Targeted cancer therapy

Charles Sawyers

Howard Hughes Medical Institute, David Geffen School of Medicine at UCLA, Jonsson Comprehensive Cancer Center, 10833 LeConte Avenue, Los Angeles, California 90095, USA (e-mail: csawyers@mednet.ucla.edu)

Disruption of the normal regulation of cell-cycle progression and division lies at the heart of the events leading to cancer. Complex networks of regulatory factors, the tumour microenvironment and stress signals, such as those resulting from damaged DNA, dictate whether cancer cells proliferate or die. Recent progress in understanding the molecular changes that underlie cancer development offer the prospect of specifically targeting malfunctioning molecules and pathways to achieve more effective and rational cancer therapy.

The reviews in this Insight summarize our current understanding of how oncogene and tumour suppressor gene networks influence the decisions of cancer cells to proliferate or die (Fig. 1). These decisions are further influenced by the tumour microenvironment and stress signals, such as DNA damage. Moreover, recent work suggests that a sub-population of cancer cells with stem-cell-like properties may be critical for triggering tumour development. Together, these studies provide a conceptual framework within which practitioners of experimental cancer thera-peutics can consider the design of targeted agents. The term 'targeted therapy' refers to a new generation of cancer drugs designed to interfere with a specific molecular target (typically a protein) that is believed to have a critical role in tumour growth or progression. The identification of appropriate targets is based on a detailed understanding of the molecular changes underlying cancer. This approach contrasts with the conventional, more empirical approach used to develop cytotoxic chemotherapeutics — the main-stay of cancer drug development in past decades. Here, I summarize current progress in targeted therapy, and review the potential targets that are emerging. I focus in particular on kinases, which have so far proved to be a promising class of targets for cancer therapy.

Targeting mutant kinases

The clinical success of the small molecule kinase inhibitor imatinib mesylate (Gleevec) in chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GIST) has established a paradigm for the treatment of tumours whose growth is acutely dependent on specific kinase targets (Table 1). CML is driven by the mutant kinase fusion protein Bcr–Abl, which displays constitutive activation of the Abl kinase, whereas GIST is caused by activating point mutations in the c-Kit or platelet derived growth factor receptor (PDGFR)-α kinases. Imatinib effectively blocks the activity of all three kinases and produces dramatic clinical responses in all three situations in a manner that correlates precisely with the presence of these mutations in the tumour[1]. In lung cancer, clinical responses to epidermal growth factor receptor (EGFR) inhibitors are associated with point mutations in the EGFR kinase domain[2,3] (thereby explaining the rather modest 10% response rate in all patients). The clear prediction from this experience is that clinical responses to kinase inhibitors occur in tumours bearing activating mutations that drive tumour progression. Extending this paradigm to larger numbers of cancer patients would require establishing the frequency of kinase mutations in human cancer on a much broader scale — presumably through global gene sequencing efforts analogous to the genome project. Indeed, initial efforts from groups at the Sanger Centre, the Eli Broad Institute and Johns Hopkins have found previously unsuspected kinase mutations in human tumours[4–7].

Given that the fraction of human cancers known to have kinase-domain mutations is currently small, can we realistically expect a substantial percentage of all cancer patients to benefit from kinase inhibitors? There are several reasons for optimism. First, the high frequency of B-Raf kinase mutations in melanoma was completely unrecog-nized until they were uncovered by a systematic sequencing effort[5]. Hence, many more surprises may follow. Second, serendipitous clinical responses in patients with rare con-ditions has led to the discovery of previously unrecognized mutant kinases in diseases such as the FIPL1–PDGFRα fusion in hypereosinophilic syndrome[8]. Third, clinical responses are also observed when tumours contain mutations in genes that activate the kinase indirectly. For example, a chromosome translocation causes overproduction of the kinase ligand PDGF in dermatofibrosarcoma protuberans[9]. This last example underscores the fact that global surveys intended to define the frequency of kinase-dependent cancers must also consider the multitude of indirect mechanisms that could lead to constitutive kinase activation.

