principles and methods for education, with a personal and self-directed system which avoids a CME bureaucracy, provides assistance and is, above all, oriented around patient needs.

(2) Funding should be accepted from different bodies, including private sources, provided that it is channelled through independent bodies.

(3) CME should be voluntary with incentives and controls in accordance with the regulations and the cultural mores in the different European countries.

(4) Given the uniqueness of oncology, which includes specialists and non-specialists and is not organ-specific but has a singular philosophy and approach to the management of disease (prevention, treatment, palliative care), a consensus conference on CME is recommended.

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European School of Oncology Task Force Report

New Approaches in Cancer Pharmacology: Drug Design and Development (Part 2)

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INTRODUCTION

IN A PREVIOUS REPORT [1], the ESO Task Force reviewed recent progress in the following areas: (1) DNA sequences and gene-specific drugs; (2) anti-sense and anti-gene oligonucleotides targeted to oncogenes; (3) prospects for biological therapies; (4) membrane and signal transduction targets; (5) development of anti-tumour ether lipids; (6) design of novel anti-endocrine agents; (7) design of novel bioreductive drugs; (8) pharmacokinetics, pharmacodynamics and dose optimisation; and (9) screening for new anti-tumour drugs. We now turn our attention to the latest advances in (1) pharmacological evaluation of new agents; (2) principles in the design of DNA-interactive molecules: (3) discovery of improved platinum analogues; (4) inhibitors of intracellular signalling; (5) discovery of inhibitors of oncogenic tyrosine kinase signalling and downstream targets; (6) apoptosis and cancer therapy; and (7) engineering antibodies for targeted cancer therapy.

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PHARMACOLOGICAL EVALUATION OF NEW ANTI-CANCER AGENTS

(Discussion leader: Maurizio D'Incalci)

To discover innovative new anti-cancer agents, we must use the most appropriate primary test systems; subsequent evaluations must then be carried out in additional relevant models to provide further information on molecular specificity and therapeutic selectivity prior to clinical trials [2, 3]. The successful identification and evaluation of novel anti-cancer drugs effective against human tumours will depend on the degree of predictivity of the experimental models which are selected. We can schematically divide the experimental systems for the evaluation of a new anti-cancer agent into: (1) *in vitro* cell-free models; (2) *in vitro* cellular models and (3) *in vivo* tumours growing in animals.

In the last few years, a number of novel molecular targets for potential new anti-cancer drugs have been identified. These targets include receptors of hormones and growth factors; DNA sequences which are presumed to be involved in the malignant behaviour of cancer cells and key enzymes or other control proteins (e.g. oncogene products) which play a crucial role in the abnormal proliferation of cancer cells [2, 4]. The *in vitro* investigation of drugs which block a specific target can now take advantage of our rapidly growing knowledge of the biology and biochemistry of cell proliferation in normal and neoplastic cells, as well as benefiting from the application of new methods to

investigate drug interactions at the molecular level. For example, it is becoming increasingly feasible to determine the binding of a drug to bases located in specific sequences and in specific genes [5, 6]. Several enzymes involved in signal transduction pathways, in the synthesis of macromolecules, and in the regulation of gene transcription are now sufficiently well characterised to develop specfiic *in vitro* assays to screen for the inhibitory activity of a drug. It should be pointed out that, in most cases, these targets are not restricted to malignant cells, so that the possibility of obtaining highly selective compounds is often still speculative. There are, however, plausible reasons to believe that some degree of therapeutic selectivity can be achieved. This is particularly evident in situations where the oncogenic change involves a mutation, as for example, with the *ras* genes and p53, which are the most frequently altered in human cancer. In addition, quantitative differences in the level of expression of growth factor receptors in tumour versus normal cell membranes could be exploited to obtain relatively selective antiproliferative agents against certain types of neoplasms. In other cases, a molecular target may be selected for screening because it is the putative locus for action for existing active anti-cancer agents. For example, a number of anti-cancer agents appear to act as inhibitors of DNA topoisomerase I and II, suggesting that these enzymes may be good potential targets for improved new anti-tumour agents.

Many pharmaceutical companies now run their screening assays in high throughput mode, allowing several thousand compounds per week to be tested. These screens will often use recombinant proteins or gene transcription systems. Recently, the three-dimensional structure of several target proteins have been characterised by means of X-ray crystallography and nuclear magnetic resonance spectroscopy. These include dihydrofolate reductase, thymidylate synthetase, protein kinase A, p21 *ras* and various *src* homology domains. It is, therefore, possible to use this structural information to design molecules which are chemically distinct and theoretically more specific than previous enzyme inhibitors. However, even if rationally designed on the basis of detailed structural information or identified by functional or chemical *in vitro* assay, all potential new drugs must be subject to further investigation in more complex biological systems in order to assess their selectivity and potential anti-tumour activity.

Turning to *in vitro* cellular models, a wide range of established cell lines derived from mouse or human tumours are currently being used for the evaluation of drug-induced cytotoxicity and differentiation. Almost 5 years ago, the U.S. National Cancer Institute (NCI) dropped the use of the P388 leukaemia *in vivo* primary prescreen and initiated a large screening programme using 60 human cancer cell lines growing *in vitro* [7, 8]. Although this approach makes it possible to test a very large number of compounds in a relatively short time against various tumour types, there are some possible drawbacks. If an inactive drug requires biotransformation in the liver to active metabolites, it will be missed using this type of screening. Moreover, no attempt has been made to consider the more complex biological aspects that might be relevant for each individual tumour type. For example, epithelial cells may behave in a different way if they are attached to plastic compared to substrates simulating the basement membrane. The addition of serum and the various other components of the tissue culture medium could enhance or inhibit the activity of a drug. Thus, the regulation of the transport of some compounds is dependent on the medium composition: for example, a low or high folate concentration can cause an up- or downregulation of membrane folate-binding proteins which are relevant carriers for certain anti-folates. It should also be underlined that the assumption that human cell lines maintain all the relevant features of the human tumour they are derived from is undoubtedly simplistic. Tumour heterogeneity is of particular concern in this respect. We know that established cell lines arise from a selected small fraction of tumour cells originally present in a tumour which is able to adapt and grow *in vitro*. Therefore, they may not be representative of the heterogeneous cancer cell populations which exist in the original human malignant tumour.

Primary cultures are better able to mimic the cancer cell populations present in a tumour biopsy, but this approach is too expensive and time-consuming to be applied routinely on a large scale. Thus, when random screening of compounds is performed, it does seem reasonable to use a large number of cell lines derived from different human tumours of the same histiotype in order to minimise the number of false negative results. Alternatively, if a compound has been identified as an active inhibitor of a specific target, a smaller number of appropriate experimental models must be used for the evaluation of its potential anti-tumour activity. For example, if a drug is selected because of its ability to bind a certain receptor, it is expected that it will be active only against tumour cells expressing that specific receptor. This means that the cell lines used to evaluate potential new anti-cancer agents should be characterised in detail with respect to the relevant targets in order to perform a more scientifically rigorous assessment of new drugs. Cells which do not possess the appropriate target or are less dependent upon it can be used as controls. It is interesting to note that initial results from the new NCI human tumour screening panel have shown that mechanistically similar agents of diverse structure can display a common pattern of activity or 'fingerprint' across the 60 cell lines. Thus, a particular fingerprint can be used prospectively to ascribe a putative mechanism of action. An example of the possible application of the specific pattern of sensitivity to anti-mitotic agents is illustrated in [9], and other examples have been obtained recently for various other classes of anti-tumour drugs. The reason for the cross-sensitivity among different agents within a given class, e.g. alkylators or topoisomerase II inhibitors, is not yet known, but some interesting and testable hypotheses can be formulated in order to explain this phenomenon. For example, it may be that the sensitivity to a given class of compounds is due to some common pattern of oncogene expression or to the deficiency of certain repair mechanisms. In any case, this finding deserves to be investigated further and opens up the possibility that the pattern of activity of an unknown compound across a large cell line panel may indicate its probable mode of action.

