scientists from politically and geographically diverse nations. If broadened in scope, this project has the opportunity to clarify and elucidate additional determinants of leukaemia.

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Anticancer Drug Screening and Discovery in the 1990s: A European Perspective

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INTRODUCTION: THE NEED FOR CHANGE

ALTHOUGH ANTICANCER drug discovery efforts over the past 4 decades have resulted in the curability of some forms of disseminated malignancies, such as acute childhood leukaemias, Hodgkin's disease and germ cell tumours, the overall results of cytotoxic therapy in the most frequent solid tumours of the adult, particularly in the advanced metastatic stage, have remained far from satisfactory [1]. The lack of success in identifying new active agents against those common cancers with the most significant impact on the mortality rate generated a great deal of pessimism amongst drug screeners and clinicians in the 1980s [2–4]. Contrast this with the remarks of Nobel prizewinner and successful drug hunter Sir James Black on the opening of the Cancer Research Campaign Beatson Laboratories, Glasgow in April 1991: "There has never been a time like this for cancer research. There is a feeling of irrepressible optimism among scientists." These comments should apply no less to the development of new anticancer drugs than they do the extraordinary advances in our understanding of the molecular basis of changes which take place when a normal cell becomes a cancer cell. We shall argue here that the understandable pessimism referred to earlier is in fact entirely inappropriate [5]. Several new agents are showing impressive early clinical results despite acting on conventional drug targets, and perhaps more importantly, a host of more mechanistically innovative molecules are emerging which will exploit the various products of cancer genes.

Up to now, the identification of active agents against human cancers has relied essentially upon the following strategies: (1) random screening of natural and synthetic products in experimental tumours; (2) rational development of new compounds on the basis of observed biochemical properties of tumour vs. normal cells; (3) synthesis of analogues of known agents, usually seeking to eliminate an undesirable feature such as an unwanted side-effect or to incorporate an advantageous property such as broader spectrum of action or maintenance of activity in resistant tumours; and (4) serendipitous observation [4, 6, 7]. In practice, a combination of these approaches is often used.

In this review, we will take a critical look at the various approaches to new drug discovery in the 1990s and beyond. Unless we are extremely lucky, the new anticancer agents we

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take into Phase I trials will only be as good as the tumour models we use to select them. Because of this, emphasis will be on the various established and emerging preclinical test systems. Random screening (used here in the sense that no specific molecular target is attacked) will be looked at especially carefully and its role *vis-à-vis* mechanism-based drug development will be addressed in particular. Examples of opportunities to exploit our knowledge of molecular alterations in the structure and expression of cancer genes will be described. Ideas on strategies for European drug development will be discussed in the light of the substantial *in vitro* human tumour screening initiative established by the United States National Cancer Institute (NCI) as well as the current efforts from the pharmaceutical industry.

In order to see the development of our perspective on anticancer drug hunting over the last 15–20 years and of our forward view, it is appropriate to begin with a look at the changes which have taken place in the NCI random screening programme, not least because it has been so influential and, more recently, controversial within the field of oncolytic drug discovery.

EARLY NCI SCREENS

Since the 1950s, the bulk of preclinical testing of new compounds has been conducted through the screening programme of the United States NCI [8–11]. Compounds were selected for screening based on an examination of the world's literature and via submission from investigators. The strategy applied for drug discovery in the NCI at that time was based on the initial *in vivo* screening of new compounds in highly sensitive, rapidly-growing murine leukaemias, such as the L1210 and latterly the P388 models. These were referred to as prescreens. They were designed to weed out inactive agents. Active compounds in the prescreen were then evaluated further in a panel of *in vivo* murine solid tumours and more recently also, a selected group of human tumour xenografts growing in immune-deprived mice [10, 11].

In 1975, compounds active in the P388 prescreen were evaluated subsequently in four mouse solid tumours (B16 melanoma, CD $8F_1$ mammary carcinoma, colon carcinoma 38 and the Lewis lung carcinoma), three human solid tumour xenografts (CX-1 colon carcinoma, LX-1 lung carcinoma and MX-1 breast carcinoma) and also the L1210 mouse leukaemia [10–12]. A bypass channel was established which allowed compounds to proceed directly for testing across the entire panel because of demonstrated antitumour activity in alternative models or because of special biochemical or biological characteristics. However, in the vast majority of cases it was necessary to demonstrate activity in the leukaemia prescreen.

One of the main conceptual problems with the above-mentioned approach to random drug screening, with its emphasis on the leukaemia prescreens, is that experimental tumours tend to exhibit high variability in their sensitivity to different cytotoxic agents and that they are in most instances far from representative of the spectrum of solid tumours occurring in man. It would be unrealistic to expect that a single tumour line would have the power to accurately reflect the range of responses seen with these different solid tumours in the clinic [11]. Also, it seems particularly unlikely that a murine leukaemia would be predictive for human solid tumour activity [2, 4], yet almost all drugs were funnelled through this test.

With the benefit of hindsight, the establishment of a panel of experimental tumours, comprising various cell lines of the major solid tumour histotypes and exhibiting distinct biological behaviours and sensitivities to cytotoxic agents (see later), would have been much more appropriate as an initial screen for new compounds. Of course, such a panel would have had serious limitations in terms of costs and capacity to evaluate a large number of compounds [13].

There were also additional problems associated with the leukaemia prescreen and these concerned further intrinsic practical differences between the model and the clinical situation. For the testing of new compounds, tumour cells were inoculated intraperitoneally and the experimental agent was also administered through the same route. Obviously, this approach does not reflect the actual manner in which cytotoxic therapy is generally administered to patients. In the clinic, investigational agents are mostly given by injection into a peripheral vein. This demands that the agent crosses numerous physiological barriers to reach the tumour site. Peak levels and area under the curve will tend to be lower than when the drug is administered directly on to the tumour cells growing as an ascites. Furthermore, solid tumours in man tend to double much slower than experimental murine leukaemias and they are treated mostly at a later stage, when the tumour burden and the presence of mutant cells resistant to therapy is in general much higher.

