

# Tyrosine Kinase Blockers: New Hope for Successful Cancer Therapy

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**Abstract:** Tyrosine kinases (TKs) are attractive targets for cancer therapy, as quite often their abnormal signaling has been linked with tumor development and growth. Constitutive activated TKs stimulate multiple signaling pathways responsible for DNA repair, apoptosis, and cell proliferation. During the last few years, thorough analysis of the mechanism underlying tyrosine kinase's activity led to novel cancer therapy using TKs blockers. These drugs are remarkably effective in the treatment of various human tumors including head and neck, gastric, prostate and breast cancer and leukemias. The most successful example of kinase blockers is Imatinib (Imatinib mesylate, Gleevec, STI571), the inhibitor of Bcr/Abl oncoprotein, which has become a first-line therapy for chronic myelogenous leukemia. The introduction of STI571 for the treatment of leukemia in clinical oncology has had a dramatic impact on how this disease is currently managed. Others kinase inhibitors used recently in cancer therapy include Dasatinib (BMS-354825) specific for ABL non-receptor cytoplasmic kinase, Gefitinib (Iressa), Erlotinib (OSI-774, Tarceva) and Sunitinib (SU 11248, Sutent) specific for VEGF receptor kinase, AMN107 (Nilotinib) and INNO-406 (NS-187) specific for c-KIT kinase. The following TK blockers for treatment of various human tumors are in clinical development: Lapatinib (Lapatinib ditosylate, Tykerb, GW-572016), Canertinib (CI-1033), Zactima (ZD6474), Vatalanib (PTK787/ZK 222584), Sorafenib (Bay 43-9006, Nexavar), and Leflunomide (SU101, Arava). Herein, we discuss the chemistry, biological activity and clinical potential of new drugs with tyrosine kinase blockers for cancer treatment.

**Key Words:** Tyrosine kinases, Cancer treatment, ATP-blockers, Monoclonal antibodies, Drug resistance.

## 1. INTRODUCTION

Approximately 20% of human genes code products which participate in cell signaling pathways. Principal regulators of these pathways are phosphorylation/dephosphorylation reactions. There are 518 kinase sequences encoded in the human genome, of which 430 are expected to be catalytically active [1, 2]. The tyrosine kinases (TKs) determine a separate class of enzymes which are responsible for phosphorylation of tyrosine residue on targeted proteins. Tyrosine kinases stimulate multiple signaling pathways responsible for basic cells functions such as growth, proliferation, migration, synthesis and apoptosis. Several oncogenic tyrosine kinases have been detected in human malignancies, one of the most extensively studied being Bcr/Abl, considered as the pathogenic principle of Philadelphia (Ph) chromosome-positive human leukemia's [3]. The Bcr/Abl fusion generated by a t(9;22) translocation mediates its biological effects through deregulated, constitutively active tyrosine kinase activity [4]. Others oncogenic forms of tyrosine kinases including: Tel/Abl, Tel/Jack2, Tel/PDGFR $\beta$  or Npm/Alk can be expressed in different types of tumors, therefore regulation of TKs may play significant role in cancer treatment [5-8].

There are two main approaches to inhibit activity of oncogenic tyrosine kinases. The first strategy uses monoclonal antibodies called 'mabs' to block the extracellular domain of tyrosine kinase receptors. The second approach is based on small molecules called 'nibs', with the ability to interact with ATP-binding domain, and inhibit the intracellular kinase activation. Some of the tyrosine kinase blockers show promising results in preclinical studies while others have already been used for years as a highly effective drugs

in human cancer treatment (e.g. Imatinib mesylate) [9]. However, some patients developed resistance to the treatment resulting in a general step-back in TKs blockers strategy [10]. Recent studies have shown several mechanisms of resistance to TKs blockers include: drug binding to inactive kinase, TK gene amplification, point mutations in ATP binding domain or clonal cytogenetic evolution of cancer cells [11, 12]. In this review, we described a mechanism of oncogenic tyrosine kinase activation that may provide a concept design for effective TKs blockers that can overcome pharmacological resistance. More importantly we discuss the clinical potential of novel therapeutic TKs blocker that may be used in human cancer treatment.

## 2. TYROSINE KINASES

There are two families of tyrosine kinases – transmembrane receptor kinases and cytoplasmatic no-receptor kinases [13,14]. Transmembrane receptor kinases are enzymes which play a role in intracellular signaling pathways by transmitting signals from membrane receptors to the cell interior and are anchored to cellular membranes by a hydrophobic transmembrane domain. Extracellular signals are received by this family of enzymes through ligand binding with the membrane receptor's exterior domain which stimulates cytoplasmic domain activation. The activation process displays two essential stages. The first stage relies on the dimerization of receptors which results in their conformation modification. In the second stage, TKs are autophosphorylated which is modulated by regulatory ligands. These processes initiate a cascade of phosphorylation reactions which activate serial proteins until the signal reaches the nucleus and causes changes of expression of specific targeted genes. Receptor tyrosine kinases may be classified in following 10 subfamilies [15]:

- Epidermal growth factor receptor EGFR subfamily: EGFR, ERBB2, ERBB4;
- Insulin receptor InsR subfamily: IGF1R, INSR;

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- Platelet-derived growth factor receptor PDGFR subfamily: FLT3;
- Vascular endothelial growth factor VEGFR subfamily: KIT, FLT1, KDR.
- Fibroblast growth factor receptor FGFR subfamily: FGFR1, FGFR2, FGFR3;
- Hepatocyte growth factor receptor Met subfamily: MET;
- Eph subfamily: EPHA1, EPHA2, EPHA4, EPHA8, EPHB2;
- Musk (Muscle-specific receptor tyrosine kinase); non-membrane spanning protein tyrosine kinase subfamily: MUSK;
- Tie (tyrosine kinase with Ig and EGF homology domains); membrane spanning protein tyrosine kinase subfamily: TIE2;
- Trk (Tropomyosin-related kinase) subfamily: TRKA, TRKB, TRKC.

