CMLS, Cell. Mol. Life Sci. 62 (2005) 2092–2099
1420-682X/05/182092-8
DOI 10.1007/s00018-005-5205-1
© Birkhäuser Verlag, Basel, 2005

CMLS Cellular and Molecular Life Sciences

Review

Genetic analysis of the *kinome* and *phosphatome* in cancer

S. Arena, S. Benvenuti and A. Bardelli*

The Oncogenomics Center, Institute for Cancer Research and Treatment (IRCC),
University of Torino Medical School, Candiolo 10060, (Italy), e-mail: a.bardelli@unito.it

Received 13 May 2005; received after revision 30 May 2005; accepted 22 June 2005
Online First 26 August 2005

Abstract. Protein phosphorylation is a well-characterized biochemical process for reversible regulation of protein activity. Protein kinases and protein phosphatases are the key complementary players in this process, and through their coordinated activity cell homeostasis is tightly controlled. If these enzymes display aberrant activity, cells may undergo unrestrained growth, thus giving rise to complex diseases such as cancer. The technological platform gathered during the Human Genome Project recently allowed the systematic identification of the genetic alterations present in the kinase (the *kinome*) and the phosphatase (the *phosphatome*) gene families. These studies suggest that most if not all human tumors carry genetic alterations in at least one phosphatase or kinase gene. Here we integrate the biochemical knowledge on the properties of these molecules with the information collected through their systematic genetic analysis in cancer. We also analyze why the molecular profiling of the *kinome* and *phosphatome* in individual cancers is revolutionizing basic and clinical oncology.

Key words. Phosphorylation; kinase; phosphatase; cancer; mutational analysis.

Phosphorylation and the control of cell homeostasis

The existence of phosphoproteins was already known at the end of the 19th century, when they were considered to be the biological carriers of nutrients, as they were found in milk, as caseins, or in egg yolk, as phosvitins. It was only in the 1950s that phosphoproteins were recognized as key mediators and regulators of cell function. In 1954 the first phosphorylation activity catalyzed by a liver enzyme on casein protein was identified [1], and from then on the term protein kinase was used in the biochemical research field.
A year later the role of phosphorylation took centre stage thanks to Wosilait and Sutherland's experiments [2] demonstrating that glycogen phosphorylase activity was regulated by the addition or removal of a phosphate group: this suggested that enzyme activities could be achieved through reversible phosphorylation. Later, Fisher and Krebs's studies on protein phosphorylation were of such great importance and had such an impact in medical research that the two scientists received the Nobel Prize in 1992.
Phosphorylation is a well-characterized biochemical process in which a phosphate group is added through a phosphoester bond (O-phosphate) to the hydroxyl side chain of serine, threonine or tyrosine residues (fig. 1). These are the most commonly phosphorylated amino acids in mammalian cells; however, in nature phosphate groups may also be added enzymatically to other amino acids, such as aspartate (A-phosphate), histidine and arginine (N-phosphate), or to specific lipids, such as phosphoinositidylinositol 4,5-biphosphate [3] (table 1).
Phosphates are negatively charged groups, and their addition to the protein can determine a conformational change of the protein itself. This process can be reversed through dephosphorylation: after phosphate removal, the protein switches back to its original conformation. If a

* Corresponding author.

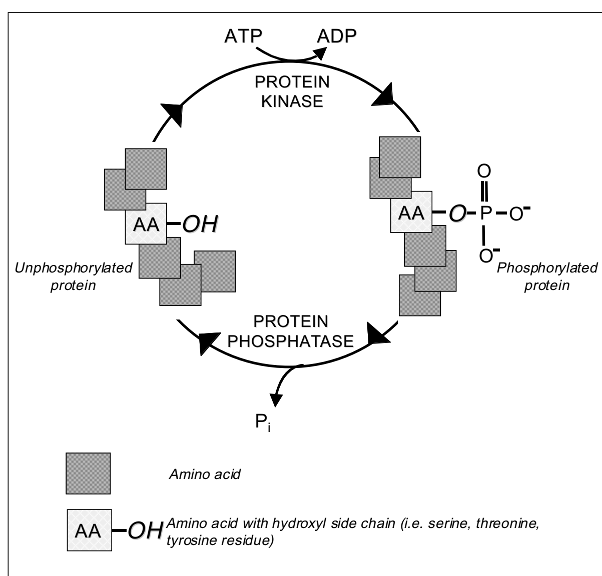

Figure 1. The process of protein phosphorylation/dephosphorylation: kinases transfer a phosphate group from an ATP molecule to the hydroxyl side chain of the target amino acid (i.e. serine, threonine or tyrosine), while phosphatases remove the phosphate group from the protein. The addition of the negatively charged phosphate group to the protein may cause the conformational change of the protein itself.

particular protein conformation is associated with its function, phosphorylation may then be considered a sort of molecular switch, turning the activity on or off.