It is important to emphasise, however, that the L1210 and P388 leukaemias were not only valuable in developing important principles such as the fractional cell kill concept and drug scheduling, the use of these models as a prescreen in the early days was also very effective in the sense of directing drug development towards the discovery of active agents against human leukaemias, lymphomas and also germ cell tumours which particularly afflict children and young adults. As examples we could cite vincristine and daunomycin, arabinosyl cytosine, 6-mercaptopurine and thioguanine in acute leukaemia; nitrogen mustard and vincristine in Hodgkin's disease; vincristine and doxorubicin in non-Hodgkin lymphomas; and cisplatin and etoposide in germ cell tumours. Had such rapidly growing human malignancies been the main target of the programme it would be hailed as a great success. Although certain agents, such as cisplatin in particular, that have been developed with the aid of the old screens do show useful activity in certain solid tumours such as ovarian and small cell lung cancer, drug resistance invariably develops (see later). In retrospect, it would surely be unrealistic to expect a high rate of success in the discovery of drugs with efficacy in advanced human solid tumours of the lung, breast and bowel by the above approach. Indeed, during the past 4 decades over 600 000 new compounds were screened for antitumour activity in various preclinical models, but only a few of these are currently used by clinicians treating patients with solid tumours and in those cases, curability is the exception, not the rule [14]. In this respect, the most important drugs arising directly from the NCI screen have been described as "modestly useful" and have "limited roles in the effort to treat and cure advanced malignancies"—chloroethylnitrosoureas, mitoxantrone, hydroxyurea and deoxycoformycin being examples cited [3].

A further confounding factor in earlier screening approaches is the long time needed for a candidate compound to have its efficacy assessed in the clinic. Even for highly effective agents such as cisplatin or etoposide which were identified as active in animal models, several years of preclinical and early clinical development were required before their value in the therapeutic armamentarium could be clearly established. Thus, the pessimism that dominated anticancer drug development over the last decade was exacerbated by the series of negative trials with

compounds in which basic scientists and clinicians had invested substantial amounts of time, funds and patient numbers. Clinicians, pharmacologists and chemists became frustrated by the combination of our inability to identify new agents with improved clinical activity and the time and resources required to generate negative data.

THE NEW NCI SCREEN

The frustration of the scientific community led the NCI to reconsider its drug screening policies in 1985. The decision was made to abandon the *in vivo* screen and to establish an *in vitro* screening panel comprising a large number of human cell lines which were hopefully more representative of the various common solid malignancies [13, 15, 16]. The concept behind this strategy was that a disease-oriented human tumour panel might have a higher chance of identifying innovative compounds showing preferential cytotoxicity against a specific human tumour type and might also uncover new molecular targets located in such tumours.

It is important to stress the point that the new NCI screen places great emphasis on this selectivity of action against particular human tumour types: broad spectrum activity, previously considered a desirable feature, will not be sought. In addition, preferential activity against a number of human solid tumours vs. human and rodent leukaemias might also be considered attractive. Focal to the success of this approach is the assumption or hope that the biochemical differences between, say, human colon, lung and breast tumours will also be reflected in the representative cell lines. Compounds which are identified as interesting by the screen might then be expected to exploit such differences. Indeed, implicit in the new development strategy is a vision that molecules might be identified which would kill tumour cells by hitting a mechanistically unique molecular target—perhaps one as yet unknown to the biochemists. An especially rich source of such agents might be the myriad of structurally complex and biologically potent natural products selected by eons of evolutionary pressure [17, 18].

The concept behind the new screen is beautifully simple and its originators are to be congratulated for taking a brave step based on logical argument in the face of considerable criticism. However, the logistic difficulties underlying its establishment and subsequent operation are immense. To give an idea of the scale of the effort, it is expected that duplicate tests will be performed on 10 000 compounds per year on each of 120 cell lines. The initial panel consists of a total of 60 human tumour cell lines covering lung, colon, melanoma, renal, ovarian, brain and leukaemia histologies [16]. This will be expanded to encompass 12 different tumour types with a minimum of 10 lines in each.

In order to enable the evaluation of a high number of compounds in such a large *in vitro* tumour cell panel, rapid, high throughput and semi-automated colorimetric assays have been utilised. This initially involved using the MTT and XTT tetrazolium dye reduction reactions to measure viable cell number, but these are now replaced by the more manageable sulphurhodamine blue stain for cell mass [19]. Sophisticated computer programs have also been required to permit an efficient and intelligent analysis of the huge volume of data generated by the screen [20]. Notably, the data base compiled so far appears to be capable of identifying cytotoxicity patterns which are shared by structurally distinct molecules, having a common intracellular target. As an illustration, the entry of a specific tubulin-interacting agent such as vincristine or taxol into the *in vitro* screen data base allows the identification of other compounds such as rhizoxin having tubulin as the primary intracellular target. Similarly, by comparing the cytotoxicity profile of a topoisomerase II inhibitor such as doxorubicin against the data base, other topoisomerase II inhibitors such as mitoxantrone or etoposide are identified [20]. Thus, it does seem likely that the data analysis would detect patterns of mechanistically related, therapeutic selectivity that it was established to identify. A key question is whether any truly novel targets will be discovered by this means.

As would be expected for any radical departure from the existing dogma, the new screen has met with considerable criticism [4, 6, 7]. Some understandable frustration has developed over the time taken for the new system to become operational, and with the resulting build up of new chemical entities waiting to be screened. However, a highly sophisticated procedure is now in place for the random screening of chemicals targeted to the biochemistry of human tumour cells. Thus it can be expected that a significant number of compounds of interest will be identified by the new *in vitro* screening system. In fact, a large number of compounds have already been considered to be of interest for further testing ([16] and M. Grever, personal communication). For example, 9-methoxy-2-methylelliptinium iodide was much more active in several solid tumour lines, including brain and non-small cell lung cancers, than it was in the leukaemias, including the P388 and L1210. Interestingly, a similar profile was seen for bleomycin, with the important exception that the ellipticine compound was very active in the brain tumour lines. A possible problem is that the high throughput primary screen may generate substantially more "actives" than can be coped with readily by a secondary *in vivo* screen. Concerns are also circulating that the human tumour panel screen would not have predicted the known patterns of clinical activity of certain established agents [21] nor those of certain newly emerging agents, such as anthrapyrazoles in breast cancer [22] and temozolomide in brain tumours (see later). However, it would seem more appropriate to assess the predictive utility of the screen with respect to such novel compounds when we have a more complete picture of their clinical profile. Moreover, although the concerns about the performance with established agents should not be ignored, the important issue is how well the screen predicts with the new agents that it uncovers.

