Background Chemotherapy has limited efficacy against brain metastases (BM) because heterogeneous tumour architecture and abnormal interstitial fluid pressure limits passive delivery of drug via blood stream. Most cytotoxic agents are associated with adverse toxicity, poor biological half-lives and non-specificity for tumour cells. Furthermore, BM are often detected late when patients become symptomatic. Theranostic nanomedicines can provide earlier detection of BM, as well as improving therapeutic efficacy and side-effect profiles of existing chemotherapeutics through tumour targeting, delayed drug clearance and microenvironment-mediated drug release. HER2 and HER3 protein receptors are implicated in tumour growth and are upregulated in a subset of breast cancer brain metastases. Here, we investigate the therapeutic potential of targeting HER2/3 using a polyethylene glycol (PEG)-based hyperbranched polymeric drug-carrier system (HBP) (depicted in Fig 1a)

**Aim** We aim to: (1) develop HER2 and 3 –targeting antibody fragments (scFvs), (2) attach HBP with scFvs and imaging moiety (Cy5) to facilitate ex vivo analysis of tissue distribution. (3) Functionalise HER2/3-targeted HBP with doxorubicin (DOX) via an acid-labile linker for pH-sensitive release in the hypoxic tumour microenvironment, and the intracellular compartment after internalization, (4) develop a BM mouse model, (5) and investigate the therapeutic potential of the HER2 and -3 targeted HBP using in vitro and in vivo models.

**HER2/3 antibody fragments are specific with strong binding affinities**

**HER2- and HER3-targeted scFvs based on ligand-binding sequences of clinically-approved antibodies were synthesized. Binding affinities were found to be stronger than parent antibodies (Fig 1b).**

**Internalisation of targeted HBPs and pH dependent drug-release**

**HER2/3-targeting HBPs were internalized into breast cancer cells and released DOX after 4 hours (Fig2). DOX-release was greatest at acidic pH (5.5) compared to physiologic neutral pHs, 6.8 and 7.4 (Fig 3).**

**Targeted HBPs were cytotoxic to cancer cells**

Relative to untreated cells, all targeted HBPs treatments were cytotoxic to BT474 with HER3-targeted HBP being the most cytotoxic treatment.

**HBP accumulation in brain metastases**

PET/MR imaging showed that radioabeled (89Zr) HBPs accumulated predominantly in BM region (MRI: red circle, PET: Red orange regions)

There were greater DOX signal detected in brains of mice treated with targeted HBPs, compared to free doxorubicin. Unlike HBP-treated mice (green, yellow lines), DOX-treated mice experienced drastic drop in body mass post-treatment (purple)

**Conclusions** Efficacy in vivo study is currently in progress. Available data shown that HER2/3-targeted-HBP are tumour-specific and cytostatic, with the ability to localize and deliver doxorubicin into brain metastases. HBP treatment was also more tolerable compared to conventional doxorubicin.