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The role of ERBB3 inhibitors as cancers therapeutics

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THE ROLE OF ERBB3 INHIBITORS AS CANCER THERAPEUTICS

By

ANKUSH CHANDRA

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Approved By

First Reader_____

Gwynneth D. Offner, Ph.D.
Director, M.A. Medical Sciences Program
Associate Professor of Medicine

Second Reader _____

Johanna Lahdenranta, Ph.D.
Associate Investigator
Merrimack Pharmaceuticals
Cambridge, MA

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THE ROLE OF ERBB3 INHIBITORS AS CANCER THERAPEUTICS

ANKUSH CHANDRA

ABSTRACT

Cancer is the most fatal disease after cardiovascular disease with over 8.2 million deaths worldwide each year. Ever since the serendipitous discovery of mustard gas as an anti-cancer therapeutic in the 1940s, serious efforts have been put into discovering more chemotherapies. Chemotherapies can be categorized into different groups such as alkylating agents (cisplatin, cyclophosphamide), antimetabolites (5-fluorouracil, Ara-C) and mitotic inhibitors (taxanes, vinca alkoids) among others. While chemotherapies have proven to kill cancer cells by targeting cell division processes, over time, tumor cells can adapt and become resistant to these drugs. With a growing understanding of cell signaling networks, targeted therapies are being developed to overcome the issue of chemotherapy resistance. Targeted therapies are highly specific molecules that bind to a specific cellular protein or molecule and block signaling networks associated with biological processes. One of the most frequently dysregulated receptor systems in cancers is the receptor tyrosine kinase family with ErbB being one of the most studied receptors families.

ErbB or HER receptors consists of four structurally related receptor tyrosine kinases namely, EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. The ErbB family of receptors plays a major role in morphogenesis of the human body as well as various cellular responses such as cell growth, differentiation and proliferation.

Overexpression and dysregulation of these receptors, particularly EGFR and HER2, have been linked to a number of cancers such as breast cancer, gastric cancer, ovarian cancer and non-small cell lung cancer, to name a few. One of the most successful therapies against ErbB related cancers have been targeted therapies. Targeted therapies for ErbB related cancers are of two kinds: (i) Small molecule tyrosine kinase inhibitors (such as erlotinib and gefitinib against EGFR) and, (ii) Monoclonal antibodies (such as trastuzumab against HER2 and cetuximab against EGFR). These drugs function either by inhibiting the kinase activity of the receptor and preventing phosophorylation of tyrosine residues, or binding to some other site on the extracellular domain of the receptor and preventing ligand binding and heterodimerization of ErbB monomers. These drugs have proven to have limited efficacy as monotherapy, but are more effective in combination with standard chemotherapies. However, tumor cells can adapt their signaling networks developing resistance to targeted therapies over the course of treatment and lead to cancer progression.

While overexpression and dysfunction of EGFR and HER2 are implicated in most ErbB driven cancers, recent studies have found HER3 playing a pivotal role in inducing resistance to EGFR and HER2 targeted therapies in various cancers and has been found to be the most sensitive node in driving the PI3K pathway leading to tumorigenesis. Thus, there is an urgent need to develop drugs targeted against HER3 and bring them into the clinic. Since HER3 lacks kinase activity, only monoclonal antibodies can be developed against it. Currently, there are a number of molecules in clinical development that target HER3. For example, patritumab and MM-121 are humanized monoclonal

antibodies that target the extracellular domain of HER3 receptor and leads to inhibition of HER3-PI3K signaling followed by rapid internalization of the receptor. MM-111 and MM-141, two different bispecific monoclonal antibodies that bind to HER2, HER3 and IGFR-1, HER3, respectively, are currently in clinical development.

HER3 inhibitors provide hope to effectively overcome HER3 induced tumor resistance and successfully treat several ErbB driven cancers. However, further development of HER3 inhibitors is necessary by taking strategic approaches. One of these approaches it the utilization of systems biology, a branch of biology that involves computational and mathematical modeling of complex biological systems with the aim of discovering emergent properties of biological systems. Systems biology enables researchers to get a deeper understanding of biological networks such as that of ErbB and make predictive models and test outcomes. This approach was used by Merrimack Pharmaceuticals to develop novel monoclonal antibodies against HER3. Computational outcomes were successfully validated by *in vitro* and *in vivo* experiments. Thus, this suggests that systems biology might be the future of designing and developing HER3 inhibitors that would successfully overcome HER3 resistance and cancer progression.

