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PRECLINICAL AND CLINICAL RESULTS IN BREAST CANCER OVEREXPRESSING HER-2

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The HER-2/*neu* proto-oncogene encodes a growth factor receptor which is overexpressed in 20-30% of human breast cancers. This overexpression is associated with a decreased relapse free as well as overall survival in those patients whose tumors contain the alteration. The overexpression is most often due to amplification in a significant number of cases. This association between HER-2/*neu* amplification/overexpression and outcome suggests that the alteration may play some causal role in the pathogenesis. To test the potential role of HER-2/*neu* overexpression in altering the biologic activity of human breast normal and malignant epithelial cells, a number of in vitro studies were conducted in which single-copy, low expressing cell lines were converted to multiple copy, high expressing cell lines. The biologic effects of HER-2/*neu* overexpression were then measured including effects on DNA synthesis, cell growth, anchorage independent growth, and tumorigenicity. Overexpression of HER-2/*neu* resulted in an increase in those parameters in the malignant cell lines as well as the non-transformed immortalized breast cell lines. In the normal primary breast cells there was no evidence of these effects with HER-2/*neu* overexpression alone.

Monoclonal antibodies directed against the extra cellular domain of the receptor can suppress all of the biologic effects induced by HER-2/*neu* overexpression both in vitro and in vivo. Pre clinical studies indicate that these antibodies can be effective in completely suppressing growth of human tumor cells as well as malignant breast tissue xenografts when either are growing in vivo. The suppression is specific to cells and tissues overexpressing the HER-2/*neu* gene. Strategies using these antibodies in combination with other therapeutic modalities indicates that this cytostatic effect can be converted into a cytocidal effect. These observations have led to the development of new treatment strategies directed at this molecular alteration and these strategies are now in clinical testing. In addition, the recent identification, cloning and sequencing of a ligand for the HER-2/*neu* receptor has allowed for its recombinant expression. The availability of this ligand has led to further insights into the role of the HER-2/*neu* protein in the pathogenesis of human breast cancer.

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Antibodies from phage libraries: clinical imaging trial of a filamentous bacteriophage-derived single chain Fv anti-CEA expressed in E.coli.

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Specific in vivo localisation of antibodies to human cancer is established in principal but the efficiency of targeting for imaging or therapy is low. Single chain Fv (scFv) antibodies selected from large libraries of antibody genes expressed in filamentous bacteriophage have the potential to improve this situation. Their small molecular mass (27kD versus 150kD for IgG) improves tumour penetration and accelerates blood clearance. Antibodies of improved affinity and specificity can be selected from large libraries ($<10^{12}$ clones) of antibody VH and VL genes derived from B cells. These are expressed in filamentous bacteriophage which contain the VH and VL genes and display the corresponding scFv antibody on their surface. Antibodies with optimal characteristics for targeting can be selected from these libraries by binding of phage to antigen in conditions which select for desired characteristics such as high affinity. The ability to select from such great diversity gives an advantage over hybridoma technology in which it is only practical to screen a relatively small number (10^3-10^4) of clones. A high affinity scFv antibody to carcinoembryonic antigen has been generated in this way, produced in E.coli (Chester et al Lancet, 1994, 343, 455-456) and prepared for clinical use under Cancer Research Campaign guidelines. The results of a clinical imaging trial of this antibody (MFE-23) are reported. 0.5mg of MFE-23 labelled with 74-220MBq of ^{123}Iodine was given IV to 9 patients with colorectal cancer and 1 with breast cancer. One patient had flushing and palpitation lasting 1 hour. There were no other adverse effects. b half life of clearance from the blood was 5.7 hours. SPECT and planar gamma camera imaging after 1, 4 and 22 hours showed antibody clearance via the kidneys, liver and bile. There was rapid tumour uptake and all known tumour sites were positive on imaging. Mean tumour concentration 1 hour after injection was 2% injected activity kg^{-1}. Tumour to blood ratios increased with time to reach 3:1 after 22 hours compared with 1:1 typically achieved with whole antibodies at this time. These data are consistent with the proposed advantages of phage-derived scFv. There is thus an improved prospect for antibody imaging and for genetically engineered therapeutic molecules incorporating scFv and the targeting moiety.

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MONOCLONAL ANTIBODY (MAB) TREATMENT OF RESECTED DUKES C COLORECTAL CARCINOMA (CRC): A PROSPECTIVE RANDOMIZED TRIAL.

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Instead of targeting MABs to large solid tumors a trial was designed with the goal to aim an anti-epithelial cancer MAB at minimal residual disease after radical tumor resection. For this purpose 189 patients with CRC Dukes C_1 and C_2 were randomly assigned to either an observation regimen or to post op. infusion with 500 mg MAB 17-1A followed by 4 monthly doses of 100 mg. The 5-year follow-up showed a reduction of death rate by 30 percent and of recurrence rate by 27 percent in the treatment group. (Cox p=0.04 and p= 0.03 respectively). No effect of MAB was seen on incidence of local relapses (Cox p=0.74) while distant recurrences as first events were reduced (Cox p=0.0014). Toxic and allergic side effects were minor only (Lancet 343, 1177, 1994). Six year follow-up data will be presented that confirm the benefit for overall survival and disease free survival. Micrometastasis of epithelial cancer thus appears to be a worthwhile target for future immunotherapy trials with MABs.

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CLINICAL TRIALS OF ANTI-ANGIOGENIC AGENTS. MJ Hawkins. Georgetown University, Lombardi Cancer Center, Washington, D.C.

Complete inhibition of angiogenesis should be well tolerated in most adults since, under physiologic conditions, angiogenesis is required only for wound healing and reproduction. However, angiogenesis is required for malignant solid tumor growth beyond 1 to 2 cubic millimeters and microvessel counts in tumor specimens have been correlated with prognosis in patients with malignancies of the breast, prostate and central nervous system. We are currently studying antiangiogenic agents that bind to heparin binding growth factors (Pentosan Polysulfate), inactivate matrix metalloproteinases (Batimastat) or inhibit endothelial cell proliferation (TNP-470) as single agents in Phase I clinical trials. To date these agents have been well tolerated with little systemic toxicity although local reactions have complicated the administration of Pentosan and Batimastat. Laboratory correlates for these studies include measurement of urinary growth factors and plasma matrix metalloproteinase levels before and during therapy and are currently in process.