

Genetic Markers and Evolution of Targeted Therapy in Cancer

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The advances in biotechnology including high throughput platforms, and bioinformatics has resulted in detailing molecular pathology of various cancers, identifying targets such as fusion genes, chimeric RNA, fusion proteins, amplified gene, genes with point mutation, overexpression or down regulation of RNA, microRNA (miRNA) and aberrant DNA methylation. The genetic markers provide diagnostic, prognostic and therapeutic markers, and may also provide predictive markers. Several targeted molecules have been identified as cell surface antigens and tyrosine kinases e. g. FLT3, NPM1, CEBPA and PRAM1 in acute myeloid leukemia (AML); BCR-ABL1 in chronic myeloid leukemia; JAK2 in chronic myeloproliferative disorders; ALK, EGFR, K-RAS and BRAF in lung cancer; BRAF, KIT in melanoma; HER2 in breast cancer. The driver molecules and their mechanism of actions revealed various oncogenic pathways in the development of effective inhibitor molecules/proteins as targeted therapy, and novel mutations in the genes associated with the inhibitor protein. Targeted cancer therapy aimed to antagonize the deregulated molecule/s, commonly comprises therapeutic monoclonal antibodies and small molecule inhibitors. *In vitro* studies and clinical trials of the inhibitory molecules showed promising results as single drug therapy or in combination with conventional chemotherapy. Further, multiple mutations associated with resistance to targeted therapy were identified, leading to treatment with second line drugs and consequent better prognosis. Further advancements of biotechnology with identification of genetic variation, multiple resistant mutations which help discovery of a cascade of genetic markers with deeper understanding of biology of disease that offers hopes towards identification of development of more efficient targeted therapy with reduced toxicity and resistance.

Genetic Markers and Evolution of Targeted Therapy in Cancer

Advances in genomic technologies have resulted in remarkable progress in molecular diagnosis of cancer with identification of various unique genetic markers of pathogenic significance as

targeted molecules. The targeted molecules comprise fusion genes, chimeric RNA, fusion/chimeric proteins, amplified genes, genes with point mutation, overexpressed/down regulated RNA and miRNA (Ali *et al.*, 2010; Pavlovi *et al.*, 2014; Shtivelman *et al.*, 1985,

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Sjogren *et al.*, 1998). The genomic alterations have led to precise WHO classification of hematological malignancies resulting in differential diagnosis and stratification of patients for appropriate treatment protocols. The routine methods used in cancer include FISH, PCR, ARMS-PCR, RFLP, Real Time PCR, capillary electrophoresis, Sanger sequencing/pyrosequencing, microarrays for whole genome/transcriptome/protein analysis, mRNA and methylotype analysis (Ku *et al.*, 2013; Sethi *et al.*, 2013; Staehler *et al.*, 2012).

Several target molecules in cancer are tyrosine kinases, as the tyrosine kinase signaling initiates molecular cascades leading to cell proliferation, differentiation, apoptosis, migration, invasion, and angiogenesis in the malignant tissues. Hence, identification and development of tyrosine kinase inhibitors as therapeutic agents has revolutionized cancer therapy (Sawyers, 2002). Epidermal growth factor receptor (EGFR) is the first receptor tyrosine kinase (RTK) played an important role in the identification of significance of tyrosine kinases in cancer (Carpenter *et al.*, 1978). The tyrosine kinases are primarily RTKs e. g. EGFRs (EGFR-1, EGFR-2, EGFR-3), platelet-derived

growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor (VEGF) receptor, and non-receptor tyrosine kinases (NRTK), e. g. SRC, ABL1, Janus kinase. The RTKs are activated by ligands, epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) by binding to the extracellular domain of the receptors (Fig. 1).

The identification of the pathogenic molecules led to development of inhibitors as targeted drugs, impacting pharmacogenomics and personalized medicine. Targeted therapy directly interacts with pathognomic molecule, as against the cytotoxic drugs that primarily kill mitotic cells by interfering with cell cycle.

Targeted cancer drugs are generally monoclonal antibodies and small molecule inhibitors. Therapeutic monoclonal antibodies target specific antigens on the cell surface, such as transmembrane receptors, or extracellular growth factors, CD20, CD33, and CD52, present on leukemic and lymphoproliferative cells. Molecules associated with the immune mechanisms led to monoclonal antibodies – Rituximab, against CD20 (Table 1) in non-Hodgkin

