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New targets and challenges in the molecular therapeutics of cancer

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The past 20 years have seen an explosion of information on the molecular changes that lead to cancer. The pathways that have been uncovered include many targets for the development of novel therapeutics. Several such drugs have been approved for clinical use and many additional drugs and targets are now being evaluated in preclinical studies. These new drugs may exhibit impressive therapeutic activity, but this is often restricted to a subpopulation of cancers with a particular molecular change. Moreover, toxicity or even antagonism may result from off-target effects of the drugs. Accordingly, it will be critical to stratify patients for treatment based on the propensity of their tumours to respond. In addition, defining the appropriate dose of targeted agents to administer is challenging; early clinical trial designs must include assays to define the effective biological dose, in addition to more traditional end-points such as the maximum tolerable dose. These and many other challenges exist in the preclinical and clinical development of these drugs.

Introduction

We have entered a new and exciting era of 'molecular therapeutics' for cancer, also called 'targeted therapeutics'. But is this really a new concept? Every drug ever developed has a molecular target, so why is this terminology only now being used? Perhaps the difference lies in the way the earlier drugs were discovered. Many of these drugs were selected because they were cytotoxic to cancer cells *in vitro*, even though their specific molecular target was not known. For example, both vinblastine and paclitaxel were discovered by serendipity and their target was only later defined as the microtubules. However, development of drugs designed to inhibit a known target is not unique to the modern era of targeted therapeutics. The earliest antimetabolites were designed with a specific target in mind and the resulting drugs were effective at inhibiting DNA or RNA synthesis, even though inhibition of the anticipated target is not always responsible for the efficacy of some of these agents.

For over 50 years, screening efforts identified numerous drugs that elicited cytotoxicity in preclinical models, although this was rarely selective for tumour cells [1]. Some of these drugs had significant impact in certain cancers, but the number of major classes of drugs remains limited, consisting primarily of alkylating agents, antimetabolites, tubulin and topoisomerase inhibitors.

