User project report

Targeted radiotherapy of radioresistant cancers – a radiobiological study with advanced cell models

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1. Authors

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

Taking advance of the complementary expertise of both teams this collaborative project aims to further investigate the potential of Targeted Radionuclide Therapy by exploring the radiobiological effects of 64/67Cu-based radiopharmaceuticals in advanced cell models of radioresistant cancers. The project will allow to compare the relative contribution in cell killing of radioresistant cancer stem cells of Auger versus beta emission in a 3D model cell culture, which allows to consider properly the volume effect of the tumor. The results could be also of interest for example for selecting 177Lu vs 64Cu. Besides the achievement of the major scientific objectives, this user project will also contribute for the advanced training of young researchers in the biological evaluation of TRT.

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

To accomplish our goal of comparing the radiobiological effects of the two potential therapeutic radioisotopes of Cu, ^{64}Cu and ^{67}Cu, a survival assay was performed with cells derived from glioblastoma spheroids incubated with both radionuclides in their salt form ([$^{64/67}$Cu]$CuCl_2$). Two different glioblastoma cell lines were selected for this purpose: U87 and U373. Spheroids were exposed for 3 h with either ^{64}Cu or ^{67}Cu. Additionally, to have a similar dose between the radioisotopes, spheroids were exposed to ^{67}Cu for 1 h.

The results obtained (Fig. 1) revealed that there might be an increased sensitivity of U87 cells to ^{64}Cu than to ^{67}Cu. However, more independent assays should be performed to confirm this. Conversely, U373 results suggest a similar effect when cells are incubated with the highest activities, independently of the incubation period. Furthermore, results also indicate that incubating U373 spheroids with [^{64}Cu]$CuCl_2$ for 3 h and with [^{67}Cu]$CuCl_2$ for 1 h has a similar effect.

4. Conclusions (800 characters max. including spaces)

Previous studies performed by us with [^{64}Cu]$CuCl_2$ in spheroids suggested that it had the potential to be used as a theranostic agent for glioblastoma. With this project, we further demonstrated that the theranostic radioisotope ^{64}Cu has a comparable anti-proliferative effect to the standard therapeutic radioisotope ^{67}Cu. Additionally, spheroids exposed to the same conditions mentioned were flash-frozen and sectioned for a future immunofluorescence study to analyse cell death and DNA damages to further compare the two radioisotopes.

Overall, despite the need for more studies to give us definite conclusions, we conclude that [^{64}Cu]$CuCl_2$ and [^{67}Cu]$CuCl_2$ have similar radiobiological effects in glioblastoma spheroids, having a huge potential for the treatment of this devastating disease.

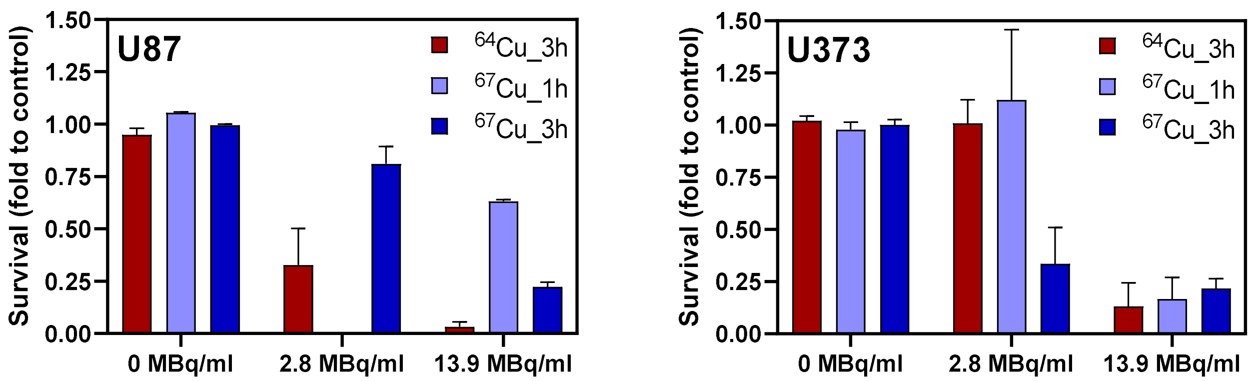

Figure 1. Survival fraction of spheroid-derived cells from U87 (left) and U373 (right) cell lines after 3 h of incubation with [^{64}Cu]CuCl$_2$ and [^{67}Cu]CuCl$_2$ and 1 h of incubation with [^{67}Cu]CuCl$_2$, presented as a fraction of the untreated control (0 MBq/ml), determined through the clonogenic assay. Data are presented as mean values ± S.E.M of 2 to 5 independent assays.

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

The PRISMAP services were fundamental in the project, with the supply of [67Cu]CuCl2, allowing the comparison of the two different Cu radioisotopes.

6. Feedback to PRISMAP (600 characters max. including spaces)

Only comment would be the short notice for the interview (nov 2022).

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

2025

Catarina I. G. Pinto, Laura Ordas, Alexandra Fonseca, Maria C Carreira, Antero J. Abrunhosa, , Joana F. Guerreiro, Sophie Poty, Claudia L D Silva, Jean-Pierre Pouget, Filipa Mendes "Advanced 3D glioblastoma models for investigating radiopharmaceutical therapy" <u>submitted</u> to the EACR 2025 Congress 16-19 June

Catarina I. G. Pinto, Laura Ordas, Alexandra Fonseca, Maria C Carreira, Antero J. Abrunhosa, , Joana F. Guerreiro, Sophie Poty, Claudia L D Silva, Jean-Pierre Pouget, Filipa Mendes "Radiobiological evaluation of the therapeutic potential of [64/67Cu]CuCl2 in cancer 3D models" 3rd International Workshop on Radiobiology of Molecular Radionuclide Therapy (IWRMR) Rotterdam, Netherlands, <u>to be presented</u> as a oral communication 8-9 April

2024

Catarina I. G. Pinto, André Dargen de Matos Branco , Alexandra Fonseca, Laura Ordas, Antero J. Abrunhosa, Cláudia L. da Silva, Joana F. Guerreiro, Sophie Poty, Jean-Pierre Pouget, Filipa Mendes "3D cancer models for the theranostic evaluation of 64CuCl2". 18th ENEN PhD Event & Prize 2024, September 11-14. Oral presentation Catarina Pinto

2023
Catarina I. G. Pinto, Laura Ordas, Alexandra Fonseca, Antero J. Abrunhosa, , Joana F. Guerreiro, Sophie Poty, Jean-Pierre Pouget, Filipa Mendes "Evaluation of the therapeutic potential of 64CuCl2 in 3D models of glioblastoma". 10th International Symposium on the Physical, Molecular, Cellular and Medical Aspects of Auger Electron Processes, September 6-8. – Oral presentation Filipa Mendes

2023
Catarina I. G. Pinto, Sara Bucar, Alexandra Fonseca, Vitor Alves, Antero J. Abrunhosa, Cláudia L. da Silva, Joana F. Guerreiro, Filipa Mendes " Theranostic potential of 64CuCl2 in 3D cell culture models of glioblastoma" , Poster at the 2nd International Workshop on Radiobiology of Molecular Radionuclide Therapy (IWRMR) London, UK, 13-14 March - best poster award

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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