Caveats for therapies targeting mutant kinases

Assuming that cancer genome surveys reveal that a large fraction of human cancers have kinase-pathway abnorm-alities amenable to pharmacological blockade, additional complexities may temper expectations for Gleevec-like results in all cancers. One consideration is whether a kinase-pathway mutation occurs early or late in the life history of the tumour, as this may affect the degree to which tumour growth is dependent on these changes. It is generally agreed that the Bcr–Abl mutation in CML serves as the initiating event, raising the question of whether the dramatic clinical responses seen in this disease are, in part, attributable to attacks on the earliest oncogenic lesion. In contrast, there is some evidence to suggest that another kinase abnormality involving the Flt3 receptor in acute myeloid leukaemia occurs late in disease progression. Phase I clinical data with at least three different Flt3 inhibitors has provided clear evidence of tumour response, but the magnitude of the response appears less impressive than that induced by Gleevec in late-stage CML (refs 10–12). Of course, a large number of other variables, such as efficacy of target inhibition, could explain this difference, but the chronology of where the targeted lesion abnormality occurs in the scheme of tumour pro-gression could have a role in clinical outcome.

An additional consideration is disease relapse due to drug resistance. Perhaps the best understanding of this problem at a molecular level comes from studies of Gleevec resistance in CML patients. Relapse is caused by the expansion of tumour
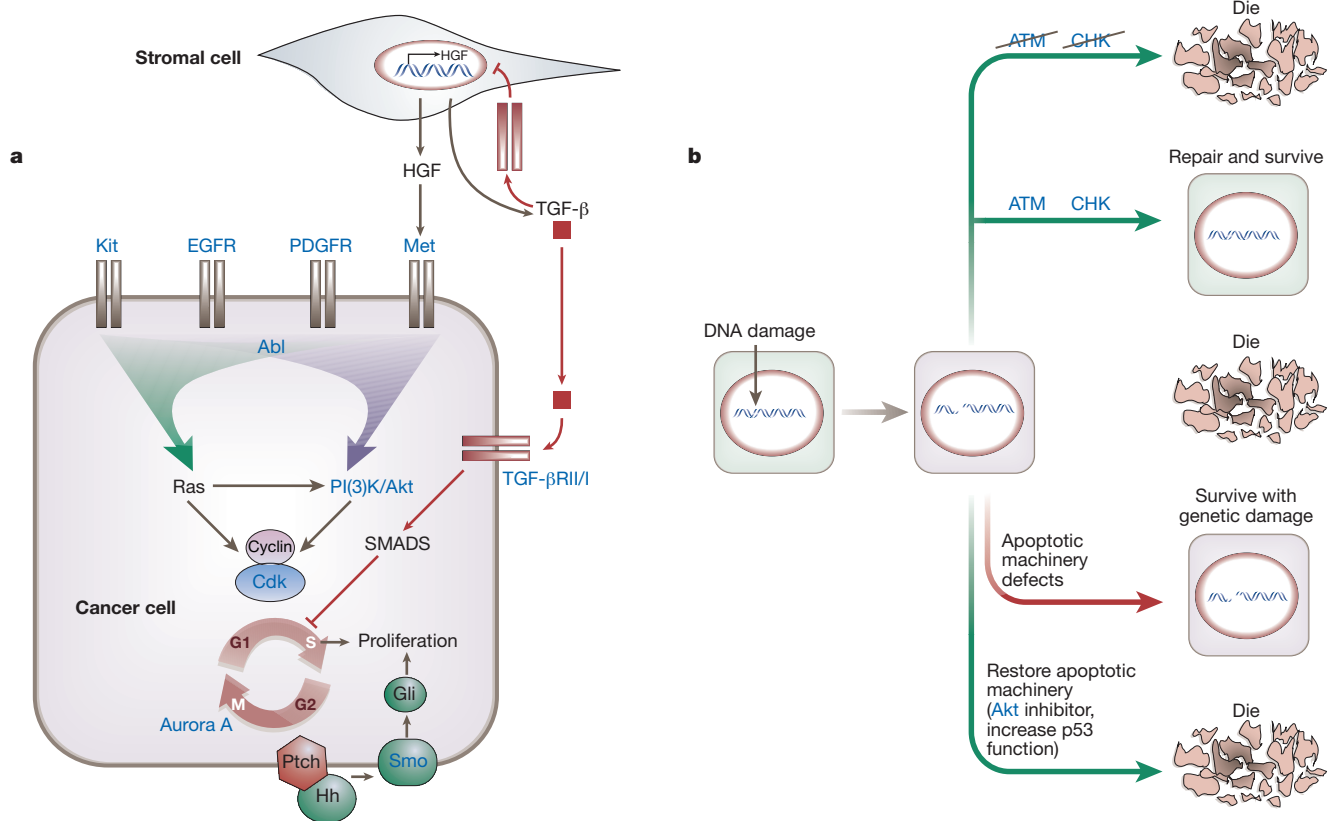