There is increasing use of cell lines, both mammalian and, for example, yeast, which have been genetically modified to display a particular molecular feature. Examples would include a mutated oncogene or high levels of a growth factor receptor. Such manufactured cell lines can be linked to an appropriate and simple biochemical readout for use in high throughput format against mechanism-based targets. Control cell lines lacking the genetic modification or possessing different modifications can be used as specificity checks.

With respect to *in vivo* testing, a number of experimental models can be used, including rodent tumours, human xenografts in immune-deprived mice and also genetically manipulated animals (e.g. the 'oncomouse'). In some instance, it may be appropriate to use the same cell for *in vivo* as was used for

in vitro screening. In comparing the results, pharmacokinetic behaviour may be the major difference. Murine tumours are still important to evaluate anti-tumour compounds since they are relatively well characterised both biologically and pharmacologically. For example, the thorough characterisation of the pattern of metastasis seen with different mouse tumours makes them very suitable for evaluating compounds which have been designed as anti-metastatic agents. Human xenografts are interesting and valuable experimental models since many of the pathological features of the human tumour they are derived from are maintained in the xenograft. However, differences will certainly exist between a human tumour growing in a patient and the same tumour growing in nude mice, not least because of the presence of murine stroma and vasculature, and we should be cautious with over-facile extrapolations. It is possible, however, to obtain tumours from patients who are either sensitive or resistant to certain drugs, or at an early versus an advanced stage, and to validate the models by comparing the drug sensitivity observed in the patient with that observed in the xenograft [10].

To promote a more scientific use of these *in vivo* experimental models, we need as much information as possible on their biological properties. Although human leukaemias are not the most frequent neoplastic disease, they certainly represent a very good model with which to investigate new and more rational approaches. In fact, for many human leukaemias, we have detailed knowledge of the typical abnormalities present at the chromosomal and molecular level. The recently obtained human leukaemias which grow in immunodeficient mice show the persistence of the particular genetic abnormalities previously observed in the patients' leukaemic cells. The molecular characterisation of the breakpoints of the typical translocations makes it possible to identify DNA sequences which are exclusively present in the tumour cells, and thus explore therapies targeted specifically towards those sequences (e.g. anti-sense and anti-gene strategies). These models are also highly suitable for an evaluation of alternative approaches including, for example, differentiation therapy (e.g. retinoic acid in promyelocytic leukaemia).

The search for new drugs must rely on the sensible integration of different approaches and the relevant experimental models. For drugs designed as inhibitors of a specific target, the test systems should be selected and optimised accordingly. With compounds for which no biochemical or pharmacological knowledge is available (e.g. extracts of natural products), screening against many *in vitro* cell lines and *in vivo* experimental tumours is the only realistic approach. Certainly for some human tumours we are beginning to obtain adequate animal experimental models which do appear to reproduce the peculiar molecular and biological abnormalities of the particular human disease. These models are certainly very attractive for the testing of mechanism-based strategies which are now emerging from rational design based on a sound scientific exploitation of advances in molecular oncology.

PRINCIPLES IN THE DESIGN OF DNA-INTERACTIVE MOLECULES

(Discussion leader: Stephen Neidle)

The targeting of DNA by low molecular weight anti-cancer agents has been, and continues to be, a major element of cancer chemotherapy. Several new drugs derived from conventional cytotoxic agents, yet with clinically useful activities, are being successfully developed. Some, such as the third-generation platinum analogues and the anthrapyrazoles, may well provide significant clinical advances over the next few years. New anti-tubulin drugs like taxol, taxotere and rhizoxin and novel topoisomerase inhibitors also appear promising.

However, it is generally accepted that the classic DNA-interactive drugs (and their more recent descendants), are essentially non-selective in their molecular action and do not discriminate in their binding between tumour cell and normal DNA. Their anti-tumour effects are dependent on events consequential to DNA binding, such as stabilisation of a "cleavable complex" with DNA topoisomerase II, or on differences in drug–DNA adduct repairability between normal and some tumour cells. All such drugs can be classified as *information-neutral* nucleic acid binding agents in that they do not show a high degree of preferential binding to particular DNA sequences. It is a common view that quantum leaps in effectiveness of treatment for the majority of common human cancers are unlikely to come from such compounds. Nonetheless, there are a few cases where selectivity for particular DNA sequences, even when they are short ones, can lead to useful drugs. A well-characterised example is the high degree of preference shown by several of the nitrogen mustards for runs of guanine nucleosides in DNA, in both *in vitro* and in intact cells [11]. This appears to be related to their extraordinary high activity in Burkitt's lymphoma, which contains multiple copies of the very GC-rich Epstein–Barr virus genome.

Knowledge of the cell and molecular biology of many cancers has now provided very considerable information on the underlying genetic abnormalities that give rise to neoplastic cells, and on the roles played by the gene products encoded by these mutations and translocations. This has profound implications for future therapeutic intervention strategies for many human cancers that are directed at the nucleic acid level, since one can now envisage moving beyond the classic DNA-interactive agents with their problems of lack of specificity and toxicity, to direct exploitation of, for example, DNA sequences for oncogenes that have arisen from point mutations or translocations. As a class, future drugs directed against such targets can be termed *information-reading* nucleic acid binding agents, because they are actually taking DNA sequence into account.

In principle, given the size of the human genome, it is necessary to recognise some 15–18 contiuous base pairs of DNA (or mRNA) in order to have total specificity against a chosen nucleic acid sequence [12]. There are currently three principal strategies for achieving this level of recognition: (i) by anti-sense oligonucleotides to mRNA [13], (ii) by triple-helix-forming nucleotides to genomic DNA [14] and (iii) by natural products or synthetic molecules in part derived from them, again targeted to DNA [15, 16]. The two oligonucleotide approaches share problems of stability, delivery and economic viability, and also have distinct conceptual problems. The synthetic approach has not, to date, been able to achieve recognition of lengths of DNA sufficient to specifically recognise a single gene, although it may have chemical and pharmacological advantages over (i) and (ii).

In molecular structural terms, the anti-sense concept is straightforward. The oligodeoxynucleotide probe is complementary to the target mRNA sequence and hybridises with it to form a DNA–RNA duplex. Duplex stability depends on the stringency of hybridisation (i.e. no or very few mispairings), as well as on the nature of the nucleotide backbone modifications used to confer improved nuclease stability and cell transport properties. Much remains to be done to ensure the optimisation and rational molecular design of such modifications in order to enhance the effectiveness of hybridisation: some alterations

clearly improve stability and intracellular uptake at the cost of a significant loss of RNA–DNA hybrid stability. The other major structural consideration that needs to be taken into account is that of mRNA secondary and tertiary structure formation. In principle, if the target mRNA folds in a stable manner, then it will not be readily accessible to an anti-sense oligonucleotide. It is posssible to predict the likely extent of secondary structure stems and hairpins using semi-empirical energy functions. However, reliable estimates of tertiary folding are rather more elusive.