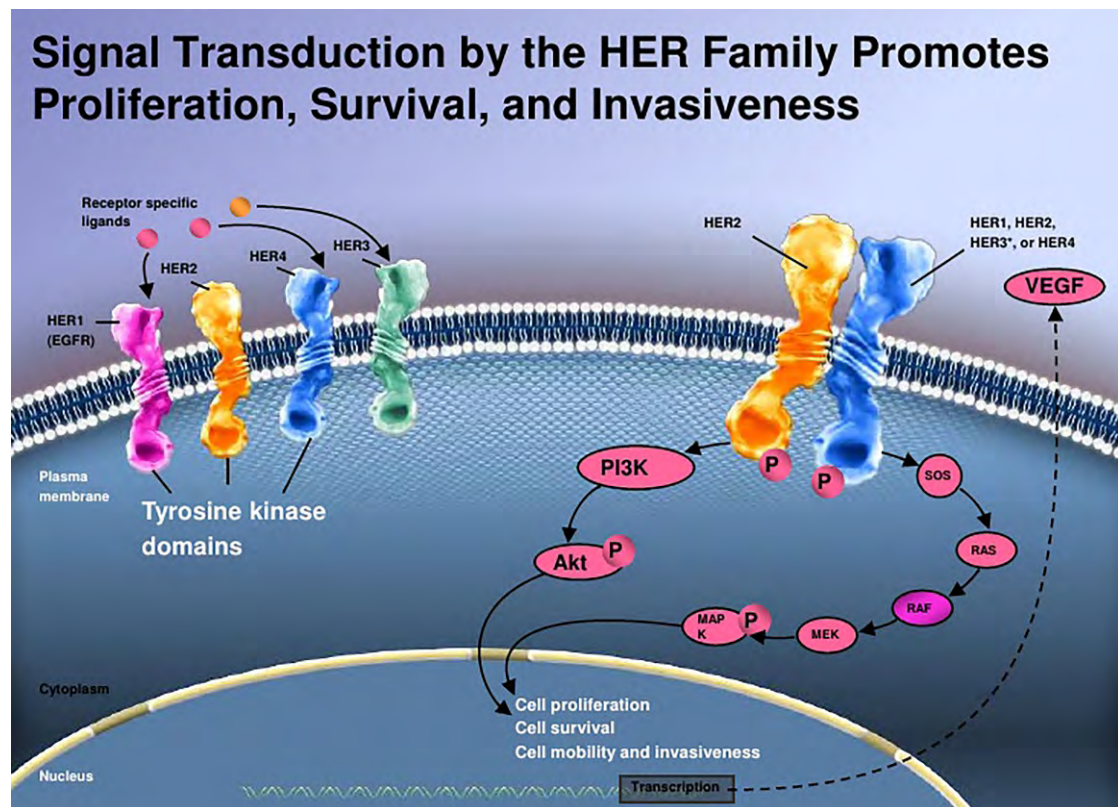


Figure 1: Activation of HER family receptors and signaling pathways (Adapted from: Hudis, 2007).

lymphoma (Silverman, 2007), and several monoclonal antibodies used in cancer treatment resulting in better prognosis (Fig. 1; Table 1). The monoclonal antibodies also target extracellular components of signaling pathways, including ligands and receptor binding domains blocking receptor signaling and downstream intracellular proteins involved in cellular proliferation, angiogenesis and invasion.

Small molecule inhibitors penetrate the cell membrane interacting with enzymatic activity of proteins, thereby blocking receptor signaling and interfering

with downstream intracellular molecules (Fig. 2). Several growth factor receptors with intrinsic tyrosine kinase activity are constitutively active in cancers and inhibition of the kinases using small molecule inhibitors sensitizes the tumor cells to apoptosis.

RTKs are preferred key targets for anti-cancer drugs as aberrant activation of the RTKs usually result in downstream signaling with activation of pivotal cytoplasmic serine/threonine kinases (STKs). Small molecule cancer inhibitors targeting extracellular RTKs and cytoplasmic STKs are extensively studied

Table 1. Molecular targets and targeted therapies in cancer (Abramson, 2015)

