Pergamon

European Journal of Cancer, Vol. 35, No. 14, pp. 2010–2030, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00280-4

European Journal of Cancer Millennium Review 2000

Discovering Novel Chemotherapeutic Drugs for the Third Millennium

M.D. Garrett and P. Workman

CRC Centre for Cancer Therapeutics at The Institute of Cancer Research, Block E, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, U.K.

Michelle D. Garrett is a Team Leader at the CRC Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, U.K. She received her post-doctoral training in the Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, U.S.A. (1991–1994). Michelle then moved to Onyx Pharmaceuticals, Richmond, California, U.S.A., where she joined the Cell Cycle Program, and went on to become Team Leader of Cell Based Assay Development for all programs at Onyx (1994–1999). The goals of her research at the Centre are to understand how cell cycle progression is regulated and to apply this knowledge to the development of drugs for cancer treatment.

Paul Workman is Director of the Cancer Research Campaign (CRC) Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, U.K. and is Harrap Professor of Pharmacology and Therapeutics at the University of London. He holds visiting chairs at the University of Manchester and Leeds and is a CRC Life Fellow. Senior positions were held previously at Zeneca Pharmaceuticals, Macclesfield, U.K. (1993–1997), the CRC Beatson Laboratories, Glasgow, U.K. (1991–1993) and the Medical Research Council Centre, Cambridge, U.K. (1976–1990). His research interests are in the discovery and development of novel mechanism-based anticancer drugs.

There is enormous potential for the discovery of innovative cancer drugs with improved efficacy and selectivity for the third millennium. In this review we show how novel mechanism-based agents are being discovered by focusing on the molecular targets and pathways that are causally involved in cancer formation, maintenance and progression. We also show how new technologies, from genomics through high throughput bioscience, combinatorial chemistry, rational drug design and molecular pharmacodynamic and imaging techniques, are accelerating the pace of cancer drug discovery. The process of contemporary small molecule drug discovery is described and progress and current issues are reviewed. New and potential targets and pathways for therapeutic intervention are illustrated. The first examples of a new generation of molecular therapeutics are now entering hypothesis-testing

Correspondence to P. Workman, e-mail: paulw@icr.ac.uk
Received and accepted 27 Oct. 1999.

clinical trials and showing activity. The early years of the new millennium will see a range of exciting new agents moving from bench to bedside and beginning to impact on the management and cure of cancer.

Key words: contemporary drug discovery, novel molecular targets, small molecule mechanism-based drugs, high throughput bioscience assays, combinatorial chemistry, drug design and screening, molecular pharmacodynamics and imaging, hypothesis-testing clinical trials
Eur J Cancer, Vol. 35, No. 14, pp. 2010–2030, 1999

In its own microscopic way, becoming cancerous is about the most glamorous and successful thing a cell can do.
(John Diamond, 'C: Because cowards get cancer too', Vermillion, London, 1998.)

INTRODUCTION

DECADES OF modern treatment have taught us that cancer is a smart disease. Patients with many types of cancer may experience little or no significant response to even the best available combination of surgery, radiation and drug therapy. When responses are obtained, cancer cells most commonly develop resistance mechanisms that lead to the failure of subsequent treatments. Though glamorous may be an overstatement, our appreciation of the smartness of cancer cells has increased enormously with the progressive elucidation of the genetic basis for the initiation of malignant transformation and the stepwise progression to an increasingly malignant, locally invasive and metastatic phenotype. Our respect is further enhanced by our growing knowledge of the biochemical networks that cancer genes exploit during their natural history. To beat 'smart' cancer cells we will need even smarter therapies. This is a considerable challenge. The challenge is matched, however, by the real sense of excitement that has developed in the field of new anticancer drug discovery over the last couple of years [1]. That excitement is based on the premise that we can design much smarter and more effective therapies by aiming to counteract or exploit the very genetic and biochemical abnormalities that drive the disease itself [2].

A vast array of opportunities are now opening up for such mechanism-based cancer therapies. They include the use of small molecule drugs, antisense oligonucleotides, peptide and protein therapeutics including monoclonal antibodies (MAbs), gene therapy, vaccines and cell therapy. The list is too long to cover in the space constraints of this review. Given that other topics (such as gene therapy—see article by Ilyas and colleagues, pp. 1986–2002) are covered by other reviews in this series, we have chosen to focus on innovative small molecule approaches targeted to aspects of the malignant cell phenotype that are not dealt with elsewhere. Small molecules, generally defined by a molecular weight cut-off of < 500 Da, have pros and cons. On the one hand, small molecules are favoured by the pharmaceutical industry because of their attractive pharmacokinetic properties, especially tumour penetration, and their relative ease in terms of development and pharmaceutical production [3–5]. On the other hand, while small molecules have a tremendous track record in delivering effective drugs directed at targets such as enzymes (e.g. from dihydrofolate reductase to novel kinases), it is a formidable challenge to design small molecules that disrupt large domain protein–protein interactions (such as those involving SH2 domains) or that interfere with transcription factor-DNA complexes.

We will approach this review by first considering what exactly it is that we seek to attain from our new drugs for the Third Millennium. We will then review the two principal means by which our objectives can be met, namely by focusing on the novel molecular targets that are causally involved in the formation, maintenance and progression of the smart cancer cell and by the optimal utilisation of the most modern technologies. Progress and issues will be illustrated by selected examples. Finally we will address the prospects and challenges ahead as our new millennium drugs enter clinical practice.

THE 'FROM–TO ANALYSIS'

Cancer drug discovery is re-inventing itself, in order to exploit the latest intellectual technological developments. This re-engineering process is consuming large amounts of the considerable but inevitably finite resources that are available from industry, government and charitable sources. Before embarking on such re-engineering projects in industry, it is standard practice to start by hiring a team of management consultants who then carry out what is known as a 'From–To Analysis'. This can be an extremely valuable exercise, as it seeks to identify answers to two key questions: 'Where are we now?' and 'Where do we need to get to?' By defining the 'Gap' between the 'From' and the 'To' positions, it enables a rational strategy to be developed in order to achieve the desired goal and provides a clear basis for the prioritisation and allocation of the resources that are needed to deliver the strategy. Of course science is a high-risk enterprise in which outcomes are difficult to predict, and this is particularly so in new drug discovery and development. But this uncertainty, coupled to the dissatisfaction with the current situation that is shared by scientists, clinicians and patients alike, makes it all the more important to move forward with a radical but rational strategy for improvement. Let us conduct our own 'From–To Analysis'.

First the 'From'. The current state of play in which roughly 1 in 4 people in the developed world will die from cancer is clearly unacceptable. The World Health Organisation [6] predicts a rise in worldwide incidence from 10 to 20 million per annum and an increase in deaths from 6 to 10 million by the year 2020. For society this represents a huge unmet medical need and immense personal and social cost; for the pharmaceutical and biotechnology industry it represents a major market opportunity; and for science and medicine it represents an enormous and daunting challenge. What about the current role of drug treatment [6–8]? Excellent results can be obtained with chemotherapy in a small range of cancers. For example, cures are now achievable in several

childhood cancers and certain adult malignancies such as lymphoma and leukaemia. Improved survival can also result from adjuvant drug treatment of breast and colorectal cancer. Useful palliation should be the norm in all patients. But we have a long way to go to achieve the necessary jump in the long-term survival and curability of the major solid cancers. This is particularly so for cancers that are in their advanced metastatic forms. In fact most patients who die from cancer have sub-clinical metastatic disease present at the time of diagnosis, so it is essential that therapies are developed that can be given systemically to treat cancer cells located throughout the body.

The latest data available from the US Food and Drug Administration (FDA) [9], shows that 92 anticancer agents have received marketing approval in the U.S.A. Yet a recent WHO consultation exercise [8] categorised only 17 drugs (plus two antiemetics) as having a high priority for widespread use, and a further 12 as having some advantages in particular clinical situations. 13 were viewed as not essential for effective delivery of cancer care. Why are our current drugs, though useful, so limited in their effectiveness? The majority of these agents are cytotoxics that act by inhibiting DNA synthesis or the mechanics of cell replication. They are 'antiproliferative' rather than 'anticancer', so biochemical selectivity for tumour versus normal cells is modest, side-effects are frequently severe and drug resistance is common. It seems unlikely that more than minor incremental benefit will be gained by producing analogues of existing cytotoxics, or by developing novel cytotoxics of natural origin, irrespective of their complexity of structure and obscurity of provenance. Instead, we should direct our energies towards developing truly innovative therapies that will have a major impact on survival and quality of life. This means developing drugs that are much more selectively effective on cancer cells and considerably less harmful to normal cells.

We have now defined the 'To' situation: we need innovative agents which can sustain major survival benefit and have a much better therapeutic index, perhaps more like but even better than our current hormonal agents or drugs used in other chronic disorders, such as cardiovascular disease. The cost effectiveness of new therapies is an issue that governments and society must face but we will not discuss it here, since we take the view that means should be found to pay for genuinely effective cancer treatments. Having defined the 'From' and the 'To' we can now understand the size of the 'Gap' and develop a strategy to overcome it. Although tac-

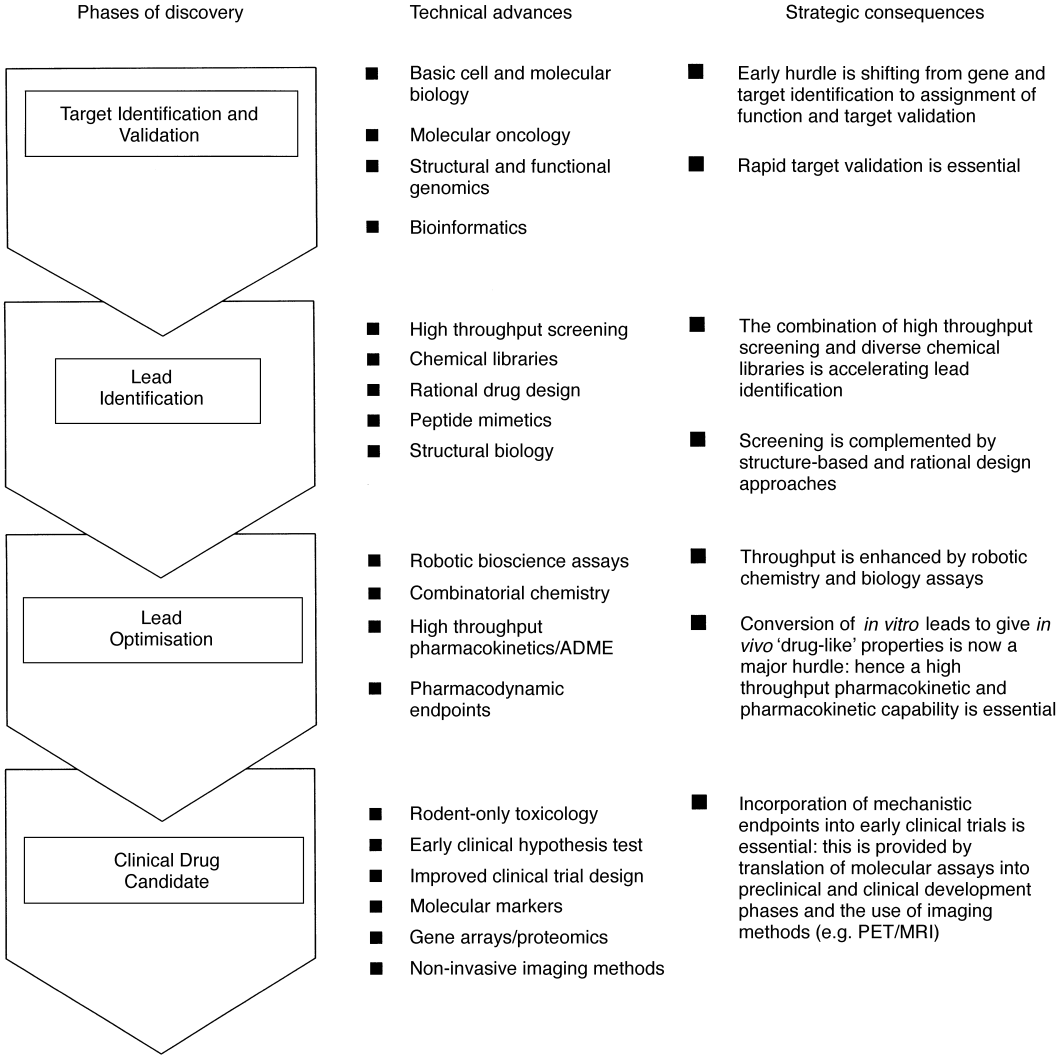

Figure 1. Scheme showing the phases of contemporary small molecule drug discovery, the technical advances that are impacting these phases and the consequences arising from their implementation, particularly in terms of removing bottlenecks.

tical details vary, a clear 2-fold strategy has been emerging for innovative, contemporary drug discovery.

2-FOLD STRATEGY FOR INNOVATION

The 2-fold strategy involves on the one hand, a focus on novel genes and pathways to improve therapeutic index and on the other, the implementation of innovative technologies to enhance the efficiency of the drug discovery and development process (Figure 1). The first component of the strategy commits us to focus on novel targets that are responsible for driving cancer progression. Such agents will block or exploit various aspects of the malignant phenotype. These include genetic instability, proliferative signal transduction, aberrant cell cycle control, deregulated survival, invasion, angiogenesis and metastasis (Figure 2). By attacking or exploiting such targets, we have the best chance to develop drugs that are truly anticancer in nature. This represents a paradigm shift that can be summarised as:

New genes → Novel targets → Innovative medicines

The second component of the strategy addresses the issue that as well being more innovative, we also need to be more efficient in drug discovery and development. Data as recent as the 1990s show that it typically takes 15 years to move a drug from initial discovery to marketing approval and hence widespread patient availability [9]. This is made up of 6.5 years in preclinical research, 1.5 years in phase 1, 2 years in phase 2, 3.5 years in phase 3 and 1.5 years awaiting FDA approval. Take the example of the taxane antitubulin agent Taxol® (paclitaxel). This was discovered as a crude extract activity as early as 1963 but did not gain regulatory approval until 1992 [10]. Similarly the discovery of the *Ras* oncogene was published in 1982 [11], but drugs designed to act at this locus (e.g. antisense and possibly farnesyl transferase inhibitors) are only now entering the clinic. Such long timescales are not acceptable. We must reduce discovery and development down to a minimum of 5–7 years or less, while at the same time increasing the therapeutic value of the drugs involved. Also important, the attrition rates in discovery and development must be reduced. Available figures show that out of 5 to 10 000 compounds tested preclinically, only 5 enter clinical trials and of these only one will gain regulatory approval [12] at an overall cost of around $500 million. Most industry sources will cite current clinical attrition rates closer to 1 success in 10. So drug development needs to be more innovative, faster paced, efficient and less expensive. Fortunately we are now benefiting enormously from the implementation of a range of impressive new technologies that have the potential to overcome the critical hurdles in contemporary drug discovery and development.

OVERCOMING HURDLES WITH NEW TECHNOLOGIES

Figure 1 shows the sequential phases of contemporary small molecule drug discovery, from the identification and validation of a novel drug target, followed by identification and optimisation of a small molecule lead acting on that target, through to selection and development of a clinical drug candidate. Alongside each phase are shown the technological advances that are enhancing the efficiency and success of that phase, together with the strategic impact that arises from implementing them.

