Enhancing Immune Therapies: Extracellular Vesicle-based Strategies for Cancer Treatment

Brittany Doran[1], Rachelle Duffin[1], Andrew French[1,2,3], Samira Hosseini[1], Jing Yang Tee[1], Vera Evitmov[1,2], Nhu-Y Nguyen[1,2] Walid Azar[1,2], Ian Nisbet[1], Richard Boyd[1,2], and Alan Trounson[1,2].

[1]Cartherics Pty Ltd, Notting Hill, Australia. [2]Australian Regenerative Medicine Institute, Monash University, Clayton, Australia. [3]Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Australia

Background

Immune evasion, therapeutic resistance, and the immunosuppressive tumour microenvironment (TME) are major barriers to achieving effective responses to engineered CAR-T and CAR-NK cell therapies in solid cancers. Extracellular vesicles (EVs) from engineered immune cells may offer a solution to overcome these issues through their inherent resistance to immunosuppressive mechanisms active in the TME (e.g., immune checkpoints such as PD-L1), along with an enhanced ability to penetrate tumours, modulate endogenous immune responses and deliver cytotoxic payloads[1,2]. Here, we have isolated EVs from induced-pluripotent stem cell-derived natural killer cells expressing a chimeric antigen receptor (CAR-iNK cells) directed towards tumour associated glycoprotein-72 (TAG-72).

The aims of this study were:

a) To isolate and characterise EVs derived from CAR-iNK cells (CAR-iNK-EVs).
b) To examine the potential cytotoxicity/efficacy against ovarian cancer cell lines.