Cytoplasmic tyrosine kinases include enzymes activated by ligands which bond with cellular receptors and unbound kinases activated by ion transport across cellular membrane or between cell cycle phases. Activation mechanisms of cytoplasmic unbound kinases are similar to receptor kinases which have reciprocal catalytic domains. Each catalytic domain of tyrosine kinase contains a specific ATP binding site which is a phosphate residue donor and also a substrate binding site, that transfers phosphate residues from ATP. Classification of tyrosine kinases is based on their structural analysis [18]. Cytoplasmic tyrosine kinases are classified in 7 subfamilies as follows [15]:

- Src subfamily which contributes to mitogenesis, activation of MAPK kinases, activation of B and T cells, signal transmission via Fc immunoreceptors associate with PDGFR and EGFR receptors: FYN, HCK, LCK, LYN, SRC;
- FAK (Focal adhesion kinase); non-membrane spanning protein tyrosine kinase subfamily: FAK;
- Jak subfamily (Janus Kinase family) associated with cytokine's receptors which activate STAT factors (Signal Transducers and Activators of Transcription): Jak2, Jak3;
- Abl (Abelson tyrosine kinase); non-membrane spanning protein tyrosine kinase subfamily: Abl;
- Csk (Carboxyl-terminal src kinase); non-membrane spanning protein tyrosine kinase subfamily: CSK;
- Syk (Spleen tyrosine kinase) subfamily: SYK, ZAP70;
- Tec (T-cell-restricted tyrosine kinase) subfamily: BTK, ITK.

### 3. TYROSINE KINASES IN HUMAN CANCER DEVELOPMENT

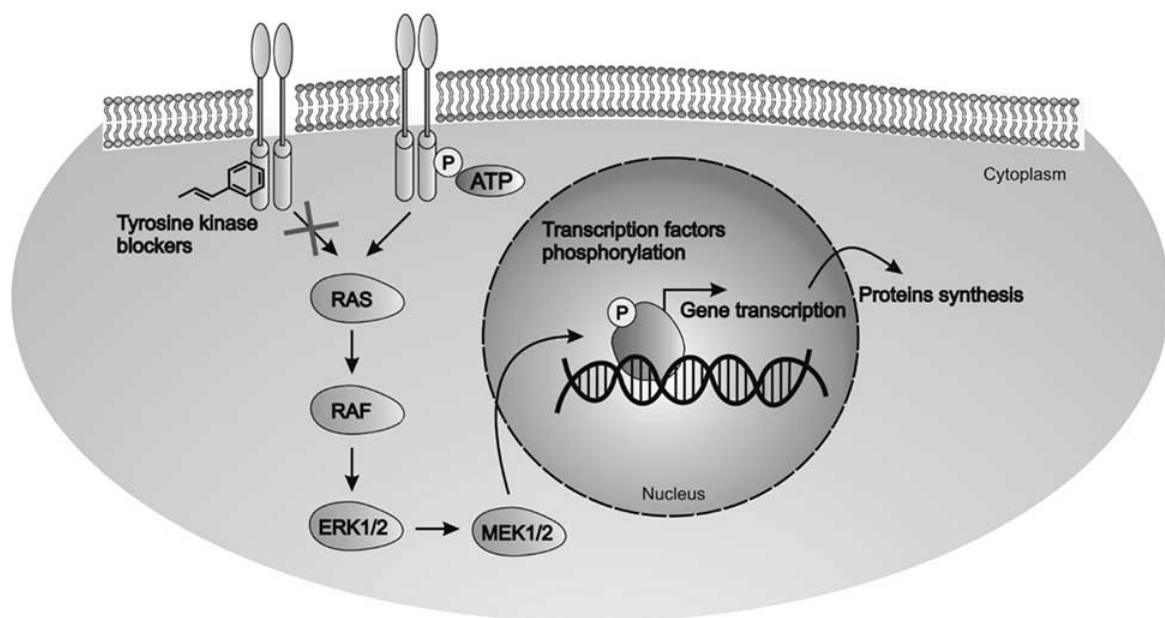
Phosphorylation's activity and signal transduction by tyrosine kinases in cells is tightly controlled [17-19]. However there are some mechanisms which allow tyrosine kinases to escape this control and enable signaling pathways including DNA repair, apoptosis and proliferation. In this case, a cell's homeostasis might be disturbed and the oncogenic transformation processes can be induced. Tyrosine kinases may obtain oncogenic specificity via mutations in their encoding genes (these genes are cellular proto-oncogene's). During chromosomal translocation tyrosine kinase's genes defer

fusion and form fusion genes (e.g. Bcr/Abl) which encode altered proteins. These proteins frequently display increased phosphorylation activity when compare to wild type kinases. Point mutations in extracellular, transmembrane and cytoplasmic domains can promote dimerization and/or autophosphorylation without binding between ligand and receptor [20,21]. In the tyrosine kinase family there are proteins responsible for biochemical conversion, which regulate cells growth, differentiation and death. They can also play a significant role in cancer development. Cancer may develop in a multi-stage process resulting in accumulation of inherited and/or acquired defects in genes affecting cell cycle regulation [22]. Therefore tyrosine kinases might increase uncontrolled proliferation of neoplastic cells, cancer development or cancer malignant resulting in metastasis without direct initiation of cancer development processes. Tyrosine kinases also stimulate angiogenesis processes directly involved in the growth of the cancer. In some types of the cancer, including leukemia, expression of proteins with tyrosine kinase specificity such as Bcr/Abl or c-KIT was observed. In comparison with normal cells, these tyrosine kinase proteins demonstrate elevated enzymatic activity, resulting in abnormal intracellular signaling pathways, modification for transcription, proliferation and apoptosis. Therefore, oncogenic tyrosine kinases disrupt regular cells processes which cause pathological phenotype development [23-25]. It appears that these specific proteins with tyrosine kinase activity might be attractive targets for effective cancer therapy Fig. (1).