Agent	Target (s)	Malignancy
Ado-trastuzumab emtansine(Kadcyla)	HER2 (ERBB2/neu)	Breast cancer (HER2+)
Lapatinib (Tykerb)	HER2 (ERBB2/neu), EGFR(HER1/ERBB1)	Breast cancer (HER2+)
Trastuzumab (Herceptin)	HER2 (ERBB2/neu)	Breast cancer (HER2+), Gastric cancer (HER2+)
Afatinib (Gilotrif)	EGFR(HER1/ERBB1), HER2(ERBB2/neu)	Non-small cell lung cancer (NSCLC)(with EGFR exon 19 deletions or exon 21 substitution)
Ceritinib (Zykadia)	ALK	Non-small cell lung cancer
Crizotinib (Xalkori)	ALK, MET	Non-small cell lung cancer
Gefitinib (Iressa)	EGFR (HER1/ERBB1)	Non-small cell lung cancer with known prior benefit from Gefitinib
Ramucirumab (Cyramza)	VEGFR2	Gastric cancer, adenocarcinoma, Non-small cell lung cancer
Axitinib (Inlyta)	KIT, PDGFR β , VEGFR1/2/3	Renal cell carcinoma (RCC)
Bevacizumab (Avastin)	VEGF ligand	Cervical cancer, Colorectal cancer, Glioblastoma, NSCLC, Ovarian cancer, RCC
Pazopanib (Votrient)	VEGFR, PDGFR, KIT	Renal cell carcinoma
Temsirolimus (Torisel)	mTOR	Renal cell carcinoma
Panitumumab (Vectibix)	EGFR(HER1/ERBB1)	Colorectal cancer (KRAS wild type)
Cetuximab (Erbix)	EGFR (HER1/ERBB1)	Colorectal cancer (KRAS wild type), Squamous cell cancer of head and neck
Everolimus (Afinitor)	mTOR	Pancreatic neuroendo tumor, RCC, Breast cancer (HR+, HER2-)
Erlotinib (Tarceva)	EGFR (HER1/ERBB1)	NSCLC, Pancreatic cancer
Bortezomib (Velcade)	Proteasome	Multiple myeloma, Mantle cell lymphoma
Carfilzomib (Kyprolis)	Proteasome	Multiple myeloma
Brentuximab vedotin (Adcetris)	CD30	Hodgkin lymphoma, Anaplastic large cell lymphoma
Alemtuzumab (Campath)	CD52	B-cell Chronic lymphocytic leukemia (CLL)
Dabrafenib (Tafinlar)	BRAF	Melanoma (with BRAF V600 mutation)
Ibritumomab tiuxetan (Zevalin)	CD20	Non-Hodgkin's lymphoma
Idelalisib (Zydelig)	PI3K δ	CLL, Follicular B-cell NHL, Small lymphocytic lymphoma
Rituximab (Rituxan, Mabthera)	CD20	Non-Hodgkin's lymphoma, Chronic lymphocytic leukemia
Imatinib (Gleevec)	KIT, PDGFR, ABL	GI stromal tumor (KIT+), Hematologic malignancies including, Ph +ve ALL and CML
Nilotinib (Tasigna)	ABL1	CML
Ponatinib (Iclusig)	ABL1, FGFR1-3, FLT3, VEGFR2	CML, ALL- Ph positive
Ruxolitinib (Jakafi)	JAK1/2	Myelofibrosis

(Arora *et al.*, 2005)) Deregulated activation of RTKs results in increased cell growth and survival, and contributes to progression of cancer.

Targeted cancer drugs are designated as per the content of basic compound like monoclonal antibodies that end with "-mab", e.g., Rituximab, whereas small

molecules end with the stem "-ib" indicating protein inhibitory action of targeted drug. For example, the small molecule STI-571 known as Imatinib (generic name) in which tinib indicated tyrosine kinase inhibitor (TKI). Drug with stem "-zom-" indicates proteasome inhibitors, e.g., Bortezomib. Small

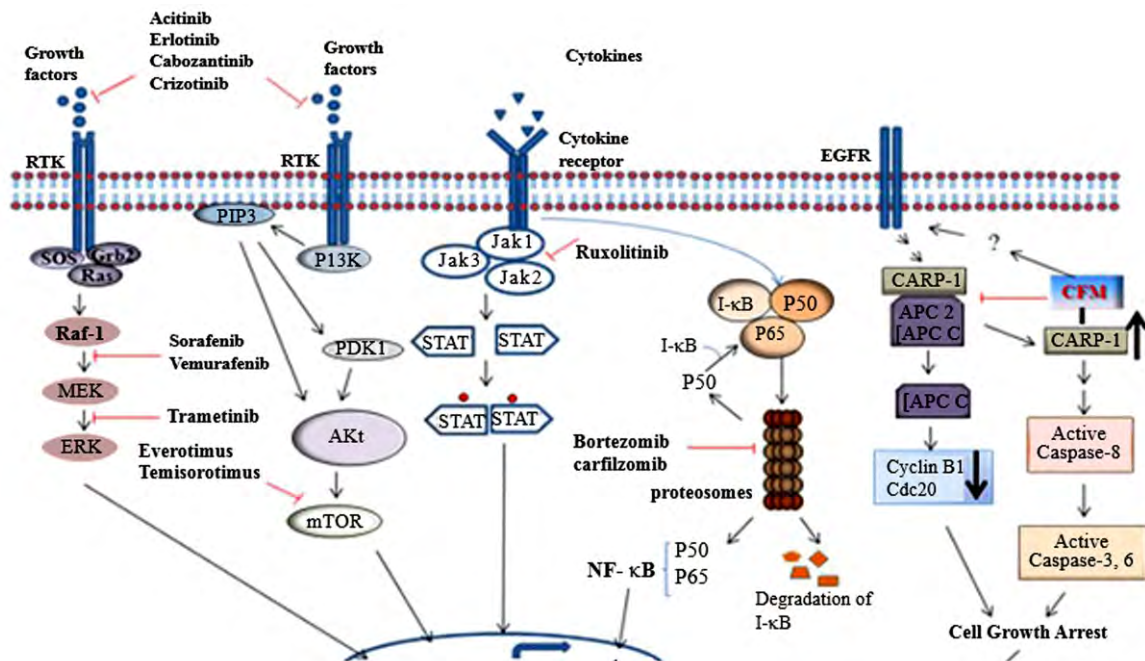


Figure 2: Schematic representation of activated cellular pathways in cancer and mechanism of small molecule inhibitors (Source: Lavanya *et al.*, 2014).

molecule inhibitors, tyrosine kinase inhibitors interrupt various intracellular signaling pathways of tyrosine kinases (Table 1).