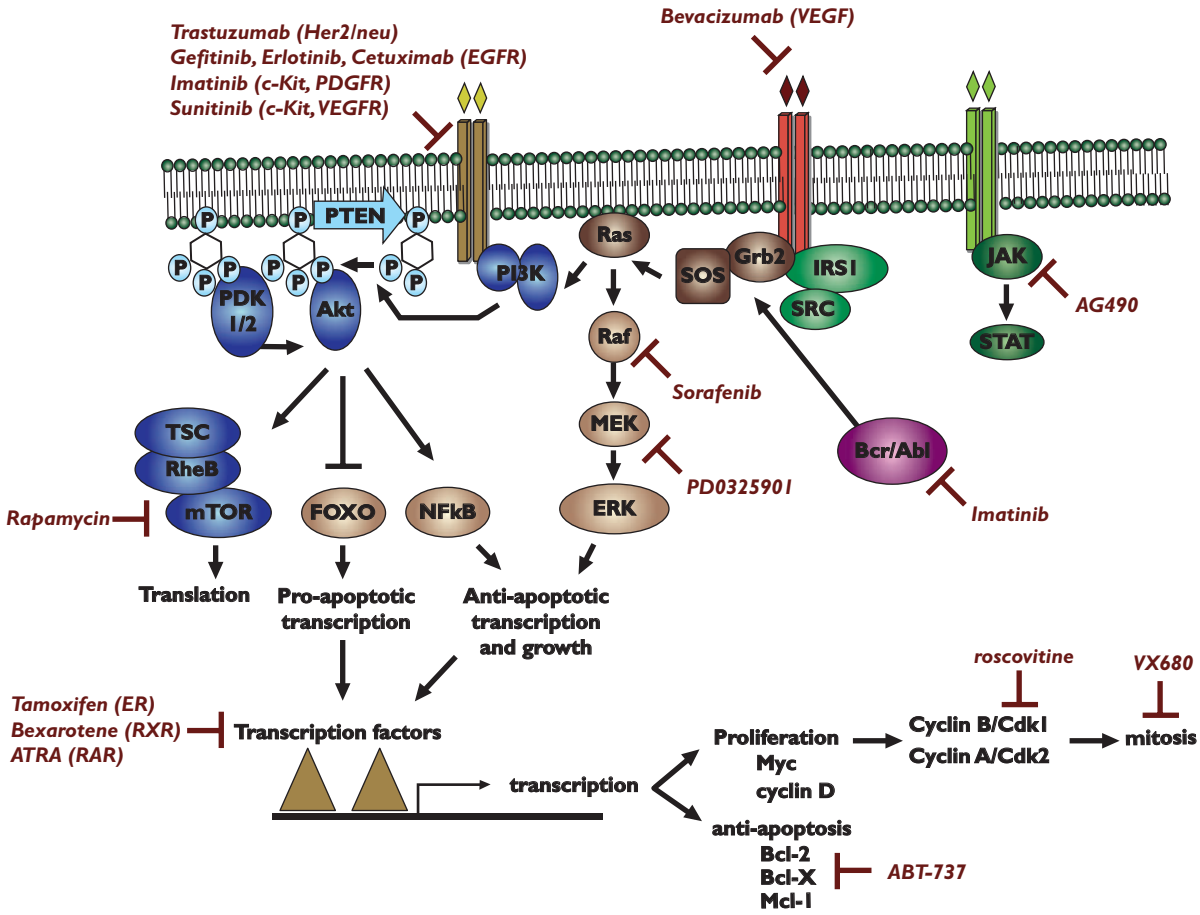

Figure 1

An overview of the major signal transduction pathways regulating cell proliferation and survival. The sites of inhibition by many of the drugs listed in the Tables are identified

Numerous analogues of these drugs were developed and tested and, whereas a few proved more effective and/or more tolerable than the parent compounds, most did not have significantly better efficacy than the parent drug.

By 1990, the cancer biology revolution had taken hold and new information was rapidly accumulating on the alterations in signal transduction pathways that explain the propensity of tumour cells to survive and grow. Proteins in these pathways were validated as targets and new drugs were developed, some of which had impressive clinical activity which generated the excitement of this new era of cancer therapeutics (Figure 1). As we learn more about tumours, many new targets are being realized; examples can be found in the pathways and processes of transcription, mitosis, apoptosis, cell cycle checkpoints and protein turnover. Greater understanding of these targets, and their roles in cancer biology, has great potential to yield therapies that significantly impact patient outcomes.

Once a new target is identified, high-throughput *in vitro* screening assays, often involving a million or more compounds, are commonly undertaken to find a lead compound that inhibits the reaction. Initial lead compounds are often active in the micromolar range and further analogue synthesis is required to develop more potent and selective inhibitors with other desirable properties such as prolonged plasma half-life or oral bioavailability. Many years of work are required to get a compound through cell culture models, animal tumour models, animal toxicity and into clinical trials. Few compounds survive this tortuous journey, but several have now been approved by the United States Food and Drug Administration and are used in clinical practice (Table 1). Many other targets are being evaluated and candidate drugs are at various stages of preclinical and clinical development (Table 2). A recent review of the history of cancer chemotherapy noted a survey in 2003 that identified at least 395 agents in cancer clinical trials

Table 1
Established signal transduction inhibitors in clinical practice

Target	Drug	FDA approval	Comments
Oestrogen receptor	Tamoxifen	1977	Used primarily to prevent recurrence in oestrogen-positive breast cancer
Retinoic acid receptor (RAR)	All-trans-retinoic acid (ATRA, Tretinoin)	1995	Approved for acute promyelocytic leukaemia
CD20 (surface receptor on B cells)	Rituximab	1997	Approved for refractory B-cell nonHodgkin's lymphoma
Her2/neu receptor	Trastuzumab (Herceptin)	1998	Approved for Her2+ metastatic breast cancer
Retinoid X receptor (RXR)	Bexarotene (Targretin)	1999	Approved for cutaneous T-cell lymphoma
Bcr/Abl kinase	Imatanib (Gleevec, Glivec, STI571)	2001	Approved for chronic myelogenous leukaemia
c-Kit	Imatanib (Gleevec, Glivec, STI571)	2002	Approved for gastrointestinal stromal tumours
Proteasome	Bortezomib (Velcade, PS341)	2003	Approved for multiple myeloma
Epidermal growth factor receptor (EGFR/ErbB1)	Gefitinib (Iressa)	2003	Approved for metastatic nonsmall cell lung cancer but subsequent trials showed no response
	Erlotinib (Tarceva, OSI-774)	2004	Approved for metastatic nonsmall cell lung cancer (The efficacy of both gefitinib and erlotinib are now thought to be limited to tumours with mutant EGFR)
	Cetuximab (Erbitux)	2004	Approved for metastatic colorectal cancer
Vascular endothelial growth factor (VEGF)	Bevacizumab (Avastin)	2004	Approved for metastatic colorectal cancer in combination with 5-fluorouracil
RAF kinase (BRAF)	Sorafenib (Nexavar)	2005	Approved for renal cell carcinoma. Inhibits many receptor kinases and the most important action has not been established
Vascular endothelial growth factor receptor (VEGFR)	Sunitinib (Sutent, SU11248)	2006	Approved for GIST. Also inhibits c-Kit, PDGFR and FLT3. Phase II activity in renal cell, breast, and neuroendocrine tumours

A note on terminology: a name ending in 'mab' is a monoclonal antibody; if ending in 'nib' it is a small molecule inhibitor.

