Evaluating targeted therapy resistance in breast cancer through mTORC1 imaging

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Our aim is to develop mTORC1 specific small molecule imaging probes that are capable of predicting breast cancer resistance to treatment utilising optical microscopy and Positron Emission Tomography (PET) imaging modalities. The combination of these approaches allows for clinically relevant imaging experiments to be conducted alongside detailed cellular microscopy. There is an unmet clinical need to identify HER2-positive breast cancer patients that are sensitive to targeted therapy. During the BOLERO-3 phase III clinical trial, the addition of everolimus to trastuzumab plus vinorelbine significantly prolonged progression free survival in patients with trastuzumab-resistant and taxane-pretreated, HER2-positive, advanced breast cancer. The common PTEN loss or PI3K mutation seen in these patients has been shown to induce the activation of the mTOR pathway, potentially mediating trastuzumab resistance.

To identify and predict the onset of these resistance mechanisms, we have developed a panel of potent small molecule inhibitors specific for mTOR complex 1. These were derived from the benzofuran based compound ChemBridge 5219657. Alkyne derivatives suitable for conjugation with fluorescent and radioisotope labels via Cu(I)-catalysed click chemistry were developed. Synthesis of these click precursors was achieved over 5 linear steps with a 24% isolated yield. Quality control was performed using NMR, MS, UV- and radio-HPLC analytic methods.

Initial in-vitro sensitivity to everolimus was performed via SRB proliferation analysis following treatment on HER2-positive human breast cancer cell lines (BT-474 and HCC-1419) and HER2 low expressing control cell lines (MDA-MB-468 and MDA-MB-231). In in-vitro binding experiments our 18F-labeled analogues demonstrated increased uptake in BT-474 and HCC-1419 cells, 1.5 and 2.5-fold compared to the negative controls, respectively. NSG mice bearing BT-474, MDA-MB-231 and MDA-MB-468 xenografts were explored for in-vivo dynamic PET imaging experiments confirming this selective uptake of radiotracer in everolimus sensitive cells. Alterations in cellular internalisation and localisation conferring sensitivity or resistance for these in-vitro models utilising a fluorescently labelled derivative are ongoing.

Our synthetic strategy identified a reliable approach capable of producing sufficient amounts of small molecules for both in-vitro and in-vivo investigations. The increased uptake in mTOR treatment sensitive cell lines in-vitro as well as in-vivo provides encouragement, warranting further preclinical studies for the investigation of HER2-positive therapy resistance in mTOR sensitive models.

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