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New Approaches in Cancer Pharmacology: Drug Design and Development

Report of a European School of Oncology Task Force comprising: P. Workman (Chairman), M. D'Incalci (Co-chairman), W.E. Berdel, M.J. Egorin, C. Hélène, J.A. Hickman, M. Jarman, G. Schwartsmann and K. Sikora

INTRODUCTION

CANCER PHARMACOLOGY is now a highly sophisticated business. The techniques available to us are infinitely more powerful than before, and the standards required in the drug development process are similarly more demanding. Perhaps even more important is the enormous leap in our understanding of the molecular basis of the multiple changes which take place in the natural history of cancer cells.

All this is very timely since it is increasingly recognised that we have probably come to something of a dead-end with more traditional approaches to cancer chemotherapy. Over the last 50 years, we have developed a range of useful drugs whose main function is to interfere rather non-specifically with the biochemistry of cell replication. These have been discovered mainly by screening for antiproliferative activity, often in cell types of dubious biochemical relevance to that of the major human solid tumours. Perhaps not surprisingly, the therapeutic index of these agents is fairly narrow, and there has been greater success in the leukaemias and lymphomas than is the case for breast, lung and gastrointestinal cancer.

The traditional approaches of screening—enlightened empiricism and analogue development—have been invaluable to us, but may have run their course. It is appropriate therefore that we should review where we stand. How should we design and develop new drugs as we approach the year 2001? How can we use our new technologies to exploit the intellectual breakthroughs in our comprehension of tumour biology and physiology? Furthermore, when exciting new drugs come through from the laboratory to the clinic, how do we optimise their subsequent development?

Our Task Force was created to look at just these issues. This report presents the first summary of our deliberations. Not all the important topics have been covered and a second report will appear in a year's time. It is not a consensus paper since at such an exciting time and with representation of various different interests it is not surprising that a variety of diverse opinions should be expressed. Indeed that is very welcome. There was, however, clear consensus in favour of maximising all available resources to develop novel drugs and exploit new targets in the most rational ways available to us.

DNA SEQUENCE AND GENE SPECIFIC DRUGS

(Discussion leader M. D'Incalci)

The recent molecular biological characterisation of important genetic and biochemical abnormalities of several human neoplasms has encouraged us to envisage hitting specific DNA sequences involved in the proliferation and malignant behaviour of cancer cells. Most drugs which are currently used in cancer chemotherapy cause DNA damage, but we have only limited knowledge of the precise mechanisms of cell death. Although structural differences in chromatin between normal and tumour cells are important, together with differences in DNA replication and repair, it is not unreasonable to hypothesise that antitumour activity is due to the drug-induced functional alteration of expression of crucial cancer cell genes. It is possible that relatively few DNA lesions are involve in antitumour activity, whereas the majority of non-specific DNA damage is responsible for normal tissue toxicity. We can therefore conceive of greatly improving the selectivity of DNA-interactive drugs by targeting the damage to critical cancer genes and minimising the effects on non-specific sequences.

The development of improved techniques for analysing the molecular basis of drug–DNA sequence specificity has been particularly important in recent years. Most sequence specificity data have been obtained using methods which detect sites of DNA alkylation, specifically N^7alkyl guanine or N^3alkyl adenine present in a determined sequence, by their quantitative conversion into strand breaks under certain physico-chemical conditions (with hot piperidine or at high temperature) [1]. More recently a DNA-polymerase stop assay has been developed to evaluate DNA sequence-specific covalent modifications induced by anticancer agents, using Taq DNA polymerase and synthetic labelled primers [2]. The technique has potential advantages over other methods in that it works also for DNA lesions which are not convertible into cleavable sites (e.g. those caused by

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cisplatin). It is also extremely sensitive. Indeed this technique can potentially be applied to determine the sequence selectivity of antitumour agent damage and repair in single copy gene sequences in intact normal and tumour cells.

Non-covalent interactions of drugs with DNA can be examined by using foot-printing assays, and the ability of drugs to inhibit the specific DNA-binding of transcription factors can be assessed by gel shift assays. The evaluation of drug effects on the transcription of genes can also be examined using assays in which the expression of a reporter gene is under the control of specific promoter sequences. Such reporter genes include chloramphenicol acetyl transferase and growth hormone. Binding of drugs or proteins to regulatory sequences can be determined using these reporter genes, but effects on the structural gene may also occur. The use of two reporter genes in combination can be helpful to rule out effects of non-specific toxicity. Techniques are also available which would allow the determination of the effects of drugs on the initiation of transcription and hence to differentiate between structural and promoter effects.

By the intelligent application of these various methods, it appears realistic to develop experimental systems for the selection of potential novel anticancer (and antiviral) agents based on the assessment of: (1) DNA sequence-specific binding, (2) preferential inhibition of the binding of particular transcription factors and (3) selective inhibition of the transcription of a given gene. In this context it should be emphasised that the information available is still very limited. Some differences in the pattern of guanine N^7 alkylations have been described for particular nitrogen mustards using isolated DNA. However, the relevance of these differences to the pharmacological properties of the drugs is still a matter of speculation. A greater degree of sequence specificity has been found for some recently developed alkylating agents which bind to adenine N^3 in the minor groove of DNA. Two protoypes of this new type of antineoplastic agent have been identified: CC-1065 and *N*-deformyl-*N*-2-chloroethylaminobenzoyl distamycin A (FCE 24517) [3, 4]. Both drugs exhibit a highly preferential akylation on adenines located in specific sequences of 5 or 6 bases. By understanding the structure requirements for this type of preferential binding using computational chemistry and computer graphics techniques combined with biochemical verification, it may be possible to increase further the preferential binding to longer DNA sequences. Physical characterisation techniques such as high-field NMR and X-ray crystallography are also becoming increasingly valuable in the design of sequence-specific agents.

Although considerable sequence recognition can be attained, it seems unlikely that this approach will lead to the discovery of drugs which are capable of specifically inhibiting a single gene, as is the case for antisense oligonucleotides (see next section). For example, DNA target sequences of 10 base pairs would be predicted to occur 2080 times in the human genome, whereas a sequence of 15 base pairs may occur only once or twice. Nevertheless, it does seem realistic to anticipate drugs which possess much greater molecular selectivity than is true for DNA-binding drugs in current clinical use, since the latter have limited though definite efficacy, but a very low degree of DNA sequence selectivity. The currently achievable specificity for sequences of 5–6 base pairs may be sufficient to antagonise the binding of specific transcription factors to promoter sequences in oncogenes. These can often be particularly G–C rich, but this is also true for many housekeeping genes. It remains to be established to what extent such an increase in DNA sequence specificity will result in a greater degree of selectivity towards tumour versus normal tissue. However, progress is being made at the molecular level. Distamycin derivatives have been shown to inhibit the DNA binding of two specific transcription factors, octamer binding factor OTF 1 and erythroid-specific GATAAG protein NFE 1. Moreover these agents inhibit the transcription of histone H2B mRNA (dependent on binding of OTF 1 to the octa motif), but not that of histone H4 or actin mRNAs which do not depend on OTF 1.

An additional feature which may contribute to tumour specificity is that damage may be enhanced in transcriptionally active genes (e.g. oncogenes in tumours) because of improved DNA accessibility, independent of sequence specificity. Also, active genes are repaired more rapidly than bulk DNA and the transcribed strand can be repaired in preference to the non-transcribed strand.

Another area that can now be addressed is the specificity of cellular proteins which recognise damaged DNA. For example, proteins have now been identified which bind specifically to platinated DNA and these can be hyperexpressed in resistant cells. Pharmacological interference with such proteins may be possible.

Drugs with improved sequence specificity such as the distamycin mustard and CC-1065 analogues are now entering clinical trial. It is expected that our growing knowledge of the molecular biology of tumours will enable us to identify crucial genes which should be selected as targets for the design of improved sequence-specific drugs for individual human neoplasms. In addition to the development of improved molecular techniques and *in-vitro* models, it will be important to utilise relevant *in vivo* tumour systems to verify the pharmacological activity and therapeutic selectivity of DNA-sequence-specific drugs. It is to be expected that these types of drugs will be effective only in tumours where the molecular targets for which the drug has been designed play a crucial role in the malignant phenotype.

