# Apalutamide radio-sensitises prostate cancer cells *in vitro* and *in vivo*

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## Introduction

Prostate cancer, at least at the first stages of the disease, depends on androgens for its growth and development. Bicalutamide is the most studied first generation antiandrogen and its combination with Radiation Therapy has been used as standard treatment for high risk and locally advanced prostate cancer. Apalutamide is a newer anti-androgen that exhibits a much higher affinity for the androgen receptor than bicalutamide. Replacement of bicalutamide with a more potent anti-androgen, like apalutamide, may provide significant clinical benefits for locally advanced disease.

## Methods

• The 22Rv1 hormone-sensitive prostate cancer cell line was used for this study.

•For proliferation experiments cell viability was measured using Alamar-Blue®.

•For immunofluorescence staining and monitoring nuclear catastrophe 22Rv1 cells were treated with the appropriate drug, and irradiated using 4Gy. Then, cells were fixed and stained with anti -gamma H2AX, anti- alpha-tubulin and anti-Ki67 antibodies.

•For the *in vivo* experiments fluorescent 22Rv1 cells were injected at the flank of R2G2 mice followed by detection of the mCherry signal by the IVIS Kinetics®.

## Results

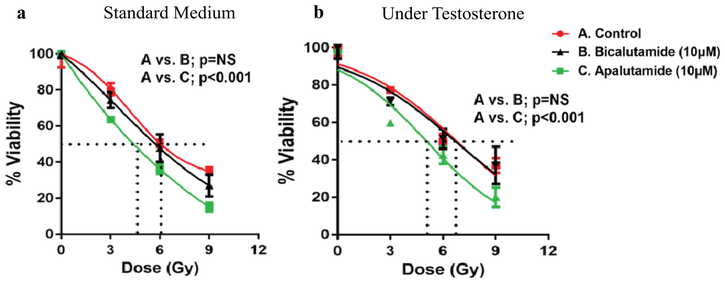


**Figure 1. Apalutamide radio-sensitises PCa cells in vitro.** Viability radiation dose–response curves of the 22Rv1 prostate cancer cell line, exposed to bicalutamide (BIC), apalutamide (APA), or no drug (control), in standard medium (a) and testosterone-enriched medium (b).

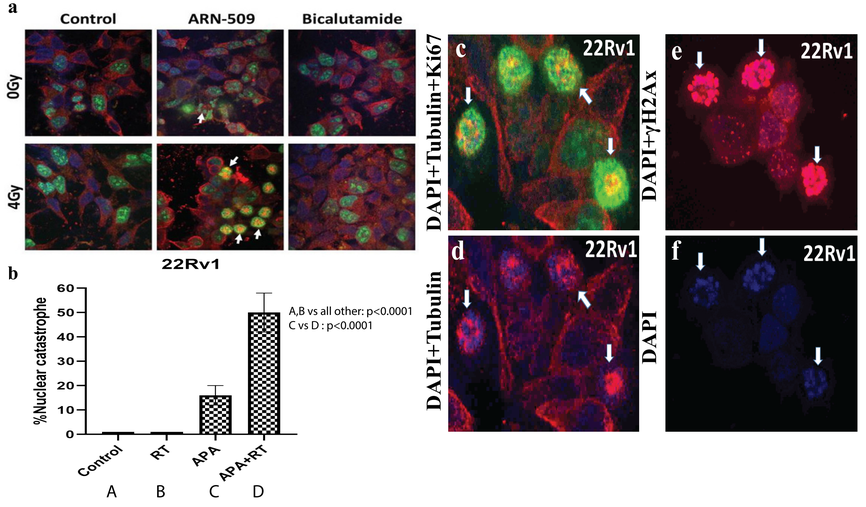


**Figure 2. γH2Ax abnormal localisation and nuclear catastrophe following apalutamide (APA) exposure and cell irradiation.** (a) Typical double immunostaining with tubulin/Ki-67 of 22Rv1 cell line, treated with APA or BIC, with or without irradiation. (b) Quantification of cells with karyorrhexis in 22Rv1 cell line, exposed to RT and/or anti-androgens. (c) Double immunostaining for Ki67 and tubulin in 22Rv1 cells exposed to RT plus apalutamide; (d) same images with DAPI and tubulin only staining. (e) γH2Ax immunostaining for 22Rv1 cells 24 h after exposure to APA and radiation; (f) same images with DAPI only. Arrows show the pyknotic cells with karyorrhexis that are evident in the apalutamide/ irradiation-treated cells.