Nonetheless, we are still left with several important limitations. The observation of cytotoxic effects *in vitro* does not mean necessarily that these effects will be translated into efficacy, considering the pharmacokinetic and normal tissue toxicity problems that might be encountered *in vivo*. For this reason, compounds with promising *in vitro* cytotoxicity profiles would move on to a further round of evaluation against an appropriate selection of the human tumour panel growing as xenografts in nude mice [16]. Agents lacking *in vivo* activity would be discarded or more likely sent back to the chemistry laboratory for analogue development. Rather than deploying a rigidly predefined series of tests, it is anticipated that the *in vivo* drug evaluation protocols will be tailored to the individual agents concerned and to the nature of the particular tumour models involved [16]. This will require integrated mechanistic, pharmacological and toxicological investigations. In addition to subcutaneous xenograft testing, orthotropic models may also be used (e.g. intrabronchial implants for lung tumours). Microencapsulated tumour models have been evaluated but found to be unsuitable for routine use [16].

It is also clear that the *in vitro* human tumour screen would

fail to detect agents that need metabolic activation by host tissues, those that act through the immune system, and those that interact with other non-tumour elements, such as the stroma. With respect to metabolic activation, the counter-argument would be that the desirable specific bioactivation of a drug in tumour tissue certainly would be identified in the new system, and that we can afford to miss a proportion of potentially active compounds that require metabolism by the liver. Examples of agents which would be missed include cyclophosphamide, and perhaps morpholinyl anthracyclines [23], but an agent like 4-ipomeanol which is metabolically activated in particular lung cell lines [24] would be picked up. Similarly, we would expect that new bioreductive agents such as EO9 which are activated by tumour DT-diaphorase would also be selected [25, 26] although in some cases hypoxic conditions would be required to see a full effect. Where a particular host metabolic pathway is of special interest a different biological evaluation strategy would be developed. The same would be true where molecules interfering with the immune system or tumour–stromal interactions are sought. For example, flavone acetic acid appears to exert its effects on solid tumours in mice through a combination of vascular shutdown plus immune effects, both of which may involve tumour necrosis factor release [27]. These are important, but special and separate issues in drug development, and in our view do not detract from what is a fundamentally simple and sound approach to random screening for cancer drugs.

Because of the sheer scale of the new NCI screen, the radical change in direction, and the inevitable controversy that has surrounded the whole operation, it would be quite easy to assume that it represents a single unified approach to new drug discovery in the United States. Of course, this would be a mistake. In addition to running the new "enlightened" screen, the NCI also promotes separate mechanism-based rational drug development projects through normal grant procedures and special requests for applications. Much of this additional work is mechanism-based (see later). Compounds which exhibit interesting selectivity profiles in the human tumour panel will also be pursued mechanistically. Furthermore, other academic and pharmaceutical industry groups in the United States continue to utilise various alternative screening strategies [4, 28]. Some of these are mechanism-oriented and others are based on more conventional *in vitro* and *in vivo* models.

ALTERNATIVE USA SCREENING SYSTEMS

Although apparently unsuitable for large scale screening [21], the human tumour colony assay is able to provide valuable information [29–31]. Using fresh human tumour samples growing in soft agar and employing various end points at different times [21], this was in a sense a forerunner of the new *in vitro* human tumour panel. Its advantage for selected studies is that fresh human tumour material is used, thus minimising the possibility that the biochemical properties will diverge from those of the primary tumour. A disadvantage is the difficulty of establishing a well-defined historical data base to which novel agents can be compared. Under the auspices of the NCI, 1200 compounds were evaluated between 1979 and 1986, with a later emphasis on agents which were inactive in rodent screens [21, 32]. Of the two drugs from these studies selected for clinical trials (in non-small cell lung cancer) dihydrolenperone was inactive whereas chloroquinoxaline sulphonamide may be efficacious [21, 32–34]. It has been argued [21] that screening assays based on fresh human tumours have been discarded prematurely, particularly in the cases of non-clonogenic assays which can measure the killing of largely non-dividing cells. Others have considered the case for and against not proven [35], and were concerned about disparities between the assay predictions and clinical Phase II results with bisantrene and mitoxantrone. However, studies with fresh human specimens continue. A recent large study analysed the activity of suramin and found much higher potency in endometrial cancer, ovarian cancer and non-small cell lung cancer compared with melanoma and renal cancer [36] and it will be interesting to compare these predictions with forthcoming clinical data.

Another interesting system involves the use of an *in vitro* soft agar disc diffusion assay to identify agents with selective activity against solid mouse carcinomas (rather than leukaemias), followed by secondary screening against the murine solid tumours *in vivo* [37–41]. Flavone acetic acid and the ellipticine analogue detalliptinum were identified as having narrow, but significant therapeutic indices in this system [39–41]. It is noteworthy that a number of pharmaceutical companies have retained the use of *in vivo* screens. A carcinoma-selective panel similar to that just described picked up the interesting pyrazoloacridines [42, 43].Carcinoma-selective activity *vis-a-vis* lymphoid cell lines was initially sought in culture followed by confirmation of this property and broad spectrum activity in *in vivo* solid tumour models. Other desirable properties included equal activity against non-proliferating and proliferating cells, selective toxicity against hypoxic cells and activity in multidrug resistant lines. When currently used cancer drugs were graded for activity against the *in vivo* murine tumour and xenograft panel, only cyclophosphamide, melphalan, doxorubicin and cisplatin showed reasonable activity while the antileukaemic drugs ranked bottom. The related anthrapyrazoles showed very broad spectrum solid tumour activity, as well as P388 positivity, and were taken into successful clinical use based on this property [22].

The criterion of broad spectrum of activity in two murine solid tumours (primary screen) and two human tumour xenografts (the LX-1 lung and CX-1 colon carcinomas from the previous NCI panel as the secondary screen), coupled with subsequent identification of novelty of mechanism of action, has led to the development of the diarylsulphonylureas (e.g. sulfenur), the deoxycytidine analogue gemcitabine (which inhibits DNA polymerases, ribonucleotide reductase and cytidine 5′-triphophosphate synthetase) and lometrexol (a folate inhibitor of glycinamide ribonucleotide formyl transferase) [4]. Whereas gemcitabine shows interesting activity in human solid tumours, sulfenur appears disappointing.