ANTISENSE AND ANTIGENE OLIGONUCLEOTIDES TARGETED TO ONCOGENES

(Discussion leader: C. Hélène)

Synthetic oligonucleotides can bind selectively to either mRNA molecules or the DNA double helix [5, 6]. In the first case they inhibit translation of the message into protein ("antisense" oligonucleotides). In the second case they inhibit gene transcription or replication ("antigene" oligonucleotides). The original idea behind the antisense strategy was that binding of a complementary sequence to an mRNA would block its translation by ribosomes, but it is now known that ongoing translation cannot be prevented in this way. Instead the mechanism of inhibition involves the recognition and cleavage of the mRNA-antisense oligonucleotide hybrid by ribonuclease H. In addition, binding of an antisense oligonucleotide to the 5′-untranslated region can inhibit binding or sliding of the 40 *S* ribosomal subunit and/or association of translation initiation factors. Antisense oligonucleotides have been used successfully in cell culture to inhibit selectively particular gene expression. The antisense oligonucleotide requires a minimum length to guarantee adequate specificity for the target mRNA and for strong binding. However, in contrast to the common view, overlong antisense oligonucleotides also show reduced efficiency because of mismatch binding. The theoretical and practical optimum is usually in the range 12–20 nucleotides. Shorter oligonucleotides can be used if the hybrids are stabilised by

covalent attachment of an intercalating agent (e.g. antitumour intercalators such as acridine or anthracycline derivatives).

For cancer treatment, oncogenes represent excellent targets for both antisense and antigene oligonucleotides [7]. Oncogenes can be discriminated from proto-oncogenes when activation arises as a result of point mutations (e.g. *ras*), translocation (e.g. *bcr-abl* in chronic myeloid leukaemia), aberrant initiation of transcription (e.g. *c-myc* in Burkitt lymphomas) or alternative splicing (e.g. epidermal growth factor receptor in gliomas). Antisense oligonucleotides can also be used to reduce overexpression of proto-oncogenes due, for example, to gene amplification or deregulation of transcription.

Inhibition of negative regulatory factors by antisense oligonucleotides could be used to enhance the expression of genes playing an important role in the control of tumour development, such as tumour suppressor genes. This approach will be less selective because transcription factors are usually involved in controlling the expression of several genes. This is true also of short double-stranded oligonucleotides that can be used to trap transcription factors and thus prevent them interacting with regulatory sequences. Other possible approaches to oligonucleotide therapies include insertion of DNA fragments placed in the reverse orientation to the gene of interest in order to produce antisense mRNAs *in situ*, and ribozymes which are short RNAs that induce catalytic cleavage of their target RNAs.

Successful and specific inhibition of oncogene expression and of tumour cell growth has been demonstrated with antisense oligonucleotides against the message for the Ha-*ras* oncogene in bladder cancer cells (see next paragraph), the *c-myc* proto-oncogene in Burkitt lymphoma and in leukaemia cells, and the *c-myb* proto-oncogene in leukaemia cells. HL-60 leukaemic cells can be induced to undergo myeloid differentiation by antisense down-regulation of *c-myc*. Antisense oligonucleotides to the colony stimulating factor CSF 1 and its receptor (encoded by the *c-fms* oncogene) can inhibit the corresponding autocrine-driven cell proliferation, as is also the case with antisense oligonucleotides for interleukins 2, 4 and 6 and basic fibroblast growth factor. A common feature is that a heterogeneous response is often seen, presumably due to a combination of variable dependence on a given oncogene and inconsistent uptake of the antisense oligonucleotide. It should also be kept in mind that the kinetics of the oligonucleotide effect will depend on mRNA and protein lifetimes within cells.

A particularly good example of the potential for a high degree of selectivity is afforded by the successful use of short modified antisense oligonucleotides directed against mutated Ha-*ras* [8]. The oligonucleotides (5'-CCACACCGA-3') were targeted to a region of the Ha-*ras* mRNA including the G to T point mutation at the 12th codon which leads to a Gly to Val substitution and activation of the *ras* p21 protein. They were linked to an intercalating agent (e.g. 5'-acridine) and/or a hydrophobic tail (e.g. 3'-dodecanol). RNase H-dependent specific inhibition of activated *ras* p21 protein synthesis was demonstrated in a cell-free system and growth inhibition was observed with T24 bladder carcinoma cells which carry the mutated Ha-*ras*, but not with cells containing two copies of the normal Ha-*ras* gene.

The antigene strategy is currently less advanced than the antisense strategy. Promising results have been obtained recently showing that it should be possible to block transcription of a single gene inside a living cell. This could be applied to control oncogene expression in tumour cells. Antigene oligonucleotides bind to DNA by triple helix formation. This is presently limited to homopurine-homopyrimidine sequences and further efforts will be devoted to extending the target sequences. A triple helix forming oligo has been shown to block *c-myc* gene transcription by preventing the binding of a transcription-factor to a regulatory sequence upstream of the start site.

Although antisense and antigene oligonucleotides appear highly promising using *in vitro* test systems, many problems remain to be overcome, particularly with respect to metabolic stability and cellular uptake efficiency. A large effort has been devoted to synthesising modified oligonucleotides that will resist degradation by nucleases and hence be more stable for *in vivo* application. Phosphorothioate, phosphoramidate and methyl phosphonate modifications have been used. All inhibit nuclease degradation. The last two do not sustain RNase H activity and are expected to be active only when targeted to non-coding regions. Cell uptake is not improved by these changes despite the elimination of charge from the methyl phosphonate. It is not clear yet what kind of modification will give the best compromise between nuclease resistance, cell uptake specificity and efficacy of biological effects. Also, detailed toxicological studies have yet to be carried out with modified oligonucleotides and their degradation products. Oligonucleotides are able to distribute into all tissues (except brain) when injected into mice, and also penetrate into live cells. However, intracellular distribution will need to be improved for them to reach their targets either in the cytoplasm (mRNAs) or in the nucleus (DNA, pre-mRNAs) with optimal efficacy. Uptake of oligonucleotides across cell membranes occurs predominantly by energy-dependent endocytosis and particular attention will need to be paid to promoting access from endocytotic vesicles to intracellular sites of action. The addition of a hydrophobic tail improves uptake of oligonucleotides. Appropriate oligonucleotide delivery systems should be explored. These will include targeted liposomes, nanoparticles and lipoproteins. Methods to scale up the synthesis of therapeutic oligonucleotides are in development.

The antisense and antigene approaches to control gene expression are very attractive because of their potential for unequalled specificity in recognising unique nucleic acid sequences. However, we should keep in mind that oligonucleotides can also bind to targets other than nucleic acids, especially proteins. Whether oligonucleotide binding to proteins will lead to unexpected therapeutic or toxic effects is still an unexplored area. Part of the effect of antisense oligophosphorothioates against HIV has been attributed to sequence non-specific effects related to binding to the cell surface receptor CD4 and viral proteins such as reverse transcriptase and gp120. Alternatively, such interactions might be used to achieve biological effects which are different from those arising through binding to nucleic acids. This might include trapping transcription factors involved in gene transcription regulation.

Many properties of oligonucleotide behaviour need to be elucidated before they can be developed as antitumour drugs for clinical use. *In vivo* delivery is perhaps the biggest issue, although applications in the bone marrow purging area may be more immediate. Unless the therapeutic oligonucleotide produces an irreversible effect such as the induction of differentiation (as in the HL-60 leukaemia model) it is likely that these will need to be administered repeatedly at appropriate intervals to ensure a continuous effect. In most cases it can be expected that oncogene inhibition will block proliferation but not kill cells, i.e. a cytostatic rather than cytotoxic effect will be seen. However, in some cases apoptosis might be induced (e.g. if the *bcl-2* gene is targeted). It is possible that inhibition of oncogene

expression might be efficient only in the relatively early stages of tumour development, i.e. after the initial step of oncogene activation but before additional events such as chromosome rearrangements have occurred. However, if a growth factor or its receptor is the target, the effect might be less dependent on the age of the tumour.

