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Trifunctional bispecific antibody treatment of ascites due to ovarian cancer: A phase I/II study

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Klinikum Großhadern.....

In a pilot clinical trial patients with malignant ascites have been treated intraperitoneally with trifunctional bispecific antibodies removab (EpCAM x CD3) and rexomab (Her2/neu x CD3). Treatment consisted of 4-5 i.p. infusions for 6 hours of removab or/and rexomab at doses from 10 to 200 µg in a 14 day course of therapy. The time interval between the infusions was at minimum 3 days. All patients responded to therapy. The response could be demonstrated (i) by the elimination of tumor cells in the ascitic fluid and (ii) the relief of ascites production not only during antibody therapy, but in the maintenance of this effect for time intervals between 1-6 months. This observation implies that locoregional intraperitoneal application of these antibodies was able to induce a therapeutic durable effect on symptomatic malignant ascites production.
Because of the limited experience until now a pivotal clinical study will be initiated. The trial is designed as a dose escalation study with 4 groups of patients with malignant ascites due to ovarian cancer and will be performed at two clinical centers (Klinikum Großhadern / Klinikum Rechts d. Isar). Included patients will receive 4 different increasing dose panels of removab (EpCAM x CD3) during 14 days of hospital stay. During treatment the elimination of tumor cells from ascitic fluid and the maintenance of relief of ascites production will be monitored.

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A trifunctional bispecific antibody Bi20 (CD20xCD3) efficiently kills B-cell lymphoma cells by unstimulated peripheral blood mononuclear cells (PBMC)

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The trifunctional bispecific antibody (bsAb) Bi20 is a promising tool for immunotherapy of CD20-positive B-cell lymphomas, especially in a minimal residual disease situation. One specificity of Bi20 is directed against the CD3 antigen on T lymphocytes, the other binding arm targets the B-cell marker CD20. The CD20 antigen is expressed on B-cell malignancies, is not shedded or internalised upon antibody binding and is therefore an excellent target for antibody-mediated immunotherapy. Furthermore, the bsAb additionally binds to and activates $Fc\gamma R^+$ accessory immune cells via its Fc-region. We used the hybrid-hybridoma-technique for the generation of Bi20. Purification was performed by affinity chromatography (Protein A column) and ion-exchange chromatography. The high purity of the bsAb fraction was shown by isoelectric focusing. Antibody binding was demonstrated by flow cytometry using Jurkat (CD3) and Ramos (CD20) cell lines. Specific B-cell lysis mediated by Bi20 was shown with unstimulated PBMCs containing 8% autologous B cells. After 48 hours of incubation killing of all B cells was observed with an antibody concentration of 50 ng/ml by flow cytometry. Further killing experiments were performed adding 2% of malignant B cells (Daudi) to unstimulated PBMCs, demonstrating that tumour and normal B cells were completely eliminated by Bi20. In conclusion, the trifunctional bispecific antibody Bi20 might represent a novel immunotherapeutic agent for the treatment of B-cell lymphomas.

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Lysis of Prostate Carcinoma Cells by Bispecific Antibodies (αEpCAM x αCD3) – Visualization by a Video Incubation System

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Bispecific monoclonal antibodies (bsAb) are a promising immunotherapeutic option for treatment of cancer, especially in minimal residual disease situations. The combination of an anti-CD3 and anti-tumor-associated-antigen antibody redirects cytotoxic T lymphocytes towards malignant cells. Using a trifunctional bsAb against EpCAM x CD3, that additionally activates $Fc\gamma R^+$ accessory cells via its Fc region, we investigated the interaction between three $EpCAM^+$ prostate carcinoma cell lines and peripheral blood mononuclear cells (PBMC). Visualization was performed using a computerized video microscopy incubation system (which enables creation of time lapse video clips from cell culture) and double immunocytochemical methods.
Tumor cells and PBMC supplemented with αEpCAM x αCD3 in 16 well chamber slides resulted in lysis of tumor cells within 1-3 d. In contrast, control tumor cells without expression of EpCAM showed undisturbed growth. The characteristic morphological process of osmotic lysis could be observed in computerized sequences of video frames. Simultaneously, there was no evidence for apoptosis using three different apoptotic markers (TUNEL, M30 CytoDEATH, anti-active caspase 3). Within the first 48 h we observed typical PBMC cluster formation with increasing cell proliferation. PBMC surrounding the tumor cells were not dominated by $CD4^+$, $CD8^+$ or $CD14^+$ cells.
In summary, bsAb can induce specific cytotoxic immunoreactivity against prostate carcinoma cells. Video incubation systems can contribute information about morphology of cell-cell-interaction.

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Induction of a long-lasting anti-tumor immunity by a trifunctional bispecific antibody

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Bispecific antibodies (bsAb) can efficiently mediate tumor cell killing by redirecting pre-activated or costimulated T-cells to disseminated tumor cells especially in a minimal residual disease situation. Here, we demonstrate that the trifunctional bispecific antibody BiLu (anti-CD3Xanti-EpCAM) is able to kill tumor cells very efficiently without any additional costimulation of effector cells *in vitro* and *in vivo*. Remarkably, this bsAb also induces a long-lasting protective immunity against the targeted syngeneic mouse tumors (B16 melanoma and A20 B-cell lymphoma, respectively). We observed a strong correlation between the induction of a humoral immune response with tumor-reactive antibodies and the survival of mice. This humoral response was at least in part tumor-specific as shown in the A20 model by the detection of induced anti-idiotype antibodies. Notably, the detected anti-Id response demonstrate that trifunctional bsAb can induce potent anti-tumor responses against antigens not bound by the bsAb directly. Both, the survival of mice and anti-tumor titers were significantly diminished when $F(ab')_2$-fragments of the same bsAb were applied, demonstrating the importance of the Fc region in this process. Using T-cell depletion, we could also demonstrate a contribution of a cellular anti-tumor response. These results reveal the necessity of the Fc region of the bsAb with its potent Ig subclass combination mouse IgG2a and rat IgG2b. The antigen presenting system seems to be crucial for achieving an efficient tumor cell killing and induction of long-lasting anti-tumor immunity. Hereby, the recruitment and activation of accessory cells by the intact bsAb is essential.