#### 3.1. Oncogenic Activation of Tyrosine Kinases

In normal signaling pathways ligand binding to receptors induces oligomerization and activation of tyrosine kinase [26]. Conformation changes which result in bound ligands and receptor dimerization, initiate reciprocal interaction between cytoplasmic domain of tyrosine kinases. Conformation changes of receptors can be generated by monomeric ligands (e.g. activation of epidermal growth factor EGF) or bivalent ligands which induce dimerization of receptors (e.g. platelet-derived growth factor PDGF and PDGFR receptor) [27-31]. However heterodimerization of receptors may result in transforming growth factor alpha (TGF $\alpha$ ) which activates EGFR and c-ErbB2 kinases [32, 33]. Activation of cytoplasmic tyrosine kinases may be a response to extracellular signaling, intracellular changes of Ca<sup>2+</sup> concentration or mitogenesis [26]. Cytoplasmic Jak kinases are activated in an oligomerization pathway [34-37], whereas activation of the Src kinase family requires dephosphorylation of the tyrosine residue in position 527 in Src kinase and their equivalent position in other kinases belonging to this family of enzymes [38]. Kinases which belong to Src family contain three SH (SRC homology domain) domains at the N-terminal end. The SH1 domain is responsible for enzymatic activity; SH2 domain is responsible for binding of phosphotyrosine residue and SH3 regulates enzyme activity [38, 39]. SH2 domain of non-active form of SRC kinase binds to phosphorylated Tyr572 residue, which is essential for active conformation of enzyme [40]. Activation of receptor and cytoplasmic tyrosine kinases induces the phosphorylation reaction cascade including enzyme autophosphorylation or phosphorylation of cytoplasmic substrates and adaptor proteins (e.g.: phosphorylation of Crk-L specific protein for Bcr/Abl kinase). Signals generated by kinase can be transferred to the nucleus, where induced target gene expression results in a cell response (e.g.: cells division).

Activation of oncogenic tyrosine kinases disorders cell stability processes and establishes constitutive enzyme activity. This process can be induced in several pathways and by different mechanisms [41]. One mechanism includes activation via mutations causing constant tyrosine kinase activity. This pathway results in enzyme activation independently of ligand binding and is observed in receptor tyrosine kinases isolated from animal viruses. This enzyme mutagenic activation pathway includes: chromosomal translocations or point mutations which induce modification in extracellular domain, changes in ATP binding complex and modification in the



**Fig. (1).** Tyrosine kinase intracellular signaling pathway activation and inhibition by TKs blockers. The MAPK/Erk intracellular signaling pathway is an example of the pathways activated by binding of a ligand (mostly growth factors) to the receptor tyrosine kinase.

catalytic part of kinase [42-44]. A second category of mechanisms includes series of incidents which can be related to ligand duplication, inhibition of inverse regulation pathways and overexpression or amplification of kinase.

### 3.2. Kinases Activation Via Mutations

There are several known tyrosine kinases which are expressed in human cancer and activated via mutations. These mutations are easily identified unlike modifications in an enzyme expression. Many of these mutations were discovered during research in different types of cancers. Oncogenic forms of tyrosine kinase can play a significant role in cancerogenesis. Chronic myeloid leukemia (CML) is characterized by a reciprocal chromosomal translocation between chromosome 9 and 22 [t(9;22)] [45] resulting in the *bcr/abl* oncogene. The encoding protein with constitutive tyrosine kinase activity is considered a pathogenic principle of the Philadelphia chromosome (Ph). Chromosomal translocation is responsible for the appearance of oncogenes encoding fusion tyrosine kinases (FTK's) such as *Bcr/Abl*, *Tel/Abl*, *Tel/Jak2*, *Tel/PDGFR* and *Npm/Alk* [41]. These TKs share structural similarity, including an amino-terminal oligomerization and activation of the associated tyrosine kinases of the carboxy-terminal fusion partner. Point mutations and chromosomal aberrations determine the second potential source of oncogenic tyrosine activation. Modifications in the nucleotide sequence of genes for transmembrane domain can induce ligand independent kinase activation (e.g.: *NEU/c-erbB-2*) [46-48]. Mutations in the transmembrane domain specifically result in expansion of substrates (e.g.: *v-ROS*) [49]. Deletions in the extracellular regulatory domain of tyrosine kinases, which induce oncogenic activation of kinase have also been observed [42]. Other mutations may result in negative regulation of tyrosine kinase (e.g.: mutation in Tyr527 of *Src* kinase) and mutations occurring in phosphor-binding sites may result in enzyme deregulation.

## 4. TYROSINE KINASE INHIBITORS USE IN CANCER THERAPY

20 years ago the discovery that some of the oncogenic viruses can encode tyrosine kinase, started a new investigation in finding directed therapy against classes of tyrosine kinase enzymes. Experiments on cancer cells with overexpression of TKs have shown anti-proliferation activity of two inhibitors: herbimycin [50] and staurosporine [51], which inhibit activity of whole complexes of

kinases. Because of the extensive activities spectrum and toxicity of these inhibitors, it was necessary to increase specificity of their effect. Initially intensive studies using chemical reagents and highly specific synthetic drugs for a narrowly defined class of kinase were accepted sceptically, because the activity of these reagents relied on competitive inhibition of ATP binding. The ATP binding domain is highly conservative and form fundamental motive of all kinases [52].

In 2001 Herceptin, a new anti-cancer inhibitor of tyrosine kinases was introduced for clinical trials. Herceptin was the first humanized monoclonal antibody against tyrosine kinase receptor *Her2/neu*. This antibody, in combination with paclitaxel, was indicated for treatment of patients with metastatic breast cancer with overexpression of the *Her-2* protein. In November 2006 the U.S. Food and Drug Administration (FDA) approved Herceptin as part of a treatment regiment containing doxorubicin, cyclophosphamide and paclitaxel, for adjuvant treatment of patients with *Her-2*-positive and node-positive breast cancer [53].

Use of Herceptin in chemotherapy in patients with metastatic breast cancer increased their chance of survived. The discovery of Imatinib (Imatinib mesylate, Gleveec, STI-571), which specifically inhibited activity of *Bcr/Abl* oncogene tyrosine kinase was a breakthrough in leukemia treatment. This drug revolutionized pharmacological strategy for patients with Philadelphia chromosome (Ph) translocation. Imatinib was approved by FDA in 2001 for the treatment of patients with chronic myelogenous leukemia (CML) [20]. Gefitinib was the first drug from a new class of anti-cancer agents formerly known as receptor tyrosine kinases blockers. Gefitinib inhibits activity of the epidermal growth factor (EGFR) intracellular tyrosine kinase. In 2003 FDA approved Gefitinib as monotherapy treatment for patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies [54]. Hopefully, future study of human cancer may open the way for new generation of blocker-drugs directly targeting tyrosine kinases. Searching for high specific blockers which may selectively interact with oncogenic tyrosine kinases is currently one of the major goals in molecular pharmacology Table 1.

### 4.1. Small Molecule Blockers Of Tyrosine Kinases

Chronic myeloid leukemia (CML) was the first human malignant disease to be linked to a single, acquired genetic abnormality.

