Development and Preclinical Evaluation of Docetaxel-loaded Micelles for Glioblastoma Treatment

Júlia German-Cortés [1,2,3], Raquel Herrero [1], Natalia Torroglosa [1], Alexandra Pumarola [1], Narine Fischer-Albiol [1], Sofia Campos-Moreno [1], Sofia Sabaté [1], Àngels Alcina [1], Sandra Mancilla [1,2], Belén García [1,2], Monserrat Llaguno-Munive [1], Zamira V. Diaz-Riascos [1,2,4], Cláudia Martins [5,6], Simó Schwartz Jr [1,7], Roser Ferrer Costa [1,7], Ibane Abasolo1,[2,4], Pilar Sanchez-Gomez[8], Bruno Sarmento [5,6,9], Diana Rafael [1,2,4], Fernanda Andrade [1,2,3*]

***Presenting author:**

fernanda.dasilva@ub.edu

Affiliation:

1 Clinical Biochemistry, Drug Delivery & Therapy, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

2 CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Barcelona, Spain

3 Faculty of Pharmacy and Food Sciences, School of Pharmacy, Universitat de Barcelona (UB), Barcelona, Spain

4 Functional Validation & Preclinical Research (FVPR)/U20 ICTS Nanobiosis, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

5 i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, Porto, 4200-135, Portugal.

6 INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen 208, Porto, 4200-135, Portugal.

7 Clinical Biochemistry Service, Vall d'Hebron University Hospital, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

8 Unidad de Neurobiología Molecular, Instituto de Salud Carlos III, Madrid, Spain

9 ICS-CESPU, Rua Central de Gandra 1317, Gandra, 4585-116, Portugal.

Acknowledgements:

CIBER-BBN, ISCIII, European Commission (ReachGLIO project, No. 964264), Generalitat de Catalunya (2021 SGR 01173), FAECC (INVES211530DASI, PRDBA258393GERM), MSCA-PF (ID 101107735). *In vivo* studies were performed by FVPR)/U20 ICTS Nanbiosis.

Introduction and Objective

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, associated with poor prognosis, high recurrence, and limited survival. Treatment failure is largely due to restricted drug penetration across the **blood-brain barrier (BBB)** and rapid therapeutic resistance[1]. **Docetaxel (DTX)** is a potent antimitotic agent but its application in GBM is limited by low aqueous solubility and insufficient brain exposure[2]. **Nanomedicine-based strategies** integrating rational carrier design, *in silico* tools, and biological targeting may help overcome these barriers and improve therapeutic efficacy.

Synthesis and characterization

DTX-loaded Soluplus® micelles **(SM)** produced by the film-hydration technique **(SM-D)** were spherical, with a diameter of around 60 nm, low PdI, and slightly negatively charged **(Fig 1A-C)**, with drug encapsulation efficiency above 90%[3]. ***In silico*** studies demonstrated that DTX-polymer association is predominantly governed by van der Waals and hydrophobic interactions **(Fig 1D-E)**[4].

Soluplus® Micelles (SM) for GBM Therapy

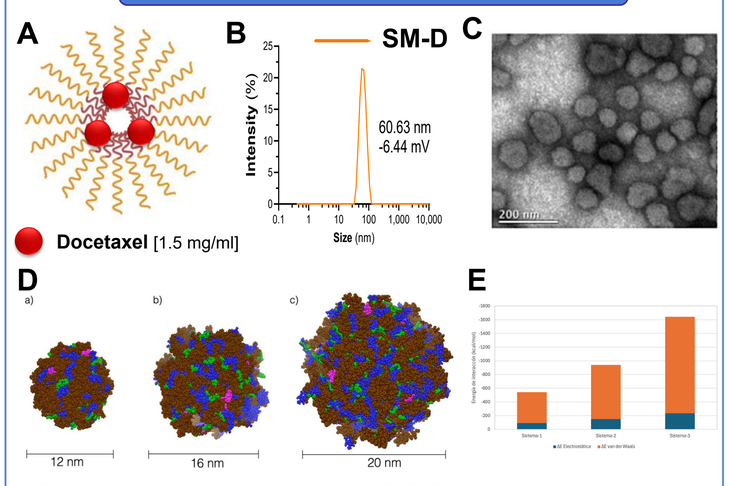


Fig 1. A. Schematic representation of SM-D, B. DLS graph with mean diameter and Z-potential. C. TEM microphotographs. D. Molecular dynamic simulations representation of micelles structure. E. Soluplus®-Docetaxel interaction energy contribution graph.

In vitro validation

3D *In vitro* assays

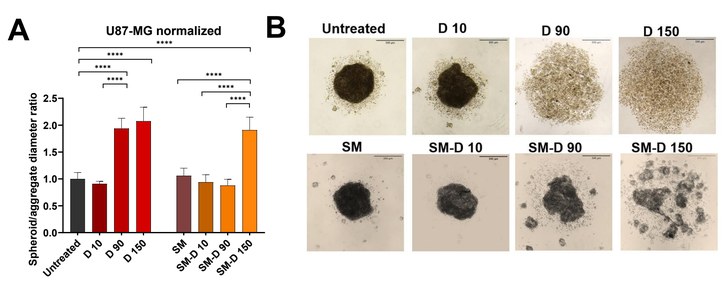


Fig 3 A. Spheroid diameter for U87-MG cells treated with different doses of SM-D and D doses (µg/mL). B. Optical microscopic images of the U87-MG cells spheroid formation capacity with the different treatments. Results as mean ± SEM, n≥3. * p<0.05, ** p<0.01, *** p< 0.001, and **** p< 0.0001.