Figure 1 Cancer pathways and targeted therapy. **a**, Multiple signalling pathways — upregulated in cancer cells owing to specific alterations in oncogenes or tumour suppressors — stimulate tumour-cell proliferation, often by promoting G1–S cell-cycle progression (see reviews in this issue by Massagué, page 298; and by Beachy *et al.*, page 324). Signals from the tumour microenvironment, including stromal fibroblasts (see review in this issue by Bhowmick *et al.*, page 332), can positively or negatively shape cancer-cell proliferation. The Inhibition of growth-promoting pathways by therapy tailored to the specific genetic alterations found in cancer offers a new therapeutic approach: an example is the recent approval of drugs targeting the Abl and EGFR kinases. These and other potential drug targets are shown in blue. CIN and other mitotic defects leading to aneuploidy (see progress article in this issue by Rajagopalan and Lengauer, page 338) may cause the accumulation of genetic defects endowing cancer cells with a selective growth advantage and providing another possible route for treatment. One potential therapeutic target is the Aurora A kinase, which is implicated in mitotic progression and CIN. **b**, Classical chemotherapy and radiotherapy eliminates cancer cells by inducing DNA damage and subsequent apoptosis. DNA-damage-response pathways (which employ the ATM and CHK1/2 kinases) promote repair and survival (see review in this issue by Kastan and Bartek, page 316). Defects in the apoptotic machinery can allow cancer cells to survive DNA damage, which may lead to the acquisition of further mutations (see review in this issue by Lowe *et al.*, page 307). Inhibition of DNA-damage-response pathways or restoration of defective apoptosis pathways may render cancer cells more susceptible to DNA-damaging agents and provide potential avenues for more efficient and tumour-specific future therapies in the future.