On the subject of the move towards human tumours for drug testing, it has been argued that mouse tumour models should not be rejected prematurely, simply because they are murine rather than human [44]. Clearly, where these models have biochemical or biological relevance they should continue to play an important role. It would certainly be unwise to discard the more significant ones. There may well be situations in which particular mouse tumours express a certain biochemical or molecular characteristic which is especially suited to a particular purpose. Equally well it should not be assumed that because a tumour model is of human origin it will necessarily exhibit all the relevant features of the clinical disease. It would not be unreasonable to suggest that where important biological correlates are known or suspected, the tumour model selected should be the one giving the closest match to the clinical counterpart. Whether this is human or rodent is probably less relevant. On the other hand, where such biological correlates or targets are

unclear, as in random screening, it may be safer to select a human model—since this may on balance be more likely to reflect the biochemistry of the human tumour. However, we strongly suggest that greater attention be paid to the selection of tumour models according to their measured biology rather than their species of origin.

EUROPEAN SCREENING: EORTC AND CANCER RESEARCH CAMPAIGN

In Europe, preclinical drug screening has followed at least an equally diverse pathway. There has been no major central screening house equivalent to that of the NCI. However, a significant proportion of the preclinical testing is carried out under the auspices of the New Drug Development Coordinating Committee (NDDCC) of the European Organization for Research and Treatment of Cancer (EORTC), the EORTC New Drug Development Office (NDDO), the EORTC Screening and Pharmacology Group and the EORTC Preclinical Therapeutic Models Study Group (formerly EORTC Clonogenic Assay Study Group). The origins of EORTC drug screening go back to 1962 with the creation of EORTC itself. The NDDCC, which oversees all preclinical and early clinical activities carried out under the auspices of the EORTC, was set up in 1981 and the NDDO, which is responsible for the day to day running of EORTC drug development, was established in Amsterdam in 1984. Until relatively recently much of the drug screening was carried out at the Institut Jules Bordet in Brussels. It involved the use of transplantable mouse tumours with the P388 leukaemia as the primary screen and was very similar to that of the NCI [45]. A range of antitumour test systems are available via the Screening and Pharmacology Group [46]. Testing is frequently integrated with detailed mechanism of action and pharmacokinetic studies via the EORTC Pharmacology and Molecular Mechanisms (formerly the Pharmacokinetics and Metabolism) Group, who in turn liaise closely with the EORTC Early Clinical Trials Group in the conduct of Phase I and II studies.

Considerable expertise has been gained in Europe with the use of human tumour xenografts in nude mice [47–51]. Under the auspices of the NDDO, this has developed into the establishment of a drug screening laboratory at the University of Freiburg [50–51]. The primary screen focuses on activity in six human tumour xenografts which are analysed for their chemosensitivity *in vitro* using a modified colony forming assay. The six tumours are the LXFL 529 non-small cell lung (sensitive); the LXFS 538 or 605 small cell lung and MAXF 401 breast (intermediate); and the OUXF 899 ovarian, MEXF 514 melanoma and CXF 886 or CXF 1103 colorectal (resistant). The results are compared with concomitant evaluation of *in vitro* myelosuppressive effects using fresh human marrow in the CFU-GM assay. Compounds active in this primary evaluation (defined as treated/control $< 30\%$ at 100 μg/ml or less) are further tested in a total of 20 xenografts in the clonogenic assay. When activity is confirmed, studies are performed using two or more selected examples of the same xenograft lines as *in vivo* solid tumours in nude mice. If a remission or acceptable growth inhibition is observed *in vivo* the agent is selected for disease-oriented testing in usually 14 but potentially up to 40–60 xenografts of various histologies. So far, a total of 138 agents have been studied in the system and these include classical cytotoxics, antibiotics and other natural products, ether lipids, biological-response modifiers and rationally designed agents [45]. Of these, 33 (24%) went on to *in vivo* testing and 10/138 [7%] exhibited regression $\leq 75\%$ in nude mice. A total of 8 (6%) were selected for clinical trials by the EORTC. These include indoloquinone EO9, the tubulin drug rhizoxin, the topoisomerase 1 inhibitor topotecan, the unusual alkylator dabis maleate, and the antimetabolite brequinar sodium. These interesting agents are now in various stages of clinical evaluation. For example, clinical trials with rhizoxin [52] and the taxol-related tubulin binder taxotere [53] appear extremely promising.

The above strategy appears to be of value in predicting antitumour activity of standard and experimental agents in the clinic, as long as certain differences in drug metabolism between mice and man are taken into consideration [50]. It will now be important to compare the Phase II clinical activity of these agents with their performance in the xenograft test models in order to determine the success of the selection system.

Individual laboratories in the EORTC and other parts of Europe have focused on various different aspects of screening for anticancer activity. The importance of developing *in vivo* screening systems which focus on therapeutic index has been emphasized [54, 55]. This involves the use of screening models in which the response of disease-oriented *in vivo* tumours can be assessed in the context of toxicity to normal tissues. The relatively chemoresistant mouse colon (MAC) tumours are a good example [54, 55].Based on results in such models, the case has been made that a more thorough consideration of the relative toxicity to dose-limiting normal tissues may have argued against carrying out clinical trials with the nitrosourea-like chloroethylating agent mitozolomide and also with flavone acetic acid [54, 55]. Although mitozolomide did have an impressive spectrum of preclinical solid tumour activity [56] this was in fact very comparable to the clinically limited chloroethylnitrosoureas and the severe dose-limiting myelosuppression was therefore predictable. The limited clinical value of nitrosourea-like agents, in contrast to their impressive broad spectrum utility in rodent tumours, did much to focus the minds of the oncology community towards more innovative models. On the other hand, this work led on to the development of the analogue temozolomide [57] which is now showing clinical activity in brain and other tumours, probably via a distint mechanism [58]. It is unclear whether temozolomide actually functions by the planned pathway of spontaneously releasing the same methylating species which is produced on metabolic activation of dacarbazine. However, it would seem appropriate to incorporate into the evaluation of all potential methylating or chloroethylating agents the measurement of the O^6-alkyl guanine repair protein in tumour tissue [59].

The MAC tumours have also been valuable in the development of the bioreductive indoloquinone EO9 [60]. In the light of their general chemoresistance, the relative responsiveness of a high DT-diaphorase MAC tumour was important in providing *in vivo* data in support of the involvement of this enzyme in EO9 bioactivation [25, 26]. Flavone acetic acid was also quite active in some normally refractory preclinical solid tumour models, including the MAC tumours, and exhibits a novel mechanism of action involving effects on either the tumour vasculature or the host immune system [27], possibly by affecting the nitric oxide signalling system [61]. However, no responses were seen in the clinic for reasons that remain unclear. This may relate to differences in vasculature between human tumours and transplanted models, and it seems inappropriate to pursue analogues of flavone acetic acid until this issue is resolved.

