



Received on 28 August, 2013; received in revised form, 27 October, 2013; accepted, 15 December, 2013; published 01 January, 2014

## TYROSINE KINASE AS TARGET FOR CANCER TREATMENT

Abdisa Tufa Bedada\*<sup>1</sup> and Reta Tsegaye Gayesa<sup>2</sup>

Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University<sup>1</sup>, Addis Ababa, Ethiopia

Department of Nursing, College of Health Sciences, Samara University<sup>2</sup>, Samara, Ethiopia

### Keywords:

Tyrosine kinase, Chronic myeloid leukemia, Non-small cell lung cancer

### Correspondence to Author:

#### Abdisa Tufa

B. Sc., M. Sc. candidate, Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University, P. O. Box 9086, Addis Ababa, Ethiopia

Email: abditufa95@gmail.com

**ABSTRACT:** Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental control. Cancer is treated with surgery, radiation, chemotherapy, hormones and immunotherapy. But, due to side effects such as toxicity, death of normal cells near to cancer cells, second formation of cancers; these treatment was not successful. Targeted gene therapy is the most acceptable for cancer treatments because the above shortcomings overcome by it. Targeted therapy refers to a new generation of anticancer drugs that are designed to interfere with a specific molecular target, usually a protein with a critical role in tumor growth or progression. This approach differs from the more empirical approach used in conventional cytotoxic chemotherapy, which has remained the mainstay of anticancer drug use over the past several decades. Targeted therapy has the potential to reduce or eliminate many of the present problems in the field of cytotoxic chemotherapy, such as the production of serious host-cell toxicity. Several types of targeted therapy are available such as targeted stem cells, but this review focuses in particular only on tyrosine kinase inhibitors (TKI). Some have been approved for use in cancer therapy, and several others are in various stages of clinical trials. This paper provides a summary of some specific tyrosine kinases (TKs) and their inhibitors which are used in cancer therapy. While in normal cell regulation, TK plays a crucial signaling role, but in cancer cells it gets dysregulated. Thus, in recent times for therapy oncologists target TKs for regulating cell growth and proliferation; through their inhibitors which are small molecules with low molecular weight.

**INTRODUCTION:** Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental control.

Neoplastic cells may have numerous acquired genetic abnormalities including aneuploidy, chromosomal rearrangements, amplifications, deletions, gene rearrangements, and loss-of-function or gain-of-function mutations. Recent studies have also highlighted the importance of epigenetic alterations of certain genes that result in the inactivation of their functions in some human cancers. These aberrations lead to the abnormal behavior common to all neoplastic cells like dysregulated growth, lack of contact inhibition, genomic instability, and propensity for metastasis

	<b>QUICK RESPONSE CODE</b> <b>DOI:</b> 10.13040/IJPSR.0975-8232.5(1).1-15
	<b>Article can be accessed online on:</b> <a href="http://www.ijpsr.com">www.ijpsr.com</a>
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.5(1).1-15">http://dx.doi.org/10.13040/IJPSR.0975-8232.5(1).1-15</a>	

The genes affected by mutations in cancer may be divided into two main classes: genes that have gain-of-function mutations which are known as oncogenes and genes for which both alleles have loss-of-function mutations, which are known as tumor suppressor genes. Close to 100 genes have been shown to play a role in the development or progression of human cancers, some of which have been implicated in a broad spectrum of malignancies, whereas others are unique to a specific type. Cancers can arise via the aberration of different combinations of genes, which in turn may be mutated, over expressed or deleted. The order in which these events occur has also proved to be important. For example, in breast cancer it has been proposed that at least 10 distinct gene alterations may be involved in disease initiation and progression<sup>2</sup>.

According to estimates from the International Agency for Research on Cancer (IARC), there were 12.7 million new cancer cases in 2008 worldwide, of which 5.6 million occurred in economically developed countries and 7.1 million in economically developing countries. The corresponding estimates for total cancer deaths in 2008 were 7.6 million (about 21,000 cancer deaths a day), 2.8 million in economically developed countries and 4.8 million in economically developing countries.

By 2030, the global burden is expected to grow to 21.4 million new cancer cases and 13.2 million cancer deaths simply due to the growth and aging of the population, as well as reductions in childhood mortality and deaths from infectious diseases in developing countries. The estimated future burden could be much larger than given above due to the adoption of western lifestyles, such as smoking, poor diet, physical inactivity and reproductive factors, in economically developing countries.

Cancers related to these factors, such as lung, breast and colorectal cancers, are increasing in economically transitioning countries. Rates of cancers common in Western countries will continue to rise in developing countries if preventive measures are not widely applied. In economically developed countries, the three most commonly diagnosed cancers were prostate, lung and bronchus, and colorectal among men, and

breast, colorectal and lung among women. In economically developing countries, the three most commonly diagnosed cancers were lung, stomach and liver in men and breast, cervix uteri, and lung in women. In both economically developed and developing countries, the three most common cancer sites were also the three leading causes of cancer death<sup>3</sup>.

Surgery or radiations are the most important methods of treating early stage cancers, including cancers of the breast, colorectum, cervix, head and neck, esophagus, stomach, and prostate. However, the availability of such treatments in Africa is limited because of lack of skilled manpower, surgical equipment, and radiation facilities. On the basis of data from the International Atomic Energy Agency (IAEA) that have been updated through 2010, only 24 of 53 countries in Africa have reported the availability of radiation treatment centers.

It is evident that, even where such facilities exist, the number of centers is inadequate in relation to the size of the catchment population. For example, >80 million people in Ethiopia are served by a single radiotherapy center in the capital city, Addis Ababa. The actual supply of radiation treatment in Africa in 2002 was only 18% of the total needed. The IAEA, through its program of action for cancer therapy, has been working with the WHO and other interested international and national organizations to establish safe and effective cancer therapy facilities such as targeted gene therapy to deliver high-quality treatment to cancer patients in Africa and in other developing areas<sup>4</sup>.

### Targeted gene Therapy:

1. **Tyrosine Kinase:** Multicellular organisms live in a complex milieu where signaling pathways contribute to critical links, for their existence. TKs are important mediators of this signal transduction process, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death. TKs are a family of enzymes, which catalyzes phosphorylation of select tyrosine residues in target proteins, using ATP. This covalent post-translational modification is a pivotal component of normal cellular communication and maintenance of homeostasis<sup>5,6</sup>.

Protein TKs are enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides. The human genome contains about 90 TK and 43 TK-like genes, the products of which regulate cellular proliferation, survival, differentiation, function, and motility. More than 25 years ago, TKs were implicated as oncogenes in animal tumors induced by retroviruses. However, they were largely ignored in drug development because of a paucity of evidence for a causative role in human cancer and concerns about drug specificity and toxicity<sup>5</sup>.

Cancers do not necessarily arise solely as a result of an accelerated rate of cell proliferation. Rather, they are the consequence of an imbalance between the rate of cell-cycle progression and cell growth on one hand and programmed cell death on the other. Researchers now recognize that aberrant cellular signal transduction pathways play a vital role in driving this imbalance and hence in malignant transformation.

Perhaps one of the most critical groups of signaling molecules involved in normal and abnormal cellular regulation are the TKs. These proteins constitute a family of enzymes that catalyze the phosphorylation of the tyrosine residues of various target molecules. This process controls fundamental cellular processes including cell cycle, migration, metabolism, proliferation, differentiation, and survival. Importantly, several tyrosine kinases are aberrantly expressed in malignancies. The underlying defects may include, but are not limited to, mutation, hybrid gene formation, amplification, and perturbation of transcriptional machinery<sup>6</sup>.

