Description for the General Public

Our understanding of the molecular pathogenesis of gliomas is only now beginning to improve. High grade gliomas (GBM) appear to be both preferentially and differentially driven by EGFR signalling. Mutations and focal amplifications of the receptor are present in 60-90% of GBM patients and are associated with more aggressive tumour behaviour, poorer prognosis and shorter survival. However, the results obtained in early phase clinical trials with inhibitors directed against this receptor were very disappointing, in part due to acquired resistance as well as lack of reliable methods for pre-selection of patients likely to respond to these therapeutics. This is not surprising since there are, as of yet, no validated predictive and response biomarkers in glioma tumours.

Therefore, novel and more sensitive diagnostic tools and new treatment paradigms are necessary to lead to durable remissions or even cures.

Recently, there has been considerable interest surrounding photoimmunotherapy (PIT), which utilises the targeting ability of a highly specific monoclonal antibody (mAb) conjugated to a photosensitiser (PS) e.g. phthalocyanine. The conjugate benefits from the targetable property of the mAb, but relies on the cytotoxicity generated via reactive oxygen species and localised heating of water when the PS is irradiated (conventional photodynamic therapy, PDT). Therefore, such an approach may not only allow for precise identification of tumour margins during the operative period, but also simultaneous eradication of cancerous cells that will spare patients from the added morbidity associated with additional surgery or chemoradiotherapy.

While using mAb as the targeting moiety offers exquisite selectivity of binding to designated targets, making PIT a promising treatment, their relatively large molecular size (150 kDa) hinders access to the target *in vivo* and reduces penetration into tumour parenchyma, dramatically limiting the extent of therapy. **We therefore, aim to develop an EGFR specific affibody-based IR700DX activated conjugate and investigate its mechanism of action using GBM primary patient-derived cell lines that recapitulate the genetic profiles and histologic features of GBM.** Furthermore, we will additionally test the PIT efficacy in novel models of GBM *in vivo* by investigating the major mechanisms involved in the treatment that may maximise target-cell killing.

Affibody molecules are low molecular weight (~6.5 kDa), three-helix proteins which were originally derived from IgG-binding domain B of staphylococcal protein A. Using various display formats including phage or staphylococcal display, affibody molecules can be selected against a particular protein of interest via randomisation of 13 surface residues. The lack of di-sulphide bonds and internal cysteines, rapid folding properties and high stability facilitate the conjugation to different fluorophores or radioisotopes. Moreover, high binding affinity (pM to nM range) of the molecules to selected targets, their small size resulting in rapid clearance from circulation with predominantly renal excretion *in vivo*, as well as good tumour penetration, make them ideal targeting agents for cancer diagnosis and therapy. Therefore, in the long term, our approach has tremendous translational potential that could lead to novel and effective therapeutic strategies for GBM patients with EGFR+ve cancers.