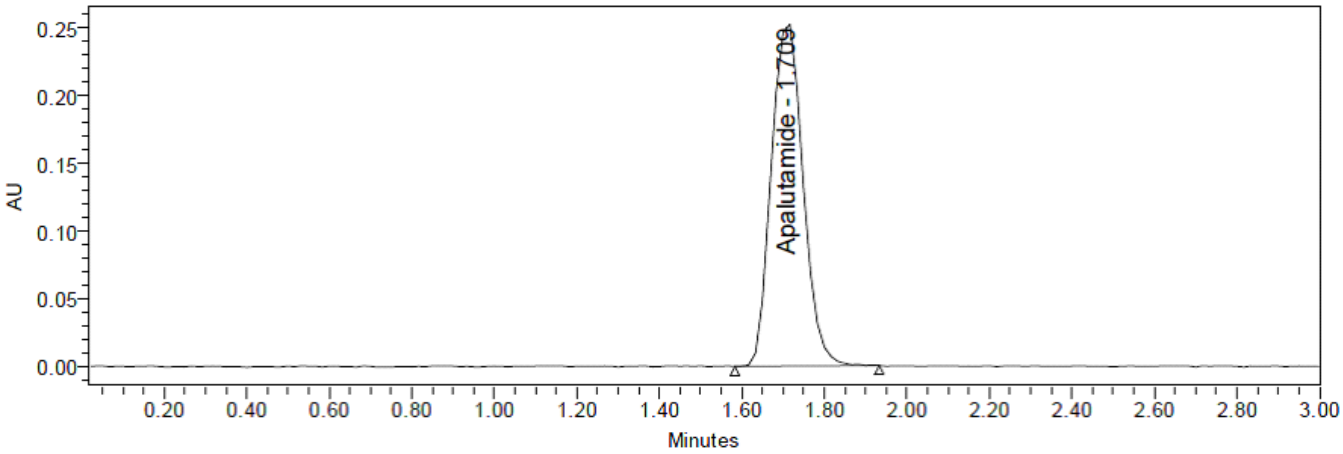


fig:1

Table 1. **Optimization Data**

Peak Name: Apalutamide

	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Apalutamide	1.709	1357473	100.00	2261	1.13
2	Apalutamide	1.709	1350040	100.00	2353	1.12
3	Apalutamide	1.714	1370390	100.00	2284	1.13
4	Apalutamide	1.715	1367344	100.00	2325	1.13
5	Apalutamide	1.716	1368357	100.00	2391	1.12
6	Apalutamide	1.717	1363074	100.00	2408	1.13
Mean			1362780			
Std. Dev.			7751.2			
% RSD			0.6			

4. METHOD VALIDATION:

4.1 LINEARITY:

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 15 ppm to 90 ppm of Apalutamide. Plot a graph to concentration versus peak area. Slope obtained was 23273 Y-Intercept was 15956 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig 2

Table 2 : Linearity Concentration and Responses

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	15	391552
50	30	728269
75	45	1053249
100	60	1374433
125	75	1773804
150	90	2121290

4.2 Precision:

Preparation of Standard stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (600µg/ml of Apalutamide).

Repeatability: Six working sample solutions of 60ppm are injected and the % Amount found was calculated and %RSD was found to be 0.5 and chromatogram was shown in fig 3

Table 3: Repeatability data:

S.No	Peak Area
1	1353177
2	1361994
3	1365293
4	1364527
5	1359048
6	1349813
AVG	1358975
STDEV	6283.6
%RSD	0.5

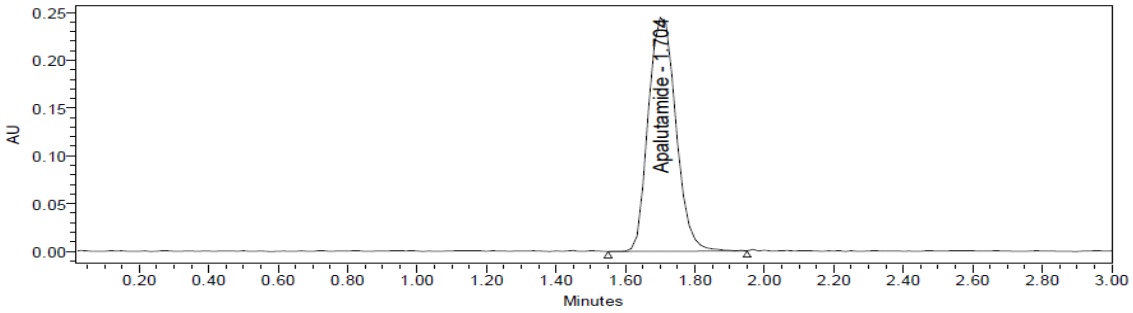


Fig:3

Intermediate precision: Five working sample solutions of 60ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.7and chromatogram was shown in fig 4