The process of phosphorylation/dephosphorylation shows several peculiar features: it is rapid (it takes just few seconds), reversible and doesn't require new protein synthesis or degradation; these aspects were probably central to its selection during evolution as a key mechanism for the control of cell homeostasis.

The key players in this biochemical scenario are two different protein superfamilies (kinases and phosphatases), that are characterized by distinct sequences and structures. Importantly, members belonging to the same family share a conserved catalytic domain. Kinases are in charge of transferring a phosphate group from an ATP molecule to the specific target, while phosphatases remove the phosphate group from the substrate.

Recent studies have described a non-enzymatic mechanism for protein phosphorylation. It appears that the inositol pyrophosphate IP7 may in fact be able to donate a phosphate group to the serine residue of eukaryotic target proteins [4].

The target substrates for kinases and phosphatases consist of proteins or, less frequently, lipids. The former comprise different members in terms of structure, function and cellular localization. Common targets of protein phosphorylation include enzymes, receptors, signaling proteins, ion channels, transcriptional factors. For example, a well characterized process, tightly controlled

Table 1. List of kinase sustrates and relative products after phosphorylation.

Kinase	Substrate	Phosphate	Product
Protein kinase	serine	O-phosphate	phosphoserine
	threonine		phosphothreonine
	tyrosine		phosphotyrosine
	histidine	N-phosphate	phosphohistidine
	arginine		phosphoarginine
	aspartate	A-phosphate	phosphoaspartate
Lipid kinase	PIP2	O-phosphate	PIP3

Grey indicates the substrates commonly utilized in human cells.

by a network of phosphorylation switches, is the transfer of a stimulus from the cell surface to the nucleus. This process is regulated through a cascade of signals between proteins whose phosphorylation state is strictly dependent on the activity of kinases and phosphatases.

A second remarkable outcome of protein phosphorylation is the generation of binding sites for interacting proteins; this does not affect the enzymatic activity of the target protein, but creates docking sites for other specific proteins, thus favoring the relocalization of effector molecules whose proximity drives activation of the signal transduction cascade.

Through phosphorylation, the cell regulates complex functions such as proliferation, differentiation, adhesion, metabolism and apoptosis; it is therefore not surprising that its aberrant activation correlates strongly with the development of cancer and other complex diseases.

The kinase and phosphatase gene families

As discussed above, protein phosphorylation is a biochemical process regulated by two large gene superfamilies, the kinases and the phosphatases. Until recently, basic and translational research has mainly focused on kinases. One of the reasons is that while the first protein tyrosine kinase was isolated at the end of 1970 [5], the first tyrosine phosphatase was purified only in 1988 [6] and cloned in 1990 [7]. The completion of a variety of genome projects has allowed a comparative analysis of the kinase and phosphatase genes. As a result, the kinases and phosphates genome complements have been systematically defined and are typically referred to as the *kinome* and the *phosphatome*.

Kinases represent a significant fraction (1.5–2.5 %) of all eukaryotic genes, thus confirming the prominent role of these enzymes in controlling key cellular functions [8]. Kinases are usually characterized by the presence of a

conserved catalytic domain. Atypical kinases have also been identified which display catalytic activity and share structural similarities with classic kinases but lacking sequence similarities with them. 478 classical kinases and 40 atypical kinases genes have been identified, for a total of 518 human kinases twice as many as are found in flies and worms.

The crystal structure of the catalytic domains of several kinases has been solved. They typically display a common bilobal architecture characterized by the active site in the cleft between the lobes and critical differences in the catalytic domains accounting for individual substrate selectivity. On the basis of residue specificity, kinases can be classified into four main groups: tyrosine kinases, tyrosine kinase-like, serine-threonine kinases and lipid kinases (fig. 2).

All types of kinases share the same ability to transfer the gamma phosphate group from the energy-carrying molecule ATP to the target substrate. Serine-threonine kinases (STKs) constitute the large majority of kinases, accounting for roughly 400 members; while tyrosine kinases (TKs) include about 90 elements. Despite their relatively low number, TKs are involved in key signaling mechanisms, including the transduction of external stimuli to the cell nucleus. This feature is an important prerogative of multicellular organisms, and from an evolutionary point of view it is supported by the development of TKs only in metazoans.