Tyrosine Kinase Deregulation and Targeted Therapy in Hematolymphoid Malignancies

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder associated with reciprocal translocation between chromosomes 9 (*BCR*) and 22 (*ABL1*) juxtaposing *BCR* sequences to *c-ABL*. *c-ABL* is a tyrosine kinase located at chromosome 9q34, resulting in constitutive production of fusion chimeric protein p210 with increased tyrosine

kinase activity. The deregulated kinase activity usurps the physiologic functions of normal ABL enzyme by interacting with a variety of effector proteins, resulting in deregulated cellular proliferation, decreased adherence of leukemic cells to the bone marrow stroma and a reduced apoptotic stimuli (Deininger *et al.*, 2000).

In acute lymphoblastic leukemia (ALL), TEL-ABL protein is constitutively phosphorylated due to reciprocal translocation t(9;12) (Hannemann *et al.*, 1998). Chronic myelomonocytic leukemia (CMML) with t(5;12) produces TEL-PDGFRB fusion protein, leading to tyrosine kinase activation (Golub *et al.*, 1994). NPM1-ALK fusion product of

t(2;5) is constitutively activated in anaplastic large cell lymphoma (Shiota *et al.*, 1995).

Imatinib mesylate, a tyrosine kinase inhibitor in CML (Druker *et al.*, 2001), acts via competitive inhibition at the ATP-binding site of the BCR-ABL1 protein, resulting in inhibition of phosphorylation of the downstream cascade of proteins in signal transduction pathways. Imatinib mesylate prevents BCR-ABL enzyme from permanent deactivation, thus inhibiting proliferation of leukemic cells and leading to apoptosis (Table 1) (Deshmukh *et al.*, 2005; Druker *et al.*, 2001). Imatinib mesylate efficiently inhibits BCR-ABL kinase, blocks platelet-derived growth factor receptor, and c-kit tyrosine kinase (Druker *et al.*, 2000). However, about 90 kinase domain mutations have been identified in *ABL1*, which prevents binding of the drug and thus induce resistance to the drug. Consequently, second generation tyrosine kinase inhibitors, Nilotinib, Dasatinib (Jabbour *et al.*, 2014; Kantarjian *et al.*, 2010) and Bosutinib (Khoury *et al.*, 2012) were developed to overcome resistance to Imatinib mesylate due to kinase domain mutations. Second generation TKIs overcome resistance of Imatinib.

However T3151, “gatekeeper” mutation, displays resistance to all second generation TKIs. Ponatinib, a third generation TKI, has overcome resistance due to kinase mutation T3151 (Jabbour *et al.*, 2014; O'Hare *et al.*, 2009) (Table 1).

In acute promyelocytic leukemia (APL), fusion gene *PML-RARA* of t(15;17) leads to a differentiation block in the abnormal promyelocytes. The targeted drug all-trans-retinoic acid (ATRA) leads to conformational change of PML-RARA protein followed by activation and regulation of RARA-responsive genes leading to differentiation of promyelocytes to granulocytes (Advani *et al.*, 1999; Grignani *et al.*, 1998). The remission rates were significantly high in APL patients treated by ATRA. However, resistance to ATRA was observed in 25–30% of APL patients (Estey *et al.*, 2006), and arsenic trioxide (ATO) was found to be more efficient than ATRA as it induced apoptosis in addition to differentiation.

Besides, translocations, epigenetic silencing is an important genetic alteration leading abnormal expression of genes involved in cell cycle control and differentiation in AML. The replacement of cytosine by 5-aza-cytidine, a cytidine analogue, acts as a block to DNA methyl

transferases, causing demethylation of DNA and consequent differentiation (Egger *et al.*, 2004). Histone deacetylase (HDAC) inhibitors Vorinostat (Zolinza) and Panobinostat are additional agents for modulation of transcriptional repression of tumor suppressor proteins (Bolden *et al.*, 2006).