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ABBREVIATIONS

5-FU	5-Fluorouracil
CDC	Center for Disease Control
EGF-R	Epidermal Growth Factor Receptor
FGFR	Fibroblast Growth factor receptor
FOLFIRI	Irinotecan, 5-FU and lecovorin
HBOC	Hereditary breast and ovarian cancer
HPV	Human Papilloma Virus
HRG	Heregulin
JM	Juxtamembrane Region
mAb	Monocloncal Antibody
MAPK	Mitogen-activated protein kinase
NSCLC	Non-small cell lung cancer
pAkt	phosphoAkt
PDGF	Platelet-derived growth factor
PDK	3-phosphoinositide dependent kinase
PFS	Progression free survival
PI3K	Phosphatidylinositol 3-kinase
PIP2	phosphophatidylinositol 4,5-bisphosphate
PIP3	phosphophatidylinositol 3,4,5-triphosphate
PTB	Phosphotyrosine Binding Domain
RTK	Receptor Tyrosine Kinase

SH2	Src homology-2
SoC	Standard of Care
TKI	Tyrosine Kinase Inhibitor
ToGA	Trastuzumab for gastric cancer
VEGFR	Vascular Endothelial growth factor receptors
vIII	Variant III
WHO	World Health Organization

INTRODUCTION

Cancer, as defined by the World Health Organization (WHO), is a term used for a large number of diseases characteristic of abnormal growth beyond a cell's usual cell division capability. These cells can metastasize or invade other parts of the body, even far away from the primary source and can cause death[1].

Based on a report by the WHO and CDC, cancer caused 8.2 million deaths in 2012 worldwide, making it the second most fatal disease in the US (Figure 1) and worldwide falling just behind cardiovascular diseases[2].

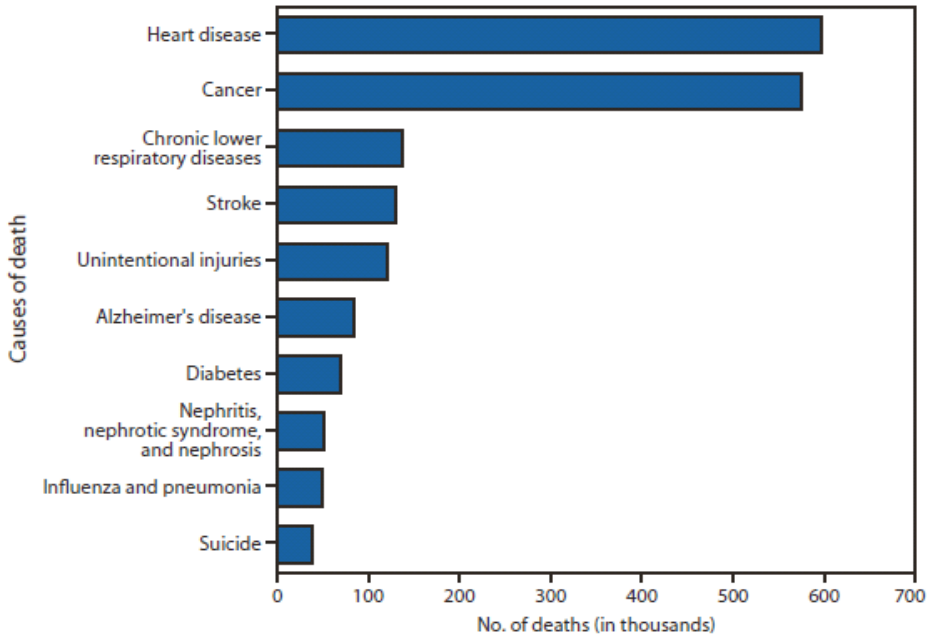

Figure 1: Leading causes of death in 2010
Cancer caused the second most deaths in the U.S with 574,743 fatalities.
Figure taken from CDC Morbidity and Mortality Weekly Report 2010(3).