The efficiency of the anti-sense method depends on being able to suppress perhaps several hundred mRNA molecules per cell, in the hope that the transcription apparatus for the parent gene will not, at some later stage, produce more mRNA copies. By contrast, the anti-gene approach directly targets genomic DNA. In principle, this has a considerable advantage, compared to anti-sense, in that there are only one or a few DNA copies per cell so that significantly lower levels of oligonucleotide will be required. It is now clear that triplex formation can result in impaired transcription [17]. The transcriptional activity of the target gene is a major factor, since the target sequence itself must be accessible. The classic target DNA sequence for triplex formation is required to be an oligopurine or oligopyrimidine, restricting specific recognition to certain sequences. Molecular modelling and other studies are beginning to indicate ways of extending this recognition code to more general sequences.

Natural products or synthetic DNA-binding compounds that have significant sequence selectivity (albeit for short <6 base-pair sequences) are exemplified by the minor-groove non-covalent binders netropsin and distamycin and the covalently-bonding drug CC1065. Analogues of the latter two compounds (e.g. distamycin mustard, adozelesin and carzelesin) are currently in early clinical trial, and it will be of some interest to see whether this class of compounds has real potential as anti-tumour agents. Although a great deal is now known about their DNA interactions at the molecular level from X-ray crystallographic, nucleic magnetic resonance and other studies, the relationship between their sequence selectivity and biological activity is currently not established. Accordingly, in common with other DNA information-reading molecules, it will be of increasing importance to use mechanistic and sequence-specific screens for future evaluations, rather than the biological black-boxes of, for example, the P388 leukaemia and similar assays.

Non-covalent minor-groove interactions are generally restricted in AT-rich sequences, in spite of considerable effort to extend the recognition code for such molecules. Covalent binding to guanines is an efficient way of solving this problem, although it is still not possible to design a molecule for any desired sequence. An extension of this approach is with cross-linking dimers of such guanine-recognising molecules [16]. These compounds can achieve very high levels of cytotoxicity, and moreover can recognise significant lengths of DNA sequence. Extension of this concept to trimers and tetramers may well lead to sequence-specific agents that can rival the oligonucleotides in their ability to specifically target 12–16 base pairs of DNA, with the advantage of irreversible binding to this target.

DISCOVERY OF IMPROVED PLATINUM ANALOGUES

(Discussion leader: Ken R. Harrap)

Pyrone's chloride, known chemically as *cis*-diamine dichloroplatinum II and now more commonly as cisplatin, was first synthesised in 1845. Its important biological properties were to lie dormant until Barnet Rosenberg induced filamentous growth in bacteria exposed to an electric field across platinum electrodes. Rosenberg and colleagues soon identified the known co-ordination complex, cisplatin, as the cytotoxic species generated by the field. Despite its severe mycloproliferative and nepthrotoxicities, cisplatin was shown to possess potent anti-tumour properties, both *in vitro* and in the animal tumour models of the NCI screen. These early discovery events have been reviewed elegantly by Rosenberg [18].

Because of its potent preclinical anti-tumour properties and despite its toxicities, cisplatin was developed as a clinical trials candidate and the first phase I studies were conducted in the early seventies in the U.K. Dose-limiting nepthrotoxicity, subsequently shown to be ameliorated by hydration and diuresis, was accompanied by severe nausea and vomiting, peripheral neuropathy and ototoxicity. These observations were confirmed in wider phase I studies in the U.S.A. However, the clinical anti-tumour value of cisplatin soon became apparent, and it is now clear that this valuable drug is curative in the treatment of testicular teratoma, induces long-term remissions in advanced ovarian cancer, and plays a major role in the treatment of head and neck, bladder and cervical cancers (see [19] for a review of early preclinical and clinical studies).

Subsequently, much effort was devoted to discovering alternatives to cisplatin which retained its useful anti-tumour properties but which were, so far as possible, devoid of its toxic limitations. Large screening programs were set up; cisplatin acquired resistant tumour models were adopted in an attempt to discover compounds possessing a wider spectrum of anti-tumour activity and structure–toxicity studies were initiated.

In broad summary, two major structural themes have emerged from this effort and several examples of each have now achieved clinical resolution. Platinum complexes carrying a diamino cyclohexane (dach) ligand retain activity against cisplatin acquired resistant mouse leukaemias. Several compounds have been developed in the hope that this property might also confer a wider spectrum of clinical activity. However, in the cases of JM82 (1,2-diaminocyclohexane-[4-carboxyphthalato]platinum II) and JM20 (1,2-diaminocylcohexane[sulphato]platinum II) these hopes were not confirmed in clinical practice. Nonetheless, tetraplatin (1,2-diaminocyclohexane tetrachloro platinum IV) has been developed according to the same thesis and is currently at a late stage of phase I evaluation. The results of this study are awaited with interest.

The second class of structures to receive wide attention includes compounds containing malonate or malonate-type leaving ligands. Such compounds are substantially more chemically stable than cisplatin, a property which appears to confer an absence of nephrotoxocity. The most important member of this class is carboplatin (Paraplatin). Following cisplatin, this is the the second platinum-based anti-cancer drug to receive worldwide regulatory approval and acceptance (see [20] for a review of developments leading to the recognition of dach- and malonate-based platinum anti-cancer drugs). Carboplatin is essentially devoid of the major toxic limitations of cisplatin. Myelosuppression, predominantly thrombocytopenia, is dose-limiting, while emesis is less severe than with cisplatin and invariably responds to conventional anti-emetic prophylaxis. There is no requirement for hydration and no evidence for ototoxicity or peripheral neuropathy. Where radomised studies have been completed, it appears that the spectrum of diseases amenable to treatment with either drug are comparable.

Some 23 platinum compounds have so far received clinical evaluation and a further nine are presently on study. Broadly, these latter can be classified as dach-containing [20] or carboplat-

in-like [21]. Of the dach-containing compounds, the further development of tetraplatin and oxaliplatin may be compromised by the incidence of neuropathy; L-NDDP, a liposome-entrapped *cis*-bis-neodecanoato-trans-R, R-1,2-diaminocyclohexane platinum II complex, has shown myelosuppression to be dose-limiting and is without nephrotoxocity, neurotoxicity and ototoxicity. Current developments with this compound are focused on improving the intra-lysosomal stability of NDDP and optimising the composition of the liposomal drug carrier. Of the "carboplatin-like" complexes, the incidence of nephrotoxicity may preclude the further development of Zeniplatin and Enloplatin. Four compounds (245-S, DWA2114R, NK121 and Lobaplatin) appear to possess acceptable "carboplatin-like" toxicities (myelosuppression) in phase I; their putative therapeutic utilities remain the subject of ongoing clinical evaluation.

A new departure has been the development of a platinum drug designed specifically for oral administration, the rationale being both to improve quality of life in the outpatient setting and to reduce treatment costs [22]. Novel chemistry was developed in order to circumvent the generally poor absorption of platinum complexes from the gastrointestinal tract. JM216 (cyclohexylamine ammine trans diactato dichloro platinum IV) is the lead compound in a large class of ammine/amine transdicarboxylates, selected for good absorption, potent anti-tumour activity in human ovarian carcinoma xenografts, low toxicity (myelosuppression) and low emetogenic activity [22, 23]. JM216 is currently the subject of phase I study.