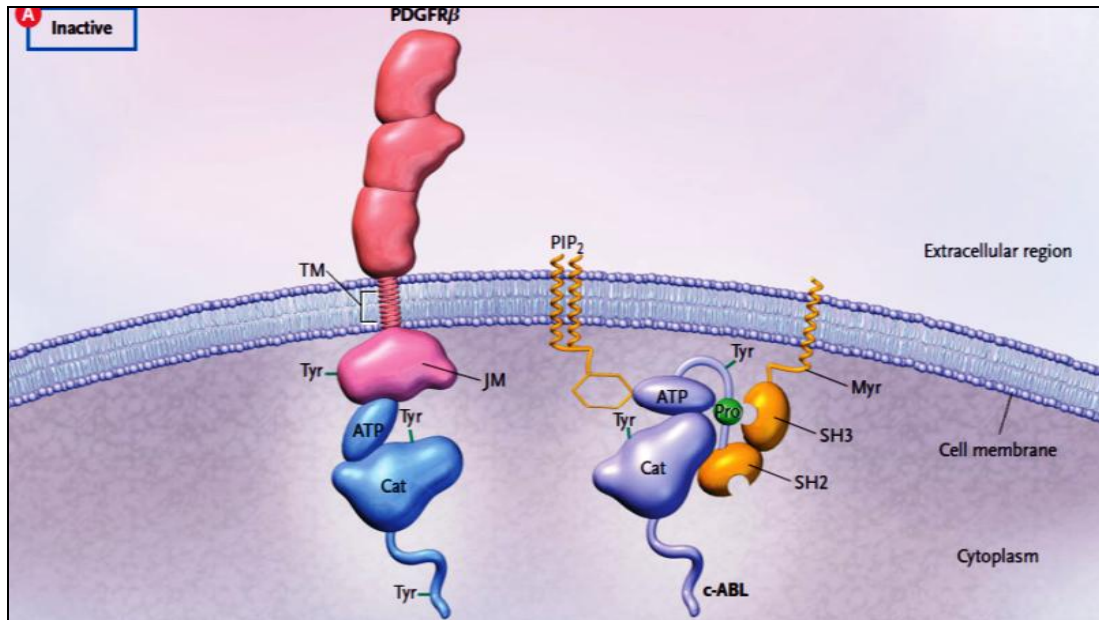
- a. **Classification of Tyrosine Kinase:** Tyrosine kinases are primarily classified as receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR and the IR and non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, FAK and Janus kinase. The receptor tyrosine kinases are not only cell surface transmembrane receptors, but are also enzymes having kinase activity. The structural organization of the RTK exhibits a multidomain extracellular ligand for conveying ligand specificity, a

single pass transmembrane hydrophobic helix and a cytoplasmic portion containing a tyrosine kinase domain. The kinase domain has regulatory sequence both on the N and C terminal end<sup>7</sup>. In the absence of ligand, RTKs are unphosphorylated and monomeric, and the conformation of their kinase domains is inactive as shown figure 1.7 below. In some RTKs, the cytoplasmic juxtamembrane region further inhibits the enzyme by interacting with the kinase domain.

RTKs become activated when ligand binds to the extracellular domain, resulting in receptor oligomerization, disruption of the autoinhibitory juxtamembrane interaction, and autophosphorylation of a regulatory tyrosine within the activation loop of the kinase. These changes reorient critical amino acid residues, thereby increasing the catalytic activity of the enzyme. After activation, autophosphorylation generates binding sites for signaling proteins, recruiting them to the membrane and activating multiple signaling pathways which depicted in figure 1.8<sup>5</sup>.

NRTK are cytoplasmic proteins, exhibiting considerable structural variability. The NRTK have a kinase domain and often possess several additional signaling or protein-protein interacting domains such as SH2, SH3 and the PH domain. The tyrosine kinase domain spans approximately 300 residues and consists of an N terminal lobe comprising of a 5 stranded  $\beta$  sheet and one  $\alpha$  helix, while the C terminal domain is a large cytoplasmic domain that is mainly  $\alpha$  helical. ATP binds in the cleft in between the two lobes and the tyrosine containing sequence of the protein substrate interacts with the residues of the C terminal lobe.

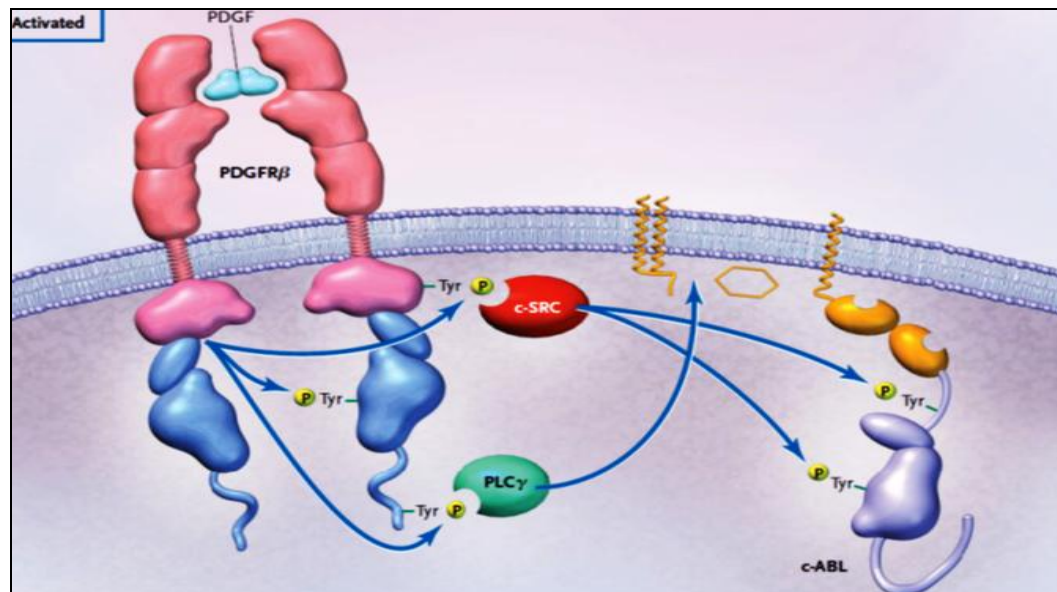
RTK are activated by ligand binding to the extracellular domain followed by dimerization of receptors, facilitating transphosphorylation in the cytoplasmic domain whereas the activation mechanism of NRTK is more complex, involving heterologous protein-protein interaction to enable transphosphorylation<sup>8</sup>.



**FIG. 1: MECHANISMS OF ACTIVATION OF NORMAL TKS SHOWS BOTH KINASES IN THEIR INACTIVE STATES**

From **figure 1** above, inactive PDGFRβ is monomeric and unphosphorylated, and the catalytic domain is inhibited by protrusion of a regulatory tyrosine (Tyr) in the activation loop into the substrate cleft and by an intramolecular interaction with the juxtamembrane (JM) domain. Inactive c-ABL is associated with the membrane through a

covalent N-terminal myristate group (Myr) and is inhibited through intramolecular interaction of the Src homology-3 (SH3) domain with an adjacent proline (Pro) residue and by direct interaction of the catalytic domain with an inhibitory membrane lipid, phosphatidylinositol-4,5-bisphosphate (PIP2).



**FIG. 2: MECHANISMS OF ACTIVATION OF NORMAL TKS SHOWS BOTH KINASES IN THEIR ACTIVE STATES**

From **figure 2** shown above, PDGFRβ is activated upon binding of the ligand (dimeric PDGF), which induces oligomerization of the receptor and intermolecular phosphorylation (P, in yellow) of the activation-loop tyrosine.

This leads to a conformational change in the catalytic domain and increased enzymatic activity, while phosphorylation of other tyrosines within the intracellular domain of the receptor creates binding sites for SH2

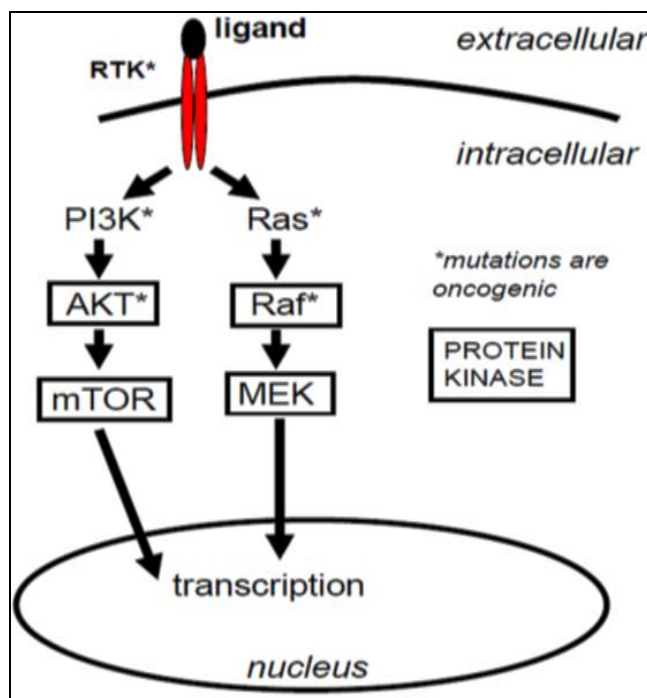
domain-containing signaling proteins, including c-SRC (red oval) and phospholipase CY (PLCY) (green oval). c-ABL is activated through the phosphorylation of two regulatory tyrosines, one in the activation loop and the other near the SH3 binding site, which can be phosphorylated by another TK, such as c-SRC. In addition, activated PLCY can hydrolyze and destroy the lipid inhibitor PIP<sub>2</sub><sup>5</sup>.