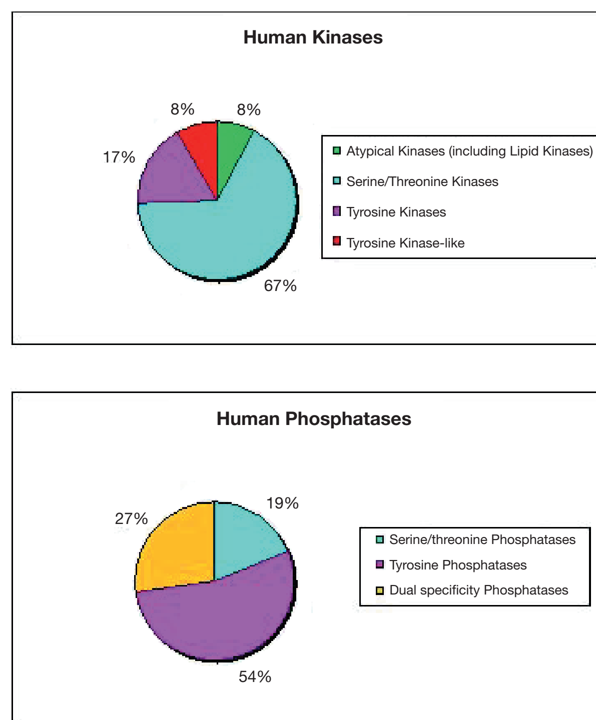

Figure 2. Classification and relative abundance of human kinase and phosphatase genes. The human *kinome* and *phosphatome* include around 530 and 180 genes, respectively [8, 10, 11, 24, 25].

TKs can furthermore be divided into receptor protein tyrosine kinases (RPTKs) and non-receptor protein-tyrosine kinases (NRPTKs). The first group includes membrane-spanning receptors, characterized by an extracellular ligand-binding domain and an intracellular kinase domain. The second group includes cytoplasmic proteins generally involved in the intracellular signaling cascade.

As phosphorylation is a dynamic and reversible process, the activity of the kinases is complemented by that of the phosphatases that catalyze the dephosphorylation reaction. Thanks also to data gathered in the postgenomic era, the phosphatases are acquiring a central connotation in the control of proliferation, differentiation, cell adhesion and motility. Phosphatases can exert both positive and negative effects on these signaling pathways, and when deregulated, they can contribute to the pathogenesis of many human diseases [9].

Protein serine/threonine phosphatases (PSTPs) specifically hydrolyze serine/threonine phosphoesters, while protein tyrosine phosphatases (PTPs) are phosphotyrosine specific [10–11]. Additionally, the subfamily of phosphatases, known as dual-specificity phosphatases, is capable of hydrolysis of both phosphotyrosine and phosphoserine/threonine residues, in addition to phospholipids [12] (fig. 2).

Like kinases, the PTPase family can be further subdivided in two classes: membrane-bound receptor phosphatases (RPTPs) and intracellular phosphatases (NRPTPs). The first group may contain one or two intracellular phosphatase domains and often immunoglobulin-like and fibronectin-like domains in the extracellular region, thus playing a role in cell-cell or cell-matrix interactions. Some RPTPs appear to participate in homophilic and heterophilic binding interactions, which suggests a role in cell guidance and contact inhibition. The NRPTPs display various intracellular localizations determined by amino acid sequences outside the catalytic domain, and some of them have been found to be associated with a variety of TKs, thus modulating the transmission of signals to the nucleus.

Kinase and phosphatase activity, or better, the balance between these activities, is extremely important for the correct execution of signal transduction: virtually all signaling pathways that have been elucidated so far involve at least one kinase or phosphatase molecule. It is therefore not surprising that genes belonging to both families are frequently altered in human cancer (see below).

Kinases and phosphatases as cancer genes

Cancer is, in essence, a genetic disease [13]. This statement represents the result of an impressive body of work that started more than a century ago but was finalized

Table 2. Selected list of kinases (grey) and phosphatases (dotted grey) genetically altered in human cancers.

Genetic alteration	Functional effect	Gene	Chromosome location	Disease
Amplification	gain of function	EGFR	7p12	glioma
		ERBB2	17q21	breast and ovarian cancer
		AKT2	19q13	ovarian and pancreatic cancer
		PRL-3	8q24	colorectal cancer
Translocation	gain of function	BCR-ABL	t(9;22) (q34;q11)	CML, ALL
Missense mutation	gain of function	BRAF	7q34	melanoma, colorectal cancer
		PIK3CA	3q26	colorectal, breast and brain cancer
		MET	7p31	HPRCC, HCC, HNSCC
		JAK2	9p24	colorectal, brain, breast, lung cancer
		PDGFRA	4q11	GIST
		Shp2/PTPN11	12q24	polycythemia vera and myeloproliferative disorders
	loss of function	PTPRG	3p21	colorectal cancer
		PTPN14	1q32	colorectal cancer
Deletion	gain of function	EGFR	7p12	NSCLC
	loss of function	PTPN13	4q21	colorectal cancer
		PTEN	10q23	glioma, prostate and breast cancer
Nonsense mutation	loss of function	PTPRT	20q12	colorectal, gastric, brain cancer
Epigenetic silencing	loss of function	PTEN	10q23	endometrial and colorectal cancer

CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukaemia; HPRCC, hereditary papillary renal-cell carcinoma; HCC, hepato-cellular carcinoma; HNSCC, head and neck squamous cell carcinoma; GIST, gastrointestinal stromal tumour; JMML, juvenile myelomonocytic leukaemia; AML, acute myelogenous leukaemia; NSCLC, non small cell lung cancer.

over the last 2 decades. Seminal studies on familiar tumor inheritance and evidence linking somatic alterations and tumors opened the way to the discovery that cancer arises from molecular alterations of the genetic code.
According to this view, cancer is a multistep process whereby cells gain properties of uncontrolled growth and proliferation as a result of the acquisition of genetic alterations that are selected through a Darwinian process of evolution [14]. In addition to unrestrained proliferation, a key property of cancer cells is their ability to invade surrounding tissues and colonize new distant sites through metastasis. The latter are ultimately responsible for the devastating effects of the disease. A variety of molecular alterations are present in the genome of cancer cells. Changes affecting single nucleotides such as point mutations are accompanied by small deletions or insertions and more complex alterations involving larger portions of chromosomes such as translocations and amplifications. Mutations can occur either in the germline, resulting in hereditary predisposition to cancer, or in single somatic cells, as occurs in sporadic tumors.
To date, almost 300 cancer-related genes, approximately 1 % of all human genes, have been identified [15]. Cancer genes can be grouped into two main categories based on their mechanism of action. When the mutations result in a dominant gain of function of the targeted protein, the corresponding gene is usually referred to as an oncogene. In this case a single mutated allele is usually sufficient to contribute to oncogenesis. When the mutations result in recessive loss of function, the corresponding gene is generally defined as a tumor suppressor gene. In this case both alleles need to be inactivated to promote tumor progression.
Among cancer genes, kinases and phosphatases play a central role, and several of these enzymes have been found altered in cancer by a variety of molecular mechanisms. The corresponding mutations have been demonstrated to be associated with cancer development in a causative fashion (table 2). Some illustrative examples

of kinases and phosphatases which have been linked to cancer in the last 20 years are the BCR-ABL translocation in chronic myelogenous leukaemia [16], the HER-2 amplification in breast tumors [17], the KIT receptor point mutations in gastrointestinal stromal tumors [18], the PTEN deletions in multiple advanced cancers [19] and the SHP2 mutations in leukaemias and solid tumors [20]. In the last 3 years the availability of the human genome sequence coupled with the development of high-throughput genomic approaches has allowed the systematic profiling of genetic alterations affecting kinases and phosphatases in cancer. These studies have unveiled a genetic snapshot of the role of protein and lipid phosphorylation in cancer cells.

Mutational profiling of gene families: lessons from resequencing the *kinome* and *phosphatome* in human cancer

The availability of the human genome sequence is renewing clinical medicine. Oncology has been one of the fields more immediately impacted by this revolution. The possibility of comparing the normal and the cancer genome sequences enables systematic identification of all the genetic alterations associated with this disease for the first time. In particular, the systematic hunt for somatic mutations in cancer genomes is now possible thanks to the development of new bioinformatics tools and sophisticated high-throughput sequencing technologies. The former are required to mine and analyze large numbers of nucleotide sequences, while the latter allow the processing of hundreds of samples at a time.

As previously discussed, cancer cells often display deregulation of signaling pathways that are controlled by the addition or removal of phosphate groups to or from protein or lipid substrates. Accordingly, members of the kinase and phosphatase gene families are frequently altered in cancer. Interestingly, however, in spite of their importance, many kinases and most phosphatases have not been characterized in detail. Furthermore, it remains unknown how many members of this gene family are altered in any particular type of cancer. A number of recent studies have started to bridge this gap using the information provided by the genome project and high-throughput DNA analysis to systematically identify cancer-associated mutations.

One approach calls for the methodical search of mutations in the molecules involved in signaling pathways in which at least one gene has previously been found to be mutated in human cancer [21]. Another approach entails sequencing entire gene families. This enables systematic genetic dissection of the specific biochemical function (for example protein phosphorylation) that is controlled by the members of the candidate gene family [22].