subclones in the face of continued therapy; these subclones contain single amino-acid mutations in the Bcr–Abl kinase domain that prevent enzyme inhibition by Gleevec[13–15]. Recent preclinical studies have identified second generation dual Src/Abl kinase inhibitors that retain activity against nearly all the Gleevec-resistant mutants[16]. These compounds are now in early clinical testing, but the expectation is that future therapy will rely on cocktails of inhibitors to prevent the emergence of resistant subclones.

Extending lesion-specific therapy to pathways

The above discussion exemplifies recent clinical success in cancer therapy by directly targeting the oncogenic lesions responsible for tumour initiation and progression. But can this approach be exploited more broadly, by inhibiting pathway components that are not themselves mutated, even though the oncogenic lesion occurs in a pathway regulator? The example of dermatofibrosarcoma protuberans discussed above represents one proof-of-concept example in a rare disease, but can we realistically expect a more generalized impact for this approach? Several of the reviews in this issue provide reason for optimism.

Patients with Gorlin's syndrome have an inherited predisposition to develop cancers because of germline mutation in the Patched

Table 1 Targeted agents and their current status in clinical testing

Drug	Target	Disease	Clinical trial status
Imatinib (Gleevec)	Abl Kit PDFGR	CML GIST HES CMML DFSP	Approved
Gefitinib (Iressa)	EGFR	Lung cancer	Approved
Bevacizamab (Avastin)	VEGF ligand	Colon cancer	Approved
CCI-779 RAD-001	mTOR	Various cancers	Phase I, II, III
BMS-354825	Abl KIT	CML GIST	Phase I
PKC-412 MLN-518 CEP-701	FLT3	AML	Phase I/II
BAY 43-9006	VEGFR RAF	Kidney cancer Melanoma	Phase I/II
SU-011248	VEGFR	Kidney cancer	Phase I/II

AML, acute myeloid leukaemia; HES, hypereosinophilic syndrome; CMML, chronic myelomonocytic leukaemia; DFSP, dermatofibrosarcoma protuberans.

(Ptch) gene, a cell surface receptor that functions in the Hedgehog (Hh) pathway (see Fig. 1 in review by Beachy *et al.*, page 324). The natural product cyclopamine interferes with the downstream Hh-pathway protein Smoothened (Smo) and impairs the growth of such tumours in model systems. Remarkably, the usefulness of this approach may not be limited to the small fraction of tumours with Ptch mutation. In many epithelial tumours, such as those arising in the pancreas and prostate, Hh-pathway ligands are produced, resulting in constitutive pathway activation and, most importantly, cyclopamine sensitivity. The molecular lesion(s) leading to ligand-dependent activation in these tumours have not been defined. In at least some cases, there seems to be a tumour-specific molecular event that alters the availability of Smo for downstream signalling. Regardless of the mechanism underlying pathway activation, these findings have already motivated the pharmaceutical industry to search for Hh-pathway inhibitors, one of which has recently been shown to be effective in a Hh-pathway-dependent mouse model of medulloblastoma[17].

Since the elucidation of the role of cyclin-dependent kinases (CDKs) in cell-cycle regulation, these proteins have been extensively explored as potential drug targets. Highly selective and potent CDK inhibitors exist, but the overriding question is how we define those tumours in which the inhibition of CDK activity provides a favourable therapeutic index. If we use the pathway-mutation paradigm to address this question, tumours with molecular lesions in the primary cell-cycle machinery should be susceptible to CDK inhibition. At a minimum, this might include rare familial melanomas with mutations in CDKs (ref. 18), mantle cell lymphomas with translocations leading to increased cyclin D1 expression[19], and tumours with loss-of-function mutations in p16 or retinoblastoma protein (Rb). However, the list could be much larger as a number of the signalling pathways more commonly mutated in human cancers (Myc, Ras, transforming growth factor beta (TGF-β) through receptor mutations or Smad 4 loss), impinge directly on cell-cycle regulation (see review in this issue by Massagué, page 298). The challenge in clinical evaluation of these inhibitors is to design trials that enroll patients for whom there is a detailed knowledge of the molecular phenotype of the tumour, so that appropriate correlations with clinical responses can be made.

A third example is to use inhibitors of kinases in the PI(3)K/Akt/mTOR pathway to treat tumours with mutations in the tumour suppressor gene *PTEN* — the negative principal regulator of this pathway (see reviews in this issue by Massagué, page 298, and by Lowe *et al.*, page 307). Proof of concept for this approach has been demonstrated in numerous murine models using rapamycin analogues that block mTOR activity[20]. Because mTOR receives signalling inputs from several signalling pathways, tumours with a number of distinct molecular lesions could be sensitive to treatment. As with CDK inhibitors, the current challenge is to correlate clinical activity with molecular phenotype.