The ultimate objective must be to discover a platinum drug which is better able to counter the problems of refractory and relapsed disease than are cisplatin/carboplatin. It is likely that such initiatives will depend, in large measure, on an improved understanding of the biochemical mechanisms underpinning intrinsic and acquired resistance, and in the availability of clinically relevant laboratory models of human malignant disease [21, 24]. There is a gathering consensus that cisplatin resistance can be a multifactorial problem, contributed individually or severally by impaired cellular accumulation, elevation in cellular nucleophiles, such as glutathione and metallothioneins, or enhanced DNA repair mechanisms. However, this classical picture of platinum resistance is becoming complicated by the discovery of proteins which may mediate intracellular cisplatin reactivity. For example, the high mobility group protein, HMG1, has been shown to bind to platinum-damaged DNA, possibly promoting DNA repair in the intact cell. Further, a membrane-associated glycoprotein is generated in acquired resistant cells which exhibit reduced platinum uptake. In addition, some oncogenes, in particular *ras*, *myc*, *fos* and *jun* can be hyperexpressed in acquired cisplatin-resistant cells. There is also evidence for the expression of the chaperonin heat shock protein hsp 60 in such cells and for an involvement of the p53 tumour gene produce [25]. It is not yet clear whether these genetic events are causally related to the resistance process or are simply companion artifacts, but they provide an exciting stimulus for further work which might lead to new drug discovery programmes.

INHIBITORS OF INTRACELLULAR SIGNALLING: PROMISE AND PROBLEMS

(Discussion leader: Garth Powis)

Considerable effort is being devoted by academic and pharmaceutical laboratories to developing drugs that are active against growth factor-activated intracellular signalling pathways in the hope that this approach might yield intrinsically new classes of anti-proliferative drugs [3, 26, 27]. These signalling pathways are the sites at which most known oncogenes produce their effects on cell proliferation and transformation through the over-expression or mutation of proto-oncogene products. There is the reasonable expectation that if drugs can be developed that specifically inhibit oncogene-activated signalling pathways, it should be possible to reverse the efforts of the oncogene and return the cell to a non-transformed state.

While the oncogene itself might provide a good target for pharmacological intervention through the use of DNA triple helix-based drugs (anti-gene therapy), this approach currently has severe practical limitations. The same is true of the mRNA anti-sense strategies based on unmodified or ribozyme-modified anti-sense RNA constructs (see earlier and [28]). The oncoprotein molecule can be targeted, as with cell surface-expressed oncoproteins, such as HER-2, where specific antibodies may be useful, or with the oncoprotein tyrosine kinases, where small-molecule, cell-permeant drugs are being developed that inhibit the enzyme activity of the oncoprotein or receptor (see next section). An alternative approach to targeting the oncogene, the oncogene mRNA or the oncoprotein products is to target other points in intracellular signalling pathways that are activated by the oncogene. Since there may be many enzymes or transducer proteins that are components of these pathways, this approach offers a number of potential target sites for drug discovery. The problem that remains is clearly one of selectivity, because the target may be used by multiple signalling pathways and all these will be inhibited. Even if a target specific to the signalling pathway to be inhibited is identified and a selective drug developed, the same pathway will be inhibited in the normal cell. Thus, the drug may be toxic to both normal and transformed cells. Fortunately, it appears that the degeneracy of growth factor signalling may allow a specific pathway to be inhibited (thus removing the growth stimulus provided by the oncogene) while leaving intact the alternative pathways necessary for normal cell function. A caveat to this hypothesis is that if several oncogene-activated signalling pathways have to be inhibited to reverse cell transformation, there may not be sufficient redundancy to save the normal cell function.

A feature of anti-signalling cancer drugs is that they would be expected to be cytostatic rather than cytotoxic. This does not mean, however, that such drugs will fail to cause tumour regression. It is well known that the size of a tumour represents a balance between new tumour cell growth and tumour cell death [29]. Thus, inhibiting tumour cell growth should be sufficient to cause tumour regression. However, it may be that the natural immune mechanisms are unable to remove residual tumour cells [30], in which case anti-signalling drugs will have to be given over extended periods. This increases the likelihood that drug resistance will develop. The use of several anti-signalling drugs in combination may overcome that problem. Indeed, such an approach may be essential both to circumvent potential resistance and to inhibit multiple oncogene-activated pathways that are likely to be present in cancer cells (but see next paragraph).

It is possible that the oncogene that is being targeted does not act continuously to promote cell transformation but rather functions as a switch: for example, allowing expansion of a premalignant clone of cells during early tumour genesis. If so, inhibition of that oncogene may be too late to reverse the transformed phenotype. Fortunately, work with experimentally-transformed cells suggests that transformation can be reversed by inhibiting an oncogene, such as mutant *ras* by

microinjection of antibody agaisnt *ras* p21 or with anti-*ras* ribozyme [31]. This work also suggests that it might, in some instances, be sufficient to administer just one anti-oncogene drug in order to reverse the transformed phenotype. This may not always be the case, however, since we know that it takes several oncogenes and/or loss of tumour suppressor genes to cause full malignant transformation [32]. Alternatively, the proliferation and malignant behaviour of human cancer cells with multiple genetic changes can be corrected by introducing a single chromosome or a specific tumour suppressor gene such as p53 or RB [33–36]. Cell death may even ensue. Nevertheless, we do not yet understand with any certainity how many oncogene or tumour suppressor gene pathways will have to be affected by drugs in order to reverse the transformed phenotype in the clinical situation. If several drugs, each inactive by itself, have to be given together, this will present a problem since we have very little experience of developing cancer drugs that lack single-agent activity. Clinical trials would be especially challenging. Finally, it is clear that to adequately test the hypothesis that anti-signalling drugs could be useful clinical anti-tumour agents, we have to be certain that the target oncogene or protein is present and active not only in the preclinical models used, but also in the tumour of the particular patient being treated; thus a biopsy sample may be required to aid patient selection. It will also be important to ensure that the target is indeed inhibited effectively at the dose of drug being given. Thus, it will be necessary to have relevant biological end points built into clinical trials so that appropriate, but not excessive doses of drugs can be defined.

There are several specific anti-signalling agents currently in development and some of these are already in clinical trial (e.g. suramin, bryostatin, ether lipids, 8-chloro-cyclic AMP and a carboxyamidotriazole agent which inhibits calcium entry). It will take considerable effort to ensure that such drugs receive proper preclinical development and clinical evaluation. Both discovery and development must, therefore, be tailored to the unique properties of individual signalling inhibitors.

DISCOVERY OF INHIBITORS OF ONCOGENE TYROSINE KINASES SIGNALLING AND DOWNSTREAM TARGETS

(Discussion leader: Paul Workman)

Of the many signal transduction mechanisms which are emerging as potential targets for drug hunting in cancer, the tyrosine kinase activities associated with growth factor receptors and oncogene products are receiving particular attention from both pharmaceutical companies and academic groups [37–43].

Protein kinases are vital for all aspects of cell function. There are at least 200 of these enzymes and up to a thousand have been predicted [44]. They catalyse the transfer of the γ-phosphate of ATP or GTP, in general, to either serine/threonine or tyrosine residues located within a particular peptide sequence in the protein substrate. While serine/threonine kinases, particularly protein kinase C, are also targets for drug development [45, 46], we will focus here on tyrosine kinases, of which over 40 vertebrate forms are now known [41].