TARGET IDENTIFICATION AND VALIDATION

A variety of approaches, which collectively are termed molecular oncology, have led over the last few years to the elucidation of a large number of genes involved in malignant progression (Figure 2). These approaches have involved genetic and biochemical analysis of tumour cell lines and clinical tumour material from familial and sporadic cancers. The genetic causation of colorectal cancer is particularly well understood [13, 14] with adenomatous polyposis coli (*APC*) gene mutations occurring relatively early, Kirsten *Ras* mutations being seen at an intermediate stage and *TP53* mutations a relatively late event. Accumulation of these genetic defects, rather than their precise order, is the key. In addition, mutations in mismatch repair genes in colorectal cancer can lead to error-prone DNA repair and the accumulation of mutations in other genes which may contribute to tumour progression. Indeed, genetic instability is now seen as a hallmark and driving force of the cancer cell. Ultimately we will understand the genetics of the initiation and progression of all tumours in greater detail than we currently do, even than for colorectal cancer.

The elucidation of the function of cancer genes is generally seen as an essential component of target validation. There has been a remarkably effective interplay between human genet-

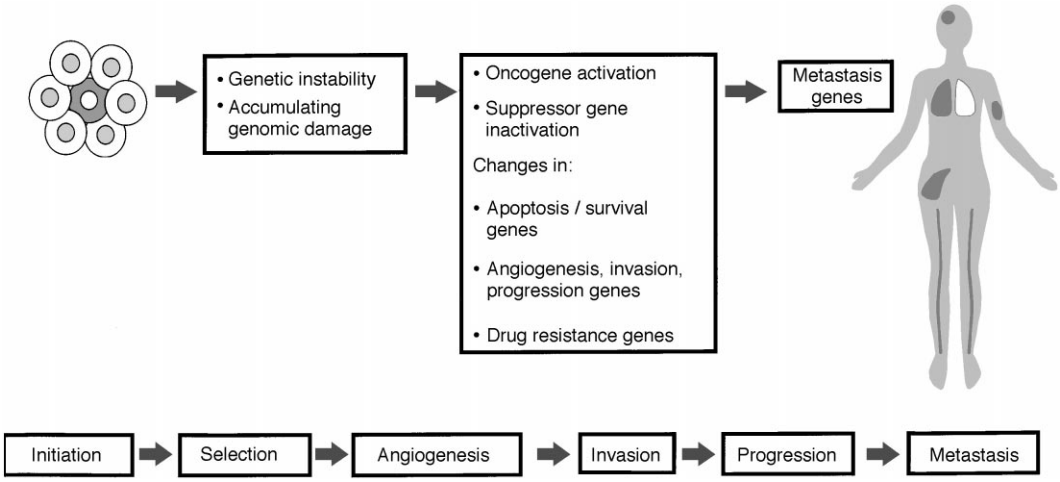

Figure 2. Genes and potential drug targets in multistep oncogenesis.

ics and studies in basic cell and molecular biology, particularly the use of model organisms such as bacteria, yeast, fruit fly, nematode worm and mouse [15]. For example, the elucidation of cell cycle control, which is deregulated in most cancers, has been heavily dependent on yeast genetics; the *Ras* pathway was elucidated with considerable help from studies in *Drosophila melanogaster*; key regulators of programmed cell death such as *bcl2* were identified in the nematode; and human mismatch repair genes were discovered by homology with the bacterial system. Target discovery and validation in cancer is now undergoing a second wave of success due to the ability to study structural and functional genomics at an accelerated rate and on a genome-wide scale. Recent estimates suggest that there may be as many as 142 000 genes in the genome of *Homo sapiens* [16]. It is predicted that the Human Genome Project will release a rough draft of the sequence of all 3 billion base pairs in March 2000 and a completely accurate version in 2003 [16, 17]. Based on a more conservative estimate of 100 000 human genes, it has been calculated that we may discover as many as 3000 to 10 000 drug targets across all non-microbial diseases, as compared with 400–500 genes that are targets of therapy today [18]. Given the number of oncogenes, tumour suppressor genes, and genetic instability genes that are already known to be involved in cancer (at least 50 or more), and taking into account the number of proteins that they interact with in biochemical networks, it seems not unreasonable to speculate that there may be several hundred new oncology drug targets and at least an order of magnitude more than we have today.

As high throughput sequencing, mutation detection and general genomic and bioinformatic advances accelerate the rate of delivery of potential drug targets, the assignment of biological function to a gene and its validation as a drug target becomes a rate limiting step. Assignment of precise function can be a long process, as we can see by the time taken to figure out even a fraction of the biochemical roles that genes such as *Ras* and *TP53* play in normal and tumour cells. What is required for pharmacological intervention is not a complete understanding, but sufficient information to provide some degree of confidence that hitting a given target will generate a selective therapeutic effect for tumour versus normal tissue. There is a difference between such experimental target validation, i.e. sufficient to mount a drug discovery programme and clinical validation, which can only be achieved when patients are treated with a drug.

Although there is no substitute for the painstaking unravelling of biological function, there are even high throughput methodologies that can help accelerate this phase. In addition to gene array technologies for profiling gene expression in tumour and all normal tissues [19], protein technology (proteomics) is emerging as a complementary technique [20], along with more sophisticated gene knock-outs in mice [21] which can now be generated in high throughput.

Various additional types of information can be used to aid target selection in cancer. There are no absolute guidelines but a number of rules of thumb have been developed with experience. The frequency of pathological deregulation, e.g. mutation or aberrant expression, is very important and this is discussed in more detail below. Moreover, any linkage of the genetic or biochemical deregulation to disease progression and clinical outcome is taken to represent especially strong evidence. Knowledge of the expression and function of the target locus in normal tissues can be helpful. New technologies are accelerating and expanding our capability to study gene expression. To illustrate the power of such technologies, in one of the first papers reporting differences in global gene expression patterns between tumour and normal tissues, in excess of 300 000 transcripts derived from a minimum of 45 000 genes were studied using serial analysis of gene expression (SAGE) [22]. There was in fact a surprising degree of commonality between gastrointestinal tumours and their normal non-malignant counterparts. Nevertheless, 548 transcripts (1.5%) showed differential expression. Again, perhaps surprisingly, the principal disparities were not in oncogenes but in genes associated with protein synthesis, ribosomal proteins, elongation factors, glycolysis and also differentiation markers. Extensive data on gene expression patterns in cancer are now beginning to emerge from the use of microarrays (Nature Genetics Microarray Meeting, Phoenix, Arizona, U.S.A. 22–25 September 1999). Massive benefit in the oncology drug discovery field should be gained from the US NCI's Cancer Genome Anatomy Project (CGAP) and related initiatives elsewhere that seek to catalogue all cancers at the level of gene structure expression pattern and function (http://www.ncbi.nlm.nih.gov/ncicgap/). All of the data generated in these projects will be placed rapidly into the public domain.

Creation of some aspect of the malignant phenotype by mutation or abnormal expression is usually considered essential for target validation. This is generally supported by the opposite experiment in which the normal phenotype is restored by 'correction' of the abnormality. This can be done using gene knockout, antisense or dominant negative (interfering mutant) constructs, antisense oligonucleotides, intrabodies or microinjected antibodies, inhibitory peptides including penetratin type conjugates, and finally any reasonably selective small molecule chemical inhibitors that may be available.

An important parameter in target selection that is often neglected, particularly outside the pharmaceutical industry, is the technical feasibility or pharmacological tractability of developing a drug that acts on the target, as distinct from a proof of principle peptide for example. As mentioned earlier, enzyme inhibitors are readily attainable where protein domain blockers or shape modifiers are extremely challenging. Hence we have a number of molecular therapeutics that target enzymes entering clinical trials, but as yet no drugs that block intracellular protein–protein interactions. This does not mean that such targets should not be selected. But it does mean that they are extremely challenging and suggests that new strategies are necessary to tackle such targets. In some cases, the decision may be taken to undertake a screening campaign against a target even in the absence of a typical validation 'package'. One reason for this is that it may be more straightforward to identify leads that act on the target and use these for validation, rather than conduct several years of biological experimentation.

LEAD IDENTIFICATION AND OPTIMISATION

Having selected a sufficiently well validated target, or accepted the increased risk that accompanies a poorly validated target in order to move quickly, the complementary options available are screening and rational design (Figure 1). The two are often used together. Screening requires the construction of an assay that is reproducible, robust, rapid and

informative. It is common to screen using a biochemical assay with recombinant proteins. Alternatively, one can screen against mammalian or yeast cells that have been engineered, for example, to have a reporter gene readout. The likelihood of finding a hit is increased in relation to the chemical diversity of the compounds screened. This has led to high throughput screening becoming almost mandatory [23–25].

High throughput screening has undergone a series of technical improvements including fluorescence technology, automation and miniaturisation. There is a live debate in the field regarding the pros and cons of moving to ultra high throughputs of around 100 000 assays per day to allow screening chemical collections of half a million or more compounds against multiple targets in a factory-like environment. The alternative is to employ more modest throughputs against smaller libraries containing around 20 000 compounds that are carefully selected to cover chemical diversity space. Large companies favour the former. Biotech companies and our own centre favour the latter, and consensus at recent screening meetings seems to be shifting away from the need for extremely high numbers. Computational chemistry methods can be employed to maximise the occupation of chemical diversity space in an efficient manner [26]. In addition, the availability of large numbers of chemicals is massively increased with the advent of the revolutionary technology of combinatorial chemistry [27, 28]. This allows thousands or even millions of compounds to be prepared very rapidly using robotic methods. It is advantageous to exclude from screening libraries chemicals that represent poor starting points for a lead optimisation programme, e.g. highly charged compounds, heavy metals, alkylating agents, Michael acceptors and compounds that have proven to be highly toxic. Chemical reactivity causes particular problems and should be generally avoided in mechanism-based drug discovery [29], although it can sometimes prove hard to move away from such compounds in cancer because of past success with reactive DNA-damaging agents.

Whereas the general trend is firmly towards screening compound libraries against a particular molecular target, the National Cancer Institute (NCI) *in vitro* 60 human tumour cell panel provides an interesting and complementary screening tool that is available to the scientific community [30, 31]. The main objective is to identify compounds with a unique pattern of cellular activity, in the hope that this would indicate activity on a distinct, potentially novel cell target. The issue here is that deconvolution of an unknown molecular target can be a major challenge, as seen with two recent chemical series that display unique cellular profiles [32, 33]. However, this can now be assisted by the molecular characterisation of the panel [34], which has latterly been extended to include gene expression profiling by tissue microarrays. At least ten mechanistically interesting agents arising from testing 70 000 compounds in the 60-cell panel screen are currently being progressed through preclinical and clinical development [30].

Another approach is to screen compounds for activity in yeast cells containing defined genetic abnormalities that parallel those in human tumour cells [35]. By this means it may be possible to identify chemicals that exert a selective therapeutic advantage in cancer cells. This would occur when a compound hits a target that results in selective action against a yeast or tumour cell because it already harbours a deficiency at another locus, e.g. a cell cycle checkpoint defect (see later).

Rational design approaches are very complementary to screening (Figure 1). They may be based on a known lead such as a natural metabolite, substrate or inhibitor. Strategies based on peptide mimics can be useful, although removal of peptidic character to create more drug-like structures can be challenging and peptide size is often limiting (e.g. smaller than 8 or 9 mers is generally required). Design based on the structural biology techniques of X-ray crystallography and nuclear magnetic resonance (NMR) can be a very powerful tool [36, 37]. There are new strategic initiatives to greatly accelerate the solving of protein structures in high throughput in order to keep pace with the output from the Human Genome Project [38]. This has the potential to enhance our ability to predict structure from genomic sequence, and thereby impact on rational drug design. The technique of SAR (structure-activity relationships) by NMR can be a valuable way to assemble separate weakly active chemicals into a more active combined structure.

A fascinating new approach involves a combination of screening of libraries of natural product-like compounds, organic synthesis, site-directed mutagenesis and X-ray crystallography in a creative meld known as 'chemical genetics' [39]. Chemical compounds are used to probe protein function in a fashion that is analogous to genetic mutation. Another useful tactic is to make mutants of drug target proteins that remain biologically active but are insensitive to the inhibitors [40].

The output from the lead identification phase is a small molecule with some activity on the target and the potential to be converted into a more potent and selective agent with drug-like character in the intact animal. In the subsequent lead optimisation phase (Figure 1) compounds discovered by screening or design are optimised by iterative cycles of synthetic chemistry refinement, based on rapid feedback from biological testing in both target protein and cell-based assays. This facilitates the building up of SAR for the desirable and undesirable features of the lead molecule [41].

Combinatorial chemistry can again play a role in this phase, particularly at an early stage where the production of libraries of a few hundred compounds focused around the lead molecule using multi-parallel synthesis can be especially valuable, an activity referred to as lead explosion. Ideally, guided by information on the structure of emerging leads complexed with the target, optimisation through sequential rounds of medicinal chemistry refinement will usually generate compounds with nanomolar potency on the selected target, 10-fold selectivity for that target versus a target to be avoided (or some appropriate counterscreen) and micromolar to submicromolar activity in intact cells by the desired mechanism. At this point the leads become suitable for optimising behaviour in the intact animal.

Translation from activity in the cell to that in the intact animal is currently the most important rate-limiting step in preclinical drug development. Poor pharmacokinetics properties are usually responsible for this [42]. Many discovery projects are stalled or stopped as a result. Problems occur in every aspect of pharmacokinetic behaviour, i.e. absorption, distribution, metabolism and excretion (ADME), and concerns often centre around poor oral bioavailability or excessively rapid clearance. This can result in failure to achieve biologically active blood and tumour concentrations. It should be noted that in contrast to the 'hit and run' properties that could be tolerated with many cytotoxics, prolonged

exposure, often necessitating chronic oral administration, will be required for many of the new molecular therapeutics. It is now common that some degree of molecular target potency and selectivity must be sacrificed in order to achieve the necessary gain in ADME properties.

Rules of thumb concerning physicochemical properties, such as the so-called Lipinski 'rule of five', can be used to improve bioavailability [43]. Maintaining molecular mass to < 500 Da, restricting hydrogen bond donors to ≤ 5, the number of nitrogen plus oxygen atoms to ≤ 10 and C log P (a measure of lipophilicity) to ≤ 5 is one means. However, apart from guidelines based on experience within a particular lead series [44, 45], the development of predictive structure-pharmacokinetic relationships is very primitive [46–49]. Because of this, the bottleneck at the cell to animal transition is currently addressed using so-called cassette or cocktail dosing to increase the throughput of pharmacokinetic analysis. This involves administering compounds together in a cassette or mixture at low doses, followed by detection using the very sensitive and selective techniques of HPLC-MS-MS [50, 51]. Higher throughput *in vitro* methods are also under development for prediction of ADME properties [24]. Subject to such information being released from pharmaceutical companies into the public domain, which should be encouraged, the future must lie in the construction of chemoinformatic algorithms to predict structures having robust, drug-like character and pharmacokinetic behaviour.

Having overcome the pharmacokinetic/ADME hurdle, the next objective is to demonstrate a mechanism-related biological effect in the intact animal. For this phase of lead optimisation, when several hundred compounds may need to be evaluated with quick feedback to the chemists, we urgently require good model systems that can provide a simple, rapid and robust readout of pharmacodynamic activity. More biologically sophisticated and pharmacologically challenging models can be introduced later as lead optimisation approaches completion. The hollow fibre assay is essentially an '*in vitro–in vivo*' hybrid or transition model that can be run in relatively high throughput [52]. However, it should be noted that while the hollow fibre assay has been validated for cytotoxic agents such as cisplatin and taxol, it has yet to prove its utility with the newer molecular therapeutics, for example, those acting on signal transduction targets. Moreover it only is useful for targets where the drug will exert a direct antitumour effect (e.g. antiproliferative, cell cycle arrest, apoptosis induction) and not if the expected biological effect involves, for example, angiogenesis, host-stromal interactions, metastasis, etc. It is not essential that the *in vivo* pharmacodynamic model involve tumour cells. A normal tissue endpoint that demonstrates activity on the desired target or pathway in the intact animal is acceptable. Genetically engineered models, possibly involving simple reporter gene readouts, may have utility.