Methodology

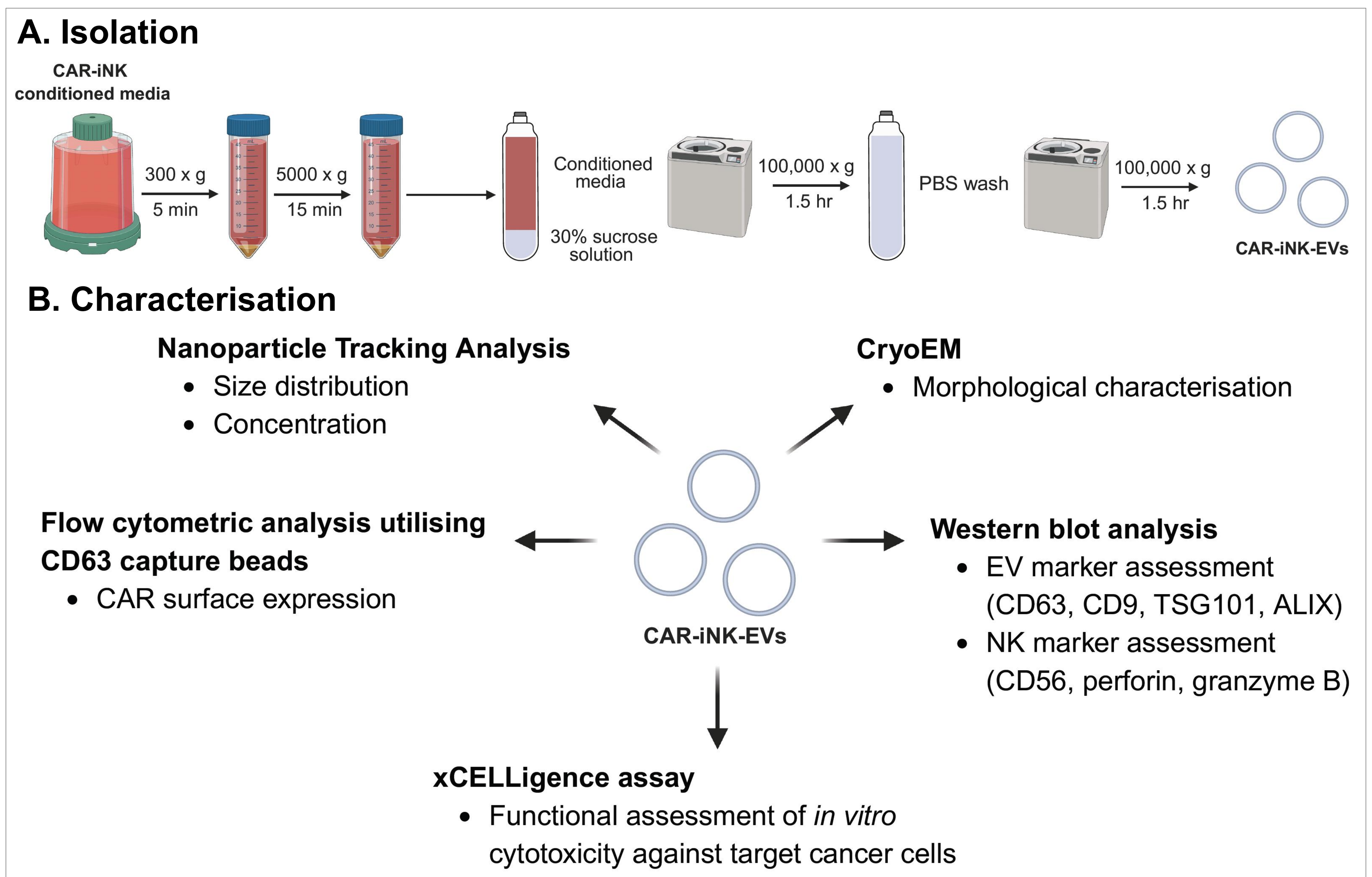


Figure 2: Isolation and characterisation of CAR-iNK-EVs from TAG-72 CAR-iNK conditioned media. (A) Pro-iNK cells were expanded and matured for 7 days and EVs were isolated from conditioned media by density gradient ultracentrifugation. (B) Isolated CAR-iNK-EVs were characterised using Nanoparticle Tracking Analysis (NTA) and cryogenic electron microscopy (CryoEM) to assess EV size, structure and concentration. Protein composition and functionality of CAR-iNK-EVs was assessed using western blot analysis, flow cytometry and xCELLigence assays, respectively.

