The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title Page
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Introduction
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	See attached
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	See attached
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	See attached
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	See attached and Results
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	See attached

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	See attached
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	See attached
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	See attached
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Results and attached
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	See attached
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	See attached
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	See attached
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	See attached
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	See attached
DISCUSSION			
Interpretation/ scientific implications	18	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 any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Discussion Conclusion See attached
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Conclusion
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	See attached

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

4. The objective of the study is to develop and identify potent site-specific antibody drug conjugate utilizing a novel cytotoxic with a unique mechanism of action involving splicing inhibition. For more detail see introduction.

5. All studies using animals are approved by the Institutional Animal Care and Use Committee (IACUC) and complied with the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Acadamies, 2011)

6. a. Control groups n=10, treatment groups n=8

   b. Animals are randomized based on tumor size

   c. Treatments were given to animals in groups

7. a. ADCs were formulated in PBS; Dosed via IV route

   b. Dose administration and tumor measuring were generally performed in the morning (8AM-12 noon) or afternoon (12 noon-4 PM) hours

   c. Home cages in the designated study rooms

   d. Antibody conjugates is routinely dosed IV to avoid degradation in GI, and to have better accessibility in patients than IP

8. a. Young adult nude (athymic nu/u) mice; female;

   Age: Mean (range) 12 weeks (9-15 weeks)

   Weight: mean (range) 26 g (22-32g)

   b. Acquired from Charles River Laboratories (Wilmington, MA)

9. a. Animals were located in the vivarium with controlled airflow, temperature (72±2C □), and humidity (50±5%); Animals were group housed (maximum 5 mice per cage) in the sterile cages of the Tecniplast Greenline rodent caging system. Mice were provided enrichment devices such as polycarbonate shelters, tunnels or huts, as well as Nylabones and wood blocks. The processed corncob product was used for bedding material.

   b. Animal room lighting is set with a diurnal cycle (light on at 6 pm and off at 6 pm). Animal feed used: Irradiated Low Isoflavone 5V02 IF50 Purina Chow; animal access to food and water ad libitum.

   c. General conditions of study animals were checked daily, and tumor growth was monitored twice weekly. Animals with health conditions were removed from studies and euthanized following an IACUC approved animal use protocol.

10. a. Described in study results (n=7-10 per group)

b. Based on our historical data, the number of animals was determined so that statistical power to detect a 50% tumor reduction is >80% using the Anova P analysis

c. NA

11. a. Animals were randomized into treatment groups weighted on the tumor size.

b. Doses were given in the order of treatment groups the animals assigned on.

12. Efficacy of ADC in mouse tumor xenograft model by monitoring tumor sizes. More details in Results.

13. For the in vivo efficacy studies, average and standard deviation were calculated using all the animals in the study. Statistical analysis was performed using ANOVA method. See supporting information for representative individual animal tumor volume.

14. Animals in all experimental and control groups appeared healthy before the treatment, weight range 23.5-31.6 g, no prior treatment.

15. All animals were included in the study.

16. Standard Deviation is shown in all the in vivo efficacy figures.

17. No adverse events were reported in any study.

18. Please see Discussion and Conclusion for the interpretation and detailed discussion. The limitation of mouse xenograft tumor models for development of cancer therapeutics is related to differences in tumor heterogeneity, antigen expression and pharmacokinetics between species.

We used minimum number of animals to test the hypothesis of the study.

19. See Conclusion

20. The authors received no specific funding for this work.