As elsewhere, considerable attention has been paid to the establishment of model systems for drug resistant disease in Europe, in order to screen selectively for analogues which would

retain efficacy both in the resistant model and in clinically insensitive tumours. The use of resistant cell lines *in vitro* and *in vivo* to identify platinum compounds and anthracycline analogues with activity in drug-refractory human tumours has been particularly successful [62–66]. Anthracyclines modified by 9-alkyl or morpholinyl substitution retain activity in multidrug resistant lines by avoiding the P-glycoprotein [62, 63, 65]. They may also be active in "atypical" drug resistance, e.g. involving changes in topoisomerase or alternative drug efflux pumps [64]. As such they represent a useful alternative approach to resistance modulating agents. One of these anthracyclines, methoxymorpholinyl doxorubicin [65], is now in clinical trial in Europe. Results will be awaited with interest since the morpholinyl anthracyclines undergo metabolic activation [23] as well as exhibiting improved cell uptake characteristics. Orally effective platinum compounds with activity in cisplatin resistant disease are now entering clinical trial [66].

It is important to ask the question: 'What is the purpose of drug screening in the 1990s? Is it, as suggested by Grindey [4], to select a small number of new chemical entities for clinical trial with the expectation of a relatively high level of success? If that is indeed the case then our models must be very highly predictive of the clincial situation. Alternatively, is the goal to identify a larger number of interesting compounds for entry into early clinical trials, based on the premise that human tumours are the best test bed for new agents? The former philosophy places greater demands on the predictability of the model system. The latter simply asks for a selection system which will filter the input to the clinical trials to a manageable level, without rejecting potentially active agents. Although our models are continually undergoing refinement, or even complete overhaul, it seems unlikely that we are anywhere near having a perfect random screen. Indeed it is our view that such a perfect screen is an impossible ideal.

Recognising the deficiencies of the available preclinical screens to come up with new chemicals active in human solid tumours, the UK Cancer Research Campaign (CRC) Phase I/II Clinical Trials Committee was formed in 1980 with the express aim of expediting the number of new agents which might undergo clinical testing in man [67, 68]. In this system, there is no formal screening system whatsoever. Emphasis is on novelty of structure and a sound rationale, coupled with an ability to interest clinicians, and of course, a suitable drug supply position and an acceptable pharmaceutical and toxicity profile. Demonstration of antitumour activity in a model system is desirable but not essential if there is a sound reason why this would not be expected. Up to six new drugs may enter clinical trials each year. Particular successes have been obtained with the anti-endocrine aromatase inhibitors, hydroxyandrostenedione and pyridoglutethimide [69, 70].Temozolomide has been referred to earlier as showing very encouraging activity in gliomas [58], a property that would not have been predicted from laboratory studies. The anthrapyrazole DuP-941, also mentioned previously, has shown striking activity in breast cancer [22]. The success of the approach is therefore clear.

Like the CRC Committee, the EORTC NDDCC has also made a decision to increase its commitment to the identification and development of compounds with a novel mechanism of action and a distinct rationale. Indeed the CRC and EORTC drug development committees are working closer than ever before on these ideas. Further international harmonisation came with the signing of a joint agreement for collaboration in drug development between the EORTC, CRC and NCI in 1986 and the establishment of a steering committee of representatives from the three organisations. In addition to the drawing up of guidelines for formulation, toxicology and early clinical trials (which are outside the scope of this review), the agreement encourages sharing of compounds and resources in order to improve the efficiency of new drug development and to avoid duplication of effort or backlogging of priority agents. It is hoped that a similar close collaboration would be encouraged with any other European national drug development group, for example, the one now emerging in Germany.

There is a strong feeling in Europe, certainly in the EORTC and CRC new drug development committees, that random screening should receive a lower priority and rational drug design be given an even greater opportunity. In keeping with developments elsewhere, there is clear recognition that rigid protocols are positively harmful to creative drug development. Instead the development process should be geared to the individual requirements of the particular agent and, of course, to the biology of the target tumours. Although there is a movement away from random screening, there is a continuing requirement for some form of efficacy testing. On the basis of the above discussion it seems inappropriate for the EORTC, CRC or any other group to invest heavily in primary screening. The scale of the NCI effort is such that it is unlikely that a distinct contribution could be made. Primary screening efforts should be targeted to particular mechanism-based projects; for example, as in the identification of agents with activity in resistant tumours. Instead, given the long experience and considerable expertise with the human tumour xenograft model in EORTC, it seems more appropriate to concentrate our screening efforts in this area. Indeed, a recent agreement has been reached which will involve the EORTC and CRC groups sharing the secondary evaluation of some of the agents identified as interesting by the NCI primary human tumour panel. This will be coordinated by the EORTC NDDO, NDDCC, the Screening and Pharmacology Group and the Pharmacology and Molecular Mechanisms Group. It is also important to mention that under the auspices of the tripartite agreement described above, a mechanism is now established whereby new chemical entities arising in Europe can gain rapid access to the new NCI human tumour screen via submission through the NDDO. This further reduces the requirement for a large-scale European random screen.

MECHANISM-BASED DRUG DEVELOPMENT

If we are to concentrate on mechanism-based concepts in Europe, what are the hot tips to focus on? It should almost go without saying (but we will say it anyway) that the emphasis should be on developing novel chemical structures which will exploit known biochemical and molecular differenes between normal and cancer cells. But what are these differences?

Obviously there can be no particular European policy on which targets are the most appropriate. Moreover, a detailed listing of these is impossible because of space constraints, and drug design will be the subject of a subsequent review (S. Neidle and P. Workman, in preparation; see also [28]). Instead, we will select a class of novel agents which are of special interest to the authors and for which there has been particular activity in Europe of late: these are the signal transduction inhibitors. Other areas of actual or potential interest will also be mentioned.

In the last few years there has been a truly staggering revolution in our understanding of the molecular changes involved in proliferation and malignant transformation. The functional roles of oncogenes and tumour suppressor genes are

becoming clearer. The mechanism by which the protein products of these genes transmit molecular signals in tumour cells are becoming understood. Dominantly active cancer genes encode protein products which are growth factors, growth factor receptors, molecules involved further downstream in signal cascades or transcription factors [71, 72]. Tumour suppressor genes are likely to have directly opposing functions (e.g. phosphatases vs. kinases, see later) [73].

