**Evaluation of novel intratumoural oxygen delivery platform for improving radiotherapy response in hypoxic solid tumours**

Xuehua Lin[1], Maitiú Ó Murchú[1,2], Ian Sullivan[3], Brenton Cavanagh[4], Fiona O'Connell[2], Jacintha O'Sullivan[2], Helena Kelly[1]

[1]School of Pharmacy and Biomolecular Sciences, RCSI University of Medicine and Health Sciences, Dublin, Ireland.
[2]Trinity St. James's Cancer Institute, Department of Surgery, St. James's Hospital and Trinity College Dublin, Dublin, Ireland.
[3] Dept. of Physiology and Medical Physics, RCSI University of Medicine and Health Sciences, Dublin, Ireland
[4] Office of Research and Innovation, RCSI University of Medicine and Health Sciences, Dublin, Ireland

**Background:** Over the past decades, the prognosis of pancreatic ductal adenocarcinoma (PDAC) remains the worse among solid tumours with a 5-year survival rate of below 5%. Radiotherapy plays a role in the curative and palliative treatment of PDAC. The primary mechanism of action of radiation is the creation of reactive oxygen species, which in turn irreversibly damage cell DNA resulting in apoptosis and death. Molecular oxygen is therefore a potent chemical radiosensitizer in this scenario. However, hypoxia is a characteristic hallmark of PDAC due to the abnormal tumour stroma and vasculature and is an important factor limiting response to radiation treatment. Hypoxic tumours require about x3 fold higher radiation dose than normoxic tumours for comparable effect. Perfluorocarbons (PFCs) which are capable of dissolving large amounts of oxygen offer a promising approach to deliver oxygen to hypoxia tumours. Systemic delivery of PFC emulsions has been explored clinically but is limited by dose related toxicity. Intratumoural delivery of PFC could address this problem.This project aims to develop a PFC oxygen-loaded nanoemulsion for direct intratumoural injection to alleviate the hypoxia status of PDAC.

**Methods:** Two PFCs-based nanoemulsions, incorporating a commercial iodinated contrast agent (Visipaque™) were prepared via sonication and characterised via droplet size (Z-average) and polydispersity index (PDI) using Malvern's Zetasizer. Their morphologies were observed by transmission electron microscope (TEM). Viscosity was assessed using a HR-1 Discovery Hybrid Rheometer with the relationship between viscosity and oxygen storage capacity evaluated. Oxygen concentration over time was measured by Firesting $O_2$ optical oxygen meter. The imaging potential and distribution of nanoemulsions were preliminarily assessed on ex vivo mouse livers via Quantum GX2 MicroCT Imaging System. The viability of PANC1 and BXPC3 cultured in PFCs nanoemulsions at various concentrations was evaluated using LIVE/DEAD kit.

**Results:** Two lead formulations (NE_15VP and NE_30VP) showed stability for up to 6 month with a droplet size range of 140-160nm and a PDI range of 0.2-0.3. TEM imaging showed a well-defined spheroid morphology. The viscosity study indicated that the presence of increased contrast agent resulted in increased viscosity, with NE_30VP more viscous than NE_15VP. Higher viscosity resulted in slower oxygen loading and prolong oxygen release. The presence of the contrast agent enabled these nanoemulsions successfully visualised via microCT following injection into *ex vivo* mouse livers, with a greater retention at the intratumoural injection site as viscosity increased. NE_15VP and NE_30VP exhibited no toxicity to PANC1 and BXPC3 cells.

**Conclusions:** Two stable nanoemulsions incorporating a commercial imaging agent have been successfully developed. Oxygenation of this nanoemulsions offers a promising approach to alleviate hypoxic conditions in PDAC. The relationship between viscosity and oxygen storage as well as drug distribution provide a new approach for oxygen delivery to hypoxia tumours. The Visipaque™ incorporated enables excellent imaging of the nanoemulsions, offering potential use in combination with hypoxia imaging of tumours for precise intratumoural administration.