The first strategy was devised by Davies and colleagues who skillfully used denaturing capillary gel electrophoresis to search for mutations in members of the RAS-RAF-MEK-ERK-MAP signaling pathway. Using this approach, BRAF, a serine threonine kinase, was identified as being frequently mutated in melanoma and other cancers and represent a novel candidate for targeted cancer therapy [21, 23].

The second strategy was devised by Bardelli and colleagues to systematically analyze the mutational profile of all tyrosine kinase genes (tyrosine kinome) in colorectal cancer genomes [22]. The same strategy was later used to analyze the lipid *kinome* [24], the serine-threonine *kinome* [D. W. Parsons et al., Nature, in press] and the tyrosine *phosphatome* [25].

A number of noteworthy lessons can be drawn from these studies. The first is that more than 70 % of colorectal cancers carry a mutation in a kinase or phosphatase gene. Interestingly, although the *kinome* and *phosphatome* comprises more than 600 genes, somatic mutations were found in only a handful of them [26]. This suggests that a small number of genes are genetically altered in cancers, even in gene families such as kinases and phosphatases that are thought to play a central role in tumorigenesis. This is surprisingly different from what is obtained by genome-wide analysis of gene expression, whereby a large fraction of genes are usually found to be differentially expressed between normal and neoplastic tissues. Importantly, somatic mutations that have been selected during tumorigenesis are, by definition, causally related to tumor formation and therefore often represent valuable therapeutic targets [13].

The second lesson is that systematic approaches have allowed the identification of somatic mutations in genes that had already been the subject of decades of work. This is the case of the PI3KCA gene mutations that were found in colorectal, breast, brain, gastric and other tumor types. This establishes PI3KCA lipid kinase as one of the most commonly mutated oncogenes in human cancer [24].

The third lesson is that the types of mutations found in kinases and phosphatases are qualitatively different. Kinases tend to be altered by heterozygous missense mutations that primarily affect residues involved in the control of their enzymatic activity. This suggests that the mutations are activating and operate by increasing the catalytic activity of the corresponding proteins. This also supports the hypothesis that mutated kinase genes act as dominant oncogenes [22, 24]. On the other hand, tyrosine phosphatases are frequently altered by nonsense mutations that often affect both alleles. This suggests that the mutated phosphatase genes could act as tumor suppressors [25].

The fourth lesson is that the mutational profiling of all genes controlling the execution of a particular biochemi-

cal reaction (in this case phosphorylation) may be very informative in reconstructing signaling pathways active in human tumors. In fact, if the mutated genes display overlapping functions (for example they share the same substrate), the mutations will be mutually exclusive, thus providing additional information on the model. This is the case of the mutations affecting the BRAF and K-RAS genes and mutually exclusive in cancers, indicating that these molecules operate within the same signaling pathway [27]. Similarly, mutations affecting kinases and phosphatases involved in the phosphoinositol-3-kinase pathway also occur in a mutually exclusive fashion [D. W. Parsons et al., Nature, in press].

Molecular profiling of kinase genes and targeted cancer therapies

Molecular alterations affecting genes involved in cell homeostasis are the hallmark of cancer. Until recently, this paradigm has had little impact on the clinical treatment of this disease. Exciting results showing that therapeutic targeting of cancer is more effective in the context of specific genetic alterations has brought the genetic profiling of tumors to center stage [28, 29, 30]. Although results in this area have been incremental, they have allowed for the first allowed time the selection of patients who are likely to benefit from specific drugs, thus introducing individualized/targeted cancer therapies. In this context, targeted therapy refers to a new generation of cancer drugs designed to interfere with a specific molecular target supposed to have a critical role in tumor growth or progression.

Pharmaceutical companies started to devote efforts to the development of signal transduction modulating drugs at the end of 1980s. At that time only a few kinases were characterized, and even fewer were thought to play a role in cancer. Among them were BCR-ABL and the EGF receptor; both kinases played a central role in initial efforts towards drug discovery projects aimed at specific molecular targets. Interestingly, although in the meantime knowledge has increased dramatically, most large pharmaceutical companies are still focused on a relatively small portfolio of molecular targets. The attention has been mainly on kinases, whose molecular and crystal structure is well defined and described in the literature, while phosphatase-targeted drugs are just now beginning to be developed. Among the first attempts to specifically interfere with the activity of tyrosine kinases was the development of a family of inhibitors, such as tyrphostins, that are low molecular weight tyrosine mimics [31]. However, because of their low specificity, tyrphostins have not found large application in clinical studies. Presently, although selectivity remains a central issue, it appears clear that kinase inhibitors can be effectively developed by targeting the ATP binding site with small molecules. The availability of the crystal structure of several kinases represents a significant booster for these approaches and is leading to faster-track development of these drugs.