There is a strong feeling that this area is now particularly ripe for pharmacological intervention [74], not least because it is a new and rational approach which is in tune with the current molecular zeitgeist. A meeting jointly sponsored by EORTC along with the British and American Associations for Cancer Research was instrumental in focusing interest in this area and bringing it to the attention of the wider oncology community [75, 76]. Progress has been rapid and a number of promising compounds are now in development. We still therefore focus on this aspect to illustrate the rational drug design approach.

Suramin complexes with growth factors such as basic fibroblast growth factor and platelet-derived growth factor and inhibits the binding of these ligands to their membrane receptors [77]. Binding of growth factors to the extracellular matrix may also be important. Although toxic (possibly via an unrelated mechanism), with careful monitoring of plasma levels this agent is showing activity in prostatic and ovarian cancer [77] and a series of analogues and related agents are being evaluated.

A number of oncoproteins and growth factor receptors are protein kinases and transformed cells generally show a higher level of protein phosphorylation compared with their normal counterparts. Under the control of protein kinases [79], these phosphorylated proteins then act as downstream effector molecules, by amplifying the signal transduction cascade which results in the altered transcription of critical genes. For example, a number of growth factors, including the epidermal growth factor receptor, have tyrosine kinase activity associated with their cytoplasmic domains [80]. Similarly, there are non-receptor tyrosine kinases such as the *src* oncogene product. In addition, the *raf*-1 oncogene product is a serine-threonine kinase, as are the members of the protein kinase C family [81] and both of these are important signalling enzymes in cancer. Mutations of oncogenes and growth factors which disable their phosphorylation ability results in the eradication of their signalling activity. In general, this blocks proliferation. Thus protein kinases are seen as especially important targets for drug development.

Certain natural products such as quercetin, genistein, herbimycin and lavedustin act as tyrosine kinase inhibitors and antiproliferative agents. Based on the structure of erbstatin, tyrphostins are basically tyrosine mimics and act competitively with the protein substrate to inhibit tyrosine kinases [82]. A surprising degree of selectivity can be seen between individual tyrosine kinases, for example the epidermal growth factor receptor kinase vs. the insulin receptor enzyme, or the former kinase vs. the closely related erbB2 enzyme. The *bcr-abl* oncogene tyrosine kinase and the *src* oncogene tyrosine kinases can also be inhibited fairly specifically. As yet these agents have not entered clinical trial, but they are undergoing intense preclinical scrutiny. Certain flavone derivatives are also of special interest because of their ability to inhibit the cdc kinases which are intimately involved together with cyclins in regulating the cell cycle [83, 84]. The key signalling enzyme protein kinase C can be inhibited by agents like the staurosporines [85] and one of these is undergoing preclinical studies with NCI. Moreover, the complex macrocyclic lactone bryostatin I [86], which acts as a partial agonist of protein kinase C as well as down-regulating it, is currently in clinical trial with the CRC [87]. Ether lipids exert a range of effects on cell signalling, including inhibition of protein kinase C and also phospholipase Cγ. Several ether lipid-type agents are in various stages of development in association with the EORTC and the CRC and also in the United States, and some responses have been seen [88]. Numerous other cell signalling targets for drug development are being uncovered at an exhilarating pace. Upon activation of receptor tyrosine kinases, for example, various signal transduction proteins bind to the phosphorylated tyrosines by virtue of their *src* homology (SH) domains to form a putative signal transduction particle [71]. These include phospholipase Cγ, phosphatidylinositol 3'-kinase and ras-GAP, all of which are excellent targets for drug hunting [74]. A number of agents have been shown to inhibit the essential farnesylation of the ras oncoprotein, a post-translational process which is essential for its membrane localisation and signalling function. One of these, limonene [89], is likely to enter clinical trial shortly in the UK. Others are under intense study.

Various insights are now beginning to emerge which provide the long-sought after link between the aforementioned membrane-localised signalling events and the subsequent nuclear changes which cause transcription of specific genes associated with proliferation and malignancy. Ras-GAP, a putative downstream effector for p21 ras, was recently shown to associate with two proteins [90]. One of these, called p62, is able to bind to mRNA and may be involved in messenger processing. The second, p190, contains a sequence almost identical to a transcriptional repressor. An even more direct link between the membrane and nucleus might be provided by the product of the *vav* oncogene [91]. Not only does this protein contain SH2 domains to permit binding to activated tyrosine kinase receptors, it also exhibits a helix–loop–helix domain, a zinc finger and a leucine zipper—all three motifs being commonly found in transcription factors. Thus this molecule may become activated by phosphorylation in the signal transduction particle, detach from the receptor, and proceed to the nucleus to directly alter specific gene expression. This remains controversial but, if confirmed, such factors represent yet more exciting targets for drug development.

In addition to their protein products, mutated cancer genes themselves can also be considered as drug targets. The required specificity for a mutated *ras* oncogene vs. the normal counterpart can be achieved with antisense/antigene oligonucleotides [92]. Although these approaches work well in tissue culture, considerable difficulty remains with respect to drug delivery *in vivo*. Despite the fact that comparable specificity of DNA-sequence recognition cannot yet be approached using drugs [93], a considerable improvement in the sequence-selectivity over conventional alkylating agents can be made with highly potent minor groove alkylating agents such as adozelesin [94] which is related to CC-1065 [95] and with distamycin mustard [96]. These are now undergoing clinical trials on both sides of the Atlantic, and it will be important to assess whether their enhanced base-sequence specificity translates into improved antitumour selectivity. Also of recent interest are the highly potent enediynes [97].

It is not only proliferative genes and signals that offer fertile ground for drug hunting. Similar signalling pathways are also involved in differentiation, metastasis, angiogenesis and programmed cell death or apoptosis. The latter process is attracting considerable current interest [98], not only because of the

potential to switch on a cell death pathway, but also because a molecular understanding of this process may finally shed light on an enigma which has puzzled chemotherapists for many years: why some cells but not others die in response to the same degree of cytotoxic damage [98, 99]. Genes are now being discovered which block programmed cell death in tumour cells, including the *bcl 2* oncogene, and tantalising links are emerging with other cancer genes. These represent exciting targets for therapeutic attack. Mutations in the p53 pathway leading to inactivation of its tumour suppressor gene activity occur with an extremely high incidence in human cancers [100]. p53 appears to be involved in numerous processes, not only involving tumour causation, but also DNA repair, apoptosis, and the generation of aneuploidy and drug resistance [100]. This makes p53 an extremely interesting target for new drugs. There is a difference in the conformation of the protein between the wild type and the mutated oncogenic form, and an attractive prospect would be to search for a drug which would reverse this structural change, quite possibly with lethal effect on the tumour cell.