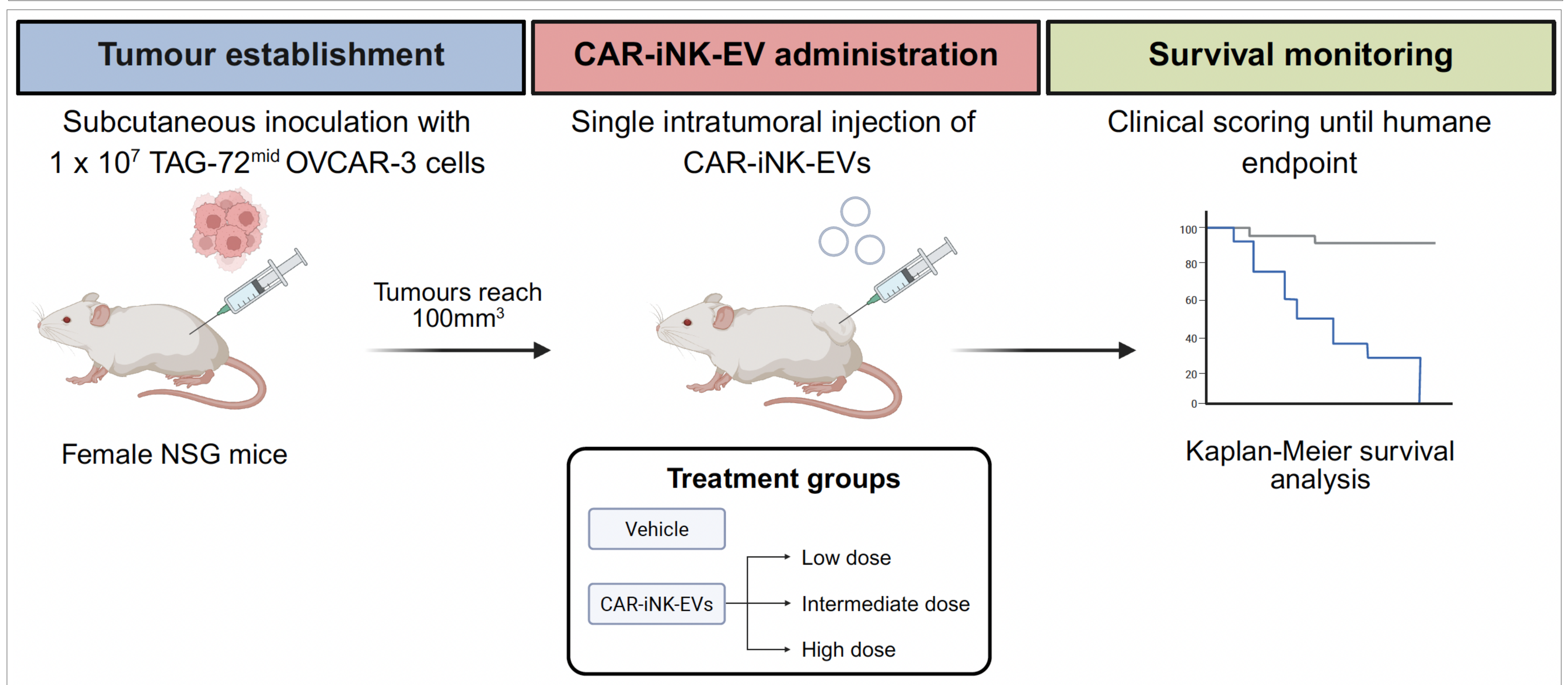


Figure 3: *In vivo* assessment of TAG-72 CAR-iNK-EV efficacy in a subcutaneous ovarian cancer mouse model. NSG mice subcutaneously inoculated with 1 x 10^7 OVCAR-3 ovarian cancer cells received a single intratumoral injection of the vehicle (PBS), or CAR-iNK-EVs at one of three concentrations. Mice were assessed via clinical scoring throughout study duration and Kaplan-Meier survival analysis was performed.

References & Acknowledgements

1. Bar et al., *Journal of Extracellular Biology.*, 2024
2. Si et al., *Mol Cancer.*, 2024

Schematics were created using BioRender (BioRender.com)