**Table 1. Tyrosine Kinase Blockers: Currently Registered or in Clinical Development for Solid Tumors**

Agent	Target Receptors	Development Stage
Low molecular weight blockers Imatinib (Gleevec, STI571)	cAbl, PDGFR $\beta$ , cKIT	Licensed for GIST, (CML) Orphan drug request for DFSP
Erlotinib (Tarceva, OSI774)	EGFR	Licensed for 2dor 3rd line NSCLC, advanced pancreatic cancer
Canertinib (CI1033)	EGFR, Her2, Her3, Her4	Phase I/II (ovarian cancer)
Vatalanib (PTK787/ZK 222584)	VEGFR, PDGFR, cKIT	Phase II/III (colorectal carcinoma)
Gefitinib (Iressa, ZD1839)	EGFR	Licensed for 2dor 3rd line NSCLC (Asia, United States)
Sunitinib (Sutent, SU11248)	PDGFR, VEGFR, KIT, FLT3	Licensed for advanced RCC, and imatinibresistant/intolerant GIST
Dasatinib (BMS354825) Nilotinib (AMN107) INNO406 (NS187)	cAbl, nonRTK Src cAbl BcrAbl and Lyn	Phase I/II (clinical trial in CML) Phase II (CML and other bloodrelated cancers) Phase I (leukemia: acute, chronic)
Lapatinib (Tykerb, GW572016) Zactima (ZD6474)	EGFR, Her2 VEGFR, EGFR	Phase I/II/III (breast cancer) Phase I/II/III (NSCLC)
Sorafenib (Nexavar, Bay 439006)	cRaf1, BRaf, VEGFR, PDGFR	Licensed for advanced RCC, Phase II/III ( melanoma, HCC)
Leflunomide (Arava, SU101)	PDGFR (EGFR, FGFR)	Phase II/III (prostate cancer, GBM)
AP23464	cAbl	Preclinical trial
SKI606	NonRTK Abl and Src	Phase I (advanced malignant solid tumours, including breast, colorectal, pancreatic, CML and NSCL cancers)
PD166326	cAbl, Src	Preclinical trial
ON012380	BcrAbl	Preclinical trail
MK0457 (VX860)	Flt3, cAbl, Aurora kinase A, B and C	Phase I (colorectal cancer)
BIRB796	p38 MAPK	Preclinical trial

Identification of the Bcr/Abl oncogenic fusion kinase and its pivotal role in the pathogenesis of CML provided new opportunities to develop molecular-targeted therapies. Blockers of Abl tyrosine kinase are divided into two groups, ATP-competitive and non-competitive inhibitors.

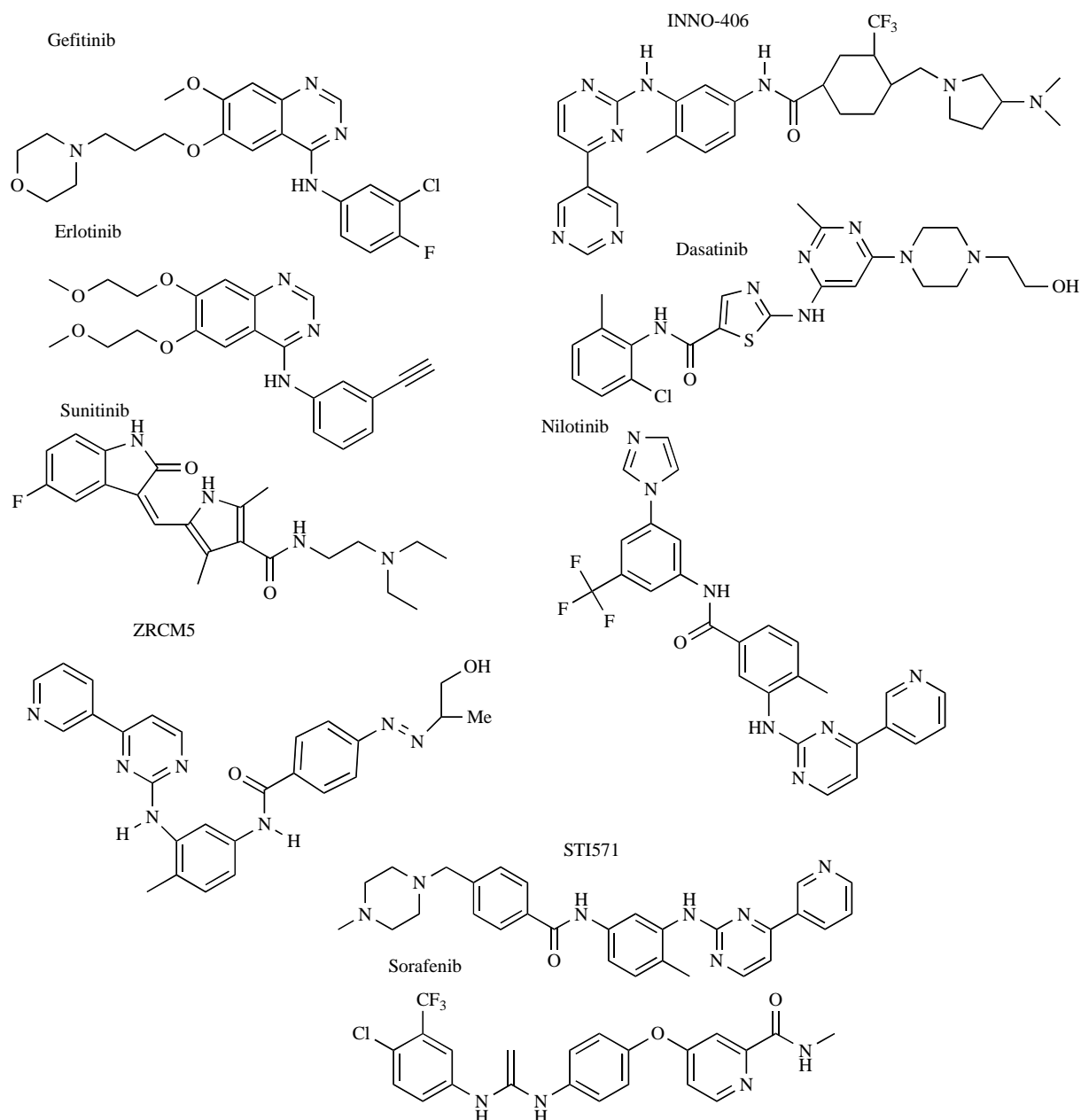
Small molecule blockers of ATP domain are a group of inhibitors, which can selectively inhibit the activity receptor and non-receptor tyrosine kinases. Crystallography studies of Imatinib structure have shown its directed interaction with a highly conserved domain of TKs which binds ATP. STI571 is chemically derived from 2-phenylaminopyrimidine. It was shown that STI571 binds preferentially with the non-active conformation of kinase (not-phosphorylated) resulting in prevention of enzyme activation and autophosphorylation [55]. STI571 competes with ATP molecule by blocking its binding site, stopping the transfer of phosphate groups to tyrosine residues. All kinases in active form display high conformational similarity; however their non-active forms displaying different structures. These differences relate mostly to the active loop. In active conformation the loop strikes opened shape with the C-terminal end pulled away from the catalytic center forming a platform for substrate binding. Non-active conformations form complexes with STI571. STI571 was identified as an effective blocker of c-ABL, c-Kit [56] and PDGFR $\beta$  kinases [57]. This drug is used in treatment of CML patients with *bcr/abl* expression and patients with gastrointestinal stomal tumors (GIST) with *c-KIT* gene expression.