SM were shown to be **safe,** and **SM-D showed higher efficacy** in both 2D and 3D U87-MG and U251-MG cells **(Fig 2-3)**. SM-D **interferes with spheroid formation (Fig 3A-B)**[3]. **SM-D impacts key GBM-related pathways,** affecting markers like E-cadherin, EPCAM, L1CAM, or EGFR **(Fig 4)**[3].

2D *In vitro* assays

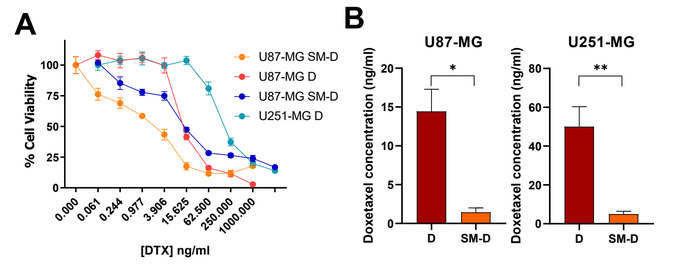
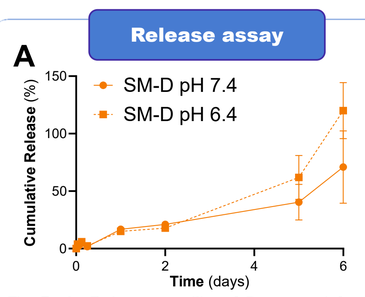


Fig 2 A. Cell viability curves in both GBM cell lines. B. IC_{50} values in both GBM cell lines. Results as mean ± SEM, n≥3. * p<0.05, ** p<0.01, *** p< 0.001, and **** p< 0.0001.

Release assay



BBB crossing assay

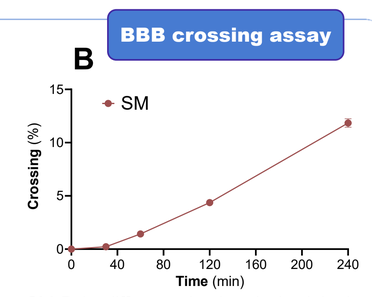


Fig 5. A. Release profile of Docetaxel from SM-D in different simulated physiologic conditions (pH 7.4 and pH 6.4). B. BBB crossing assay in hCMEC-D3 monolayer model over 4h. Results are expressed as mean ± SD, n=3.

SM-D showed **efficient and preferential drug release** in the tumoral microenvironment (pH 6.4 > pH 7.4) **(Fig 5A)**[3].

12% of the SM without surface modification were able to **cross the BBB** using an hCMEC-D3 monolayer cell model **(Fig 5B)**[3].

Quantitative PCR and Western Blot

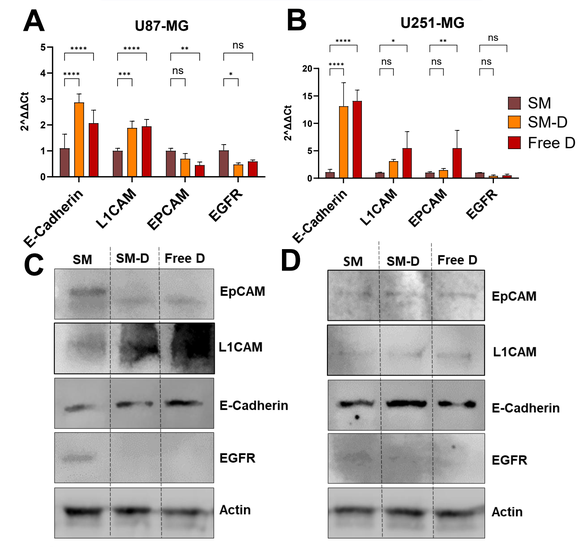
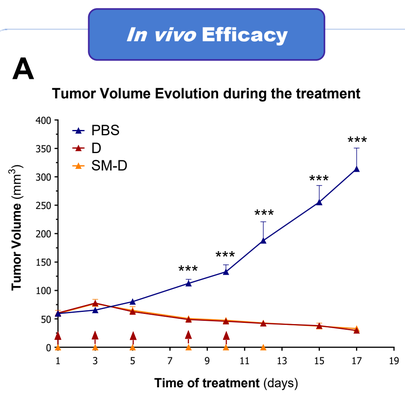


Fig 4. Gene and protein expression analysis. Quantification of gene expression levels of adherence and GBM pathways in U87-MG (A) and U251-MG (B) cells. The results are represented in fold change relative to untreated cells. GADPH was used as a housekeeping gene. Protein levels in U87-MG (C) and U251-MG (D).

