

Over the last decades, many discoveries have been made concerning the genetic defects involved in tumor formation and growth. This has launched the development of novel anticancer agents, interfering with the proteins encoded by the identified mutated genes. The bcr-abl gene is considered to be a prototypic tyrosine kinase oncogene. Signal transduction inhibitors target deregulated intracellular pathways. Uncontrolled signal transduction has important deregulatory consequences and is responsible for deranged cellular proliferation, apoptosis, cytoadhesion and ultimately oncogenesis. The efficacy of a recently approved drug named STI571 (Gleevec) in treating patients with chronic myeloid leukaemia gives new hopes to the scientific community to combat cancer. STI571 has proved to be very effective in killing Bcr-Abl-positive cells in vitro and in vivo, both in chronic or stable phase disease and in blast crisis phase of chronic myelogenous leukemia as well as in Ph+ acute lymphocytic leukemia (ALL). The discovery of STI571 is considered as the hallmark of a new beginning in drug therapy. It is believed that a growing number of new molecular targets will soon emerge. In this article we present a brief review of the signal transduction pathway and explain the philosophy of the signal transduction inhibitors, focusing on the newly discovered drug STI571 (Gleevec).

Key words: signal transduction, Gleevec, tyrosine kinase, carcinogenesis.

Tyrosine kinase inhibitors and signal transduction: molecular biology and latest data

Inhibitory kinazy tyrozynowej i przekazywanie sygnału: mechanizmy molekularne w aspekcie najnowszych danych

George Constantine Zografos, Philip John Domeyer

1st Propaedeutic Surgical Clinic, University of Athens, Ippokrateio Hospital, Greece

Introduction

Target-based drugs are a new category of anticancer drugs that have recently been developed thanks to the enormous progress in molecular biology and biochemistry. These include inhibitors of signal transduction, cyclin-dependent kinase, angiogenesis, and matrix metalloproteinases. Other new therapies include immunotherapy and gene therapy. There are multiple steps in the signal transduction cascade. Growth factor binds to its cognate receptor tyrosine kinase and the phosphotyrosines on the receptor serve as attachment sites for substrates or adapter molecules. Grb2 functions by directly coupling activated receptor tyrosine kinases to the Ras or Ras family members, which in turn leads to activation of the mitogen-activated protein kinase (MAPK) cascade pathway. This has been implicated as a necessary component of intracellular signaling to elicit a range of cellular responses including mitogenesis, differentiation, and cell survival [1]. Based on this knowledge, novel drugs have been developed that specifically target and block these critical molecules. The tyrosine kinase inhibitor, imatinib mesylate (formerly STI571, Gleevec; Novartis Pharmaceuticals Corp, East Hanover, NJ), recently approved for the treatment of chronic myeloid leukemia (CML) in various countries, is a good example of the positive impact of basic science discoveries on clinical practice [2]. Other examples include Herceptin (trastuzumab) for the treatment of advanced breast cancer and Iressa (gefitinib, ZD1839, in Japan) for the treatment of lung cancer. Very interestingly, these are the first examples of drugs which have successfully translated basic research on oncogenes into cancer therapeutics and have been considered as the most significant development toward a new era of target-directed therapies [3, 4].

This article reviews the links between the basic principles of the molecular biology of the signal transduction inhibitors and the molecular, pharmacological and clinical significance of the novel tyrosine kinase inhibitor, imatinib mesylate, which could prove to be extremely important in the treatment of chronic myelogenous leukemia [5]. The development of the research in this field and the pertaining literature is so fast that we had to operate a very subjective selection. We decided to focus our attention on the tyrosine kinase oncogene bcr-abl and the novel signal transduction inhibitor Imatinib Mesylate, given their major clinical significance that we attempt to point out.