The more demanding models are needed to prioritise compounds as the project approaches the final stages and in particular nomination of a candidate and backups for clinical development. Activity in at least a couple of human tumour xenograft models [53] is generally considered necessary to progress to the clinic. However, no model is ideal [54] and none have been validated by clinical experience with the new molecular therapies. Our own view is that demonstration of activity by the desired mechanism *in vivo* is more important than a slavish adherence to standard practice. Transgenic models have been used with success with signal transduction inhibitors but it is too early to assess their predictive value [55]. It seems likely that orthotopic and metastatic models [56] will be used increasingly as discovery projects target invasion, angiogenesis and metastasis. Although human tumour models have the obvious advantages of being driven by human genes, there may well be instances in which syngeneic rodent tumour models could have value [53, 57]. Particular attention should be paid to the nature of the tumour vasculature or the host–tumour interaction, especially where these processes are involved in the target mechanism. Above all else, the linkage of the target mechanism to the tumour response must be secure.

Many groups find that it is valuable to define a target drug profile for the new therapeutic entity that is being sought in the project. This describes the essential and desirable features of the drug, including mechanism, potency, selectivity, therapeutic index, schedule and route of administration and pharmacokinetic criteria. This target drug profile allows the research team to measure progress towards the overall objective and to be clear about when a clinical development candidate can be nominated. The target drug profile also allows the test cascade, i.e. the series of hierarchical tests discussed above, to be constructed so as to direct the project specifically towards the correct goal.

The importance of having in place one or more assays that can be used to repeatedly check that activity is being maintained by the desired mechanism throughout all phases of the project, from cells to animal models, cannot be overestimated. Without this there is a danger that activity can deviate into unwanted mechanisms, e.g. DNA damage. Appropriate mode of action assays will ideally be translated from the preclinical to clinical development and examples will be discussed in the following section.

DRUG CANDIDATE

Following the nomination of a potential clinical trial candidate (plus back-up compounds) based on meeting the target profile, preclinical toxicological studies must be carried out to ensure acceptable safety in humans. Excessive toxicology studies can cause delay and in the cancer field these are often poorly predictive of the qualitative nature of particular organ toxicities. Two not-for-profit organisations in Europe, the Cancer Research Campaign (CRC) and European Organisation for Research and Treatment of Cancer (EORTC) utilise a system involving a fairly simple programme of rodent-only (mouse and rat) toxicology [58]. The rodent only toxicology is carried out using the clinical formulation and as close as is feasible to the intended clinical schedule. Formulation itself can be a rate-limiting step and is something of a black art. Improved formulation technology is needed to aid all aspects of *in vivo* drug evaluation but is especially critical in the clinical candidate selection and toxicology phase. Because of this CRC and EORTC have established formulation facilities. CRC has taken around 50 new drugs into phase 1 clinical trials in cancer patients since 1980. The rodent only toxicology approach has proved remarkably safe and effective, while also avoiding the use of larger animals [59, 60]. CRC aims to progress five drugs annually into phase 1 trials and to progress any given agent through formulation and toxicology in 12–18 months. A novel approach to investigate whether the therapeutic and toxicological effects of drugs are related to 'on-target' versus 'off-target' effects is to

create transgenic mice containing drug resistant mutants of target proteins [40].

In the past, phase 1 studies of anticancer agents have nearly always been carried out in patients with advanced cancer rather than normal volunteers, an exception being the hormonal agents. The intention was generally to define the nature of the toxicity of the agent, the maximum tolerated dose, the recommended starting dose for phase 2, and usually the pharmacokinetic profile. This is now changing rapidly, particularly with the introduction of the new molecular therapeutics.

A number of approaches are being used to improve the speed and quality of phase 1 clinical trials [61, 62]. There is insufficient space to discuss this in detail, but the reader is referred to the recent commentary by Gelman and colleagues [63]. Phase 1 strategies include pharmacokinetically/pharmacologically-guided dose escalation [64, 65] continual reassessment [66] and accelerated titration designs [62]. Of major importance is the need to introduce pharmacodynamic endpoints which will allow us to decide whether a particular molecular target or pathway is being affected in the treated patient, as it was earlier in the animal model and tissue culture cell. It is essential that early clinical trials of today's molecular therapeutics contain a strong component of hypothesis testing. We need to answer such questions as the following: is the desired molecular target being modulated (e.g. kinase or farnesyl transferase inhibition)? Is the biochemical pathway undergoing modulation (e.g. the ERK/MAP kinase or PI3 kinase pathway)? And finally is the intended biological affect being achieved (e.g. inhibition of proliferation, cell cycle arrest, apoptosis induction or angiogenesis inhibition)? If some or all of these effects are seen, it provides confidence to move forward to the increasingly expensive later phases of clinical development. Such a structured, rational and mechanism-based approach can greatly improve decision making. If problems arise these can be addressed or resources can be allocated to other more promising agents. If a kinase assay, for example, suggests that the molecular target is not being inhibited despite good pharmacokinetics, this might suggest a pharmacological limitation with the current agent which could be overcome with an alternative drug candidate. If modulation of the molecular target to the required degree is seen, but the intended biological effect is not obtained, this might indicate that the particular target is not valid in the human, but modulation of alternative targets in the same pathway might be worth pursuing to achieve the biological effect. Suppose, however, that the molecular target is hit, the biological effect is achieved but there is no effect on the disease process, then it would be hard to avoid the conclusion that the pathway and biological effect are not linked to the disease in humans and hence further approaches in that area would not be worthwhile. Given the plethora of new target opportunities available, such early go/no go decisions will be essential.

Pharmacodynamic assays include the full range of modern molecular techniques, including Northern and Western blotting, proteomics, RNase protection and quantitative polymerase chain reaction (PCR). Gene microarray technology is now beginning to be used and we recommend that this be introduced into all early clinical trials so that an interrogatable database can be constructed for future use. Access to appropriate clinical material, especially tumour tissue, can be a challenge in phase 1 and 2 clinical trials. For this reason non-invasive technologies such as positron emission tomography (PET) or magnetic resonance imaging (MRI) will play an increasingly important role as their hypothesis testing power increases [67–71].

It should be noted that for certain molecular therapeutics, e.g. some kinase inhibitors, the decision is being made to initiate human studies in normal volunteers rather than patients. These studies, which require a good safety profile, are carried out at low doses to obtain early pharmacokinetic and possibly pharmacodynamic information.

Following satisfactory completion of phase 1 and subsequent efficacy studies, regulatory review has often been slow. This now appears to be speeding up. FDA approval for Herceptin (trastuzumab), a humanised MAb targeted to the erbB2 receptor [72], was achieved in a record 4.5 months [73].

In conclusion, there are an enormous number of opportunities for new drug discovery projects. The first wave of molecular therapeutics are now entering clinical trial and even showing early indications of activity. It is impossible to cover all of these opportunities here, but the following sections give a flavour of current activity and future potential. We have found it convenient to focus on molecular pathways rather than biological effects. Some topics are reviewed in some detail, while others are highlighted more briefly.

THE RECEPTOR TYROSINE KINASE→RAS→ERK PATHWAY

The Receptor Tyrosine Kinase (RTK)→Ras→Raf-1→MEK→ERK signal transduction pathway (Figure 3) has been the most highly favoured for the initial wave of molecular therapeutics. This is because it was one of the earliest pathways to be understood in some detail and because of the frequency with which it is deregulated in human cancer. For example, the erbB2/HER-2 receptor is commonly overexpressed in human breast and ovarian cancer [74]. There are now a number of inhibitors of RTKs already in clinical trials and preclinical development. These include agents targeted to epidermal growth factor receptor (EGFR), erbB2, platelet derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) and also RTK-associated tyrosine kinases such as Src (for review see [75]).

Early work focused especially on inhibitors of EGFR because of its frequent overexpression in various cancers and its involvement in autocrine loops. Proof of the principle that ATP competitive inhibitors could achieve molecular and cellular selectivity versus other kinases was demonstrated by the tyrphostin class of agents [76]. However the activity of these agents in animals was restricted by insufficient drug-like robustness and poor pharmacokinetics [77]. A combination of high throughput screening with a structure-based modelling approach led to the discovery of the anilinoquinazolines and related agents [78, 79]. These leads could be optimised into derivatives that showed excellent pharmacokinetics and good activity in animal models with little or no toxicity at active doses. Inhibitors of EGFR would be expected to produce a cytostatic effect, i.e. growth arrest, and will require chronic, probably oral administration. Clinical trials are in progress with agents such as ZD1839 and CP358774 and these agents appear to be well tolerated with signs of clinical activity emerging. Also encouraging is that the Herceptin antibody targeted to the erbB2 receptor is now approved for the treatment of breast cancer [73].

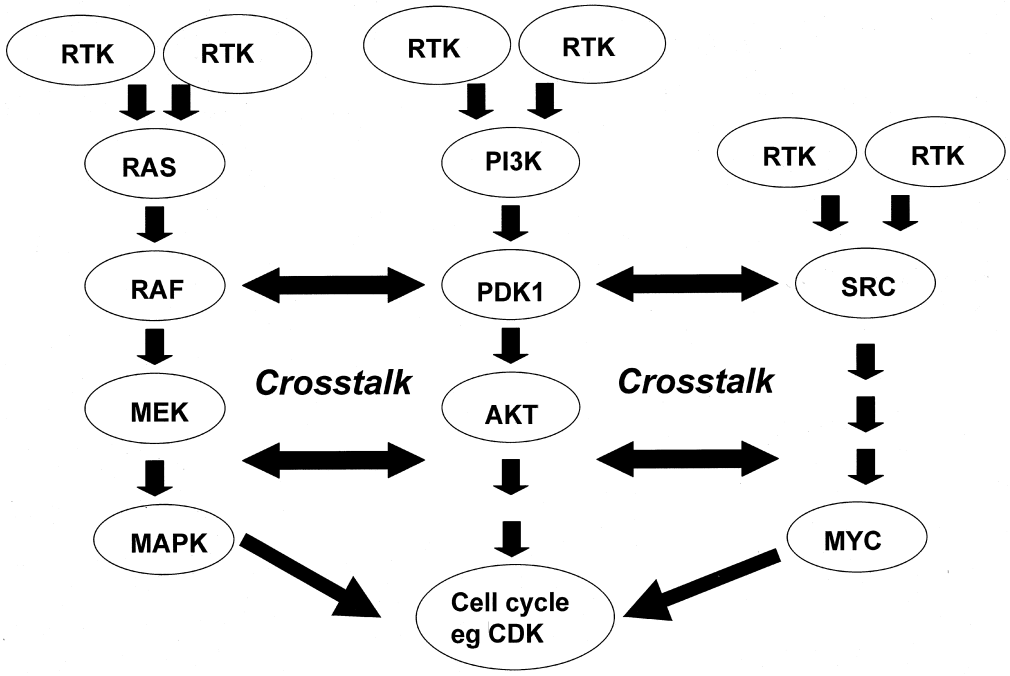

Figure 3. Antiproliferative signal transduction targets.

Moving further down the pathway, the products of the Ras genes are arguably the most well validated of new cancer targets [80]. Clinical trials of agents designed to block Ras signalling by preventing the essential membrane docking and hence the signalling function of Ras [81] are now underway.

In terms of genetic validation, *Ras* genes are frequently mutated in human cancers [82, 83] and Kirsten *Ras* mutation is linked with clinical outcome in colorectal cancer [84]. X-ray crystallography studies demonstrate at the molecular level how mutation leads to knockout of the self-inactivating GTPase function of the Ras G-protein, leading to a constitutive 'on' signalling and the transformed state [85]. Multiple experimental studies have shown that transfection of mutant *Ras* genes into normal cells (usually mouse fibroblasts) can result in malignant transformation [80]. Moreover, very recent data have shown that cancer cells can be created from normal human epithelial cells by introduction of an oncogenic Harvey *Ras* allele, in collaboration with another oncogene (simian virus 40 large-T which inactivates the retinoblastoma protein Rb as well as p53) and the catalytic subunit of telomerase [86]. Harvey *Ras* was also shown to be required for the genesis and maintenance of melanomas in a transgenic mouse model [87]. Further validation was provided by the classic studies of Stacey and colleagues and the use of interfering mutants [88, 89].

Other attempts to inhibit Ras function, such as the use of GTP mimetics, did not prove successful [90], however inhibitors of farnesyl transferase, designed to block ras farnesylation and hence membrane localisation, showed considerable promise in cells and animal models [91, 92]. Discovered by rational peptidominetic design and also by screening, these agents act as so-called CAAX box mimetics. Achieving good bioavailability proved challenging but that problem was overcome during chemical lead optimisation. This had led to at least three farnesyl transferase inhibitors entering clinical trial and there is anecdotal evidence of activity.

There are, however, a number of issues surrounding the use of farnesyl transferase inhibitors as a means to block tumours driven by mutant *Ras*. The mechanism for any potential selective effect in tumour versus normal cells is not clear, although one hypothesis is based on observations with dominant negative interfering Ras mutants [93]. Tumour cell line sensitivity to farnesyl transferase inhibitors does not however seem to relate to mutation status. At the *in vivo* level, angiogenesis inhibition may contribute to the anti-tumour effect, in addition to any direct effect on tumour cells. Moreover the precise mechanism of action of the farnesyl transferase inhibitors is now under challenge. A range of proteins undergo farnesylation in the cell. Moreover, Ras undergoes an alternative prenylation reaction known as geranylgeranylation when its farnesylation is blocked and recent evidence suggests a role for RhoB as a more likely target for farnesyl transferase inhibitors. This remains an open question.

Downstream of Ras in the RTK→Ras→Raf-1→MEK→ERK pathway, inhibitors of Raf-1 have been identified. However, those that have been disclosed have the drawback of causing a paradoxical and undesirable activation of Raf-1, which becomes apparent when the inhibitors are removed [94, 95]. Inhibitors of the dual-specificity kinase MEK were discovered in a screening cascade assay [96]. These appear to have greater therapeutic potential than Raf-1 inhibitors, particularly since one such compound, PD184352, shows good activity in xenograft models [97]. Both potency and selectivity of this compound for MEK versus other kinases are very high. The agent is not competitive with ATP or the MAP Kinase site on MEK, consistent with an allosteric mechanism of inhibition. Both growth and invasiveness of colon tumour cells are blocked, and these effects were shown to be associated with a reduction in ERK (MAP Kinase) phosphorylation, consistent with the intended mechanism. Inhibition of the MAP kinases is also feasible, as exemplified by the pharmacological effects of inhibitors of the stress-activated kinase,

p38 [40] which have therapeutic potential in inflammation and other diseases. There is also considerable interest in JNK MAP kinase inhibitors as potential therapeutic agents.

THE PI3 KINASE PATHWAY

Important signals for proliferation, survival and cell fate are catalysed by the PI3 kinase pathway. Although positioned as a pathway parallel to the Ras→Raf-1→MEK→ERK pathway in Figure 3, these and other pathways operate in complex networks with extensive crosstalk and there is evidence that PI3 kinase is both upstream and downstream of Ras [98].

Biochemically, PI3 kinase phosphorylates the hydroxyl group at the three position on the inositol ring of phosphoinositides, generating lipid signals that regulate fate. Of the three main classes of PI3 kinase catalytic subunits, the most well known in relation to cancer is the Class 1A form of p110α which is activated by RTKs via its p85 associated adapter protein [98]. Recent evidence shows that activation of PI3 kinases leads to activation of PDK1 and AKT/PKB (see Figure 3) with further downstream substrates including GSK3β, $p70^{S6K,}$ PKC, BAD and IκB [98, 99].