[1]. In addition to the major pharmaceutical industry, it has been estimated that more than 700 small biotechnology companies have been formed in the USA to develop cancer therapies based on molecular targets, all vying for a piece of a multibillion dollar world market. Here, we will review some of the lessons learned and the difficulties that still need to be surmounted.

There is no perfect drug (yet)

The perfect drug would target a characteristic that is unique to the tumour and therefore would have no impact on the patient's normal tissues. Such characteristics would be likely to derive from a mutagenic change that is required for tumour growth and survival. The best-known example in this regard is the 9;22 chromosomal translocation recognized as the Philadelphia chromosome, a hallmark of chronic myelogenous leukaemia (CML). This translocation fuses the amino-terminus of Bcr to the majority of the Abl gene, resulting in inappropriate cytosolic Abl kinase activity. Imatinib mesylate (Gleevec Glivec, STI-571) inhibits Abl kinase activity and is selectively cytotoxic to cells that depend on Bcr/Abl for survival, i.e. CML [2].

Unfortunately, imatinib is not perfect. Many tumours circumvent the efficacy of imatinib by mutating the drug-binding pocket in the Abl kinase [3]. Alternative drugs have now been developed to inhibit these mutant kinases [4], but we should equally expect mutations to arise that will elicit resistance to these drugs.

Another reason why imatinib is not a perfect drug is that it inhibits multiple targets. This is a mixed blessing. On the one hand, imatinib inhibits c-Kit, which is over-

Table 2
Selected novel targets with lead drugs in clinical trials or preclinical assessment

Target	Drug(s)	Comments
Akt	RX-0201, SR13668	In Phase I/II trials
Ataxia teclangiectasia mutated (ATM)	KU55933	A cell cycle checkpoint inhibitor that sensitizes cells to many DNA-damaging agents
Aurora kinase	VX680	Inhibits mitosis; in Phase I trials
Bcl-2 and Bcl-X	ABT-737	Blocks binding of anti-apoptotic Bcl-2 and Bcl-X to their pro-apoptotic partners. Potently suppresses tumour growth in some animal models
Chk1	UCN-01	Inhibitor of DNA damage-induced cell cycle checkpoint arrest; in Phase I/II trials
Cyclin-dependent kinase	R-roscovitine (Seliciclib, CYC682)	Induces cell cycle arrest generally in G_1 and G_2 phases. Originally thought to target Cdk1/2 but now recognized to inhibit protein translation by inhibiting Cdk 7/9; in Phase I/II trials
Farnesyl transferase	Tipifarnib (Zarnestra, R115777)	Prevents trafficking of G proteins (e.g. Ras, Rho) to plasma membrane; in Phase III trials (haematological malignancies)
Histone deacetylase	SAHA, Pivanex (AN-9)	In Phase II trials.
	Valproic acid (divalproex, Depacon, Depakote)	Approved as an anticonvulsant
Heat shock protein 90 (Hsp90)	17-allylaminogeldanamycin (17-AAG)	In Phase II trials
Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase	Atorvastatin (Lipitor)	Approved as anticholesterolaemics. All statins inhibit farnesylation and geranylgeranylation of G proteins such as Ras and Rho, preventing trafficking to plasma membrane
Janus kinase (JAK)	AG490	Inhibits the JAK/Stat signaling pathway; used primarily as a laboratory tool
MEK	PD 0325901	Inhibit the Raf/MEK/ERK signaling pathway; in Phase I/II trials
	ARRY-142886 (AZD6244)	
Molecular target of rapamycin (mTOR)	Rapamycin (sirolimus)	Approved as an immunosuppressant. Inhibits downstream in the Akt pathway. Rapamycin analogues in Phase I/II trials
	CCI-779, RAD001, AP23573	
p53	Nutlin	Inhibits p53:MDM2 binding leading to activation of p53
	Onyx-015	Oncolytic virus, selective replication in p53-deficient cells
Smothened (Hedgehog pathway)	Cyclopamine analogues	In preclinical development; leads have shown promise in animal models
Survivin	LY2181308	Antisense inhibits expression of survivin; in Phase II trials
	Tetra-O-methyl norhydro-guiaretic acid	Inhibits survivin transcription; in Phase I/II trials