W ostatnich dziesięcioleciach nastąpił gwałtowny rozwój wiedzy dotyczącej zaburzeń genetycznych warunkujących powstawanie i wzrost guzów nowotworowych. Wiedza ta z kolei umożliwiła opracowanie nowych, celowanych terapii przeciwnowotworowych, mających za zadanie wybiórcze hamowanie funkcji białek kodowanych przez uszkodzone geny. Gen *bcr-abl* będący onkogenem koduje białko o aktywności kinazy tyrozynowej. Inhibitory szlaków transdukcji sygnału wybiórczo hamują uszkodzone mechanizmy wewnątrzkomórkowego przekazu informacji od błony komórkowej do jądra. Zaburzenia w transdukcji sygnału mogą wybitnie zakłócić takie mechanizmy komórkowe, jak proliferacja, apoptoza, czy adhezja i w konsekwencji doprowadzić do transformacji nowotworowej danej komórki. Skuteczność preparatu STI571 (Gleevec) w terapii chorych z CML daje nadzieję na istotny postęp w celowanym leczeniu nowotworów. STI571 skutecznie eliminuje komórki wykazujące ekspresję genu *Bcr-Abl* zarówno w warunkach *in vitro*, jak i *in vivo*. Jest skuteczny zarówno w przewlekłej, stabilnej fazie choroby oraz w kryzie blastycznej w przebiegu CML, jak również w ostrej białaczce limfocytarnej Ph+. Wykrycie STI571 uznaje się za przełom w terapii nowotworów. Prawdopodobnie w krótkim czasie będziemy mieli do czynienia z dużą liczbą nowych preparatów wybiórczo działających na określone cząsteczki biorące udział w procesach transformacji nowotworowej. W niniejszym artykule, na przykładzie preparatu STI571 (Gleevec), omawiane są mechanizmy transdukcji sygnału oraz zasady działania ich inhibitorów.

Słowa kluczowe: transdukcja sygnału, Gleevec, kinaza tyrozynowa, nowotworzenie.

Signal transduction inhibition: molecular biology

Basic principles of molecular signal transduction

The cell membrane has proved to be the key site of many elements involved in the control of fundamental cell processes [6-8]. Although lipid-soluble hormones can diffuse through the plasma membrane and interact directly with transcription factors in the cytoplasm or nucleus, the second major class of hormones, peptide and protein hormones, cannot. Instead, these hormones function by binding to specific cell-surface receptors, which then transduce the signal through the cytoplasm to the nucleus. This process is called signal transduction. In many cases, the mechanism by which a hormonal signal is transduced into an activating signal for transcriptional factors involve phosphorylation [2, 9]. The latter is a basic mechanism for many signal transduction biochemical modifications, including oxidation or reduction, post-translational addition of protein modifiers such as ubiquitin and binding of nucleotides, such as ATP and GTP [1]. Phosphorylation of the substrate proteins or lipids is carried out by tyrosine and serine/threonine kinases, which leads to quantitative or qualitative changes of the substrates. The set of protein phosphorylations and dephosphorylations that take place constitutes a cascade, which allows the entire group of enzyme-catalyzed reactions to be regulated by a single type of molecule. Moreover, a huge amplification of the initially small signal is achieved [9].

Receptor tyrosine kinases (RTK): principles of structure and function

All RTKs contain a large, glycosylated, extracellular ligand-binding domain, a single transmembrane region and a cytoplasmic portion with a conserved protein tyrosine kinase domain. In addition to the catalytic domain, a juxtamembrane region and a carboxyl-terminal tail can be identified in the cytoplasmic portion. They further contain regulatory sub-domains which negatively or positively influence substrate-binding and phosphorylation, as well as sub-domains involved in the obligatory dimerization and/or structural changes during kinase activation after ligand binding [3]. Each of these structural domains could become future targets for drug development.

There is substantial evidence that ligand-induced activation of the kinase domain and its signaling potential are mediated by receptor dimerization [3, 9-12]. How ligands bind to the receptors and induce oligomerization is specific for each class of receptor tyrosine kinases [10-13]. Ligand binding is believed to cause formation of a homodimer (between two identical receptors) or heterodimer (between different members of the same receptor family), triggering the binding and activation of a cytosolic protein tyrosine kinase. However, although receptor dimerization plays a pivotal role, it may not be sufficient for RTK activation. Experimental modifications of the transmembrane domain of some RTKs showed that the main function of the transmembrane domain is to anchor the receptor in the plain of the plasma membrane, thereby connecting the extracellular environment with internal compartments of the cell [3].

