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Immune monitoring of tumor cell elimination from malignant ascites during immunotherapy with trifunctional bispecific antibodies

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Malignant ascites is an abnormal accumulation of fluid within the peritoneal cavity caused by a malignant process and represents a difficult clinical problem. It is a manifestation of advanced malignant disease, is associated with a poor prognosis and causes symptoms like discomfort, restricted mobility, aneurexia, abdominal swelling and abdominal pain.
Here we describe the establishment of immunohistochemical and molecular methods to monitor tumor cell elimination from malignant ascites during immunotherapy with trifunctional bispecific antibodies EpCAM x CD3 and/or Her2/neu x CD3. The practicability of the intraperitoneal application of the bispecific antibodies is dependent on the presence of at least one of the tumour associated antigens EpCAM or Her2/neu. In order to determine the expression of these antigens at the surface of the tumour cells in ascites fluid of the patients we carried out FACS and Cytospin analysis. So far 4 patients with advanced peritoneal carcinomatosis and malignant ascites were stained positive for either EpCAM and/or Her2/neu and were treated by intraperitoneal application with the corresponding trifunctional bispecific antibodies. Before, during and after therapy tumor cell elimination in malignant ascites (or peritoneal lavages) was analyzed by FACS analysis, RT/nested PCR analysis, cytospin analysis and clonogenic assays. After 2 weeks of treatment all 4 patients showed a complete elimination of tumor cells in the peritoneal fluid with immunohistochemical methods and even in RT/nested PCR analysis. These data impressingly demonstrate the *in vivo* efficacy of trifuncional bispecific antibodies against intraperitoneal spread of tumor cells.

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Trifunctional bispecific antibody BiLu induces long-lasting anti-tumor immunity

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Bispecific antibodies have been shown to be a promising tool for the destruction of disseminated tumor cells by redirecting pre-activated or costimulated immune effector cells. Here, we demonstrate that the trifunctional bispecific antibody BiLu with specificities directed against human EpCAM X murine CD3 is able to kill EpCAM transfected mouse tumor cells very efficiently without any additional costimulation of effector cells *in vitro* and *in vivo*. Thereby, tumor cell elimination was specific and clearly superior to conventional monoclonal antibody therapy. Remarkably, this bispecific antibody also induces a long-lasting protective immunity against targeted A20 lymphoma cells in immune competent BALB/c mice. We observed the induction of tumor specific anti-EpCAM and anti-A20 idiotype antibodies. Both, the survial of mice and anti-tumor titers were significantly diminished when $F(ab')_2$-fragments of the same bispecific antibody were applied. Using T-cell depletion we could also demonstrate a contribution of a cellular anti-tumor response. When mice were immunized with EpCAM-negative A20 wild type cells and bispecific antibody only a partial protection could be achieved. Taken together, these results reveal the necessity of the Fc region of the bispecific antibody with its potent Ig subclass combination mouse IgG2a and rat IgG2b. The redirection and activation of both, T-cells and accessory immune cells seem to be essential for efficient tumor cell killing and induction of anti-tumor immunity. We therefore suggest a tri-cell-complex-model induced by the trifunctional bispecific antibody.

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Fc receptor-directed single chain bispecific antibodies for lymphoma therapy

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Despite major advances in chemo- and radiotherapy, low grade lymphomas or relapses of high grade lymphomas still have a poor prognosis. Over the last years, antibody treatment has become another option for lymphoma patients. Therapeutic efficacy of antibodies may be further improved by bispecific constructs. Here, genetically coupled bispecific single chain Fvs (bsscFvs) were produced, directed against one of the effector cell antigens FcγRI (CD64), FcγRIII (CD16) or FcαRI (CD89), and against HLA class II - a known effective target for effector cell-mediated lysis of malignant human B-lymphoid cells. The two component scFvs were fused via a flexible 20aa linker. ScFv fragments were generated by producing phage libraries from corresponding hybridomas, and screening the libraries with antigen-positive cells. Recombinant scFvs against HLA class II, CD16, CD64 and CD89 were thus obtained from the hybridomas F3.3, 3G8, 22 and A77, respectively. Expression of functional bsscFvs in *E. coli* was limited by inefficient transport of recombinant proteins to the periplasmic space. This problem was overcome by expression in eukaryotic cells. Functional bsscFvs were expressed and secreted both by insect and mammalian cells, and were purified via Nickel chelate chromatography. Purified bsAbs reacted with HLA class II positive target cells, and with Fc receptor-expressing effector cells. Furthermore, bsscFvs triggered potent ADCC reactions in Cr-51 release assays. For example, in conjunction with the [FcγRIII x HLA class II] bsscFv, human mononuclear cells mediated effective killing of LLM cells – pro B-leukemia cells with the t(4;11) translocation, which are resistant to apoptosis induced by most chemotherapeutic agents. Thus, these recombinant scFvs may allow the design of highly specific and effective antibody derivatives for the treatment of B-lymphoid malignancies.

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Induction of specific anti-tumor immunity in advanced gastric cancer patients by bispecific trifunctional antibodies

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The critical role of professional APC like Dendritic Cells (DC) for anti-tumor immunity has been shown using peptide or tumor-lysate pulsed DC or tumor cell-DC hybrids. A new class of bispecific antibodies (bsAb) with intact Fc-fragment is able to activate APC/DC via their FcγR1-receptor and to induce specific immunity in a mouse model. To test this new approach of anti-tumor vaccination clinically, tumor-direc-ted in situ bsAb application was performed in an immunologically privileged compartment: In 3 patients with advanced gastric carcinoma and peritoneal carcinomatosis direct intraperitoneal bsAb application was performed after gastrectomy and ineffective chemotherapy. According to the antigen expression of autologous tumor cells (auTu), the bsAb CD3x EpCAM and/or CD3xHER2/neu were used. Patient 1 received 3 doses of CD3xEpCAM (10-20μg) and 2 doses of CD3xHer2/neu (10-25μg), patient 2 and 3 received 3-4 doses of CD3x Her2/neu (10-100μg) within 15 days. After 4 weeks, patient 1 and 2 received bsAb with irradiated auTu for antigeneic restimulation. 10 days later, PBMC were analyzed for specific tumor reactive CD8+ CTL by restimulation with auTu and the Milteniy IFN-γ Secretion Assay. Antibody application was well tolerated. Patient 3 died 5 weeks after treatment before immune response evaluation could be performed. In patient 1 and 2, significant IFN-γ producing CD8+ CTL were found in PBMC compared to the situation before treat-ment (P1: 1.25 % vs. 0.1%; P2: 2.8% vs 0.2%). In conclusion, the vaccination approach using i.p. application of trifunctional bsAb was able to induce a specific immune response by tumor reactive CD8+ CTL.