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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).	Four groups were compared: Control, CAV2sh, crizotinib, and CAV2sh + crizotinib. Control mice were inoculated with empty-vector H1993 cells; crizotinib mice were inoculated with empty-vector H1993 cells and treated with crizotinib; CAV2sh mice were inoculated with CAV2sh-transduced A 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.	Eight mice were allocated to each group, with 32 mice used in total. The sample size was determined based on previous studies using similar xenograft models and treatment designs. No formal a priori sample size calculation was performed.
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.	Female BALB/c nude mice in good health and with appropriate body weight before tumor inoculation were included. Exclusion criteria were established a priori and included failure of tumor establishment, severe illness unrelated to tumor burden, or death unrelated No animals or data points were excluded from the analysis. The exact n was 8 per 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.	Mice were randomly allocated to experimental groups using a random number table. To reduce potential confounders, all mice were maintained under the same SPF conditions, and tumor measurements and sample analyses were performed by investigators blinded to group allocation.
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).	The investigator responsible for animal numbering and group allocation was aware of the allocation. Investigators responsible for tumor measurement, sample collection, and data analysis were 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.	Outcome measures included tumor volume and tumor weight. Tumor volume was the primary outcome measure. Tumor weight and survival were secondary outcome measures.
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 analyses were performed using GraphPad Prism 8. Tumor growth curves were analyzed using two-way repeated-measures ANOVA, followed by Bonferroni-adjusted comparisons between the indicated groups. Endpoint tumor weights were analyzed using one-way ANOVA followed by Bonferroni's
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.	Female BALB/c nude mice, 5 weeks old and weighing 20-22 g at tumor inoculation, were obtained from GemPharmatech Co., Ltd. The mice were immunodeficient nude mice and had not undergone previous experimental procedures before this study.
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).	9a. What was done, how it was done and what was used: H1993 cells transduced with empty-vector or CAV2sh lentivirus were mixed with Matrigel and subcutaneously injected into the left axilla 9b. When and how often: Mice were inoculated with tumor cells after acclimatisation. Crizotinib treatment was started when tumors reached approximately 50-100 mm³ and was administered once daily 9c. Where, including acclimatisation: All animal procedures were performed in the specific pathogen-free animal facility of Huazhong University of Science and Technology. Mice were acclimatised under 9d. Why: The H1993 xenograft model was used to evaluate the in vivo role of CAV2 knockdown in lung cancer tumor growth and crizotinib response. Crizotinib treatment was initiated
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.	10a. Summary/descriptive statistics: For each experimental group, tumor volume, tumor weight, and survival outcomes are reported in the Results section and figure legends. Tumor volume and tumor weight are presented as mean ± SD, with individual 10b. Effect size with confidence interval: No separate effect-size estimates with confidence intervals were calculated. The magnitude and variability of the treatment effects are presented using group means, SDs, individual data points, and longitudinal

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 Abstract summarizes the research objecti
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.	12a. The scientific background and rationale are described in the Introduction. Briefly, MET dysregulation contributes to lung cancer progression, cancer stemness, and resistance to MET-targeted therapy. This study investigated whether CAV2-mediated 12b. Female BALB/c nude mice were used because their immunodeficient status allows efficient establishment of human lung cancer xenografts. The subcutaneous H1993 xenograft model was selected to assess tumor growth and treatment response in vivo, using
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	The objective was to determine whether CAV2
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 animal study was approved by the Experimental Animal Ethics Committee of Huazhong University of Science and Technology (Project title: Mechanistic study of MET-mediated CAV2 regulation in maintaining lung cancer stemness and mediating MET-TKI resistance; Approval No. 386-20231211; Date of approval: 2024-02-01).
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Mice were housed in a specific pathogen-free
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.	Mice were monitored for tumor growth, body condition, and signs of distress. Tumor size was measured every 3 days. No expected or unexpected adverse events, including treatment-related death, severe body weight loss, tumor ulceration, impaired mobility, or signs of severe distress, were observed during the experiment. Humane endpoints included tumor volume reaching 2000 mm^3, maximum tumor diameter reaching 20 mm, tumor ulceration, marked body weight loss, impaired mobility, or severe distress. No unexpected adverse events occurred.
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 animal data support the role of CAV2 knockdown in suppressing tumor growth and enhancing crizotinib response in vivo. Limitations include the use of immunodeficient mice and a subcutaneous rather than orthotopic xenograft 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).	The findings are relevant to MET-driven lung cancer and MET-TKI resistance, but translation to human disease should consider the limitations of subcutaneous xenografts and immunodeficient mouse models.
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.	No formal protocol registration was performed. The animal experiments were conducted according to the approved institutional animal protocol.
Data access	20	Provide a statement describing if and where study data are available.	The datasets generated and/or analyzed durin
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 authors declare that they have no competing interests. This research was supported by the National Natural Science Foundation of China (Grant No. 82172716), the Beijing Xisike Clinical Oncology Research Foundation (Grant No. Y-2023AZMETMS-0013), the Hubei Provincial Natural Science Foundation of China (Grant