- b. **Mechanisms of tyrosine kinase dysregulation in cancer:** Tyrosine phosphorylation is a unique biochemical mechanism utilized by intra- and intercellular communication pathways in metazoans. Abnormal tyrosine kinase activities lead to various cancers as well as other diseases. A common mechanism of TK activation in hematologic cancers is the fusion of a receptor or non-receptor TK with a partner protein, usually as a consequence of a balanced chromosomal translocation.

A frequent feature of the partner protein is a domain that causes constitutive oligomerization of the TK in the absence of ligand binding or physiologic activating signals, thereby promoting autophosphorylation and activation. Activation of oncogenes is an important early event in the transformation of normal cells to malignant cells.

RTKs represent an important group of oncogenic protein kinases and molecular targets for cancer therapy. These include the epidermal growth factor receptor (EGFR/ErbB2) family of kinases, PDGFR and insulin-like growth factor kinases (IGF-1, IGF-2).

RTKs typically are activated by binding extracellular ligand. This mediates transmembrane signaling through dimerization and autophosphorylation on cytoplasm-facing tyrosine residues. These residues are docking sites for downstream effectors that bear phosphoselective protein-binding domains that constitutive activation of certain RTKs as depicted in **figure 3**<sup>9</sup>.

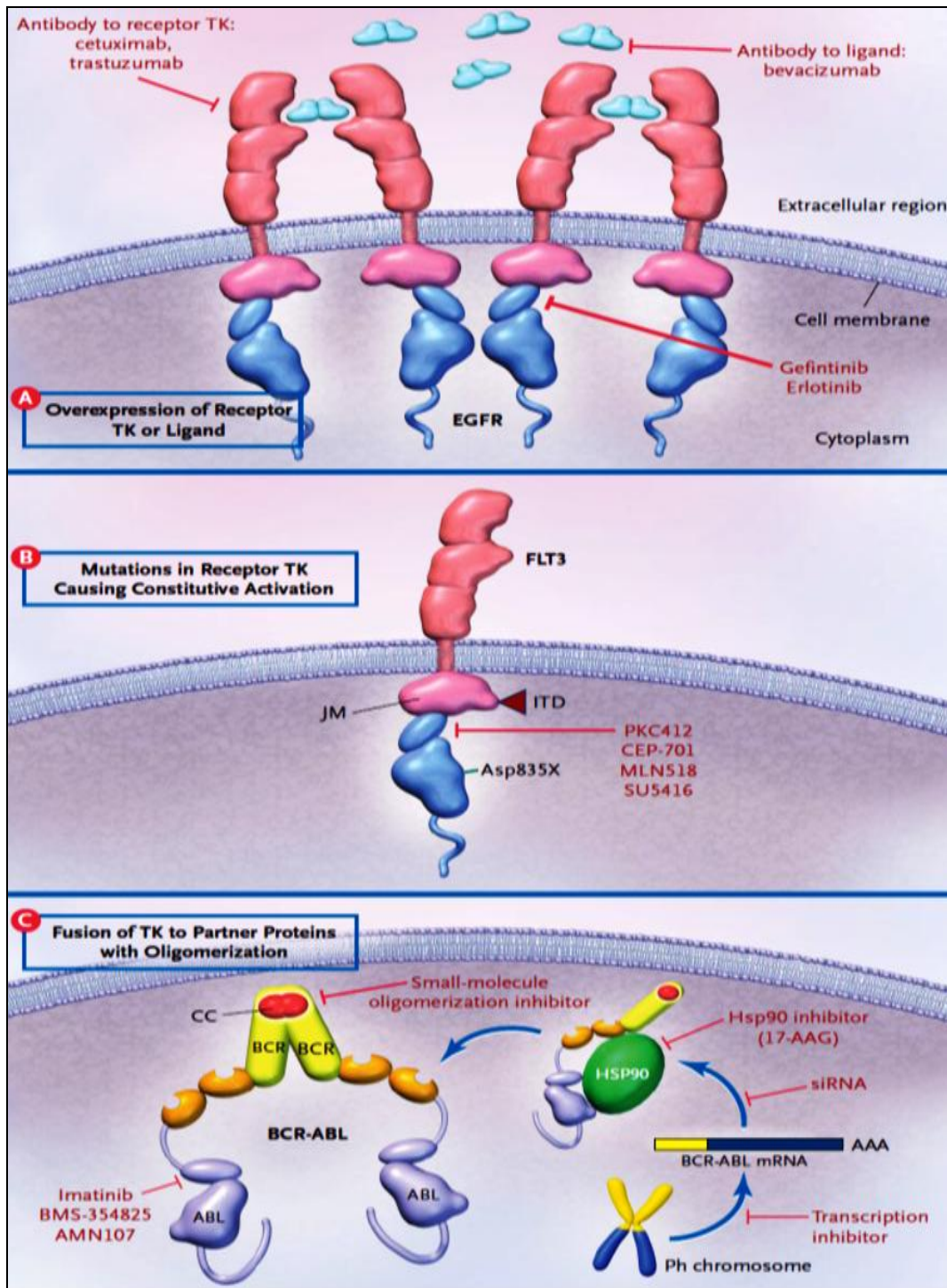


**FIG. 3: ONCOGENIC TYROSINE KINASE**

From the figure 3, RTKs are transmembrane proteins that dimerize when bound to ligand. EGFR and ErbB2/Her2 are examples of oncogenic RTKs. Downstream mitogenic signaling cascades include the canonical Ras signaling pathway and the PI3K pathway. These protein kinases typically phosphorylate serine/threonine residues. Here, mitogenic signaling protein kinases are boxed. Oncogenic mutations have been found in AKT and Raf among the protein kinases<sup>9</sup>. There is strong evidence that during tumor progression, the hyperactivation of tyrosine kinases leads to the continuous activation of downstream signaling cascades that block cellular apoptosis, promote cellular proliferation, and increase the nutrient/waste interchange by enhancing angiogenesis.

Activation of the kinase is achieved by ligand-binding to the extracellular domain, which induces homo/hetero-dimerization of the receptors. Activated receptors phosphorylate tyrosine residues outside their catalytic domain via cross phosphorylation. This phosphorylation stabilizes the receptor conformation in an active state and creates phosphotyrosine docking sites for proteins which transduce signals within the cell. In cancer, this mechanism of ligand-dependent activation can be bypassed by;

- Overexpression of the RTK, which increases the dynamics of receptor homo/heterodimerization in the absence of the ligand;
- By activating mutations, which stabilize the receptor active conformation or,
- By autocrine stimulation as depicted in **figure 4**<sup>5,10</sup>.



**FIG. 4: MECHANISMS OF TK DYSREGULATION AND THERAPEUTIC TARGETING IN CANCER**

From figure 4 above, in each case, the TKs known to be activated through that mechanism are listed. Overexpression of a normal receptor TK here, EGFR, its ligand or both is depicted in Panel A.

In Panel B, mutations that render a receptor TK constitutively active in the absence of ligand are represented by internal tandem duplications (ITDs) in JM domain and point mutations (Asp835X) in the activation loop

of Fms-like tyrosine kinase 3 (FLT3). In Panel C, BCR-ABL exemplifies the fusion of receptor and non-receptor TKs to various N-terminal partner proteins as a consequence of chromosomal translocations and deletions. A common feature of the partner proteins is a domain that mediates oligomerization, such as the coiled-coil (CC) domain of BCR. Examples of therapeutic agents targeting TKs are listed in red.

Small-molecule TK inhibitors usually act to block binding of ATP or substrate to the catalytic domain of the TK. BCR-ABL may also be targeted by compounds such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) that interfere with binding to cellular chaperones such as Hsp90, by compounds that block oligomerization, by small interfering RNA (siRNA) that induces degradation of BCR-ABL mRNA, or by inhibitors of BCR-ABL gene transcription<sup>5</sup>.

A primary example of TK dysregulation mechanism is BCR-ABL, the non-receptor fusion TK in CML, in which a tetramerization domain in BCR overcomes autoinhibition of ABL catalytic activity through oligomerization and autophosphorylation. With some RTKs, absence of the juxtamembrane inhibitory domain in the fusion protein contributes to activation. CML is an acquired hematopoietic stem cell disorder characterized by expression of the BCR-ABL oncoprotein. BCR-ABL expression alone may be enough to explain the chronic phase of the disease. The BCR-ABL oncoprotein interacts with many substrates in the leukemic cell, which ultimately leads to the CML phenotype<sup>11</sup>.

