

Cancer - The Way Forward

The Society for Medicines Research 'Cancer Therapy' meeting on 11 July 1996 covered the increasingly varied ways this disease is being approached in the search for new treatments. It is clear that beyond the headlines of British Biotech's matrix metalloproteinase inhibitors, such as marimastat, now in Phase III clinical trials, a vast array of new research is advancing. This is spurred by the sobering statistic that despite current treatments including surgical techniques, chemotherapy and radiotherapy, fewer than 50% of cancer patients with access to health-care are helped at all by modern medicine.

Professor Peter Parker (Imperial Cancer Research Fund) opened the symposium with a wide-ranging discussion of phospholipid-based enzymes and their potential role in cancer. The multiplicity of genes coding for different versions of these enzymes, such as phospholipid C which has 2-4 genes per subtype, complicates the picture. In the protein kinases, debate surrounds whether the gain or loss of function is important for therapy, since acute and chronic effects of inhibitors are different. Proof-of-principle clinical studies are clearly required to test a number of hypotheses before therapies can be developed with any confidence.

Dr Howard Cooke (MRC, Edinburgh) talked about telomeres; arrays of simple repeated sequences of DNA which stabilize chromosomes against fusion or loss. Telomeres are incompletely replicated, and become smaller by 50-200 base pairs during cellular division. Below a minimum size of telomere, normal cells are unable to divide. The link between telomere length and senescence is obvious, although it is not a simple relationship. Telomere length varies from individual to individual, and can be increased by telomerase which is present in 95% of metastatic tumours - not in normal cells. It is tempting, but in Dr Cooke's view perhaps simplistic, to conclude from this that telomerase blockers may be useful in cancer, and telomerase promoters may induce cell immortality.

Dr Mark Sopwith revealed Celltech's experiences with antibody-targeted calicheamicin, a pheno-menally potent cellular poison of the ene-diyne family that works by binding to the minor groove of DNA. One molecule of calicheamicin per cell is lethal to any target cell. American Cyanamid (now part of Wyeth-Ayerst) originally discovered calicheamicin, but were unsuccessful in finding an analogue with sufficient selectivity in its toxicology. They therefore approached Celltech for its antibody-targeting technology. In ovarian cancer, Celltech had spliced a mouse antibody to an epithelial marker called PEM into a human antibody, and linked the resultant construct to calicheamicin via a lysine group. The result, known as hCTMOI, was given at up to 1.0 mg/kg in a human clinical trial. This localized to the ovarian tumour to the extent of 9%, whereas 15% appeared in the liver. Liver uptake could be reduced by pre-dosing with the non-calicheamicin doped antibody, but unfortunately uptake in the kidney was increased commensurately. Clearly the toxicity of this product will need to be watched closely. Researchers at Celltech have also linked calicheamicin to an antibody for acute myeloid

leukaemia, but a different link had to be developed; clinical results for this treatment, hp67.6, and targeted towards CD33 are still awaited.

The next three talks referred to different applications of gene therapy to cancer.

Dr Roger Craig (Therexys) described an alternative to ADEPT (antibody-directed enzyme pro-drug therapy) which was developed at Zeneca. This technique, called GDEPT, requires the insertion of a gene encoding for an enzyme into the cancer cell; the enzyme is expressed and converts a pro-drug to an active toxin. The toxin is produced in the target cells, and then released on cell death when it may affect adjacent cells (the bystander effect). For instance, ganciclovir is a drug that can become activated by phosphorylation with thymidine kinase. This enzyme can be supplied by viral infection using Herpes Simplex Virus (HSV).

Therexys (together with ICR of Sutton, Erasmus University of Rotterdam and the Patterson Institute of Manchester) used an old drug, CB1954, discovered in 1967. CB1954 is an alkylating agent that works only after prior reduction of one of the nitro groups to an amino function. The enzyme that does this, DT diaphorase, is a nitro-reductase that exists in rat, although not human cells. Work at Therexys is incorporating the genetic material (DNA) for the nitro-reductase into a targeting particle composed of a shell of histone-like condensing peptides, which is then surrounded by a number of lipid 'spikes' that assist transfer across cell membranes. The efficiency of these particles was described as better than a liposome, but inferior to an adenovirus. The suspension of the DNA-enclosed particles is designed to be administered directly by hypodermic injection into a palpable, imageable or visible growth. Transgenic animal models suggest that CB1954 works in thymic cancerous situations, reducing thymus (and spleen) cell counts. However, efficacy is still unproven in a human clinical situation.

Dr Heung Chong from the Imperial Cancer Research Fund, London, described the work carried out to stimulate the body's natural defence mechanisms by genetic amplification of cancer cell epitopes. The epitopes are normally insufficient to induce a pronounced immune response. In animal models, expression of the co-stimulatory molecule B7.1 (CD 80) on tumours, which is a T-cell trigger, reduced the tumourigenicity of colorectal tumours. However, the enhanced immune response was not re-mounted if the animals were challenged a second time with colorectal tumour cells. Dr Chong also described a series of experiments that demonstrated, in another model with the ganciclovir/HSV system, that killing tumour cells resulted in an additional immunity of the Th1 class.

