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).	2.9 The experiment included two groups: control group (vehicle-treated) and AO group. All groups were treated identically except for the intervention. 2.9 The experimental unit was the individual animal (mouse).
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.	2.9 A total of 10 mice were used across all experiments. Each experimental group contained 5 animals. 2.9 No formal a priori power analysis was performed because no preliminary data were available to estimate the effect size and variance for this exploratory study. Based on our previous experience with similar intracranial xenograft models
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.	2.9 Animals were included if they were healthy at the start of the experiment and weighed between 16 - 18 g. Inclusion and exclusion criteria were established a priori. Inclusion criteria for mice were: successful subcutaneous tumor implantat 2.9 No animals were excluded from the final analysis. 3.5 Sample sizes varied by analysis. Tumor volume and body weight: n = 5 per group. Immunohistochemistry and other analyses: n = 3 per group. In all statistical graphs, each data point represents an i
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.	2.9 Yes. Animals were randomly allocated to experimental groups. An independent investigator not involved in the study generated the randomization sequence and allocated mice to groups. 2.9 No specific measures were taken to control for treatment order, measurement order, or cage location. Animals were housed under standard conditions.
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).	ment, bioluminescence imaging analysis, H&E staining evaluation, and immunofluorescence quantification were performed by investigators blinded to group allocation. Data analysis: Statistical analysis was performed by an investigator blinded to group allocation.
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.	3.5 Tumor volume (primary); body weight and overall survival (secondary); Ki67 expression, MDA and GSH levels, and protein markers including p-cdc2, cdc2, γ-H2AX, c-caspase3, and GAPDH (exploratory). 2.9 Tumor volume was the prespecified primary outcome for in vivo efficacy. However, no formal a priori power analysis was performed because no preliminary data were available to estimate the effect size.
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.	2.11 All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc., USA). Data are presented as mean ± standard error of the mean (SEM). The normality of data distribution was assessed using the Shapiro-Wilk test 2.11 All variables met the statistical assumptions, as reported in the manuscript.
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.	2.9 Ten SPF female BALB/c-nu nude mice (4 weeks old) were purchased from Chengdu GemPharmatech Co. Ltd (Chengdu, China). 2.9 Animals were acclimatised to the housing facility for 7 days before the start of the experiment.
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).	2.9 The U87 cell suspension (100,000 cells) was subcutaneously injected into one side of the mouse axilla using a sterile syringe. 2.9 Beginning on the second day after cell implantation, the mice were intraperitoneally administered either normal saline or AO (20 mg/kg) once daily for three consecutive weeks. and their body weieh 2.9 SPF animal house 2.9 Female BALB/c nude mice were selected because they are immunodeficient and thus permit engraftment and growth of human GB cells without immune rejection.
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.	3.5 For all quantitative experiments, data are presented as mean ± standard error of the mean (SEM) for each experimental group, unless otherwise stated. Sample sizes are indicated in the figure legends or by the number of individual data 2.9 No effect sizes or confidence intervals were calculated for the animal experiments. This was an exploratory study, and effect size reporting was not prespecified in the study design.

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.	3.1-3.5 The therapeutic effect of AO was e
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.	2.9 The U87 cell suspension (100,000 cell s) was subcutaneously injected into one s ide of the mouse axilla using a sterile s yringe.The subcutaneous model was chosen because it allows reproducible tumor volu me measurement, easy access for intraperi 2.9 BALB/c nude mice are immunodeficient and therefore support the engraftment and growth of human GB cells without immune rejection. The subcutaneous xenograft mod el is a standard preclinical model for ev aluating tumor growth and drug response b
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	3.5 The effect of AO on the in vivo growth
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.	See "Ethics Approval and Consent to Parti cipate". Ethics Approval and Consent to P articipate: The animal experiments were a pproved by the Ethics Committee of Southw est Medical University (approval number: 20240927-001) and corresponded to the pro visions in the Guidelines for the Managem ent and Use of Laboratory Animals of Sout hwester Medical University.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	2.9 Heaithy four-week-old female BALB/c nu
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.	2.9 The mice were deeply anesthetized wit h sodium pentobarbital (50 mg/kg, i.p.) b efore euthanasia by cervical dislocation. 3.5 No unexpected adverse e vents were observed during the study. 2.9 No humane endpoi nts were established .
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.	4 These results demonstrate that AO exert s its anti-proliferative and pro-apoptoti c effects on GB cells primarily through a ROS-dependent mechanism, consistent with the hypothesis that AO, as a structural analog of DOX, shares its ROS-mediated cy 5 The blood-brain barrier (BBB) penetrati on capability of AO, a critical factor fo r treating intracranial GB, was not asses sed in this subcutaneous model.
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).	5 Future studies employing orthotopic GB models are es sential to evaluate its eff icacy and pharmacokinetics within the brain parenchyma .
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.	2.9 A detailed study protocol, including the research question, key design feature s, sample size justification, randomizati on and blinding procedures, outcome measu res, and statistical analysis plan, was p repared before the experiments and approv ed by the Animal Ethics Committee of the Affiliated Hospital of Southwest Medical University. This study was not preregiste
Data access	20	Provide a statement describing if and where study data are available.	"Availability of Data and Materials". All
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.	See "Conflicts of Interest" .The authors declare that t hey have no conflicts of in terest. See "Funding Statement". This study was s upported by University Science and Techno logy Strategic Cooperation Project (2024S NXNYD03 to Zhengjun Zhou).