PI3 kinase is deregulated in various tumours. For example, the *PIK3CA* gene that encodes the p110α is amplified and overexpressed in ovarian cancer [100]. This led to the suggestion that *PIK3CA* may be an oncogene. In addition, the product of the *PTEN* tumour suppressor gene acts as a lipid phosphatase that reverses the PI3 kinase reaction [101]. These results support the search for the discovery of PI3 kinase inhibitors as anticancer agents. Among various agents that have already been identified as PI3 kinase inhibitors, the most well known and useful (as investigative probe agents) have been LY294002 and wortmanin and related agents such as demethoxyviridin [102, 103]. Although the wortmanin/viridin type of natural products exhibit potent PI3 kinase inhibitory activity and antitumour activity [104], these agents are reactive, unstable and rather promiscuous in their actions, hence there is a need for improved PI3 kinase inhibitors. Inhibitors of downstream targets such as AKT/PKB could also have therapeutic utility.

THE Wnt SIGNALLING PATHWAY

The Wnt signalling pathway is a key regulator of developmental processes in a number of organisms that include *Drosophila* and man [105, 106]. As proteins that regulate this pathway have been identified, it has become clear that Wnt signalling is also involved in tumorigenesis [107, 108]. At the cellular level, the Wnt proteins are a family of secreted glycosylated ligands that bind to the extracellular domain of the frizzled receptors [109]. This complex then activates the dishevelled protein, an event that causes inhibition of glycogen synthase kinase 3β (GSK3β), a cytoplasmic serine threonine kinase that was originally identified through its role in glycogen metabolism. At the moment it is not clear how many regulatory steps there are between dishevelled and GSK3β (if any) in mammalian cells, although studies in *X. laevis* and *C. elegans*, suggest that the serine kinase CK1 (casein kinase 1) is downstream of dishevelled and upstream of GSK3β [110]. Nevertheless, it is clear that when the Wnt signal is absent, GSK3β is active, and can phosphorylate the N-terminus of β-catenin, an effector of Wnt signalling which targets it for degradation [108, 111]. Other proteins are also implicated in regulation of β-catenin levels. These include the adenomatous polyposis coli tumour suppressor protein (APC) and axin which can be found in a cytoplasmic complex with β-catenin and GSK3β [108, 111]. Although the actual role of APC in this process remains elusive, recent reports suggest that axin is dephosphorylated in the presence of Wnt signalling [112]. In this dephosphorylated state axin binds β-catenin less efficiently so that β-catenin is no longer recruited to the complex, cannot be phosphorylated by GSK3β and thus is not targeted for proteolysis. As a result, Wnt signalling leads to accumulation of β-catenin which can bind to the Lef/Tcf family of transcription factors and this complex translocates to the nucleus, where Lef/Tcf dependent transcription can take place [113].

The connection between the Wnt pathway and cancer goes back to the actual discovery of the first mammalian Wnt ligand, which was identified in the mouse as the protein product of the cellular oncogene *int-1* (later renamed *Wnt-1*) that is activated by proviral insertion in murine mammary carcinomas [114]. In addition, overexpression of Wnt genes can cause transformation in mammary epithelial cell lines [115]. Downstream of Wnt, it has been shown that up to 80% of colon cancers have defects in the *APC* tumour suppressor gene [116]. Many of these mutations appear to block the ability of APC to downregulate the levels of β-catenin in the cell and lead to constitutive Lef/Tcf dependent transcription [108]. In some colon cancers that lack defects in APC, mutations have been identified that knock out the putative GSK3β phosphorylation sites of the β-*catenin* gene and also cause constitutive activation of Lef/Tcf transcription [108]. These types of mutations have also been identified in other forms of cancer including melanoma [117] and hepatocellular carcinoma [118]. Finally, it has recently been shown that mutated forms of β-catenin will induce neoplastic transformation of an immortalised neonatal rat epithelial line and that the ability of β-catenin to bind Lef/Tcf factors is required for this process [119].

Thus, with the large amount of genetic evidence that is available showing links between the Wnt pathway and cancer, what types of questions need to be answered in order to increase our confidence that blocking Wnt signalling is an appropriate therapeutic strategy for the treatment of colon cancer? Recent reports have described the use of antisense technology to show that downregulation of the *Wnt-1* gene in mouse mammary tumour cells reverses the malignant phenotype and that antisense β-catenin can block invasion of collagen by human melanoma cells [120]. The genetics of the pathway suggest that since both APC and β-catenin are major points of mis-regulation in cancer, an optimal therapeutic strategy would be one designed to block the pathway either at, or downstream of, β-catenin. One possibility, would be to define the site of interaction between β-catenin and the Tcf/Lef factors and then identify molecules that could block this interaction. Alternatively, if an as yet unknown target of Lef/Tcf is identified which is required for β-catenin dependent transformation, then this gene could be a specific locus for therapeutic intervention. At the present time there are a number of avenues that could be taken, and this number will probably become larger as our understanding of the Wnt pathway continues to increase.

CYCLIN-DEPENDENT KINASES AND THE CELL CYCLE

The mammalian cell division cycle is a tightly regulated cellular process [121]. At the heart of this regulation is the

cyclin dependent kinase (CDK) family of proteins [122]. For full activity, each CDK protein must associate with a regulatory subunit from the cyclin family of proteins [123]. This complex can then phosphorylate a number of substrate proteins, including the retinoblastoma tumour suppressor protein (pRb) and through this mechanism regulate cell cycle progression (see Figure 4). Since the CDKs have such a key regulatory role in the cell, it is not surprising that, along with their substrates and a number of proteins that inhibit their activity, CDKs and cyclins are frequently either direct or indirect targets of genetic lesions that promote neoplastic transformation [124–127]. With this in mind, many groups have sought ways of interfering with the cell cycle for cancer treatment.

One of the major drug targets has been inhibition of the CDKs themselves. At the present time, only one compound with inhibitory activity towards CDKs is in clinical trial [128]. This compound is flavopiridol, a synthetic flavone which can inhibit a broad range of CDKs [129, 130]. However, it is not yet clear whether CDK inhibition is the mechanism by which flavopiridol has antitumour activity, and recent reports indicate that flavopiridol can bind to proteins other than CDKs [131, 132]. Conversely, indirubin, the active ingredient of a Chinese anti-leukaemia medicine, has recently been revealed as a potent and specific inhibitor of cyclin-dependent kinases [133]. Over the last few years high throughput screening has lead to the identification of a number of CDK inhibitors. One structural class with a very high profile, is the tri-substituted purines, as exemplified by the related compounds olomoucine, roscovitine and the purvalanols. These compounds inhibit cdk2, cdc2 and cdk5 (but not the cyclin D-dependent kinases, cdk4 and cdk6) and can block the growth of tumour cell lines *in vitro* [134–137]. Likewise, the unrelated compound, butyrolactone, and a class of compounds known as paullones, also identified as an inhibitors of cdk2, cdc2 and cdk5, can inhibit the growth of cultured tumour cells [138–141]. Notably, none of the above compounds can specifically inhibit the cyclin D-dependent kinases. This has been a goal of many groups, since it became clear that mutation or aberrant expression of cyclin D1, cyclin D2, cdk4 and deletion or mutation of the cyclin D-dependent kinase inhibitor p16INK4A can be found in many human cancers [126]. However, only one group has so far reported the identification of compounds with potential as leads for a cdk4/cyclin D1 inhibitor programme [142].

It is interesting to note that all the compounds discussed above are competitive with ATP. However, several other approaches to CDK inhibition have recently come to light. One of these is the identification of peptides that can mimic the CDK-inhibitory activity of the CDK inhibitors p16INK4A and p21CIP1/WAF1 [143–147]. A second is the discovery of peptides from a combinatorial library that can bind to cdk2 and act as a substrate competitor [148, 149]. This type of strategy is particularly interesting, as it raises the possibility of increased selectivity for a particular CDK and its substrates, as compared to an ATP competitor that binds into the catalytic pocket of a CDK. The use of peptides in a therapeutic manner brings us to one last topic in this section, the E2F family of transcription factors [150]. E2F activity is actually the result of a heterodimeric association between one member from each of the E2F and DP families of proteins (this dimer is subsequently referred to as simply E2F). During early G1, the retinoblastoma gene product, pRb, is hypophosphorylated and tightly binds E2F, masking the transactivation domain of the factor and thus inhibiting its transcriptional activity [151]. As G1 progresses and pRb

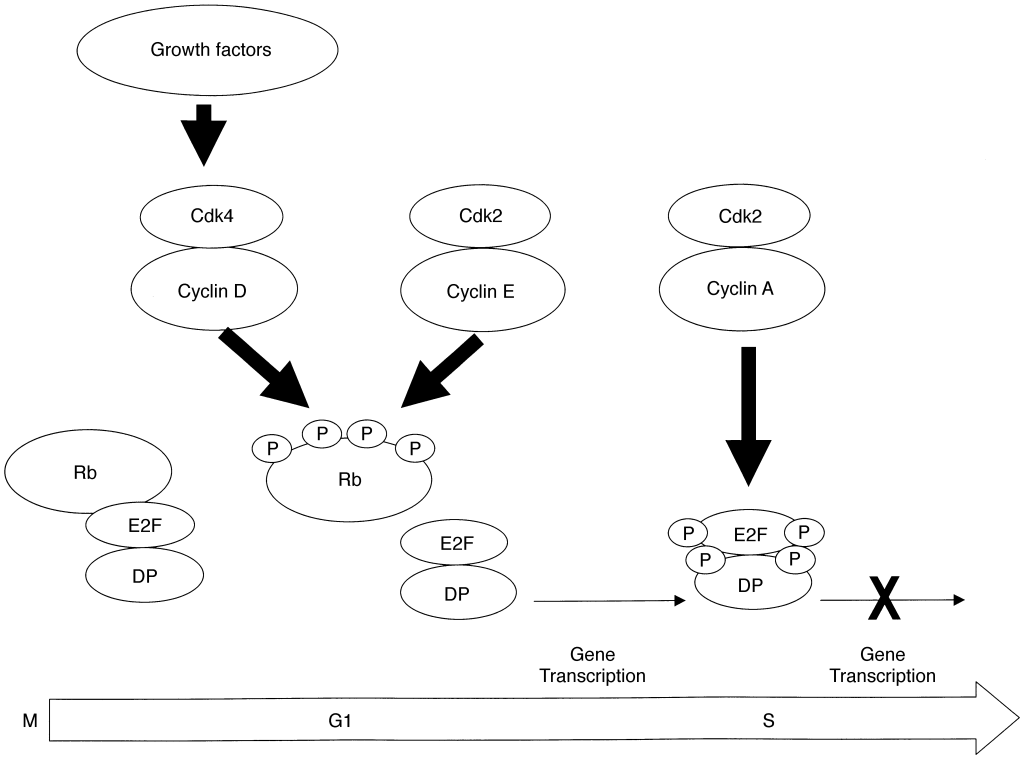

Figure 4. The role of cyclin-dependent kinases in progression through G1 into S phase of the cell cycle (see text for detailed explanation).

becomes increasingly phosphorylated, it loosens its hold on E2F, allowing transcription to occur [152]. Since the heterodimer of E2F and DP is required for E2F activity there is a possibility that blocking the association of E2F and DP, as a downstream target of the CDK/pRb pathway, will have therapeutic value as a cancer treatment. Such a strategy has been tested with a report that a peptide identified again from a combinatorial library, this time for aptamers that bind to E2F, blocks the association of DP with E2F and induces a G1 block in mammalian cells [153]. Finally, it has recently come to light that the cdk2/cyclinA complex can directly bind to E2F1 and that the role of this association is to phosphorylate E2F1/DP1 during S phase, inducing dissociation of the complex from DNA [154, 155] (see Figure 4). This event probably marks the end of a requirement for E2F dependent transcription during the normal cell cycle. Interestingly, expression of a mutant of E2F1 that can no longer bind cdk2/cyclin A will induce apoptosis during S phase [156]. Even wild type E2F1 when strongly overexpressed will induce apoptosis in S phase, suggesting that continued activity of E2F1 during S phase is inappropriate for progression through the cell cycle and so triggers apoptosis [157, 158]. This model has been put to the test by the development of peptide aptamers that block the interaction of cdk2/cyclin A with E2F, so that the CDK can no longer switch off E2F during S phase by phosphorylation [159, 160]. Curiously, these peptides appear to selectively kill normal cells versus tumour cells, which offers an exciting prospect in terms of targeting a tumour for drug treatment. One explanation of such an effect could be that many tumour cells have higher levels of E2F than normal cells and are thus harder pushed to inactivate all their E2F at the start of S phase. Addition of peptides that block cdk2/cyclin A mediated E2F shutdown may lead to sufficient residual E2F activity during S phase to induce apoptosis.

Finally, it must be said that the topics discussed in this section may be just the tip of the iceberg with regard to cell cycle targets. Other potential targets with links to cancer include the Aurora and Polo families of kinases which both appear to play a role in mitosis [161, 162]. In summary, the cell cycle field has already produced a first generation of rationally designed compounds (based on CDK inhibition), which could in the near future become therapeutic drugs. Perhaps exploitation of E2F function may generate the next wave.

THE p53 PATHWAY

The *TP53* tumour suppressor gene is mutated in over half of all sporadic cancers, making *TP53* mutations the most common genetic change in human cancer [163, 164]. The reason for this inevitably lies in its critical function in normal cells, as 'Guardian of the Genome' [165]. The p53 protein carries out this function by acting as a sensor of signals that arise from cellular stress such as DNA damage, hypoxia, and nucleotide deprivation [166]. One of the major responses to these types of signals is that the normally low amount of p53 in the cell is elevated and in some cases p53 becomes modified, to generate a protein that can now take action [167–169]. Two of the major biological responses by p53 are to induce a cell cycle arrest at the G1/S transition along with DNA repair, or if DNA repair is impossible, to activate apoptosis [167–169]. The p53 protein executes many of its functions through transcriptional activation of promoters containing p53 specific binding sites. In this way, p53 can, when required, induce the CDK inhibitor p21 CIP1/WAF which mediates the G1/S specific cell cycle arrest and in other cases upregulate the pro-apoptotic gene *bax*, which may be involved in the p53 dependent apoptotic response. Thus, mutations that inactivate p53 may contribute to tumorigenesis, by allowing a cell to grow and divide under inappropriate conditions.

This model has led to several initiatives based on the concept that reintroduction of p53 or regain of p53 function in a p53 mutant tumour could be of therapeutic value. This type of strategy is already being tested in the clinic, where retroviral and adenoviral vectors containing wild-type p53 are being introduced into patients in phase 1 and 2 clinical trials [170–173]. However, we will have to wait and see whether this type of treatment makes it out of the experimental stage. Still working along these lines, there is building evidence that reactivation of mutant p53 can be induced by interaction of synthetic peptides derived from the C-terminal domain of p53 with the mutated protein [174–176]. One such peptide has also been shown to induce growth inhibition and apoptosis in cell lines where mutant p53 is present, but not when it is absent, suggesting perhaps a therapy that will target cells with mutant p53 [177].

Research on the regulation of p53 accumulation, has led to a novel strategy for activating p53, involving MDM2, a negative regulator of p53. MDM2 seems to accomplish this regulatory activity in two ways: by repression of the transcriptional activity of p53 and by induction of ubiquitin mediated proteolysis of p53 [178]. Peptide phage display libraries have been used to identify the sites of interaction between MDM2 and p53 and subsequently to identify peptides that can block this interaction [179, 180]. This has led to the development of an MDM2 mini protein that can also block the interaction of p53 with MDM2 [181]. Finally it has recently been shown that the antifungal antibiotic leptomycin B can block the nuclear export of p53, leading to its accumulation in the nucleus and activation of the p53 response [182]. This suggests the possibility of specifically inhibiting p53 nuclear export as a way to activate p53 function. To summarise, these types of reagents can cause elevation of p53 levels in the cell in the absence of any form of genotoxic stress, a result that may be of therapeutic benefit in those tumours that still have normal p53 but in which the pathway has been inactivated by some other mechanism upstream of p53.

