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Targeted alpha particle therapy of EGFR-positive breast cancer using site-specifically labeled 225Ac–dN-SpyCatcher-SpyTag-nimotuzumab

Introduction: Targeted alpha-particle therapy is a promising approach for breast cancer treatment. Anti-EGFR antibodies e.g. cetuximab, panitumumab, and nimotuzumab are used to treat different EGFR positive cancers. Especially, nimotuzumab is better tolerated and has low skin toxicities, because it's "affinity optimized"binding characteristic ensures low transient binding to low EGFR-expressing healthy tissues such as the skin. In this study, we have radiolabeled an anti-EGFR antibody nimotuzumab with 225Ac at the Fc domain using SpyCatcher/SpyTag protein ligase system. We have evaluated the 225Ac-dN-SpyCatcher-SpyTag-nimotuzumab in EGFR-positive MDA-MB468 cells and mouse xenograft.

Methods: Nimotuzumab was site-specifically labeled by a two-step process. Firstly, dN-SpyCatcher was reduced using TCEP, which was followed by desferoxamine (DFO-maleimide conjugation to yield a reactive DFO-dN-SpyCatcher. The DFO-dN-SpyCatcher was reacted with SpyTag-nimotuzumab to obtain stable dN-SpyCatcher-SpyTag-nimotuzumab. Radiolabeling was performed with 89Zr, and the conjugate was used for imaging in vivo. Similarly, dN-SpyCatcher was conjugated to an eight-membered macrocyclic chelator SCN-macropa, and used to radiolabel the SpyTag-nimotuzumab with Actinum-225. All constructs were characterized using biolayer interferometry, flow cytometry, radioligand binding assays, HPLC and bioanalyzer. The in vitro cytotoxicity of 225Ac-dN-SpyCatcher-nimotuzumab-SpyTag was evaluated in EGFR-positive MDA-MB-468 and EGFR-negative MDA-MB-435 cells using live-cell imaging and the in vivo efficacy was studied in mice bearing MDA-MB-468 xenografts. When tumors had reached 50 −100 mm3, mice were treated with two 450 nCi doses of 25Ac-dN-SpyCatcher-SpyTag-nimotuzumab 14 days apart. Non-specific binding antibody construct was used as control.

Results: In vitro binding in MDA-MB-468 cells was specific. Radiochemical yield for 89Zr and 225Ac radioimmunoconjugates was >90 % with a purity >95 % of both tracer agents. MicroPET/CT imaging showed good tumor uptake of 89Zr-dN-SpyCatcher-SpyTag-nimotuzumab with the highest % injected activity per gram of 6 % at 48 h post injection. The IC50 of 225Ac-dN-SpyCatcher-SpyTag-nimotuzumab and 225Ac-Control-IgG against MDA-MB468 cells was 0.13 ± 0.09 and 0.43 ± 0.16 nCi/mL, respectively. 225Ac-dN-SpyCatcher-nimo significantly prolonged the survival of MDA-MB468 mice (60 days) compared to 225Ac-control IgG (33.5 days) or PBS treated mice (30 days). Further evaluation in other EGFR positive xenografts is ongoing.

Conclusion: The results showed that the conjugation and labeling by dN-SpyCatcher system to nimotuzumab did not significantly alter the receptor binding and internalization nimotuzumab compared to non-specific conjugation approach. 225Ac-dN-SpyCatcher-SpyTag-nimotuzumab was effective in vitro and showed to be promising in a breast cancer xenograft.

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