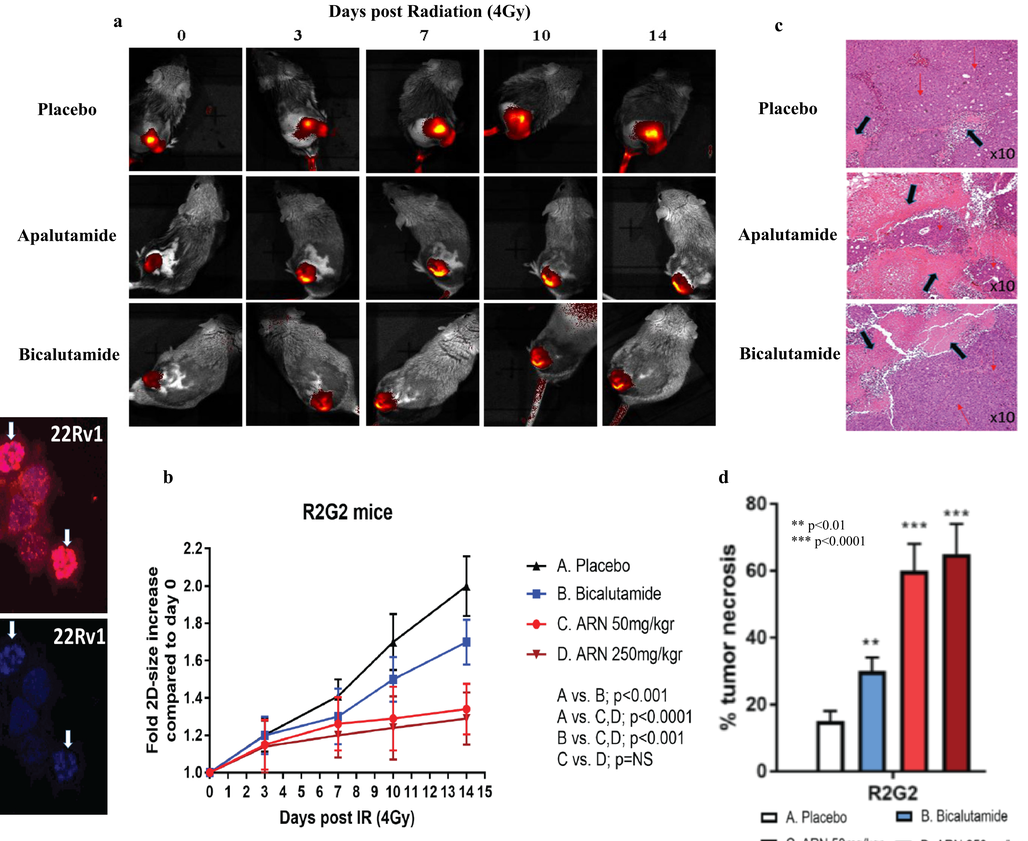


**Figure 3. Apalutamide radio-sensitises PCa cells *in vivo*.** Effect of apalutamide or bicalutamide combination with radiotherapy in 22Rv1 prostate cancer xenografts: (a) Typical images of R2G2 mice with fluorescent xenografts monitored for 14 days after therapy, with the IVIS kinetics system; (b) growth rate of xenografts growing in R2G2 mice after radiotherapy and its combination with bicalutamide or two-dose schedules of apalutamide. (c) Typical haematoxylin/eosin xenograft tissue sections after radiotherapy and its combination with bicalutamide or apalutamide, where necrosis is indicated with black arrows. (d) Quantification of the extent of necrosis in xenografts treated with radiotherapy ans its combination with bicalutamide or apalutamide.

## Conclusions

Apalutamide significantly enhances the effect of radiation on prostate cancer cells, *in vitro* and *in vivo*.
Apalutamide showed a strong radiosensitizing effect when combined with 4Gy of radiation, and this indicates its clinical significance in hypofractionated radiotherapy schemes.
The combination of Apalutamide with Radiation Therapy induces a specific type of cell death, which includes karyorrhexis and pyknosis.

**Our results provide the biological basis for the future design of clinical trials for the combined use of radiotherapy with second-generation antiandrogens.**

## References

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FUNDING

The study has been supported by a research grant (ARN-I-15-GRC-001-V02) from Janssen Pharmaceutica NV through Special Account for Research Funds (SARF) of DUTH.