

Kinase mutations in cancer: chinks in the Enemy's armour?

Federica Di Nicolantonio and Alberto Bardelli

Purpose of review

Over the past few years, a revolution has transformed the oncology field. This revolution is characterized by two main features. The first is the introduction of the concept of individualized cancer therapy. The second is the development of drugs targeting molecules selectively altered in tumours. This review analyses these aspects by looking at the role that altered kinases and their inhibitors have played in this historical process.

Recent findings

Tumour progression is the result of the sequential accumulation of mutations in genes monitoring the rates of cell birth and cell death. The molecular profiling of cancers has shown that protein and lipid kinases are frequently altered in tumour cells. In most cases, these alterations translate in constitutively active proteins, which are amenable of therapeutic targeting. Intriguingly, even 'established' cancer cells remain somewhat 'addicted' to the deregulated activity of mutated kinases. This feature appears to be the basis for the ability of kinase inhibitors in controlling the development of a number of cancers. The therapeutic efficacy of kinase inhibitors is impaired by the emergence of tumour cells carrying 'resistance' mutations.

Summary

Many oncogenes are mutated kinase genes. In most cases, the mutations result in the constitutive activation of the affected kinase that can be pharmacologically inhibited. Unfortunately, upon treatment with kinase inhibitors, resistant clones develop rapidly, impairing their therapeutic effect. Strategies to overcome resistance are discussed as well as the possibility to target kinases regulating cancer stem cells.

Keywords

amplification, deletion, EGFR, individualized therapies, kinase, kinase inhibitors, mutation, oncogene addiction, phosphorylation, targeted therapies

Abbreviations

CML	chronic myeloid leukaemia
GISTs	gastro-intestinal stromal tumours
HER-2	human epidermal growth factor receptor 2
PDGF	platelet-derived growth factor

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1040-8746

Introduction: The genetic basis of cancer and the kinase therapy revolution

From a molecular point of view, cancer is, in essence, a genetic disease. According to the Darwinian vision of tumour progression, cancer cells arise as a result of the sequential accumulation of mutations [1^{••}]. These are selected for because they provide an additional growth advantage to the tumour cell, allowing it to outgrow the precursor lesion. Multiple waves of mutation, clonal expansion and growth lead to cancer progression. The final result is the deregulation of cellular homeostasis. This occurs because the mechanisms monitoring the rates of cell birth and cell death are altered.

The cellular pathways that supervise the rates of cell birth (proliferation) and cell death (apoptosis) rely on molecular switches that control the various steps of these processes. Many of these molecular switches are controlled by the addition of phosphate moieties to proteins or lipid substrates. According to their phosphorylation status, such proteins and lipids, in turn, activate downstream effectors. At the cellular level, a large family of proteins that possess kinase activity meticulously controls addition of phosphates.

Both protein and lipid kinases are often found genetically altered in cancer cells [2[•]]. Interestingly, in most cases, these alterations translate in constitutively activated proteins, which are therefore amenable of therapeutic targeting.

Over the past few years, a revolution has started in the oncology field. This revolution is characterized by two main features. The first is the introduction of the concept of individualized cancer therapy, while the second is the development of the first drugs targeting molecules selectively altered in tumours. In both, protein and lipid kinases have played a central role. This review will describe these ongoing historical changes from a 'kinase' perspective.

Curr Opin Oncol 17:000–000. © 2006 Lippincott Williams & Wilkins.

The Oncogenomics Center, Institute for Cancer Research and Treatment (IRCC), University of Torino Medical School, Candiolo, Italy

Correspondence to Prof. Alberto Bardelli, PhD, The Oncogenomics Center, Institute for Cancer Research and Treatment (IRCC), SP142, Km 3.95, 10060 Candiolo (TO), Italy
Tel: +39 011 9933601; fax: +39 011 9933225; e-mail: a.bardelli@ircc.it

Current Opinion in Oncology 2006, 17:000–000

How many faces does the Enemy show? Molecular profiling of cancers and individualized cancer therapies

Until quite recently, oncologists were treating cancer patients with the so-called ‘one size fits all’ approach. All individuals affected by a certain tumour type (as defined by histological examination) received the same conventional therapy – often a combination of cytotoxics that were essentially killing actively replicating tumour (and normal) cells.