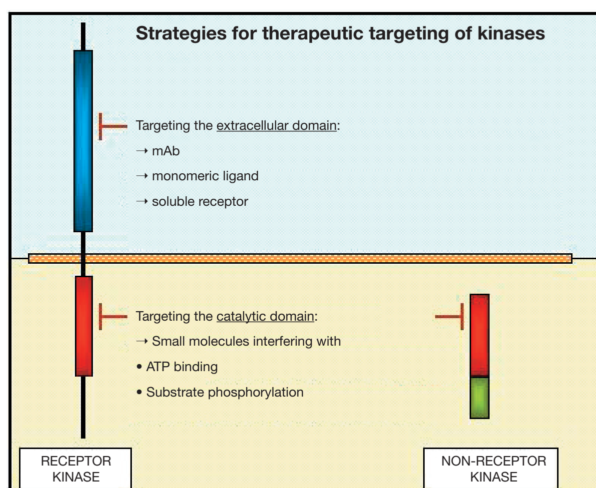

Figure 3. Rationale for therapeutic targeting of kinases. The deregulated kinase can be inhibited by a variety of strategies including: targeting the extracellular domain with monoclonal antibodies which bind to the extracellular domain and inhibit receptor activation; monomeric ligands interfering with receptor dimerization; soluble extracellular portions of the receptors to sequester the ligand. Targeting the catalytic activity with small molecules interfering with ATP binding to the catalytic site or the substrate binding site.

Clinical validation of kinase inhibitors has had a bumpy ride. In many cases this was due to the lack or inappropriate selection of patients included in clinical trials. The levels of expression of the target kinase and its involvement in specific signaling pathways were among the criteria initially used to identify the cancer type for clinical trials. Often this led to failures, some of which later turned into success when patients cohorts were stratified on the basis of the genetic profile of their tumors. It is becoming increasingly clear that responsiveness to kinase inhibitors has a genetic basis and that molecular alterations present in tumors can be used to identify patients who are likely to benefit from the treatment.

Although there is still considerable debate regarding the conceptual basis of these findings, it is clear that genetic alterations that have been selected during tumorigenesis are causally related to tumor formation. As such, these mutations should represent legitimate targets for anticancer drugs. Several representative examples indicate that this may be a promising and revolutionary paradigm for fighting cancer (fig. 3 and table 3).

The first example is imatinib mesylate (Gleevec) for the treatment of chronic myelogenous leukaemia (CML). Imatinib is a small chemical compound (approved by FDA in 2001), which at micromolar concentrations inhibits

Table 3. Selected list of kinase inhibitors.

Target kinase	**Disease**	**Drug**
ABL, KIT, PGFR	CML, GIST	imatinib (Gleevec)
EGFR	NSCLC NSCLC, pancreatic cancer colorectal cancer colorectal cancer, lung cancer	gefitinib (Iressa) erlotinib (Tarceva) cetuximab (Erbitux) panitumumab
ERBB2	breast cancer	trastuzumab (Herceptin)
EGFR, ERBB2	breast, endometrial and renal cancer	lapatinib
FLT3	AML	PKC-412
VEGFR2 PDGFR FLT3 KIT	kidney cancer lung cancer, kidney cancer AML lung cancer	SU011248
RAF VEGFR2	melanoma kidney cancer	BAY-43-9006

CML, chronic myelogenous leukaemia; GIST, gastrointestinal stromal tumor; NSCLC, non small cell lung cancer; AML, acute myelogenous leukaemia.

the activity of ABL kinase by competing with ATP for its binding site. This results in inhibition of cell growth and induction of apoptosis of the leukaemic cells carrying the BCR-ABL fusion protein caused by a pathogenetic translocation among chromosome 9 and 22 [32]. The genetic basis for molecular therapies of CML based on targeting the BCR-ABL protein is further demonstrated by the fact that in patients who become refractory to imatinib mesylate, the BCR-ABL fusion gene typically carries additional genetic alterations [33, 34]. A new combination of drugs that inhibits the different conformational variants of mutated BCR-ABL is effective in overcoming this resistance [35]. Additionally, it was recently shown that a non-ATP-competitive ABL inhibitor may also be useful in overriding Gleevec resistance [36].

Another example supporting this paradigm also involves imatinib mesylate, whose efficacy has been demonstrated in gastrointestinal stromal tumors (GISTs) [37] [38]. In this case, however, the response rate is associated with the mutational status of two tyrosine kinase receptors, KIT and PDGF [39]. Both receptors share homology with the Abl kinase and are therefore inhibited by imatinib mesylate.

