Targeted alpha-therapy with 211At-anti-mCD138 mAb in a syngeneic murine Multiple Myeloma model : can repeated doses or fractionation protocol overpass single dose efficiency?

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EGFR bsAb was radiolabeled with ^{89}Zr for microPET imaging and biodistribution. Tumor uptake of ^{89}Zr-bsAb ,control ^{89}Zr-human IgG was studied in CD-1 nude mice inoculated with EphA2+/EGFR+ MDA-MB-231 TNBC cells. BsAb was radiolabeled with ^{225}Ac for alpha particle therapy. The (radio)immunoconjugates and controls were characterized by flow cytometry, HPLC and internalization rate (live-cell imaging). In vitro cytotoxicity was studied in EphA2+/EGFR+ MDA-MB-231. In vivo radioimmunotherapy using ^{225}Ac-bcAb was studied in EphA2+/EGFR+ MDA-MB-231 model following treatment with three doses of 350 nCi/dose administered 10 days apart. A panel of EphA2-/EGFR+, EphA2+/EGFR- and EphA2-/EGFR- cells are developed to further characterize the imaging properties of ^{89}Zr-bsAb and study in vitro cytotoxicity and in vivo efficacy of ^{225}Ac-bsAb. ***Results:*** Flow cytometry showed > 90% binding to the cells. The microPET imaging data showed persistently high tumor uptake of ^{89}Zr-bsAb in EphA2+/EGFR+ MDA-MB231 xenograft. The uptake of ^{89}Zr-bsAb was clearly visible as early as 24 h p.i. (6.1 ± 0.9 %IA/g) and peaked at around 48 h p.i. (6.6 ± 1.3 %IA/g). In vitro studies showed enhanced cytotoxicity (IC_{50}) of ^{225}Ac-bsAb (1.8 ± 0.6 nM; 0.5 nCi/mL) compared with control ^{225}Ac-IgG (5.8 ± 1.9 nM; 1.5 nCi/mL) and non-labeled antibodies: bsAb (113.5 ± 0.4 nM); human IgG (374.1 ± 1 nM), respectively. ^{225}Ac-bsAb prolonged the survival of mice bearing EphA2+/EGFR+ MDA-MB231 TNBC xenograft compared with control ^{225}Ac-human IgG or non-treated mice. 1/5 mice treated injected with ^{225}Ac-bsAb had complete remission. Median survival after > 90 days since start of treatment are: ^{225}Ac-bsAb (not yet reached); unlabeled bsAb (37 days), control ^{225}Ac-human IgG (24 days), non-treated (20 days). ***Conclusion:*** ^{225}Ac-labeled anti-EGFR/EphA2 bispecific radioimmunotherapeutic shows great promise in TNBC. Validation of this radioimmunoconjugate in other TNBC xenografts is ongoing. ***References:*** None

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Comparison of intravenous and intraperitoneal dosing of the Targeted Alpha Therapy Thorium-227-anetumab corixetan in a model of peritoneal carcinomatosis of mesothelioma

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Aim/Introduction: Peritoneal carcinomatosis is common in abdominal cancers of mesothelium and ovary appendix. The treatment may include cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy. Mesothelin-targeted thorium-227 conjugate (MSLN-TTC, ^{227}Th-anetumab corixetan) is a new targeted alpha therapy (TAT; 1, 2) currently being tested in phase 1 in patients suffering from mesothelioma and serous ovarian cancer (NCT03507452) and dose administration is pursued intravenously. An alternative treatment option could be administration of MSLN-TTC into the intraperitoneal cavity. We present the differences in biodistribution, efficacy and tolerability of the two administration routes, studied in a mouse model of peritoneal carcinomatosis of mesothelioma. ***Materials and Methods:*** Nude mice with established intraperitoneal (i.p.) luciferase-tagged mesothelioma NCI-H226 tumors were allocated to 7 groups (n=10 each) after stratification using bioluminescence imaging signals (BLI). Animals were treated either intravenously (i.v.) or intraperitoneally (i.p.) with MSLN-TTC (2 x 250 kBq/kg, 8 weeks apart, or 1 x 500 kBq/kg). Tumor burden was followed by weekly bioluminescence imaging (BLI). Mice were sacrificed when a critical BLI signal intensity was reached. Tolerability was followed by hematology measurements. In a biodistribution study, MSLN-TTC accumulation was compared to a murine cross-reactive MSLN-surrogate-TTC, both administered either i.v. or i.p. at a dose of 500 kBq/kg. Organs and tumors were harvested two weeks after administration to determine accumulated thorium-227. ***Results:*** In the biodistribution study, a statistically significant higher tumor accumulation was observed for MSLN-TTC after i.p. administration when compared to i.v. Accumulation in all organs, including isolated peritoneum, was not statistically significantly different. Similarly, there was no difference in organ and tumor accumulation between MSLN-TTC and the murine cross-reactive MSLN-surrogate-TTC. In the efficacy study, animals were euthanized upon reaching a critical BLI signal. Vehicle treated animals showed a medium survival time (MST) of 25 days and the MST for radiolabeled isotype control treated animals was 55 days after i.v. or i.p. administration. However, animals treated with MSLN-TTC showed MST of 80 to 98 days after i.v. or i.p. dose administration. However, based on tumor weight data upon sacrifice, i.p. administration of MSLN-TTC appeared to be more effective than i.v. administration. Intravenous administration of MSLN-TTC resulted in longer lasting leukopenia. ***Conclusion:*** Intraperitoneal administration of MSLN-TTC in an intraperitoneal model of mesothelioma resulted in increased efficacy and decreased myelosuppression. ***References:*** 1. Hagemann UB et al; Cancer Biother Radiopharm. 2020 Apr 7. doi: 10.1089/cbr.2020.3568. 2. Hagemann UB et al; Clin Cancer Res. 2019 Aug 1;25(15):4723-4734. doi: 10.1158/1078-0432.CCR-18-3476. Epub 2019 May 7

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Targeted alpha-therapy with ^{211}At-anti-mCD138 mAb in a syngeneic murine Multiple Myeloma model : can repeated doses or fractionation protocol overpass single dose efficiency?