A second important mechanism of TK dysregulation is a mutation that disrupts autoregulation of the kinase. Small deletions and point mutations in the kinase domain of EGFR in a subset of non-small-cell lung cancers increase the sensitivity of the receptor to its ligand and alter receptor signaling. The TK activity of EGFR may be dysregulated by several oncogenic mechanisms, including EGFR gene mutation, increased gene copy number, and EGFR

protein overexpression. The receptors and ligands of the EGFR family also mediate complex interactions between tumor cells and the tumor microenvironment.

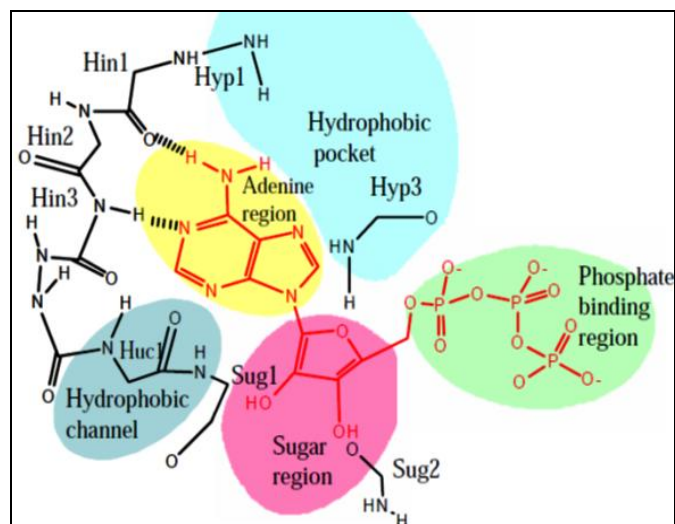
Improper activation of EGFR TK inhibits tumor cell apoptosis and contributes to tumor progression. EGFR may also interact with the integrin pathway and activate matrix metalloproteinases to alter cellular adhesion, stimulate cell motility and invasion, and promote metastasis<sup>12</sup>.

2. **Tyrosine kinases as Targets in the Treatment of Malignant Hematologic Disorders:** The role of TKs in cancer molecular pathogenesis is immense and recently kinases have come in vogue as potential anticancer drug targets, as a result a couple of anticancer drugs are in the market. The complexity and the number of TKs have greatly increased with the sequencing effort of the Human Genome Project, thus providing more opportunities for drug discovery.

The TKIs are a part of the growing anticancer targeted therapies aimed at blocking mutations responsible for the growth of cancers (growth factor receptors, intracellular signaling pathways, DNA repair and apoptosis, and angiogenesis). They are small, synthetic molecules that enter cells and inhibit critical protein kinases implicated in the growth of cancer<sup>13</sup>. Inhibiting the activity of TKs by low molecular weight compounds capable of interfering with either ligand binding in the case of RTKs or with protein substrate in case of NRTKs has proved.

The bisubstrate inhibitor approach offered promise but, with very little practical progress. Approaches to generate non-competitive or allosteric inhibitors have also failed. The ATP competitive inhibitors appear to be the target of choice. ATP binds within a deep cleft formed between the two lobes of the tyrosine kinase domain. Though the ATP binding site is highly conserved the architecture in the regions proximal to the ATP binding site does afford some key diversity for designing new drug and has potential application in drug discovery. The ATP binding sites have the following features:

- i. Adenine region – contains two key Hydrogen bonds formed by the interaction of N-1 and N-6 amino group of the adenine ring. Many potent inhibitors use one of these Hydrogen bonds.
  - ii. Sugar region – a hydrophilic region, except a few e.g. EGFR. Hydrophobic pocket – though not used by ATP but plays an important role in inhibitor selectivity.
  - iii. Hydrophobic channel – not used by ATP and may be exploited for inhibitor specificity.
  - iv. Phosphate binding region – can be used for improving inhibitor selectivity as in **figure 5**<sup>8</sup>.
- iii. Allosteric inhibitors, that bind outside of the ATP-binding site, modifying the tridimensional structure of the receptor and disrupting the interaction between the ATP and the kinase pocket; and
  - iv. Covalent inhibitors, that binds irreversibly by covalently bonding to the ATP-binding site of the target kinase<sup>10</sup>.
- a. **BCR-ABL Tyrosine Kinase Inhibitors:** CML is a disease of the hematopoietic stem cell, characterized by the t(9 ; 22) q(34 ; q11) translocation encoding the oncoprotein BCR-ABL. Patients suffering CML benefit from new-targeted therapies based on the use of TKIs<sup>14</sup>. CML is associated with an acquired cytogenetic abnormality: the Philadelphia (Ph) chromosome as shown in **figure 6A**. This translocation generates the BCR/ABL fusion gene, which is translated in an oncoprotein Bcr-Abl with highly deregulated, constitutive tyrosine kinase activity. The most commonly occurring form of Bcr-Abl is a 210-kDa protein that plays a critical role in the pathogenesis of CML. This oncogenic protein plays important roles in the proliferation and survival of myeloid progenitor cells.



**FIG. 5: MODEL OF ATP-BINDING SITE OF PROTEIN KINASES.** ATP is depicted in red, Sug1, Hyp1 & Hyc1 are residues lining the sugar region, hydrophobic pocket (Hyp), hydrophobic channel (Huc) and hinge region (Hin) respectively (redrawn).

TKIs are small molecules that inhibit the enzymatic activity of the target protein. Most of these molecules can be categorized into four groups:

- i. ATP-competitive inhibitors, which bind predominantly to the ATP-binding site of the kinase when this site is in the active conformation;
- ii. Inhibitors that recognize and bind to the non-active conformation of the ATP-binding site of the kinase, thus making activation energetically unfavorable;

As a result of the increased tyrosine kinase activity, Bcr-Abl activates several signaling pathways, including Ras, PI3K-Akt, Jak and NF- $\kappa$ B, pathways leading to proliferation, reduced growth factor dependence and apoptosis, and an abnormal interaction with extracellular matrix and stroma<sup>15</sup>. In **figure 6 B**, the fusion product BCR-ABL encodes for the BCR-ABL protein and BCR-ABL protein has constitutively activated tyrosine kinase activity which results uncontrolled myeloproliferation, decreased apoptosis, altered cellular adhesion, defective DNA repair at the end formation of CML<sup>16</sup>.

CML is a clonal myeloproliferative disorder characterized by the presence of BCR-ABL, which is an oncogene created by the fusion of BCR and ABL genes. The juxtaposition of these genes in response to genetic mutation encodes a novel fusion gene which translates into a protein with constitutive TK activity.



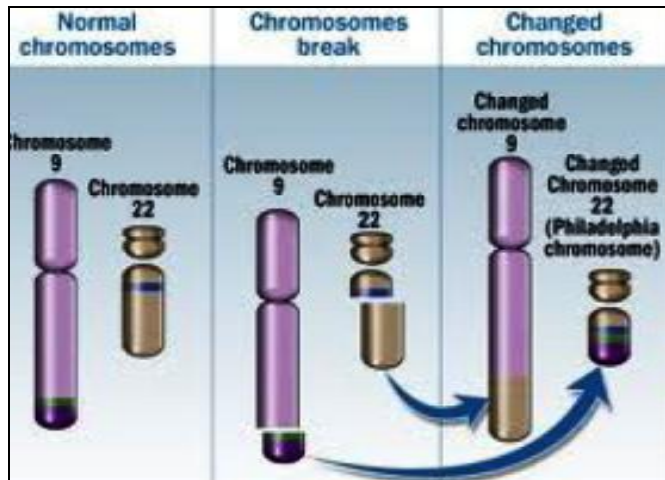


FIG. 6A: PHILADELPHIA CHROMOSOME

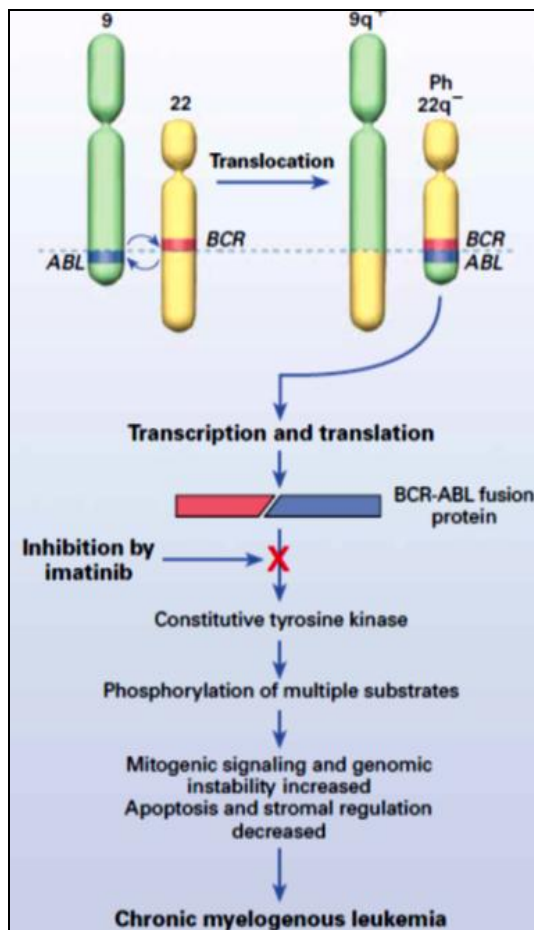


FIG. 6B: MECHANISM OF CML

This deregulated activity present in the haemopoietic stem cell population results in the pathogenicity of the disease with the over-production of mature myeloid cells in the bone marrow and peripheral circulation. The disease is characterized by three distinct phases; beginning in chronic phase, developing into accelerated and then blast crisis phases, which are progressively more aggressive<sup>17</sup>.