Targeting cancer stem cells

Even in the seemingly near-perfect world of Abl-kinase-inhibitor treatment of CML, it is becoming increasing clear that we cannot ignore the concept of cancer stem cells. As discussed by Beachy and colleagues (in this issue, page 324), there is growing evidence that some, if not all, tumours derive from a small number of stem-cell-like cells that either retain or acquire the capacity for self-renewal. Presumably, targeted therapies must eliminate tumour stem cells to prevent a later relapse. The clinical experience with imatinib in CML provides an opportunity to consider this concept in patients. Despite the fact that imatinib reliably reduces the tumour burden in CML by three to four orders of magnitude, most patients continue to have residual disease. This is detected by quantitative polymerase chain reaction (PCR) for the Bcr–Abl fusion breakpoint[21]. The risk of relapse in these patients remains low with three years of clinical follow up, but recent studies suggest that these residual CML cells

reside in the stem-cell-like CD34+ population, and may contain imatinib-resistance mutations[22–24]. Persistence in this self-renewing pool raises obvious concerns about the eventual emergence of resistant subclones. The new dual Src/Abl inhibitors discussed above, which are more than 100-fold more potent than imatinib, could theoretically represent a solution, provided that that the CML stem cell requires Bcr–Abl for survival.

On the basis of our current understanding of cancer stem cells, can we envision a strategy to eliminate them? If these cells have unique patterns of cell-surface antigen expression, monoclonal antibodies might be designed to target them specifically (assuming these antigens would not be shared by normal stem cells from the tissue of origin). Perhaps more promising is the potential to target specific signalling pathways required for stem-cell function. The most likely suspects, on the basis of current thinking, are the Hh and Wnt pathways, both of which have oncogenic potential based on known mutations in pathway components found in several human tumours (see review in this issue by Beachy *et al.*, page 324). Furthermore, pharmacological blockade of these pathways (like that demonstrated with cyclopamine) seems feasible.

How might such inhibitors perform in clinical trials? If the effects are relatively stem-cell specific, clinical responses may be slow to manifest. Consider, by comparison, a drug that affects the large mass of more differentiated tumour cells (imatinib), which shows clinical responses in days to weeks. The effects of a stem-cell drug may take longer to become clinically evident (possibly months or years), especially if the differentiated tumour cells that are not affected by the therapy have a long lifespan as is the case with some epithelial tissues. An additional consideration is safety; extended monitoring for delayed toxicity due to loss of normal stem-cell function in the relevant organ may be needed.

Targeting the microenvironment

Much attention over the years has been devoted to the notion that the tumour blood supply can be targeted with antiangiogenic agents. This has culminated in the recent approval of a monoclonal antibody directed against the vascular endothelial growth factor (VEGF) ligand (which is essential for endothelial cell proliferation) for the treatment of colon cancer, when used in conjunction with chemotherapy[25]. Part of the attraction of this approach is the near universality of its potential application, as essentially all cancers require a blood supply for their continued growth and spread. In addition, there is the notion that therapy directed against the supporting host tissue rather than the tumour itself will be less prone to resistance because the genetic plasticity of the cancer is not reflected in the stroma. Curiously, the clinical activity of the VEGF antibody in colon cancer was not anticipated and does not obviously correlate with tumour-associated angiogenesis patterns. Notably, the VEGF antibody and small molecule inhibitors targeting the VEGF tyrosine kinase receptor have both shown impressive single-agent activity in renal cancer[26–28]. These tumours are highly vascular owing to the deletion of the von Hippel–Lindau tumour suppressor gene — the primary molecular lesion in these cancers. This leads to upregulation of the HIF transcription factors and constitutive expression of VEGF in tumour cells[29]. The growth of these tumours is driven by HIF, in much the same way as activating kinase mutations drive the growth of some of the cancers discussed above, so the anti-tumour properties of VEGF-pathway drugs may not occur solely through effects on the stroma because these tumours often express VEGF receptors.