Gefitinib (Iressa, ZD1839) is the first commercially available EGFR tyrosine kinase blocker and is now registered for use in Asia and the United States in second- or third line therapy for advanced non-small-cell lung cancer. ZD1839 is a small molecule inhibitor, chemically derived from 4-anilinoquinazoline which displays specificity to the receptor tyrosine kinase group including epidermal growth factor receptor EGFR (ErbB1) [58,59]. ZD1839, like STI571, is a competitive kinase inhibitor, which blocks ATP binding to enzyme catalytic domain. Another blocker of EGFR kinase is OSI-774, derived from 4-anilinoquinazoline. ZD1839 and OSI-774 demonstrate 100-fold higher substrate specificity to ErbB1 than ErbB2. Atom N1 of quinazoline forms hydrogen bonds with the

nitrogen of the Met769 residue. This interaction is important for ATP binding [60]. Preliminary data indicated complete tumor inhibition after drug application for ovarian cancer patients [61]. Clinical studies with ZD1839 monotherapy or combination therapy have been conducted for many tumor types, including esophageal carcinoma, metastatic breast cancer, prostate cancer, head and neck cancer, colorectal cancer, renal cell carcinoma, and ovarian carcinoma [62-67]. Therefore, more beneficial effects of ZD1839 are expected [68].

Erlotinib (Tarceva, OSI-774,) is an EGFR tyrosine kinase blocker with proven efficacy in monotherapy trials in advanced non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, head and neck squamous cell cancer, and primary glioblastoma [69-71]. OSI-774 is registered for the second- and third-line treatment of patients with advanced NSCLC after failure of at least one prior platinum treatment. Since late 2005, OSI-774 is also registered for advanced pancreatic cancer. The study of OSI-74 in patients with advanced biliary cancer and hepatocellular cancer showed a potentially beneficial effect of this blocker [72,73]. Clinical trials in metastatic colorectal carcinoma patients with erlotinib alone or in combination with chemotherapy also showed promising results [74,75]. OSI-774 in combination with bevacizumab evaluated in patients with metastatic clear-cell renal carcinoma resulted in increasing ratio of survival patients as compared with treatment one of these agents [76]. Current studies with pancreatic cancer patients focus on combinations with chemotherapy, radiotherapy, and other targeted therapies including maintenance therapy of OSI-774.

Sunitinib (Sutent, SU11248) is an orally available blocker of VEGFR, PDGFR, c-KIT, and FLT-3 kinase activity. The study of patients with immunotherapy refractory metastatic renal cell carcinoma treated with SU11248 showed a statistically significant improvement in median progression free survival (47.3 vs. 24.9 weeks) and objective response rate (24.8% vs. 4.9%) for SU11248 over IFN- $\alpha$  83 [77,78]. Therefore SU11248 might now be considered the new standard first-line treatment for advanced kidney cancer. In January 2006, SU1128 was not only approved by the FDA for advanced renal cell carcinoma, but also for imatinib-resistant



**Fig. (2).** Chemical structure of tyrosine kinase blockers: Gefitinib, Erlotinib, Sunitinib, Nilotinib, Dasatinib, Imatinib, Sorafenib, INNO-406 and ZRCM5.

and imatinib-intolerant GIST. Moreover, SU1128 showed a potentially beneficial effect in previously treated advanced NSCLC and unrespectable neuroendocrine tumors [79].

Sorafenib (Bay 43-9006, Nexavar) is a novel oral Raf-1 kinase, platelet-derived growth factor receptor (PDGFR) and VEGFR kinase blocker with antitumor effects in colon, pancreas and breast cancer cell lines and in colon, breast and non-small-cell lung cancer xenograft models [80,81]. Bay 43-9006 was granted FDA fast track approval in December 2005. Clinical trials with Sorafenib in melanoma and in advanced hepatocellular carcinoma, and other multiple tumor types are ongoing [82]. Chemical structure of some tyrosine kinase blockers are presented in Fig. (2).

#### 4.2. Monoclonal Antibodies

One strategy to inhibit tyrosine kinases uses monoclonal antibodies (mAb), which are proteins of the immune system. Monoclonal antibodies are the most widely used form of cancer immuno-

therapy at this time. Monoclonal antibodies recognize and bind to foreign antigens eliciting an immune response. The first mAb used in immunotherapy directed against tyrosine kinases were antibodies which recognize *new* oncogene [83,84]. The unique structures of extracellular receptor's domains are useful to create specific monoclonal antibodies that may recognize and block tyrosine kinase. These antibodies have multiple functions which can include: blocking of ligand binding, inhibition of receptor dimerization or initiation of receptor degradation. Herceptin is the first drug from the mAb group which recognizes receptor Her-2/neu (ErbB2) of TKs. Herceptin is used in breast cancer therapy and binding of this drug prevents receptor oligomerization of oncogene Her-2/neu [53,85]. The antibody recognizes an extracellular fragment of TKs but the epitope is a C-terminal fragment of domain number IV. The mechanism of Herceptin activity on Her-2/neu is still unknown because domain IV does not participate in direct dimerization of the receptor; however it is known, that there are localized sites in this domain which can be identified by metalloproteases. It was demon-