Myeloproliferative disease resulting from a specific genetic mutation that characterized by the presence of the Ph<sup>+</sup> chromosome and BCR-ABL transcript in leukemic cells. In **figure 7**, the normal cell and CML compared comparatively with amount of clonal myeloproliferative cell<sup>16</sup>.

Depending on the breakpoint in the BCR gene, three main types of BCR/ABL genes can be formed as shown **figure 8**.

The majority of patients with CML have breakpoints in introns 1 or 2 of the ABL gene and in the major breakpoint cluster region (M-bcr) of the BCR gene, either between exons 13 and 14 (b2), or 14 and 15 (b3) (Figure 8). These breakpoints produce BCR/ABL fusion genes that transcribe either a b2a2 or b3a2 mRNA. The product of this genetic rearrangement is a 210 kDa cytoplasmic fusion protein, p210BCR/ABL, which is essential and sufficient for the malignant transformation of CML, and responsible for the phenotypic abnormalities of chronic phase CML<sup>18</sup>.

- i. **Imatinib therapy in patients with CML:** BCR-ABL1 kinase is a pivotal driver of the pathogenesis of CML through phosphorylation and activation of a broad range of downstream substrates that modulate signal transduction and transformation. Thus, BCR-ABL1 kinase represents an obvious therapeutic target. Imatinib mesylate, an orally bioavailable 2-phenyl-aminopyrimidine, was the first compound described to target BCR-ABL1 kinase in a robust and efficacious manner<sup>19</sup>.

The structure of imatinib is shown in **figure 9** below: Imatinib or Gleevec is an orally administered TKI specifically designed to inhibit the BCR-ABL fusion protein by occupying the ATP-binding pocket of the ABL-kinase domain. This prevents a change in conformation of the protein to the active form of the molecule. By blocking the ATP-binding site, imatinib reduces cell proliferation and stops disease progression<sup>20, 21</sup>.

