Clinical courses of patients with ovarian carcinomas after repeated immunoscintigraphy

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In vivo localisation of tumours can be achieved after injection of radiolabelled antibodies. We investigated the effects of ^{131}I labelled F(ab)2 fragments of the OC 125 MAb against the tumour-associated antigen CA 125. All patients were previously treated in the same manner by surgery followed by chemotherapy. CA 125 plasma concentrations decreased immediately after antibody infusion and showed an increase at 5 days p.i. or later. We observed a rapid and high increase in patients who received repeated infusions of antibodies. This increase did not conform with the clinical state of the patients and was partially caused by anti-idiotypic antibodies. In contrast to the serum diagnosis, these patients had a good survival rate as compared to patients who were not treated with antibodies, but had similar chemotherapy and FIGO staging. These results lead to the conclusion that anti-idiotypic antibodies may cause false-positive elevations in plasma CA 125 levels and might be responsible for the immunotherapeutic effect in the survival rate of patients with ovarian cancer.

Session 7. Chairman: A.A. Epenetos

Bifunctional monoclonal antibodies in activation of lymphocytes for cytokine production and lysis of human renal and ovarian cancer cells

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Under physiological conditions T cells become activated when their T cell receptor (TCR) binds to the MHC/antigen complex on another cell resulting in multiple links between these structures. The result is delivery of a lethal hit. Activation of cytotoxic lymphocytes can be induced by monoclonal antibodies (mAbs) that bridge the CD3/TCR complex to a target cell surface structure. This can be accomplished by using bifunctional mAbs which recognise activation sites on the lymphocytes and target cell structures respectively. Therefore the use of bifunctional mAbs may allow us to "retarget" the specificity at will. Activation of T cells by mAbs via T cell activation sites can result in the production of cytokines. In addition to the TCR/CD3 complex activation, signals can also be transduced via CD2 and CD16. Our recent data also demonstrate that the lymphocyte function associated antigen-1 (LFA-1) can coactivate the TCR complex. Under physiological conditions the LFA-1/ICAM-1 interaction is a prerequisite which however can be circumvented by the use of bifunctional mAbs. Using bifunctional mAbs, which recognise TCR/CD3 and renal cell or ovarian cell carcinoma associated antigens, we have shown that this retargetting of CTL represents a powerful system for adoptive immunotherapy of these cancers. Experiments were performed to investigate the recycling capacity of bifunctional mAb retargetted and cloned CTL. Reactivation of the retargetted CTL requires the addition of "fresh" bifunctional mAbs.

Immunotoxins to treat B lymphoma

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Antibodies directed against molecules on neoplastic B and T cells from humans or mice have been coupled to chemically deglycosylated ricin A chain (dgA). Extensive studies *in vitro* and *in vivo* in murine B and T cell tumour models have demonstrated that such immunotoxins are potent antitumour agents. Based on this success, we initiated studies to treat refractory B cell lymphomas in humans with anti-CD22 immunotoxins. A CD22 antibody, RFB4, was selected which recognises only B cells in a panel of 37 normal human tissues. An immunotoxin prepared by linking RFB4 to dgA by means of a hindered disulphide bond was 10–20 fold more toxic than ricin itself to the B lymphoblastoid line, Daudi. Another immunotoxin, prepared by linking F(ab') fragments of RFB4 to dgA via a cysteine bridge, was toxic as ricin to Daudi cells. Toxicology studies in Rhesus monkeys have established an effective dose for eliminating normal B cells that cause virtually no side-effects. Phase I clinical trials are in progress. To date, 18 patients have been treated. Preliminary data on toxicity, pharmacokinetics, antibody response and efficacy are favourable.

Preparation and biodistribution of ^{111}Indium and ^{90}Yttrium labelled immunoconjugates incorporating the macrocyclic chelating agent DOTA—laboratory data

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^{90}Yttrium radioimmunotherapy is currently hindered by the instability of the Ab-DTPA-^{90}Y complex. The dissociation of the Ab-DTPA and ^{90}Y *in vivo* leads to accumulation of the radioisotope in bone and subsequent myelosuppression. A more suitable chelating agent for ^{90}Y is required for successful progress in this field. The monoclonal antibodies H17E2 and HMFG1 were successfully conjugated to the macrocylic chelating agent 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetraacetic acide (DOTA). HMFG1-DOTA was labelled with ^{90}Y in a direct comparison with HMFG1-DTPA. Similar specific activities and labelling efficiences were achieved for each conjugate $\approx$ 1.8 μCi/μg ($\approx$ 75%). Biodistribution studies in nude mice compared ^{90}Y concentrations in tissues following IP administration of HMFG1-DTPA-^{90}Y and HMFG1-DOTA-^{90}Y. Further animal studies repeated this protocol incorporating IV EDTA at 2, 22 and 46 h following the original IP HMFG1-^{90}Y, in an attempt to facilitate excretion of the radioisotope as EDTA ^{90}Y. At 5 days, bone concentrations of ^{90}Y for HMFG1-DOTA-^{90} were 6–10 fold lower than for HMFG1-DTPA-^{90}Y but made no difference to the already low levels for HMFG1-DOTA-^{90}Y. Tumour localisation was demonstrated for H17E2-DOTA-^{90}Y and H17E2-DOTA-^{111}In in nude mice bearing the HEp-2 human tumour xenograft. This study demonstrates the *in vivo* stability of ^{90}Y labelled immunoconjugates incorporating the macrocyclic chelating agent DOTA.

Preparation and biodistribution of ^{90}Yttrium labelled immunoconjugates incorporating the macrocylic chelating agent dota—clinical data

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Myelotoxicity at relatively low doses has limited the use of yttrium labelled monoclonal antibodies (MAbs) for intraperitoneal radioimmunotherapy. Patients have been treated as part of a phase I–II study using a macrocycle (DOTA) to link the MAb to yttrium. This compound is stable *in vitro*, and if the same holds true *in vivo* it should permit dose escalation of ^{90}Y to a theoretically cytotoxic level. Pharmacokinetics of intraperitoneally administered ^{90}Y-DOTA MAb have been compared with those of ^{90}Y-DTPA MAb and data, so far, indicate that the use of ^{90}Y-DOTA-MAb produces less bone marrow toxicity than equivalent doses of ^{90}Y-DTPA MAb.