A relatively small proportion of phosphorylated residues on proteins involve tyrosine, and the amount is kept in close check by the opposing effects of tyrosine kinases and tyrosine phosphatases. When cellular tyrosine phosphorylation is increased by the appropriate stimulus (for example, the docking of cognate ligand to a cell membrane receptor) this initiates a cascade of downstream biochemical events, many of which involve sequential protein phosphorylation and protein–protein interactions (see later and [47]). The biological outcome can take the form of a variety of cellular responses, the nature of which can vary with both the stimulus and the cell type.

The tyrosine kinases can be divided into two categories: those associated with the cytosolic domains of cell membrane receptors on the one hand, and those that are non-receptor tyrosine kinases on the other. Examples of the former include the so-called class 1 receptor tyrosine kinases including the epidermal growth factor (EGF) and c-*erb*B2 (HER 2 or neu) receptors [47] which are often over-expressed in human tumours, including breast and ovarian cancers; moreover, this increased expression, sometimes associated with gene amplifications, can be an important prognostic factor in these settings [48, 49], consistent with (but not proving) a causal relationship. Other receptor tyrosine kinases which are over-expressed in human malignancy include those for platelet-derived growth factor (PDGF) and the fibroblast growth factors (FGFs) [47]. In addition to their mitogenic action, the FGFs, together with vascular endothelial growth factor (VEGF), are also implicated in tumour angiogenesis [50]. Examples of non-receptor tyrosine kinases (which nevertheless do associate with the cell membrane) include c-src which shows increased activity in colon, breast and other tumours [51, 52]. In patients with chronic myelogenous leukaemia, the translocation involving chromosomes 9 and 22 results in the fusion of the gene for another non-receptor tyrosine kinase, abl, with another gene known as *bcr* [53]. This creates a novel chimeric protein with distinctive enzymatic and transforming properties. Because of their association with human cancer, several of the enzymes mentioned in this paragraph are among those which have been most extensively studied in the development of tyrosine kinase inhibitors.

In addition to the association of increased expression and activity of protein tyrosine kinases with various human tumours, proof of principle that these enzymes represent viable targets for cancer drug discovery is provided by the inhibitory effects of antibodies and antisense RNA together with molecular genetic experiments. Thus when recombinant DNA techniques are used to inactivate the kinase activity of either growth factor receptor-associated tyrosine kinases or src-like enzymes, the proliferative/transforming/tumorigenic capability is simultaneously eliminated [54, 55]. It seems reasonable to conclude from this that inhibition of the kinase by a drug would have the same effect, although the extent to which the transphosphorylation activity must be depressed in cells is unclear.

The participation of tyrosine kinases in numerous signalling pathways in normal cells clearly raises concerns about the potential toxicity of such therapies. However, selectivity might be envisaged to arise by a variety of means. Tumour cells might be unusually dependent upon tyrosine phosphorylation for their proliferation. A more convincing and common view is that tyrosine kinase inhibitors could be developed with a high degree of molecular specificity for one or a few individual tyrosine kinases upon which a particular tumour type may rely heavily for proliferation/transformation signals.

A high degree of anti-tumour selectivity could also arise from the known degeneracy of signalling in normal cells, i.e. the existence of alternative or parallel transduction pathways would would protect the non-malignant tissue from the signalling blockade. In support of this concept, gene knockout experiments have shown that mice deprived of certain individual non-receptor tyrosine kinases exhibit either no abnormality (*yes* and *fyn* genes) or osteoporosis due to defective osteoclast function (*src* gene), whereas double mutants for the *src*/*yes* or *src*/*fyn* genes

died shortly after birth [56]. This indicates that various *src* gene family members can substitute for each other with respect to tyrosine kinase signalling in normal cells.

The considerable progress which has been made in the development of protein tyrosine kinase inhibitors has resulted from both high throughput screening and rational design approaches, and has involved natural product leads as well as synthetic medicinal chemistry applications [37–43]. Knowledge of the predicted sequence of the 250–300 amino acid catalytic domain [57] coupled with the recent X-ray crystal structures of the serine-threonine kinases cAMP-dependent protein kinase (PKA, murine and porcine) [58–60] and cyclin-dependent kinase 2 (CDK2, human) [61] allows homology modelling of protein tyrosine kinases and provides insights into the reaction mechanism [62].

In the EGF receptor tyrosine kinase, a conserved arginine in the catalytic loop appears to interact with the γ phosphate of ATP; in addition, a second loop generates a binding surface that positions the phenolic hydroxyl of the tyrosine for phosphotransfer while a positively charged surface contributes to substrate recognition [62]. This greatly facilitates the computer-aided molecular design of inhibitors. While the extent to which it is desirable or possible to achieve a 'clean' inhibitor of a single tyrosine kinase as opposed to a 'dirtier' inhibitor of several kinase enzymes remains to be determined, it is clear that a surprising degree of molecular specificity can be obtained.

Detailed reviews of the wide variety of chemical structures identified as active against tyrosine kinase are provided in [37–43]; here we will mention some key examples. Among the natural products, the flavonoid compounds like genistein and quercetin were important in demonstrating that inhibitors competing with ATP could nevertheless exhibit selectivity, e.g. for tyrosine kinases versus serine/threonine kinases [63]. However, the anti-proliferative effects of these agents are likely to be due, at least in part, to their inhibition of topoisomerases [64]. Other flavonoids are potent and selective inhibitors of the lck tyrosine kinase [65] or block tyrosine phosphorylation the p34cdc2-type cell cycle kinase [66]. Interestingly, the structurally-related plant product piceatannol can be competitive with peptide substrate rather than APT [67].

Demonstration of tyrosine kinase inhibition and anti-tumour activity of the sytrene-containing natural product erbstatin [68] led to the impressive benzylidene malonitrile analogues known as tyrphostins [38]. Of particular interest, is the reported ability to discriminate not only between tyrosine versus serine/threonine kinases, but also between individual kinases, e.g. EGF versus insulin receptor kinases [69] and even the closely-related EGF versus erbB2/neu receptor enzymes [70]. Tyrphostins are competitive with protein substrate and may be competitive or non-competitive with ATP. Structure–activity relationships demonstrated the importance of planarity and hydroxylation in the molecule. A further significant development was the demonstration of *in vitro* and *in vivo* anti-tumour activity with potentially more stable second generation tyrphostin derivatives lacking hydroxyl substituents [71]. Additional tyrosine mimics with good inhibitory activity include hydroxycinnamides [72] and nitrostyrenes (see below).

Another interesting approach has involved the synthesis of potential bisubstrate or transition state analogues featuring a tyrosine mimic in combination with an ATP cofactor mimic or spacer as, for example, with the sulphonylbenzoyl-nitrostyrenes which were potent and selective inhibitors of the EGF receptor kinase [73]. Other potent and selective tyrosine kinase inhibitors were provided by the thiazolidine-diones [74] and aminoalkylacrylophenones [75]. Surprisingly, whereas the natural product staurosporine was unselective across tyrosine and serine/threonine kinases and certain aglycone analogues highly specific for protein kinase C, a novel series of related dianilinophthalimides have proved recently to be highly active against the EGF receptor tyrosine kinase but inactive on protein kinase C [76, 77]. Such agents not only exhibit potent anti-proliferative activity *in vitro*, but also display promising anti-tumour efficacy *in vivo* at well-tolerated doses [77].