Revolutionising cellular therapies for cancer and beyond

CAR-iNK-EVs exhibit typical EV properties

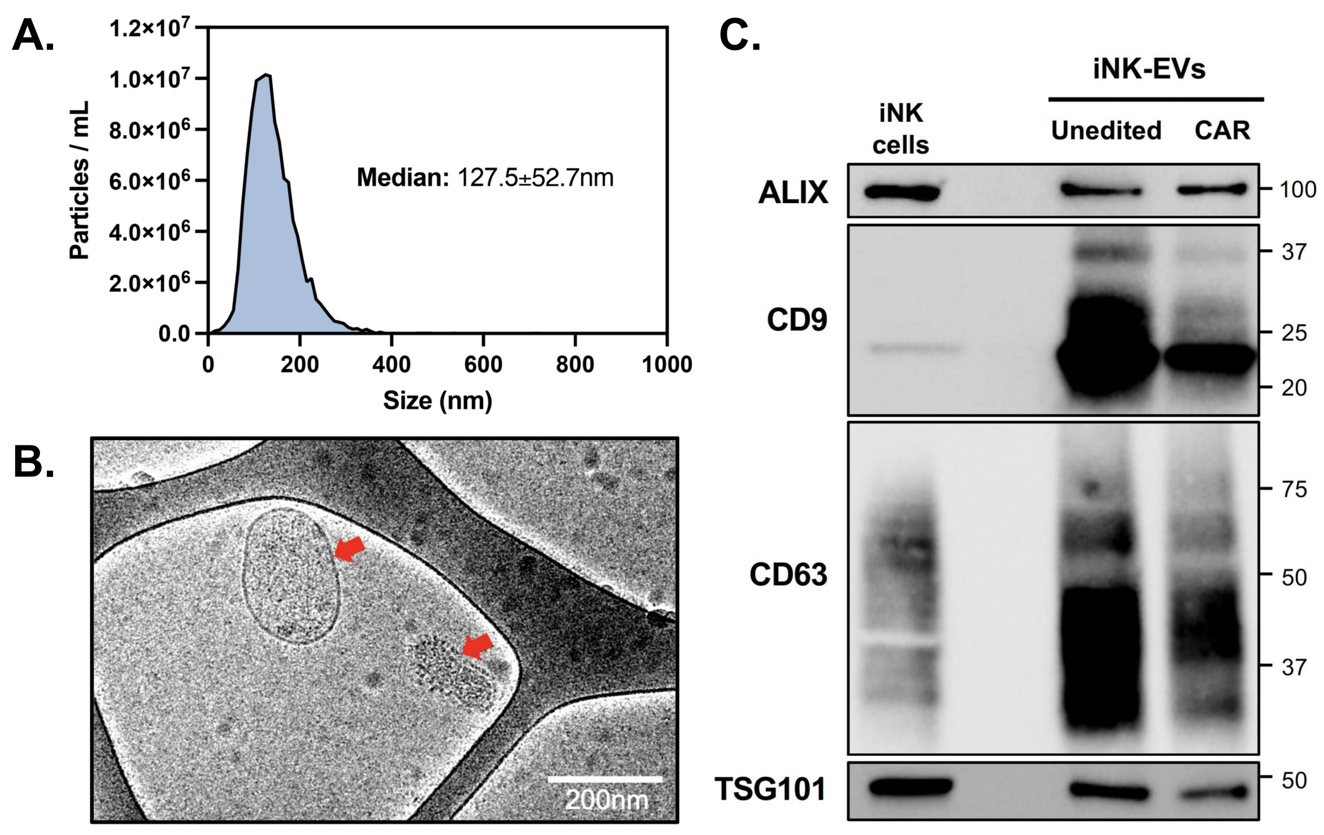


Figure 4: Characterisation & validation of TAG-72 CAR-iNK-EVs. (A) NTA size distribution of isolated CAR-iNK-EVs. (B) CryoEM images of CAR-iNK-EV morphology; red arrows indicate EVs. (C) Western blot characterisation of EV markers in iNK-EVs.