All the approaches described above attempt to reintroduce or elevate normal p53 function into a cell, in order to inhibit growth or induce death. However, another tactic taken by researchers has been to exploit the fact that many cancers harbour p53 mutations. The most well recognised of these, is the adenovirus known as ONYX-015 [183, 184]. This virus is disabled so that it can only replicate in mutant p53 cells (and thus should leave normal cells untouched), and is soon to start phase 3 clinical trials. The observation that cancers with p53 mutations are often resistant to many of the genotoxic chemotherapeutic drugs that are available for cancer treatment has also produced a novel combined drug strategy [185, 186]. This is based on the fact that response to DNA damage involves arrest of the cell cycle at both the G1/S and G2/M transition, allowing cells time to undergo DNA repair. The G1/S arrest is mediated via p53 dependent induction of the p21Cip1/Waf1 CDK inhibitor, while the G2 arrest is dependent on transient inactivation of the cdc2/cyclin B1

kinase complex whose activity is required for entry into mitosis. Cells with mutant p53 fail to arrest at the G1/S transition and give a preferential G2/M arrest. The dependence of p53 deficient cells on the G2 checkpoint, when exposed to genotoxic stress, raised the possibility that reactivation of cdc2 kinase after DNA damage may be selectively detrimental to these cells. A number of chemical agents have been identified that have this property. These include the methylxanthines, caffeine and 7-hydroxystaurosporine or UCN-01 [185–187]. The most interesting of these is UCN-01, as it is currently in phase 1 clinical trials as a potential cancer treatment and there is evidence that when combined with a DNA damaging agent it causes G2 checkpoint abrogation through reactivation of cdc2 kinase [187–189]. Recent data suggest that the locus of action of UCN-01 may be Chk1 [244], which is an interesting target for G2M checkpoint modulation. Finally, one may say that the most novel exploitation of the p53 pathway in terms of cancer treatment is the recent discovery of pifithrin-α, a chemical inhibitor that protects mice from the effects of DNA-damaging cancer therapy [190]. The identification of pifithrin-α was based on the observation that radiation and chemotherapy for cancer often have side-effects that cause damage to normal tissue which is mediated by p53. Temporary suppression of p53 function therefore may prevent this type of normal tissue damage during the treatment of a p53 deficient tumour. Pifithrin-α was identified in a screen of a chemical library for compounds that could reversibly block p53 dependent transcription and apoptosis. Its actual molecular mechanism of action though, has yet to be elucidated. To conclude, it is clear that research on p53 function and development of therapeutic strategies for cancer treatment based on p53 are progressing hand in hand. Therefore, we await the next round of discoveries in p53 research that may help the development of new strategies for cancer treatment.

PROTEIN TRANSLATION

The translation of mRNA into protein is an intricate process that is critical for the control of cell growth and proliferation [191, 192]. For example, an increase in the rate of translation is required for cells to enter and then transit through the G1 phase of the cell cycle [193]. The importance of translation in cellular growth control can be embodied by the fact that it is a target of misregulation in certain forms of cancer [194–197]. Furthermore, there is mounting evidence that many growth regulatory proteins and oncogenes are encoded by mRNAs that contain highly structured 5′ untranslated regions that are inefficiently translated and rely very much on the activity of eukaryotic translation initiation factors (eIFs) for their expression [198]. In addition, such initiation of translation appears to be regulated by mitogenic stimulation. Thus, one form of growth control on a cell may be the requirement of growth factor signalling for the translation of growth promoting proteins. So how do growth factor signalling pathways feed into translation initiation? The start of translation is characterised by the binding of the 40S ribosomal subunit to the mRNA [199] (see Figure 5). A key part of this is recruitment of the initiator Met-tRNA to the ribosome. This is mediated by eukaryotic initiation factor 2 (eIF2) which, when bound to GTP, forms an eIF2.GTP.-Met-tRNA complex that in turn binds to the 40S ribosomal subunit along with other initiation factors (eIF1, eIF1A and eIF3) to give the 43S pre-initiation complex. The GTP is hydrolysed late in initiation and eIF2 is released from the ribosome in the GDP form. Exchange of GDP for GTP on eIF2 is promoted by eIF2B. The exchange factor activity of

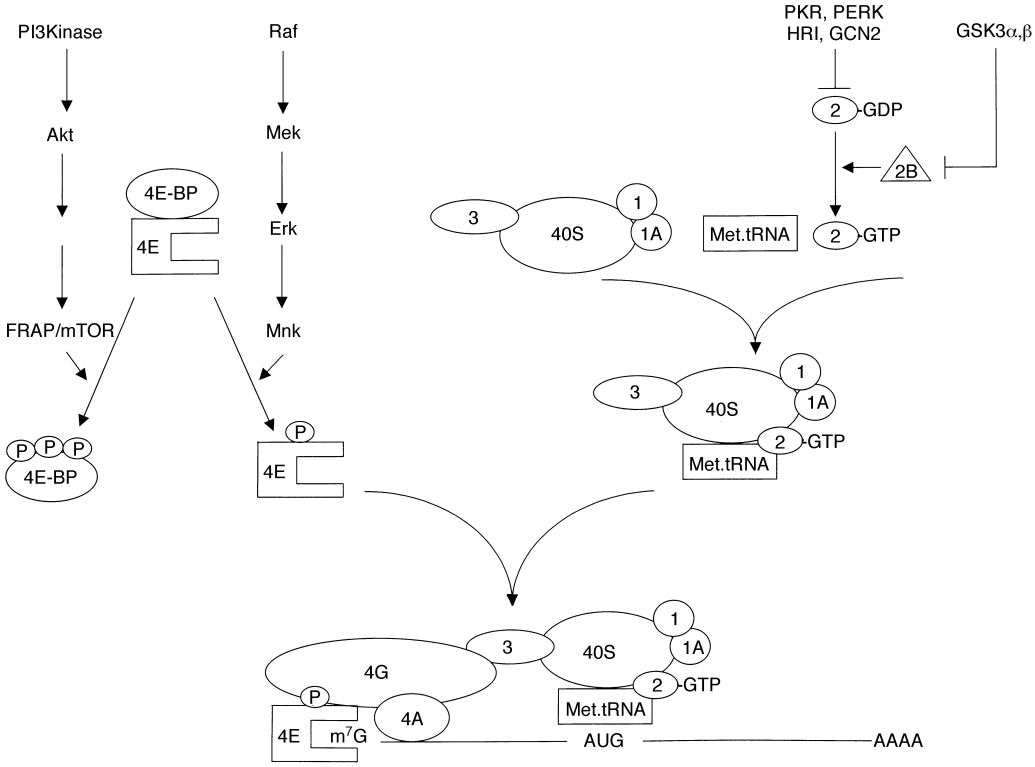

Figure 5. Initiation of protein translation: all factors shown as numbers are eukaryotic initiation factors (eIFs). (See text for other definitions and detailed explanation.)

eIF2B can be inhibited either by direct phosphorylation using GSKα and β or phosphorylation on serine 51 of the alpha subunit of eIF2. The latter turns eIF2 into a competitive inhibitor of eIF2B [204]. Four kinases have been identified that can phosphorylate eIF2 at this site. They are: haem regulated inhibitor (HRI), RNA-dependent protein kinase (PKR), PKR-like ER kinase (PERK) and a mammalian homologue of the yeast GCN2 kinase [199] (see Figure 5). Interestingly, the antimycotic drug clotrimazole (CLT), which is known to have anti-proliferative properties, can also induce phosphorylation at this site on eIF2, resulting in inhibition of translation initiation and a G1 cell cycle arrest [200]. In this instance, phosphorylation of eIF2 appears to be due to the fact CLT induces a sustained depletion of intracellular calcium stores, which leads to activation of PKR. The elucidation of the anti-proliferative mechanism of CLT action via protein translation has led to a strong interest in CLT as an anticancer drug (especially since it can be safely given to humans). It also provides further evidence that initiation of translation is a valid anticancer target and that potential cancer drug targets could be either activation of PKR, or inhibition of the exchange factor activity of eIF2B.

In order for the 43S initiation complex to bind to the mRNA near its 5′ end, the RNA must be 'prepared' for ribosome binding [199]. This is accomplished by formation of a protein complex known as eukaryotic translation initiation factor 4F (eIF4F) on the 5′ cap of the mRNA. EIF4F is made up of three subunits: eIF4E, which directly interacts with the cap, eIF4G which acts as a protein scaffold and eIF4A, a DEAD-box RNA helicase for RNA unwinding [201–204] (see Figure 5). EIF4F also interacts with the 43S preinitiation complex and is thought to aid mRNA binding to the ribosome. The formation of the eIF4F complex at the cap appears to be exquisitely sensitive to extracellular stimuli. For example, eIF4E function is tightly regulated by a family of binding proteins called 4E-BPs (4E binding proteins) or PHAS (for phosphorylated, heat and acid stable protein) which compete with eIF4G for binding to eIF4E. This interaction is regulated by sequential phosphorylation of 4E-BP, which blocks the interaction of 4E-BPs with eIF4E allowing association of eIF4E with eIF4G and translation to occur. When cells are exposed to growth factors, hormones or mitogens, phosphorylation on the 4E-BPs is elevated leading to increases in the rates of protein translation [203, 204]. The PI3 kinase/AKT pathway appears to be involved, as the PI3 kinase inhibitors LY294002 and wortmanin can block 4E-BP1 phosphorylation induced by growth factors and a kinase dead form of AKT can block insulin induced 4E-BP1 phosphorylation [203, 204] (see Figure 5). One strong candidate for a direct kinase of 4E-BPs *in vivo* is the rapamycin sensitive kinase mTOR/FRAP/RAFT, which can phosphorylate 4E-BP1 *in vitro* at sites that are found phosphorylated in cells [205]. It appears that external stimuli such as growth factors and hormones also induce phosphorylation of eIF4E itself, which is reported to increase the affinity of eIF4E for the cap structure [206]. A candidate kinase for this event has been identified as Mnk1, a MAP kinase activated kinase, thus linking the Ras/Raf/MEK/ERK pathway into translation [207]. With regard to uncontrolled cellular proliferation and tumour progression, eIF4E overexpression will induce transformation and is found overexpressed in breast, head and neck and prostate tumours [245]. In addition, the transformed phenotype of tumour cells can be reversed by antisense to eIF4E and by overexpression of 4E-BP which would sequester eIF4E. So, inhibiting eIF4E function in certain tumour types could be a target for a drug discovery programme. Several approaches to this can be envisaged. One would be to seek inhibitors of Mnk1 kinase, and another would be to identify inhibitors of kinases that phosphorylate the 4E-BPs. Interestingly rapamycin, an inhibitor of mTOR, is already in clinical trial as an immunosuppressant in heart and kidney transplantation [208]. Consequently, the connection between rapamycin, mTOR and protein translation is now being investigated from the perspective of anticancer treatment [209].

UBIQUITIN-MEDIATED PROTEOLYSIS

Proteins in the cell usually end their life by being proteolytically degraded. One of the major routes by which proteolysis occurs is the ubiquitin-proteasome pathway [210, 211]. This is a process by which proteins are initially 'tagged' for degradation by the addition of multiple monomers of a 76 amino acid molecule known as ubiquitin. This makes them a target for degradation by a multisubunit protease known as the 26S proteasome. The addition of ubiquitin to a protein is a multi-step reaction involving three classes of enzymes known as E1, E2 and E3. Initially ubiquitin is activated by the E1 enzyme, to which it becomes linked by a thiolester bond. The activated ubiquitin is then transferred to a cysteine residue on one of several known E2 enzymes (also referred to as ubiquitin conjugating enzymes or UBCs). Next, the E2 enzyme transfers the ubiquitin onto a member of the E3 family of enzymes (also known as the ubiquitin protein ligase family) to which a substrate is specifically bound. The E3 then catalyses the last step of the conjugation process, which is formation of an isopeptide bond between the C-terminal glycine of the ubiquitin and a lysine residue on the substrate. Further molecules of ubiquitin are added via the lysine at position 48 on the previously conjugated ubiquitin molecule to generate a polyubiquitin chain. The protein is then ready to be degraded by the 26S proteasome.

It is now becoming clear that ubiquitin-mediated proteolysis is responsible for the rapid turnover of many regulatory proteins. These include p53, cyclin E, cyclin D1, p27, c-FOS and a number of growth factor receptors, all proteins that are on pathways found to be misregulated in cancer [212]. Indeed, there is evidence that proteasome function may be altered upon cellular transformation by oncogenic viruses [213, 214]. A good example of this is the E6 protein of human papilloma virus, which together with a cellular E6 binding protein called E6-AP (E6-associated protein) binds to p53 and promotes its ubiquitin-mediated degradation [215]. In the case of p27, low levels of this CDK inhibitor have been found to correlate with poor prognosis in colon and breast cancer, and in one study it was shown that increased proteasome dependent degradation was responsible for low p27 levels [216–219]. In these cases, restoration of the function of these proteins by blocking their proteolysis may be a possible therapeutic strategy. Another substrate for the proteasome with therapeutic potential is the inhibitory partner of NF-κB, IκB. Activation of NF-κB by degradation of IκB is often a response to cytotoxic stress, which leads to the transcription of anti-apoptotic genes [220, 221]. NF-κB can also induce the transcription of a number of adhesion molecules involved in angiogenesis and metastasis [222]. Thus, it is possible that blocking proteolysis of IκB may make dividing cancer cells more susceptible to apoptosis and also

block tumour metastasis. At the present time, all compounds that block ubiquitin-mediated proteolysis do this by inhibiting the activity of the 26S protease. The best known is the fungal metabolite lactacystin, which acts by modifying a critical threonine residue on a subunit of the proteasome and is a specific inhibitor of this complex [223]. Lactacystin will cause cell cycle arrest in certain lines and even apoptosis, however, there have been no reports on its value as an anticancer agent in tumours. Alternatively the agent aclarubicin which is used clinically in the treatment of cancer, has been shown to inhibit the proteasome by blocking ubiquitin-ATP-dependent proteolysis [224]. However, this is probably not the only cellular target of this compound. Other reported proteasome inhibitors are peptidyl aldehydes such as N-acetyl-leucinyl-leucinyl-norleucinal (ALLN), benzyloxycarbonyl (Z)-leucinyl-leucinyl nor-leucinal (ZLLL), and unfortunately show less specificity than lactacystin [212, 225]. To bring us up to date, the most recently reported proteasome inhibitor is PS-341, a dipeptide boronic acid that can selectively and potently block the chymotryptic activity of the proteasome [226]. This agent was tested in the NCI *in vitro* screen and found to have strong cytotoxicity against a broad range of tumour lines. In human prostate PC3 cells it was found to induce a G2 arrest which was followed by apoptosis. This compound also showed antitumour activity in mice and is now undergoing phase 1 clinical evaluation in patients with advanced cancer. With the available inhibitors of ubiquitin mediated proteolysis, it would be fair to say that none have been shown to attenuate the degradation of one specific protein. In order to attain this level of specificity, and be able to block the degradation of one protein, it may be that we have to look at the other regulators of ubiquitin mediated proteolysis, such as the E1, E2 and E3 enzymes. In this regard, it is clear that there are multiple E2 enzymes and at least four families of E3 ligases with mounting evidence that there is some selectivity to the proteins that the latter associate with [210, 227]. The most recent to be identified implicates pVHL, the product of the *VHL* tumour suppressor gene, as part of a complex that displays E3 ubiquitin-protein ligase activity, demonstrating another link between cancer and ubiquitin mediated proteolysis [228]. Finally, it appears that phosphorylation may also have a role to play in selecting proteins for degradation by this pathway. There have been a number of reports indicating that phosphorylation of proteins on specific residues can be a signal for ubiquitination. One such protein that we have mentioned earlier is the cell cycle regulator p27Kip1, which requires phosphorylation at the threonine127 position in order to be a target for ubiquitination and hence proteolysis by the 26S proteasome [229]. Thus, identification of the kinases and phosphatases that regulate these events will also provide new avenues for drug discovery in the area of ubiquitin mediated proteolysis.