Another series of tyrosine kinase inhibitors which show significant anti-tumour activity *in vivo* are the benzoquinoid ansaymycin antibiotics typified by herbimycin A and geldanamycin [78]. These agents inhibit the src tyrosine kinase among others, and this has been correlated with reversion of *src* transformation [78]. Abolition of inhibitory activity by thiol reagents suggest a mechanism involving suphydryls in the kinase [79]. Other recent work suggests that herbimycin A inhibits the association p60 v-src with the cytoskeleton and with an important downstream effector, phosphatidylinositol 3'kinase (P13 kinase) [80]. A potential problem with such quinone-containing agents is the complications introduced by metabolic redox cycling leading to oxidative stress and DNA damage.

The last few months have seen a detailed elucidation of the molecular basis of the signal transduction events downstream of growth factor receptor tyrosine kinases and the src enzyme, and the nature of crosstalk with other pathways (see [81] and Figure 1). These have revealed further potential targets for pharmacological intervention. Of particular interest are the

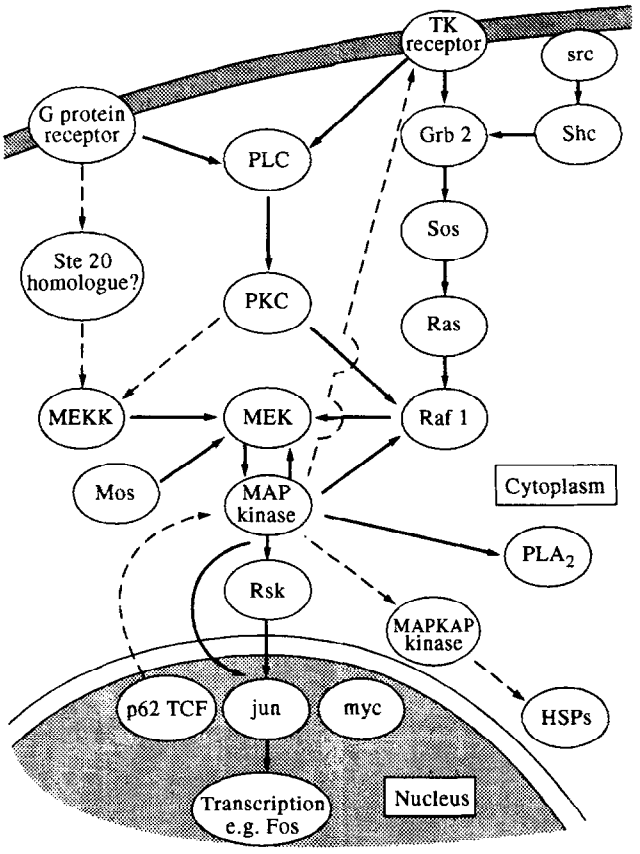

Figure 1. Convergent MAP kinase activation pathways

protein–protein interactions involving src homology domains (SH2 and SH3) [82]. These are responsible for the binding of adaptor (e.g. grb2) and effector molecules (e.g. PI3 kinase and phospholipase Cγ) to phosphorylated tyrosines in specific peptide sequences, and these interactions can be blocked by phosphorylated peptide antagonists [83, 84]. Ras acts as a crucial molecular switch downstream of tyrosine kinases, leading to activation of the raf-MAP kinase cascade and the phosphorylation of nuclear transcription factors (Figure 1). Ras mutations which are very common in human cancers cause the ras switch to be continually 'on' [85]. An exciting development, therefore, has been the demonstration of selective inhibition of the proliferation/transformation of cells containing mutant oncogenic *ras* by peptide mimetics that inhibit the farnesylation reaction essential for membrane docking and signal transduction [86, 87]. The next phase will be to achieve *in vivo* anti-tumour activity using this approach.

The development of agents capable of selectively inhibiting tyrosine kinase, ras and other key points on signalling cascade shown in Figure 1 should allow us to identify which targets are most appropriate to generate selective anti-tumour efficacy. It is possible that this may vary according to the oncogene/signal transduction profile of particular tumours.

APOPTOSIS AND CANCER THERAPY

(Discussion leader: Wilfried Bursch)

The involvement of programmed cell death by apoptosis in the regulation of tissue growth continues to attract increasing attention. Apoptosis is considered as a cellular "suicide" and the functional opposite to mitosis, both of which, in concert, determine maintenance, growth or involution of tissues. Apoptosis is controlled by a complex interaction between extrinsic and intrinsic signals (see below). Under pathophysiological conditions, such as acute tissue injury, apoptosis may also be induced by (slight) cell damage. Furthermore, precancerous cells appear to be selectively eliminated by apoptosis, and thus transmission of DNA damage to progeny cells may be prevented by this means. Apoptosis, therefore, seems to constitute one of the organism's important defence lines against cancer. Non-genotoxic carcinogens (tumour promoters) can block apoptosis and thereby accelerate the occurrence of frank neoplasia, as exemplified by phenobarbital in the liver. Likewise, inhibition of apoptosis in haemopoetic cell lines due to overexpression of the *bcl-2* gene appears to be involved in the pathogenesis of Burkitt's lymphoma. The significance of apoptosis in ontogeny, the regulation of cell number homeostatis in the adult, and also protection against disease has been extensively reviewed [88–93]; here the potential significance of apoptosis for tumour therapy will be addressed.

The occurrence of apoptosis in normal and tumour tissue may depend on the levels of organotropic hormones and growth factors such as oestrogens (mammary gland), testosterone (prostate), transforming growth factor β1 (TGF-β1; endometrium, liver) and glucocorticoids and interleukins (many haematological cells). The endogenous control of apoptosis in tissues provides targets for therapeutic intervention. In human lymphatic leukaemia, apoptosis may be induced by treatment with glucocorticoids as suggested by experiments on glucocorticord-induced death of normal and tranformed haematological cells. Recently, an antibody anti-APO 1 was found to be involved in recognition and selection of leukaemic cells for apoptosis [94, 95]. In hormone-dependent tumours, induction of apoptosis by chemical or surgical hormonal ablation appears, in addition to inhibition of cell proliferation, to be involved in tumour regression. Analogues of somatostatin and LH:RH cause massive apoptosis and regression of chemically-induced pancreatic cancer in hamsters. In the human mammary cancer cell line MCF7, *in vitro* tamoxifen and other anti-oestrogens induced both depression of DNA synthesis and increased cell death [92]. To what extent induction of cell death may contribute to the preventive effect of tamoxifen against mammary cancer development in patients at risk remains to be elucidated. Conceivably, this could result from anti-promotion by the antagonist via induction of apoptosis in cancer prestages. Recent findings suggest that TGF-β1 plays an important role within the regulatory network that controls the balance between replication and death in the endometrium and the liver [92]. Interestingly, some tumour cells were found to produce TGF-β1, but be resistant to its growth-inhibitory effects. Hypothetically, therefore, tumours could prevent growth or even induce death of their healthy neighbour cells, and at the same time escape from growth restrictions active in normal tissues.