It is becoming increasingly clear that cancers affecting the same tissue in two patients may be caused by alterations in different molecular mechanisms, thus resulting in tumours with different biological behaviour and clinical outcome. The realization that cancer has a genetic basis and the availability of the human genome sequence has been central to this step forward by allowing the comprehensive molecular profiling of tumours. Overall, the results of these analyses are reshaping the field of oncology for at least three reasons. First, a number of studies have demonstrated that cancers can be diagnosed and classified on the basis of their molecular profiles [3**]. Secondly, the pattern of genetic alterations present in individual tumours can, at least in part, be used to predict their clinical outcome [4]. Thirdly, the success of drugs aimed at inhibiting mutated genes has shown that cancer-specific genetic alterations are legitimate targets for therapy [5*,6*].

The molecular profiling of kinases in tumour genomes has played a major role in this process, as (i) kinases are frequently mutated in multiple tumour types; (ii) the corresponding mutations are often ‘dominant’ in nature, thus allowing therapeutic targeting; and (iii) the catalytic activity of kinases can be pharmacologically inhibited.

Chinks in the Enemy’s armour: the paradigm of ‘cancer addiction’ to kinases

A single cancer cell frequently contains gross chromosomal abnormalities, mutations in multiple genes and widespread changes in its gene expression profile. An axiom in cancer research is that the multistage process of tumour formation is driven by progressive acquisition of activating mutations in dominant growth-enhancing genes (oncogenes) and inactivating mutations in recessive growth-inhibitory genes (tumour suppressor genes) [7].

Cancer cells are often ‘addicted to’ (i.e. physiologically dependent) on the continued activity of specific activated oncogenes for maintenance of their malignant phenotype, even in the presence of additional tumourigenic lesions. This phenomenon is referred to as ‘oncogene addiction’ [8,9*].

Inactivation of the mutated gene can induce cancer cells to differentiate into cells with a normal phenotype or to undergo apoptosis [10]. Addiction to mutated genes represents chinks in the tumour’s armour and can be therapeutically exploited [11*,12*]. This has been convincingly shown through genetic approaches in cancer cells carrying oncogenic ras. In these cells, the continuous presence of the mutated but not the wild-type ras allele is required for their tumourigenic potential [13]. Similar results have been shown for a number of kinases. For example, the metastatic potential of colorectal cancers with PIK3CA mutations is dependent on the presence of the mutated PI3K allele [14**]. Similarly, the knock down of mutated BRAF in melanoma cells causes growth arrest, and promotes apoptosis [15].

The ‘oncogene addiction’ theory is further supported by experiments involving the pharmacological inhibition of kinases genetically altered in human cancers (see below).

Fighting the Enemy with ‘intelligent’ bullets: protein kinase inhibitors in oncology

Over the past decade, massive advances in the molecular basis of cancer have paved the way to the design of ‘selective’ anticancer agents. Most of the new drugs work by inhibition of various protein kinases (Table 1).

The first example of compounds targeting protein kinases is represented by the monoclonal antibody trastuzumab (Herceptin®), which inhibits the human epidermal growth factor receptor 2 (HER-2 or ErbB2). The amplification of *HER-2* gene or overexpression of its protein correlate with poor breast cancer prognosis, including reduced relapse-free and overall survival [16]. Drugs targeting the product of the *HER-2* gene therefore represent magic bullets for aggressive tumours showing alterations in this oncogene. Trastuzumab can be considered the first case of individualized therapy with inhibitors of tyrosine kinases. Not all patients whose tumours display overexpression of the HER-2 protein or amplification of the corresponding gene, however, respond to treatment with this agent. Actually, when trastuzumab is given as a single agent as a first-line treatment of HER-2-overexpressing metastatic breast cancer, it is associated with a 30% objective response rate [17]. In the remaining cases, no tumour regression is observed. It is possible that in these patients, tumour cells have found a way to escape their dependency on HER-2, possibly by activating alternate pathways leading to cell survival and proliferation.