strated that incision of domain IV by metalloproteases stimulates dimerization of intracellular receptors in pathway up-regulated by Her-2/neu kinase. Perhaps, in this context, binding of monoclonal antibodies can control activity of metalloproteases and block kinase Her-2/neu receptor dependent signaling pathway [60]. IMC-C225 (Cetuximab, Erbitux) is a chimeric monoclonal antibody directed against receptor tyrosine kinase EGFR (ErB1). IMC-C225 binds with the receptor domain and inhibits ligand binding. IMC-C225 is believed to operate by binding to the extracellular domain of the EGFR. This blocks downstream signaling of EGFR impairing cell growth and proliferation [60]. IMC-C225 has also been shown to mediate antibody dependent cellular cytotoxicity. IMC-C225 was approved by the FDA in March 2006 for use in combination with radiation therapy for treating squamous cell carcinoma of the head and neck (SCCHN) or as single agent in patients who have had prior platinum-based therapy. IMC-C225 is also used in metastatic colon cancer therapy [86]. It has been estimated that monoclonal antibodies currently present one fourth of the products clinically processed so they probably announce a new era in medicine.

## 5. CELLULAR RESISTANCE TO TYROSINE KINASE BLOCKERS

With the generalization of Imatinib as a medicine for CML, new problems related to cellular resistance have appeared. CML-positive patients with Philadelphia chromosome in blast crisis phase have shown increased activity of Bcr/Abl despite Imatinib treatment. Therefore, Imatinib resistance has become a serious problem in CML anticancer therapy. Most frequently the mechanism of resistance is amplification and/or mutations of the *bcr/abl* gene [87,88]. Recent studies have shown mutations in the ATP binding domain which may affect Bcr/Abl protein conformation resulting in Imatinib resistance [89,90]. Some mutations completely stop Imatinib binding to the target causing total resistance to the drug while other mutations allow partial binding which leads to moderate resistance [87,91,92]. To overcome resistance, several approaches have been studied *in vitro* and *in vivo*. They include dose escalation of Imatinib, combination with other chemotherapeutic drugs, alternative Bcr/Abl inhibitors, inhibitors of kinases downstream of Bcr/Abl, farnesyl and geranylgeranyl transferase inhibitors, histone deacetylase, proteasome and cyclin-dependent kinase inhibitors, arsenic trioxide, hypomethylating agents, troxacitabine and immunomodulatory strategies [93,94]. Finally down-regulation of the expression by small interfering RNA (siRNA) or antisense strategies for *bcr/abl* mRNA can also be used [95,96]; however, it is important to understand that these approaches differ in efficiency, which is often dependent on the mechanism of resistance.

Attempts to circumvent resistance to Imatinib led to the discovery of Nilotinib (Tasigna; Novartis AG), a novel, potent and selective oral Bcr/Abl kinase inhibitor [97]. Nilotinib, an orally bioavailable, selective Bcr/Abl tyrosine kinase inhibitor, is 30-fold more potent than Imatinib and overcomes most imatinib resistant Bcr/Abl mutations. Nilotinib was effective in patients harboring Bcr/Abl mutations associated with Imatinib resistance and also in patients with a resistance mechanism independent of Bcr/Abl mutations. Efficacy has been observed in models of CML and other myeloproliferative disorders that are driven by Bcr/Abl and related kinases. Nilotinib has been filed for approval in the US and EU for use in Philadelphia-positive leukemias in patients who are resistant or intolerant to imatinib. Nilotinib is undergoing clinical trials in patients with newly diagnosed CML, acute lymphoblastic leukemia and gastrointestinal stromal tumors, among other indications [98,99].

Recent studies report an activating mutation in another TKs gene of EGFR kinase that seems predictive for response to Gefitinib used in NSCLC treatment [100-102]. Therefore, for future clinical use, it is important to select patients that are likely to bene-

fit from this EGFR kinase inhibitor, while non-selection is likely the main cause of the disappointing results of Gefitinib.

Various cellular mechanisms may be involved in the nature of cellular resistance. Increased amount of target, alteration in structure of target proteins, decreased drug uptake and increased detoxification are well-known mechanisms of resistance. Identification of all possible mechanisms which participate in inhibitor resistance require further studies. It is known that mutations are characteristic for patients in an advanced stage of the disease. This information leads to some important questions. Why do these mutations occur? What new methods should be developed and used to inhibit disease relapse? The models show that in the last stage (blast crisis) of CML disease, cells display genomic instability and accumulate mutation during progressive development of disease. Treatment with drugs-like kinase inhibitors takes advantage of effective selection of drug resistance alleles. Future studies may help design appropriate inhibitor which can be chosen in patients treatment at an early stage of cancer disease.

### 5.1. Novel Tyrosine Kinase Blockers

Imatinib mesylate has dramatically changed the strategy for chronic myeloid leukemia (CML) therapy. However, resistance is often reported in advanced-stage CML patients. Several novel TKs blockers have been developed to overcome Imatinib resistance mechanism due to point mutations within the ABL kinase domain Fig. (3). Recently, a novel Bcr/Abl/Lyn dual inhibitor INNO-406 (NS-187), which shows a unique profile to overcome this resistance, has been successfully developed.

