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Enhanced gene therapy with TRAIL by bispecific antibody immuno therapy in advanced prostate and pancreatic cancer

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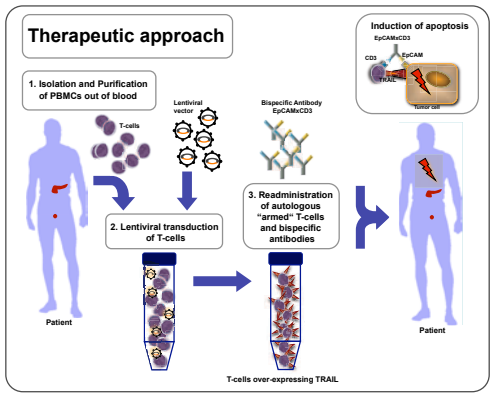
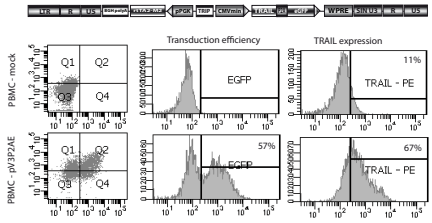
Introduction

Patients with advanced pancreatic or prostate cancer have a poor survival rate and new therapeutic strategies are needed. The overall aim of the project is the establishment and functional analysis of a new approach for the treatment of advanced prostate and pancreatic cancer. This approach will utilize a combination of the death ligand TRAIL as a pro-apoptotic agent over-expressed by T-cells and bispecific antibodies (bsAbs) targeting EpCAM on tumor cells and CD3 T-cells. The novel aspect is the combination of genetic modification with immune therapy.
The lentiviral vectors allow the modification of lymphocytes to over-express the death ligand TRAIL (TNF-related apoptosis inducing ligand). The bispecific antibodies (bsAbs) binds with one arm the T cell via CD3 and with the second arm the tumor surface antigen EpCAM, thereby bringing tumor and effector cell in closer contact. We analyzed these features in established primary prostate (PC-3) and pancreatic (BxPC-3) carcinoma cell lines, in *in vitro* three-dimensional tumor reconstructs and in mouse xenografts.

Therapeutic approach

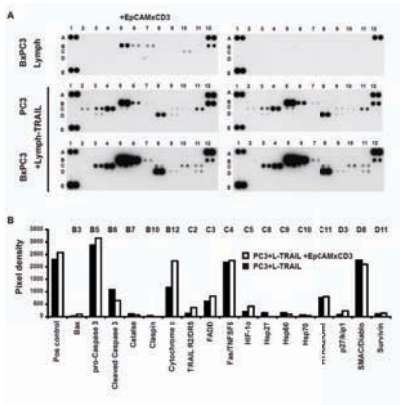


Transduction of PBMCs with lentiviral TRAIL



PBMCs were isolated from fresh healthy donor's blood, and following activation with IL-2 and OKT3 were transduced with lentiviral TRAIL. The chosen vector (pV3P2AE) is a lentiviral SIN vector of the 2nd generation, expressing TRAIL and EGFP under the control of a tet-activator. Transduction was done in 2 cycles on day 1 and 2 with MOI10 each. Efficiency and TRAIL overexpression was verified 3 days post transduction via FACS analysis. All cells were used at this day in experiments.

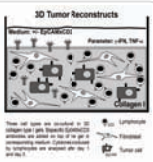
Apoptosis induction in 3D tumor reconstructs



To evaluate the efficacy of bispecific EpCAMxCD3 antibodies, we established an *in vitro* 3D tumour reconstruct system. To mimic tumour microenvironment, a mix of transduced lymphocytes were co-cultured together with tumour cells and fibroblasts in collagen type I gel. Activation of lymphocytes was analyzed by secretion of γ-IFN and TNF-α (data not shown).

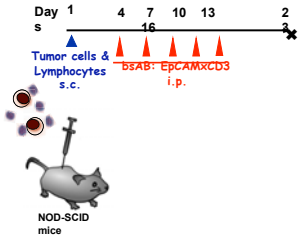
Apoptosis was analysed after 24h using the apoptosis profiler kit from R&D, detecting 40 related proteins. TRAIL-transduced lymphocytes in combination with bsAB EpCAMxCD3 showed an efficient activation of apoptosis in the 3D reconstructs (A). Detected proteins are shown in pixel density for PC-3 mixed with TRAIL-transduced lymphocytes +/- bsAb EpCAMxCD3 (B). BxPC3 gave similar results (data not shown).

TRAIL-transduced lymphocytes in combination with bsAB EpCAMxCD3 actively induce apoptosis in the reconstructs.



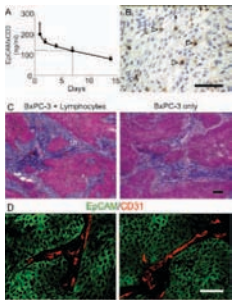
Dynamic interactions between lymphocytes and tumor cells were analyzed by time-lapse video-microscopy. The presence of EpCAMxCD3 bsAbs did not affect speed and motility of lymphocytes However, duration of contacts between lymphocytes and tumor cells was three-times longer in the presence of EpCAMxCD3 (data not shown) .