The results presently available with antisense and antigene oligonucleotides provide a rational basis for the development of highly selective therapeutic approaches. Tumour-specific targets have been identified, but it remains to be demonstrated that inhibition of target genes can be achieved *in vivo*.

PROSPECTS FOR BIOLOGICAL AND GENE THERAPIES

(Discussion leader: K. Sikora)

Recombinant DNA technology has produced large numbers of biological agents in a highly purified form suitable for clinical trial. Studies of interferons, interleukin 2 and tumour necrosis factor have shown interesting responses in melanoma, renal cell cancer and certain lymphomas. Although responses may be seen in around 20% of such patients, the number of complete responses obtained with biological therapies has been low, usually of the order of 5%, and the activity in other disease sites has been disappointing. The lack of a significant breakthrough in the use of recombinant cytokines against the major solid tumours indicates the need for a more mechanistically based approach for future development. Even in the most therapeutically promising scenario of interferon-alpha in hairy cell leukaemia, the mechanism of cytokine action is totally unclear.

The discovery of the importance of oncogenes and tumour suppressor genes in human tumours has clearly provided novel targets for logical drug design [9]. The *c-erb-B2* protein provides a good example [10]. The gene encodes a transmembrane receptor for which the ligand is as yet undefined. The gene is amplified in around 20% of human breast tumours and the protein overexpressed in about 30%. Importantly, the presence of excess *erb-B2* protein conveys a poor prognosis, independently of other clinical and laboratory parameters. The activation of the *erb-B2* protein and related receptors (see also next section) requires an obligatory dimerisation step. The molecular basis for this process is now becoming clear through a combination of elegant recombinant DNA and molecular modelling studies. Peptide inhibitors have been designed which block the dimerisation of the transmembranous regions of the *erb-B2* protein. These amphipathic peptides have so far been shown to have inhibitory activity in cell-free systems and further development is underway.

A number of exciting approaches are now being pursued to enhance selective tumour destruction and which can be categorised under the general heading of gene therapy [11]. The sensitivity of tumour cells to cytotoxic agents can be increased by inserting foreign genes into *in-vitro* models. An example is increased cisplatin sensitivity in ovarian cancer cells containing an epidermal growth factor (EGF) expression vector. Similarly, the sensitivity of critical normal tissues to cytotoxic agents can be decreased by the insertion of resistance genes. This approach has been illustrated by the successful protection of bone marrow cells against agents in the multidrug resistance (MDR) category by transfection of the *mdr*-1 gene which encodes the P-glycoprotein efflux pump protein. Expression of this gene confers haemopoietic cell resistance to MDR drugs in transgenic mice. The gene for the alkyl transferase repair protein could be used to protect the bone marrow from methylating agents and nitrosoureas.

A more sophisticated gene therapy system involves the insertion of deleterious genes into retroviral shuttle vectors containing promoters which result in expression only within the tumour. More likely, expression could be restricted to the tumour together with the corresponding normal cells because tumours may share transcriptional control systems with the tissue of origin. This would be acceptable provided that the normal cells were not essential for survival. Such selective expression of the toxic product would be driven off the corresponding tissue-specific promoter, e.g. for genes such as carcinoembryonic antigen, alpha-fetoprotein, amylase, calcitonin and prostate-specific antigen. A highly novel approach involves the use of virally directed enzyme prodrug therapy (VDEPT). Here the gene product expressed specifically in the tumour or particular normal tissue is a viral enzyme which can activate a systemically administered prodrug to a toxic product. An interesting model system exists for hepatoma. The alpha-fetoprotein promoter is linked to a viral thymidine kinase gene, resulting in a high level of enzyme activity in the hepatoma cells. This can then be used to convert appropriate nucleoside analogues to their cytotoxic metabolites in the tumour.

Another approach at the genetic level is to enhance the immune recognition of weakly immunogenic tumours by the expression of "immunoattractant" proteins *in vivo*. Such proteins include TNF, IL2, IL4, interferon-gamma and foreign MHC peptides. Although tumour cells may not normally be able to elicit a cytotoxic T-cell response, the presence of such factors following transfection of the genes into tumour cells may encourage a more powerful recognition process.

A particularly exciting aspect of gene therapy involves a further extension of cellular immunotherapy with lymphokine-activated killer (LAK) cells and tumour-infiltrating lymphocytes (TILs) [12]. Responses have been described in melanoma and renal cell cancer with this approach, but the toxicity associated with such therapy is considerable. The new development is to use TILs as a cytokine vectoring system. One of the reasons for the failure of TNF as a systemic agent may be its greater toxicity in humans compared with rodents, in which impressive antitumour activity is seen. By achieving high local concentrations in tumour via delivery in TIL cells, enhanced responses may be possible and systemic toxicity may be reduced. The feasibility of targeting genetically altered cells using a marker gene for neomycin resistance has already been demonstrated. Clinical trials of TNF-containing TIL cells are now underway.

In addition to the dominantly active transforming oncogenes, the discovery of tumour suppressor genes presents a new challenge for innovative therapy, including gene replacement therapy. *In vitro* there are many examples of the successful reversal of tumour phenotype by the insertion of functional tumour suppressor genes. These include wild type p53, the retinoblastoma RB gene and the Wilm's tumour gene.

Currently there are 5 protocols involving gene therapy for cancer approved by the recombinant DNA Advisory Committee of the US National Cancer Institute. The technology is likely to improve considerably over the next decade, increasing the possibilities for further development.

MEMBRANE AND SIGNAL TRANSDUCTION TARGETS

(Discussion leader: J.A. Hickman)

The potential for the plasma membrane and cellular signal cascades to be targets for anticancer drugs is largely accepted

[13, 14]. The membrane became the focus for the attention of experimental chemotherapists and pharmacologists during a phase when it was considered unlikely that significant numbers of new drugs would emerge from continuing programmes targeted at modifying DNA synthesis. Additionally, there was an opinion that advances in the treatment of slow growing tumours required strategies which were not aimed at the biochemistry of proliferation.

The discoveries of the past decade defined the molecular basis of many of the events initiated at the plasma membrane in response to growth factors. This provided a new biochemical framework for the investigations of pharmacologists. Initial studies were iconoclastic attempts to demonstrate that existing antitumour drugs exerted some or all of their activity at the plasma membrane. Perhaps surprisingly, nearly all of them had effects at this locus: they induced changes in the biophysical nature of the membrane, modulated ion flux and altered the activity of membrane-associated protein kinases. The growing definition of the function of growth factors in driving tumour cell proliferation and the understanding of the role that membrane-bound oncogene products had in subverting cell signalling cascades then began to stimulate *de novo* synthesis of inhibitors. Antagonists of autocrine growth factors such as bombesin/gastrin releasing peptide (GRP) in small cell lung cancer (SCLC) emerged from elegant studies in model systems. SCLC cells not only generate GRP, but also a myriad of other hormones with the potential to stimulate growth. It was shown that many of these mitogenic hormones were capable of raising intracellular calcium via interactions with their specific membrane receptors. It was perhaps not surprising, therefore, that specific blockade of the bombesin/GRP receptor with a highly selective peptide antagonist did not inhibit SCLC growth *in vitro*. Instead, non-specific inhibitors of a range of receptors (bradykinin, substance P and bombesin/GRP) proved to be more effective [15]. These pioneering studies point to the limitations of the perception that a single receptor cascade is responsible for driving the growth of an individual tumour. The opportunity for selective chemotherapy will clearly be limited by this. Blockade of multiple receptors with important roles in normal tissue signalling is likely to cause greater toxicity than would be the case with more specific inhibition. Nevertheless, broad spectrum mitogenic peptide receptor antagonists will undergo clinical trial in the near future.

