## Background to the project:

Diagnosis of pancreatic cancer tends to be at a late stage and as a result only 20% of patients have surgically resectable disease. Currently the best option for patient survival is resection which can improve the five-year survival rate from 4% to 15-20%. For those patients with LAPC or BRPC, neo-adjuvant therapy (chemotherapy before surgery) can be used to downstage the tumour and increase the likelihood of resection. This normally involves the systemic administration of toxic chemotherapy drugs that often possess no form of tumour targeting, resulting in significant off-target toxic side effects.

FOLFIRINOX is a combination chemotherapy treatment used to treat pancreatic cancer. Unfortunately, it is an extremely toxic treatment meaning it is only indicated for those patients who are otherwise fit and healthy. We have previously demonstrated that it is possible to utilise ultrasound responsive microbubbles to enable the targeted delivery of drugs to pancreatic tumours in mice. However, our microbubble design limits this to the delivery of only two agents. In this project, we will incorporate ultrasound responsive liposomes with microbubbles to enable the delivery of the four component drugs used in FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan and oxaliplatin). If successful, this should reduce off-target toxicity and provide a potential targeted neo-adjuvant treatment for LAPC or BRPC patients.

### Objectives of the research project:

**Objective 1: Preparation of microbubbles and liposomes.**

Avidin functionalised lipid stabilised microbubbles and biotin functionalised liposomes will be prepared with liposomes attached to the microbubbles using the avidin-biotin interaction. Various approaches for drug loading will be investigated such as incorporation within the MB or liposome shell through hydrophobic interactions, encapsulation in the hydrophilic core of the liposome for the hydrophilic drugs or by covalent attachment through modification of the shell chemistry.

**Objective 2: Stability of MB-liposome conjugates and in vitro drug release upon ultrasound irradiation.**

Stability of the formulations will be tested by MB counting over time. Drug release from the MB or liposome components will be tested ± ultrasound to determine release kinetics.

**Objective 3: Cytotoxicity of the conjugates in vitro**
The cytotoxicity of the drug loaded MB-liposome conjugates will be tested in a panel of pancreatic cancer cells lines and compared against the free drugs alone.

**Objective 4: Cytotoxicity of the conjugates in vivo.**
The best performing candidate from the in vitro studies will be tested in a murine model of pancreatic cancer.

**Methods to be used:**

**Objective 1:** Avidin functionalised lipid stabilised MBs will be prepared following protocols developed in our laboratory.¹⁻³ Ultrasound responsive liposomes will be prepared following a procedure developed by Rizzitelli et al⁴ but modified to incorporate DSPC-biotin in the outer lipid layer. The drugs will be incorporated at various stages during both MB and liposome manufacture to load either the MB shell, the liposome shell or liposome core. We have already prepared several biotinlated chemotherapeutics including biotin 5-Fluorouracil and will also investigate loading 5-FU on the surface of the MB using the biotin-avidin interaction.

**Objective 2:** The stability of the MB conjugates will be determined following incubation at 37°C by direct MB counting using optical microscopy. The response of the conjugates to ultrasound exposure will be determined using physiological flow conditions and a modified flow cell set-up. Ultrasound will be delivered at an intensity of 1 MHz, a pulse repetition of 100 Hz with the duration, power density and duty cycle modified to identify the optimum conditions. Drug release from the liposomes will be determined following dialysis and quantifying using UV-Vis spectroscopy or HPLC.

**Objective 3:** The cytotoxicity of the conjugates will be tested in a panel of pancreatic cancer cell lines [Panc01, BxPC3, MiaPaCa and KPC] using conventional 2D tissue culture-based systems as well as 3D spheroids for the BxPC3 and KPC cell lines. Conventional cell viability assays will be used to determine toxicity ± ultrasound irradiation.

**Objective 4:** The best performing formulation from objectives 2-3 will be administered IV to SCID mice bearing human xenograft ectopic MiaPaCa-2 tumours with ultrasound applied to the tumour during and for a fixed period after administration. Tumour volume will be measured using callipers on a daily basis for 14 days following treatment. Control groups using untreated animals and animals treated with conjugates only in the absence of ultrasound will be used for comparative purposes.

**Skills required of applicant:**
- Understanding of / or willingness to learn basic synthetic chemistry techniques.
- Understanding of / or willingness to learn cell culture techniques.
- Willingness to undertake animal experimentation.

**References:**