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The therapeutic potential of anti-HER2 2Rs15d nanobody labeled with 225Ac –an in vitro and in vivo evaluation

Objectives: Nanobodies (Nbs) are the smallest antibody-derived fragments with beneficial pharmacokinetic properties for molecular imaging and radionuclide therapy. Human Epidermal Growth Factor Receptor type 2 (HER2) is overexpressed in numerous carcinomas and portends a poor prognosis. Therefore, HER2-targeting nanobodies are very attractive vectors for TRT, especially when labeled with α-particle emitters. The aim of this study was to evaluate the therapeutic potential of the anti-HER2 Nb 2Rs15d labeled with 225Ac.
Methods: Anti-HER2 Nb 2Rs15d was coupled with the bifunctional chelate p-SCN-Bn-DOTA and further was labeled with 225Ac. Its binding affinity and specificity for HER2, together with immunoreactive fraction (IF), were evaluated on SKOV-3 (HER2+) and MDA-MB-231 (HER2-) cells. Its in vitro cytotoxicity was assessed using MTS and clonogenic assays. In vivo, 225Ac-DOTA-Nb 2Rs15d and a non-targeting control 225Ac-DOTA-Nb R3b23 were evaluated in female athymic nude mice subcutaneously xenografted with SKOV-3 and MDA-MB-231 tumors, both alone and with molar excess of unlabeled 2Rs15d. After determination of maximum tolerated dose (MTD), the therapeutic efficacy of 225Ac-DOTA-Nb 2Rs15d was investigated in mice bearing intraperitoneal SKOV-3.IP1/luciferase+ xenografted metastases against several controls, a trastuzumab regimen and a combination of both 225Ac-DOTA-Nb 2Rs15d and trastuzumab.
Results: The yield of DOTA-Nb 2Rs15d labeling was high (>90%), with radiochemical purity ≥95%. 225Ac-DOTA-Nb 2Rs15d bound specifically to HER2+ cells with ~75% IF, a KD of 3.50±0.17nM and lack of competition with trastuzumab or pertuzumab in vitro. Cytotoxicity studies demonstrated that 225Ac-DOTA-Nb 2Rs15d significantly reduced SKOV-3 cell viability in a dose-dependent and HER2-mediated manner, compared to 225Ac-DOTA or 225Ac-DOTA-Nb R3b23 as controls. Tumor uptake in SKOV-3 xenografted mice was high and specific (~8%), whereas in MDA-MB-231 was <0.5% already 1h pi. Its accumulation in kidneys was reduced almost 3-fold by coinjection 225Ac-DOTA-Nb 2Rs15d with 150 mg/kg Gelofusine. Therapy studies indicated that 225Ac-DOTA-Nb 2Rs15d increased Median Survival significantly, which measured 83 days compared to about 49 days for animals treated with controls PBS and 225Ac-DOTA-Nb R3b23, and to 72 days in case of trastuzumab regimen. The most extensive therapeutic effect (MD~97 days) was observed for the combination of both 225Ac-DOTA-Nb 2Rs15d and trastuzumab.
Conclusions: 225Ac-DOTA-Nb 2Rs15d efficiently targets HER2+ cells both in vitro and in vivo. Strong signs of therapeutic potential were observed in vitro, which were confirmed also at in vivo setting in mice bearing SKOV-3 xenografts. This study underlines the strong potential of 225Ac-DOTA-Nb 2Rs15d as a new radioconjugate for TAT and supports its further development towards the clinic.
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