Development of lipid-polymer hybrid nanoparticles for the co-encapsulation of 6-bromo-indirubin-3'-oxime and copper diethyldithiocarbamate for synergistic cancer therapy

Radu Alexandru Paun[1,4], Daciana Catalina Dumut[2,4], Danuta Radzioch[2,3,4], Maryam Tabrizian[1,5]

[1]Department of Biomedical Engineering, McGill University, Montreal, Canada
[2]Department of Medicine, McGill University, Montreal, Canada
[3]Department of Human Genetics, McGill University, Montreal, Canada
[4]Research Institute of the McGill University Health Centre, Montreal, Canada
[5]Faculty of Dentistry and Oral Health Sciences, McGill University, Montreal, Canada

Statement of Purpose: The recent approval of immune checkpoint inhibitors (ICI) for clinical use, such as ipilimumab and nivolumab, highlights the survival advantage they provide to patients who previously had untreatable late-stage cancer like advanced metastatic melanoma.[1] However, not all patients respond to the therapy due to a lack of tumor immune infiltrates and cancer cell resistance to immune-mediated clearance.[2] Tumors that are resistant to ICI typically exhibit aberrant IFNγ signaling via the JAK/STAT pathway.[3] The compounds 6-bromo-indirubin-3'-oxime (6BIO) and the metal complex copper diethyldithiocarbamate (CuET), have been shown to exhibit anti-neoplastic activity by interfering with JAK/STAT signaling and causing endoplasmic reticulum stress, respectively, leading to cancer cell death.[4,5] The proposed drug combination is a strong candidate to help improve the response of immunologically cold tumors to ICI. However, due to the strong hydrophobicity of 6BIO and CuET, a stable and injectable nanoparticle formulation has yet to be developed for in vivo use. Herein, we show the synthesis of a novel lipid-polymer nanoparticle formulation to encapsulate both drugs at a predetermined ratio. We also demonstrate that the combination of 6BIO and CuET (6BC) synergizes to improve cancer cell death when compared to either compound in isolation.

Methods: The synergistic effect of 6BIO and CuET was studied using the Chou-Talalay model of synergism in multiple cancer cell lines (A375, B16F10, YUMM1.7, YUMMER 1.7). Cytotoxicity assays were performed using the previously established sulforhodamine B protocol.[6] The lipid-polymer nanoparticles encapsulating 6BIO and CuET (LP-6BC) were synthetized using the solvent injection method into ultrapure water. The physicochemical properties of the obtained nanoparticles were characterized using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and dynamic light scattering (DLS). The encapsulation efficiency and formulation stability were evaluated using HPLC and DLS, respectively.

Results: For the human melanoma cell line A375 (Fig. 1a), 6BIO has an IC_{50} of 1.48 µM, while for CuET the IC_{50} is 41.11 nM. When the cells are treated with the drug combination at a molar ratio of CuET to 6BIO of 1:25 in dimethyl sulfoxide (DMSO diluted in media <1%), the IC_{50} of both drugs is shifted to 0.25 µM and 10 nM for 6BIO and CuET, respectively. The drug combination results in a combination index (CI) of 0.91, 0.83, and 0.77 when the affected cell fraction (Fa) is 0.75, 0.9, and 0.95, respectively (Fig. 1b). These results are replicated in other cancer cells, supporting the notion of combining 6BIO and CuET for the treatment of cancer. To produce a clinically relevant formulation, we employed solvent injection to produce lipid-polymer hybrid nanoparticles with a mean size of 125.8 ± 3.1 nm, a polydispersity index of 0.043 ± 0.025, and zeta potential of -18.43 ± 1.3 mV, highlighting the colloidal stability of the system when PVP40 is added as an excipient (Fig. 1c). The encapsulation efficiency of both drugs is over 80% at a consistent and reproducible ratio, while maintaining its synergistic effect on cancer cells.

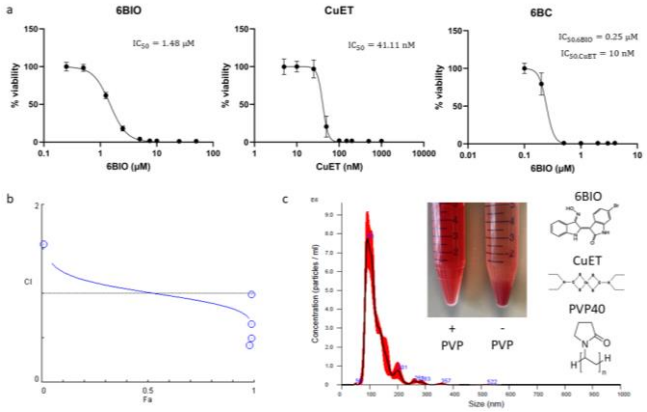

Figure 1. (a) Cell toxicity assays to determine the IC_{50} value of each compound individually and in combination for the A375 cell line. **(b)** The combination index (CI) with respect to the affected cell fraction (Fa). **(c)** NTA measurement showing the nanoparticle size distribution. Image shows the colloidal stability when PVP40 is added, and the chemical structures of relevant compounds.

Conclusions: Treatment of multiple cancer cells with the combination of 6BIO and CuET demonstrates synergistic anti-neoplastic activity. Furthermore, the development of a stable nanoparticle formulation was achieved using the highly reproducible and scalable solvent injection method, without compromising the drugs' efficacy.

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