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Aim/Introduction: Multiple myeloma (MM) is a B-cell malignancy of terminally differentiated plasma cells developing in the bone marrow. Despite a considerable improvement over the past 30 years, cure is almost never achieved. We have already demonstrated the usefulness of Targeted Alpha-Therapy (TAT) to eradicate residual cells in a MM preclinical syngeneic model by administrating a single high dose of monoclonal antibody anti-CD138 radiolabeled with astatine-211 as alpha particle-emitting radionuclide. The aim of this study was to evaluate the benefit of dose fractionation or repeated doses of an astatine-211-labelled anti-mouse CD138 antibody (^{211}At-9E7.4) in term of survival or side effects in the same syngeneic mouse MM model compared to one injection therapy. ***Materials and Methods:*** C57BL/KaLwRij mice were grafted with 10^6 5T33 MM cells by intravenous injection and received a first injection of ^{211}At-9E7.4 ten days after. A group of mice was used to evaluate the fractionated doses protocol in which a 660 kBq efficient activity was administered in one injection, in 2 fractions of 330 kBq or in 3 fractions of 220 kBq with 1 or 2 weeks interval. A second group was used to evaluate the repeated doses protocol: a 660 kBq activity was administered once or twice with 1 or 2 weeks interval. Hematologic parameters were monitored as well as survival. Data were compared to our previously data obtained with single-dose. ***Results:*** As a reminder, a single-dose of 740 kBq of ^{211}At-9E7.4 was able to cure 65% of mice until 150 days after engraftment while 1100 kBq provoked radiotoxic lethality 14 days after treatment injection. Without any treatment, mice died around 42 days after engraftment presenting paraplegia in most of the cases. In this study, a single-dose of 660 kBq cured 40% of mice at D100. The fractionation in 2 doses treated only 20% of mice regardless of the time interval between the 2 doses. By fractionating the dose of 660 kBq in 3 parts of 220 kBq, even less survival benefit was observed. Concerning the repeated dose protocol, an interval of one week between 2 injections of 660 kBq appeared lethal while an interval of 2 weeks appeared possible in term of early radiotoxicity but gave no benefit in term of survival. ***Conclusion:*** In the particular context of MM disseminated microlesions treatment, it appears that neither the split-dose protocol nor the repeat-dose protocol provided better results than the single-dose treatment. ***References:*** None

OP-830

Increased SST_2 Expression on Pulmonary Neuroendocrine Tumor Cells by Epigenetic Drug Treatment with Histone Deacetylase Inhibitors

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Aim/Introduction: Our aim is to increase the expression of somatostatin type-2 receptors (SST_2) on the pulmonary neuroendocrine tumor (NET) cell line NCI-H727 by using epigenetic drugs. We tested the effect of six histone deacetylase inhibitors (HDACis), aiming to promote the euchromatin state supporting SST_2 gene transcription. This approach may possibly open up opportunities for NET patients with insufficient tumoral SST_2, thereby increasing the number of patients eligible for SST_2-targeted therapies such as peptide receptor radionuclide therapy (PRRT). We also compared our results with similar studies performed in the pancreatic NET cell line BON-1. ***Materials and Methods:*** EC_{50} values on cell growth were determined in NCI-H727 after 7-day HDACi treatment. Valproic acid (VPA), tacedinaline (TAC), entinostat (ENT), LMK-235, mocetinostat (MOC) and panobinostat (PAN) were evaluated. After a 7-day treatment, (1) samples were collected for RT-qPCR to determine SST_2 mRNA expression levels and (2) internalization studies were performed by incubating NCI-H727 with 1mL of 1nM [^{111}In]In-DOTA-TATE (50MBq/nmol), +/- 1μM unlabeled DOTA-TATE. To study the reversibility of the observed effects, samples were collected for RT-qPCR 1 and 3 days after HDACi-withdrawal. ***Results:*** For all HDACis, except MOC, the uptake of [^{111}In]In-DOTA-TATE was significantly increased, reaching a maximum upregulation of 4.2-fold for VPA. In line with this, SST_2 mRNA levels were upregulated as well, although statistical significance was not reached for LMK-235 and PAN. For MOC, both SST_2 mRNA levels and uptake of [^{111}In]In-DOTA-TATE were significantly downregulated. Already 1 day after HDACi-withdrawal, SST_2 mRNA levels were significantly lowered for 5 of the 6 HDACis. Only VPA-treated cells reached control levels at this time. Three days after drug withdrawal, SST_2 mRNA levels reached control levels for all treatments. In comparison, control levels were not reached yet in BON-1 7 days after HDACi-withdrawal for 4 of the 6 HDACis. Moreover, the percentage increased uptake of [^{111}In]In-DOTA-TATE was higher in BON-1. ***Conclusion:*** In conclusion, SST_2 expression is evidently upregulated by HDACi treatment in NCI-H727, characterized by intermediate basal SST_2 expression levels. Effects were more prominent in BON-1, probably due to lower basal expression levels of this cell line. Our results support the possibility for a combined treatment approach of HDACi and SST_2-targeted therapies. Furthermore, our in vitro results predict the importance of precise timing as SST_2 upregulation is reversible. Future studies focus