It is now well known that many oncogenes encode products with an essential tyrosine kinase activity. These include genes for transmembrane receptors like *erb*-B2 and those for other membrane-associated proteins such as *src* and *abl*. Studies with mutated receptors have shown very clearly that if the kinase is inactive the signal cascade does not fire. This discovery attracted medicinal chemists to design inhibitors of tyrosine kinase activity. Two major classes have emerged. The first are the flavones and isoflavones such as genistein. Although these compete with ATP they exhibit surprising selectivity for tyrosine kinase *vis à vis* the serine/threonine kinases. The second type is the tyrphostin class of molecules [16]. These are substituted benzylidenemalononitriles based on erbstatin. They are different from the first class in that they are competitive with respect to the protein substrate and would therefore be expected to have a much greater potential for selective activity against the kinase activities of individual gene products. Although growth-inhibitory, there is some controversy as to whether this effect depends on blockade of tyrosine kinase activity alone. Recent *in vivo* studies have suggested that some of these compounds can inhibit human tumour xenograft growth, but it remains to be determined whether blockade of tyrosine kinases is primarily responsible.

The pioneering work in this area was to generate tyrosine kinase inhibitors selective for the epidermal growth factor (EGF) receptor enzyme. Inhibitors with relative specificity towards other receptors have also been made. For example recent elegant studies have used genetically engineered chimeric receptors to generate selective inhibitors of the *erb*-B2 receptor, despite the fact that the ligand for this receptor is currently unknown.

Many attractive targets for drug development exist downstream from receptor activation. These include the *src* homology or SH2 domain interactions, which couple receptors to processes involved in the next stage of the signal cascade: inositol lipid metabolism and protein kinase C activation of GTP binding proteins such as the *ras* gene product. All these areas are subject to intense activity and will certainly generate exciting new compounds for clinical trial. For example, the *ras* p21 protein undergoes post-translational modification by isoprenylation and palmitoylation to ensure membrane localisation and optimal transforming activity. The specific farnesyl transferase responsible for this activity has now been identified, paving the way for specific inhibitors to be developed. But there is a caveat: the framework of biochemistry giving rise to these developments is one which seeks to define the events following the stimulation of a quiescent cell into the cell cycle and DNA synthesis. It is proliferation biochemistry, albeit new and exciting. Drugs targeted at this biochemistry may therefore turn out to be a new class of antiproliferatives and might not be as effective as we would like against slow-growing solid tumours which express little of this biochemistry.

The growth of a functional tissue depends on the coupling of proliferation with differentiation. A fundamental aspect of the malignant cell is that differentiation and proliferation become uncoupled. The stem cells of a solid tumour may not be proliferating, but when they do they generate progeny with the same proliferative potential rather than daughter cells with a greater degree of differentiation and a reduced proliferative potential. Thus, in considering signal cascades as targets for drugs, it would seem attractive to inhibit those signal cascades which subvert differentiation or to activate those which promote differentiation. Such signals may be initiated by growth factors and cytokines, but nothing is known about them. Signals of this type, which initiate changes in gene transcription and phenotype, also come from the extracellular matrix of epithelial cells. Again, although the integrin receptors which are involved in extracellular matrix interactions are now defined, we are ignorant about the signals they generate.

Another important consideration in tumour growth is that proliferation is not matched by an equal cell loss as it is in a normal tissue. Growth factors and cytokines have recently been shown to provide signals not only for proliferation and differentiation, but also for cell survival. There is thus the exciting possibility that the selective attenuation of survival signals could actively promote cell loss, probably in a programmed way via apoptosis. This is virgin territory for drug development. However, it has been shown that the membrane-bound oncogene protein *bcl-2* is able to prevent engagement of apoptosis and to promote survival, and this is certainly an attractive possibility for therapeutic intervention.

Strangely, we still do not know how our current drugs make some cells die, but not others. A recent idea [17] is that cells are able to detect damage and then to elicit a signal to respond in

various ways. We presume that the response is phenotypically determined. In some cells the response is death by apoptosis, in others it may be to differentiate, in others to up-regulate damage limitation programmes, e.g. multidrug resistance genes. Modulation of the signals that determine this fundamental response may lie at the centre of our ability to make cells lose their proliferation potential, either through death or differentiation responses.

DEVELOPMENT OF ANTITUMOUR ETHER LIPIDS

(Discussion leader: W.E. Berdel)

Ether lipids and their derivatives represent an interesting and novel class of anticancer drugs [18]. Part of the antineoplastic activity of these agents *in vivo* is mediated through an enhancement of non-specific cellular immune response. In addition, however, they also kill cancer cells directly, and they exhibit a surprising degree of selectivity against individual cell lines. Antitumour ether lipids are of particular interest because they do not appear to interfere directly with the structure, function or synthesis of DNA. Instead they exert a range of effects on tumour cell membranes and the associated signal transduction pathways. The precise mode of action remains unclear, however, and it seems possible that numerous effects combine to cause cytotoxicity. Despite this apparent lack of molecular target specificity, several ether lipids are undergoing clinical trials and others are in preclinical development. Progress with antitumour ether lipids will therefore be monitored very carefully, and many lessons will be learned that will be useful in development of other membrane-active agents and cell signalling inhibitors.

Antitumour ether lipids are related structurally to the naturally occurring phospholipids lysophosphatidyl choline and platelet activating factor and were synthesised originally as a new class of small molecular weight immunopotentiating biological response modifiers. Good activity was seen with these agents against *in vivo* models where immunomodulation can be detected. Macrophage-mediated effects have featured especially prominently, but other immune cells may also be involved here. Further studies then revealed activity in leukaemias and the ability of these agents to purge bone marrow of leukaemic cells was identified. Moreover, responses were seen in experimental solid tumours, including certain human xenografts.

The antitumour ether lipids which have entered clinical trials include ET-18-OCH_3 (edelfosine), the thioether BM 41.440 (ilmofosine) and the related agent hexadecyl phosphocholine (miltefosine). A cyclic analogue SRI 62-834 will shortly enter clinical trial with Cancer Research Campaign in the UK. As far as direct antiproliferative activity is concerned, structure–activity investigations originally indicated the requirement for an ether linkage in the *sn*-1 position of the glycerol and a metabolically stable substitution at *sn*-2, but this does not now seem to be correct as a rigorous rule. It is clear, however, that some small changes in structure can give rise to major differences in cytotoxicity, possibly by influencing metabolic stability, while other quite major alterations have very little effect.

The development of improved antitumour ether lipids has mainly involved the synthesis of large numbers of chemical analogues coupled to a variety of antitumour screens. Metabolic stability seems to be important, although degradation to more active metabolites may also occur. Drug design has been hindered by the difficulty in modelling the three-dimensional structure of ether lipids and by the lack of a precise molecular target. However, several potential sites of action are emerging. Numerous changes have been noted in cell membranes in response to antitumour ether lipids, ranging from alterations in fluidity, through increasingly serious membrane permeabilisation through to frank cell lysis. It is still not clear whether these are caused by a detergent-like effect, but the long hydrophobic alkyl chain clearly favours membrane localisation while the phosphocholine head group will promote polar interactions. Although it has sometimes been considered that direct membrane-damaging effects would be difficult to reconcile with selectivity, it is possible that differences in membrane composition may explain this. Variations in endogenous ether lipids and cholesterol may be important. Recent studies suggest that a major factor influencing ether lipid sensitivity is the rate of uptake by endocytosis [19, 20] and further work is required in this area.

Interference with a variety of signal transduction targets has been demonstrated [14, 21]. Antagonism of some growth factor receptor interactions, including epidermal growth factor and platelet-derived growth factor, have been reported. Inhibition of the binding of granulocyte–macrophage colony stimulating factor to human leukaemic cells is also seen. Blockade of growth factor-induced calcium signalling occurs. However, antitumour ether lipids can also induce a rise in cell-free calcium directly and yet this latter effect does not appear to involve a functional platelet activating factor receptor. This appears to induce cytotoxicity rather than mitogenesis. Changes in phospholipid metabolism are seen and such key signalling enzymes as phospholipase C and protein kinase C are inhibited.

A valuable feature of the antitumour ether lipids is their general lack of cross-resistance with conventional cytotoxic agents, including cisplatin and drugs involved in multidrug resistance.

