

## **Biparatopic targeting of epidermal growth factor receptor positive breast cancer cells using domain I/II and domain III specific antibody conjugates**

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**Background:** Epidermal growth factor receptor (EGFR) is overexpressed in > 50% of breast cancer. EGFR consists of an extracellular or ligand binding domain which consists of four binding epitopes (domains I, II, III, IV). Anti-EGFR therapeutic antibodies such as nimotuzumab and cetuximab bind to domain III of EGFR. For the first time we have developed an antibody that binds to epitope I & II of EGFR called FabH. Our hypothesis is that simultaneous targeting of domains I/II and III using immunoconjugates that are specific to these epitopes can lead to enhanced therapeutic outcome in EGFR positive cancers. To accomplish this we aimed to deliver potent alpha particle to domain I/II using 225Ac-FABH (radioimmunotherapy) and potent cytotoxic agent (PEGylated maytansine using nimotuzumab-PEG6-DM1 antibody drug conjugate (an ADC) to domain III.

**Methods:** FABH was conjugated to an eight-membered macrocyclic chelator SCN-macropa, and then radiolabeled with 225Ac. The radiochemical yield of 225Ac-FABH was >90%. We investigated the cytotoxicity of this biparatopic approach in EGFR-positive breast cancer cells. 225Ac-FABH and nimotuzumab-PEG6-DM1 were developed and characterized by flow cytometry, bioanalyzer, HPLC and internalization rate (live-cell imaging). In vitro cytotoxicity was studied in MDA-MB-468, MDA-MB-231 and TrR1 EGFR-positive triple negative breast cancer cells. In vitro cytotoxicity using the biparatopic approach (225Ac-FABH + nimotuzumab-PEG6-DM1) was compared with 225Ac-FABH, + nimotuzumab-PEG6-DM1 or control non-specific immunoconjugates.

**Results:** Bioanalyzer showed the purity and size of FabH, nimotuzumab, nimotuzumab-PEG6-DM1, and their respective macropa conjugated immunoconjugates. Flow cytometry showed nearly > 90% binding to the cells. In all three cell lines, in vitro studies showed enhanced cytotoxicity of 225Ac-FABH + nimotuzumab-PEG6-DM1 compared with the single agents FABH, nimotuzumab, nimotuzumab-PEG6-DM1, 225Ac-FABH or non-specific (radio)immunoconjugates which was evident from low IC50 values ranging from 12nM<<47nM<<65nM<<105nM<<125nM respectively.

**Conclusions:** The delivery of multiple cytotoxic agents to EGFR using this biparatopic approach is very promising in vitro. In vivo studies using mouse xenografts are planned.

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### **Presentation Type**

Poster

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