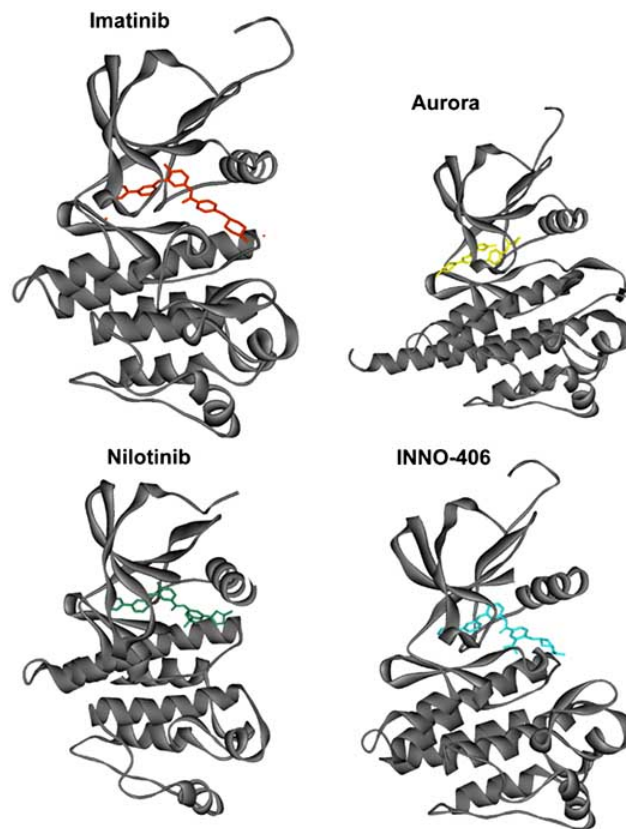
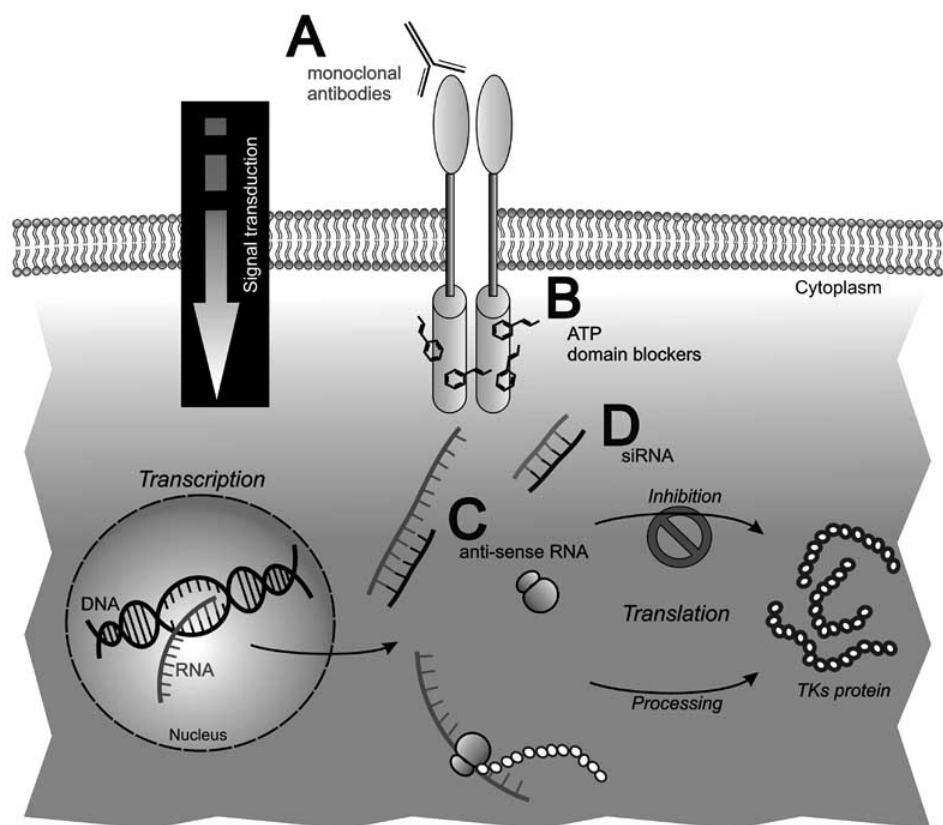


Fig. (3). Molecular structure of oncogenic tyrosine kinase Bcr/Abl and its inhibitors: Imatinib, Aurora, Nilotinib and INNO-406.



**Fig. (4).** Rationale engineering of tyrosine kinase blockers. Mechanism of signal transduction inhibition by (A) monoclonal antibodies, (B) ATP domain blockers, (C) anti-sense RNA and (D) siRNA.

Dasatinib (BMS-354825), AP23464, SKI-606, and PD166326 are classified as Src/Abl inhibitors, while Nilotinib (AMN107) and INNO-406 (NS-187) belong to the 2-phenylaminopyrimidin-based subclass of inhibitors. Clinical studies of Nilotinib and other compounds, including Dasatinib, SKI-606 and INNO-406, have been performed in rapid succession. Because of their strong affinities for the ATP-binding site compared to Imatinib, most ATP-competitive inhibitors may be effective in Imatinib-resistant patients. However, an ATP-competitive inhibitor that can inhibit the phosphorylation of T315I Bcr/Abl has not yet been developed. Instead, ATP non-competitive inhibitors, such as ON012380, Aurora kinase inhibitor MK0457 (VX-680), and p38 MAP kinase inhibitor BIRB-796, have been developed to address this problem [93].

The activity of the novel TKs inhibitor INNO-406 against human cells with mutated KIT was investigated. Human mast (HMC)-1.1 cells with juxtamembrane domain mutation V560G, and HMC-1.2 cells with both V560G and the kinase domain mutation D816V, were treated with INNO-406. INNO-406 was potent against HMC-1 cells regarding cell proliferation, inhibition of KIT phosphorylation, and induction of apoptosis. These results suggested clinical potential for INNO-406 in KIT V560G-expressing malignancies [103].

## 6. RATIONALE ENGINEERING OF TYROSINE KINASE BLOCKERS

The introduction of Imatinib for the treatment of CML in clinical oncology has had a dramatic impact on the way this disease is managed [45]. Studies have shown that Imatinib acts by binding the inactive form of the Abl kinase domain of Bcr/Abl, and the structural basis for the autoinhibition of the c-ABL tyrosine kinase has been solved [104]. Imatinib has rapidly become the first-line therapy for CML, however the acquired resistance in patients incurs a

serious clinical problem [105]. The development of resistance to Imatinib is a frequent setback, particularly in patients in advanced phases of the disease. Recently it has been found that some mutations in the ATP-binding domain of *ABL* gene lead to conformational changes in the Bcr/Abl oncoprotein resulting in impairment of Imatinib binding and leading to clinical resistance [106].