The activated kinase phosphorylates specific tyrosine residues in the cytoplasmic portion of the receptor. Moreover, tyrosine phosphorylation in the kinase domain stimulates the intrinsic catalytic activity of the receptors; substrate proteins then bind to these phosphotyrosine residues and are phosphorylated [9]. This autophosphorylation is believed to occur in *trans* by a second receptor tyrosine kinase after dimerization and/or conformational changes induced by ligand binding. In the unphosphorylated state, the receptor has a low catalytic activity due to the particular conformation of the activation loop, which takes part in the phosphorylation event. Phosphorylation of the kinase domain removes this inhibition and the catalytic activity is enhanced and persists for a while, independently of the presence of the ligand [3, 14, 15].

Although the juxtamembrane sequence that separates the transmembrane and cytoplasmic domains is not well conserved between different families or

receptors, the tyrosine kinase domain is the best conserved among tyrosine kinase receptors and, therefore, an intact protein tyrosine kinase domain is believed to be absolutely necessary for receptor signaling. The carboxyl-terminal tail sequences are the most divergent between known RTKs and the carboxyl-terminal domain is thought to play an important role in regulating the kinase activity.

The role of tyrosine kinases and their receptors in oncogenesis

As a result of protooncogene to oncogene transformation, kinases that recognize and phosphorylate a broad range of target proteins are formed. These kinases are now rendered constitutively active without the requirement of growth factor stimulation by ligand and can drive deregulated cell growth; receptor tyrosine kinases such as the insulin-like growth factor 1 (IGF 1) receptor are key components of the cell death control machinery. Angiogenesis-initiating signaling also involves receptor tyrosine kinases such as the vascular endothelium growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) receptors [3]. These activated tyrosine kinase oncogenes and their corresponding receptors are becoming more and more interesting as drug targets for many reasons. First of all, the number of known oncogenes coding for activated kinases, such as Bcr-Abl, EGF-R is important and ever increasing. Second, small-molecule drugs can easily fit into the enzymatic kinase domains. Finally, these domains are so specific that drugs can distinguish and selectively target the enormous amount of kinases in cells [2]. Proof-of-concept for the drugability of these molecular targets is already established with the approval of the anti-erbB2 antibody – Herceptin and the tyrosine kinase inhibitors – Gleevec and Iressa.

A prototypic tyrosine kinase oncogene: the bcr-abl gene

Virtually all patients suffering from chronic myelogenous leukemia (CML) have in their cells the hybrid Philadelphia chromosome. This chromosome is a fusion of most of chromosome 9 to a piece of chromosome 22. The reciprocal translocation is also present, although it is not easily recognized. *Abl* was first identified as an oncogene in human cancer when it was discovered that the *c-abl* protooncogene lies at the break point on chromosome 9, and the translocation causes the formation of an mRNA derived partly from chromosome 22 and partly from *c-abl*. Therefore, in this fusion gene, a large portion of the *c-abl* tyrosine kinase gene from chromosome 9 is joined to sequences derived from the breakpoint cluster region (*bcr*) gene on chromosome 22. This newly formed gene is transcribed into a chimeric 8.5 kilobase mRNA that is translated into the p210 Bcr-Abl protein. The loss of the *c-abl* first exon disrupts the normal regulatory mechanisms controlling the *Abl*-derived kinase domain of Bcr-Abl. This protein is leukemogenic because of its constitutive tyrosine kinase activity, which drives numerous downstream signal transduction pathways [16].

Activation and transformation of Bcr-Abl as a kinase is thought to occur because a C-terminal „coiled-coil” oligomerization domain in Bcr causes dimerization or

aggregation of many molecules. When Bcr-Abl come close, they autophosphorylate their tyrosine residues, which now serve as docking sites for the SH2 domains of adapter proteins such as GRB2 or CRKL. These adaptor molecules bind to Bcr-Abl and to other substrates that are brought close to each other in similar manners, creating large signaling complexes. These complexes are now capable of transducing multiple cellular signals or pathways in an uncontrolled fashion. Pathways affected by this abnormal transformation are, among others, the *c-Myc* and *ras* family oncogenes, the MAP kinase, cell cyclins and tyrosine phosphatases [2].