Additional examples involve targeting members of the epidermal growth factor receptor (EGFR) family, which has been achieved among two strategies. Monoclonal antibodies have been developed to block binding of the ligand to the extracellular domain of Erbb1 (cetuximab and panitumumab) and Erbb2 (trastuzumab) receptors. Furthermore, small molecules that antagonize ATP binding represent another class of EGFR inhibitor drugs. Two of these drugs, gefitinib (Iressa) and erlotinib (Tarceva), are already in clinical use. Tumor responsiveness to the anti-EGFR monoclonal antibodies is associated with the copy number of the corresponding gene present in individual tumors [40]. On the other hand, sensitivity to gefitinib correlates with mutations of the EGFR catalytic domain [41].

In conclusion, the molecular profiling of cancer patients on the basis of the genetic analysis of kinase genes has contributed to the creation of a new paradigm in basic and clinical oncology. This paradigm consists of the individualized analysis and treatment of cancer patients according to the mutated genes present in their tumors. Given that the presence of *kinome* and *phosphatome* mutations has been assessed in very few cancer types, it is likely that this approach will provide additional promising therapeutic avenues.

Acknowledgements. We apologize to colleagues whose work was not cited owing to space constraints or our oversight. We thank Dr Asha Balakrishnan for critical reading the manuscript. Supported by the Italian Association for Cancer Research (AIRC) and the Ministero dell'Istruzione, dell'Università e della Ricerca (MURST) and from the Regione Piemonte. S. B. is supported by an AIRC fellowship.

1 Burnett G. and Kennedy E. P. (1954) The enzymatic phosphorylation of proteins. J. Biol. Chem. **211** (2): 969–980
2 Sutherland E.W. Jr and Wosilait W. D. (1955) Inactivation and activation of liver phosphorylase. Nature **175** (4447): 169–170
3 Toker A. (1998) The synthesis and cellular roles of phosphatidylinositol 4,5-bisphosphate. Curr. Opin. Cell Biol. **10** (2): 254–261
4 Saiardi A., Bhandari R., Resnick A. C., Snowman A. M., Snyder S. H. (2004) Phosphorylation of proteins by inositol pyrophosphates. Science **306** (5704): 2101–2105