Many academic institutions and pharmaceutical companies are generating inhibitors to many of these targets, and numerous analogues and other lead compounds are in development.

expressed in gastrointestinal stromal tumours (GIST); these tumours are particularly responsive to the drug. Imatinib also inhibits the tyrosine kinase activity of platelet-derived growth factor (PDGF), which potentially extends its efficacy to even more tumours. On the other hand, however, none of these targets is unique to tumours. Inhibition of tumour-specific Bcr/Abl must be put into context with the fact that imatinib equally inhibits the normal Abl kinase, which may protect cells from DNA damage [5]. This may complicate its use in combination with some conventional cytoxic agents. Furthermore, Abl, c-Kit, and PDGF are expressed on many normal cells. Although imatinib is well tolerated, it does produce clinical toxicities in many patients, including

fluid retention/oedema (~ 70% of patients), cutaneous reactions (~ 30%), mild nausea or diarrhoea (~ 50%) and musculoskeletal discomfort (~ 35%) [6]. Fluid retention and oedema may be related to inhibition of the Abl or PDGFR kinases, as mice with homozygous deletion of either kinase are prone to oedema [7, 8]. Likewise, it is speculated that diarrhoea may be related to inhibition of c-Kit in intestinal cells.

Oncogene addiction

Considering that imatinib inhibits many molecular targets in both tumour and normal cells, it is perhaps surprising that it has such a high therapeutic index for CML. Probably none of the currently targeted pathways is unique to tumours, hence there is always concern that inhibition of the target in normal cells will elicit toxicity to the patient. However, selective drug effects may be elicited in tumours that have become addicted to the particular signalling pathways. Such addiction can occur when the activated pathway is required for tumour growth and survival, but simultaneously induces feedback inhibition of alternate survival pathways. As a result, a tumour cell may die when the pathway is inhibited, while a normal cell retains the ability to survive by using alternate and/or redundant pathways.

The therapeutic efficacy of imatinib presumably results from addiction of CML cells to Bcr/Abl. Imatinib inhibits the Abl kinase which is present in both tumour and normal tissues, while the aberrant form, fused to Bcr in the tumour, results in addiction to this kinase. This is probably due to the resulting activation of several signalling pathways, including MEK → ERK and PI3-kinase → Akt (Figure 1) [2]. This addiction hypothesis is seen in other models, most notably in response to inhibitors of EGFR, mTOR and MEK. The story of the EGFR inhibitor gefitinib (Iressa) in patients with nonsmall cell lung cancer is enlightening in this regard. In these patients, response to gefitinib was infrequent, did not correlate with levels of EGFR and was higher among Japanese patients (27.5%) compared with a Western population (10.4%) [9]. Responding patients were found to have a gain-of-function mutation in the receptor and it is assumed that this makes the tumours addicted to this pathway [10, 11]. Cells with mutant EGFR preferentially activate the PI3-kinase → Akt and STAT pathways, which may explain their addiction [12]. However, more than 40 different mutations have been reported in EGFR, many with differential effects on downstream pathways, so the specific impact of each activated pathway remains to be defined [13]. Furthermore, gefitinib is clearly not selective for the mutant EGFR in the tumour, as most patients exhibit a skin rash which is thought to result from inhibition of normal EGFR in the skin.

The second example of addiction is illustrated by inhibition of mTOR, a downstream step in the PI3-kinase → Akt pathway, by rapamycin. Following receptor activation, PI3-kinase phosphorylates a membrane-bound inositol-lipid (Figure 1). The phosphoinositol provides a docking site for Akt at the membrane so it can be activated by other membrane-associated kinases. Dephosphorylation of inositol is caused by the phosphatase and tensin homologue (PTEN), which turns the signal off. Loss of PTEN is a frequent event in cancer and such tumours lack the ability to turn off this signal, thereby resulting in constitutively active Akt signalling. It has been shown that cells defective in PTEN are particularly sensitive to rapamycin [14]. This may be the easiest addiction model to understand because the loss of PTEN leads to constitutive activation of Akt and Akt is known to repress alternate survival pathways. The activated, and now unregulatable, Akt is dependent on mTOR to elicit its growth and survival effects, so that these cells may die when mTOR is inhibited.

