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Bispecific EpCAMxCD3 antibody eradicates tumors *in vivo* and potently stimulates lymphocytes in 3D tumor reconstruct system *in vitro*

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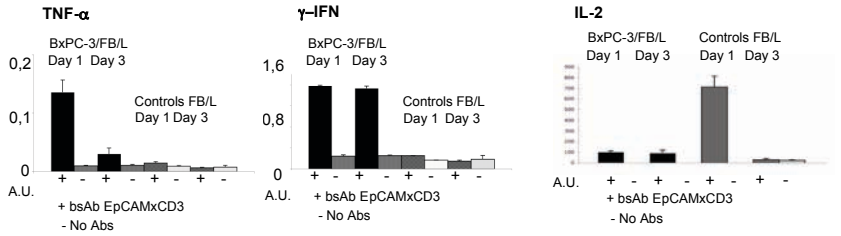
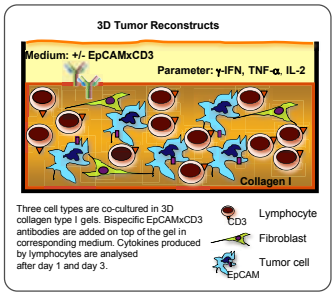
Prostate and pancreatic cancers remain among neoplasms with the worst survival rate. New therapeutical strategies are needed for patients with both local and metastatic disease. We evaluated the in vivo and in vitro efficiency of novel bispecific EpCAMxCD3 antibody targeting EpCAM antigen on tumor cells and CD3 molecules on lymphocytes.

Bispecific EpCAMxCD3 antibody stimulates production of γ–IFN, TNF-α and IL-2 by lymphocytes in 3D tumor reconstructs





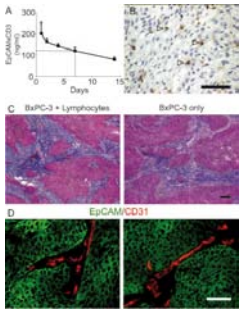
BxPC-3 - Pancreatic tumor cells
L - Activated lymphocytes
FB - Fibroblasts

BxPC-3 - Pancreatic tumor cells
L - Non-activated lymphocytes
FB - Fibroblasts

To evaluate the efficacy of bispecific EpCAMxCD3 antibodies we established an *in vitro* 3D tumor reconstruct system.
In this system extracorporally pre-activated (IL-2/anti-CD3) human lymphocytes or non-stimulated PBMCs are co-cultured together with tumor cells and fibroblasts in collagen type I gel to mimic tumor microenvironment. Anti-tumor effects and activation of lymphocytes were analyzed by secretion of γ-IFN, TNF-α and IL-2. EpCAMxCD3 potently stimulated secretion of γ-IFN and TNF-α by both pre-activated lymphocytes and non-stimulated PBMCs.

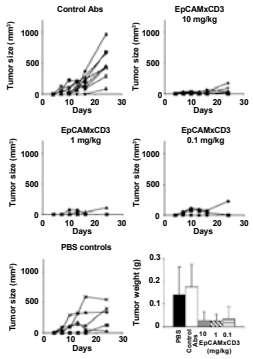
BxPC-3 pancreatic and PC-3 prostate tumor models

Tumor cells were mixed with extracorporally pre-activated human lymphocytes and s.c. transplanted to NOD SCID mice.

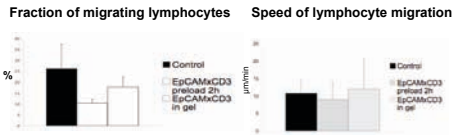


A. Pharmacokinetics of bispecific EpCAMxCD3 antibody
B. CD45+ lymphocytes in tumor tissues
C. Collagen content in BxPC-3 tumor stroma
D. EpCAM antigen is expressed by tumor cells in vivo

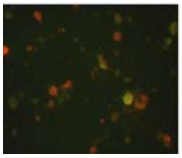
Anti-tumor efficiency of EpCAMxCD3 in BxPC-3 xenograft pancreatic carcinoma model



EpCAMxCD3 does not affect migratory properties of lymphocytes



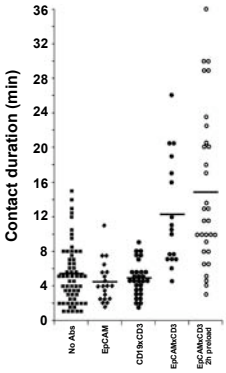
Apoptosis induction in tumor reconstructs



Red: active caspase 3
Green: pan cytokeratin

Influence of inhibitory cytokines (TGF-β, IL-10) produced in 3D reconstructs was also investigated.

EpCAMxCD3 increases contact time between lymphocytes and tumor cells



Dynamic interactions between lymphocytes and tumor cells were analyzed by time-lapse video-microscopy. Duration of contacts between lymphocytes and tumor cells was three-times longer in the presence of EpCAMxCD3.

Results & Conclusion

In NOD SCID mice, EpCAMxCD3 had a long serum half-life ($t_{1/2}$ ~ 7 days). EpCAMxCD3 significantly reduced growth of BxPC-3 pancreatic and PC-3 prostate cancinoma xenografts. For mimicking the pancreatic cancer microenvironment in vitro we developed a 3D tumor reconstruct system, in which lymphocytes were co-cultured with tumor cells and fibroblasts in a collagen matrix. In this in-vivo-like system EpCAMxCD3 potently stimulated production of the effector cytokines IFN-γ and TNF-α by extracorporally pre-activated lymphocytes. Moreover, EpCAMxCD3 activated production of TNF-α, IFN-γ and IL-2 by non-stimulated PBMCs more effectively than a bivalent anti-CD3 antibody. Most excitingly, EpCAMxCD3 induces prolonged contacts between lymphocytes and tumor cells, which may be the main reason for the observed anti-tumor effects. As important prerequisite for future use in patients, EpCAMxCD3 did not alter lymphocyte migration velocity as measured by time-lapse video microscopy.

Our data may open a way to improve the immune response and treatment outcome in patients with pancreatic or prostate cancer.

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