CAR-iNK-EVs express NK cell markers and retain surface CAR expression

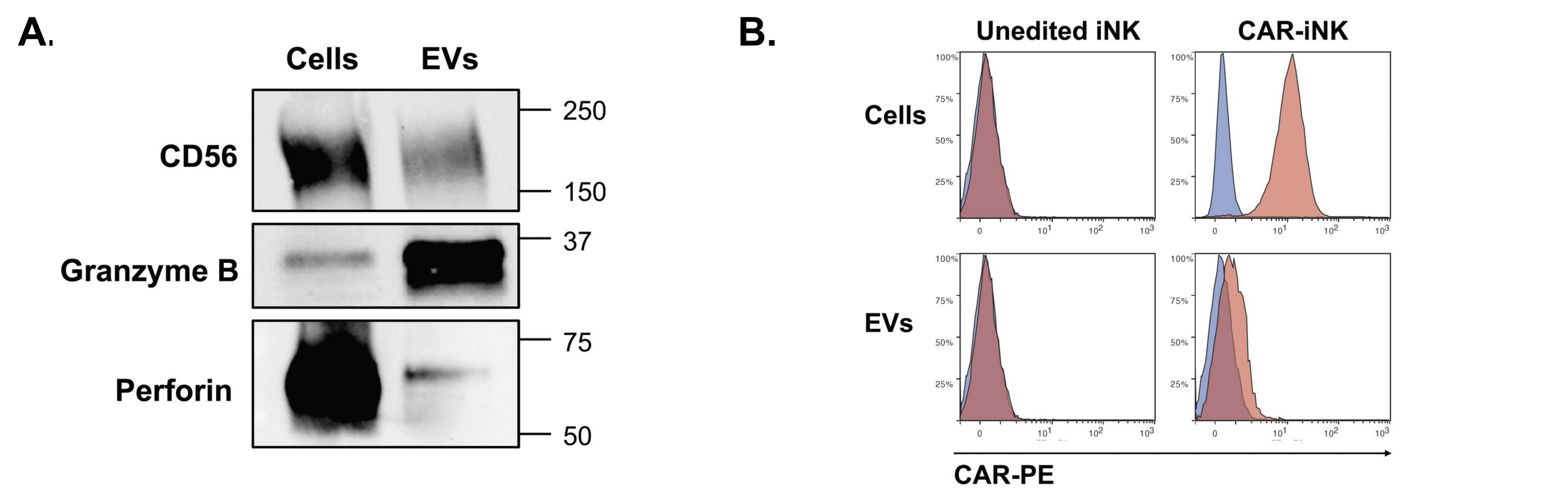
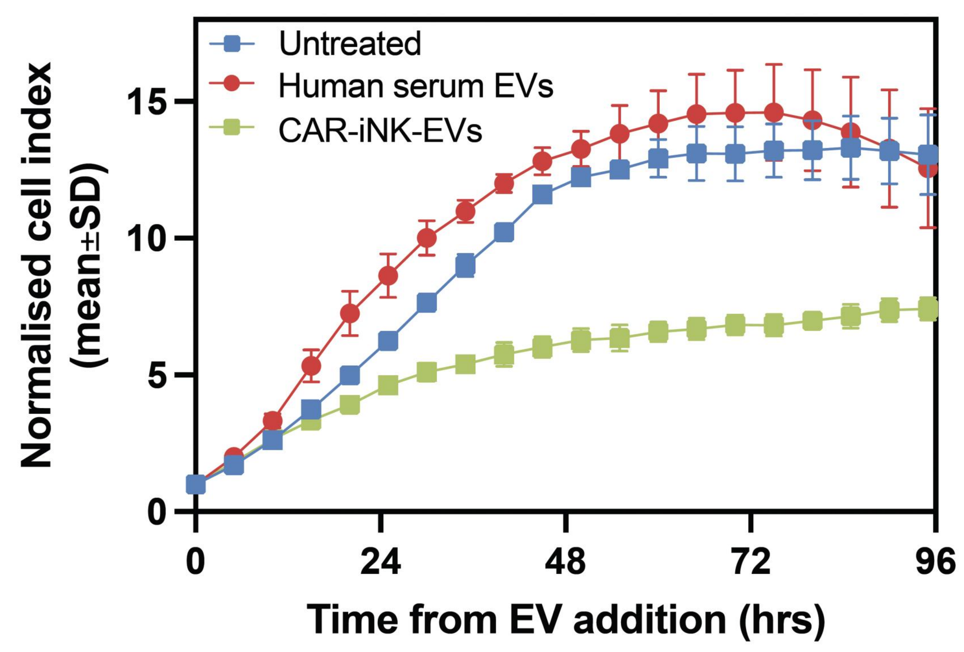


Figure 5: Demonstration of key CAR-iNK marker expression in TAG-72 CAR-iNK-EVs. (A) Western blot analysis of CD56, granzyme B and perforin expression in CAR-iNK cells and EVs. (B) Flow cytometry demonstrating surface expression of the CAR. Blue histograms represent isotype controls, Red histograms represent CAR expression.