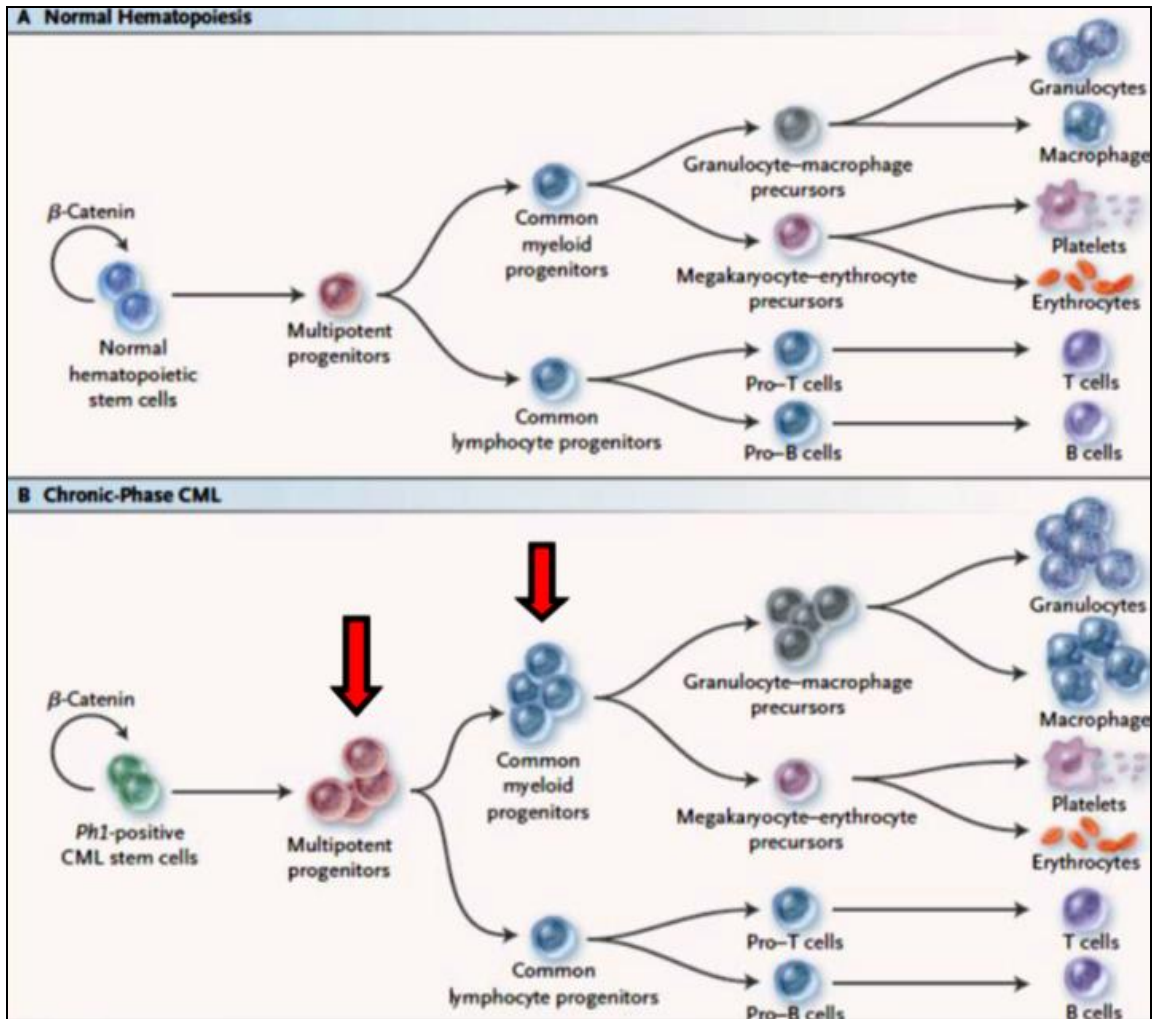


FIG. 7: CHRONIC MYELOID LEUKEMIA

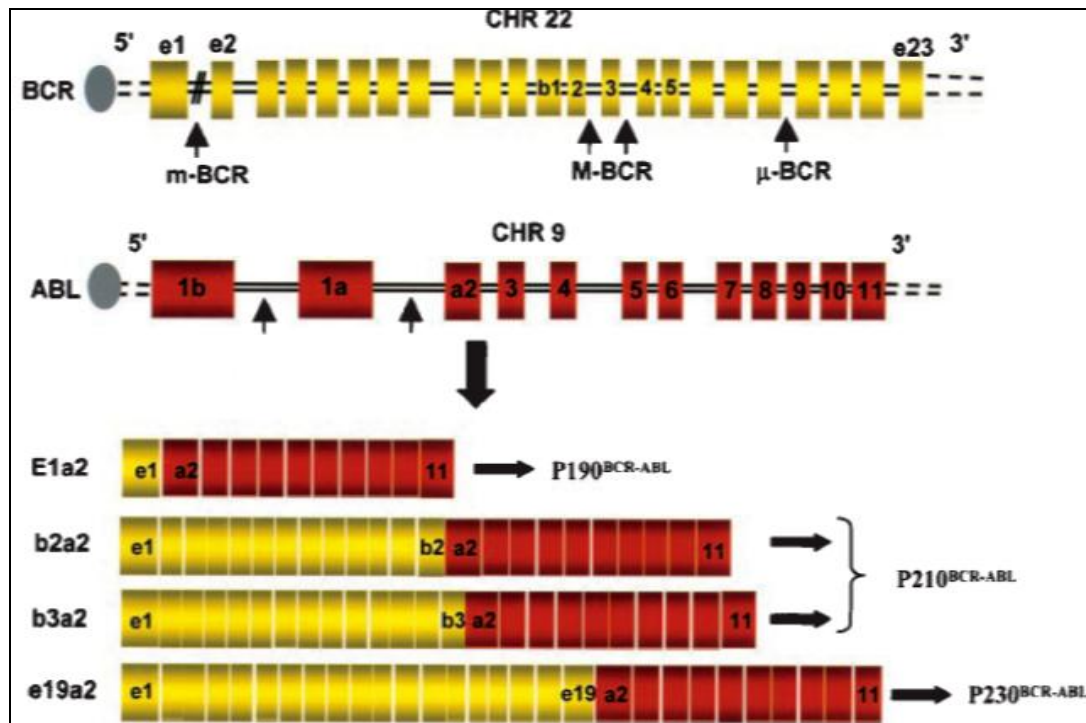
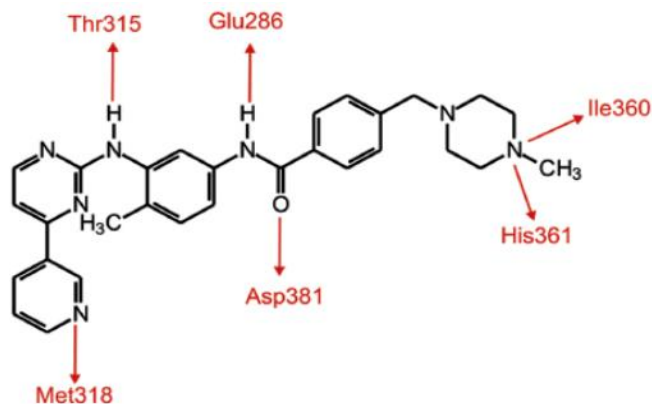


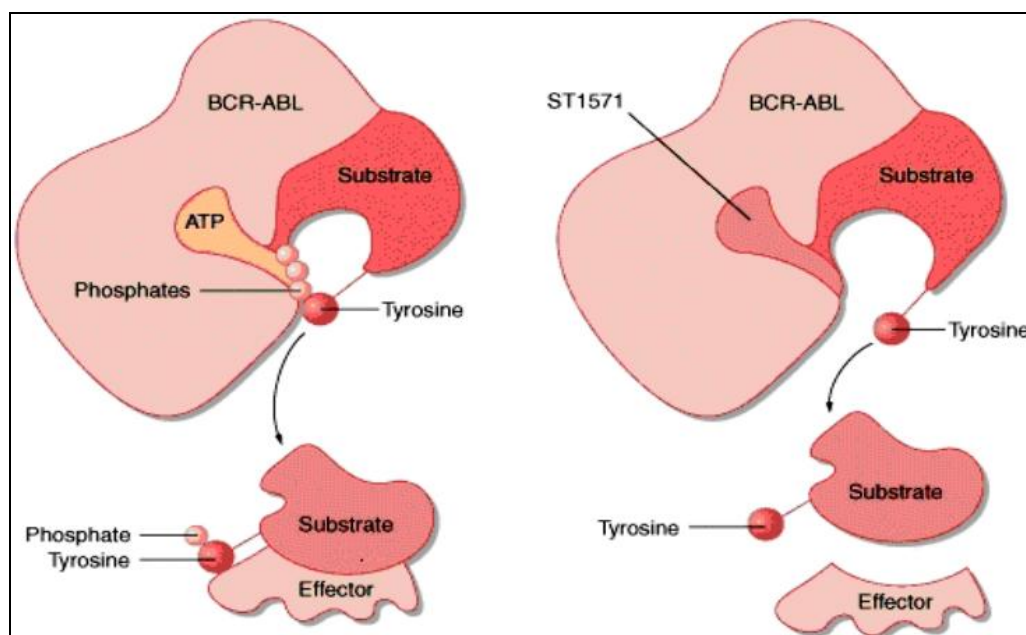
FIG. 8: LOCATIONS OF THE BREAKPOINTS IN THE ABL AND BCR GENES AND STRUCTURE OF THE CHIMERIC BCR/ABL MRNA TRANSCRIPTS DERIVED FROM THE VARIOUS BREAKS



**FIG. 9: CHEMICAL STRUCTURES OF IMATINIB**

STI-571(Imatinib) is an effective inhibitor of c-Abl and Bcr-Abl protein-tyrosine kinase activity. Using X-ray crystallography, found that STI-571 binds to an inactive conformation of c-Abl. The drug binds in the cleft between the amino- and carboxy-terminal lobes of the kinase

domain. Only the left most portion of STI-571 is found where the adenine base of ATP normally binds. The rest of the compound penetrates further into the hydrophobic core of the kinase, inserted between the activation loop and helix  $\alpha$  C, thereby keeping the kinase in an inactive conformation. Recent steady-state kinetic studies show that STI-571 is a competitive inhibitor with respect to ATP, which is consistent with the X-ray studies showing that the drug binds to the ATP-binding site. In chronic myeloid leukemia, c-Abl is fused with Bcr causing it to be constitutively active. Thus, the activation loop in Bcr-Abl would be in the open conformation and phosphorylated. When the activation loop is transiently dephosphorylated, STI-571 can bind and inactivate the kinases as illustrated in **figure 10** below<sup>22</sup>.



**FIG. 10: MECHANISM OF ACTION OF IMATINIB.** (A) The constitutively active BCR-ABL tyrosine kinase functions by transferring phosphate from ATP to tyrosine residues on various substrates to cause excess proliferation of myeloid cells characteristic of CML. (B) Imatinib blocks the binding of ATP to the BCR-ABL tyrosine kinase, thus inhibiting kinase activity

Nilotinib is an orally active phenylamino-pyrimidine derivative of imatinib developed using rational drug design based on the crystal structures of inhibitors in complexes with ABL. Nilotinib is approximately 30 times more potent than imatinib at inhibiting BCR-ABL. It does not inhibit the SRC family of TKs. Studies performed in vitro suggest that nilotinib inhibits 32 of 33 mutant BCR-ABL forms resistant to

imatinib at physiologically relevant concentrations. It, like imatinib, binds to the inactive conformation of ABL, but with a slightly better topographical fit. It has a higher binding affinity and selectivity for BCR-ABL with respect to imatinib and is an effective treatment of CML after imatinib failure<sup>23</sup>. The chemical structure of nilotinib is shown in **figure 11** below:

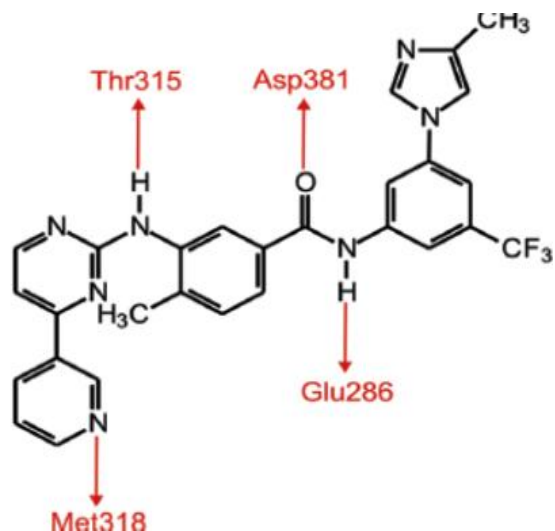


FIG. 11: MOLECULAR STRUCTURES OF NILOTINIB

Dasatinib binds to the ATP-binding site in a position that is similar to imatinib. The central cores of dasatinib and imatinib share overlapping regions, the difference being that they extend in opposite directions. It differs from imatinib and nilotinib in that it binds to both the active and inactive conformation of Abl kinase and inhibits a broader range of kinase targets<sup>24</sup>. It is a highly potent, orally active inhibitor of SRC and the SRC-family kinases.

The SRC family of TKs modulates multiple intracellular signal transduction pathways involved in cell growth, differentiation, migration and survival, many of which are involved in oncogenesis, tumour metastasis and angiogenesis. It is an inhibitor of FYN and YES, which are ubiquitously expressed, and of FGR, HCK, LCK and LYN, which are found mainly in hematopoietic cells. It is also a potent BCR-ABL kinase inhibitor and has additional activity against the KIT, PDGFR and ephrin RTKs.

