

## Article

## **Cross-Resistance to Abiraterone and Enzalutamide in Castration Resistance Prostate Cancer Cellular Models is Mediated by AR Transcriptional Reactivation**



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**Figure S1.** Development process of Resistance to ADT and establishment of the second line treatment (**A**) ADT Resistance development of LNCaP and 22RV1 cells lines (R-ADT). Wild-type cell lines, sensitive cells to ADT, were exposed to medium with charcoal stripped serum (CSS) for 6 months. (**B**) Second line treatment of R-ADT cells. R-ADT cells were treated with 40  $\mu$ M Enzalutamide (Enz) (R-ADT +E), 20  $\mu$ M Abiraterona (AA) (R-ADT + AA) or 40  $\mu$ M Enz plus 20  $\mu$ M AA (R-ADT + E+A) for five days.



**Figure S2.** Development process of Resistance to ADT and new antiandrogens (Enz and/or AA) and establishment of the second line treatment. Novel Hormonal Agents (NHAs) resistance development of LNCaP and 22RV1 cells lines: (**A**) R-ADT/Enz; (**B**) R-ADT/AA and (**C**) R-ADT/E+AA. Treatment with Enz and/or AA was increased in a stepwise manner up to 4 times until reaching the highest final concentrations (20  $\mu$ M for AA and 40  $\mu$ M for Enz). All the cells were grown with charcoal stripped serum (CSS). Second treatment line of R-ADT/NHAs cells line: (**D**) R-ADT/E cells were treated with 20  $\mu$ M AA for five days (R-ADT/E + AA); (**E**) R-ADT/AA cells were treated with 40  $\mu$ M Enz (R-ADT/AA + ENZ) for five days.

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**Figure S3.** Alignment of the CDS of the *AR-V7* and *AR-V9* isoforms and the sequenced qPCR products. Examples of the positive alignments obtained between the cloned qPCR products. (**A**) Sequence of *AR-V7* and (**B**) sequence of *AR-V9*.



**Figure S4.** Cell cycle analysis with flow cytometry in wild-type PCa cell lines grown in ordinary medium and hormone-reduced medium (CSS). A representative cell cycle profile is included for each cell line and growth condition. Cells were stained with propidium iodide and cell cycle phases were determined using ModFit LT<sup>TM</sup>. Every cell cycle phase was remarked in a different color:  $G_0/G_1$  peak (white),  $G_2/M$  (Light grey), S (dark grey) and Sub-G<sub>0</sub> (black). (A) LNCaP wild-type cells. (B) 22RV1 wild-type cells. (C) PC3 wild-type cells.



**Figure S5.** Heatmap representation of the expression levels of all the isoforms of AR (AR TOTAL), *AR full length, AR-V7* and *AR-V9* and their target genes in all cellular models. In addition to the transcription levels for AR, AR isoforms and AR target genes, we also included the concept of AR Activity (ACTIVITY) considering the average fold of the 9 AR target genes analyzed. The scale ranges from very low expression (less than 0.25-fold change), to low expression (from 0.25 to 0.5-fold change), no change (from 0.5 to 2-fold change), overexpressed (from 2 to 5-fold change) and highly overexpressed (more than 5-fold change).

AR Total FW	5'-CCACTTGTGTCAAAAGCGAA-3'
AR Total RV	5'-AAGACCTGCCTGATCTGTGG-3'
AR full length FW	5'-TGTCCATCTTGTCGTCTTCG-3'
AR full length RV	5'-TTCAGATTACCAAGTTTCTTCAG-3'
AR-V7 FW	5'-TGTCCATCTTGTCGTCTTCG-3'
AR-V7 RV	5'-TAGTCTGGAGAAACCT-3'
AR-V9 FW	5'-TGTCCATCTTGTCGTCTTCG-3'
<i>AR-V9</i> RV	5'-ACGTGATCCCAAAAGATGTG-3'
CDK1 FW	5'-TTTTCAGAGCTTTGGGCACT-3'
CDK1 RV	5'-CCATTTTGCCAGAAATTCGT-3'
CDK2 FW	5'-CATTCCTCTTCCCCTCATCA-3'
CDK2 RV	5'-CAGGGACTCCAAAAGCTCTG-3'
FGF8 FW	5'-GACCTACCAACTCTACAGCCG-3'
FGF8 RV	5'-CTCCTCGGACTCGAACTCTG-3'
FKBP5 FW	5'-TCCCTCGAATGCAACTCTCT-3'
FKBP5 RV	5'-AAACATCCTTCCACCACAGC-3'
KLK3 FW	5'-GTTGTCTTCCTCACCCTGTCC-3'
KLK3 RV	5'-GCAGCTGTGAGGACCCACT-3'
NDRG1 FW	5'-ACAACCCTGAGATGGTGGAG-3'
NDRG1 RV	5'-TGTGGACCACTTCCACGTTA-3'
PMEPA1 FW	5'-AAGATGCCCTGTCCTCAGAA-3'
PMEPA1 RV	5'-GTGCTGCAGGTACGGATAGG-3'
TMPRSS2 FW	5'-CACTGTGCATCACCTTGACC-3'
TMPRSS2 RV	5'-ACACGCCATCACACCAGTTA-3'
UBE2C FW	5'-ACCCAACATTGATAGTCCCTTG-3'
M13 FW	5'-GTAAAACGACGGCCAG-3'

Table S1. Primer list.