More recently, another monoclonal antibody has reached clinical use: cetuximab (Erbix®), which inhibits the subtype 1 of the HER family receptors, also known as EGFR. When given to unselected colorectal cancer

Table 1. Inhibitors of kinases genetically altered in human cancer

Kinase	Chromosome location	Tumour type	Genetic alterations	Inhibitor	Secondary drug-resistance mutations	Clinical status
ABL	9q34	CML	Translocation/fusion with BCR or ETV6	Imatinib mesylate	T315I, M351T	Approved
BRAF	7q34	Melanoma	Point mutation	Sorafenib (BAY 43-9006)		Phase III
EGFR (ERBB1)	7p12	NSCLC	Point mutations and small in-frame deletions/insertions	Gefitinib, Erlotinib	T790M	Approved
FLT3	13q12	Colorectal	Increased copy number	Cetuximab, Panitumumab		Approved
HER-2 (ERBB2)	17q21	AML	Point mutation	Sutent (SU11248)	CEP-701, MLN581	Phase I/II
		Breast	Amplification	Trastuzumab		Approved
		Lung	Point mutation			
KIT	4q11	GIST	Point mutation and small in-frame deletions	Imatinib mesylate	V654A, T670I	Approved
MET	7q31	HPRCC, HCC	Point mutation	PHA665752		Pre-clinical
NTRK1	1q21	PTC	Translocation/fusion with multiple partners			
NTRK3	15q25	Congenital fibrosarcoma, secretory breast carcinoma	Translocation/fusion with multiple partners			
PDGFRA	4q12	CMPD, CEL, GIST	Translocation/fusion with BCR or F1P1L1	Imatinib mesylate		Approved
PDGFRB	5q33	CMML, CMPD, AML	Translocation/fusion with multiple partners	Imatinib mesylate		Approved
RET	10q11	MEN-2A, FMTC MEN-2B	Point mutation		T674I	Approved

For each gene, the chromosomal location, types of tumours involved, types of mutations observed and the specific inhibitors are indicated. Types of alterations affecting these genes include missense, nonsense, deletion, amplification and translocation mutations. CML, chronic myelogenous leukemia; CMPD, chronic myeloproliferative disorder; NSCLC, non-small-cell lung cancer; ALL, acute lymphocytic leukaemia; GIST, gastrointestinal stromal tumour; HPRCC, hereditary papillary renal-cell carcinoma; HCC, hepatocellular carcinoma; AML, acute myelogenous leukaemia; CMML, chronic myelomonocytic leukaemia; FMTC, familial medullary thyroid carcinoma; MEN-2A and MEN-2B, multiple endocrine neoplasia types 2A and 2B.

patients, cetuximab shows modest activity. Consistently with the paradigm of oncogene addiction, dramatic responses have been reported in patients whose tumours display amplification of the *EGFR* gene [18^{••},19[•]].

Small molecule inhibitors of the *EGFR* gene, such as gefitinib (Iressa[®]) and erlotinib (Tarceva[®]), are also in clinical use, while other compounds with similar properties are being developed. These inhibitors are particularly effective in patients with lung cancers harbouring genetic alterations of the *EGFR* gene. These typically consist of in-frame deletions, including amino acids at codons 746–750 in exon 19 or an amino acid substitution at codon 858 (L858R) in exon 21 [20^{••}–22^{••}]. According to these three initial reports, however, four of 24 gefitinib-responsive tumours did not contain *EGFR* mutations, suggesting that other mechanisms might contribute to *EGFR* inhibitors. More recent studies have correlated response to gefitinib and erlotinib to *EGFR* gene amplification [23^{••}–25^{••}] and *HER-2* gene amplification [26^{••}]. These observations further support the concept of oncogene addiction, as both gene amplification and heterodimerization with *HER-2* result in activation of the *EGFR* signalling pathway [27].