OTHER NEW TARGETS

An emerging new area for potential therapeutic intervention is the control of gene transcription via the acetylation and deacetylation of chromatin and transcription factors such as p53 and E2F1. These reactions are catalysed by families of acetylases and deacetylases that act as transcriptional co-activators and co-repressors [230, 231]. There is an increasing link with cancer, since acetylases, for example, are frequently found to be deregulated by mutation or translocation and are also targeted by viral proteins. Early natural product inhibitors of histone deacetylases include trichostatin A, trapoxin and depudecin. Recently a series of synthetic benzamide derivatives were found to exhibit histone deacetylase activity and show potent inhibition of human tumour xenografts [232]. Together with the related area of DNA methylation, this appears to be an area with considerable therapeutic potential.

Another area receiving considerable interest at the moment is the inhibition of the Hsp90 molecular chaperone [233]. Hsp90 appears to function as a general chaperone in response to cellular stress, assisting in the refolding of mis-folded proteins. But it is also involved in specialised functions in unstressed cells, including control of the folding, function and location of particular sets of signalling molecules, especially hormone receptors, oncogenic kinases (e.g. erbB2 and Raf-1) and mutant p53. Potent natural product inhibitors of Hsp90 include radicicol and the geldanamycins such as 17AAG [234]. Inhibition results in the degradation of the above mentioned client proteins and a resulting antitumour effect *in vitro* and *in vivo* [234]. Sensitivity to 17AAG, but not geldanamycin or radicicol is greatly increased in cells containing high levels of the *NQO1* gene product, DT-diaphorase, an enzyme that is overexpressed in a high proportion of tumour cells [235].

Modulation of DNA repair following treatment of tumours with DNA damaging agents and radiation continues to be of interest. Target repair proteins include DNA alkyl guanine alkyl transferase [236], poly (ADP-ribose) polymerase (PARP) [237] and DNA-dependent protein kinase [238]. With regard to DNA itself, an area of great interest in terms of both research and cancer treatment has been telomeres and the enzyme telomerase. Recent results suggest that telomerase has potential as a specific cancer target for drug discovery [239–241]. Inhibitors of telomerase, involving both catalytic blockade and G-quadruplex binding, are now emerging [242]. The bcr/abl oncoprotein results from a translocation seen in the majority of patients with chronic myeloid leukaemia (CML). The constitutively active tyrosine kinase function of bcr/abl is inhibited by the 2-phenylaminopyridine agent CGP57148B and treatment with this agent resulted in cures of leukaemic mice [247]. This drug has now entered phase 1 clinical trials and there are anecdotal reports of excellent cytogenetic and clinical responses in CML patients.

CONCLUSION

We would reiterate again that space has allowed us to cover only a proportion of the exciting new small molecule approaches designed to attack or exploit the molecular pathology of tumours. The intention was to illustrate and exemplify the enormous potential for a new generation of innovative therapies based on novel targets, and at the same time to stress the impact of the new technologies on the cancer drug discovery process. There is extraordinary potential for a rapid and rational approach all the way from the *in silico* genome sequence and laboratory bench through to the hypothesis-testing clinical trial at the patient's bedside.

Discussion will continue regarding the advantages and disadvantages of carrying out cancer drug discovery in relatively small to medium sized, highly focused academic centres and biotechnology companies as opposed to the increasingly enormous capacity of large pharmaceutical companies. Economies of scale and the very high screening throughputs of large pharmaceutical companies can be important, but it

has been argued that this can be counterbalanced by such factors as short term market considerations, the distractions of mergers and acquisitions and challenge of linking industrial throughputs to the development of novel cutting edge concepts [243]. The biotech industry has been extremely innovative and academic institutes have an excellent track record in cancer drug discovery and development. For example, in our own centre we have taken eight new drugs, derived from totally in-house or partnered discovery projects, into phase 1 clinical trials over the past 6 years, with a further agent scheduled for entry during 2000. The costs associated with establishing on a reasonable scale the new technologies described in this review are no longer prohibitive for small biotech and academic centres. Again, in our own centre we have established most of the technologies shown in Figure 1 and we can gain access to the remainder within our Institute and a broader network of collaborators across CRC and the extended academic community. From a personal perspective, having experienced cancer drug discovery in academic, biotech and large pharma settings, the present authors favour strategic partnerships that play to the relative strengths of all three sectors. The initial measure of success should be how quickly we can bring to the cancer clinic hypothesis-testing trials on the plethora of innovative agents targeted to the molecular pathology of cancer. The ultimate test will be whether the new molecular therapeutics deliver the goods in terms of improved patient management and survival.

There are many unanswered questions. How active will the new molecular-targeted drugs be when given as single agents, and how well will they be tolerated? Will resistance arise because of the emergence of redundant pathways? Will the biological effects seen involve cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, or modulation of invasion and metastasis? How best should the new molecular therapies be combined with existing cytotoxic therapies, surgery and radiotherapy? To what extent will the prescribing of the new therapies be linked to tumour genotype—for example, will we be able to dial up a combination of drugs that specifically target an individual patient's tumour, e.g. one that has a mutant *Ras* oncogene, together with mutated *TP53* and *p16* suppressor genes and deregulated *Myc* expression? How will gene expression microarray and proteomics technologies be used in the clinic? These and many others are important and fascinating questions that will be answered during the next 5 to 10 years. As indicated in this review, there is no shortage of stunning new ideas and targets. The annual review of the Pharmaprojects database published in May 1999 [246] shows that anticancers represent the single most active therapeutic area for drugs in development worldwide, with a total of 1422 projects currently underway. The first few years of the third millennium will be exciting for all of us involved with cancer drug discovery as we strive to realise the enormous therapeutic potential of our intellectual and technological achievements.

1. Baring M. From bench to bedside. *Science* 1997, **278**, 1035–1039.
2. Workman P. The potential for molecular oncology to define new drug targets. In Kerr DJ, Workman P, eds. *New Molecular Targets for Cancer Chemotherapy*. CRC Press Inc., 1994, 1–29.
3. Jain RK. Delivery of novel therapeutic agents in tumors: physiological barriers and strategies. *J Natl Cancer Inst* 1989, **81**, 570–576.
4. Stevens MFG. Is there a future for the small molecule in developmental cancer therapy? In Waring MJ, Ponder BAJ, eds. *The Search for New Anticancer Drugs*. Dordrecht, Kluwer, 1992, 1–17.
5. Workman P. Emerging molecular therapies: small molecule drugs. In Bronchud MH, Foote M, Peters WP, Robinson MO, eds. *Principles of Molecular Oncology*. Totowa, Humana Press, 1999, 413–429.
6. World Health Report. *Life in the 21st Century. A Vision for All*. Geneva, Switzerland, World Health Organisation, 1998.
7. Tannock IF. Conventional cancer therapy: promise broken or promise delayed? *Lancet* 1998, **351**(Suppl. II), 9–16.
8. Sikora K, Advani S, Koroltchouk V, *et al*. Essential drugs for cancer therapy: a World Health Organisation consultation. *Ann Oncol* 1999, **10**, 385–390.
9. Food and Drug Administration Web Site. http://www.fda.gov/oashi/cancer/cdrug.html.
10. Rowinsky EK, Donehower RC. Drug therapy: paclitaxel (Taxol). *N Engl J Med* 1995, **332**, 1004–1114.
11. Cooper GM. *Oncogenes*. 2nd edn. Boston, Jones and Bartlett Publishers, 1995.
12. PhRMA Web Site. http://www.phrma.org.
13. Ilyas M, Straub J, Tomlinson IPM, Bodmer WF. Genetic pathways in colorectal and other cancers. *Eur J Cancer* 1999, **35**, 335–351.
14. Haber DA, Fearon ER. The promise of cancer genetics. *Lancet* 1998, **351**(Suppl. II), 1–8.
15. Lane D. The promise of molecular oncology. *Lancet* 1998, **351**(Suppl. II), 17–20.
16. Dickson D. Gene estimate rises as US and U.K. discuss freedom of access. *Nature* 1999, **401**, 311.
17. Collins FS, Patrinos A, Jordan E, Chakravarti A, Gesteland R, Walters L. New goals for the US Human Genoma Project: 1998–2003. *Science* 1998, **282**, 682–689.
18. Drews J. Genomic sciences and the medicine of tomorrow. *Nat Biotechnol* 1996, **14**, 1518–1519.
19. Debouck C, Goodfellow PN. DNA microarrays in drug discovery and development. *Nature Genetics* 1999, **21**, 48–51.
20. Page MJ, Amess B, Rohlff C, Stubberfield C, Parekh R. Proteomics: a major new technology for the drug discovery process. *Drug Discovery Today* 1999, **4**, 55–62.
21. Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K. Deletion of a DNA polymerase β gene segment in T cells using cell type-specific gene targeting. *Science* 1994, **265**, 103–106.
22. Zhang L, Zhou W, Velculescu VE, *et al*. Gene expression profiles in normal and cancer cells. *Science* 1997, **276**, 1268–1272.
23. Bevan P, Ryder H, Shaw I. Identifying small-molecule lead compounds: the screening approach to drug discovery. *Trends in Biotechnology* 1995, **13**, 115–121.
24. Dove A. Drug screening—beyond the bottleneck. *Nature Biotech* 1999, **17**, 859–862.
25. Archer R. The drug discovery factory: an inevitable evolutionary consequence of high-throughput parallel processing. *Nature Biotech* 1999, **17**, 834.
26. Willett, ed. *Computational Methods for the Analysis of Molecular Diversity. Perspectives in Drug Design*. Dordrecht, Kluwer, 1997, 7/8.
27. Terrett NK, Gardner M, Gordon DW, Kobylecki RJ, Steele J. Combinatorial synthesis—the design of compound libraries and the application to drug discovery. *Tetrahedron* 1995, **51**, 8135–8173.
28. Hogan JC. Combinatorial chemistry in drug discovery. *Nature Biotechnology* 1997, **15**, 328–340.
29. Rishton GM. Reactive compounds and *in vitro* false positives in HTS. *Drug Discovery Today* 1997, **2**, 382–384.
30. Monks A, Scudiero DA, Johnson GS, Paull KD, Sausville EA. The NCI anti-cancer drug screen: a smart screen to identify effectors of novel targets. *Anticancer Drug Des* 1997, **12**, 533–541.
31. Workman P. Towards intelligent anticancer drug screening in the post-genome era? *Anti-Cancer Drug Design* 1997, **12**, 525–531.
32. Skelton LA, Ormerod MG, Titley J, Kimbell R, Brunton LA, Jackman AL. A novel class of lipophilic quinazoline-based folic acid analogues: cytotoxic agents with a folate-independent locus. *Br J Cancer* 1999, **79**, 1692–1701.

33. Bradshaw TD, Wrigley S, Shi DF, Schultz RJ, Paull KD, Stevens MF. 2-(4-Aminophenyl) benzothiazoles: novel agents with selective profiles of an *in vitro* anti-tumour activity. *Br J Cancer* 1998, **77**, 745–752.
34. Weinstein J, Myers T, O'Connor PM, *et al.* An information-intensive approach to the molecular pharmacology of cancer. *Science* 1997, **275**, 343. For update of molecular target information see http://dtp.nci.nih.gov/ click on molecular targets.
35. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. *Science* 1997, **278**, 1064–1086.
36. Blundell TL. Structure-based drug design. *Nature* 1996, **384**, 23–25.
37. Kubinyi H. Structure-based design of enzyme inhibitors and receptor ligands. *Current Opinion in Drug Discovery and Development* 1998, **1**, 4–15.
38. Burley SK, Almo SC, Bonanno JB, *et al.* Structural genomics: beyond the human genome project. *Nature Genetics* 1999, **23**, 151–157.
39. Crews CM, Splittgerber U. Chemical genetics: exploring and controlling cellular processes with chemical probes. *TIBS* 1999, **24**, 317–320.
40. Eyers PA, van den Ijssel P, Quinlan RA, Goedert M, Cohen P. Use of a drug-resistant mutant of stress-activated protein kinase 2a/p38 to validate the *in vivo* specificity of SB 203580. *FEBS Lett* 1999, **21**, 191–196.
41. King FD, ed. *Medicinal Chemistry: Principles and Practice*. Cambridge, Royal Society of Chemistry, 1994, 179–188.
42. Workman P. Pharmacokinetics and cancer. Successes, failures and future prospects. In Workman P, Graham MA, eds. *Pharmacokinetics and Cancer Chemotherapy. Cancer Surveys, Vol. 17*. New York, Cold Spring Harbor Laboratory Press, 1993, 1–26.
43. Current Drugs Drug metabolism and pharmacokinetics symposium. IF weekly highlights. Current Drugs Ltd, September 1998, 25.
44. Brown JM, Workman P. Partition coefficient as a guide to the development of radiosensitizers which are less toxic than misonidazole. *Radiat Res* 1989, **82**, 171–190.
45. Workman P, Brown JM. Structure-pharmacokinetic relationships for misonidazole analogues in mice. *Cancer Chemother Pharmacol* 1981, **6**, 39–49.
46. Rowland M. Pharmacokinetics-QSAR: definitions, concepts and models. In Dearden JC, ed. *Quantitative Approaches to Drug Design*. Amsterdam, Elsevier, 1983, 155–161.
47. Mayer JM, van de Waterbeemd H. Development of quantitative structure-pharmacokinetic relationships. *Environmental Health Perspectives* 1985, **61**, 295–306.
48. Blakey GM, Nestorov IA, Arundel PA, Aarons LJ, Rowland M. Quantitative structure-pharmacokinetics relationships: I. Development of a whole-body physiologically based model to characterize changes in pharmacokinetics across a homologous series of barbiturates in the rat. *J Pharmacokinetics and Biopharmaceutics* 1997, **25**, 277.
49. Nestorov I, Aarons L, Rowland M. Quantitative structure-pharmacokinetics relationships: II. A mechanistically based model to evaluate the relationship between tissue distribution parameters and compound lipophilcity. *J Pharmacokinetics and Biopharmaceutics* 1998, **26**, 521.
50. Ohah TV, McLoughlin DA, Gilbert JD. The simultaneous determination of mixtures of drug candidates by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry as an *in vivo* drug screening procedure. *Rapid Communications in Mass Spectrometry* 1997, **11**, 17–23.
51. Rodrigues AK. Preclinical drug metabolism in the age of high throughput screening: an industrial perspective. *Pharmaceutical Research* 1997, **14**, 1504–1515.
52. Hollingshead MG, Alley MC, Camelerier RF, *et al. In vivo* cultivation of tumour cells in hollow fibers. *Life Sciences* 1995, **57**, 131–141.
53. Kallman RF. Rodent Tumor Models. In *Experimental Cancer Therapy*. New York, Pergamon Press, 1987.
54. Gura T. Systems for identifying new drugs are often faulty. *Science* 1997, **278**, 1041–1042.
55. Kerbel RS. What is the optimal rodent model for anti-tumor drug testing? *Cancer Metastasis Rev* 1998, **17**, 301–304.
56. Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. *Cancer Metastasis Rev* 1998, **17**, 279–284.
57. Martin DS, Balis ME, Fisher B, *et al.* Role of murine tumor models in cancer treatment research. *Cancer Res* 1986, **46**, 2189–2192.
58. Burtles SS, Newell DR, Henrar REC, Connors TA. Revisions of general guidelines for the preclinical toxicology of new cytotoxic anticancer agents in Europe. *Eur J Cancer* 1995, **31A**, 408–410.
59. Burtles SS, Jodrell DI, Newell DR. Evaluation of ''rodent only'' preclinical toxicology for phase I trials of new cancer treatments—the Cancer Research Campaign (CRC) experience. *Proc AACR* 1998, **39**, 363.
60. Newell DR, Burtles SS, Fox BW, Jodrell DI, Connors TA. Evaluation of rodent-only toxicology for early clinical trials with novel cancer therapeutics. *Br J Cancer* 1999, **81**, 760–768.
61. Graham MA, Kaye SB. New approaches in preclinical and clinical pharmacokinetics. *Cancer Surv* 1993, **17**, 27–49.
62. Simon R, Freidlos B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I trials in oncology. *J Natl Cancer Inst* 1997, **89**, 1138–1147.
63. Gelman KA, Eisenhauer EA, Harris AL, Ratain MJ, Workman P. Anticancer agents targeting signaling molecules and cancer cell environment: challenges for drug development. *J Natl Cancer Inst* 1999, **91**, 1281–1287.
64. Collins JM, Zaharko DS, Dedrick EL, *et al.* Potential roles for preclinical pharmacology in phase I clinical trials. *Cancer Treat Rep* 1986, **70**, 73–80.
65. Graham MA, Workman P. The impact of pharmacokinetically guided dose escalation strategies in phase I clinical trials: clinical evaluation and recommendations for future studies. *Ann Oncol* 1992, **3**, 339–347.
66. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase I clinical trials in cancer. *Biometrics* 1990, **46**, 33–48.
67. Maxwell RJ. New techniques in pharmacokinetic analysis of cancer drugs III: nuclear magnetic resonance. *Cancer Surv* 1983, **17**, 415–423.
68. Tilsley DW, Harte RJ, Jones T, *et al.* New techniques in the pharmacokinetic analysis of cancer drugs. IV. Positron emission tomography. *Cancer Surv* 1993, **17**, 425–442.
69. Workman P, Maxwell RJ, Griffiths JR. Non-invasive MRS in new anticancer drug development. *NMR Biomed* 1992, **5**, 270–272.
70. Workman P. Bottlenecks in anticancer drug discovery and development: *in vivo* pharmacokinetic and pharmacodynamic issues and the potential role of PET. In Komar D, ed. *PET for Drug Development and Evaluation*. Dordrecht, Kluwer, 1995, 277.
71. Weissleder R. Molecular imaging: exploring the next frontier. *Radiology* 1999, **212**, 609–614.
72. Pergram MD, Lipton A, Hayes DF, *et al.* Phase II study of receptor-enhanced chemosenitivity using recombinant humanised anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 1998, **16**, 2659–2671.
73. Scrip No 2374 30 September 1998, 20.
74. Slamon DJ, Godolphil W, Jones LA, *et al.* Studies of the *HER-2/neu* proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
75. Strawn LM, Shawver LK. Tyrosine kinases in disease. *Exp Opin Invest Drugs* 1998, **7**, 553–573.
76. Levitski A, Gazit A. Tyrosine kinase inhibition: an approach to drug development. *Science* 1995, **267**, 1782–1788.
77. McLeod HL, Brunton VG, Eckardt N, *et al. In vivo* pharmacology and anti-tumour evaluation of the tyrphostin tyrosine kinase inhibitor RG13022. *Br J Cancer* 1996, **74**, 1714–1718.
78. Fry DW, Kraker AJ, McMichael A, *et al.* A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* 1994, **265**, 1093–1095.
79. Ward WHJ, Cook PN, Slater AM, Davies DH, Holdgate GA, Green LR. Epidermal growth factor receptor tyrosine kinase. *Biochem Pharmacol* 1994, **48**, 659–666.
80. Marshall CJ. Opportunities for pharmacological intervention in the ras pathway. *Ann Oncol* 1995, **6**(Suppl. 1), S63–67.