Signalling inhibitors may be identified in mechanism-based screens. These can involve whole cells, possibly transfected with appropriate cancer genes, or purified proteins, such as receptors or enzymes. It is feasible that a highly specific and potentially antineoplastic antisignalling drug would test as inactive if evaluated against an inappropriate cell line—for example, one which was not driven by the oncogene targeted by the particular drug discovery programme. It is therefore essential that such sophisticated agents be screened against biologically relevant tumour models. Anticipating potential problems with the unavailability of molecularly defined screens, the University of Freiburg xenograft panel is being classified for the expression of key growth factor receptors and oncogenes for subsequent use by EORTC.

If signalling pathways stand out with respect to key exploitable *molecular* differences between tumour and normal cells, hypoxia is probably the principal exploitable *physiological* difference [101]. Evaluation of compounds that are activated preferentially under hypoxic conditions continues to be of major interest in Europe. Hypoxia is mainly limited to solid tumours and this physiological difference with respect to normal tissues can be exploited by bioreductive agents which are activated selectively by enzymes in hypoxic tumour cells [102]. The novel bioreductive indoloquinone EO9, referred to earlier, is an example and clinical trials with this agent have just begun following promising preclinical activity [103]. Since the drug is activated by the enzyme DT-diaphorase, tumours rich in this enzyme may be particularly susceptible to therapy [25, 26, 60, 102]. Measurement of DT-diaphorase in tumour biopsies should therefore be built into Phase II studies. A further valuable feature is its lack of myelosupression in contrast to the related quinone mitomycin C [103]. Other interesting bioreductive agents approaching or entering clinical trial include the benzotriazine di-*N*-oxide WIN 59075 or SR 4233 [104], now in clinical trial in Europe and the USA, and the dual-function nitroimidazole RB 6145 [105], an enantiomer of which is likely to enter Phase I in association with the CRC. The traditional approach to the development of bioreductive agents has involved screening for cytotoxic activity in hypoxic vs. oxic cells in culture [106]. It is now clear that an extra level of mechanism-based sophistication can be built in by considering the enzyme profile of both human and experimental tumours and by taking an enzyme-directed approach. This will allow drug design to optimise activation by individual reductases together with the selection of patients most likely to respond [102, 107]. It is, however, crucial that the enzymology of the tumours used to test such agents is relevant to that of the human tumours of interest.

Of course, mechanism-based drug discovery can also be carried out on more conventional targets. There can be no doubt that efforts to eliminate the potentially cardiotoxic, free-radical generating, redox cycling potential of anthracycline-like agents has generated agents with highly promising clinical activity [22]. Whether these actually work via DNA intercalation, topoisomerase II or some other means is not clear but it seems likely. Considerable effort has gone into mechanism-based screening and design of topoisomerase inhibitors [108] and clinical interest is now shifting to topoisomerase I. Camptothecin arose via conventional screening methods, but clinical trials by the NCI in the 1960s were hindered by toxicity and poor water solubility, possibly related factors. Introduction of water-soluble groups led to CPT-11 [109] and topotecan or SK&F 104864 [110] which appear highly promising, and the less soluble 9-aminocamptothecin will also undergo clinical evaluation [111]. Interestingly, these agents show striking activity against human tumour xenografts. A number of sophisticated yet relatively straightforward functional assays are now available for evaluating the various different consequences of drug binding to topoisomerases. Inhibitors of topoisomerase II are now appearing which do not stabilise the cleavable complex or induce DNA strand-breaks [28].

Cell-free assays are also available to assess the molecular effects of various anti-mitotic agents which act by interfering with tubulin. Most interfere with the colchicine site, including the highly potent combretastatin family of natural products that inhibit polymerization of tubulin [112]. Dolastatin 10 is a linear pentapeptide that inhibits microtubule assembly at the vinca alkaloid binding domain [113]. After earlier experimental and clinical studies, taxol was rediscovered as an agent with highly promising activity in treatment-refractory in ovarian cancer and other sites. It exerts its antitubulin effects through a unique mechanism involving the promotion of pathological microtubule polymerisation [114]. While studies with taxol continue, there are problems with supply since the drug is obtained from the bark of the rare Pacific yew tree. Considerable efforts are going into the search for alternative sources and the synthesis of taxol and its congeners. The derivative taxotere is obtained from the needles of a more abundant European yew and is now entering Phase II studies via the EORTC [53, 115]. The macrocyclic lactone rhizoxin acts at the maytansine-binding site of tubulin [52, 116]. This drug is also now entering Phase II studies with EORTC, CRC and NCI. It is interesting that although molecular studies with tubulin have supported the development of this class of agent, in general these potent natural products have been discovered by conventional cytotoxicity screening.

CONCLUDING REMARKS: WHAT NEXT?

As a general development strategy we feel that it is critical that rational drug discovery gains progressively more support in Europe. This should be seen as one of our main priorities in the years ahead. At the present moment in European anticancer drug development, many of the essential ingredients for success are being gathered together. First, recent developments in molecular oncology have generated a wealth of new interesting avenues to explore in experimental therapy. Second, the scientific community has realised the importance of an integrated effort, whereby different groups share their abilities and expertise to accelerate the drug development process. The current

strategy of integrating the clinical monitoring programmes and the instigation of collaborative strategies for clinical development between EORTC, CRC and the NCI are successful examples of these efforts. Similar integration of our work in the area of screening and discovery is now developing. Moreover, it is important to stress that international collaborations stretch beyond the EORTC, CRC and NCI. Strong links are established with Japan, particularly in the area of natural products. Ties with what used to be referred to as Eastern Europe are strengthening and a South American New Drug Development Office has opened in Brazil.

In terms of the new drugs which are coming through, it is heartening that topoisomerase I inhibitors and the novel tubulin-interacting agents (taxol, taxotere and rhizoxin) are producing a significant number of tumour regressions even in early clinical trials, a situation that has not been the case for many years [117]. It is not clear why this should be, since in general these agents were discovered by traditional screening methods and are hitting conventional cellular targets. While we are hopeful that the clinical utility of these agents will be sustained, we should nevertheless prepare ourselves for the probability that resistance will develop to these agents as it does with the old ones. That means two things. Firstly, efforts to modulate drug resistance must be maintained. Secondly, agents which attack truly novel targets must be discovered.