Uncontrolled signal transduction has important deregulatory consequences and is responsible for deranged cellular proliferation, apoptosis and cytoadhesion [16]. Regarding CML, the deregulated kinase activity of Bcr-Abl is probably one of the first steps that finally leads to the disease. The pivotal role that Bcr-Abl kinase plays in the progression of this disease has been intensively studied. Recently, those efforts bore fruit with the development of a new drug targeting this kinase.

The novel signal transduction inhibitor – Imatinib Mesylate

The development of this new drug, called imatinib mesylate, seems to be extremely important for the treatment of chronic myelogenous leukemia, although its therapeutic capacities are probably much broader and include other sensitive solid tumors such as gastrointestinal stromal tumors and malignancies in general. Imatinib mesylate, also called STI571 (Gleevec) is a 2-phenylaminopyrimidine derivative and emerged from an effort by Novartis Pharmaceuticals to inhibit PDGF-R but was also found to inhibit the *abl*-related tyrosine kinases including p210*bcr-abl*, p185*bcr-abl*, v-*abl* and *c-abl* as well as *c-kit* tyrosine kinase and the *Abl*-related kinase, Arg [2, 17, 18]. STI571 is thought to bind to a distinctive „inactive” conformation of the centrally located activation loop of *abl* kinase, preventing its catalytic utilization of ATP and therefore activation [19]. After inhibition of p210*bcr-abl* has taken place, cytochrome c is released from mitochondria to the cytosol and caspases 3 and 9 are activated. DNA fragmentation and apoptosis follow [20]. In order to maintain the inhibition of *bcr-abl* kinase activity, continuous drug exposure is necessary [21]. Moreover, STI571 has been proved to inhibit the growth of glioblastoma (perhaps by PDGF-R effects) [22] and small cell lung cancer known to express *c-kit* [23]. Finally, in Ph⁺ leukemia cell lines, STI571 was shown to have synergistic effects with α -interferon and vincristine as well as an additive effect in combination with cyclophosphamide, hydroxyurea, cytarabine, doxorubicin and etoposide, whereas an antagonistic effect was exerted when combined with methotrexate [17, 24].

STI571 is obviously considered a drug with a lack of absolute target specificity, which was originally considered a disadvantage. However, it is now appreciated that the growing repertoire of potential targets of imatinib mesylate contributes to the drug's potential usefulness as a therapeutic agent for cancer [2].

Resistance to STI571 has already been noted and several mechanisms have been proposed to explain it. These include reduced drug uptake, overexpression of multidrug resistant P-glycoproteins, a *bcr-abl* amplification and others. STI571 has also been shown to bypass chemotherapy resistance and seems to have a synergic effect with other drugs including interferon- α , daunorubicin, cytarabine and etoposide [25, 26].

Pharmacological properties

Imatinib mesylate is given orally and well absorbed. A single daily dose of 400 mg achieved a steady-state mean maximal concentration of 2.3 $\mu\text{g/ml}$ and 0.72 $\mu\text{g/ml}$ 24-hour concentration, exceeding the threshold value for *bcr-abl* kinase inhibition [27]. The drug's half life is of 13 to 16 hours. STI571 is metabolized in the liver mainly through CYP3A4 enzyme and excreted through the kidneys.

Clinical response

STI571 has been proved to possess a great ability to kill Bcr-Abl-positive cells *in vitro* and *in vivo*, both in chronic or stable phase disease and in blast crisis phase of chronic myelogenous leukemia as well as in Ph⁺ acute lymphocytic leukemia (ALL) [2, 16, 26]. Ninety-eight percent of patients suffering from chronic phase CML that received a dose of 300 mg/day or more achieved complete hematologic response at 3 weeks after the beginning of treatment. The positive result was maintained for a median of 265 days in 96% of patients. Additionally, thirty-one percent of patients achieved major or complete cytogenetic response [28]. Complete cytogenetic response was also noted in 28%, 44% and 62% in phase II studies of chronic phase, accelerated phase and myeloid blast crisis phase of CML, respectively [17, 29].

