Nanoparticle delivery of synergistic doxorubicin and olaparib combinations for triple negative breast cancer

Robert Cavanagh[1], Patrícia Monteiro[1], Cara Moloney[2], Alessandra Travanut[1], Fatemeh Mehradnia[1], Vincenzo Taresco[3], Ruman Rahman[2], Stewart Martin[2], Anna Grabowska[2], Marianne Ashford[3] and Cameron Alexander[1]

[1] School of Pharmacy, University of Nottingham, UK; [2] School of Medicine, BioDiscovery Institute, University of Nottingham, UK; [3] School of Chemistry, University of Nottingham, UK; [4] Advanced Drug Delivery, Pharmaceutical Sciences, AstraZeneca, Macclesfield, UK

Background: Multidrug combination therapy represents a promising opportunity to target multiple means of generating anticancer activity. However, a challenging aspect of combination therapy remains the distinct pharmacokinetics of independent compounds within the body. The use of nanoparticle delivery systems for drug combinations represents an approach to overcome this challenge via the simultaneous intracellular delivery of rationally selected drugs. In such a manner, the synergistic effects of the multidrug combinations can be fully realised.

Here we perform *in vitro* investigations on combinations of the topoisomerase II inhibitor doxorubicin and the poly (ADP-ribose) polymerase inhibitor olaparib for the treatment of triple negative breast cancers (TNBC); a disease with a poor prognosis and limited treatment options. Following identification of synergism, drug combinations have been loaded into polymeric nanoparticle carriers containing an oxidatively-cleavable thioketal group crosslinking the micellar-like cores and tested *in vitro*.

Methods: In the current work we performed *in vitro* screening of combinations of doxorubicin and olaparib, administered directly or encapsulated within polymer nanoparticles, in both 2D and in 3D spheroid models of breast cancer. A variety of assays were used to evaluate single and combination drug potency, and synergy quantified using the Chou and Talalay method. Moreover, drug mechanisms have been investigated using measures of DNA damage, apoptosis and measures of mitochondrial health. Assessment of nanoparticle uptake was performed with Cy5 encapsulated particles, lysosome co-localisation and tracking doxorubicin intracellular delivery. Multidrug nanoparticle potency has been assessed and compared to free drugs in 3D spheroid models.

Results: The data demonstrate that doxorubicin and olaparib combinations act synergistically in an anti-cancer manner on triple negative (MDA-MB-231, MDA-MB-468 cells), but interestingly not on luminal breast cancer cells (MCF-7). Moreover, synergistic or antagonistic effects of this drug combination are induced in a molar ratio dependent manner. Mechanisms of synergy are related to enhancement of DNA damage as shown by the level of double-strand DNA breaks. One the other hand, mechanisms of antagonism are associated with mitochondrial mediated cell survival, as indicated by reactive oxygen species (ROS) generation and monitoring of mitochondrial membrane potential. Enhanced drug delivery was observed with nanoparticle formulations, with a greater extent of doxorubicin localised to cell nuclei as evidenced by fluorescent microscopy. Furthermore, nanoparticle formulations of drug combinations demonstrate higher cytotoxicity at the compared to free drugs.

Conclusions: Together, the work presented identifies specific molar ratio combinations of doxorubicin and olaparib which were most effective in a panel of TNBC cell lines, explores the mechanisms by which these combined agents might act, and shows that formulation of these drug combinations into polymeric nanoparticles retain the advantages of specific drug : drug ratios in synergistic cytotoxicity in TNBC cell lines.