Clinical studies have involved both intravenous and oral dosing, often with chronic administration. Plasma concentrations achieved with oral dosing are rather low and gastrointestinal toxicity is limiting. The parenteral route may be preferred. Some clinical responses have been seen, and topical administration of hexadecylphosphocholine in breast cancer has appeared especially promising, as does the use of ET-18-OCH_3 in purging bone marrow of leukaemic cells *ex vivo* prior to autologous bone marrow transplantation.

Optimal routes and therapeutic schedules for ether lipids remain to be defined. They continue to be studied in the laboratory as fascinating pleiomorphic membrane-active signal transduction inhibitors, and as a basis from which even more selective anti-signalling molecules may emerge.

DESIGN OF NOVEL ANTI-ENDOCRINE AGENTS

(Discussion leader: M. Jarman)

Carcinoma of the breast and prostate are unique among major cancers in that in a high proportion of cases growth is driven by the action of oestrogenic or androgenic steroids, respectively, on their respective receptor proteins. Consequently, these tumours can respond to surgical or chemotherapeutic manoeuvres designed to interfere with hormone biosynthesis or action. In principle this provides the opportunity for highly selective therapy.

Since the early 1970s, hormonal antagonism by antiendocrine drugs has been an alternative to surgical ablation (e.g. oophorectomy, adrenalectomy, orchidectomy). Therapies with natural or synthetic oestrogens are now becoming treatments of choice for these cancers [22, 23]. Drugs have been discovered which are variously targeted towards steroid receptors (antioestrogens and antiandrogens) and the enzymes of steroid biosynthesis (e.g.

of oestradiol and oestrone by aromatase, of testosterone via steroid 17α-hydroxylase/$C_{17,20}$-lyase, and of dihydrotestosterone by testosterone-5α-reductase). None of the clinically established treatments is curative, and existing drugs have real or potential drawbacks, not surprisingly since many were discovered serendipitously to possess hormone antagonistic activity whilst being developed for other therapeutic applications. This has prompted the design of new agents with improved potency or specificity of action for their chosen target.

Tamoxifen is a major drug for the treatment of breast cancer. The main thrust in the search for a successor to this antioestrogen arises from the fact that tamoxifen is not a pure antagonist of oestrogen action at the level of the receptor, but has some residual agonist activity. The recent development of steroidal compounds which are pure antagonists, such as ICI 182780, should answer the question of whether or not partial agonism is in fact truly disadvantageous. Aromatase inhibitors, the longest established being aminoglutethimide, are complementary to tamoxifen since they may produce second responses in patients who relapse on tamoxifen. Modest potency and lack of specificity for the target enzyme are two drawbacks of aminoglutethimide which have prompted the design of more potent and selective agents such as CGS 20267. However it remains in question whether total oestrogen ablation can in practice be achieved by such agents since there appears to be an additional source of circulating oestrogens other than the direct action of aromatase upon their precursors.

Turning to prostatic cancer, luteinising hormone releasing hormone (LHRH) agonists already provide a highly effective alternative to orchidectomy for the depletion of circulating testosterone. However, some 5% of testosterone derives from adrenal synthesis and this component is not ablated by these treatments. Accordingly the concept of total androgen ablation has been promulgated. This combines LHRH agonists or castration either with an antiandrogen such as flutamide (to antagonise residual adrenal testosterone or dihydrotestosterone derived therefrom) or with other agents acting at the level of adrenal steroid biosynthesis, e.g. hydroxylase/lyase inhibitors. Controversy still exists as to the efficacy of the combined treatment over testicular ablation alone. Nevertheless the development of antiandrogens and hydroxylase/lyase inhibitors which are potential improvements on the respective lead compounds, flutamide and ketoconazole, has proceeded apace. For example, the discovery that the activity of flutamide is mediated through its hydroxylated metabolite has provided a starting point for the rational design of the newer antiandrogens anandron and casodex. Testosterone-5-α-reductase inhibitors are also potentially attractive as agents for the treatment of prostatic cancer, since the product dihydrotestosterone is the principal active androgen in the prostate. Potent inhibitors such as the transition-state analogue finasteride have been developed by rational consideration of the mechanism of action of enzymatic reduction, though clinical development of 5-α-reductase inhibitors is at an early stage.

A growing understanding of the other enzymatic mechanisms mentioned here has also impacted on the design of potentially improved antiendocrine drugs. Aromatase and hydroxylase/lyase are both cytochrome P-450 enzymes of known sequence, but unknown tertiary structure. Based on sequence homologies between the bacterial enzyme cytochrome P-450cam, the only such enzyme for which the complete three-dimensional structure is known, a structure for human hydroxylase/lyase has been proposed which could prove useful in inhibitor design. Meanwhile molecular modelling studies have attempted to rationalise the observed activities of inhibitors of these enzymes in terms of mimicry between their structures and those of the natural steroid substrates. Such models have also proved predictive and could therefore be useful in the *de novo* design of inhibitors.

The availability of a new generation of potent and specifically acting antiendocrine drugs should enable the ultimate potential, and limitations, of this type of approach to the treatment of steroid hormone-dependent cancers to be assessed definitively.

DESIGN OF NOVEL BIOREDUCTIVE DRUGS

(Discussion leader: P. Workman)

Bioreductive agents are prodrugs which are essentially inactive unless they undergo metabolic reduction [24]. The high degree of specificity of bioreductive drugs is dependent upon preferential metabolic activation in tumour cells as compared with critical normal tissues. This can arise in two ways. First, tumour cells may express bioreductive enzymes to a comparatively higher level of activity than in normal cells. Second, solid tumours develop physiological hypoxia to a greater extent than normal tissues. Current opinion favours the existence of two kinds of tumour hypoxia. The form described as chronic hypoxia has been known for some time. It arises as a result of tumour growth outstripping the supportive vasculature. Metabolic consumption by tumour cell respiration then exceeds oxygen delivery at around 200 μm from capillaries, causing foci of hypoxia to develop. Chronically hypoxic cells develop at the interface between the viable oxic cells and the anoxic dead population. The chronically hypoxic cells are resistant to radiation (because of the participation of oxygen in the radical mechanism) and also to conventional chemotherapy (because they may be non-cycling or in some cases receive inadequate drug delivery). They can, however, repopulate the tumour if the oxic population is sterilised by radiation or drug treatment, and thereby contribute to treatment failure. In contrast to chronic hypoxia, acute hypoxia arises because of the more transient opening and closing of tumour capillaries. This is believed to occur because the tumour vessels are less amenable to normal regulatory control mechanisms.

Evidence for acute hypoxia is restricted to experimental tumour models in which it can be demonstrated very elegantly by the fluorescent dye mis-match technique. A large body of data supports the existence of chronic hypoxia in both experimental and human solid tumours. This evidence ranges from the classic histological observations of Thomlinson and Gray, through radiation response data and bioreductive chemical probe binding studies to the very recent direct observations using oxygen electrodes in human tumours. This showed that in 15 breast cancer patients the median oxygen partial pressure (pO_2) was 30 mmHg compared with 65 mmHg in the normal breast tissue of patients [25]. Moreover 6 of 15 breast cancers contained areas of tissue with pO_2 values between 0 and 2.5 mmHg, whereas in the normal breast values $\leq$ 12.5 mmHg could not be detected. Therefore 40% of the tumours contained hypoxic areas with pO_2 values which would confer less than half-maximal radiosensitivity. Thus it seems likely that hypoxic cells do occur at a level equal to or greater than that of the 15–30% seen in many rodent and in human xenograft models, where the therapeutic advantages of bioreductive drugs have been clearly demonstrated. What is not yet clear is whether hypoxic cells are truly treatment-limiting in human tumours.

Various attempts have been made to improve the response of

hypoxic tumour cells, particularly to radiation therapy. These include enhancing oxygen delivery to tumours and administering electron-affinic chemicals such as misonidazole and etanidazole. Experimental and clinical work is continuing with these approaches, but recent calculations favour the use of agents which kill rather than to sensitise the hypoxic population. Bioreductive agents are generally much more toxic towards hypoxic cells because low oxygen conditions encourage a sustained reduction of the drug. Thus the problem of tumour hypoxia is actually turned to therapeutic advantage.