Attempts have also been made to inhibit the MEK → Erk pathway, but the initial clinical results have not been encouraging [15]. This disappointment is probably due to inadequate inhibition of the signal transduction pathway at the administered dose. However, it has recently been shown that these inhibitors may be particularly active in tumours that have an activating Raf mutation, something that is fairly common in melanoma [16]. These tumours are presumably addicted to the MEK → Erk pathway. We must now wait to see whether MEK inhibitors will have efficacy in this patient population.

Synthetic lethal

Many of the genetic changes in tumours may not provide good targets for therapy, particularly in the case of tumour suppressors that have been deleted. Fortunately, loss of one gene/pathway can result in dependence on a second pathway. If this second pathway is inhibited, it is possible to kill tumour cells selectively. This strategy is well known in yeast genetics, where it is termed synthetic lethal [17]. Specifically, this term refers to two non-essential but redundant genes; if either gene is lost, then deletion of the second gene is lethal. Extending this concept to cancer treatment, identification of a drug that selectively inhibits one such pathway should only kill cells that are already defective in the second pathway.

Many defects that occur in cancer are found in pathways that respond to DNA damage. These pathways normally respond to DNA damage by activating cell cycle checkpoints that prevent cell cycle progression.

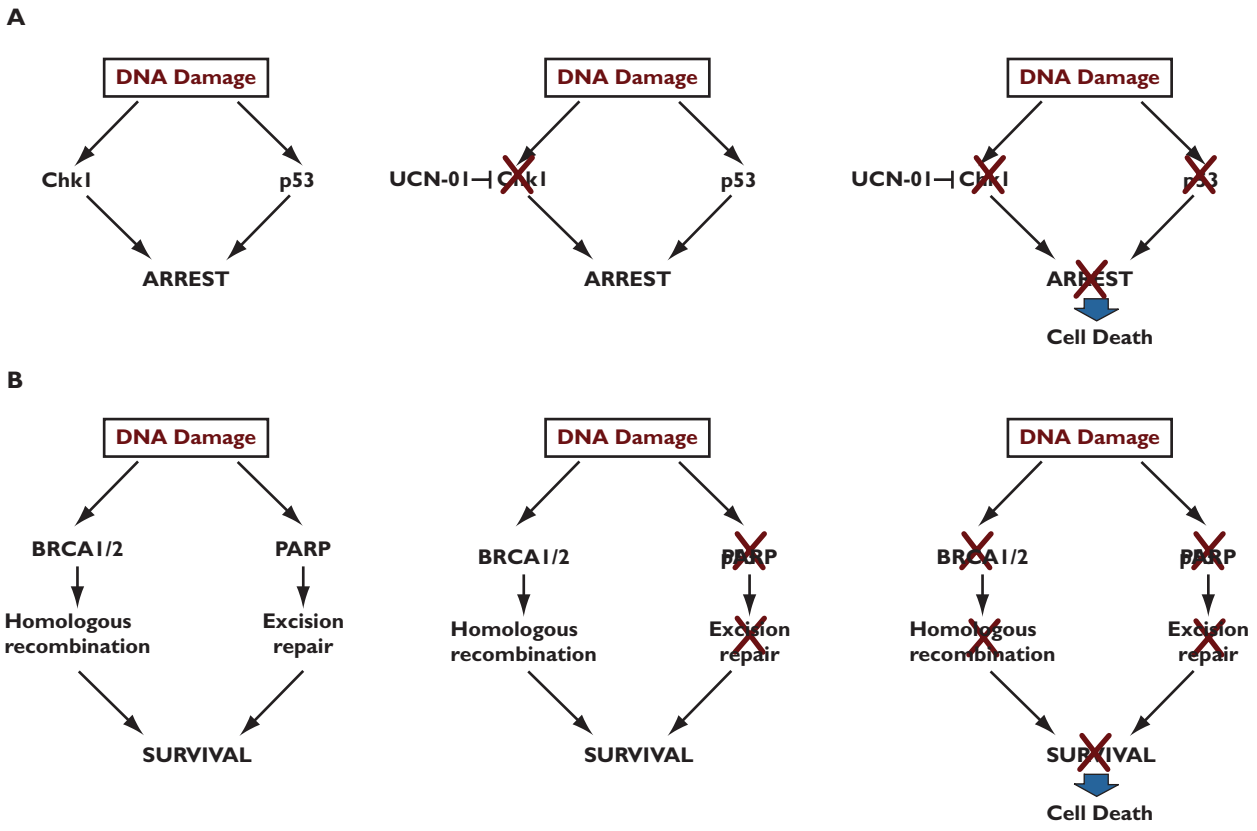

Figure 2

Two examples of a synthetic lethal strategy to kill tumour cells. (A) Chk1 and p53 are redundant, non-essential pathways, where inhibition of Chk1 can selectively kill p53-defective tumours. (B) Homologous recombination and excision repair can be redundant pathways for DNA repair, where inhibition of poly(ADP-ribose) polymerase (PARP) with one of a number of inhibitors can selectively kill BRCA1- or BRCA2-defective tumours

The arrest permits time for the cell to repair the damage, after which it can recover, survive and grow. The best-characterized DNA damage response pathway involves the p53 tumour suppressor protein, which is defective in more than 50% of human cancers. p53 plays two important roles: suppressing cell cycle progression when damage is detected, and activating apoptosis when cells have sustained lethal damage. The first of these pathways can be used in a synthetic lethal strategy (Figure 2). When DNA is damaged, p53 facilitates the induction of the cyclin-dependent kinase inhibitor p21waf1, which causes cell cycle arrest. However, p53 is a non-essential gene because there is an alternative, p53-independent pathway that can also arrest cells in response to DNA damage. This pathway involves the checkpoint proteins ATM/ATR and Chk1. Whereas neither pathway is essential for cell survival, the loss of both pathways makes cells hypersensitive to DNA damage. This situation can be exploited with inhibitors of ATM/ATR (caffeine) or Chk1 (UCN-01). Consequently, these drugs are particularly effective at sensitizing p53-defective cells to DNA damage [18, 19]. Caffeine is not tolerated at the required doses in humans, but ongoing Phase I clinical trials with UCN-01 suggest that adequate concentrations to inhibit Chk1 can be obtained *in vivo* [20].

