Immediate impact of Herceptin targeting nanoparticles on plasma membrane dynamics in HER2+ breast cancer cells

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Background: Nanoparticles decorated with various ligands offer the opportunity to target plasma membrane receptors that are often upregulated in different types of cancer cells. This includes the receptor tyrosine kinase HER2 that is upregulated and driving cell growth and division in 20% of breast cancer cases. Despite significant leaps in cancer cell biology and nanoparticle design, translation of targeting nanoparticles as nanotherapeutics delivering therapeutic payloads, through to the clinic has been disappointing. This includes those targeting HER2 that is characterised by having very poor endocytic capacity compared to other family members such as EGFR. Pre-clinical in vitro studies with cell models of disease allow for high content mechanistic analysis of the initial binding of the nanoparticle to the cell surface and also endocytosis and intracellular traffic to various subcellular compartments or recycling back to the plasma membrane. For nanoparticle image analysis, typical studies explore timepoints in the range 1-24hrs thus potentially overlooking critical events that may happen immediately after exposure of the cells to the nanoparticle. As ligand binding to growth factor receptors generally results in very rapid downstream signalling and often endocytosis, there is a need to capture nanoparticle cell dynamics at very early time points following their addition to cells. Previous research by our lab discovered that crosslinking HER2 on breast cancer cells stimulated its endocytosis and trafficking to lysosomes for enzymic degradation. This leads to the hypothesises that ligand decorated nanoparticles can be tailored to crosslink HER2 receptors and to drive endocytosis and thus selectively deliver a payload.

Methods: We employed SEM and live cell confocal microscopy to monitor plasma membrane dynamics, cellular binding and uptake of poly(lactic-co-glycolic acid (PLGA) nanoparticles decorated with the clinically approved HER2 targeting monoclonal antibody Trastuzumab (Tz) labelled with Alexa488 (PLGA-Tz488); including a variant encapsulating the fluorescent dye Rhodamine (PLGA-Tz488-Rho). These were analysed in HER2 high (SKBR3, BT474) or low (MCF-7) expressing breast cancer cells

Results: Initial analysis after 5 hour incubations show selective PLGA-Tz488-Rho binding to the HER2 overexpressing cells with evidence of the aggregation of the particles on the plasma membrane. Confocal time lapse Imaging and SEM at very early time points (<15 minutes) after addition of PLGA-Tz488 revealed extensive reorganisation of the plasma membrane that we postulate is caused by particle mediated HER2 crosslinking. Endocytosis of PLGA-Tz488 was analysed in cells having previously had their lysosomes labelled with the fluid phase endocytosis and macropinocytosis probe Alexa647-dextran. Confocal analysis revealed clear colocalization of both probes confirming that the events observed at the plasma membrane was driving particle uptake to this organelle. Ongoing studies will allow for determination of the fate of HER2 following nanoparticle mediated crosslinking and uptake, mechanistic analysis of proteins responsible for plasma membrane reorganisation and the capacity of the NPs to deliver bioactive cargo to the cytosol and beyond.

Conclusions: Our studies highlight very early events that occur on the plasma membrane that are likely to have significant effects on downstream processes such as signalling, internalisation, endocytic traffic and fate that will ultimately influence delivery efficiency of any therapeutic cargo into the cytosol to mediate a therapeutic effect.