Clinical evidence for the benefit of gene therapy was produced by Lindy Durrant (Cancer Research Campaign, Nottingham). She revealed work on an anti-idiotypic antibody, 105AD7, that bound in the same way as the gp72 antigen found on a number of solid tumours. This technique has successfully induced anti-tumour immune responses. Results from a Phase I study showed increased survival in colorectal cancer patients. In a Phase II adjuvant study, there was no antibody toxicity or recurrence of disease. Dr Durrant saw this type of treatment as being useful in treating residual disease following surgical

removal of the bulk of a tumour; patients would receive a series of treatments to boost the immune reaction, some before their surgery.

Dr Cliff Murray, also from the CRC described anti-angiogenic strategies. Solid tumour growth is angiogenesis dependent and it also requires the presence of a number of positive (vascular endothelial growth factor (VEGF) and human basic fibroblast growth factor (bFGF)) and negative (thrombospondin and angiostatin) regulators. There is evidence to show that suppression of solid tumour growth occurs by an immuno-neutralizing mAb against human bFGF and that inhibition of VEGF suppresses tumour growth. Thus new drugs and biological modifiers may suppress angiogenesis, thereby inhibiting tumour expansion. Agents which inhibit endothelial cell proliferation include: AGM 1470, linomide, suramins, anti-FGF, minocycline, anti-integrins, BB94 and retinoids. Other strategies include the use of toxins or catalytic fragments of coagulation proteins conjugated to antibodies which selectively recognize tumour-associated endothelium. One group of molecules which represents important potential targets are the endothelial-specific receptor tyrosine kinases. The excitement surrounding these receptors: KDR, flt-1 (VEGF receptors) and flt-4 (receptor for VEGF-C) is that they are up-regulated in tumour cells (and in the embryonic state) and are thus amenable to a number of therapeutic approaches including antibody-directed and gene therapy approaches.

Dr Mike Dukes from Zeneca Pharmaceuticals described that company's pursuit of extreme oestrogen ablation in the treatment of breast cancer. One approach is the selective inhibition of aromatase, a cytochrome P450 which converts C19 androgens to aromatic C18 oestrogens. Inhibitors include anastrozole (Arimidex™) which shows high potency and selectivity. At 1mg/day orally, it has been shown to inhibit whole-body aromatase activity in post-menopausal women by >95% and to reduce circulating oestrogens to a corresponding degree. Absence of hormonal activity provides a greater disease-free interval than tamoxifen and an acceptable long-term side-effect profile; hot flushes are not exacerbated. This is also the case with the second approach using anti-oestrogens such as ICI 182,780 and ZM 189,154. These compounds have high affinity for oestrogen receptors and show no agonist effects. Complete blockade of natural and synthetic oestrogens is achieved in experimental models. In Phase III trials in post-menopausal patients with advanced breast cancer that had progressed despite prior tamoxifen, anastrozole was as effective as a standard hormonal treatment (megestrol acetate). A small Phase II trial ICI 182,780 has shown very promising efficacy in similar patients.

Dr Jacques Robert from the Institut Bergonié and Université Victor Ségalen, Bordeaux, discussed the present status and future prospects for combating multi-drug resistance (MDR). MDR emanates from a phenotype occurring in tumour cells and is characterized by:

- cross-resistance to a variety of structurally and functionally unrelated natural product
- anti-cancer drugs;

- reduced accumulation of cytotoxic drugs due to increased active efflux;
- over-expression of a high molecular weight membrane glycoprotein, P-glycoprotein (P-gp), encoded
- by a gene, MDR1;
- reversibility by a number of agents from different families sharing only a high lipophilicity and
- a positive charge at neutral pH.

Chemical agents that can reverse MDR include: the Ca⁺⁺ channel blocker verapamil, calmodulin antagonists (phenothiazines), cyclosporin A (CsA), quinolines (quinine), hormonal compounds (tamoxifen) and inactive analogues of anti-cancer drugs (N-methyl-daunorubicin). It is not known whether all modulators share the same binding sites on P-gp and whether there is a common pharmacophore. Administration of these drugs has sometimes required physical tools of drug encapsulation in liposomes or nanospheres. Biological approaches involve immuno-targeting of mAbs against P-gp and antisense RNAs against MDR1 messengers. A modulator of MDR must be easy to obtain and formulate, be devoid of toxicity and its pharmacokinetics must be compatible with the drug. For example, concomitant administration of CsA enhances cancer cell bioavailability of etoposide, doxorubicin and taxol by 50%. CsA is of potential interest in haematological malignancies, but its toxicity restricts clinical evaluation - a failing that other MDR modulators share. Normally, one would give the conventional dose of the drug and progressively increase the dose of the modulator (except CsA). Verapamil has proven the modulator concept where responses were observed among myeloma and lymphoma patients. No positive results were registered in solid tumours with any of the modulators tested. The failure of MDR modulators in solid tumours suggests alternatively that MDR may not be a relevant means of resistance in such cases; that doses of modulators may not have been high enough; that the schedule of the administration of anti-cancer drug and MDR modulator may not have been optimized and finally, the design of the trials, which have often led to inconclusive results, was flawed.

Current research is aimed at better defining the pharmacophore of P-gp and identification of the other modulator targets. Better guidelines have been established for the design of more rigorous clinical trials.

More than many areas, cancer exemplifies tremendous ingenuity in new therapeutic approaches, and in the increasing role of molecular biology and biotechnological techniques: even without the convenience of oral administration, medical need makes new medicine imperative