Tumours defective in either BRCA1 or BRCA2 provide another example where a synthetic lethal strategy may be effective (Figure 2). These proteins participate in homologous recombination repair of DNA damage. It is now established that excision repair and homologous recombination repair can be non-essential, redundant pathways. Excision repair can be inhibited with novel drugs targeting poly(ADP-ribose) polymerase. Surprisingly, these drugs are selectively cytotoxic to cells defective in either BRCA1 or BRCA2 even in the absence of exogenous DNA damage, presumably because endogenous DNA damage is inadequately repaired [21, 22]. Whether the effectiveness of this approach can be enhanced by introducing exogenous DNA damage remains to be determined.

Selectivity

The need to search for drugs with increased potency is frequently overrated, as the more important issue is the selectivity of a drug for its target. Potency is really only meaningful in the context of drug toxicology, in reference to concentrations that can safely be achieved in patients. As an example, many people achieve plasma concentrations of caffeine in excess of 100 μM on a daily basis, achieving pharmacodynamic effects which, though inadequate to inhibit the checkpoints discussed above, are desired by those individuals.

Many newer anticancer drugs are not particularly selective, often inhibiting other activities in addition to the target within the same concentration range and commonly inhibiting many additional targets at higher concentrations. This point is highlighted in two papers, which showed many common inhibitors possessed unanticipated activities against an extensive array of protein kinases [23, 24]. The major reason many of these drugs lack selectivity is that they are ATP-mimetics, i.e. they compete with ATP for binding to the ATP binding pocket in the kinase. Although this pocket varies between kinases, it is architecturally well conserved, so that it is a rare compound that inhibits only one kinase. Some of these nonselective or 'dirty drugs' might possibly be more effective than a pure inhibitor, but only if the alternate targets are biologically relevant (e.g. many kinase inhibitors also inhibit VEGF signalling). Unfortunately, the real concern is that the lack of selectivity is more often due to 'off-target' effects that may lead to toxicity in patients. Hence, a selective drug that lacks the undesirable off-target effects, even if its potency is in the μM range (assuming appropriate solubility and pharmacokinetic properties), should have priority over less selective compounds with potencies in the low nM range. Furthermore, the search for more selective drugs should emphasize those that interact with the target at nonconserved sites and, in particular, avoid the ATP binding pocket. In this regard, one approach that is showing unexpected promise has been to target the interface between two proteins. These interacting surfaces were previously thought to be large areas that were not amenable to small molecule inhibitors, but recent developments of compounds that inhibit the binding of Bcl2 or Bcl-X to Bax [25], p53 binding to MDM2 [26] and Myc binding to Max [27] suggest that such pessimism was unwarranted.