Whether or not a cell enters apoptosis in response to extrinsic stimuli also depends on the state of differentiation. Evidence for cell cycle-related factors that can modulate apoptosis is provided by the following observations. In mouse intestine, after radiation or certain chemotherapeutic agents, crypt cells of the proliferating cell pool preferentially undergo apoptosis, whereas most cells from the non-proliferating pool survive. Furthermore, Chinese hamster ovary cells undergo apoptosis in response to chemotherapeutic agents after G_2M arrest [92, 96]. The basis for this selectivity is not known (see below). Receptors may also become targets for therapeutic modulation of the apoptosis rate in tumours. Glucocorticoids induce apoptosis of lymphatic cells via steroid receptors, and in various haematological cell lines, apoptosis cannot be induced by glucocorticoids because of reduced receptor affinity or a lack of receptors. Cell surface target molecules, such as fas and apo-1, appear to be involved in antibody-mediated apoptosis [94, 95]. Furthermore, downregulation of hormone receptors might be involved in the selection of hormone-independent tumour cells which limits the effectiveness of endocrine therapy for tumours of endocrine organs. Nevertheless, hormones and growth factors interact with specific receptors in their target cells, and this property may become a useful approach to kill tumour cells via selective pathways.

Detailed work in the nematode has identified a large number of genes involved in the regulation of programmed cell death [97]. In mammalian fibroblasts, overexpression of the *myc* proto-oncogene product induces apoptosis when strain or certain mitogenic factors, such as IGF, are withdrawn [98]. Furthermore, over-expression of the *bcl2* oncogene product in this model confers resistance to the apoptotic pathway [98, 99]. This provides a fascinating new mechanism of oncogene collaboration operating, for example, in lymphomas.

DNA-targeted drugs and radiation may also activate apoptosis. As indicated from studies with epithelial cells, DNA-damaging compounds, such as arabinosylcytosine, nitrogen mustard, cisplatin and etoposide and many others, can induce programmed cell death [93]. In the case of lymphatic cells, DNA fragmentation readily occurs after cisplatin or etoposide, but it is not clear whether this reflects activation of the complete apoptotic program [96]. Until recently, there was little information on how cell injury could trigger the programme of events eventually resulting in apoptosis. DNA damage may induce a recognition process and DNA repair and poly(ADP-ribose) synthesis, leading to nucleotide depletion and cell death. Inhi-

bition of poly(ADP-ribose) synthesis and DNA repair was found to increase the rate of apoptosis in glucocorticoid-treated mouse lymphoma cells. Interestingly, *bcl2* overexpression inhibits DNA damage-induced apoptosis as well as programmed cell death caused by growth factor withdrawal [100, 101]. Moreover, there has recently been an explosion of information which demonstrates a role for the wild type p53 suppression gene protein in DNA-damage-induced apoptosis, although it is not involved in apoptotic programmes initiated by, for example, glucocorticoid, calcium or aging in the thymocyte [102–104]. Moreover, the role of p53 may be cell-type dependent. Overexpression of the wild type p53 tumour suppressor gene has been found to induce apoptosis of both myeloid leukaemia and epithelial tumour cells [34, 105, 106], but growth inhibition without apoptosis is seen in other cell systems [107]. The lack of direct involvement of p53 in normal physiological apoptosis is illustrated by the fact that p53 knock-out mice are developmentally normal [108]. However, p53 levels are elevated after DNA damage, leading to a G1 block in the cell cycle, an effect which is lacking in the absence of wild type protein [109, 110]. Thus, the current model visualises p53 as a component of a DNA damage-limitation pathway, in which the cell cycle block allows either repair of damage or an apoptotic suicide. This protects the cell against genomic instability, including gene amplification [110]. The molecular mechanism is thought to involve p53 acting as a sequence-specific transcription factor [111], and it is now important to identify the genes downstream of p53. There is evidence for the GADD 45 gene as a candidate downstream target for activation by wild type p53, while an ataxia telangiectasia gene product(s) lies upstream of p53 [112]. Very recent results show that WAP1/CIF1 gene expression is induced by p53, the product of which binds to cyclin complexes and inhibits cyclin-dependent kinases [113, 114]. This suggests a highly plausible model in which a major effect of p53 is to block cell cycle progression by preventing phosphorylation of critical cyclin-dependent kinase substrates, including Rb. The importance of this for cancer therapy is that these apoptotic mechanisms are blocked in the presence of p53 mutations, which occur in more than half of all human cancers.

Other apoptosis-controlling genes are also likely to be identified shortly, for example, there is evidence that *fos* may be involved [115]. These "master genes", such as p53 and *bcl*-2, will provide a major challenge for the development of gene-specific drugs.

Signal transduction after exposure to cytotoxic drugs may include numerous biochemical responses, including increases in cytosolic Ca^{2+} levels, inositol trisphosphate synthesis and activation of protein kinase C [93]. Whether a given cell eventually enters the apoptotic or necrotic pathway appears to be determined by dose and type of damage, in addition to the genetic make-up and signal transduction pathways operating in the particular cell type. It is probable that a critical dose of injury may exist, beyond which necrosis occurs in a given cell. However, our knowledge about the metabolic changes induced by cytotoxic drugs which lead to apoptosis is scarce. Elucidation of the underlying mechanism may pave the way for the development of more selective and potent drugs or other therapeutic strategies.

The morphological and functional features of apoptosis are well characterised. However, as outlined above, relatively little is known about the biochemical events in the apoptotic process, resulting in a paucity of specific markers. Therefore, the classification of cell death as apoptosis is still difficult, and this may impede approaches in drug development using the occurrence of apoptosis as an endpoint. Apoptosis was defined originally on the basis of morphological and functional criteria [88]. Today the morphological features still provide the most reliable markers of apoptosis, i.e. condensation of chromatin at the nuclear membrane as detected by electron and light microscopy and the presence of intact organelles in dead cells or cell fragments. Recent findings suggest that immunostaining for TGF-β1 precursor, which seems to be present in apoptotic but not in necrotic hepatocytes, may become a useful marker of apoptosis, and even of pre-apoptotic cells [92].

The condensation of chromatin has been associated with the activation of a non-lysosomal endonuclease, which cuts chromatin into oligonucleosomes. The resulting chromatin fragmentation yields a characteristic "ladder" pattern after gel electrophoresis, and is frequently used to detect apoptosis, so far mainly in thymocytes and lymphocytes. However, the general occurrence of this pattern during apoptosis in different cell types has not been established. Morphologically-proven apoptosis in hepatocytes and other epithelial cells can occur without classical DNA fragmentation into oligonucleosomes [92]. Furthermore, endonuclease is constitutively present in intact, non-apoptotic nuclei, and may also be activated by mechanisms *not* related to apoptosis. Therefore, endonuclease activation should not be considered as a *general* marker of apoptosis.

The discrimination between apoptosis and necrosis may also be rendered difficult by incomplete expression of the genes involved in apoptosis which may result in a necrotic appearance of dead cells. Of practical importance, because of the widely used cell culture systems used to study apoptosis, is the possible occurrence of transitions between both processes: a cell may begin on a pathway to apoptotic death, but then collapse completely and end in necrosis ("secondary necrosis"); this readily occurs in cell cultures where apoptotic cells are not phagocytosed *in situ*.

Unfortunately, no biochemical or molecular alteration is currently known that could serve as a general marker of apoptosis in different cell types. The various morphological criteria are still the most appropriate for identification and quantification of cell death by apoptosis, and functional and biochemical characteristics may serve as supportive evidence.

It is clear that there is exciting potential to understand the diverse aspects of cell death and, specifically, the role of apoptosis in cancer therapy. This should provide important new opportunities, both to modulate responses to existing treatments and to devise novel therapies for new apoptotic targets.

