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# A NANOMEDICINE, CHEMISTRY AND TUMOR BIOLOGY THERAPEUTIC RECIPE MADE @ UK-PORTUGAL TO DEFEAT GLIOBLASTOMA

Cláudia Martins[1,2], Catarina Barbosa[1], Marco Araújo[1], Stuart J. Smith[3], Maria Oliveira[1], Dimitrios Lamprou[4], Ruman Rahman[3], Jonathan W. Aylott[2], Bruno Sarmento[1]

[1]University of Porto, 4200-135 Porto, Portugal; [2]School of Pharmacy, University of Nottingham, NG7 2RD Nottingham, UK; [3]School of Medicine, University of Nottingham, NG7 2RD Nottingham, UK; [4]School of Pharmacy, Queen's University Belfast, BT9 7BL Belfast, UK

**Background:** The 5-year survival of glioblastoma (GBM) patients is limited to a dismal 5%, highlighting the need to advance more effective therapies. GBM tissue abnormally overexpresses the L-type amino acid transporter 1 (LAT1), for which L-histidine (His) is an inexpensive and powerful targeting ligand. Thus, we propose the chemical modification of a conventional chemo-immunogenic drug, docetaxel, into a nanomedicine with surface-His (nano-DTX-His) to target GBM tissue via LAT1 adhesive binding and further augment localized cell death. Since nano-DTX-His cannot be used for IV therapies due its inability to cross the blood-brain barrier (BBB) *per se*, we further propose its modification with an acid-cleavable Angiopep-2 layer (nano-DTX-His-*clv*-Angiopep2) to favor BBB translocation. It is of important note that the choice of DTX is based on its $IC_{50}$, which is around 20.000-times lower than the standard temozolomide (in https://www.cancerrxgene.org/).

**Methods:** Carbodiimide and carbamate hydrolysis were employed to synthesize a poly(lactic-co-glycolic) acid (PLGA) and His-functionalized polyethylene glycol (PEG) conjugate, to serve as the nano-DTX-His matrix. Nano-DTX-His was further manufactured through a large-scale microfluidic technique. To evaluate cell uptake and viability, 2D and 3D models of conventional GBM cell lines (U87, U251, U373) and primary lineages isolated from the GBM invasive margin of human tumors (GIN lineages) were used. To investigate a possible immunogenic cell death, a novel 3D high-throughput model of the GBM microenvironment was developed, including U251 cells, buffy coat-isolated monocytes, and brain primary endothelial cells. Finally, nano-DTX-His was modified into nano-DTX-His-*clv*-Angiopep2 though inclusion of a PLGA-acetal-PEG-Angiopep2 conjugate (Angiopep-2 coupled with a PLGA-acetal-PEG polymer via Thiol-Michael addition) into the final nanomedicine formulation. BBB translocation was evaluated in hCMEC/D3 Transwell® systems.

**Results:** Monodisperse nano-DTX-His was manufactured with c.a. 250 nm and a controlled DTX release over 48 h. The uptake of nano-DTX-His was 3.5-times higher than nano-DTX-ØHis in U87, U251 and U373 cells. In GIN lineages, cell uptake was 8-times higher than the controls. 2D studies of cell viability in GIN lineages demonstrated an anti-cancer potential of nano-DTX-His 50% superior compared to the controls, after 4 h flash and 96 h treatments. In a heterotypic GIN 3D culture, this anti-cancer potential was kept. Moreover, in the 3D GBM microenvironment model, nano-DTX-His presented 1) a 60% cytotoxicity increase compared to the controls, and 2) the capacity to polarize macrophages into an anti-tumor phenotype. Finally, nano-DTX-His-*clv*-Angiopep2 was able to provide higher BBB translocation of DTX compared to nano-DTX-His and nano-DTX-His-Ø*clv*-Angiopep2 in hCMEC/D3 Transwell® systems.

**Conclusions:** Nano-DTX-His demonstrated better properties of cell uptake and cytotoxicity compared to the conventional therapy, in reliable 2D and 3D models of GBM. Moreover, its modification into nano-DTX-His-*clv*-Angiopep2 allowed the nanomedicine to acquire an ameliorated BBB translocation capacity. Ongoing work focuses on *in vivo* studies.