Causes of this deadly disease can be attributed to environmental and genetic factors.

Environmental factors can be split into 3 categories: (i) Chemical carcinogens (ii) Physical carcinogens, and (iii) Infectious agents. Chemical carcinogens often include agents that damage DNA via electrophilic attack on the tissue nucleophile such as coal tar and tobacco.

Physical carcinogens generally include UV radiation, X-rays and other ionizing radiations[4]. These carcinogens cause physical damage to the DNA and interferes with the DNA replication process. This can lead to mutations potentially leading to carcinogenesis [4].

Last of the environmental factors are infectious agents that include viruses such as Human papilloma virus (HPV) that is associated with cervical cancer; bacteria such as *Helicobacter pylori* associated with stomach cancer and other microorganisms[5].

On the other hand, genetic factors that cause cancer involve two types of mutations (i) inherited, and (ii) somatic mutations. Inherited mutations are those mutations that are present in the genetic material of the egg or sperm that lead to the formation of the child and is passed on from one generation to another[6]. This is often also known as Family Cancer Syndrome such as in the case of hereditary breast and ovarian cancer (HBOC) due to mutations in *BRCA1* and *BRCA2* genes [4,6].

Somatic mutations are those mutations that are acquired over the course of a lifespan. Thus, the mutated cell passes on the mutations only to its daughter cells [5].

Whether mutations are inherited or somatic, it is well understood that cancer is caused as a result of alteration in genes that are responsible for cell growth regulation, differentiation and DNA repair[8]. Such genes are often classified into two groups, (i) tumor

suppressor genes: genes that are responsible for limiting cell division and survival, and (ii) oncogenes: genes that are responsible for normal cell growth and differentiation.

Different type of mutations in either of these genes can lead to malignant transformations such as an inactivation or under expression of tumor suppressor genes, or overexpression or activation mutation of oncogenes[7]. These mutations give a growth or pro-survival advantage to the cells and could lead to malignancies[8].

Oncogenes can also be classified into several categories such as transcription factors, chromatin remodelers, signal transducers, apoptosis regulators, growth factors, and growth factor receptors[8]. Of the oncogenes listed above, the last two oncogenes, growth factors and growth factor receptors, are of special interest because of their close connection with one another and their role in cell growth and tumorigenesis.

GROWTH FACTORS AND GROWTH FACTOR RECEPTORS

Growth factors, typically proteins or steroids, are molecules produced by cells to modulate growth, proliferation and differentiation on neighboring cells (paracrine) or on the cell itself (autocrine). They may positively or negatively modulate the cell growth and proliferation and play a pivotal role in overall development of the body[17]. Growth factors are ligands for growth factor receptors, which on binding lead to downstream activation of intracellular signaling cascades further leading to activation or inactivation of certain genes which modulate growth and differentiation [17]. Constitutive activation of growth factor genes that are positive modulators of cell differentiation and proliferation can lead to malignancies as seen in the case of platelet derived growth factor (PDGF) [6]. PDGF, secreted by platelets

during coagulations, stimulates proliferation of various cell types and fibroblasts during wound healing[6]. In vitro studies have indicated that excess PDGF can lead to fibroblast transformation leading to uncontrolled cell growth and may cause malignant transformations in humans as well. Furthermore, an antibody against PDGF, its receptor or small molecule antagonist blocking the receptor prevents the fibroblast transformation confirming the hypothesis in *in vitro* models.

The primary drivers of growth factors are growth factor receptors and these receptors are altered in several types of cancers. Certain families of these receptors are related to tumor cell resistance, which is a major topic of therapeutic concern today.

Growth factor receptors are typically receptor tyrosine kinases (RTKs), which are a large family of membrane spanning receptors with intrinsic tyrosine kinase activity that share a similar structure: an extracellular ligand binding domain, a cytoplasmic single transmembrane domain, and an intracellular tyrosine kinase domain[18]. The ligand-binding domain is connected to the cytoplasmic domain by a single transmembrane helix as shown in Figure 2 [19, 20].

