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A New Class of Trifunctional Bispecific Antibodies Mediated Efficient Immunological Purging of Peripheral Blood Stem Cells

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Tumor cells can be detected in up to 50% of bone marrow collections or peripheral blood stem cell (PBSC)grafts from solid cancer patients. As some groups argue, this might be the limiting factor in the success of stem cell transplantation, as tumor cells are mobilized together with stem cells during G-CSF application. We showed previously, that specific and efficient carcinoma cell killing can be mediated by simultaneous retargeting and activating T-cells and Fc□-receptor positive accessory cells by trifunctional bispecific antibodies (triomabs). Here we investigated the efficiency and feasibility of PBSC-purging using a combination of two antibodies with the specificity's CD3xEpCAM and CD3xHer 2/neu. Experiments on the in vitro growth inhibition of tumor cells revealed a highly effective killing in concentrations as low as 0,2-0,5 ng triomabs/1,5 x 10^5 peripheral blood mononuclear cells (PBMCs). 28 PBSC aliquots from 22 patients with metastatic breast cancer, two patients with advanced ovarian carcinoma and one patient with a metastatic leiomyosarcoma were then subjected to immunological ex vivo.purging. Using immunocytochemical detection and highly sensitive nested-RT-PCR, tumor cells were detectable in 10/29 (34%) of the samples before purging. After incubation with triomabs none of the 10 samples remained positive for tumor cells. In contrast to previous publications, in this protocol, PBSCs were not prestimulated, allowing a feasible approach for clinical use. Monitoring the kinetic of triomabs we showed, that the antibodies were completely digested by accessory cells within the incubation period.The results of the present study indicate that specific purging tromabs provides the advantage of an easy to handle methodology, that is highly effective in removal of contaminating tumor cells.

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Persistent Occult Metastatic Cells in Bone Marrow of Breast Cancer Patients May Require Antibody Therapy

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The presence of occult metastatic cells in bone marrow (BM) of breast cancer patients at the time of diagnosis indicates occult hematogenous tumor cell dissemination and increases the risk of subsequent distant disease. Currently, there are no data available on the influence of different adjuvant therapies on the survival of these cells.

We analyzed bone marrow aspirates of 161 patients without evidence of recurrence at the time of primary diagnosis and a median interval of 13 months (range: 6 – 74) thereafter. Carcinoma cells were detected using a standardized immunoassay with monoclonal antibody A45-B/B3 directed against cytokeratin (CK).

At the time of primary diagnosis, 46 of 161 patients (29 %) had a positive BM finding . Of these , 45 (28 %) had a positive BM finding at the time of the second BM analysis. Among those patients with an initially negative BM finding, 21 patients (13 %) had a positive BM finding at the second aspiration, while 24 patients (15 %) remained BM-positive. Of the 46 patients with ITC at the time of primary diagnosis, 23 patients (50 %) received adjuvant chemotherapy, 7 patients (15 %) received endocrine therapy and 16 (35 %) patients had no systemic treatment at all. 56 % of the patients without systemic therapy (n=7) converted to a negative BM status at time of follow-up examination, while 43 % of the patients with endocrine (n=4) or cytostatic (n=13) therapy became negative (P= .70).

In a considerable number of patients with primary breast cancer, minimal residual disease can be detected by follow-up BM analysis. Despite cytotoxic therapy, about half the patients are remain BM-positive, suggesting the need for non cell cycle dependent therapy, such as antibody therapy.

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CHIMERIC IgA ANTIBODIES FOR LYMPHOMA THERAPY

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Antibody therapy has become a new treatment option for lymphoma patients. Antibodies against HLA class II variants - such as 1D10 or Lym-1 - are actively investigated in clinical trials whilst antibodies against "classical" HLA class II were associated with systemic complement activation and severe toxicity. IgA antibodies are promising candidates to overcome this problem, because IgA was documented not to activate the classical complement pathway, and FcαRI- directed bispecific antibodies very effectively recruited polymorphonuclear cells – the most populous effector cells in human blood. Therefore, we chimerized murine HLA class II antibodies F3.3 and Lym-1, resulting in antibody panels with identical antigen- specificities and human constant regions of IgA1, IgA2, IgG1-4 isotype, respectively. After demonstrating of their integrity and proper binding to target antigens, functional activity of antibodies was tested in ^{51}Cr- release assays against ARH-77 mature B- cells, and freshly isolated tumor cells from patients with B-CLL. Killing by the IgG1 construct was mainly triggered by human NK cells and complement, whereas both the IgA1 and IgA2 variants mediated effective lysis by PMN, but did not activate complement, or recruit NK cells. Importantly, IgA antibodies were similar effective as respective IgG1 antibodies in killing freshly isolated human CLL cells. In conclusion, IgA antibodies may be an attractive alternative to commonly used human IgG1 antibodies, especially for targeting HLA class II as tumor antigen.

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Intracellular domains of target antigens influence their capacity to trigger ADCC

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Antibody- mediated signalling in tumor cells, and antibody- dependent cellular cytotoxicity (ADCC) were both considered as relevant effector mechanisms for antibodies (Ab) in tumor therapy. To address potential mutual interactions, we generated HER-2/neu- and CD19- derived chimeric target antigens, which were expressed in experimental tumor target cells. HER-2/neu- directed Ab were documented to mediate effective ADCC with both, mononuclear (MNC) and neutrophil (PMN) effector cells, while Ab against CD19 were effective only with MNC. We generated cDNA encoding HER-2/CD19 (extracellular/intracellular), or CD19/HER-2 chimeric fusion proteins, by combining cDNA encoding extracellular domains of HER-2/neu or CD19 with intracellular domains of CD19 or HER-2/neu, respectively. After transfecting HER-2/neu, or HER-2/CD19 into RAJI Burkitt's lymphoma cells, or CD19, or CD19/HER-2 into SK-BR-3 breast cancer cells, target cell lines were selected for high membrane expression. We investigated the efficacy of tumor cell lysis by PMN and MNC with CD19- or HER-2/neu- directed Ab. MNC triggered effective ADCC against target cells expressing wildtype or either chimeric target antigen. As expected, PMN killed HER-2/neu-, but not CD19- transfected target cells. PMN were also effective against CD19/HER-2, but – interestingly - not against HER-2/CD19- transfected target cells. In conclusion, these results demonstrate that intracellular domains of target antigens contribute substantially to effective antibody- mediated tumor cell killing by PMN.