[^{64}Cu/^{67}Cu]Radiolabelled exosomes as a theranostic tool for lung metastasis

Antero Abrunhosa

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1. Authors

Alexandra I. Fonseca[1]
José Sereno[2]
Liliana Santos[3,4]
Célia M. Gomes[3,4]
Antero J. Abrunhosa[1,2]

[1]ICNAS Pharma, University of Coimbra, Portugal
[2]CIBIT/ICNAS – Institute for Nuclear Sciences Applied to Health, University of Coimbra, Portugal
[3]iCBR - Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra, Portugal
[4]CIBB - Center for Innovative Biomedicine and Biotechnology Consortium, University of Coimbra, Portugal

2. Context of the project (800 characters max. including spaces)

In this project, we aimed to develop a ^{67}Cu-based therapeutic radiopharmaceutical for the treatment of lung metastases of osteosarcoma (OS), using exosomes derived from an OS cell line as theranostic nanocarriers. This work extents an ongoing project in which a diagnostic radiopharmaceutical for PET imaging was developed using copper-64, which resulted in a publication prior to the results in this report (Almeida, 2022). The project was carried out at ICNAS, University of Coimbra, Portugal. The main objectives were (1) to develop a ^{67}Cu-based therapeutic tool; (2) to evaluate in vitro cytotoxicity and DNA damage; and (3) to assess in vivo efficacy in a metastatic lung model.

3. Results and discussion (1000 characters max. including spaces)

Radiolabeling was conducted using the same optimized protocol previously established for copper-64, involving functionalization with NODAGA followed by reaction with copper-67. Radiochemical yields of approximately 100% were consistently achieved after 30 minutes, with radiochemical purity exceeding 95%, confirmed by radio-TLC.

OS cells were incubated with increasing activities of [^{67}Cu]-EXO (1–10 MBq/mL), with free ^{67}Cu serving as a control. Cell viability was measured using the CellTiter-Glo Viability Assay, and DNA double-strand breaks were assessed via γ-H2AX foci accumulation. The results demonstrated a dose-dependent decrease in cell viability along with increased DNA damage.

Mice bearing metastases were divided into three groups and treated with a single therapeutic dose of [^{67}Cu]-EXO. Treatment efficacy was monitored twice a week by PET/MRI. The radiopharmaceutical significantly delayed metastatic progression, confirmed by MRI. This treatment markedly improved overall survival compared to both control mice and those treated with free ^{67}Cu (figure 1 and 2).

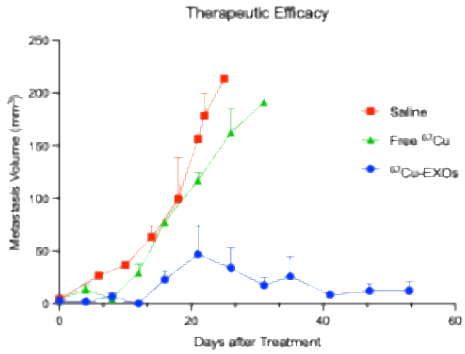

Figure 1. Changes in metastatic lesions volume over time in mice treated with ^{67}Cu-EXs (~26 MBq), free ^{67}Cu, or vehicle control.

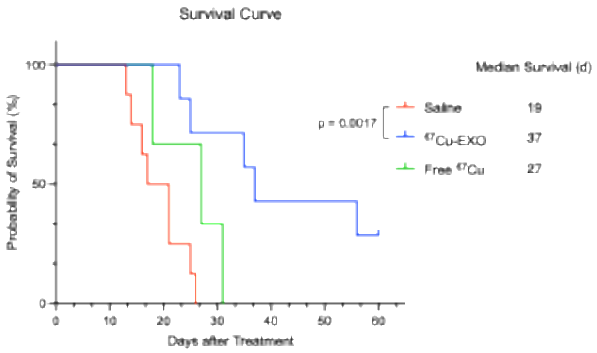

Figure 2. Kaplan–Meier survival analysis for the three groups, demonstrating an improvement in overall survival in the ^{67}Cu-EXs-treated group.