To date, there are about 20 different families of RTKs identified which include Epidermal Growth Factor receptors (EGFR/ErbB/HER), fibroblast growth factor receptors (FGFR) and vascular endothelial growth factor receptors (VEGFR) as seen in Figure 2. While the RTKs are essential for growth and proliferation of cells, mutations and deregulation of these receptors are implications seen in different cancers.

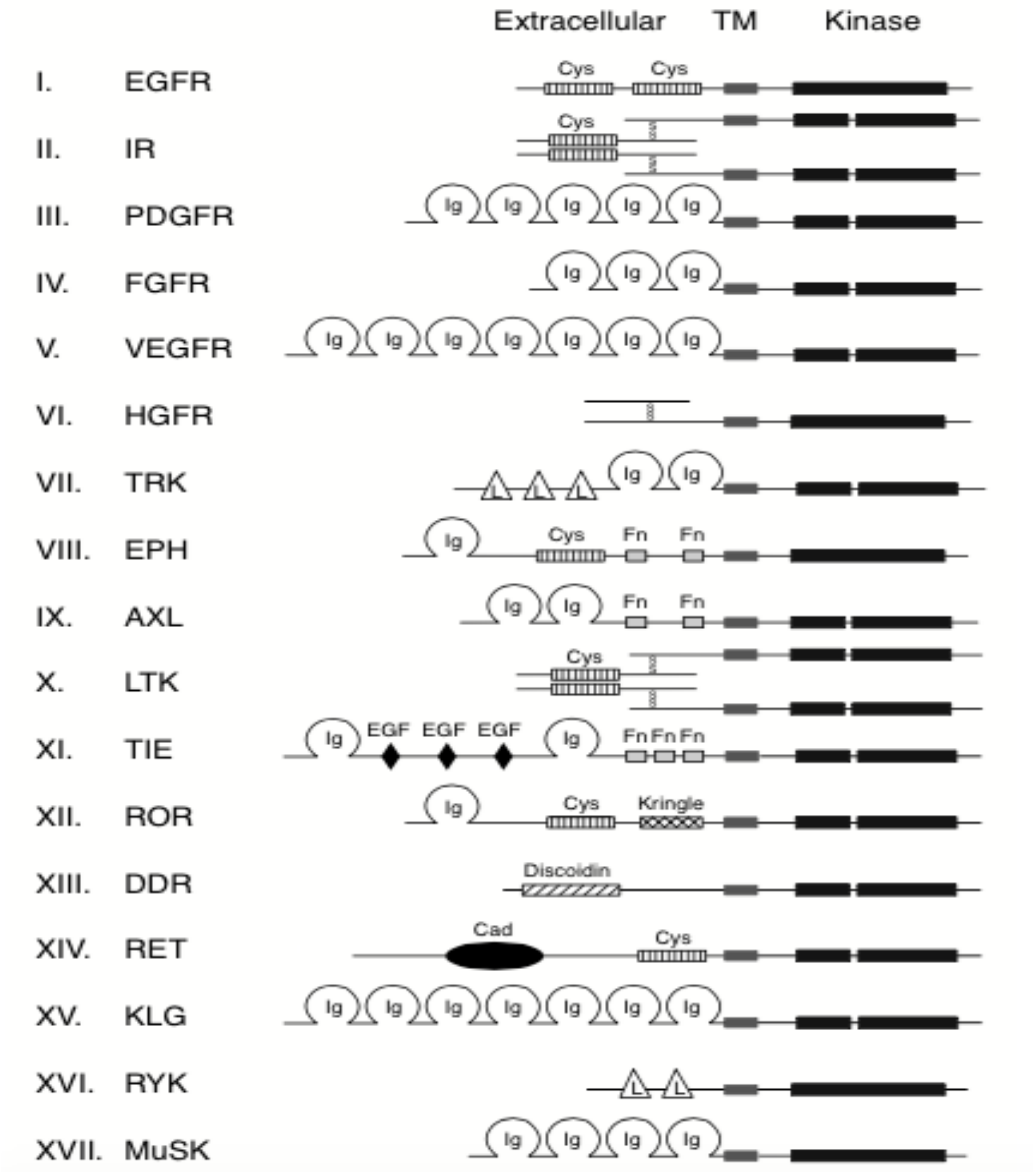

Figure 2: Family of RTK along with the 2 different domains.