Table 4: Intermediate precision data:

S.No	Peak Area
1	1319612
2	1311423
3	1301815
4	1320846
5	1328057
6	1322743
AVG	1317416
STDEV	9354.2
%RSD	0.7

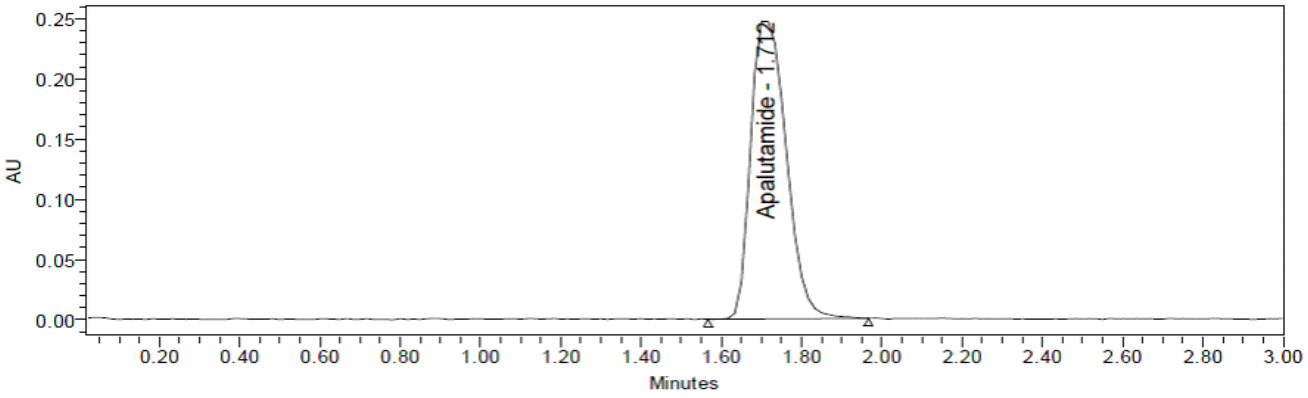


fig: 4

4.3 Accuracy:

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.14. And chromatograms were shown in fig **5**

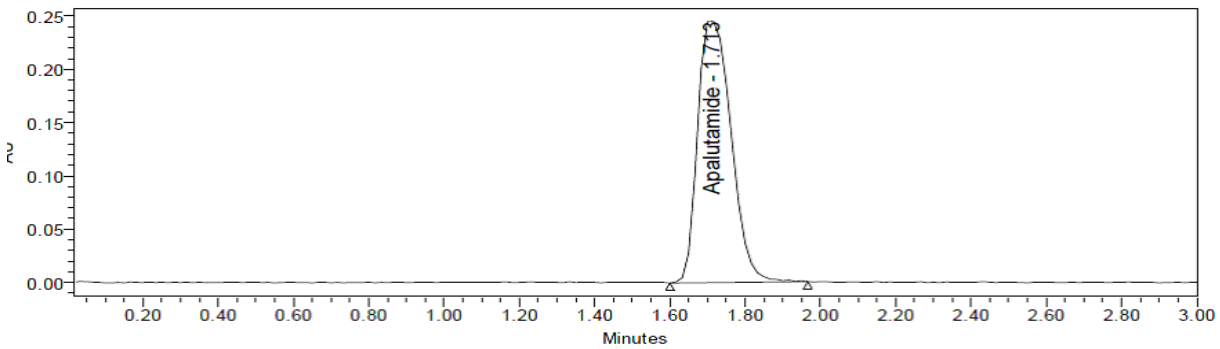


fig: 4

Table:5 Accuracy data:

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	30.01	100.02	100.14%
	30	29.96	99.88	
	30	30.02	100.06	
100%	60	59.91	99.85	
	60	60.38	100.63	
	60	60.08	100.13	
150%	90	90.10	100.12	
	90	89.66	99.62	
	90	90.82	100.91	

4.4 Robustness:

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated.

Table:6 Robustness Data

Parameter	%RSD
Flow Minus(0.27ml/min)	0.8
Flow Plus(0.33ml/min)	0.3
Mobile phase Minus(55:45)	1.0
Mobile phase Plus(45:55)	0.6
Temperature minus (25^0C)	0.9
Temperature plus (35^0C)	1.0

4.5 Limit of detection and limit of quantitation:

LOD: Detection and limit of the Apalutamide in this method was found to be 0.24µg/ml.