Three classes of bioreductive agent are currently known: nitro aromatic and heterocyclic compounds, quinones and the newly identified *N*-oxides. Until recently the design of bioreductive agents was dominated by considerations of redox potential and to a lesser extent lipophilicity and charge. These factors cannot always predict accurately for cytotoxicity however, and it is now realized that the ability of the prodrug to act as a substrate for various reductase enzymes is also a major factor. This has led to the concept of "enzyme-directed bioreductive drug development" [26], which is likely to be increasingly important in the future.

The prototype bioreductive agent in clinical use is mitomycin C. This drug shows only a modest preferential activity against hypoxic cells (around 2-fold). It is activated by various reductases, including cytochrome P-450 reductase, resulting in a crosslinked adduct located in the minor groove of the DNA helix. Recent work suggests that the interesting quinone reductase DT-diaphorase may also be involved, although this reaction proceeds only at very low pH. However, at neutral pH DT-diaphorase bioreductively activates both the aziridinylbenzoquinone diaziquone or AZQ and the novel indoloquinone EO9 [27]. This latter agent exhibits preferential activity against mouse and human colon tumours expressing high DT-diaphorase activities. Additional attractive features include an improved hypoxic cell specificity (about 5-fold), a unique spectrum of activity against the US NCI human tumour panel and a lack of myelosuppression (in contrast to mitomycin C).

Among the nitro compounds, the most recent innovation has been the introduction of an alkylating aziridine moiety into the nitroimidazole side chain to produce the DNA crosslinking derivative RSU 1069. This confers both potency and very marked hypoxic cell specificity (around 100-fold). Gastrointestinal toxicity limited the plasma concentrations in man to suboptimal levels. However, masking the aziridine in the form of the bromoethylamino derivative RB 6145 [28], which spontaneously releases RSU 1069, gives an improved therapeutic index in mice. RB 6145 is also scheduled for clinical trial. The major reductive enzymes involved are not known, but by analogy with other nitroimidazoles these are likely to include cytochrome P450, P450 reductase, xanthine oxidase and aldehyde oxidase.

The new *N*-oxide approach is typified by the benzotriazine di-*N*-oxide SR 4233 (WIN 59075) [29]. Like RSU 1069 and RB 6145 this agent displays a marked preferential killing of hypoxic cells (again about 100-fold), but damages DNA by a quite different mechanism. The active species appears to be a unique one-electron-reduced oxidising nitroxide radical which abstracts hydrogen from DNA without forming covalent adducts. The enzymes involved are those listed above for nitroimidazoles. The individual cytochrome P450 subfamilies responsible are currently being identified. The drug is also reduced by DT-diaphorase, which may be a bioprotective pathway in this case. Further structural analogues of SR 4233 are under evaluation.

The previously successful chemistry and screening approaches to bioreductive development may now have limited potential. Designing drugs for specific reductases expressed at high level in human tumours (e.g. DT-diaphorase) is one way ahead [26]. Elucidation of X-ray crystal structures combined with molecular modelling is an especially attractive approach for the future. Recent results suggest that bioreductive drugs and enzymes might be used in antibody-directed enzyme prodrug therapy (ADEPT). The dinitrophenylaziridine CB 1954 is activated more efficiently by the rat DT-diaphorase than by the human counterpart. Thus the rat enzyme could be targeted to human tumours with appropriate antibody prior to administration of the drug. Administering vasoactive drugs to increase tumour hypoxia has been successful in animals, but not in patients (raising concerns about the transplantable tumour models). But other approaches such as haemoglobin left-shifting agents and photodynamic therapy might be possible to increase the effect of bioreductive agents. Ideally patients will be selected for bioreductive therapy on the basis of a demonstrated level of tumour hypoxia combined with tumour enzyme profile. SR 4233 has now entered phase I clinical trial and similar studies are expected to begin shortly with RB 6145 and EO9.

It is important to emphasise that in the clinical development of hypoxia-specific bioreductive agents, it is necessary to remember that not all tumour cells will be sensitive. Thus sensible incorporation into radiation or chemotherapy combinations will probably be essential.

PHARMACOKINETICS, PHARMACODYNAMICS AND DOSE OPTIMISATION

(Discussion leader: M.J. Egorin)

Pharmacokinetic studies play an increasingly important part in new drug development, both in the preclinical and clinical phases. Moreover, the sophistication and quality of such investigations is progressively increasing. Particularly important is the welcome trend towards utilising pharmacokinetic information in a positive way rather than simply collecting and publishing them. Another valuable development is the increasing integration of pharmacokinetics with measurements of toxicity and tumour response.

Administration of any drug to a patient carries with it the implicit assumption that the drug will do something to the patient. In a schematic sense, treatment of a patient involves: (1) making a diagnosis and selecting a drug; (2) administering the drug to the patient, in whom it is distributed and eliminated; and then (3) observing the patient for target organ effects, both therapeutic and toxic. Two separate but interrelated aspects of clinical pharmacology are involved in this process. The first of these is "pharmacokinetics" which is a mathematical description of the behaviour of the drug and its metabolites in a system, i.e. a concentration × time history. The second of these is "pharmacodynamics", i.e. drug effect, both therapeutic and toxic. Another basic and implicit assumption is that there is some relationship between drug concentration and the response elicited. This is frequently drawn as a sigmoidally shaped curve, beginning at zero and plateauing at some asymptote. This relationship has been described with a variety of models, the most popular of which is a sigmoid E_{max} model described by the modified Hill equation.

The curve can take a variety of shapes. It is important to understand that in all of these sigmoidal descriptions relating drug dose or concentration to effect there is no consideration of the element of time. In contrast, it is obvious that administration

of a drug rarely elicits an immediate or instantaneous effect. Reasons for the temporal delay between administration of a drug and observation of effect include: (1) time for distribution for drug to distribute to its site of action located in tissues outside the plasma; (2) time for generation of a primary or secondary messenger; (3) the inability to monitor drug effect at its initial site; (4) the question of whether active metabolites are involved, etc. This issue of a temporal delay between drug administration and measurement of a plasma concentration and correlation with a pharmacodynamic effect has been dealt with through the concept of reverse hysteresis. With antineoplastic agents, one is probably dealing with the ultimate hysteresis loop, i.e. concentration increases and decreases with minimal, if any, observable effect, and then 2–4 weeks later toxicity or response becomes manifest at a time when little or no plasma concentration of drug is observed.

Relationships between pharmacokinetics and toxic pharmacodynamic consequences of antineoplastic therapy have now been demonstrated for a number of drugs including carboplatin, menogaril, hexamethylene bisacetamide, 5-fluorouracil, doxorubicin, vinblastine, busulfan and etoposide. In each case, the relationships have utilised area under the curve or its surrogate, steady state concentration during a continuous infusion. Similarly, in each case the models employed have been used in the Hill equation or a subset of the Hill equation, i.e. effect = 100 $(1-e^{kAUC})$. This latter subset is of note because it is the same model used by Skipper and Schabel to describe survival fraction of cells after exposure to non-cell cycle specific agents *in vitro*. Both the Hill equation and its subset have proven applicable for relating pharmacokinetics to both toxicities such as myelosuppression, which can be described as a continuous variable, and toxicities such as mucositis which can be described as semiquantitative or discontinuous variables being present or absent to some degree. More recently relationships between pharmacokinetics and the therapeutic pharmacodynamic consequences of their administration have been described for a paediatric population receiving the podophyllotoxin teniposide and a population of women with ovarian carcinoma treated with carboplatin.

These defined pharmacokinetic/pharmacodynamic relationships can be and have been utilised in a variety of fashions. The most obvious of these involves optimal dosing for individual patients in whom the aim is to maximise the likelihood of a therapeutic response while minimising the likelihood of a toxic pharmacodynamic outcome. These strategies have included renal function-based adaptive control dosing for carboplatin or more sophisticated adaptive control with feedback dosing for hexamethylene bisacetamide, 5-fluorouracil, suramin and methotrexate. In this latter dosing strategy, measurements of plasma concentration of drug are utilised to adjust dosing. In order to implement this strategy, there are requirements for: (1) known and careful sample handling; (2) rapid, affordable, and applicable methodology; (3) a defined population pharmacokinetic model; and (4) an applicable limited sampling strategy. Evaluation of such strategies can be carried out: (1) on a pharmacokinetic basis, comparing how close actual concentrations come to desired concentrations; (2) on a pharmacodynamic basis, determining whether or not the the desired drug effect expected for a given drug exposure was actually obtained; and finally (3) on a cost-benefit basis, in which the question is whether any actual patient benefit is derived from employing more expensive and complex dosing strategies.