We have emphasised an emerging consensus that signal transduction targets, in all their myriad forms, are particularly exciting new possibilities for drug hunting. How will progress be made in this area? Clearly the first generation anti-signalling drugs (not counting the existing anti-hormonal agents) are now entering the clinic and from these we will learn a tremendous amount about the potential pitfalls with such agents. We will see new types of toxicities, for sure. Probably also new mechanisms of resistance. We may need to use more than one drug to arrest the growth of tumours driven by more than one cancer gene or to circumvent the signalling degeneracy seen in many tumour cell types. Alternatively, we may find ourselves developing drugs with low molecular specificity—to inhibit multiple kinases, for example. On the other hand, as we learn more about the individual members of signalling protein families, we may find ourselves going the other way—towards particular isoform-specificity.

We could continue with numerous examples. The important point is that a mechanism-based approach allows various hypotheses to be tested and new directions to be followed in a rational way.

A recent editorial [21] called for antineoplastic drug screening to be carried out primarily in the laboratory rather than at the bedside, citing the large number of patients who have failed to respond in Phase II studies. The approach recommended was to screen in the laboratory using fresh tumour specimens. However, this criticism needs to be tempered by the more recent experience described above. Nevertheless, we believe that the strategy for 1990s should be to harness all the intellectual and technological advances of the last 20 years to support mechanism-based development of drugs, particularly those that will antagonise the effects of aberrant cancer genes. Individualisation of patient treatment could then be based on the use of assays to determine the spectrum of pathological molecular changes associated with each individual tumour.

How will mechanism-based drug hunting change in the coming years? The educated layman thinks we already design our drugs on the computer screen. In fact, we cannot yet do this from scratch. The elucidation of the X-ray crystal structure of cancer-inducing proteins is a worthy goal. Yet the structure of oncogenic ras p21 [118] has not immediately suggested a novel therapeutic approach. Moreover, membrane proteins are notoriously difficult to crystallise and X-ray crystallographic structures of proteins may take years to solve. High-field nuclear magnetic resonance spectroscopy is quicker and may give more relevant results but is restricted to around 10–20 K molecular weight [119]—about the size of an SH2 domain. It is possible that the prediction of protein structures using computational methods from deduced aminoacid sequence of cloned genes alone may become reality [120] but is currently blocked by the "protein folding problem".

What we already have is the actual or potential availability of large quantities of our new molecular targets by recombinant DNA technology, together with their structural and functional manipulation by site-directed mutagenesis [121, 122]. More than ever before, anticancer drug discovery requires the interdisciplinary cooperation of cancer pharmacologists, molecular biologists and medicinal chemists.

If anticancer drug discovery proceeds along similar lines to other branches of pharmacology where antagonists of defined molecular targets such as receptors are pursued, then it is likely that an element of screening will be retained as the most efficient means of identifing a new lead structure. Further refinement can then be made by medicinal chemistry and the computational chemistry approaches mentioned earlier. However, such screening approaches will be more sophisticated and specific than hitherto in the cancer field. High throughput robotic assays with pure recombinant target proteins are now being used in some laboratories in place of L1210 or P388 leukaemia cells. Moreover, new techniques employing combinatorial peptide libraries have astonishing potential to identify small molecules that bind to a given receptor or enzyme [123–125]. Because many potential drug targets are membrane-based or even unknown, the approach of antibody-directed drug discovery has been advocated [126]. This involves raising a monoclonal antibody to the target (e.g. an unpurified receptor), next making an anti-idiotype antibody which will be a positive image of the target site, then reading out the structural information by X-ray crystallography or otherwise, and finally producing a peptidomimetic drug [126]. Of course, conversion from an identified peptide to a drug is far from trivial.

This review has been restricted to lower molecular weight anticancer drugs—an approach we favour because of pharmacokinetic delivery problems with the like of antibodies, oligonucleotides and even gene therapy. And yet these strategies are also benefiting enormously from the advances in molecular biology techniques. The approach of antibody-directed enzyme prodrug therapy or ADEPT seems particularly promising [127]. Here the antibody is used to target a non-mammalian enzyme to the tumour and this in turn activates a systemically administered prodrug. An advantage is the potential for killing bystander tumour cells which lack the antigen, following local diffusion of the activated prodrug. An even more sophisticated development involves targeted expression of the drug-activating gene by retroviral transfection alongside a tissue-specific promoter. This has been applied successfully by introducing the herpes simplex thymidine kinase gene into dividing rat brain tumour cells, after which ganciclovir is given and is selectively activated by the transfected gene in the tumour [128].

However sophisticated or hi-tech the molecular screening system for new drug discovery, some demonstration of *in vitro*

activity against tumour cells and of beneficial effects against actual tumours *in vivo* will normally be an essential requirement. As we have stressed throughout this review, it is essential that these models be chosen intelligently so as to minimise the likelihood that an active agent is not lost to an ignorant screen.

We will close with a question to ponder: who should carry out these new approaches to anticancer drug discovery? As we have said, it is clear that the modern state of the art drug development process requires many different types of expertise. To do the work efficiently, some aspects will be automated. It may be that certain elements of the new anticancer drug hunting strategies will be more suited to pharmaceutical companies than to academic institutes. Certainly some of the most challenging developments will be expensive. For example, the proposed "teraflop" computer—a machine which will be 1000 times faster than today's supercomputers and is heralded by some as the answer to rational drug design—is estimated to cost $50–100 million [129]. Already the first drugs designed *de novo* on the basis of an X-ray crystal structure are entering clinical trial—in this case a series of thymidylate synthetase inhibitors [130, 131]. Crystal structures have also now been published for the first protein kinase (a serine-threonine cAMP-dependent protein kinase) and its peptide inhibitor [132, 133]—but as for the ras p21 oncoprotein no drugs have yet appeared based on such knowledge. As we go to press, X-ray and NMR structures have appeared for SH2 domains of the *src* and *abl* oncogene products and for phosphatidyl -3′ kinase [134]. This approach will not necessarily be faster than modern molecular screening strategies and some would argue that it should be seen as a complementary approach—to help and inform other efforts [129].

Best estimates of the cost of introducing a new drug to the market are in excess of $250 million. Academic cancer researchers are now working increasingly closely with the pharmaceutical industry and it is important that this healthy trend should continue to the benefit of both sides. The US system of Cooperative Research and Development Agreements (CRADA) appears to be working well and may be a scheme we should emulate in Europe. The big question is: will we at the end of the day be able to deliver totally new kinds of potent and selective anticancer drugs? Only time will tell—but it may be sooner than we think.

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