It has been shown to directly inhibit 21 out of 22 mutant forms of BCR-ABL resistant to imatinib. In pre-clinical comparisons with imatinib, dasatinib was 325 times more potent than imatinib against cells expressing wild-type BCR-ABL. This may be due in part to the ability of dasatinib to bind to both the active and inactive conformations of ABL<sup>21</sup>. The structure of dasatinib shown in **figure 12** below:

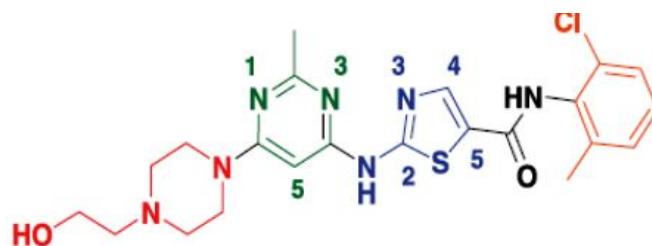


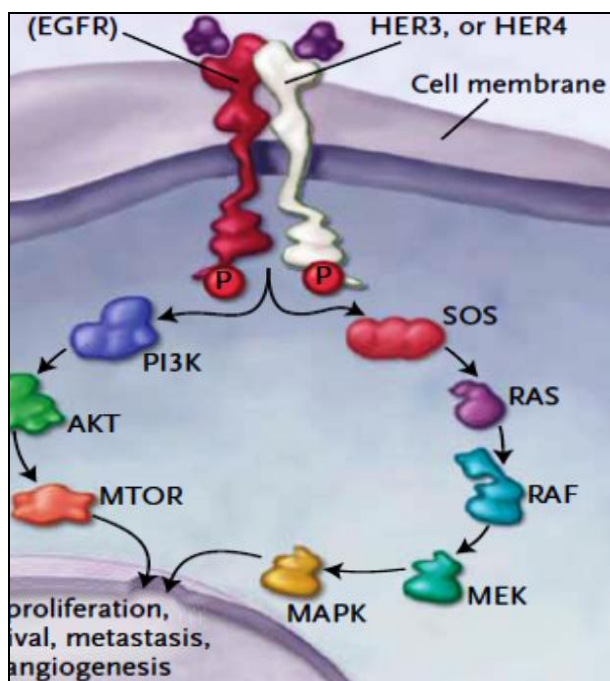
FIG. 12: TWO-DIMENSIONAL STRUCTURE OF DASATINIB

- ii. **EGFR Tyrosine Kinase Inhibitors:** Lung cancer is the leading cause of cancer-related deaths, accounting for one-third of worldwide deaths from cancer. Non-small cell lung cancer (NSCLC) represents 80 % of lung cancers. The EGFR is an important therapeutic target in NSCLC<sup>25</sup>.

The signaling pathway of the EGFR, a cell-surface receptor, is activated in more than half of patients with NSCLC, and this activation can be the result of protein overexpression, increased gene copy number, or genetic mutations. Binding of secreted growth factors, such as the epidermal growth factor (EGF) and other EGF-like growth factors, including transforming growth factor  $\alpha$  and epiregulin, induces receptor dimerization, resulting in the phosphorylation of tyrosine residues in the kinase domain. These phosphotyrosines recruit partner proteins that trigger intracellular signaling cascades, chiefly through the mitogenactivated protein kinase (MAPK) and PI3K pathways, which are involved in the induction of cell proliferation, protection from apoptosis, activation of angiogenesis, and development of metastasis as depicted in **figure 13**<sup>26</sup>.

**Figure 13** below shows that binding of ligands to the HER family of receptors induces either homodimerization or heterodimerization of the receptors. Dimerization results in phosphorylation of the tyrosine residues of the EGFR kinase domain. The activated receptor may then phosphorylate a wide array of intracellular signaling cascades, such as the RAS-RAF-MEK-ERK and PI3K-AKT pathways that induce cellular proliferation, angiogenesis and metastases.

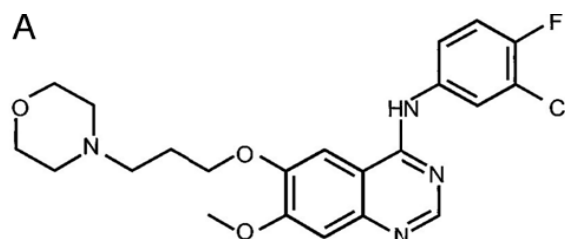
EGFR amplification can obviate the requirement for ligand-induced dimerization<sup>26</sup>.



**FIG. 13: EGFR SIGNALING PATHWAY.** Where, MTOR Mammalian Target of Rapamycin, P Phosphorylation, and SOS Son of Sevenless.

The EGFR signaling network plays a central role in the growth and maintenance of epidermal derived tissues, and alterations of this network can lead to malignant transformation. As in other epithelial tumors, EGFR expression is detectable in most NSCLC cases, and it is present in premalignant bronchial epithelium as well as in up to 90% of tumors of the squamous cell subtype of NSCLC and 30%–65% of tumors of the adenocarcinoma subtype. Based on its central role in cellular growth and its ubiquitous expression, EGFR was posited as a priority target for the development of anti-NSCLC treatments. Small molecules were developed to inhibit EGFR signaling by blocking the intracellular TK domain. The first two of these EGFR TKIs, gefitinib (Iressa) and erlotinib (Tarceva), made their way into clinical trials in the early 2000s. Both were orally administered agents that bound at the catalytic cleft of EGFR in competition with ATP, causing suppression of receptor phosphorylation and downstream signaling<sup>27</sup>.

iii. **Gefitinib therapy in patients with NSCLC:** Gefitinib is a reversible inhibitor of the EGFR tyrosine kinase. The chemical structure of the drug is shown in **Figure 14**. Gefitinib inhibits autophosphorylation and downstream signaling by EGFR by competing with ATP for binding in the ATP-binding pocket of the EGFR kinase domain. Gefitinib was the first targeted therapy drug used for the treatment of NSCLC, receiving FDA approval for the treatment of advanced NSCLC. The drug is utilized as a second- or third-line agent in patients for whom first line salvage chemotherapies have failed<sup>28</sup>.

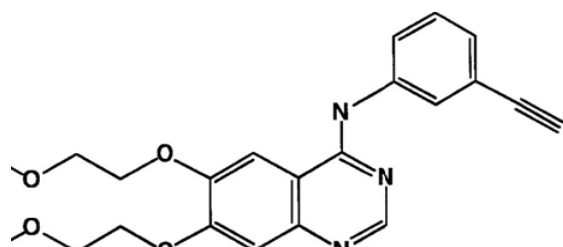


**FIG. 14: STRUCTURES OF GEFITINIB**

Gefitinib is metabolized principally by cytochrome P450A4, while CYP3A5 and CYP2D6 are less involved. This is the reason why gefitinib metabolism can differ from patient to patient; meaning, in consideration of inter-individual variability of CYP3A4 expression and activity. Therefore, inducers or inhibitors of this cytochrome can also influence the pharmacokinetics of this drug.

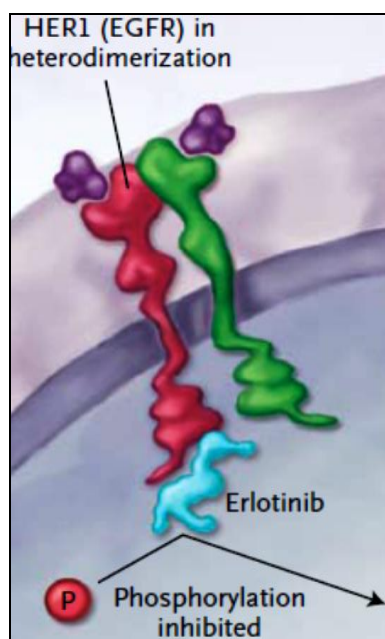
Some studies demonstrated that gefitinib blocks selectively EGFR tyrosine-kinase (if compared with TKs of different receptors) and that it does not inhibit serine threonine-kinases. Its activity determines an up regulation of a cell cycle inhibitor (p27) and a down regulation of a transcription factor (c-fos), resulting in arresting the cell cycle in G1 phase. EGFR works through two different downstream signaling pathways: MAP kinase cascade, that activates different genes linked to cell proliferation and survival, and PI3K-AKT cascade, in which phosphorylated AKT (p-AKT) inactivates pro apoptotic proteins<sup>29</sup>.

- iv. **Erlotinib therapy in patients with NSCLC:** Erlotinib is another reversible inhibitor of the EGFR tyrosine kinase used for the targeted therapy of NSCLC patients. The chemical structure of the drug is shown in **Figure 15**. Similar to gefitinib, erlotinib selectively and reversibly inhibits the tyrosine kinase activity of EGFR, competing with ATP for binding in the EGFR kinase domain as in **figure 16**.