Molecular pharmacodynamics

As mentioned above, ATP-mimetic drugs commonly inhibit many protein kinases in addition to the intended target. These off-target effects can elicit toxicity and some can even antagonize the desired therapeutic activity. The traditional paradigm of escalating drugs to their maximum tolerated dose (MTD) in Phase I clinical trials may not be the most appropriate end-point for early trials of targeted agents. It is equally, if not more important to know that a drug inhibits the desired target *in vivo*. With drugs that inhibit multiple targets, as they all appear to do, it is possible that a high dose may antagonize the desired effect and administration of a drug at its MTD may be counterproductive. This point is exemplified by our experience with the Chk1 inhibitor UCN-01 [28–30]. Chk1 inhibitors enhance cell killing by abrogating DNA damage-induced cell cycle arrest and driving the damaged cells through a lethal mitosis. Alone, Chk1 inhibitors are predicted to be nontoxic. However, UCN-01 also inhibits other targets and induces cell cycle arrest at concentrations above those that abrogate damage-induced arrest. Hence, it is critical that the administered dose is low enough that it inhibits Chk1 but does not elicit the antagonistic arrest.

Clinical trials to assess target-directed activity are complicated by the need to develop assays to quantify inhibition of the target. A major issue is the source of material for such assays and the tumour is clearly the most appropriate. However, the need for sequential tumour biopsies limits the number of patients who can readily be accrued to these trials. We have been able to obtain four daily biopsies from patients with cutaneous metastasis (primarily melanoma) in our clinical trial with UCN-01 [20], but the requirement for biopsiable tumour has severely limited the rate of accrual. Another approach we have used successfully is to obtain tissue specimens at clinically indicated surgeries or endoscopies, following prior administration of investigational drugs at defined times. In many instances, biopsies obtained previously for diagnosis and/or staging can provide a pretreatment baseline. A single dose of a drug given in this manner may not impact the course of the patient's disease, but should pose minimal risk. The drug may also be administered subsequently in the postoperative setting, justifying treatment on the basis of potential therapeutic benefit. The downside of this approach is that it can provide a tumour sample only at a single time point following treatment. It is probable that most signal transduction inhibitors will need to suppress a pathway for a reasonable time period, so that assessment of effect at a single time point will be inadequate.

These problems may be surmountable if some appropriate parameter can be assessed in a surrogate tissue such as blood. One critical issue rarely addressed in trials is validation of the biomarker in the surrogate

tissue. In other words, one must establish that expression of the marker in surrogate tissue, and its response to treatment, corresponds to the behaviour of the marker in the tumour. For example, there is a particular concern for assuming that if a drug inhibits its target in circulating cells that it is also inhibiting it in the tumour. This is true even if the circulating cells are tumour cells which are probably already in circulation at the time the drug is administered. Plasma concentrations of drug, particularly those administered by the intravenous route, may be very different from the concentrations that penetrate into a solid tumour. If it is possible to validate a marker in surrogate tissue, then accrual to molecular proof-of-principle trials can be greatly accelerated.

Non-invasive imaging technologies [for example, functional magnetic resonance imaging (fMRI), and positron emission tomography (PET)] are increasingly applied for assessment of target-directed drug activity in Phase I trials. In these applications, it is important to know whether the assay directly measures target-directed activity or whether it measures a surrogate end-point. For example, PET imaging is most commonly used to assess changes in glucose uptake. Many drugs can modify glucose uptake, but this frequently reflects drug-induced cell death rather than a direct effect on glucose metabolism. Alternative approaches use fMRI to quantify the incidence of apoptosis, but this does not confirm that the drug under investigation killed the cells as a consequence of interacting with the predicted target. While confirming cell death in a tumour is a valid end-point, it does not confirm the mechanism of action and is an irrelevant end-point for many drugs such as UCN-01 whose desired mechanism of action is nontoxic. Without rigorous validation, such functional studies could have costly and misleading consequences for drug development. For example, it could be costly to continue developing a lead compound in the belief that it worked by a particular mechanism *in vivo*. Alternatively, a novel kinase might be considered a poor therapeutic target if a potent inhibitor did not have the desired efficacy *in vivo*, yet this would be an irrational conclusion in the absence of direct evidence that it did indeed inhibit the target *in vivo*. Surprisingly, such data are only rarely being obtained in current trials: a recent overview of Phase I trials of targeted agents shows that molecular pharmacodynamic end-points were the basis for determination of the recommended Phase II dose in only 3% of trials [31].