A 400 mg dose of STI571 was also given to a patient suffering from metastatic gastrointestinal stromal tumor (GIST) resistant to other chemotherapeutic agents and expressing *c-kit*, with very good results [30]. *c-kit* is thought to contribute to the pathogenesis of several solid tumors such as germ cell tumors, melanoma, ovarian tumors and breast cancer. An EORTC phase I study concerning GIST and other solid tumors using STI571 has been encouraging so far [16, 31]. Positron-emission tomography (PET) has been proved of valuable use to assess the metabolic response to STI571 [30, 31].

Toxicity

Treatment with STI571 with a dose range of 25-1,000 mg was associated with a tolerable toxicity profile. This could be explained by the following observations. First, Kit, Arg and platelet-derived growth factor receptor, all inhibited by STI571, are not important in adult tissues and secondly, inhibition conveyed by STI571 is partial (50% to 99%) [2].

The most commonly observed toxic effect was nausea (43-55%). Edema (39-41%), myalgias (21-41%), vomiting (18-41%), diarrhea (17-25%), rash (17-19%), fatigue (10-20%), arthralgias (13%) and anorexia (10%) were also noted [28, 32]. Other rare toxic effects possibly related to STI571 include anemia, elevated liver enzymes, gastric hemorrhage, congestive heart failure, renal failure and exfoliative dermatitis

[16]. Tumor lysis syndrome was also reported and resulted in solid tumor bleeding. Finally, thrombocytopenia (30-69%) and neutropenia (34-66%) were noted and treated with dose reduction or temporary treatment interruption [16].

New molecular targets for drug therapy

The discovery of STI571 was the hallmark of a new beginning in drug therapy. This drug is now being evaluated as treatment for metastatic gastrointestinal stromal tumors. The overall position of receptor tyrosine kinases (RTK) at the very heart of many of the cellular processes that are deregulated in cancer makes them even more attractive since inhibition of one RTK pathway may yield effects on more than simply cell proliferation [3]. For example, Herceptin has recently been shown in a mouse model of breast cancer to exert also its beneficial effects by inhibiting angiogenesis [33]. Moreover, it is believed that a growing number of new molecular targets will soon emerge, given the already increasing interest of pharmaceutical industry in the development of new drugs targeting biochemical pathways. Drug targets include mutated tyrosine kinases, signaling molecules that take part in important signal transduction as well as kinases that are structurally intact but overexpressed in tumor tissues.

The FLT3 receptor exclusively expressed on hematopoietic cells is considered a good candidate target. FLT3, a protooncogene capable of transforming cell lines *in vitro* to growth factor independence, is associated with myelodysplastic syndrome and is frequently mutated in patients with acute myeloblastic leukemia [2, 16]. Another excellent target molecule is the *c-kit* protooncogene. This encodes a cell surface tyrosine kinase that functions as the receptor for stem cell factor or steel factor. It is expressed on normal cells such as hematopoietic stem and progenitor cells, germ cells, mast cells and melanocytes. Finally, papillary renal carcinoma is known to be associated with mutated forms of the *c-Met* receptor, another promising molecular target [2].

Conclusion

Molecular pharmacology has entered a new era where development of therapeutic anticancer drug plays a pivotal role and the discovery of STI571 proves the truth of this statement. The development of these anticancer drugs depends on the identification of suitable targets within the pathophysiologic pathway of carcinogenesis, such as tyrosine kinases, and on developing agents which act on them. STI571 is undoubtedly an important multi-target drug that proves once more that molecular biology and pharmacology can be successfully combined for the fight against cancer.

References

1. Ueda Y, Saijo N. Signal transduction inhibitor. *Gan To Kagaku Ryoho* 2001; 28 (5): 591-600.
2. Griffin J. The biology of signal transduction inhibition: basic science to novel therapies. *Semin Oncol* 2001; 28 (5 Suppl 17): 3-8.
3. Bennisroune A, Gardin A, Aunis D, Cremel G, Hubert P. Tyrosine kinase receptors as attractive targets of cancer therapy. *Crit Rev Oncol Hematol* 2004; 50 (1): 23-38.