4. Conclusions (800 characters max. including spaces)

This project successfully demonstrated the potential of [^{67}Cu]-EXO as a promising therapeutic radiopharmaceutical for the treatment of OS lung metastases. The optimized radiolabeling protocol achieved high radiochemical yield and purity. In vitro studies confirmed the radiopharmaceutical's cytotoxic on OS cells in a dose-dependent manner, while in vivo experiments revealed significant therapeutic efficacy and improved overall survival. These findings provide a strong preclinical basis for further investigation of [^{67}Cu]-EXO as a therapeutic agent in OS management and highlight the potential of exosome-based nanocarriers in targeted radionuclide therapy.

5. Involvement of the PRISMAP services (600 characters max. including spaces)

The key results obtained during the project, which are summarized in this report, were achieved between November 2023 and February 2025. They were made possible by the delivery of five batches of copper-67, each with an activity of 200 MBq, which enabled the completion of the project and its experimental work.

6. PRISMAP (600 characters max. including spaces)

The overall process with PRISMAP, from the project application to the implementation of the experimental work, was straightforward and well-organized. The application procedure was clear and user-friendly, and communication with the PRISMAP team was efficient throughout the project. Although we experienced some initial difficulties with the first delivery of the radioisotope from Denmark, these issues were promptly addressed, and the process was subsequently optimized. Overall, our experience with PRISMAP was very positive, and we appreciate the support provided by the team.

7. Publications and other dissemination activities (conferences etc.)

An abstract has been submitted to the 8th Theranostics World Congress (TWC), entitled *"Theranostic Potential of [$^{64/67}$Cu]Cu-NODAGA-Exosomes in Lung Metastasis of Osteosarcoma."* This submission represents an important step in disseminating our recent findings to the international scientific community. In addition, we are currently preparing a manuscript based on these results, which is expected to be submitted for publication in a peer-reviewed journal next year. These dissemination activities aim to enhance the visibility and scientific impact of our research within the field of theranostics and nuclear medicine.

Appendix 1. Dissemination guidelines for user projects as agreed in the signed User Agreement

Dissemination rules

Only user groups that are allowed to disseminate the results which they have generated under the project may benefit from the access, unless they are working for SMEs.

For each user group project, a publishable project summary and a publishable summary of the results will be published on the European Union Horizon 2020 PRISMAP project website www.prismap.eu. The publication of results in journals or at conferences is strongly encouraged.

To ensure the long-term sustainability of the PRISMAP initiative, proper recognition of the contributing facilities, their services and the involved persons is necessary. All participating PRISMAP facilities shall be acknowledged in the publication. Acknowledgement and co-authorship of PRISMAP staff members who participated in the experiment shall be considered according to the research field best practices and verified with the PRISMAP Technical Manager before any publication.

The user group shall contact the PRISMAP Technical Manager 30 days prior to submission of publications or other communications of results that were obtained by making use of services provided by PRISMAP (radionuclides delivered or medical services provided). The Technical Manager will communicate to the user group the list of PRISMAP facilities and persons that have contributed to each specific project and the way this contribution must be acknowledged in the publication/communication or where co-authorship is required to reflect specific scientific contributions.

Users must comply with Horizon 2020 dissemination rules (i.e. acknowledge that their work was financially supported by the European Union's Horizon 2020 Research and Innovation Programme by including the following acknowledgement: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP)"), and grant open access to resulting publications and related data.

Dissemination shall take place only once legitimate interests regarding intellectual property have been safeguarded. A maximum publication delay of 90 days may be granted for this purpose.

Acknowledgements

The list of name(s) to be mentioned in the acknowledgment section is sent to the technical manager by the main contact of the involved facilities.

A general sentence will be added by the corresponding author of the article (user side):

"The authors would like to thank the members of the PRISMAP consortium and of the PRISMAP user selection panel, coordination and management team for their advice and support."

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