NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	a.In this study 4 groups of NOD.SCID mice were compared to each other. Likewise, 4 groups of R2G2 mice were compared to each other. The control group consists of animals treated with placebo (vehicle control) plus Radiotherapy. The b.Single animal
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	a.In the present study 4 groups consisting of 7 animals each for the NOD.SCID mouse model and 4 groups consisting of 7 animals each for the R2G2 mouse model were used. The total number of animals being used was b. The calculation of sample size was held using the UCSF Biostatistics: Power and Sample Size Programs for the comparison of the mean values with continues variables
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Exclusion criteria: weight loss of 20% due to Radiotherapy or drug medication, any observed side effect due to drug medication (ulceration, intestinal obstruction, pain), any anxious behavior (decreased activity, No animals were excluded since none of the above exclusion criteria were observed. 7 mice in each experimental group
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	There was no randomization procedure applied. Random healthy mice were allocated to each experimental group.All mice were housed in ventilated cages, under the same conditions (continuous supply of standard rodent chow and water, 12 hour light-dark photoperiod at ambient temperature of 23
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	CK (co-author) was aware of group allocation and the treatment randomization. CK performed all the experimental procedures. IL(co-author) and EX (co-author) carried out the imaging session without knowing the treatments groups. AG performed the histopathological analysis
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Tumor growth in cm2 at days 0, 3, 7, 10, 14 post treatment with Radiotherapy. Tumor necrosis was assed 10 days after Radiotherapy via IHC. The primary outcome measurement was the tumor growth in correlation with the given treatment.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Statistical analysis and graph presentation were performed using the GraphPad Prism 7.0 (GraphPad Software Inc., USA). The unpaired two-tailed t-test or the Wilcoxon matched-pairs test were used to compare he calculation of sample size was held using the UCSF Biostatistics: Power and Sample Size Programs for the comparison of the mean values with continues variables
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Species: Mus musculus. Subspecies: Mus musculus musculus. Two GEMM (Genetically Engineered and Muted Mice) strains, the NOD SCID (NOD.CB17-Prkdcscid/J, Genotype: HOM Both strains are models of immunosuppressed mice, which lack functional T and B lymphocytes. In addition, the R2G2 mouse model lacks natural killer
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Species: Mus musculus. Subspecies: Mus musculus musculus. Two GEMM (Genetically Engineered and Muted Mice) strains, the NOD SCID No anaesthesia was used in this study as the only invasive procedures were the subcutaneous injections. Xenografts iniections as well as the 4 iniections of the For the euthanasia, the animals were anesthetized using ketamine (70mg/kg via Intraperitoneal injection) followed by cervical dislocation
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	SCID MICE Placebo-Bicalut -ARN 50-ARN 250 Number - 5- 5- 5 - 5 ---- Minimum- 1- 1- 1 - 1 R2G2 Placebo-Bicalutamide -ARN50-ARN250 Number- 5- 5- 5- 5 Minimum- 1- 1- 1 - 1

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	The research objectives of the in vivo experime
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Anti-androgenic hormone therapy with bicalutamide, in combination with local radiotherapy, is the golden standard treatment for high-risk and locally advanced prostate cancer (1,2). Apalutamide is a Both mouse models used in the current study are highly immunodefficient, rendering them an appropriate model to examine xenografts growth patterns and responses to treatments. Our in vitro results were
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	The main hypothesis of this study is whether th
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	The study has been approved by the local Animal Experimentation Research Committee and the Ethics Committee of the Democritus University of Thrace. The Veterinary Direction also approved all experimental procedures for Animal Research in the Department of Experimental Surgery at the Democritus University of Thrace.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Mice were housed in individual ventilated cages
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	All mice were familiarized in advance with the researcher that conducted all the experiments. None of the experimental procedures were stressful or painful. Interventions are not required, since the No unexpected adverse effect were recorded throughout the experiment. An expected side effect was a minor loss of body weight (5-10%) due to Radiotherapy, which did not affect the well-being of the animals. Humane endpoints: weight loss of 20% due to Radiotherapy or drug medication, any observed severe side effect due to drug medication, painful symptoms or another adverse effect on their general condition due
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	The in vivo confirmation of the important in vitro radiosensitization conferred by apalutamide over bicalutamide is original and has not been tested in the past. It opens a new window to clinical trials on the Experiments with xenografts and immunocomprimised animals have profound limitations as these models do not represent the actual tumor microenvironment (no adequateblood supply and angiogenesis, no
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	The combination of Radiotherapy with Bicalutamide is used commonly in clinical practice offering better results than Radiotherapy alone for the treatment of prostate cancer. In the present study we showed that Apalutamide exhibits a significantly stronger radio-sensitizing effect than Bicalutamide against human-derived
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	The experimental protocol was designed in advance and it was submitted to the ethical committees for approval and to Janssen Pharmaceutical for funding.
Data access	20	Provide a statement describing if and where study data are available.	A report with all the results included in this stud
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	The study has been supported by a research grant (ARN-I-15-GRC-001-V02) from Janssen Pharmaceutical (Principal Investigator MI Koukourakis). There are no other conflicts of interest to report. The study has been supported by a research grant (ARN-I-15-GRC-001-V02) from Janssen Pharmaceutical. Janssen Pharmaceutical was not involved in the