The two most prominent mechanisms in Myelodysplastic syndromes (MDS), DNA methylation and histone acetylation play a role in hematopoiesis. Methylation is focally increased around tumor suppressors and other mitogen inhibitors. DNA methyl transferases (DNMTs) play a role in increased methylation and hence a key target for treatment of MDS (Shih *et al.*, 2012). In high risk MDS, a number of genes associated with DNA repair, cell-cycle control, regulation of development, differentiation and apoptosis are hypermethylated in 70% of patients. The critical hypermethylated genes are *ALOX12*, *GSTM1*, *HIC1*, *FZD9*, *TET2* and *HS3ST2* (Jiang *et al.*, 2009). These hypermethylated genes are potential targets for demethylating agents. Patients with hypermethylated *TET2* showed better response rates (82%) on treatment with demethylating drug azacytidine than those with wild-type *TET2* (45%) (Itzykson *et*

al., 2011).

JAK2 mutation has been reported in myeloproliferative disorders Polycythemia Vera, Primary Myelofibrosis and Essential Thrombocythemia. *JAK2* encodes an on-receptor tyrosine kinase associated with signal relays for hemopoietic cell growth, development and differentiation (Neubauer *et al.*, 1998). Ruxolitinib, a JAK inhibitor showed promising results in patients with Myelofibrosis (Harrison *et al.*, 2012).

BRAF is a potent activator of MAP/ERK kinase pathway associated with regulation of cell cycle, differentiation and cell survival. *BRAF* mutations have been reported in solid cancers and hematopoietic cancers (Davies *et al.*, 2002; Holderfield *et al.*, 2014). The most common *BRAF* mutation is the V600E mutation (Holderfield *et al.*, 2014). Vemurafenib, a small molecule inhibitor showed anti-melanoma activity against the *BRAF* V600E mutant protein (Tsai *et al.*, 2008). Hematolymphoid malignancies including hairy cell leukemia and multiple myeloma with *BRAF* V600E mutation, showed favourable clinical response on treatment with Vemurafenib (Machnicki *et al.*, 2014) (Table 1).

Fms-like tyrosine kinase 3, CD135 (FLT3) a tyrosine kinase receptor is activated when bound by the FLT3 ligand (FL), subsequently promoting homodimerization. This switches tyrosine kinase activity of FLT3 followed by recruitment and phosphorylation of intracellular proteins SHC, GRB2, SHIP, CBL, CBLB-related protein domain, further leading to activation of MAP kinase, STAT and AKT/PI3 kinase signal transduction pathways. The proteins are transported to the nucleus regulating cellular proliferation, differentiation and apoptosis (Zhang *et al.*, 1999). *FLT3-ITD* (Internal tandem duplication) is a common mutation in 15–35% AML (Stirewalt *et al.*, 2006) and 5–10% MDS. *FLT3-ITD* and allelic variation in patients influences prognosis of AML patients (Meshinchi *et al.*, 2006). *FLT3-TKD* (Tyrosine kinase domain) mutation occurs in codon 835 (D835). Sorafenib, a tyrosine kinase inhibitor specifically targets the leukemic blasts in AML (Williams *et al.*, 2012) (Table 1).

Upregulation of JAK2 in AML cells results in resistance to *FLT3-TKI* inhibition (Ikezoe *et al.*, 2011). Second generation drug, Quizartinib (AC220) was potent in *FLT3-TKI* resistant cases due to

upregulation of JAK2 (Cortes *et al.*, 2011). Pacritinib (SB 1518) is another potent JAK2/FLT3 inhibitor, in combination with Pracinostat (SB939), an oral HDAC inhibitor, showed synergy in inducing remission and better survival in the patients (Novotny-Diermayr *et al.*, 2012).

Nucleophosmin (NPM1) mutations result in overexpression of the phosphoprotein in 27–35% of adult AML and 40–60% of adult AML with normal karyotype (Falini *et al.*, 2005). *NPM1* mutation occurs due to four base sequence TCTG duplication at position 956–959 in *NPM1* gene (Falini *et al.*, 2005). Inhibitors of *NPM1* oligomerization such as NSC348884 increase apoptosis when exposed to the ATRA plus cytarabine combination (Balusu *et al.*, 2011).

CCAAT/enhancer binding protein alpha (*CEBPA*) protein is a key regulator of granulocytic differentiation (Rosenbauer *et al.*, 2007). Hence, *CEBPA* mutations induce proliferation and block differentiation of myeloid lineage. *CEBPA* mutation occurs due to N-terminal frameshift mutations and secondly due to C-terminal in-frame insertions or deletions. *CEBPA* mutations frequently (70%) occur in AML patients exhibiting a normal karyotype. AML patients with a normal

karyotype and *CEBPA* mutation in the absence of *FLT3* show favorable prognosis (Green *et al.*, 2010).

