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NOVEL ANTIBODY-BASED FUSION PROTEINS IN CANCER TREATMENT

RHJ Begent, KA Chester, RB Pedley, SJ Sharma, L Robson and J Bhatia. CRC Targeting and Imaging Group, Dept of Clinical Oncology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

Antibody targeting of biological agents has potential for therapy of cancer but chemical conjugation of antibody from hybridomas with a therapeutic molecule is cumbersome, difficult to reproduce, may contain unnecessary and potentially immunogenic protein and have limitations in targeting efficiency. Making a genetic fusion of single chain Fv (scFv)antibody from a phage library with the therapeutic molecule and expressing the fusion protein in bacteria appears to have significant advantages which are illustrated with a fusion protein of a phage-derived scFv antibody to CEA (MFE-23) with carboxypeptidase G2 designed for use in antibody-directed enzyme prodrug therapy. The fusion protein was produced in bacteria and purified either using a CEA affinity column or by immobilised metal affinity chromatography through a hexahistidine tag. The product localised specifically in human colon carcinoma xenografts in nude mice giving sufficient enzyme concentration in tumour for effective ADEPT and higher tumour to normal tissue ratio s than could be achieved with a chemical conjugate. The genetic construct makes a suitable base for further improvements in affinity or reduction of immunogenicity of the enzyme. The bacterial production is cheap and avoids introduction of mammalian or viral DNA as in eukaryotic production. the system has general application to production of antibody based fusion proteins for a variety of therapeutic applications.

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ZD2767, AN IMPROVED SYSTEM FOR ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY THAT RESULTS IN TUMOUR REGRESSIONS IN COLORECTAL TUMOUR XENOGRAFTS.

[1]Blakey, D. C., [2]Burke, P. J , [1]Davies, D.H., [1]Dowell, R.I., [1]East, S. J., [4]Melton, R. G., [3]Niculescu-Duvaz, I. A., [2]Sharma, S. K. and [3]Springer, C. J. [1]Cancer Metabolism and Endocrine Research Dept., Zeneca Pharmaceuticals, Alderley Park, Cheshire, UK, [2]Charing Cross Hospital, London, UK, [3]Institute of Cancer Research, Sutton, Surrey, UK, [4]PHLS, CAMR, Porton, UK.

Antibody-directed enzyme prodrug therapy (ADEPT) is a two step targeting strategy for the treatment of cancer. The first step involves administration of a tumour selective antibody linked to an enzyme. Following tumour localisation of the conjugate and clearance of conjugate from blood and normal tissues, the second step involves administration of an inactive prodrug which is converted by the targeted enzyme at the tumour site into a potent cytotoxic drug.

We have developed an ADEPT system, ZD2767, consisting of a conjugate of the $F(ab')_2$ fragment of the anti-CEA antibody, A5B7, and the bacterial enzyme carboxypeptidase G2 and a novel prodrug of p-[N,N-bis(2-iodoethyl)amino]phenol mustard linked to L-glutamic acid. The prodrug is a good substrate for CPG2 and cleavage releases the potent bis-iodo phenol mustard drug. The ZD2767 conjugate specifically localised to CEA expressing LoVo colorectal tumour xenografts growing s.c. in athymic nude mice. Tumour levels of the conjugate were 10-50 fold higher than in blood and normal tissues after 72 hr and administration of ZD2767 prodrug at this time point, at a dose which caused little toxicity as judged by body weight loss (6-7 %), resulted in approximately 50 % of the tumours undergoing complete regressions and tumour growth delays > 30 days. In contrast, ZD2767 prodrug in combination with a control conjugate which does not bind LoVo cells only resulted in tumour growth delays < 5 days, confirming the tumour specificity of this approach. These studies demonstrate the potential of this ADEPT system for the treatment of colorectal cancer and ZD2767 is now in pre-clinical development.

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Tumor immunotherapy with bispecific antibodies: from bench- to bedside

Gundram Jung, Ludger Große-Hovest, Martina Brandl, Achim Pfosser and Wofgang Wilmanns. Medizinische Klinik III der Universität München and Institut für Klinische Hämatologie, GSF-Research Center for Environment and Health, D-81377 München, Germany

Bispecific antibodies (bsAbs) directed to tumor associated antigens and to the TCR/CD3 complex on T-cells are capable to induce T-celll activation and tumor cell killing if bound at the surface of tumor target cells. If supported by a second bispecific construct triggering the T-cell costimulation receptor CD28 this effect is markedly increased.

In vitro, anti-tumor activity of those bsAbs is easily demonstrable measuring tumor cell killing during cocultivation of tumor cells and freshly isolated autologous or allogeneic lymphatic effector cells. In SCID- or nude mice injected with tumor cells and human effector cells as well as in several syngeneic mouse models impressive anti-tumor effects of anti-tumor X anti-CD3-bsAbs have been observed.

Here we summarize those results with emphasis on our own work and report preliminary data of an experimental study, in which bispecific antibodies and autologous lymphocytes are locally applied to patients with malignant glioblastoma.

Despite promising results the supply of bispecific antibodies in sufficient quality and quantitity is still a major obstacle to the clinical evaluation of these reagents. Genetically engineered antibody constructs may help to overcome this problem in the near future.

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ANTIBODY MEDIATED THERAPY OF MINMAL RESIDUAL LEUKEMIA: USE OF HUMANIZED MONOCLONAL ANTIBODIES AND TARGETED ALPHA PARTICLES

David A. Scheinberg, M.D., Ph.D.Memorial Sloan-Kettering Cancer Center

Humanized monoclonal antibodies reactive with CD33, a cell surface protein found on myeloid leukemia cells and a subset of myeloid progenitor cells, and alpha particle emittin radioimmunoconjugates of the anti-CD33 antibodies are under investigation for the elimination of minimal residual leukemia. HuM195 (anti-CD33) is non-immunogenic and capable of ADCC against human leukemia cells *in vitro*. In phase II studies of this agent alone in patients with acute promyelocytic laukemia who have been induced into clinical remission using retinoic acid, but who still retain evidence of minimal leukemia by RT-PCR assay for the product of the PML/rar alpha gene product, infusions of the unlabeled antibody are capable of eliminating the PCR signal in approximately 50% of patients treated. In the remaining patients a single additional round of chemotherapy is capable of eliminating the PCR signal. With a total of 11 patients treated thus far, using retinoic acid followed by monoclonal antibody consolidation and then 3 cycles of idarubicin and cytarabine, there have been no relapses and disease free survival is 100% (median 19 months; range 2 to 30 month). In orther studies to render the antibody more potent, bifunctional radiometal chelates containing bismuth-213 have been conjugated to the HuM195. Bismuth-213 has a half-life of 47 minutes and a emits high LET alpha particles with an energy of 8 million electron volts and a path lenght of 50-80 microns. Therefore, these constructs have the potential to allow single cell kill with minimal bystander cell kill with as few as 1-2 atoms delivered to the leukemia cell. A phase I dose escalation study was initiated using ^{213}Bismuth-HuM195 in patients with refractory and relapsed myeloid leukemias. The constructs appear to be stable *in vivo*and rapidly target within minutes to sites of leukemia throughout the body with significant target to background ratios (>10,000). Evidence for anti-leukemic effects has been seen at the early dose levels.