81. Hancock JF, Magee AI, Childs JE, Marshall CJ. All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell* 1989, **57**, 1167–1177.
82. Bos JL. The ras gene family and human carcinogenesis. *Mutat Res* 1988, **195**, 255–271.
83. Kiaris H, Spandidos DA. Mutations of ras genes in human tumours. *Intl J Oncol* 1995, 7, 413–421.
84. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998, **90**, 675–684.
85. Wittinghofer A, Pai EF. The structure of Ras protein: a model for a universal switch. *Trends Biochem Sci* 1991, **16**, 382–387.
86. Hahn WC, Counter CM, Lundberg AS, Bijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature* 1999, **400**, 464–468.
87. Chin L, Tam A, Pomerantz J, *et al*. Essential role for oncogenic Ras in tumour maintenance. *Nature* 1999, **400**, 468–472.
88. Mulcahy LS, Smith MR, Stacey DW. Requirement for ras proto-oncogene function during serum-stimulated growth of NIH 3T3 cells. *Nature* 1985, **313**, 241–242.
89. Smith MR, DeGuidicibus SJ, Stacey DW. Requirement for c-ras proteins during viral oncogene transformation. *Nature* 1986, **320**, 540–543.
90. Gibbs JB, Oliff A. Pharmaceutical research in molecular oncology. *Cell* 1994, **79**, 193–198.
91. Lerner EC, Hamilton AD, Sebti SM. Inhibition of Ras prenylation: a signalling target for novel anti-cancer drug design. *Anti-Cancer Drug Design* 1997, **12**, 229–238.
92. Sebti S, Hamilton A. Inhibitors of prenyl transferases. *Curr Opin Oncol* 1997, **9**, 557–561.
93. Stacey DW, Feig LA, Gibbs JB. Dominant inhibitory Ras mutants selectively inhibit the activity of either cellular or oncogenic Ras. *Mol Cell Biol* 1991, **11**, 4053–4064.
94. Hall-Jackson CA, Eyers PA, Cohen P, *et al*. Paradoxical activation of Raf by a novel Raf inhibitor. *Chem Biol* 1999, **6**(8), 559–568.
95. Hall-Jackson CA, Goedert M, Hedge P, Cohen P. Effect of SB 203580 on the activity of c-Raf *in vitro* and *in vivo*. *Oncogene* 1999, **18**(12), 2047–2054.
96. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci USA* 1995, **92**(17), 7686–7689.
97. Sebolt-Leopold JS, Dudley DT, Herrera R, *et al*. Blockade of the MAP kinase pathway suppresses growth of colon tumors *in vivo*. *Nat Med* 1999, **5**(7), 810–816.
98. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci* 1997, **22**(7), 267–272.
99. Khwaja A. Akt is more than just a bad kinase. *Nature* 1999, **401**, 33–34.
100. Shayesteh L, Lu Y, Kuo WL, *et al*. *PIK3CA* is implicated as an oncogene in ovarian cancer. *Nat Genet* 1999, **21**(1), 99–102.
101. Stambolic V, Suzuki A, de la Pompa JL, *et al*. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* 1998, **95**(1), 29–39.
102. Vlahos CJ. Phosphatidylinositol 3-kinase inhibitors and their effects on cell signalling pathways. *Drugs of the Future* 1995, **20**(2), 165–171.
103. Cross MJ, Hodgkin MN, Plumb JA, *et al*. Inhibition of phospholipid signalling and proliferation of Swiss 3T3 cells by the wortmannin analogue demethoxyviridin. *Biochim Biophys Acta* 1997, **1362**(1), 29–38.
104. Schultz RM, Merriman RL, Andis SL, *et al*. *In vitro* and *in vivo* antitumor activity of the phosphatidylinositol-3-kinase inhibitor, wortmannin. *Anticancer Res* 1995, **15**(4), 1135–1139.
105. Dierick H, Bejsovec A. Cellular mechanisms of wingless/Wnt signal transduction. *Curr Top Dev Biol* 1999, **43**, 153–190.
106. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Ann Rev Cell Dev Biol* 1998, **14**, 59–88.
107. Bullions LC, *et al*. The role of beta-catenin in cell adhesion, signal transduction, and cancer. *Curr Opin Oncol* 1998, **10**(1), 81–87.
108. Polakis P. The oncogenic activation of beta-catenin. *Curr Opin Genet Dev* 1999, **9**(1), 15–21.
109. Dierick H, Bejsovec A. Cellular mechanisms of wingless/Wnt signal transduction. *Curr Top Dev Biol* 1999, **43**, 153–190.
110. Peters JM, McKay RM, McKay JP, Graff JM. Casein kinase I transduces Wnt signals. *Nature* 1999, **401**, 345–350.
111. Bienz M. APC: the plot thickens. *Curr Opin Genet Dev* 1999, **9**, 595–603.
112. Willert K, Shibamoto S, Nusse R. Wnt-induced dephosphorylation of axin releases beta-catenin from the axin complex. *Genes Dev* 1999, **13**(14), 1768–1773.
113. Eastman Q, Grosschedl R. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. *Curr Opin Cell Biol* 1999, **11**(2), 233–240.
114. Nusse R, van Ooyen A, Cox D, Fung YK, Varmus H. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature* 1984, **307**, 131–136.
115. Brown AM, Wildin RS, Prendergast TJ, Varmus HE. A retrovirus vector expressing the putative mammary oncogene int-1 causes partial transformation of a mammary epithelial cell line. *Cell* 1986, **46**(7), 1001–1009.
116. Miyoshi Y, Nagase H, Ando H, *et al*. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1992, **1**(4), 229–233.
117. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science* 1997, **275**, 1790–1792.
118. Miyoshi Y, Iwao K, Nagasawa Y, *et al*. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 1998, **58**, 2524–2527.
119. Kolligs FT, Hu G, Dang CV, Fearon ER. Neoplastic transformation of RK3E by mutant beta-catenin requires deregulation of Tcf/Lef transcription but not activation of c-myc expression. *Mol Cell Biol* 1999, **19**, 5696–5706.
120. Kim K, Daniels KJ, Hay ED. Tissue-specific expression of beta-catenin in normal mesenchyme and uveal melanomas and its effect on invasiveness. *Exp Cell Res* 1998, **245**(1), 79–90.
121. Morgan DO. Principles of CDK regulation. *Nature* 1995, **374**, 131–134.
122. Nasmyth K. Viewpoint: putting the cell cycle in order. *Science* 1996, **274**, 1643–1645.
123. Roberts JM. Evolving ideas about cyclins. *Cell* 1999, **98**(2), 129–132.
124. Bartkova J, Lukas J, Bartek J. Aberrations of the G1- and G1/S-regulating genes in human cancer. *Prog Cell Cycle Res* 1997, **3**, 211–220.
125. Tsihlias J, Kapusta L, Slingerland J. The prognostic significance of altered cyclin-dependent kinase inhibitors in human cancer. *Ann Rev Med* 1999, **50**, 401–423.
126. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer. *Adv Cancer Res* 1996, **68**, 67–108.
127. Sherr CJ. Cancer cell cycles. *Science* 1996, **274**, 1672–1677.
128. Senderowicz AM, Headlee D, Stinson SF, *et al*. Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. *Clin Oncol* 1998, **16**(9), 2986–2999.
129. Losiewicz MD, Carlson BA, Kaur G, Sausville EA, Worland PJ. Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochem Biophys Res Commun* 1994, **201**, 589–595.
130. Meijer L. Chemical inhibitors of cyclin-dependent kinases. *Trends in Cell Biology* 1996, **6**, 393–397.
131. Schnier JB, Kaur G, Kaiser A, *et al*. Identification of cytosolic aldehyde dehydrogenase 1 from non-small cell lung carcinomas as a flavopiridol-binding protein. *FEBS Lett* 1999, **454**, 100–104.
132. Hooijberg JH, Broxterman HJ, Scheffer GL, *et al*. Potent interaction of flavopiridol with MRP1. *Br J Cancer* 1999, **81**, 269–276.
133. Hoessel R, Leclerc S, Endicott JA, *et al*. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nat Cell Biol* 1999, **1**(1), 60–67.
134. Gray N, Detivaud L, Doerig C, Meijer L. ATP-site directed inhibitors of cyclin-dependent kinases. *Curr Med Chem* 1999, **6**(9), 859–875.
135. Gray NS, Wodicka L, Thunnissen AM, *et al*. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* 1998, **281**, 533–538.
136. Chang YT, Gray NS, Rosania GR, *et al*. Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors. *J Med Chem* 1999, **42**(15), 2909–2919.