C-KIT, a stem cell gene, encoding tyrosine kinase, demonstrated c-KIT mutations in AML patients with core binding factor rearrangement. Upon binding of the ligand stem cell factor, to c-kit, phosphorylation of several cytoplasmic proteins occur followed by activation of downstream MAP kinase, JAK/STAT, and PI-3 kinase pathways (Linnekin, 1999). Mutations in *c-KIT* receptor result in constitutive phosphorylation and activation of the receptor in absence of the ligand. Mutations in *c-KIT* and *FLT3* genes are associated with unfavorable prognosis in patients with t(8;21). In particular, patients with *c-KIT* mutation have been reported to have a higher incidence of relapse (80% versus 13.5%) (Pascka *et al.*, 2004). *In vitro* studies have shown sensitivity to Imatinib for a mutation in exon 8 and exon 17. APcK110, with potent proapoptotic and antiproliferative activities has shown promising results in AML cell lines and primary samples (Faderl *et al.*, 2011).

BCL2, an anti-apoptotic protein, is overexpressed in hematological malignancies and is a possible molecule

for targeted therapy. AML patients treated with Bcl-2 antisense oligonucleotide based therapy inhibit Bcl-2 overexpression, promote apoptosis and reduce drug resistance (Marcucci *et al.*, 2003).

Targeted Therapy in Solid Tumors

According to National Comprehensive Cancer Network (NCCN) guidelines, several molecular markers have been identified as targets for therapy in solid tumors. The molecular markers include *HER2 (ERBB2)* amplification in breast cancer, *K-RAS* and *BRAF* mutations in colorectal cancer, and *BRAF* v600 mutation in melanoma, *EGFR* mutation/*ALK*/rearrangement in non-small-cell-lung-cancer (NSCLC), and *c-KIT* in gastrointestinal stromal cancer. The following section discusses the markers in specific cancers.

Molecular Markers and Targeted Therapy in Lung Cancer

Lung cancer is the most common cancer in men globally with about 15% five year survival rates. Based upon various driver mutations, NSCLC is stratified based on the molecular lesions as NSCLC with *K-RAS* mutation, *EGFR* mutation, echinoderm microtubule-associated

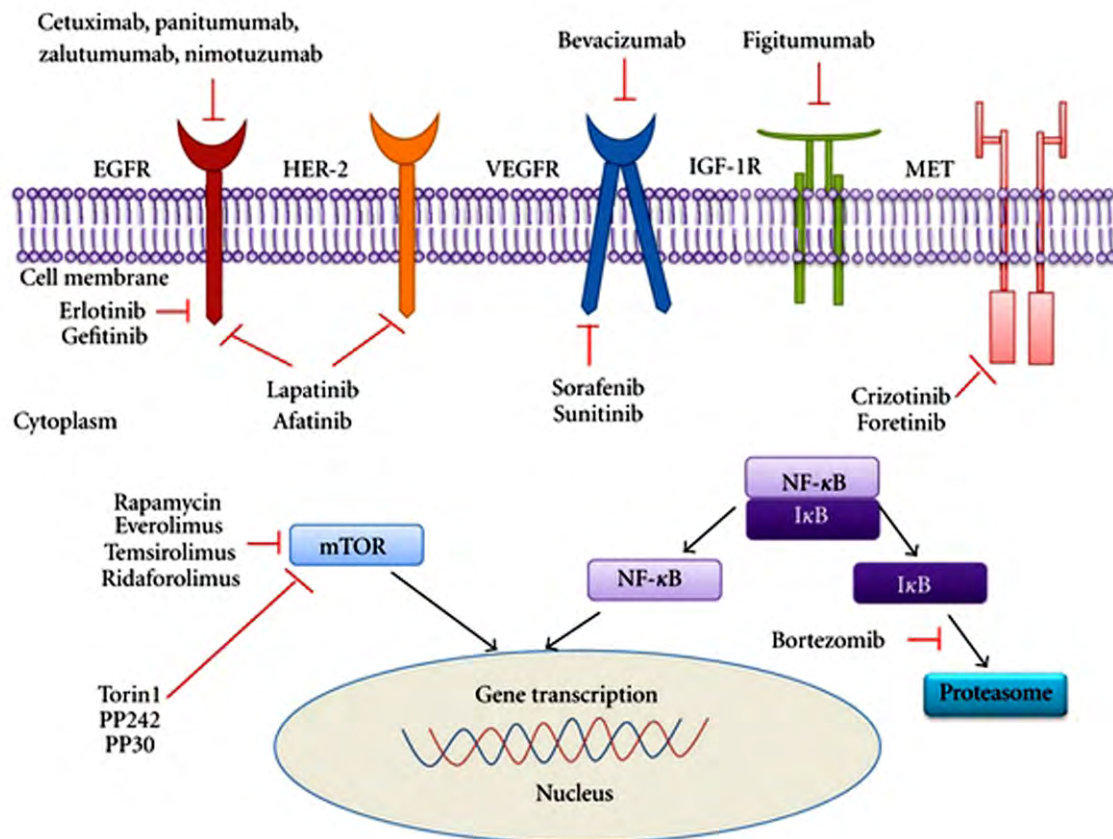


Figure 3: Mechanism of targeted therapies in patients with HER2 and EGFR, VEGFR and IGF-1R mutation (Source: Gao *et al.*, 2012).

protein like 4-anaplastic lymphoma kinase (*EML4-ALK*) mutation, *herceptin 2* (*HER2*) mutation, *v-raf* murine sarcoma (*BRAF*) mutation, mesenchymal epithelial transcription factor (*Met*) mutation, protein kinase B (*PKB/AKT1*), *phosphatidylinositide 3 kinase catalytic subunit* (*PI3KCA*) mutation (Pao *et al.*, 2011).

