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Immunotherapy of breast cancer with an anti-idiotypic antibody mimicking a human cell substrate adhesion molecule in an in-vivo model

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The idiotypic network concept offers an elegant method to transform epitope structures into idiotypic determinants, which are expressed on the surface of antibodies. Anti-idiotypic antibodies (Ab2b), which are mimicking tumour-associated antigens and express this antigen in a different molecular environment have been shown to overcome the immunosuppression in the cancer patient by stimulating "silent clones" and/or allowing T cell help to become active making the immune response stronger than the nominal antigen. We generated a monoclonal anti-idiotypic antibody (IgG1), designated ACA14C5, against the cell substrate adhesion molecule CA14C5 on breast cancer cells and introduced the mAb ACA14C5 in an in-vivo model to prove his capacity for inhibition of invasion and metastasis. 6 day old Sprague-Dawley rats (n=3x12) received tumor cells (2x10^6) subcutaneously. After one week series Ab2 received mAb ACA14C5 intraperitoneally at a dosage of 100 µg weekly (n=12). A control group received polyvalent mouse IgG at the same dosage intraperitoneally weekly (n=12) and a negative control received only tumor cells (n=12). The tumor incidence in the used model was > 90%. The tumor growth was evaluated over a period of 60 days. 8 applications were administered in total. At the end of the experiment animals were evaluated for T-cell responses against the HH16cl.1/2. The results showed a highly significant difference in the tumor growth as the ACA14C5 treated group developed a mean tumor size of 6.5±12.7 mm and the IgG control showed a mean diameter 37.2±14.9 mm ($p<0.005$) and the tumor control group showed a diameter 15.3±16.3 ($p<0.05$). In the anti-idiotype treated animals 10 of 12 anials were cured from their tumor burden and a T-cell response (lysis of HH16cl.1/2) could be evaluated, which was not present in the controls.
In summary, this is the first report of an inhibition of tumor growth in-vivo caused by an anti-idiotypic antibody (ACA14C5) reacting with a human cancer antigen, which is a cell substrate adhesion molecule of 90 kd and is expressed on different invasive tumors, especially on invasive breast cancer.

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RAPID GENERATION OF HUMAN ANTIBODIES AGAINST TUMOUR ASSOCIATED ANTIGENS

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Our development of novel antibody-based cancer therapeutics is based on the effect of the 17-1A (Panorex) antibody in the treatment of minimal residual disease. Taking the heterogeneity of disseminated tumor cells into account we have identified several targets against which antibodies are currently isolated from our human combinatorial antibody library (HuCAL).

HuCAL is a fully synthetic antibody library based on human consensus frameworks and designed for obtaining high affinity antibodies. A first library was created by cloning one HuCAL antibody gene as a single chain Fv-fragment (scFv) and randomizing the heavy chain CDR3. It was screened against a variety of antigens including targets involved in inflammation and cancer. In almost all cases binders were obtained that recognize the antigens specifically and with high affinity. Moreover, all scFvs were functionally produced in the multi-mg range in *E.coli*.

To create multivalent antibodies displaying high affinities for cell surface antigens, scFvs have been fused to a self-assembling polypeptide based on the tetramerization domain of human p53 (scFv4). A scFv4 against the tumor-associated carbohydrate antigen Le^Y was cloned. For comparison, the corresponding scFv and dimeric scFv2 were prepared. Both dimeric and tetrameric scFv exhibited an enormous gain in binding affinity compared to the scFv. The scFv4 recognizes specifically cancer cells overexpressing Le^Y compared to cells overexpressing Le^X or with low expression of Le^Y.

The HuCAL antibody library combined with multimerization techniques will enable us to rapidly create high affinity human antibodies for selectively targeting cells overexpressing disease-associated antigens.

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Immunological Responses to CA125 induced by the anti-idiotypic antibody MAb ACA125 - Results of a clinical phase Ib study

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The idiotypic network offers a method for immunotherapy by presentation of tumor antigens as an idiotypic determinant in a different environment. Therefore, we have generated an IgG1 murine monoclonal anti-idiotype antibody (Ab2) designated ACA125, which mimics a specific epitope on the tumor-associated antigen CA125. This antigen is expressed by most of malignant ovarian tumors. Patients with CA125 positive tumors are immunologically tolerant to CA125. We used ACA125 as a surrogate for the tumor-associated antigen CA125 for vaccine therapy of 18 patients with advanced epithelial ovarian cancer (n=5) or recurrences (n=13). Each of the patients received a minimum of three injections up to nineteen injections of the complete anti-idiotype MAb ACA125 at a dosage of 2 mg per injection. 11 of 18 patients developed anti-anti-idiotypic (Ab3) responses to the ACA125. All 11 patients generated specific anti-CA125 antibody demonstrated by reactivity with purified CA125. 9 of 18 patients developed a CA125 specific cellular immune response by their PBL. Toxicity was limited to abdominal pain in one case, which led to the withdraw of further immunizations. The median progression free survival in those patients, who showed a specific immune response to the tumor-associated antigen CA125, was 10.3 months without any other therapy, in contrast to 7.1 months in the anti-anti-idiotype negative group. In 3 patients local regression of tumors could be detected, accompanied by CD8 infiltrations in different tumor sites.
This is the first clinical trial of the induction of a specific active immunity to the tumor-associated antigen CA125 in patients with advanced ovarian cancer treated with an anti-idiotype antibody that "mimics" CA125. Patients showed the development of a specific humoral and cellular immune reponse to an otherwise non-immunogenic tumor antigen. The immune responses in patients treated with this anti-idiotype vaccine, the low rate of side effects, and the improved time to progression after the induction of a specific immune response against the tumor-associated antigen CA125 justify follow-up clinical trials in advanced ovarian cancer patients with minimal residual disease in an adjuvant approach (supported by DFG Wa 740/1-3).

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A BISPECIFIC ANTIBODY INDUCES EFFICIENT KILLING OF TUMOR CELLS

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Bispecific antibodies (BsAb) have been shown to be powerful tools to induce T cell mediated antitumor responses both *in vitro* and *in vivo*. We have constructed a BsAb which targets Ep-CAM present on head and neck squamous cell cancer and T lymphocytes via CD3. Incubation of tumor cells with peripheral allogeneic blood mononuclear cells with this antibody resulted in activation of T cells and efficient killing of the tumor cells. Activated T cells kill tumor cells in a second round experiment even w/o the addition of BsAb.

Tumor cell killing was observed much less efficiently when both parental antibodies were added. T cell activation as measured by an increased expression of CD25, 28, 40L and 95L, production of IL-2 and induction of perforin expression was much higher with BsAb. Tumor cell killing and T cell activation can be inhibited by the addition of either anti MHC class I or class II antibodies.

PBMCs which have been depleted of monocytes /macrophages (=PBL) are much less efficient in tumor cell killing. This implicates that monocytes/macrophages are activated by binding to the Fc-gamma part of the BsAb and may contribute to full T cell activation. This is proven by the induction of CD25, 40, and 80 on CD14+ cells and the production of IL-6 only by PBMCs. Especially, the expression of CD80 (B7.1) may be mandatory for T cell activation by providing a co-stimulatory signal which is not present on target tumor cells.

Our approach using BsAb for the killing of residual tumor cells may be used *in vivo* after surgical resection of the tumor in a minimal residual disease situation to target and eliminate metastases. The use of a BsAb cocktail targeting several different antigens present on the tumor cells may multiply the efficiency of this immunological trial.