These comments may appear to suggest that increased emphasis on molecular pharmacodymanics of targeted agents makes determination of MTD in Phase I trials obsolete. Yet, there may still be value in defining MTD, particularly as such agents will probably be given in combination. Development of rational regimens may be facilitated by knowledge of the clinical toxicities and therapeutic index for each drug in the combination. However, it is critical that proof-of-concept clinical trials be performed with the intent to establish that the drug truly does impact its intended target.

Why have these new drugs been elusive until now?

The fact that drugs against most of these new targets were not identified by earlier screening approaches is important. First, many of the new drugs may not kill cells and as such would not show positivity in a cytotoxicity assay. Some of these drugs are expected to have impact only in drug combinations and this would clearly be missed when scoring drugs for efficacy as single agents. Another very important reason why such compounds were not seen in early screens is that they impact only a defined subset of tumours with a particular molecular alteration and the absence of appropriate screening models means the compounds would have been missed. Perhaps one or two cell lines in the NCI 60 cell line panel do have the appropriate responsive phenotype, but this could easily have been overlooked when the primary goal was to find drugs with a broad therapeutic efficacy. This latter point highlights an extremely important issue in developing novel targeted therapeutics: it is essential not only to use appropriate screening models, but also to stratify patients so that the drug is tested primarily in patients who are predicted to respond based on the mechanism of action of that particular drug.

Patient stratification

It is self-evident that presence of the target is essential, not just in screening models but also in patients. Early trials of targeted agents should ideally be done in patients who are predicted to respond, based on the mechanism of action of a particular drug; indeed, failure to do so risks missing the activity of a promising agent altogether [32]. Yet, sometimes the presence of the target may be insufficient to predict which patients will respond. It is worth reconsidering the story of gefitinib discussed above. Very few patients responded initially despite the over-expression of the receptor, but then it was discovered that responding patients had a mutation in the receptor. If the presence and impact of this mutation had been known prior to the start of the clinical trial, it would have been possible to stratify patients and administer drug only to those with a mutation. As a result, much higher response rates would have been seen and far fewer patients would have been required for the

study. This illustrates that more basic information could dramatically reduce the cost and increase the efficiency of early clinical trials of targeted agents. One cannot help but wonder how many other promising drugs have failed clinically because they were tested in inappropriate patient populations.

The gefitinib example also illustrates that translation of new therapies into the clinic is an iterative process, not unidirectional. The mutations that predict response were identified only after the drug was found to have a lower than expected response rate in patients known to express high levels of EGFR. In this case, observations in the clinic helped focus laboratory science, ultimately yielding a biomarker which improved our ability to stratify patients as candidates for treatment with the drug.

It is also important to recognize that most drugs considered 'effective' are still ineffective in many patients. The recent excitement about the efficacy of trastuzumab (Herceptin) in the adjuvant treatment of patients with breast cancer should be tempered by the realization that it remains ineffective in 50% of patients who receive it [33]. Hopefully, we will soon determine what predicts response to trastuzumab, so that those who will not respond can be considered for alternate therapies and/or clinical trials.

The development of gene expression microarrays dramatically enhances the ability to identify predictors of response and thereby facilitate the stratification of patients. This approach has already identified novel subsets of patients with particular molecular characteristics and has improved the diagnosis of some forms of cancer [34, 35], and it is being applied more frequently to identification of prognostic markers for therapeutic response. The microarray approach provides far more information than current cytology and immunohistological methods, but whether this will eventually become the standard of care depends on whether the methodology can be adapted to routine pathology laboratories.

Together, these considerations suggest that it is imperative that targeted agents be developed as rigorously as possible. This entails use of valid preclinical models, stratification of patients for early trials based on the presence of the target and iterative interaction between laboratory and clinical investigators. The costs of drug development and of lives lost to ineffective therapies are simply too great to do otherwise.

Conclusion

Cancer biology has taught us that each tumour has its Achilles heel, but that it is different for each particular subset of tumours. It is essential to develop therapeutic approaches that selectively target these weaknesses in tumours. We can now look forward to the day when molecular diagnostics, biomarkers and targeted therapeutics are sufficiently developed so that physicians can prescribe drugs that are known to be effective against the patient's particular tumour. Therapy will be individualized for each patient and the days of administering highly toxic drugs to unresponsive patients will soon be history.

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