5 Czernilofsky A. P., Levinson A. D., Varmus H. E., Bishop J. M., Tischer E., Goodman H. M. (1980) Nucleotide sequence of an avian sarcoma virus oncogene (src) and proposed amino acid sequence for gene product. Nature **287** (5779): 198–203
6 Tonks N. K, Diltz C. D, Fischer E. H. (1988) Purification of the major protein-tyrosine-phosphatases of human placenta. J. Biol. Chem. **263** (14): 6722–6730
7 Guan K. L., Haun R. S., Watson S. J., Geahlen R. L., Dixon J. E. (1990) Cloning and expression of a protein-tyrosine-phosphatase. Proc. Natl. Acad. Sci. USA **87** (4): 1501–1505
8 Manning G., Whyte D. B., Martinez R., Hunter T., Sudarsanam S. (2002) The protein kinase complement of the human genome. Science **298** (5600): 1912–1934
9 Zhang Z. Y. (2001) Protein tyrosine phosphatases: prospects for therapeutics. Curr. Opin. Chem. Biol. **5** (4): 416–423
10 Alonso A., Sasin J., Bottini N., Friedberg I., Osterman A., Godzik A. et al. (2004) Protein tyrosine phosphatases in the human genome. Cell **117** (6): 699–711
11 Plowman G. D., Sudarsanam S., Bingham J., Whyte D., Hunter T. (1999) The protein kinases of *Caenorhabditis elegans*: a model for signal transduction in multicellular organisms. Proc. Natl. Acad. Sci. USA **96** (24): 13603–13610
12 Lyon M. A., Ducruet A. P., Wipf P, Lazo J. S. (2002) Dual-specificity phosphatases as targets for antineoplastic agents. Nat. Rev. Drug Discov. **1** (12): 961–976
13 Vogelstein B., Kinzler K. W. (2004) Cancer genes and the pathways they control. Nat. Med. **10** (8): 789–799
14 Vogelstein B., Kinzler K. W. The multistep nature of cancer. (1993) Trends Genet. **9** (4): 138–141
15 Futreal P. A., Coin L., Marshall M., Down T., Hubbard T., Wooster R. et al. (2004) A census of human cancer genes. Nat. Rev. Cancer **4** (3): 177–183
16 Saglio G., Cilloni D. (2004) Abl: the prototype of oncogenic fusion proteins. Cell. Mol. Life Sci. **61** (23): 2897–2911
17 Hynes N. E., Lane H. A. (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat. Rev. Cancer **5** (5): 341–354
18 Rubin B. P., Singer S., Tsao C., Duensing A., Lux M. L., Ruiz R. et al. (2001) KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res **61** (22): 8118–8121
19 Parsons R., Simpson L. (2003) PTEN and cancer. Methods Mol. Biol. **222**: 147–166
20 Bentires-Alj M., Paez J. G., David F. S., Keilhack H., Halmos B., Naoki K. et al.(2004) Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. Cancer Res. **64** (24): 8816–8820
21 Davies H., Bignell G. R., Cox C., Stephens P., Edkins S., Clegg S. et al.(2002) Mutations of the BRAF gene in human cancer. Nature **417:** 949–954
22 Bardelli A., Parsons D. W., Silliman N., Ptak J., Szabo S., Saha S. et al. (2003) Mutational analysis of the tyrosine kinome in colorectal cancers. Science **300** (5621): 949
23 Tuveson D. A., Weber B. L., Herlyn M. (2003) BRAF as a potential therapeutic target in melanoma and other malignancies. Cancer Cell **4** (2): 95–98
24 Samuels Y., Wang Z., Bardelli A., Silliman N., Ptak J., Szabo S. et al. (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science **304** (5670): 554
25 Wang Z., Shen D., Parsons D. W., Bardelli A., Sager J., Szabo S. et al. (2004) Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science **304** (5674): 1164–1166
26 Bardelli A., Velculescu V. E. (2005) Mutational analysis of gene families in human cancer. Curr. Opin. Genet. Dev. **15** (1): 5–12
27 Rajagopalan H., Bardelli A., Lengauer C., Kinzler K. W., Vogelstein B., Velculescu V. E. (2002) Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature **418** (6901): 934
28 Lynch T. J., Bell D. W., Sordella R., Gurubhagavatula S., Okimoto R. A., Brannigan B. W. et al.(2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N. Engl. J. Med. **350** (21): 2129–2139
29 Paez J. G., Janne P. A., Lee J. C., Tracy S., Greulich H., Gabriel S. et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science **304** (5676): 1497–1500
30 Pao W., Miller V., Zakowski M., Doherty J., Politi K., Sarkaria I. et al. (2004) EGF receptor gene mutations are common in lung cancers from never smokers and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc. Natl. Acad. Sci. USA **101** (36): 13306–13311
31 Levitzki A., Gazit A., Osherov N., Posner I., Gilon C. (1991) Inhibition of protein-tyrosine kinases by tyrphostins. Methods Enzymol. **201**: 347–361
32 Druker B. J., Tamura S., Buchdunger E., Ohno S., Segal G. M., Fanning S. et al. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med. **2** (5): 561–566
33 Shah N. P., Sawyers C. L. (2003) Mechanisms of resistance to STI571 in Philadelphia chromosome-associated leukemias. Oncogene **22** (47): 7389–7395
34 Gambacorti-Passerini C. B., Gunby R. H., Piazza R., Galietta A., Rostagno R., Scapozza L. (2003) Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. Lancet Oncol. **4** (2): 75–85
35 Shah N. P., Tran C., Lee F. Y., Chen P., Norris D., Sawyers C. L. (2004) Overriding imatinib resistance with a novel ABL kinase inhibitor. Science **305** (5682): 399–401
36 Gumireddy K., Baker S. J., Cosenza S. C., John P., Kang A. D., Robell K. A. et al. (2005) A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. Proc. Natl. Acad. Sci. USA **102:** 1992–1997
37 Tuveson D. A., Willis N. A., Jacks T., Griffin J. D., Singer S., Fletcher C. D. et al.(2001) STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene **20** (36): 5054–5058
38 Chen H., Isozaki K., Kinoshita K., Ohashi A., Shinomura Y., Matsuzawa Y. et al. (2003) Imatinib inhibits various types of activating mutant kit found in gastrointestinal stromal tumors. Int. J. Cancer **105** (1): 130–135
39 Heinrich M. C., Corless C. L., Demetri G. D., Blanke C. D., von Mehren M., Joensuu H. et al. (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J. Clin. Oncol. **21** (23): 4342–4349
40 Moroni M., Veronese S., Benvenuti S., Marrapese G., Sartore-Bianchi A., Di Nicolantonio F. et al. (2005) Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. Lancet Oncol. **6** (5): 279–286
41 Pao W., Miller V. A. (2005) Epidermal growth factor receptor mutations, small-molecule kinase inhibitors and non-small-cell lung cancer: current knowledge and future directions. J. Clin. Oncol. **23** (11): 2556–2568