EGFR plays a critical role in cell proliferation, angiogenesis, and inhibition of apoptosis. *EGFR* mutation is reported in 10% of NSCLC in US, and 35% in Asian population (Pao *et al.*, 2011). The *EGFR*

mutation is observed in less than 5% squamous cell cancer patients and 15–20% adenocarcinomas including females (never smokers) (Pao *et al.*, 2010). *EGFR* mutations are located in the kinase domain at exons 18–21 (Kosaka *et al.*, 2009). *EGFR* amplification has also been reported in NSCLC patients and associated with bad prognosis. Patients stratified as NSCLC with *EGFR* mutation are effectively treated with targeted therapy Erlotinib or Gefitinib targeted to the deregulated *EGFR* (Lazarus *et al.*, 2013) (Figs. 3 and 4) (Table 1). *EGFR* TKI,

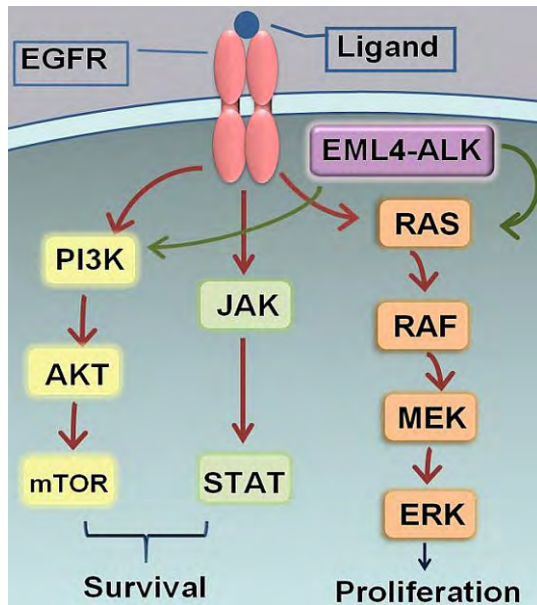


Figure 4: EGFR mutation, EML4-ALK translocation and Signaling (Source: Wu *et al.*, 2012).

a small molecule inhibitor, therapy also shows better response to patients with *EGFR* amplification as compared with *EGFR* mutation.

An additional molecular lesion in lung adenocarcinomas is the point mutation in *K-RAS* gene, codon 12 or 13 (Knickelbein and Zang, 2015). *EGFR* mutation activates *RAS* signaling pathway downstream, hence patients with *K-RAS* mutation are resistant to *EGFR* TKI (Raponi, 2008).

ALK encodes a tyrosine kinase receptor normally expressed in selected neuronal cell types. *ALK-EML4* rearrangement/translocation and balanced translocations retain *ALK* kinase domain with constitutive activation of tyrosine kinase, leading to transformation of cells

(Soda, *et al.*, 2007) (Fig. 4). In Lung cancer, *ALK* rearrangement is detected by FISH with an *ALK* break-apart probe (Soda *et al.*, 2007). Lung cancer patients with *EML4-ALK* translocation show sensitivity to TKI inhibitor Crizotinib (Shaw *et al.*, 2011). However, resistance to the targeted therapy has been reported in patients with secondary mutations in *ALK* (Ettinger *et al.*, 2012; Sasaki *et al.*, 2011).

FGFR1, Fibroblast growth factor receptor 1 encodes a member of the *FGFR* tyrosine kinase family, with a critical role in cell development. *FGFR1* is deregulated either by point mutation, translocation or amplification (Turner *et al.*, 2011). Preclinical trials with *FGFR1* inhibitors have shown encouraging results in lung cancer (Weiss *et al.*, 2010). *FGFR1* amplifications are also observed in 20% in smokers with squamous cell sarcoma.