137. Garrett MD, Fattaey ACDK. inhibition and cancer therapy. *Curr Opin Genet Dev* 1999, **9**, 104–111.
138. Nishio K, Ishida T, Arioka H, *et al*. Antitumor effects of butyrolactone I, a selective cdc2 kinase inhibitor, on human lung cancer cell lines. *Anticancer Res* 1996, **16**(6B), 3387–3395.
139. Yamamoto H, Monden T, Miyoshi T, *et al*. Cdk2/cdc2 expression in colon carcinogenesis and effects of cdk2/cdc2 inhibitor in colon cancer cells. *Int J Oncol* 1998, **13**(2), 233–239.
140. Schultz C, Link A, Leost M, *et al*. Paullones, a series of cyclin-dependent kinase inhibitors: synthesis, evaluation of CDK1/cyclin B inhibition, and *in vitro* antitumor activity. *J Med Chem* 1999, **42**(15), 2909–2919.
141. Zaharevitz DW, Gussio R, Leost M, *et al*. Discovery and initial characterization of the paullones, a novel class of small-molecule inhibitors of cyclin-dependent kinases. *Cancer Res* 1999, **59**(11), 2566–2569.
142. Kent LL, Hull-Campbell NE, Lau T, Wu JC, Thompson SA, Nori M. Characterization of novel inhibitors of cyclin-dependent kinases. *Biochem Biophys Res Commun* 1999, **260**(3), 768–774.
143. Fahraeus R, Paramio JM, Ball KL, Lain S, Lane DP. Inhibition of pRb phosphorylation and cell-cycle progression by a 20-residue peptide derived from p16CDKN2/INK4A. *Curr Biol* 1996, **6**(1), 84–91.
144. Ball KL, Lain S, Fahraeus R, Smythe C, Lane DP. Cell-cycle arrest and inhibition of Cdk4 activity by small peptides based on the carboxy-terminal domain of p21WAF1. *Curr Biol* 1997, **7**(1), 71–80.
145. Fahraeus R, Lain S, Ball KL, Lane DP. Characterization of the cyclin-dependent kinase inhibitory domain of the INK4 family as a model for a synthetic tumour suppressor molecule. *Oncogene* 1998, **16**(5), 587–596.
146. McConnell BB, Starborg M, Brookes S, Peters G. Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts. *Curr Biol* 1998, **8**(6), 351–354.
147. Kato D, Miyazawa K, Ruas M, *et al*. Features of replicative senescence induced by direct addition of antennapedia-p16INK4A fusion protein to human diploid fibroblasts. *FEBS Lett* 1998, **427**(2), 203–208.
148. Colas P, Cohen B, Jessen T, Grishina I, McCoy J, Brent R. Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. *Nature* 1996, **380**, 548–550.
149. Cohen BA, Colas P, Brent R. An artificial cell-cycle inhibitor isolated from a combinatorial library. *Proc Natl Acad Sci USA* 1998, **95**(24), 14272–14277.
150. Johnson DG, Schneider-Broussard R. Role of E2F in cell cycle control and cancer. *Front Biosci* 1998, **3**, d447–448.
151. Helin K, Lees JA, Vidal M, Dyson N, Harlow E, Fattaey AA. cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. *Cell* 1992, **70**(2), 337–350.
152. Dyson N. The regulation of E2F by pRB-family proteins. *Genes Dev* 1998, **12**(15), 2245–2262.
153. Fabbrizio E, Le Cam L, Polanowska J, *et al*. Inhibition of mammalian cell proliferation by genetically selected peptide aptamers that functionally antagonize E2F activity. *Oncogene* 1999, **18**(30), 4357–4363.
154. Dynlacht BD, Flores O, Lees JA, Harlow E. Differential regulation of E2F transactivation by cyclin/cdk2 complexes. *Genes Dev* 1994, **8**(15), 1772–1786.
155. Krek W, Ewen ME, Shirodkar S, Arany Z, Kaelin Jr WG, Livingston DM. Negative regulation of the growth-promoting transcription factor E2F-1 by a stably bound cyclin A-dependent protein kinase. *Cell* 1994, **78**(1), 161–172.
156. Krek W, Xu G, Livingston DM. Cyclin A-kinase regulation of E2F-1 DNA binding function underlies suppression of an S phase checkpoint. *Cell* 1995, **83**(7), 1149–1158.
157. Kowalik TF, DeGregori J, Schwarz JK, Nevins JR. E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis. *J Virol* 1995, **69**(4), 2491–2500.
158. Qin XQ, Livingston DM, Kaelin Jr WG, Adams PD. Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. *Proc Natl Acad Sci USA* 1994, **91**(23), 10918–10922.
159. Chen YN, Sharma SK, Ramsey TM, *et al*. Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc Natl Acad Sci USA* 1999, **96**(8), 4325–4329.
160. Lees JA, Weinberg RA. Tossing monkey wrenches into the clock: new ways of treating cancer. *Proc Natl Acad Sci USA* 1999, **96**(8), 4221–4223.
161. Nigg EA. Polo-like kinases: positive regulators of cell division from start to finish. *Curr Opin Cell Biol* 1998, **10**(6), 776–783.
162. Bischoff JR, Plowman GD. The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis. *Trends Cell Biol* 1999, **9**(11), 454–459.
163. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994, **54**(18), 4855–4878.
164. Carson DA, Lois A. Cancer progression and p53. *Lancet* 1995, **346**, 1009–1011.
165. Lane DP. Cancer. p53, guardian of the genome. *Nature* 1992, **358**, 15–16.
166. Giaccia AJ, Kastan MB. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev* 1998, **12**(19), 2973–2983.
167. Prives C, Hall PA. The p53 pathway. *J Pathol* 1999, **187**(1), 112–126.
168. Agarwal ML, Taylor WR, Chernov MV, Chernova OB, Stark GR. The p53 network. *J Biol Chem* 1998, **273**(1), 1–4.
169. Ko LJ, Prives C. p53: puzzle and paradigm. *Genes Dev* 1996, **10**(9), 1054–1072.
170. Habib NA, Hodgson HJ, Lemoine N, Pignatelli M. A phase I/II study of hepatic artery infusion with wtp53-CMV-Ad in metastatic malignant liver tumours. *Hum Gene Ther* 1999, **10**(12), 2019–2034.
171. Clayman GL, Frank DK, Bruso PA, Goepfert H. Adenovirus-mediated wild-type p53 gene transfer, as a surgical adjuvant in advanced head and neck cancers. *Clin Cancer Res* 1999, **5**(7), 1715–1722.
172. Roth JA, Nguyen D, Lawrence DD, *et al*. Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. *Nat Med* 1996, **2**(9), 985–991.
173. Estreicher A, Iggo R. Retrovirus-mediated p53 gene therapy. *Nat Med* 1996, **2**(11), 1163.
174. Selivanova G, Ryabchenko L, Jansson E, Iotsova V, Wiman KG. Reactivation of mutant p53 through interaction of a C-terminal peptide with the core domain. *Mol Cell Biol* 1999, **19**(5), 3395–3402.
175. Selivanova G, Kawasaki T, Ryabchenko L, Wiman KG. Reactivation of mutant p53: a new strategy for cancer therapy. *Semin Cancer Biol* 1998, **8**(5), 369–378.
176. Wiman KG. New p53-based anti-cancer therapeutic strategies. *Med Oncol* 1998, **15**(4), 222–228.
177. Selivanova G, Iotsova V, Okan I, *et al*. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nat Med* 1997, **3**(6), 632–638.
178. Prives C. Signaling to p53: breaking the MDM2-p53 circuit. *Cell* 1998, **95**(1), 5–8.
179. Picksley SM, Vojtesek B, Sparks A, Lane DP. Immunochemical analysis of the interaction of p53 with MDM2;- fine mapping of the MDM2 binding site on p53 using synthetic peptides. *Oncogene* 1994, **9**(9), 2523–2529.
180. Bottger V, Bottger A, Howard SF, *et al*. Identification of novel mdm2 binding peptides by phage display identification of novel mdm2 binding peptides by phage display. *Oncogene* 1996, **13**(10), 2141–2147.
181. Bottger A, Bottger V, Sparks A, Liu WL, Howard SF, Lane DP. Design of a synthetic Mdm2-binding mini protein that activates the p53 response *in vivo*. *Curr Biol* 1997, **7**(11), 860–869.
182. Lain S, Midgley C, Sparks A, Lane EB, Lane DP. An inhibitor of nuclear export activates the p53 response and induces the localization of HDM2 and p53 to U1A-positive nuclear bodies associated with the PODs. *Exp Cell Res* 1999, **248**(2), 457–472.
183. Bischoff JR, Kirn DH, Williams A, *et al*. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996, **274**, 373–376.
184. Kirn D, Hermiston T, McCormick F. ONYX-015: clinical data are encouraging. *Nat Med* 1998, **4**(12), 1341–1342.
185. O'Connor PM. Mammalian G1 and G2 phase checkpoints. *Cancer Surv* 1997, **29**, 151–182.
186. O'Connor PM, Fan S. DNA damage checkpoints: implications for cancer therapy. *Prog Cell Cycle Res* 1996, **2**, 165–173.

187. Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA, O'Connor PM. UCN-01: a potent abrogator of G2 checkpoint function in cancer cells with disrupted p53. *J Natl Cancer Inst* 1996, **88**(14), 956–965.
188. Yu L, Orlandi L, Wang P, *et al*. UCN-01 abrogates G2 arrest through a Cdc2-dependent pathway that is associated with inactivation of the Wee1Hu kinase and activation of the Cdc25C phosphatase. *J Biol Chem* 1998, **273**(50), 33455–33464.
189. Sausville EA, Lush RD, Headlee D, *et al*. Clinical pharmacology of UCN-01: initial observations and comparison to preclinical models. *Cancer Chemother Pharmacol* 1998, **42**(Suppl.), S54–59.
190. Komarov PG, Komarova EA, Kondratov RV, *et al*. A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. *Science* 1999, **285**, 1733–1737.
191. Conlon I, Raff M. Size control in animal development. *Cell* 1999, **96**(2), 235–244.
192. Leevers SJ. All Creatures Great and Small. *Science* 1999, **285**, 2082–2083.
193. Brooks RF. Continuous protein synthesis is required to maintain the probability of entry into S phase. *Cell* 1977, **12**(1), 311–317.
194. Sonenberg N. Translation factors as effectors of cell growth and tumorigenesis. *Curr Opin Cell Biol* 1993, **5**(6), 955–960.
195. Clemens MJ, Bommer UA. Translational control: the cancer connection. *Int J Biochem Cell Biol* 1999, **31**(1), 1–23.
196. Sonenberg N, Gingras AC. The mRNA 5′ cap-binding protein eIF4E and control of cell growth. *Curr Opin Cell Biol* 1998, **10**(2), 268–275.
197. Jagus R, Joshi B, Barber GN. PKR apoptosis and cancer. *Int J Biochem Cell Biol* 1999, **31**(1), 123–138.
198. Willis AE. Translational control of growth factor and proto-oncogene expression. *Int J Biochem Cell Biol* 1999, **31**(1), 73–86.
199. Dever TE. Translation initiation: adept at adapting. *Trends Biochem Sci* 1999, **24**(10), 398–403.
200. Aktas H, Fluckiger R, Acosta JA, Savage JM, Palakurthi SS, Halperin JA. Depletion of intracellular Ca2+ stores, phosphorylation of eIF2alpha, and sustained inhibition of translation initiation mediate the anticancer effects of clotrimazole. *Proc Natl Acad Sci USA* 1998, **95**(14), 8280–8285.
201. McKendrick L, Pain VM, Morley SJ. Translation initiation factor 4E. *Int J Biochem Cell Biol* 1999, **31**(1), 31–35.
202. Keiper BD, Gan W, Rhoads RE. Protein synthesis initiation factor 4G. *Int J Biochem Cell Biol* 1999, **31**(1), 37–41.
203. Raught B, Gingras AC. eIF4E activity is regulated at multiple levels. *Int J Biochem Cell Biol* 1999, **31**(1), 43–57.
204. Rhoads RE. Signal transduction pathways that regulate eukaryotic protein synthesis. *J Biol Chem* 1999, **274**(43), 30337–30340.
205. Gingras AC, Gygi SP, Raught B, *et al*. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* 1999, **13**(11), 1422–1437.
206. Minich WB, Balasta ML, Goss DJ, Rhoads RE. Chromatographic resolution of *in vivo* phosphorylated and nonphosphorylated eukaryotic translation initiation factor eIF-4E: increased cap affinity of the phosphorylated form. *Proc Natl Acad Sci USA* 1994, **91**(16), 7668–7672.
207. Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T, Sonenberg N. Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. *EMBO J* 1999, **18**(1), 270–279.
208. Brattstrom C, Tyden G, Sawe J, Herlenius G, Claesson K, Groth CG. A randomized, double-blind, placebo-controlled study to determine safety, tolerance, and preliminary pharmacokinetics of ascending single doses of orally administered sirolimus (rapamycin) in stable renal transplant recipients. *Transplant Proc* 1996, **28**(2), 985–986.
209. Hosoi H, Dilling MB, Shikata T, *et al*. Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. *Cancer Res* 1999, **59**(4), 886–894.
210. Ciechanover A. The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J* 1998, **17**(24), 7151–7160.
211. Hershko A, Ciechanover A. The ubiquitin system. *Ann Rev Biochem* 1998, **67**, 425–479.
212. Spataro V, Norbury C, Harris AL. The ubiquitin-proteasome pathway in cancer. *Br J Cancer* 1998, **77**(3), 448–455.
213. Schwartz AL, Ciechanover A. The ubiquitin-proteasome pathway and pathogenesis of human diseases. *Ann Rev Med* 1999, **50**, 57–74.
214. Jarrousse AS, Gautier K, Apcher S, *et al*. Relationships between proteasomes and viral gene products. *Mol Biol Rep* 1999, **26**(1–2), 113–117.
215. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 1993, **75**(3), 495–505.
216. Steeg PS, Abrams JS. Cancer prognostics: past, present and p27. *Nat Med* 1997, **3**(2), 152–154.
217. Porter PL, Malone KE, Heagerty PJ, *et al*. Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. *Nat Med* 1997, **3**(2), 222–225.
218. Catzavelos C, Bhattacharya N, Ung YC, *et al*. Decreased levels of the cell-cycle inhibitor p27Kip1 protein: prognostic implications in primary breast cancer. *Nat Med* 1997, **3**(2), 227–230.
219. Loda M, Cukor B, Tam SW, *et al*. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* 1997, **3**(2), 231–234.
220. Foo SY, Nolan GP. NF-kappaB to the rescue: RELs, apoptosis and cellular transformation. *Trends Genet* 1999, **15**(6), 229–235.
221. Chu ZL, McKinsey TA, Liu L, Gentry JJ, Malim MH, Ballard DW. Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappaB control. *Proc Natl Acad Sci USA* 1997, **94**(19), 10057–10062.
222. Sharma HW, Narayanan R. The NF-kappaB transcription factor in oncogenesis. *Anticancer Res* 1996, **16**(2), 589–596.
223. Fenteany G, Schreiber SL. Lactacystin, proteasome function, and cell fate. *J Biol Chem* 1998, **273**(15), 8545–8548.
224. Isoe T, Naito M, Shirai A, Hirai R, Tsuruo T. Inhibition of different steps of the ubiquitin system by cisplatin and aclarubicin. *Biochim Biophys Acta* 1992, **1117**(2), 131–135.
225. Lee DH, Goldberg A. Proteasome inhibitors: valuable new tools for cell biologists. *Trends Cell Biol* 1998, **8**(10), 397–403.
226. Adams J, Palombella VJ, Sausville EA, *et al*. Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res* 1999, **59**(11), 2615–2622.
227. Patton EE, Willems AR, Tyers M. Combinatorial control in ubiquitin dependent proteolysis: don't Skp the F-box hypothesis. *Trends Genet* 1998, **14**(6), 236–243.
228. Lisztwan J, Imbert G, Wirbelauer C, Gstaiger M, Krek W. The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. *Genes Dev* 1999, **13**(14), 1822–1833.
229. Sheaff RJ, Groudine M, Gordon M, Roberts JM, Clurman BE. Cyclin E-CDK2 is a regulator of p27Kip1. *Genes Dev* 1997, **11**(11), 1464–1478.
230. Luo RX, Dean DC. Chromatin remodelling and transcriptional regulation. *J Natl Cancer Inst* 1999, **91**(15), 1288–1294.
231. Kouzarides T. Histone acetylases and deacetylases in cell proliferation. *Curr Opin Genet Dev* 1999, **9**(1), 40–48.
232. Saito A, Yamashita T, Mariko Y, *et al*. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked *in vivo* antitumor activity against human tumors. *Proc Natl Acad Sci USA* 1999, **96**(8), 4592–4597.
233. Mayer MP, Bukau B. Molecular chaperones: the busy life of Hsp90. *Curr Biol* 1999, **9**(9), R322–325.
234. Schulte TW, Neckers LM. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother Pharmacol* 1998, **42**(4), 273–279.
235. Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J Natl Cancer Inst* 1999, **91**(22), 1–10.
236. Dolan ME, Pegg AE. O6-benzylguanine and its role in chemotherapy. *Clin Cancer Res* 1997, **3**(6), 837–847.
237. Griffin RJ, Crutin NJ, Newell DR, Golding BT, Durkacz BW, Calvert AH. The role of inhibitors of poly(ADP-ribose) polymerase as resistance-modifying agents in cancer therapy. *Biochimie* 1995, **77**(6), 408–422.
238. Jackson SP. DNA damage detection by DNA dependent protein kinase and related enzymes. *Cancer Surv* 1996, **28**, 261–279.

239. Hahn WC, Stewart SA, Brooks MW, *et al*. Inhibition of telomerase limits the growth of human cancer cells. *Nat Med* 1999, **5**(10), 1164–1170.
240. Zumstein LA, Lundbald V. Telomeres: has cancer's Achilles' heel been exposed? *Nat Med* 1999, **5**(10), 1129–1130.
241. De Lange T, Jacks T. For better or worse? Telomerase inhibition and cancer. *Cell* 1999, **98**(3), 273–275.
242. Perry PJ, Kelland LR. Telomeres and telomerase: targets for cancer chemotherapy. *Exp Opin Ther Patents* 1998, **8**(12), 1567–1586.
243. Drews J. *In Quest of Tomorrow's Medicines*. New York, Springer-Verlag, 1999.
244. Yu L, Graves P, Tempcyzk A, Sausville EA, Piwnica-Worms H, O'Connor PM. The checkpoint kinase Chk1 is a target of the anticancer agent UCN-01. *Proc AACR* 1999, **40**, 304.
245. De Benedetti A, Harris AL. EIF4E expression in tumours: its possible role in progression of malignancies. *Int J Biochem Cell Biol* 1999, **31**(1), 59–72.
246. Pharmaprojects, Richmond, PJB Publications, 1999.
247. Le Coutre P, Mologni L, Cleris L, *et al*. *In vivo* eradication of human bcr/abl-positive leukemia cells with an abl kinase inhibitor. *J Natl Cancer Inst* 1999, **91**(2), 163–168.

Acknowledgements—The authors work is primarily funded by the U.K. Cancer Research Campaign (CRC) and the Institute for Cancer Research. We would also like to thank John F. Lyons for a discussion on protein translation and Lesley Robertson and David C. Bedford for help with manuscript preparation.