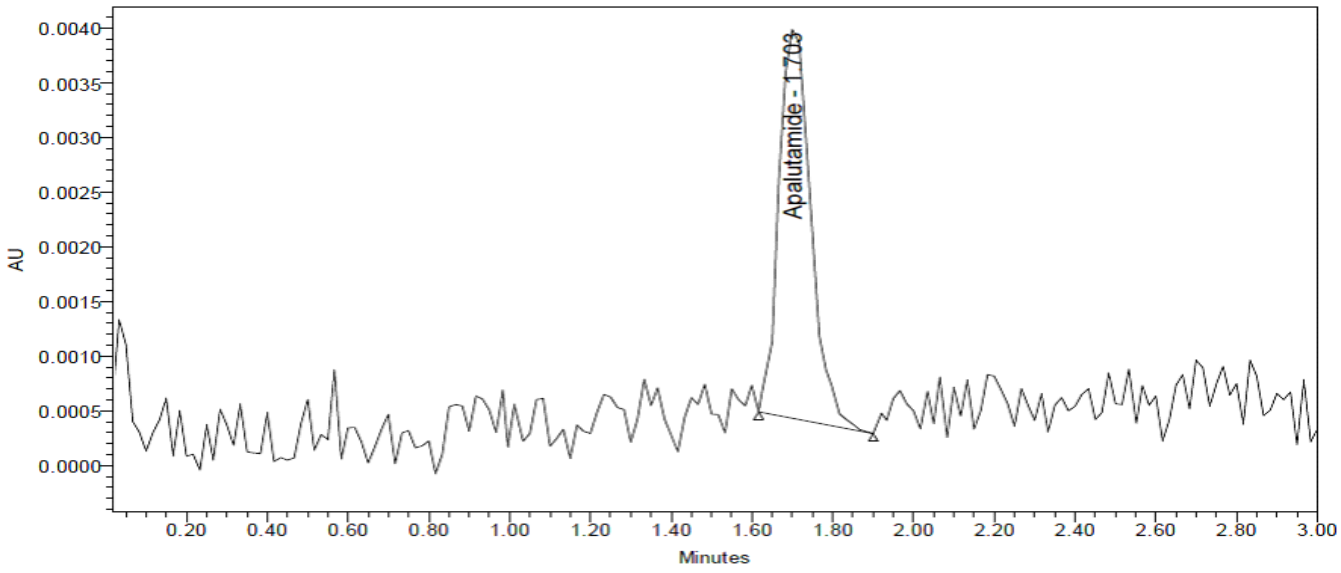


fig:6

LOD Chromatogram of Apalutamide

LOQ: Quantification limit of the Apalutamide in this method was found to be 0.72µg/ml.

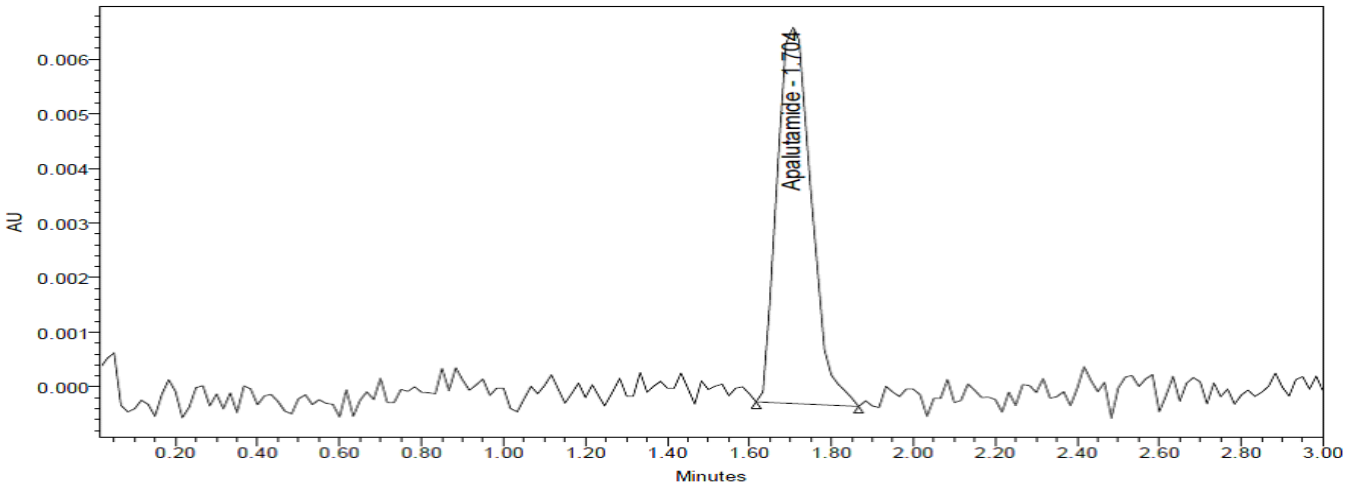


fig: 7

LOQ Chromatogram of Apalutamide

5. DEGRADATION:

5.1 Degradation Studies:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table:7 Degradation data of apalutamide.

S.NO	Degradation Condition	% Drug UN Degraded	% Drug Degraded
1	Acid	90.82	9.18
2	Alkali	92.55	7.45
3	Oxidation	94.31	9.42
4	Thermal	96.52	3.48
5	UV	98.73	1.27
6	Water	98.73	1.27

