



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: - The groups being compared, including control groups. If no control group has been used, the rationale should be stated. - The experimental unit (e.g. a single animal, litter, or cage of animals).	Groups compared: wild-type (W, control) and GABRG1-knockout (KO) mice; bladder tumours were induced in both groups using orthotopic and subcutaneous models. Wild-type mice served as the control group. The experimental unit was the individual mouse (in vivo) and biological replicates (n = 3 per group) for in vitro experiments.
Sample size	2 - 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. - Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	bladder (n = 3 per group, 2 groups; 6 mice); flow cytometry analysis of tumour-infiltrating immune cells was performed using 3 mice per group selected from the above cohorts (no additional animals). Total number of animals used: 46 mice. Sample size was determined based on previous similar studies; no formal <i>a priori</i> sample size calculation was performed.
Inclusion and exclusion criteria	3 - 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 <i>a priori</i>. If no criteria were set, state this explicitly. - 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. - For each analysis, report the exact value of <i>n</i> in each experimental group.	No specific inclusion/exclusion criteria for animals or data points were established <i>a priori</i> ; all animals that reached the experimental endpoint were included in the analyses. No animals, experimental units or data points were excluded from the analyses.
Randomisation	4 - 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. - 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.	Animals were allocated to experimental groups according to their genotype (wild-type vs GABRG1-knockout); randomisation was therefore not applicable for group allocation, and no randomisation sequence was generated. Potential confounders (e.g., order of surgery and measurements, animal/cage location) were not explicitly controlled.
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).	Blinding was not performed; investigators were aware of group allocation during the conduct of the experiment, outcome assessment and data analysis.
Outcome measures	6 - Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). - For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	gene expression (RNA-seq, RT-PCR, qPCR); flow cytometry analysis of tumour-infiltrating immune cells (CD45/CD3/CD8; CD45/F4/80/CD206); immunohistochemistry and flow cytometry; CD8 expression (transcriptome sequencing and qPCR). The primary outcome measure was tumour weight (tumour progression).
Statistical methods	7 - Provide details of the statistical methods used for each analysis, including software used. - 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.	For two-group comparisons and one-way ANOVA for multi-group comparisons. Non-normally distributed data were analysed using the Mann-Whitney U test or Kruskal-Wallis test. $P < 0.05$ was considered statistically significant. Assessed before statistical analysis; data meeting the normality assumption were analysed by parametric tests, and data not meeting the assumption were analysed by the corresponding non-parametric tests (Mann-Whitney U or Kruskal-Wallis).
Experimental animals	8 - Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. - Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Species: mouse. Strain: C57BL/6J, male mice, 6–8 weeks old, weighing 25–30 g. Experimental conditions. The KO group comprised GABRG1 gene-knockout mice, and the W group comprised wild-type mice without genetic modification. No previous experimental procedures were performed. The animals were healthy, with normal immune status.
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: - What was done, how it was done and what was used. - When and how often. - Where (including detail of any acclimatisation periods). - Why (provide rationale for procedures).	Immunohistochemistry (CD8, F4/80, CD163, FOXP3); flow cytometry of tumour-infiltrating immune cells (CD45/CD3/CD8; CD45/F4/80/CD206); macrophage depletion with clodronate liposomes (200 μ L, i.p.). At 24 h; tumours were established and samples collected 14 days after implantation; clodronate liposomes were administered on days 0 and 7 post-tumour implantation. Conducted in the animal facility of the Institute of Urology, Chinese PLA General Hospital. The animals were acclimatised for 7 days after arrival before the start of the experiment. Reliable tumour size measurement; the macrophage-depletion experiment was designed to verify the role of M2 macrophages in suppressing CD8 ⁺ T-cell infiltration.
Results	10 For each experiment conducted, including independent replications, report: - Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). - If applicable, the effect size with a confidence interval.	Summary/descriptive statistics (mean $\pm$ SD) with <i>P</i> values are reported for each experimental group throughout the Results section (e.g., activation-marker proportions, tumour weights, cytokine concentrations and immune-cell proportions). Effect sizes with confidence intervals were not reported; group comparisons are presented as mean $\pm$ SD with <i>P</i> values.

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.	11 dysfunction) and the study conclusions.
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.	12 sufficient scientific background on bladder cancer epidemiology and treatment, CD8+ T cell-mediated anti-tumour immunity, and GABA/GABA _A receptor biology, and explains the rationale and experimental approach of the study. 13 r of human bladder cancer and directly addresses the study objectives. The relevance to human biology is discussed in the Discussion (e.g., GABRA6 loss-of-function mutations occur in 4.38% of human bladder cancers).
Objectives	13 Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	13 targets for bladder cancer immunotherapy.
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.	14 Materials and methods, 'Animal experiments' (line 35): the experimental protocol was fully reviewed and formally approved by the Laboratory Animal Welfare and Ethics Committee of SinoResearch (Beijing) Biotechnology Co., Ltd. (Approval No.: ZY22 02511006S). The study endorses the ARRIVE guidelines.
Housing and husbandry	15 Provide details of housing and husbandry conditions, including any environmental enrichment.	15 11 mice had free access to food and water.
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.	16 Avertin anaesthesia was used for all surgical procedures; post-operative care included placing mice on a heating pad to prevent hypothermia and iodine disinfection of the incision (Materials and methods, line 39). No adverse events were observed during the study. Specific humane endpoints were not predefined; animals were monitored for general condition and tumour burden, and euthanised at the scheduled experimental endpoint (day 14 after tumour implantation).
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.	17 Discussion (lines 74–80): the results are interpreted in the context of the study objectives and current literature on GABA/GABA _A receptor signalling in cancer and tumour immunity. Study limitations include the relatively small sample size, the use of a single cell line (MB49) and model, and the unverified underlying mechanism.
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).	18 ssed. GABRA6 loss-of-function mutations occur in 4.38% of human bladder cancer specimens (TCGA data), and our findings suggest that combining macrophage inhibition with PD-1/PD-L1 blockade may enhance immunotherapy efficacy in patients with GABRA6 mutations. Further studies are needed to confirm generalisation to other species and experimental conditions.
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.	19 No study protocol was prepared or registered before the study.
Data access	20 Provide a statement describing if and where study data are available.	20 .nih.gov/geo/query/acc.cgi?acc=GSE347180).
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.	21 Conflicts of interest (lines 151–152): 'The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.' Funding (lines 154–155): 'None.' No external funding was received, and therefore no funder had a role in the design, analysis or reporting of the study.