Recent advances in our understanding of tumour–stroma interaction reveal a much more complex interplay that extends well beyond the simple notion of vascularity. Tumour stroma includes stromal fibroblasts and a number of different inflammatory cells that can clearly modulate tumour growth. Among the best-studied stromal factors is TGF-β, which can exert myriad effects that influence tumour growth in a positive or negative fashion (see also review in this issue by Bhowmick *et al.*, page 332). Although TGF-β has

immunosuppressive properties that may hamper host immune surveillance, it also exerts direct anti-proliferative effects on epithelial cells by engaging the TGF-β receptor (TGFβR)/SMAD signalling pathway. Remarkably, the tumour-suppressive effect of TGF-β is critical in stromal cells as well as in adjacent epithelium, as was recently demonstrated by the finding that epithelial malignancy can develop in certain organs when the TGF-β receptor is deleted only in stromal fibroblasts[30]. This result, together with earlier studies that demonstrate pro-oncogenic characteristics of cancer-associated fibroblasts isolated from human prostate tumours[31], clearly establishes that stroma should not merely be considered as supportive to the cell-autonomous growth of tumour cells. Instead, the stroma can exert profound effects on the initiation and progression of epithelial malignancies. Elucidation of the molecular circuitry of this crosstalk could profoundly influence our thinking about targeted cancer therapy, and may provide new prevention strategies.

Combining classical chemotherapy with targeted therapy

Although the above discussion offers many exciting new targets for treating and/or preventing cancer, classical chemotherapy and radiotherapy approaches remain the mainstay of cancer treatment for tumours that cannot be cured solely by surgical excision. Because of the recent success in defining the biochemical details of cells' responses to DNA-damaging agents, these conventional cancer therapies may be combined with targeted agents that disrupt the DNA-repair response; hopefully a more catastrophic cell kill in tumours will result. Proof of concept comes from the well-known hypersensitivity of ataxia-telangiectasia patients to ionizing radiation and chemotherapy. The molecular basis for this exaggerated response is deficiency in DNA-damage-induced cell-cycle arrest owing to mutation in ATM kinase in these patients (see review in this issue by Kastan and Bartek, page 316). This compromises the time needed to repair DNA lesions that are induced by chemo- or radiotherapy. It stands to reason that pharmacological inhibition of ATM or downstream CHK kinases with specific inhibitors might similarly impair the DNA-damage response to conventional cancer therapy, and provoke an even greater apoptotic response. The challenge for pursuing this approach will be to ensure an adequate therapeutic index, such that the nearly universal toxicities of chemotherapeutic agents on normal haematopoietic and gastrointestinal epithelial cells are not similarly enhanced. One scenario might be to use ATM or CHK kinase inhibitors in combination with focal radiotherapy, such that DNA damage is restricted only to cells in the radiation-therapy field.

The above discussion addresses the goal of maximizing tumour cell kill with conventional cancer therapy by poisoning the cell's ability to repair the damage induced by these agents. An alternative strategy that may achieve a similar goal is to define precisely why some tumours fail to respond to chemotherapy in the first place, and then to interfere with these resistance pathways so that cytotoxics can provoke tumour shrinkage. A vast amount of work on this question suggests that most cancers acquire defects in apoptosis pathways (see review in this issue by Lowe *et al.*, page 307), such that tumour cells fail to die despite the presence of strong pro-apoptotic signals induced by chemotherapy. The cataloguing of human tumours for such defects indicates myriad mechanisms, but there are several nodal points in the pathway that might be amenable to pharmacological intervention. These involve proteins such as Bcl2, p53 or Akt kinase. In each case, small molecule strategies have been reported in model systems that either block (Bcl2, Akt) or promote (p53) the activity of the target protein. These could potentially restore the cell-death response in the presence of DNA damage. Clinical evaluation of any of these concepts, however, has yet to be initiated.

Concluding remarks

The reviews in this Insight provide much food for thought. The era of molecular targeted cancer therapy has clearly arrived, but patients and practitioners are yearning for this approach to have a broader impact. The successes of the past few years illustrate the power of the approach and should reinforce the need to continue basic studies of the molecular underpinnings of human cancers. The failure to see clinical responses with some targeted agents teaches critical lessons as well. Of utmost importance is the need to define the relevant patient population for clinical trials and therapy through molecular characterization of the tumour. Overcoming this barrier will require the development and widespread adoption of appropriate molecular diagnostic assays. Only then will it be possible to realize the broader potential of targeted cancer therapy. □

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