During the last few years, the thorough analysis of the mechanisms underlining the Bcr/Abl pathway has led to novel strategies in leukemia treatment. It was found that Bcr/Abl induced resistance to cytotoxic drugs by upregulating DNA repair mechanisms [107]. Moreover, it was demonstrated that Imatinib sensitized Bcr/Abl expressing cells to cytotoxic DNA damaging agents by depleting the anti-apoptotic properties associated with Bcr/Abl [108]. These results support new strategies to combine a DNA damaging agent with Bcr/Abl kinase inhibition into a single molecule in order to induce the enhanced potency in CML cells. The "combi-targeting" therapy is a new approach to rationale engineering of TKs blockers [108]. The synthesis of compounds with mixed Bcr/Abl-DNA targeting properties may well represent a single kinase blocker alternative to Imatinib or a new type of agent to be used in combination with it. The first reported Bcr/Abl-DNA blocker is 3-methyl-1,2,3-triazene (ZRCM5) [92]. Chemotherapeutic agents of the triazene class had been before used in the clinical management of many tumors including brain cancers, leukemias and melanomas. Their mechanisms of action are based on the generation of an alkylidiazonium species that damages DNA at the O6 and N7 position of guanine. The binary Bcr/Abl-DNA targeting properties of ZRCM5 is based on a 2-phenylaminopyrimidine moiety targeted to Bcr/Abl kinase and a triazene tail capable of generating a methylodiazonium species upon hydrolysis. Thus, the significant potency of ZRCM5 against the leukemia cells is attributed to its ability to simultaneous blocking of Bcr/Abl and related with DNA repair activity while

including significant DNA lesion in Bcr/Abl expressing leukemia cells.

The combination of ZRMC5 with Imatinib is a promising treatment for Bcr/Abl-positive CML, especially when more aggressive therapies are required for patients in blast crisis [52,94]. The *in vitro* studies are ongoing to increase ZRMC5 affinity as a single blocker agent and the "combi-targeting" strategy may become an answer to Imatinib acquired resistance in leukemia treatment. Most likely, further investigations into the molecular mechanisms of leukemia will guide the design of new treatment modalities in clinical trials.

A new anti-cancer drug available for patients who have progression of breast cancer is a small molecule kinase inhibitor lapatinib chemically derived from quinazolin-4-amine and developed by GlaxoSmithKline Company (GSK). Lapatinib is an ATP competitive dual kinase inhibitor of EGFR and HER2/neu (ErbB-2) which inhibits receptor autophosphorylation and activation. The drug was approved by FDA on March 2007, for use in patients with metastatic breast cancer in combination with chemotherapy drug capecitabine (Xeloda). Clinical trial demonstrated that additionally therapy with lapatinib delayed the time of cancer growth compared to capecitabine alone [109, 110]. The most adverse drug-related treatment effects included diarrhea, nausea, vomiting and rash. However the survival data are not yet mature. Recently, a novel combination therapy of lapatinib and herceptin was proposed. Burris *et al.* observed a synergistic and anti-proliferative effect against ErbB2-positive breast cancer cells *in vitro*. The optimally tolerated regimen with clinical activity was lapatinib at dose 1,000 mg per day combined with herceptin treatment at 4 mg/kg loading dose then 2 mg/kg weekly [111]. Progress in the combine drug therapy base on lapatinib offers an important advance for patients with HER2 positive metastatic breast cancer. The natural product, SL0101 initially isolated from plant *Foresteronia refracta* found in the South America rainforest is a kaempferol glycoside that belongs to the class of competitive ATP binding inhibitor. SL0101 specific inhibits the serine/threonine p90 ribosomal S6 kinase (RSK) activity. RSK kinases function as downstream effectors of MAPK kinase [112, 113] and have been implicated in various human cancers including prostate, and breast cancer. The RSK family consists of four isoform RSK1, RSK2, RSK3, RSK4 products of separate genes [114]. SL0101 was reported to inhibit proliferation of the MCF-7 human breast cancer cell line but does not effect the growth of the normal breast line MCF-10A even though inhibits RSK activity in both cell lines [115, 116]. Furthermore, the growth of the human prostate cancer cell line LNCaP and PC-3 the androgen-independent more aggressive human prostate cancer than LNCaP was inhibited by SL0101. It was also found that inhibition of RSK activity in LNCaP cells by S0101 decreased expression of the prostate-specific antigen (PSA) [117]. SL0101 or a chemically modified version may be a promising drug target for these cancers.

Other therapeutic strategies were developed as a need for specific therapy, which can affect a broad range of cancer and reduce side effects Fig. (4). One of them uses long antisense RNA (asRNA) or RNA interference (siRNA) which may selectively inhibit expression of specific gene targets implicated in cancer development. asRNA and siRNA inhibit gene expression by paring to complementary mRNA [118]. asRNA transcribed in cells from plasmid or viral vectors is more efficient at long term gene silencing. It was reported that asRNA silences the anti-apoptotic Bcl-2 protein [119] and telomerase [120] in cell culture, leading to inhibition of cell growth. Moreover, asRNA was found to inhibit the tumorigenicity of cells with insulin-like growth factor 1 receptor (IGF-IR) [121] and VEGF [122]. Additionally asRNA may successfully silence EGFR *in vivo* in subcutaneous xenografts of nude mice, leading to tumor growth inhibition. These inhibitory effects will occur in healthy cells as well, leading to undecirable side effects [118]. siRNA was primarily described in the *Caenorhabditis elegans*

[123]. The advantage of siRNA molecules is high specificity to unique mRNA transcripts that may be present in cancer cells, sometimes only different from the wild type transcript by a single point mutation. siRNA may be used to target Bcr/Abl and Npm/Alk oncogenic fusion transcripts in leukemias and lymphomas [124]. In human pancreatic carcinoma siRNA was shown to inhibit the oncogenic K-ras mutant but not affect the wild-type K-ras transcript [125]. Resistance to asRNA and siRNA could results from activation of alternative signal transduction pathway and enhance independent cell proliferation viability. Also, mutation in transcripts inhibits hybridization with the asRNA or siRNA and interfere gene silencing as well. asRNA and siRNA have great potential as an anticancer agents but additional studies are required.

## SUMMARY

A new therapeutic approach is necessary to improve the treatment of cancer patients. It is possible that agents such as Imatinib or Gefitinib classified to tyrosine kinase blockers, will target and kill the cancer precursor cells, protect normal tissues, and consequently will be less toxic for patients then other anticancer agent used in cancer therapy. It is believed that preclinical studies will develop novel tyrosine kinases blockers effective in cancer treatment. Future treatment regiments are likely to include multiple tyrosine kinase inhibitors, based on biogenetics of the tumor cells, in combination with other anticancer agents. Hopefully this may provide new molecular targets for human tumors and accelerate real progress in the treatment of patients with this disease.

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