4. Sawyers CL. Rational therapeutic intervention in cancer: kinases as drug targets. *Curr Opin Genet Dev* 2002; 12: 111-5.
5. Workman P, Kaye SB. Translating basic cancer research into new cancer therapeutics. *Trends Mol Med* 2002; 8 (4): S1-9.
6. Cohen P, Honslay M (eds.). *Molecular mechanisms of transmembrane signalling, molecular aspects of cellular regulation*. Elsevier, New York 1985.
7. Rozengurt E. Early signals in the mitogenic response. *Science* 1986; 234 (4773): 161-6.
8. Verweij J. New promising anticancer agents in development: what comes next? *Cancer Chemother Pharmacol* 1996; 38 Suppl: S3-S10.
9. Lodish H, Baltimore D, Berk A, et al. *Molecular cell biology*. 3rd ed. Scientific American Books.
10. Heldin CH. Dimerization of cell surface receptors in signal transduction. *Cell* 1995; 80: 213-23.
11. Weiss A, Schlessinger J. Switching signals on or off by receptor dimerization. *Cell* 1998; 94: 277-80.
12. Brennan PJ, Kurnagai T, Berezov A, Murali R, Greene MI, Kumogai T. HER2/neu: mechanisms of dimerization/oligomerization. *Oncogene* 2000; 19: 6093-101.
13. Hubbard SR. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol* 1999; 71: 343-58.
14. Hubbard SR, Till JH. Protein tyrosine kinase structure and function. *Annu Rev Biochem* 2000; 69: 373-98.
15. Ulrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990; 61: 203-12.
16. Kantarjian HM, Talpaz M. Imatinib Mesylate: Clinical results in Philadelphia chromosome-positive leukemias. *Semin Oncol* 2001; 28 (5 Suppl 17): 9-18.
17. Elsayed YA, Sausville EA. Selected novel anticancer treatments targeting cell signaling proteins. *Oncologist* 2001; 6: 517-37.
18. Druker BJ, Lydon NB. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J Clin Invest* 2000; 105: 3-7.
19. Schindler T, Bornmann W, Pellicena P, et al. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 2000; 289: 1938-42.
20. Svingen PA, Tefferi A, Kottke TJ, et al. Effects of the bcr/abl kinase inhibitors AG957 and NSC 680410 on chronic myelogenous leukemia cells *in vitro*. *Clin Cancer Res* 2000; 6: 237-49.
21. Le Coutre P, Mologni L, Cleris L, et al. *In vivo* eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst* 1999; 91: 163-8.
22. Kilic T, Alberta JA, Zdunek PR, et al. Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenyl-aminopyrimidine class. *Cancer Res* 2000; 60: 5143-50.
23. Krystal GW, Honsawek S, Litz J, et al. The selective tyrosine kinase inhibitor STI571 inhibits small cell lung cancer growth. *Clin Cancer Res* 2000; 6: 3319-26.
24. Kano Y, Akutsu M, Tsunoda S, et al. *In vitro* cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. *Blood* 2001; 97: 1999-2007.
25. Thiesing JT, Ohno-Jones S, Kolibaba KS, et al. Efficacy of STI571, an abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against bcr-abl-positive cells. *Blood* 2000; 96: 3195-9.
26. Fang G, Kim CN, Perkins CL, et al. CGP57148B (STI-571) induces differentiation and apoptosis and sensitized Bcr-abl-positive leukemia cells to apoptosis due to antileukemic drugs. *Blood* 2000; 96: 2246-53.
27. Bruker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001; 344: 1031-7.
28. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001; 344: 1038-42.
29. Mauro MJ, Druker BJ. STI571: targeting BCR-ABL as therapy for CML. *Oncologist* 2001; 6: 233-8.
30. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with metastatic gastrointestinal tumor. *N Engl J Med* 2001; 344: 1052-6.
31. Van Oosterom AT, Judson I, Verweij J, et al. STI571, an active drug in metastatic gastrointestinal stromal tumors (GIST), an EORTC phase I study. *Proc AM Soc Clin Oncol* 2001; 20: 2a.
32. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001; 344: 1031-7.
33. Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumor biology: Herceptin acts as an anti-angiogenic cocktail. *Nature* 2002; 416: 279-80.

Corresponding author

Philip John Domeyer
 2, Agiou Andreou Str.
 Nea Smyrni
 Athens 17122
 Greece
 e-mail: jdomeyer@central.ntua.gr