Implicit in the development and implementation of each of these important concepts is the need for: (1) definition of the pharmacokinetics and pharmacokinetic/pharmacodynamic relationships of agents during phase I clinical trials; (2) the use of phase I pharmacokinetic data to develop limited sampling strategies as well as population pharmacokinetic models; (3) the use of phase II studies to validate optimal sampling strategies designed from phase I data and to confirm kinetic/dynamic relationships; and (4) the use of subsequent trials to evaluate the utility of individualised dosing.

Our understanding of pharmacokinetic–pharmacodynamic relationships is advancing rapidly [30]. Most studies have dealt with toxicity as the pharmacodynamic endpoint, but tumour response is increasingly coming under scrutiny. To date the most useful practical application of the concepts discussed here has been the development of pharmacokinetically based dosing schemes for carboplatin [31, 32]. Dosing can be optimised using a simple formula based on renal function. Results obtained with etoposide [33, 34] and 5-fluorouracil [35] also appear valuable.

A further important new development is the use of pharmacologically guided dose escalation [36]. This is a method by which dose escalation can proceed more aggressively than with conventional schemes such as the modified Fibonacci. The magnitude of the dose increment is dictated by the disparity between the measured AUC in the patient and the known AUC at the maximum tolerated dose (usually LD_{10}) in mice. The assumption is that AUC at the toxic dose will be the same in mouse and man. There is considerable support for this. If successful, the use of the new scheme will allow more rapid, but safe dose escalation to occur in phase I studies. This will save time and resources. It will reduce the number of patients required for a phase I study, and decrease the proportion who receive low and probably ineffective doses. Prospective validation of the scheme is underway. Some of the anticipated problems are indeed becoming apparent, including inadequate assay sensitivity, inter-patient variations and species differences in metabolism. However, there is considerable enthusiasm for its application in a sensible and above all flexible way, as part of an intelligently designed modern phase I and pharmacokinetic study.

SCREENING FOR NEW ANTITUMOUR DRUGS

(Discussion leader: G. Schwartsmann)

Current efforts in anticancer drug development are focused mainly on strategies to improve cure rates in the most common solid tumours of the adult. What is clear from the experience of drug screening over the past 4 decades is that the classical models utilised for the testing of new compounds are not suitable for that specific purpose. Human solid tumours are highly heterogeneous in behaviour, tend to have a slow rate of growth and are mostly refractory to the agents exhibiting high activity against rapidly growing, drug sensitive cancers such as human leukaemias or lymphomas.

New approaches to anticancer therapy should incorporate our advanced level of understanding of tumour biology, growth regulation and mechanisms of resistance, as well as alternative strategies involving tumour–host interactions or immunomodulation. An important challenge is to identify appropriate models to discover or evaluate novel agents and to develop a rational, but flexible decision-making process for drug selection.

There is considerable controversy in the field of anticancer drug development as to the role of "random" screening [13, 37–44]. By this we mean a system of drug discovery which is not based on a particular biochemical or molecular target. Thus,

this would include the new US NCI human tumour panel screen [40, 43, 44], even though it is recognised that this screen is targeted to select novel compounds with human solid tumour activity. Proponents of the new NCI screen would maintain that it should identify molecules with biochemical properties different from those selected in the earlier screening cascades, in which for example fast-growing rodent leukaemias were normally used as the "prescreen". They would argue further that new molecules might be identified which act against novel loci, as yet unknown to the rational drug designer. An additional argument is that by concentrating on natural products in particular we might harness the cumulative products of eons of selective pressure, and that we need to do this now before too many exotic plants and bizarre marine organisms become extinct.

Detractors take several different lines. Some are ideologically opposed to "random" screening and favour the logical mechanism-based approach to drug hunting to the exclusion of all else. Others would claim that the new screen would not identify some of the broad spectrum agents of current clinical value, such as the anthracyclines for example, nor would agents requiring metabolic activation be picked up. To these points, the response might be that we do not need more of such compounds. Another criticism concerns the fact that the new screen still selects for an antiproliferative response rather than a more subtle biological endpoint — differentiation or programmed cell death, for example. A related concern is that the use of highly selected cell lines grown as monolayers on plastic dishes is unlikely to approach the biological sophistication of a human solid tumour — including tumour–stromal interactions, in particular.

There are no doubt other reactions, some pragmatic, others more philosophical. A not insignificant factor is the disposition of a finite budget. The reality is that a human tumour panel screen is now in place. Perhaps we should applaud the hard work and ingenuity that has gone into its instigation and reserve judgement until such a time as the new agents begin to come through. What seems clear, however, is that we do not need further "mini-screens" to be of a similar nature to the set-up elsewhere. Efforts from academic researchers and groups such as EORTC, for example, would be channelled more sensibly into the development of more sophisticated and biologically relevant test systems and into mechanism-based drug design and development in areas such as those discussed earlier in this paper. It will be important to bring together those with expertise in different key areas to help achieve these goals.

It is very important that scientists involved in anticancer drug discovery programmes retain a broad view of the potential strategies to be taken. It is indisputable that recent advances in cancer biology have disclosed new and fascinating avenues in drug development, which certainly should be of high priority in the future. Although mechanism-based, biology-driven approaches to exploit new targets will be at the forefront, it is a sobering thought that several new agents now showing interesting activity in early clinical trials — taxol, anthrapyrazole and topoisomerase I inhibitors — are in fact derived from the traditional methods of drug screening. Another aspect to be considered is that biochemical and molecular investigations will be carried out to determine mechanisms underlying any histiospecific drug effects which are identified by the cell line panel. This may therefore provide an excellent resource for mechanism-based drug hunting.

CONCLUDING REMARKS

There are clearly many different ways to proceed in the rational design and development of new anticancer drugs. Some but not all of these have been reviewed here. Spectacular advances have been made in defining novel targets and harnessing new technologies to take advantage of these discoveries. We predict that the next decade will be very exciting for cancer chemotherapy.