**FIG. 15: STRUCTURE OF ERLOTINIB**

Erlotinib was approved by the FDA in November 2004 and by the European Medicinal Evaluation Agency (EMA) in October 2005, for the treatment of chemotherapy-resistant advanced NSCLC patients<sup>28</sup>. The above figure shows the intracellular binding of erlotinib (similar to that of gefitinib) to the ATP-binding site of the tyrosine kinase domain of EGFR, which blocks the catalytic activity of the kinase, thereby inhibiting downstream signaling of the pathways responsible for cellular proliferation<sup>28</sup>.



**FIG. 16: MECHANISM ACTION OF EGFR ERLOTINIB**

**CONCLUSION:** Targeted therapies represent an important new set of strategies under rapid development for the treatment of cancer. Tyrosine kinase is one of the targeted gene therapy involved in cell proliferation and have attracted a great deal of interest because of their comprehended signal to act as highly effective target for cancer therapy.

It is classified into two: RTK in the transmembrane and NRTK in cytoplasm. High-resolution structural studies of RTKs and NRTKs together with a wealth of biochemical and genetic data; are continuing to reveal the precise molecular mechanisms underlying in ligand recognition by RTKs, dimerization and activation of RTKs, regulation of catalytic activity in NRTKs and specificity in protein TK signaling. Among TK, ABL-BCR targeted for CML by using small molecule inhibiting like imatinib, nilotinib and dasatinib. EGFR also targeted for non-small cell lung cancer by using drug such as gefitinib and erlotinib.

A growing body of evidence provides new insight in the comprehension of the cellular and molecular mechanisms responsible for TKI in cancer treatment. However, clinical evidences suggest that the most effective way of cancer treatment by using TKIs is to consider each patient or tumor individually and to determine the strategy that specifically targets the consequences of altered (epi) genetics of the tumor, with an acceptable toxicity. There is significant variation between individuals in their response to different medications. Therefore, future personalized medicine required for the correct treatment in that individuals.

Although some evidences well address the effectiveness of TK in cancer targeted therapy; in my outlook a detailed knowledge of the mechanisms responsible for cancer treatment by TKIs and their real effectiveness *in vivo*, is necessary in order to propose them as potential target of cancer therapy and candidates for cancer treatment. It could be recommended that broad application of TK as target for cancer treatments are coupled to efficient lead finding and optimization needs more intervention in the area of cancer genome based molecular therapeutics. All these concerted effort may pave to personalized cancer therapeutics.

**REFERENCES:**

- Mills K: Molecular Analysis of Cancer, In: Methods in Molecular Medicine, Boulton J and Filder C, 1-5, Humana Press, Towata, New Jersey, 2002.
- Devilee P, Schuurin E, van de VM, Cornelisse CJ: Recent developments in the molecular genetic understanding of breast cancer. *Critical Review Oncogene* 1994; 5:247-270.
- Center M, Siegel R, Jemal A: Global Cancer: Facts and Figures. *American Cancer Society* 2011; 10(40): 320-333.
- Jemal A, Bray F, Forman D, Brien OM, Ferlay J, Center M, Parkin M: Cancer Burden in Africa and Opportunities for Prevention. *American Cancer Society* 2012; 2(4):105-123.
- Krause SD, Van Etten AR: Mechanisms of disease tyrosine kinases as targets for cancer therapy. *The new England journal of medicine* 2005; 353(2):172-190.
- Tibes P, Trent J, Kurzrock R: Tyrosine kinase inhibitors and the dawn of molecular cancer therapeutics. *Annual Reviews of Pharmacology and Toxicology* 2011; 45:357-384.
- Levitzi A: Tyrosine Kinase Inhibitors: Views of Selectivity, Sensitivity, and Clinical Performance. *Annual Review of Pharmacology and Toxicology* 2013; 53: 161-185.
- Paul KM, Mukhopadhyay KA: Tyrosine kinase – Role and significance in Cancer. *International Journal of Medical Sciences* 2004; 1(2): 101-115.
- Burkard EM, Jallepalli VP: Validating cancer drug targets through chemical genetics. *Biochemical physics acta* 2011; 1806(2): 251-257.
- Sierra RJ, Cepero V, Giordano S: Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. *Molecular Cancer* 2010; 9:75.
- Lenaerts L, Pacheco MJ, Traulsen A, Dingli D: Tyrosine kinase inhibitor therapy can cure chronic myeloid leukemia without hitting leukemic stem cells. *Haematologica* 2010; 95:900-907.
- Santos CG, Shepherd AF, Tsao SM: EGFR Mutations and Lung Cancer. *Annual Review of Pathology* 2011; 6:49-69.
- Archer M, Oderda G, Brown S, Chin M: Antineoplastic Tyrosine Kinase Inhibitors. *Antineoplastic Agents* 2012; 3-20.
- Cassuto O, Dufies M, Jacque A, Robert G: All tyrosine kinase inhibitor-resistant chronic myelogenous cells are highly sensitive to Ponatinib. *Oncology target* 2012; 3(12): 1557-1565.
- Benito R, Lumbres E, Abaigara M, and Gutierrez CN: Imatinib therapy of chronic myeloid leukemia restores the expression levels of key genes for DNA damage and cell-cycle progression. *Pharmacogenetics and Genomics* 2012; 22:381-388.
- Nguyen VD. Should nilotinib replace imatinib as first line treatment of chronic myeloid leukemia in chronic phase 2010; URL/http://test.metromomsblog.org. Accessed on 01/07/2013.
- Sinclair A, Latif LA, Holyoake LT: Targeting Survival Pathways in Chronic Myeloid Leukaemia Stem Cells. *British Journal of Pharmacology* 2013; 3-22.
- Salesse S, Verfaillie MC: BCR/ABL: from molecular mechanisms of leukemia induction to treatment of chronic myelogenous leukemia. *Oncogene* 2002; 21: 8547 – 8559
- Cardama QA, Kantarjian MH, Cortes EJ. Mechanisms of Primary and Secondary Resistance to Imatinib in Chronic Myeloid Leukemia. *Cancer control* 2009; 16(2):122-130.
- Henkes M, Kuip VH, Aulitzky EW. Therapeutic options for chronic myeloid leukemia: focus on imatinib (Glivec®, Gleevec™). *Therapeutics and Clinical Risk Management* 2008; 4(1): 163-187.
- Rogers G, Hoyle M, Coon TJ, Moxham T, Liu Z, Pitt M, Stein K: Dasatinib and nilotinib for imatinib resistant or -intolerant chronic myeloid leukaemia: a systematic review and economic evaluation. *Health Technology Assessment* 2012; 16( 22):205-4025
- Patel D, Suthar PM, Patel V, Singh R: BCR-ABL kinase inhibitors for cancer therapy. *International Journal of Pharmaceutical Sciences and Drug Research* 2010; 2(2): 80-90.
- Rosti G, Palandri F, Castagnetti F, Breccia M, Levato L. Nilotinib for the frontline treatment of Ph+ chronic myeloid leukemia. *American Society of Hematology* 2013; 114(24):1493-1456.
- Maekawa T, Ashihara E, Kimura S: The Bcr-Abl tyrosine kinase inhibitor imatinib and promising new agents against Philadelphia chromosome-positive leukemias. *International Japan Society of Clinical Oncology* 2007; 12:327-340.
- Sudo M, Chin MT, Mori S, Doan BN, Said WJ, Akashi M, Koeffler PH: Inhibiting proliferation of gefitinib-resistant, non-small cell lung cancer. *Cancer Chemother Pharmacol* 2013; 71:1325-1334.
- Cataldo DV, Gibbons LD, Perez-Soler R, Quintas-Cardama A: Treatment of Non-Small-Cell Lung Cancer with Erlotinib or Gefitinib. *New England Journal of Medicine* 2011; 10:364-943.
- Sequist VL, Lynch JT: EGFR Tyrosine Kinase Inhibitors in Lung Cancer: An Evolving Story. *Annual Review Medicine* 2008; 59:429-442.
- Xu Y, Liu H, Chen J and Zhou Q: Acquired resistance of lung adenocarcinoma to EGFR-tyrosine kinase inhibitors gefitinib and erlotinib. *Cancer Biology & Therapy* 2010; 9(8): 572-582.
- Tiseo M, Bartolotti M, Gelsomino F, Bardi P: Emerging role of gefitinib in the treatment of non-small-cell lung cancer (NSCLC). *Medical Oncology* 2010; 4: 81-98.

**How to cite this article:**

Bedada AT and Gayesa RT: Tyrosine kinase as target for Cancer treatment. *Int J Pharm Sci Res* 2013; 5(1): 1-15. doi: 10.13040/IJPSR. 0975-8232.5(1).1-15

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)