In vivo bsAb treatment experiments



NOD-SCID mice were injected s.c. with a cell mixture of lentivirally transduced lymphocytes and tumor cells (BxPC3 or PC3) and were treated i.p. with five bispecific antibodies single doses beginning on day 4 in a 3-day-intervall. The animals were monitored for tumor take and tumor growth the whole time. One week after the end of the treatment, mice were sacrificed and tumors were taken. No metastasis could be found.

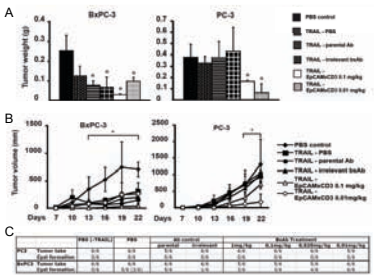
BxPC-3 pancreatic and PC-3 prostate tumour models



A. Pharmacokinetics of bispecific EpCAMxCD3 antibody
B. CD45+ lymphocytes in tumor tissues
C. Collagen content in BxPC-3 tumor stroma
D. EpCAM antigen is expressed by tumor cells in vivo

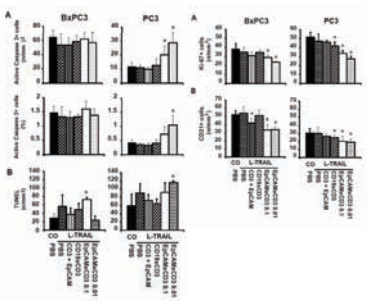
Tumor cells were mixed with extracorporally pre-activated lymphocytes and s.c. transplanted into NOD SCID mice. The bsAB had a serum half-life ot T1/2 ~ 7 days (A). Co-transplantation did not affect tumor growth and morphology (C, D). CD45+ PBMCs even could be detected in the tumours after 23 days (B).

Anti-tumor efficiency of EpCAMxCD3 in BxPC-3 pancreatic and PC-3 prostate tumor model



Efficiency of TRAIL-transduced lymphocytes and bispecific antibody EpCAMxCD3 in the in vivo xenograft PC-3 and BxPC-3 tumour model. Animal groups of n=6 were split into two with different blood donors for more variation. Data is presented as growth kinetics of individual tumors. (✳= significant with p<0,05) In (A) the weight of tumors is shown at the end-point of the bsAb treatment at day 23. The animals were monitored for tumor take and tumor growth. Tumor take and tumor growth was strongly reduced in bsAb treated mice
Compared to previous experiments, we observed a cyst formation in the BxPC-3 xenograft models in all groups with TRAIL-transduced lymphocytes (C).

Proliferation arrest, reduced vessel density, but little apoptosis in *in vivo* tumor models



Histological analysis of xenograft tumors revealed a proliferation arrest (Ki-67 in A/right) and a reduced blood vessel density (CD31 in B/right) in bsAb EpCAMxCD3 treated xenograft. However, the observed tumor growth retardation could not be explained by apoptosis induction at the xenografts at day 23 (so on day 14, data not shown). The observed effect an be explained from the in vitro data.

Results & Conclusion

We evaluated the anti-tumour efficacy of bispecific EpCAMxCD3 antibody linking tumour cells and T-lymphocytes. In NOD SCID mice, EpCAMxCD3 had a long serum half-life (*t1/2* ~ 7 days). EpCAMxCD3 significantly reduced growth of BxPC-3 pancreatic and PC-3 prostate carcinoma xenografts. Since little apoptosis could be detected in these tumours on day 23 (TUNEL, Caspase 3), but lymphocytes (CD45+) could be detected – we assumed, that the time of observation for the mechanism resulting in the growth retardation and the cyst formation, was an earlier event. To further investigate the potential mechanisms of in vivo anti-tumour effects of EpCAMxCD3, we used a collagen gel 3D tumour reconstruct system, which closely resembled the tumour microenvironment. Therefore, to mimic the tumour situation, a mix of TRAIL transduced lymphocytes was co-cultured together with tumour cells and fibroblasts in collagen type I gel *in vitro*. In this setting apoptosis related proteins were expressed indicating an apoptosis induction in this system. To further prove the anti-tumour effect of the TRAIL-lymphocytes with the bsAb EpCAMxCD3, we performed kill assays incubating tumour cells with the bsAb loaded TRAIL-lymphocytes (data in preparation). With the previously demonstrated prolonged contacts between lymphocytes and tumour cells during the bsAb EpCAMxCD3, we could demonstrate a direct apoptosis effect through TRAIL-lymphocytes and bsAb in vitro to explain the observed anti-tumour effects in vivo.
Overall, we provide a new combinatorial approach in which bsAb targeting EpCAM on the surface of tumour cells and genetically modified lymphocytes over expressing TRAIL could improve the therapy of advances prostate and pancreatic cancer in patients.

(manuscript in preparation)

(Salnikov AV, **Groth A**, Herr I, Moldenhauer G. Targeting of cancer stem cell marker EpCAM by bispecific antibody EpCAMxCD3 inhibits pancreatic carcinoma. JCMM. 2009 Apr 2. [Epub ahead of print])

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