In vivo validation

Treatment promoted **a clear decrease in tumor volume** in athymic nude mice with subcutaneous U87-MG GBM tumors **(Fig 6A)**. **SM-D treatment impacts key GBM-related and stemness pathways (Fig 6B-C)**. SM-D remarkably reduced tumor mass and cancer cell density **(Fig 7A)**. **DTX encapsulation strongly reduced the toxicity**, showing a favorable safety profile compared to the free drug, as evidenced by reduced mouse weight loss **(Fig 8A)** and histological assessment[3].

***In vivo* Efficacy**



Quantitative PCR and Western Blot

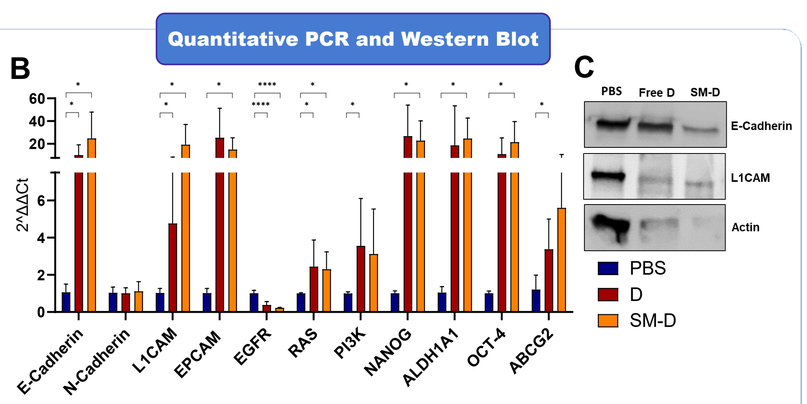


Fig 6. A. Tumor volume over time in mice treated i.v. with 7.5 mg/kg of DTX and 250 mg/kg of Soluplus®. Results are expressed as mean ± SEM, n ≥ 3 B. Gene expression levels of adherence and malignancy pathways related genes in tumors. The results are represented in fold change relative to untreated cells. GADPH was used as a housekeeping gene. C. Protein levels of E-Cadherin, L1CAM, and Actin in tumors. Results are expressed as mean ± SEM, n = 3. * p<0.05, ** p<0.01, *** p< 0.001, and **** p< 0.0001.

Encapsulation of DTX into SM **increases its half-life and the mean residence time, and reduces its clearance (Fig 8B)** when compared to the free drug[5].

Histological analysis

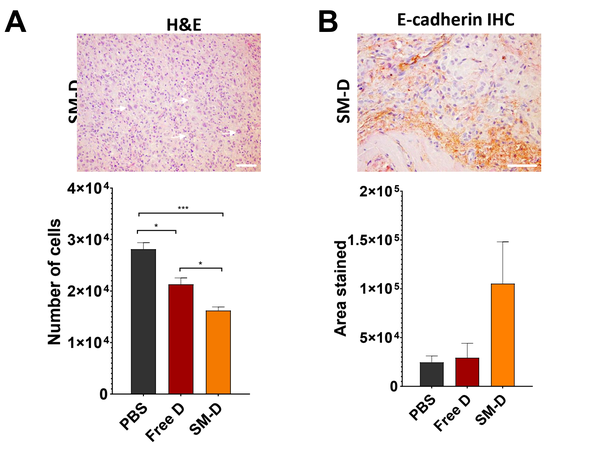
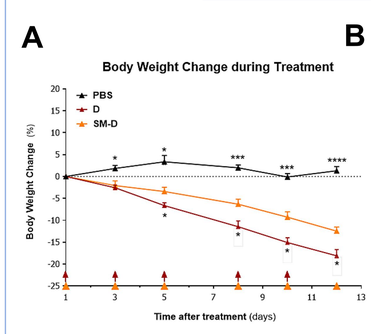


Fig 7. Histological (H&E) (A) and Immunohistochemical (IHC) (B) analysis of tumor sections for quantification of cell number and E-Cadherin expression, respectively. Scale bars correspond to 100 µm.

Conclusions

The integration of polymeric micelles, molecular simulations, and bioinformatic design represents a powerful strategy to cross the BBB and enhance DTX delivery to the brain. This multidisciplinary approach supports micelles as a versatile and rationally optimized platform for GBM therapy and provides a framework for the development of next-generation BBB-crossing nanomedicines.

Pharmacokinetic profile



B

PK parameters in serum	SM-D
$T_{1/2}$ (h)	28.89
Cl (mL/h)	4.92
AUC (µg/mLxh)	35.45
V_d (mL)	205.20
MRT (h)	58.30

Fig 8. A. Body weight changes during treatment. B. Pharmacokinetic parameters of SM-D i.v. injected.

References

1. doi.org/10.3390/ijms25053040, 2. doi.org/ 10.1016/j.jddst.2020.101959,
3. doi.org/10.1016/j.jconrel.2025.113616, 4. doi.org/10.1016/j.carpta.2026.101120,
5. doi.org/10.2147/IJN.S121881