***K-RAS* Mutations and Targeted Therapy in Colorectal Cancer**

KRAS is a membrane bound GTPase, active in the GTP-bound form and inactive when GDP-bound. *KRAS* activity mediates a cascade of intracellular signaling events initiated by the ligand-receptor binding of RTKs, including *EGFR* (Downward *et al.*, 2003). *EGFR*

upon binding to its ligand is auto-phosphorylated creating a docking site for the adaptor protein growth factor receptor bound protein 2 (GRB2), resulting in activation of KRAS GTP, which further stimulates downstream signaling pathways, RAF/MEK and PI3K (and phosphoinositide-3 kinase)/AKT controlling cell growth and survival (Downward *et al.*, 2003) (Fig. 4). *K-RAS* mutations resulting in constitutive activation of RAS with expression of RAS proteins are reported in 20–25% of several human tumors including pancreatic cancer with *K-RAS* mutation in 90% (Downward *et al.*, 2003). The potent transforming mutations are detected in codons 12 (82% of *K-RAS* mutations) and 13 (17%) in exon 2 of the *K-RAS* gene (Wang *et al.*, 2010). *K-RAS* gene mutations predict outcome of treatment with anti-EGFR antibodies in advanced colorectal cancer (CRC).

Cetuximab, a human–mouse chimeric IgG1 monoclonal antibody, EGFR-targeted agent approved for the treatment of colorectal cancer (Jonker *et al.*, 2007) (Fig. 3), and Panitumumab are commonly used in CRC therapy (Heinemann *et al.*, 2013). Bevacizumab (Avastin), Ramucirumab (Cyramza), and Ziv-aflibercept (Zaltrap) are drugs used for

colon cancer that target VEGF (Douillard *et al.*, 2014). These drugs are combined with chemotherapy to treat advanced colon cancer (Table 1). Farnesyl transferase inhibitors (FTIs) are small molecule inhibitors that selectively inhibit farnesylation of a number of intracellular substrate proteins such as RAS, an additional approach to target *K-RAS* mutations (Gysin *et al.*, 2013). However, a comprehensive understanding of RAS mediated signal transduction feedback loops, tumor heterogeneity and mechanisms of downstream targets of *K-RAS* gene on CRC is needed for optimal use of the monoclonal antibodies, small molecular inhibitors to *K-RAS* aberrations.

***HER2* Marker and Targeted Therapy in Breast Cancer**

HER2 amplification has been observed in 20% invasive breast carcinomas, and is a poor prognostic marker with an increased risk of disease progression, recurrence of disease with poor survival (Andrulis *et al.*, 1998). FISH is an efficient tool for detection of *HER2* amplification. *HER2* encodes a transmembrane tyrosine kinase receptor in the EGFR family. *HER2* stimulates growth factor signaling

pathways such as PI3K–AKT–mTOR pathway (Fig. 1). Trastuzumab (Herceptin), a humanized, recombinant monoclonal antibody that binds to the extracellular domain of HER2 is an efficient targeted therapy (Vogel *et al.*, 2002) (Fig. 3). Trastuzumab selectively blocks ligand independent HER2–HER3 dimerization and proteolytic cleavage of the extracellular domain of HER2 resulting in downregulation of PI3K pathway signaling and downstream cell cycle protein cyclin D1 (Junttila *et al.*, 2009). Herceptin resistance is seen in several breast cancer patients with mutational activation of P13K pathway through loss of *PTEN*, indicating PI3K-based treatment options. Lapatinib, an ATP-competitive inhibitor of HER2 and EGFR tyrosine kinases, have shown efficacy in Trastuzumab resistant patients (Konecny *et al.*, 2006). Pertuzumab monoclonal antibody binding to a distinct epitope on the extracellular domain of HER2 blocks ligand induced dimerization of HER2 and HER3 (Junttila *et al.*, 2009) (Table 1).

BRAF V600E mutation occurs in 60% melanoma patients. The mutation constitutively activates mitogen activated protein kinase (MAPK) pathway,

promoting cell proliferation and preventing apoptosis (Gray-Schopfer *et al.*, 2007). Hence, *BRAF* V600E mutation is considered as a promising therapeutic target in metastatic melanoma. Vemurafenib treatment in patients with *BRAF* V600mutant metastatic melanoma indicated that inhibition of MAPK pathway promoted cell proliferation and prevented apoptosis (Flaherty *et al.*, 2010). Vemurafenib induces clinical responses in 50% patients with *BRAF* V600 mutant metastatic melanoma. Vemurafenib and Dabrafenib are effective targeted drugs for melanomas with *BRAF* V600Emutation (Kim *et al.*, 2014) (Table 1).

CONCLUSION

A continuous research efforts by various genomic technologies made remarkable progress in the discovery of genetic markers which have diagnostic as well as prognostic significance in hemato-lymphoid malignancies and solid tumors as well. Driver mutations and their mechanism of actions disclosed role of various oncogenic pathways that contributed significantly in the development of effective inhibitor molecules/proteins as targeted therapy.

Clinical trials of the inhibitor molecules have shown promising results in comparison with traditional cytotoxic chemotherapy. Further advancement in genomics is expected to identify cascade of genetic markers help understanding biology of disease that offers hopes towards development of more efficient targeted therapy with reduced toxicity and resistance.

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