CAR-iNK-EVs are cytotoxic against TAG-72^mid OVCAR-3 ovarian cancer cells *in vitro*

Figure 6: *In vitro* cytotoxicity assessment of TAG-72 CAR-iNK-EVs against an ovarian cancer cell line. The TAG-72^mid OVCAR-3 ovarian cancer cell line was treated with 1 × 10^9 particles of CAR-iNK-EVs or human serum-derived EVs (as a negative control). CAR-iNK-EVs decrease the cell index over 96hrs post-treatment compared to the untreated and human serum EV controls.



CAR-iNK-EVs prolong survival in an ovarian cancer mouse model in a dose-dependent manner

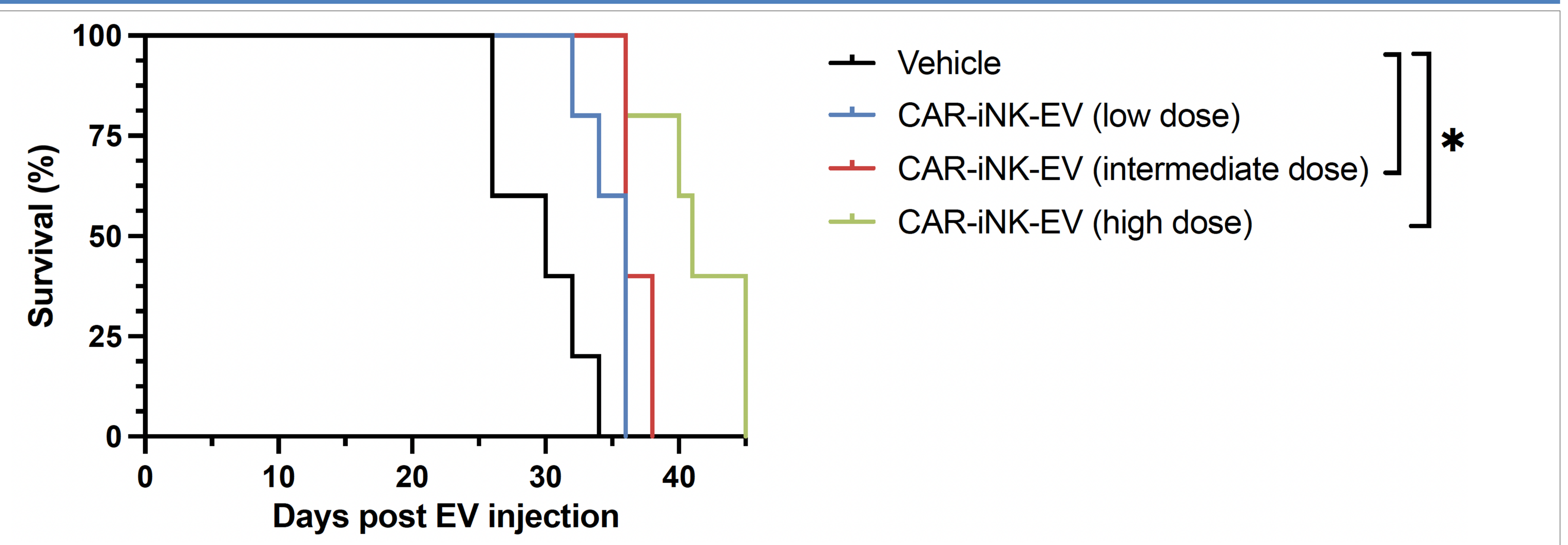


Figure 7: *In vivo* efficacy of TAG-72 CAR-iNK-EVs against subcutaneous OVCAR-3 tumours. Survival analysis demonstrated significantly prolonged survival in mice that received CAR-iNK-EVs at the intermediate and high doses compared with the vehicle control (median survival: 36 and 42 days vs. 30 days, respectively). *$p \geq 0.05$, n=5 mice/group.

Conclusion

CAR-iNK-EVs exert direct cytotoxic effects against ovarian cancer cells *in vitro* and prolong survival in tumour models *in vivo,* thereby demonstrating their potential as a novel therapeutic approach for the treatment of cancers.

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