The concept of oncogene addiction is also illustrated in chronic myeloid leukaemia (CML), in which the inhibitor imatinib mesylate (Gleevec) can cause complete regression of advanced tumours by specifically inhibiting the tyrosine kinase activity of the BCR-ABL oncoprotein [28[•]]. Imatinib effectively inhibits several additional tyrosine kinases, including the platelet-derived growth factor (PDGF) receptor kinases (PDGFRA and PDGFRB), the macrophage colony-stimulating factor receptor c-fms and the c-Kit receptor tyrosine kinase [29]. Indeed, imatinib's remarkable activity in gastro-intestinal stromal tumours (GISTs) has been linked to the presence of activating mutations in the *c-kit* and *PDGFRA* genes [30].

Although imatinib, gefitinib and erlotinib are currently the only FDA-approved small molecule kinase inhibitors available for cancer therapy, several other selective kinase inhibitors are undergoing preclinical and clinical testing for various cancers (Table 1). These include inhibitors of the vascular endothelial growth factor (VEGF) receptor, the B-Raf kinase, the PI3K kinase and the c-Met receptor tyrosine kinase [31].

The Enemy' resistance war: the molecular basis of resistance to kinase inhibitors

Acquired resistance to chemotherapy is a major obstacle to successful cancer treatment. Understanding the mechanisms by which tumours become resistant to a particular agent is key to identifying new drugs or combination regimens.

4 Cancer biology

A central mechanism of resistance is the acquisition of a secondary mutation in the targeted kinase. The outgrowth of cancer cells carrying these secondary mutations is driven by Darwinian natural selection and obliterates the therapeutic effect of the inhibitors.

For example, nearly all patients whose tumours initially respond to EGFR inhibitors eventually become resistant to the drugs and progress despite continued therapy. Two recent studies [32^{••},33^{••}] examined tumours from patients with non-small-cell lung cancer who initially responded to gefitinib or erlotinib but subsequently relapsed. Tumours from all patients carried activating mutations in the *EGFR* gene and, in a number of cases, the resistant tumour cells carried an identical second mutation in the *EGFR* gene (T790M).

Whereas the activating mutation was present in tumour cells before treatment with erlotinib or gefitinib, the second mutation was not detected in biopsies from these patients taken before treatment, nor in over 150 lung cancer samples from patients who had not been treated with either drug.

Further work is needed to explore the possibility that the secondary mutation conferring resistance is already present in a very small fraction of tumour cells before exposure to EGFR inhibitors, and is simply not detectable by current sequencing methods. Interestingly, in one study, the T790M mutation was detected clearly only after subcloning the PCR product [33^{••}], and it is not mentioned whether subcloning was performed also for the amplicon obtained from the primary tumour before treatment. In the specimen obtained at relapse, the resistance conferring mutation T790M was consistently observed with either wild-type or primarily mutated sequences (i.e. with the activating deletion L747–S752), suggesting that the tumour had two distinct populations of cells. A very recent report describes one patient who had concomitant T790M and L858R *EGFR* mutations in the original lung-biopsy specimen and showed primary resistance to gefitinib [34^{••}]. It is therefore possible that T790M mutant gefitinib-resistant clones may be already present at levels below the threshold of detection in some patients with lung cancer at presentation and then may expand selectively under gefitinib treatment, thus leading to clinical resistance.

