INFLUENCE OF THE ADMINISTRATION ROUTE ON THE TREATMENT EFFICIENCY OF TUMOR-ASSOCIATED ANTIGEN (TAA) SPECIFIC TH1 CELLS IN AN ANIMAL MODEL FOR PANCREATIC CANCER

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Introduction: TAA-specific Th1 cells could serve as a promising new approach for immunotherapy of cancer patients. For the clinical application adequate treatment schemes have to be established defining the right administration route, dosage, injection frequency or adjunction with other immunotherapeutic agents or chemotherapeutics like the leucocyte depleting agent cyclophosphamide. Furthermore, the mode of action of TAA-specific Th1 cells during immunotherapy is not well understood and has to be further characterized in preclinical settings. In this approach we compared the influence of the administration route on TAA-specific Th1 cell-treatment efficiency in an endogenous animal model for pancreatic cancer (RIP1-Tag2).

Methods: Irradiated (2Gy) RIP1-Tag2-mice were treated weekly *i.v.* or *i.p.* with 10^7 Tag2-Th1 cells until the age of 14 weeks. The tumor size progression and treatment efficiency were determined by 7 T small-animal Magnetic Resonance Imaging (MRI). In addition blood glucose levels (BGL) serve in this animal model as a marker for tumor burden and progression. Untreated animals die at 14 weeks of age due to hypoglycemia. We further analyzed homing properties of *i.v./i.p.* transferred Cy5-fluorescence labeled Tag2-Th1 cells in 14 weeks old Tag2-Th1 cell-treated RIP1-Tag2-mice with Optical Imaging (OI) and Fluorescence Microscopy (FM). To characterize the Tag2-Th1 cell-mediated antitumoral effect, we further performed H&E-histology of the T-cell homing sites and immunohistochemistry of the pancreatic tissue.

Results: BGL in *i.p.* treated mice were 82±5 mg/dl and slightly increased in *i.v.* treated mice to 93±4 mg/dl, compared to 41±3 mg/dl in SHAM-treated mice at 14 weeks of age. In SHAM-treated 14 weeks old RIP1-Tag2-mice the mean tumor volume was 30.0±8.6 mm^3, in *i.p.* treated mice 15.2±4.4 mm^3 while tumor volume was lowest in *i.v.* treated mice with 5.8±1.7 mm^3 (MRI). OI revealed strong homing to the tumor site only of *i.p.* transferred Cy5-labeled Tag2-Th1 cells, whereas *i.v.* administration leads to very low cell accumulations at the tumor site. FM analysis confirmed the OI results. As further homing sites could be the lung, liver, and spleen identified after *i.v.* administration. *I.p.* transferred Tag2-Th1 cells homed mainly to the lymph nodes, peripancreatic-lymphatic-tissue, and spleen. CD3- and B220-immunohistochemistry of the pancreatic tissue showed an infiltration of host B- and T-cells in *i.v.* Tag2-Th1 cell-treated RIP1-Tag2-mice, whereas after *i.p.* Tag2-Th1 cell-treatment host immune cells accumulated circularly around the tumors. No infiltration in the complete pancreatic tissue of host immune cells could be observed after SHAM-treatment. H&E-staining of the thymus revealed, that weekly *i.v.* treatment with Tag2-Th1 cells induced an atrophy and *i.p.* treatment resulted in a depletion. No histological altertions were found after SHAM-treatment. The treatment with Tag2-Th1 cells had no influences on other T cell homing sites (lung, liver, spleen).

Conclusions: Thus, *i.v.* administration of Tag2-Th1 cells is more efficient in RIP1-Tag2-mice than *i.p.* treatment despite a lack of migration to the tumor site. The differences in the antitumoral effects and mobilization of host immune cells to the tumor tissue are associated with the administration route. Future experiments will characterize these administration dependent effects of TAA-specific Th1 cells during immunotherapy.