ENGINEERING ANTIBODIES FOR TARGETED CANCER THERAPY

(Discussion leader: Robert E. Hawkins)

The clinical use of antibodies for targeted cancer therapy has achieved some successes, but has also revealed several problems. The main obstacles concern the use of rodent antibodies, poor tumour penetration by macromolecules and the lack of entirely specific tumour antigens. Improvements in understanding the process of oncogenesis is revealing some truly tumour-specific antigens (for review see [116]), but methods are also being developed to optimally utilise antigens which, although present on normal tissue, are selectively overexpressed on tumours. New techniques for making antibodies and novel forms of expression should lead to significant improvements in antibody-targeted therapy.

Initially the antibodies used were rodent-derived, and long-

term therapy was limited by their immunogenicity. This problem was solved by the process of 'humanisation'; initial testing of a fully-humanised antibody, CAMPATH-1H, in patients with non-Hodgkin's lymphoma gave encouraging responses [117]. Other antibodies with specificities suitable for treatment of epithelial malignancies have now been humanised. More recently, the use of bacteriophage to display antibody fragments allows the rapid isolation of human antibodies directly [118]. This method is based on the use of polymerase chain reaction to amplify repertoires of immunoglobin V-genes [119] and their subsequent cloning into bacteriophage vectors for surface display. The phage display antibody fragments on their surface, and contain the V-genes within them so they are, in some respects, very similar to a B-cell. From a large library of antibody fragments, those phages displaying the antibodies of interest can be selected simply by their binding to antigen. The numbers of phages thus selected can be amplified by re-infection of bacteria and then reselected, allowing progressive enrichment for the antibodies of interest. In addition to selecting for the specificity required, this system also allows the selection for antibodies of predefined characteristics, such as high affinity or slow "off-rate" [120]. For the production of therapeutic antibodies, there are thus a number of advantages. Certainly, human antibodies can be isolated directly. In addition, it is more rapid and efficient than cell culture-based methods so greater numbers of antibodies can be made. The availability of large numbers of antibodies will allow the use of cocktails consisting of antibodies which recognise a variety of different antigens. This reduces problems of antigenic heterogeneity and also the risk of tumour escape through selective loss of the antigen. The ability to select the binding characteristics should also improve the therapeutic effect. For example, in animal models, higher affinity radiolabelled antibodies have more potent anti-tumour activity and lead to improved survival [121]. In theory, slow off-rate antibodies should prolong retention of the antibody within the tumour, whilst the blood level decreases, leading to improved therapeutic ratios.

In parallel with these improvements, there have been advances in protein engineering which allow the production of novel forms of antibodies. One potential advantage is the production of small fragments in bacteria. Production in bacteria is convenient and cheap, and the use of small fragments improves tumour penetration [122]. Such expression systems also facilitate the production of fusion proteins consisting of antibodies with novel effector functions.

Experience of antibody therapy suggests that the ideal reagent should be small, have high affinity (and slow off-rate) and be non-immunogenic. Because of difficulty reaching all tumour cells and the generally limited amounts of antibody that target to the tumour, together with antigenic heterogeneity, it is desirable that the effector mechanism should allow amplification, have some bystander effects and be non-toxic in the unbound form. Currently, these criteria are best fulfilled by the use of natural effector mechanisms or antibody-directed enzyme prodrug therapy (ADEPT) [123]. To recruit natural effector cells, bispecific antibodies, which can redirect specific cell types, may be more effective than natural antibodies. For example, bispecific antibodies incorporating an antibody to CD3 have been used to redirect and activate T-cells to destroy a tumour [124]. With the use of appropriate human or humanised antibodies, such an approach should be non-toxic [125]. In the past, the development of bispecific antibodies has been difficult, but new methods [126] will facilitate such approaches and should allow more effective therapy. The use of bispecific antibodies to suitable combinations of antigens may also be useful as a means of increasing tumour specificity—frequently tumours express a combination of antigens, none of which are specific, but the combination is unique to tumour cells. An antibody molecule recognising such a combination of antigens would, therefore, have improved specificity.

The ADEPT approach [123] certainly has the advantage that the antibody molecule is non-toxic, the enzymatic step produces amplification, and the final cytotoxic molecule is small and easily diffusible, thus circumventing problems of antigenic heterogeneity and poor access of the antibody. The drawbacks have been that the conjugates were difficult to produce and that they were also immunogenic. The recent production of human antibody-(human) β-glucuronidase enzyme fusion proteins [127] shows that these problems can be overcome, and prodrugs consisting of glucuronides of nitrogen mustards of anthracyclines can be made. With such an approach, the effective delivery of high doses of drugs with much reduced toxicity should be possible. The therapeutic benefits appear attractive, but must await full clinical testing.

Other effector mechanisms, such as the use of toxins, drug conjugates or radiation, are also being evaluated. With radiation, it is difficult to get sufficient dose into the tumour without attendant normal tissue toxicity, and the logistic problems of widespread use are considerable. Powerful toxins, such as *Pseudomonas* exotoxin, conjugates can now be produced as antibody-fusion proteins, and these are also being humanised [128]. However, difficulties of access to cells in large deposits and of antigenic heterogeneity are likely to limit the use of such approaches.

The problems of tumour resistance to drugs or loss of antigens suggest that the best approach will be combined one. In this regard, we can envisage the use of ADEPT for bulk disease followed by prolonged utilisation of natural effector mechanisms to eradicate residual disease. The application of native antibodies or bispecific antibodies, perhaps combined with cytokine therapy, immunisation with tumour-specific antigens or adoptive immunotherapy, may be very effective in stimulating efffective immune surveillance. Finally, gene therapy techniques for the treatment of cancer are making rapid progress [129, 130]. Antibodies may be used to target relevant genes or appropriate vectors employed to allow *in vivo* production of antibody-based molecules.

There is now a plethora of new techniques available which will allow us to test therapeutic modalities involving the use of antibodies for the targeted therapy of cancer. It is certain that all possible permutations cannot be tested, and the main need now is to apply our knowledge of the principles involved to design suitable therapeutic molecules, and then evaluate them in appropriate clinical settings.

CONCLUDING REMARKS

It is frustrating but inevitable that there is necessarily a delay between any breakthrough in basic science and the exploitation of that knowledge for medical treatment. The path from a newly cloned cancer gene to a novel cancer drug is a long and tortuous one. Nevertheless, the opportunities provided by advances in molecular biology—both practical and intellectual—now provide considerable grounds for optimism that we should be able, in the next 5 to 10 years, to deliver innovative new therapeutic agents which display major advantages over conventional treatments.

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European School of Oncology Task Force Papers: Gene Therapy—a Future in Cancer Management?

Antisense Therapy for Malignant Disease

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INTRODUCTION

THE POTENTIAL to 'switch off' the genes that are implicated in oncogenesis is clearly very attractive. This goal has been the incentive for many academic and commercial research groups to apply antisense technology to various malignant diseases. Over a period of at least 15 years of antisense experimentation, many successes have been claimed, although very few clinical applications have yet been developed. This, to some extent, reflects the many difficulties that are associated with the investigation of antisense oligomers in biological systems as well as the synthesis problems that have, until recently, limited the quantity of oligomer than can be synthesised. Advances in biotechnology have now enabled the synthesis of clinically useful quantities of various oligomers, and fuelled the hope that these molecules may have a future, not only in the investigation of gene expression, but also as therapeutic agents in malignancy, viral infection, vascular disease and inflammatory processes. However, the pharmacodynamics, cellular uptake, intracellular