Resistance is unfortunately among the features shared by tyrosine kinase inhibitors, and a resistance mutation very similar to the one identified in *EGFR* has also been found in other kinase genes from tumours with acquired resistance to imatinib. In CML and GISTs, the two main mechanisms of resistance to imatinib are point mutations [35,36[•]–38[•]] or, less commonly, amplification of the *BCR-ABL* gene [39]. Interestingly, one of the most

common imatinib resistance mutations in *BCR-ABL* replaces threonine at position 315 (the amino acid structurally corresponding to T790 of *EGFR*) with isoleucine in the ABL tyrosine kinase domain (T315I), leading to a structural change very similar to that observed with *EGFR* T790M [33^{••}].

It is clear, though, that the acquisition of a secondary mutation is only one mechanism of resistance, because in other cases, resistance occurred in the absence of the second mutation [32^{••}]. Indeed, drug resistance is multifactorial, and other potential resistance mechanisms include pharmacokinetic factors [40[•],41[•]], amplification of the kinase gene and triggering of alternative pathways to compensate for the lack of kinase expression, such as activation of additional kinases or loss of tumour suppressor genes (see below).

As an example of alternative mechanisms of resistance, loss of PTEN confers resistance to trastuzumab and patients with PTEN-deficient breast cancers show significantly poorer responses to trastuzumab-based therapy than those with normal PTEN [42^{••}]. This can be, in part, explained by the fact that activation of the tumour suppressor gene *PTEN* contributes to trastuzumab's anti-tumour activity. It has been demonstrated that trastuzumab treatment quickly increased PTEN membrane localization and phosphatase activity by reducing PTEN tyrosine phosphorylation via Src inhibition [42^{••}].

Can the Enemy be defeated? Strategies to overcome resistance to kinase inhibitors

Tumours exhibit sufficient genetic heterogeneity such that a single targeting agent might not successfully eradicate an entire population of tumour cells. Combination therapies might induce durable remissions or cures by circumventing or preventing the development of drug resistance.

The first approach is to combine a kinase-targeting agent with a conventional antiproliferative therapy. This is well documented for trastuzumab, which demonstrated synergism with cytotoxics and, in particular, with anthracyclines. Sensitivity to anthracyclines has been associated with elevated levels of their target enzymes: topoisomerase II α . Indeed, the *HER-2* gene is co-localized on the same chromosome region as the topoisomerase II α gene and amplification of both genes occur often in the same tumours [43], thus offering a mighty explanation for their increased chemosensitivity [44,45]. Synergism has also been observed between trastuzumab and other drugs [46[•]], both at the preclinical and at the clinical levels, and trials are still ongoing. Similarly, a synergic effect is seen when the anti-EGFR antibodies cetuximab or panitumumab are administered with irinotecan for the treatment of metastatic colorectal cancer [47^{••}]. In contrast,

the combination of small molecules EGFR inhibitors with cytotoxics such as cisplatin and paclitaxel for the treatment of NSCLC has so far been disappointing [48^{••},49[•]]. Recent in-vitro data, however, suggest that a synergistic antiproliferative activity may be obtained when chemotherapy is followed by treatment with EGFR antagonists [50]. Further studies are therefore required to explore the full potential of these novel agents in combination/sequential therapy.

A second possibility to circumvent resistance to TK inhibitors is the association of drugs targeting multiple molecules acting on the same pathway or an alternative pathway. For example, the phosphatidylinositol-3-kinase (PI3K) pathway is genetically deregulated in human cancers at several levels; the tumour suppressor PTEN, which dephosphorylates phosphatidylinositol-3-phosphate (PIP3) to PIP2, is inactivated in several different tumours [51]. Other cancers harbour activating mutations of PI3K [52^{••}], which promotes the conversion of PIP2 to PIP3. In other cancers still, in which PI3K and PTEN are wild-type, other members of the signalling pathway (such as AKT2 or PDK1) are mutated [53^{••}]. Mutations of members of the PI3K signalling pathway are mutually exclusive, confirming their complementary effects in tumourigenesis [53^{••}]. The development of inhibitors targeting multiple effectors of the PI3K pathway may therefore represent a valuable therapeutic strategy. Interestingly, for example, PI3K inhibitors rescued PTEN loss-induced trastuzumab resistance, suggesting that PI3K-targeting therapies could overcome this resistance [42^{••}].

