Description for the general public

(objective of the project, the research carried out, reasons for choosing the research topic)

Molecularly targeted-based treatments including therapeutic antibodies, antibody-toxin conjugates, and radioimmunoconjugates offer the promise of being effective with fewer side effects than conventional cancer therapies. Recently**, photoimmunotherapy (PIT) has been established as a potential and highly selective cancer treatment.** The therapy utilises the targeting ability of a highly specific monoclonal antibody (mAb) conjugated to a photosensitiser (PS, e.g. porphyrin, IRD700). The conjugate benefits from the targetable property of the antibody, but relies on the cytotoxicity generated via reactive oxygen species when the PS is irradiated. Emerging evidence from current studies indicate that PIT induces rapid and profound damage to the outer and inner membrane structures of target cells, where the mAb-IRD700 is bound, leading to necrotic cell death. Furthermore, it was demonstrated that immediate cytotoxic effects induced by PIT could be detected by decreased glucose uptake using ^{18}F-FDG PET imaging, before changes in tumour size became evident *in vivo*. In addition, since the PS used in PIT is a fluorescence agent, this strategy allows achieving diagnostic and therapeutic effects simultaneously (theranostics approach). Even though mAbs-based immunoconjugates offer exquisite selectivity of binding to their designated targets, the relatively large molecular size of mAbs (150 kDa) hinder access to the target and reducing penetration into tumour parenchyma, which limits the extent of therapy. Therefore, **we propose using engineered affibody molecules**, which are considerably smaller (~6.5 kDa) proteins that bind with high target affinity (pM-nM range) **to selected targets** (e.g. HER2) **and could improve the intratumoural distribution of the photoconjugate.**

We hypothesise that affibody-infrared light activated conjugates targeting HER2 will have significant activity in inducing cell death selectively in HER2+ve cancer cells, while limiting IR700 toxicity in normal tissues.

The hypothesis will be tested through two research aims:

Aim. 1. We will optimise labelling strategies and determine the immunoconjugate's optimal binding kinetics, as well as therapeutic effect in a panel of HER2+ve cell lines *in vitro*.

Aim. 2. We will evaluate the therapeutic response of the immunoconjugate in HER2+ve mouse models and compare this with metabolic activity, histological characteristics and HER2 expression in tumour tissues following treatment.

HER2-specific affibody molecules will be conjugated to IR700 through maleimide group. Following conjugation optimisation, probes will be thoroughly characterized by *in vitro* binding and saturation assays to determine the receptor affinity for the probe using human HER2+ve cell lines with different HER2 expression.

Phototoxicity studies *in vitro* will be performed using cells treated with: (i) $Affi_{HER2}$IR700, (ii) IR700 for 1 and 4 hours and exposed to red light emitted at wavelengths of 670–690 nm at a fluence rate of 10 mW/cm^2. Afterwards, cells will be incubated at 37°C for the following 24, 48 and 72 h and their proliferation will be evaluated using standard colorimetric proliferation assay e.g. MTS. Since PIT induces acute stress, accompanied by bursts in molecular signalling transduction pathways in response to this damage, at each time point cell lysates will be prepared for Western blot to assess the effect of treatment on HER1/HER2/HER3 expression and downstream signalling, especially the PI3K/AKT/mTOR pathway.

Pharmacokinetic studies of the immunoconjugate *in vivo* will be performed using animal models. Cells will be implanted on the right shoulder of mice. In order to assess the systemic conjugate distribution typically three mice will be used per time-point and conjugate dose. Mice will be sacrificed and their major organs as well as tumours dissected and imaged using IVIS-Spectrum small animal imaging system. Regions of interest (ROIs) will be drawn around each organ, and the mean fluorescence intensity calculated for each. This will allow us to select the optimal conditions for the following treatment studies. The tumour growth inhibitory properties of PIT using $Affi_{HER2}$IR700 will be performed after tail vein injection of i) saline, ii) $Affi_{HER2}$IR700 or iii) IR700 alone. Mice will be subjected to PIT by exposing the tumour to light from a red-light–emitting diode at a wavelength of 670–710 nm and a power density of 100 J/cm^2.

This early stage project will provide information about the basic mechanisms involved in the treatment that maximize target-cell killing. However, in a long term, such an approach has tremendous translational potential that could lead to novel and effective therapeutic strategies for patients with HER2+ve cancers.