1. Mattes WB, Hartley JA, Kohn KW. DNA sequence selectivity of guanine N7 alkylation by nitrogen mustards. *Nucleic Acid Res* 1986, **14**, 2971–2987.
2. Ponti M, Forrow SM, Souhami RL, D'Incalci M, Hartley JA. Measurement of the sequence specificity of covalent DNA modification by antineoplastic agents using Taq DNA polymerase. *Nucleic Acid Res* 1991, **19**, 292–293.
3. Hurley LH, Reynolds VL, Swenson DH, Petzfoll G, Scahill TA. Reaction of the antitumour antibiotic CC-1065 with DNA: structure of DNA adduct with DNA sequence specificity. *Science* 1984, **226**, 843–844.
4. Broggini M, Erba E, Ponti M, Ballinari D, Geroni C, Spreafico F, D'Incalci M. Selective DNA interaction of the novel distamycin derivative FCE 24517. *Cancer Res* 1991, **51**, 199–204.
5. Hélène C, Toulme JJ. Specific regulation of gene expression by antisense, sense and antigene nucleic acids. *Biochim Biophys Acta* 1990, **1049**, 99–125.
6. Hélène C, Toulme JJ. Control of gene expression by oligodeoxynucleotides covalently linked to intercalating agents and nucleic acid-cleaving reagents. In: Cohen JS, ed. *Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression*. London, Macmillan Press, 1989, 137–172.
7. Calabretta B. Inhibition of protooncogene expression by antisense oligodeoxynucleotides: biological and therapeutic implications. *Cancer Res* 1991, **51**, 4505–4510.
8. Saison-Behmoaras T, Tocque B, Rey I, Chassignol M, Thuong NT, Hélène C. Short modified antisense oligonucleotides directed against Ha-*ras* point mutation induce selective cleavage of the mRNA and inhibit T24 cells proliferation. *EMBO J* 1991, **10**, 1111–1118.
9. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**, 759–760.
10. Gullick WJ. The role of EGFR and the *c-erb*B2 protein in breast cancer. *Int J Cancer* 1990 (Suppl. 5), 55–61.
11. Sikora K. Gene therapy for cancer. *Eur J Cancer* 1991, **27**, 1069–1070.
12. Rosenberg SA, Aebersold P, Cornetta P, *et al.* Gene transfer into humans — immunotherapy using tumour infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* 1990, **323**, 570–578.
13. Tritton TR, Hickman JA. How to kill cancer cells: membranes and cell signalling as targets in cancer chemotherapy. *Cancer Cells* 1990, **2**, 95–105.
14. Powis G. Signalling targets for anticancer drug development. *Trends Pharm Sci* 1991, **12**, 188–194.
15. Woll PJ, Rozengurt E. A neuropeptide antagonist that inhibits the growth of small cell lung cancer *in vitro*. *Cancer Res* 1990, **50**, 3968–3973.
16. Gazit A, Osherov N, Posner I, *et al.* Tyrphostins. 2. Heterocyclic and α-substituted benzylidenemalononitrile tyrphostins as potent inhibitors of EGF receptor and ErbB2/neu tyrosine kinases. *J Med Chem* 1991, **34**, 1896–1907.
17. Dive C, Hickman JA. Drug-target interactions: only the first step in the commitment to a programmed cell death? *Br J Cancer* 1991, **64**, 192–196.
18. Berdel WE. Membrane-interactive lipids as experimental anticancer drugs. *Br J Cancer 1991*, **64**, 208–211.
19. Bazill GW, Dexter TM. Role of endocytosis in the action of ether lipids in WEHI-3B, HL60 and FDCP-mix A4 cells. *Cancer Res* 1990, **50**, 7505–7512.
20. Workman P. Antitumour ether lipids: endocytosis as a determinant of cellular sensitivity. *Cancer Cells* 1991, **3**, 315–317.
21. Grunicke HH. The cell membrane as a target for cancer chemotherapy. *Eur J Cancer* 1991, **27**, 281–284.
22. Cole PA, Robinson CH. Mechanism and inhibition of cytochrome P-450 aromatase. *J Med Chem* 1990, **33**, 2933–2942.
23. Crawford ED. Hormonal therapy of prostatic carcinoma. Defining the challenge. *Cancer* 1990, **66**, 1035–1038.

24. Kennedy KA. Hypoxic cells as specific drug targets for chemotherapy. *Anti-Cancer Drug Design* 1987, **2**, 181–194.
25. Vaupel P, Schlenger K, Knoop C and Hockel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurement. *Cancer Res* 1991, **51**, 3316–3322.
26. Workman P, Walton MI. Enzyme-directed bioreductive drug development. In: Adams GE, Breccia A, Fielden EM, Wardman P, eds. *Selective Activation of Drugs by Redox Processes*. New York, Plenum Press, 1990, 173.
27. Walton MI, Smith PJ, Workman P. The role of NAD(P)H: quinone reductase (EC 1.6.99.2, DT-diaphorase) in the reductive bioactivation of the novel indoloquinone antitumour agent EO9. *Cancer Commun* 1991, **3**, 199–206.
28. Jenkins TC, Naylor MA, O'Neill P, *et al.* Synthesis and evaluation of α-(((2-haloethyl)amino)methyl)-2-nitro-1H-imidazole-1-ethanol as prodrugs of α-((1-aziridinyl)methyl)-2-nitro-1H-imidazole-1-ethanol (RSU 1069) and its analogues which are radiosensitizers and bioreductively activated cytotoxins. *J Med Chem* 1990, **33**, 2603–2610.
29. Zeman EM, Brown JM, Lemmon MJ, Hirst VK, Lee WW. SR-4233: A new bioreductive agent with high selective toxicity for hypoxic mammalian cells. *Int J Radiat Oncol Biol Phys* 1986, **12**, 1239–1242.
30. Ratain MJ, Schilsky RL, Conley BA, Egorin MJ. Pharmacodynamics in cancer therapy. *J Clin Oncol* 1990, **8**, 1739–1753.
31. Egorin MJ, Van Echo DA, Olman EA, *et al.* Prospective validation of pharmacologically based dosing scheme for the cisplatin analog, carboplatin. *Cancer Res* 1985, **45**, 6502–6506.
32. Calvert AH, Newell DR, Gumbrell LA, *et al.* Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989, **7**, 1748–1756.
33. Mick R, Ratain MJ. Modelling interpatient pharmacodynamic variability of etoposide. *J Natl Cancer Inst* 1991, **83**, 1560–1564.
34. Stewart CF, Arbuck SG, Fleming RA, Evans WE. Relation of systemic exposure to unbound etoposide and hematologic toxicity. *Clin Pharm Ther* 1989, **50**, 387–393.
35. Thyss A, Milano G, Renee N, *et al.* Clinical pharmacokinetic study of 5-FU in continuous 5-day infusions for head and neck cancer. *Cancer Chemother Pharmacol* 1986, **16**, 64–66.
36. Collins JM, Zaharko DS, Dedrick RL, Bruce BA. Potential roles for preclinical pharmacology in Phase I clinical trials. *Cancer Treat Rep* 1986, **70**, 73–80.
37. Corbett TH, Valeriote FA and Baker LH. Is the P388 murine tumour no longer adequate as a drug discovery model? *Invest New Drugs* 1987, **5**, 3–20.
38. Double JA and Bibby MC. Therapeutic index: a vital component in the selection of anticancer agents for clinical trial. *J Natl Cancer Inst* 1989, **13**, 988–991.
39. Johnson RK. Screening methods in antineoplastic drug discovery. *J Natl Cancer Inst* 1990, **82**, 1082–1083.
40. Chabner BA. In defense of cell-line screening. *J Natl Cancer Inst* 1990, **82**, 1083–1085.
41. Grindey GB. Current status of drug development: Failure or limited success? *Cancer Cells* 1990, **2**, 163–171.
42. Sikic BI. Anticancer drug discovery. *J Natl Cancer Inst* 1991, **83**, 738–740.
43. Boyd MR. Status of the NCI preclinical antitumour drug discovery screen. *Principles and Practices of Oncology* 1989, **3**, 1–11.
44. Monks A, Scudiero D, Skehan P, *et al.* Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. *J Natl Cancer Inst* 1991, **83**, 757–766.

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Cell Kinetic Alterations During Epidermal Carcinogenesis

EACR—Mühlbock Memorial Lecture, 1991

Olav Hilmar Iversen

INTRODUCTION

Normality depends on a complex biological equilibrium

THE STRUCTURE of the higher living organisms is characterised on the one hand by complexity and strict organisation, and on the other by its dynamic character. Cells differentiate, mature, die and are constantly being replaced by new cells. While alive, all cells also exchange molecules and atoms, make use of them, excrete waste products and metabolites, and send out signal substances. Order in a state of perpetual change is maintained by a steady flow of information in control systems, which operate at the molecular, cellular, tissue and organ levels. All cells play a part in maintaining body equilibrium by communicating through biochemical and electrophysiological signals. The brain is the supreme director, and through the pituitary gland the hypothalamus is the conductor of the hormone orchestra. At the cellular level, everything depends on and is regulated by the information in the genes.

In the field of growth regulation there are basically two approaches, or two opposing paradigms. One is based on the theory that, in terms of cellular proliferation, living cells are naturally quiescent, like the Sleeping Beauty. They need a life-giving kiss from a growth factor in order to be activated to cell division. This way of thinking has been greatly encouraged by the discovery of a considerable number of stimulatory growth factors, and their receptors and genes; and by the fact that some growth factors are directly or indirectly associated with the oncogenes. Some adherents to the Sleeping Beauty theory also believe that cells produce factors to stimulate themselves, a hypothesis called autocrine growth stimulation [1].

The other theory, which I consider more probable, is that healthy cells possess a strong natural aptitude for division. This is clearly shown by the exponential growth phase in cell culture.

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