Other examples of therapies aimed at targeting multiple members of the same signalling pathway are the novel combined inhibitors GW572016 (lapatinib) and AEE788 that inhibit both EGFR and HER-2 [54[•],55,56[•]], and the irreversible pan-erbB receptor inhibitor CI1033 (canertinib dihydrochloride) [57]. Their efficacy might be explained by considering that in cancer cells, the HER-2 receptor tyrosine kinase can be activated in two ways: by overexpression or by ligand-mediated stimulation of another ErbB receptor. Blocking all the isoforms of the erbB receptor can be advantageous, as this would prevent kinase activation by heterodimerization. A recent study [58] revealed how targeting ErbB receptors with a combination of the tyrosine kinase inhibitor lapatinib and the antibody trastuzumab enhanced apoptosis in cancer cell lines.

Another possible approach is the development of novel molecules inhibiting tyrosine kinases with acquired resistance to the first-generation compounds imatinib, gefitinib and erlotinib. Progress in this promising direction has been already achieved. For example, antitumour activity has been observed with the tyrosine kinase

inhibitors BMS-354825 and AMN107 in cells with acquired resistance to imatinib [59^{••}]. Similar results were obtained with inhibitor SU11248 in patients with imatinib-resistant GISTs [60]. The association of the first-generation compound imatinib with BMS-354825 could reduce the emergence of drug-resistant clones [61[•]]. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. For example, the EGFR inhibitors EKB-569 and CI-1033 potently inhibit the gefitinib and erlotinib-resistant EGFR (L858R/T790M) kinase [62^{••},63^{••}].

Conclusions: targeting the enemy main headquarters? Attacking the cancer stem cell compartment

Genetic lesions affecting kinases that have been selected during tumourigenesis are causally related to tumour formation and therefore represent legitimate targets for anti-cancer drugs. Cancer cells are often 'addicted' to the continued activity of mutated kinase genes for maintenance of their malignant phenotype even in the presence of additional tumourigenic lesions. The identification of mutated kinases to which individual tumours are 'addicted' is providing unanticipated therapeutic avenues and allows the selection of patients that are most likely to benefit from them. Despite massive investments and decades of research, however, survival of patients with solid tumours has improved only marginally. Current anticancer therapies are relatively effective in eradicating a significant fraction of the hundreds of billions of cancer cells that are present in patients with metastatic disease. As discussed above, however, present treatments fail as a result of the rapidly evolving variants that defeat any therapeutic onslaught. The task ahead therefore remains daunting. It is likely that the concomitant treatment with multiple drugs targeting altered kinase genes will reduce the chances of survival of resistant variants. Even if this strategy is successful, however, other obstacles are on the way. For example, there is increasing evidence for the existence of cancer stem cells [64^{••},65^{••},66,67^{••}]. These cells seem to constitute a very relevant, though small, subpopulation within a tumour. Cancer stem cells are thought to display clonogenic potential, to be capable of unlimited self-renewal and to be highly resistant to apoptosis [68[•],69[•]]. Assuming this hypothesis is correct, therapeutic efforts should be specifically devoted to target this crucial feature of tumours [68[•]]. Given their central role in virtually all cellular circuitries, it is likely that once again, kinase inhibitors will be relevant for therapies targeting the replication of cancer stem cells.

Acknowledgements

We apologize to colleagues whose work was not cited because of space constraints or our oversight. Supported by (AIRC) Italian Association for Cancer Research and (MIUR) Italian University, Technology and Research Ministry.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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