6. CONCLUSION:

Chromatographic conditions used are stationary phase BEH C18 (100mm 2.1mm1.8), Mobile phase 0.01N KH_2PO_4: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 0.3ml/min, detection wave length was 234nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999.Precision was found to be 0.6 for repeatability and 0.5 for intermediate precision.LOD and LOQ are 0.24µg/ml and 0.72µg/ml respectively. By using above method assay of marketed formulation was carried out 99.32% was present. Degradation studies of Apalutamide were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Apalutamide.

7. REFERENCES:

1. Rathkopf DE, Morris MJ, Fox JJ, Danila DC, Slovin SF, Hager JH, et al. (October 2013). "Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer". Journal of Clinical Oncology. 31 (28): 3525–30. doi:10.1200/JCO.2013.50.1684. PMC 3782148. PMID 24002508.

2. Al-Salama ZT (April 2018). "Apalutamide: First Global Approval". Drugs. doi:10.1007/s40265-018-0900-z. PMID 29626324.

3. Chong JT, Oh WK, Liaw BC (2018). "Profile of apalutamide in the treatment of metastatic castration-resistant prostate cancer: evidence to date". Onco Targets Ther. 11: 2141–2147. doi:10.2147/OTT.S147168. PMC 5905496. PMID 29695920.

4. Dellis AE, Papatsoris AG (June 2018). "Apalutamide: The established and emerging roles in the treatment of advanced prostate cancer". Expert Opin Investig Drugs. doi:10.1080/13543784.2018.1484107. PMID 29856649.

5. Clegg NJ, Wongvipat J, Joseph JD, Tran C, Ouk S, Dilhas A, et al. (March 2012). "ARN-509: a novel antiandrogen for prostate cancer treatment". Cancer Research. 72 (6): 1494–503. doi:10.1158/0008-5472.CAN-11-3948. PMC 3306502. PMID 22266222.

6. Schweizer MT, Antonarakis ES (August 2012). "Abiraterone and other novel androgen-directed strategies for the treatment of prostate cancer: a new era of hormonal therapies is born". Therapeutic Advances in Urology. 4 (4): 167–78. doi:10.1177/1756287212452196. PMC 3398601. PMID 22852027.

7. Leibowitz-Amit R, Joshua AM (December 2012). "Targeting the androgen receptor in the management of castration-resistant prostate cancer: rationale, progress, and future directions". Current Oncology. 19 (Suppl 3): S22–31. doi:10.3747/co.19.1281. PMC 3553559. PMID 23355790.

8. Rathkopf D, Scher HI (2013). "Androgen receptor antagonists in castration-resistant prostate cancer". Cancer Journal. 19 (1): 43–9. doi:10.1097/PPO.0b013e318282635a. PMC 3788593. PMID 23337756.

9. Kim W, Ryan CJ (February 2015). "Quo vadis: advanced prostate cancer-clinical care and clinical research in the era of multiple androgen receptor-directed therapies". Cancer. 121 (3): 361–71. doi:10.1002/cncr.28929. PMID 25236176.

10. Kawahara, Takashi; Miyamoto, Hiroshi (2014). "Androgen Receptor Antagonists in the Treatment of Prostate Cancer". Clinical Immunology, Endocrine & Metabolic Drugs. 1 (1): 11–19. doi:10.2174/22127070114019990002. ISSN 2212-7070.

11. Patel JC, Maughan BL, Agarwal AM, Batten JA, Zhang TY, Agarwal N (2013). "Emerging molecularly targeted therapies in castration refractory prostate cancer". Prostate Cancer. 2013: 981684. doi:10.1155/2013/981684. PMC 3684034. PMID 23819055.

12. Pinto Á (February 2014). "Beyond abiraterone: new hormonal therapies for metastatic castration-resistant prostate cancer". Cancer Biology & Therapy. 15 (2): 149–55. doi:10.4161/cbt.26724. PMC 3928129. PMID 24100689.

13. Anderson J (March 2003). "The role of antiandrogen monotherapy in the treatment of prostate cancer". BJU Int. 91 (5): 455–61. PMID 12603397.

14. Ya-Xiong Tao (11 June 2014). Pharmacology and Therapeutics of Constitutively Active Receptors. Elsevier Science. pp. 351–. ISBN 978-0-12-417206-7. ARN-509 is related structurally to enzalutamide with greater in vivo activity in CRPC xenograft models (Clegg et al., 2012).