There are about 20 families of RTKs discovered till date. Some of them are represented in the figure above. Legend EGFR, epidermal growth factor receptor; IR, insulin receptor; PDGFR, platelet-derived growth factor receptor; FGFR, fibroblast growth factor receptor; VEGFR, vascular endothelial growth factor receptor; HGFR, hepatocyte growth factor receptor; TRK, tropomyosin receptor kinase; AXL, anexelekto (Greek word for uncontrolled); LTK, leukocyte tyrosine kinase; TIE, tyrosine kinase with Ig and EGF homology domains; DDR, discoidin domain receptor; RET, rearranged during transformation; KLG, kinase-like gene; RYK, related to tyrosine kinase; MuSK, muscle-specific receptor tyrosine kinase. Other abbreviations: Cad, cadherin repeat; Cys, cysteine-rich region; Discoidin, discoidin or factor VIII domain; EGF, EGF-like repeat; Extracellular, extracellular domain; Fn, fibronectin III repeat; Ig, immunoglobulin-like domain; Kinase, tyrosine kinase domain; Kringle, kringle domain; L, leucine-rich motif; SS, disulfide bond; TM, transmembrane domain. Figure taken from Robertson, et al. 2000(23).

ACTIVATION OF RTKs AND DOWNSTREAM SIGNAL TRANSDUCTION

With the exception of the insulin receptor family, all other known RTKs exist as monomers in the cell membrane. Ligand binding leads to the induction of dimerization of the receptors that further results in autophosphorylation of *trans* tyrosine residues in the cytoplasmic domain [21-23]. This is the activated state of the RTK. The phosphorylation of the tyrosine residues leads to a conformational change in the receptor and stabilizes the active state of the receptor [25].

The phosphotyrosine residues of the receptor become the binding sites for other downstream signaling proteins such as adaptor proteins and enzymes, usually, through the Src homology-2 (SH2) and/or phosphotyrosine binding domains (PTB) as seen in Figure 3 below. RTK signaling pathways, as seen in figure 4 will be discussed later in the context of ErbB receptors.

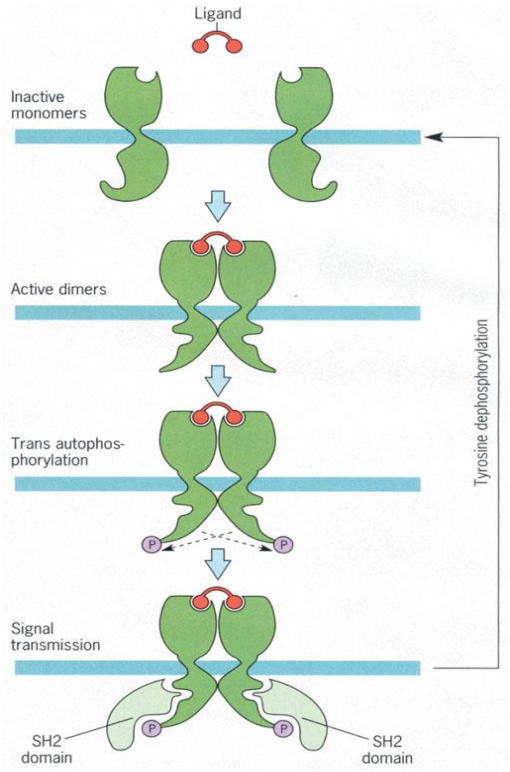

Figure 3: Mechanism of RTK activation
Ligand binding on RTKs leads to dimerization of receptors and trans auto-phosphorylation of tyrosine leading to activation of RTKs. Figure taken from http://yxsj.baiduyy.com/whole/image/chapter15/15.22.jpg

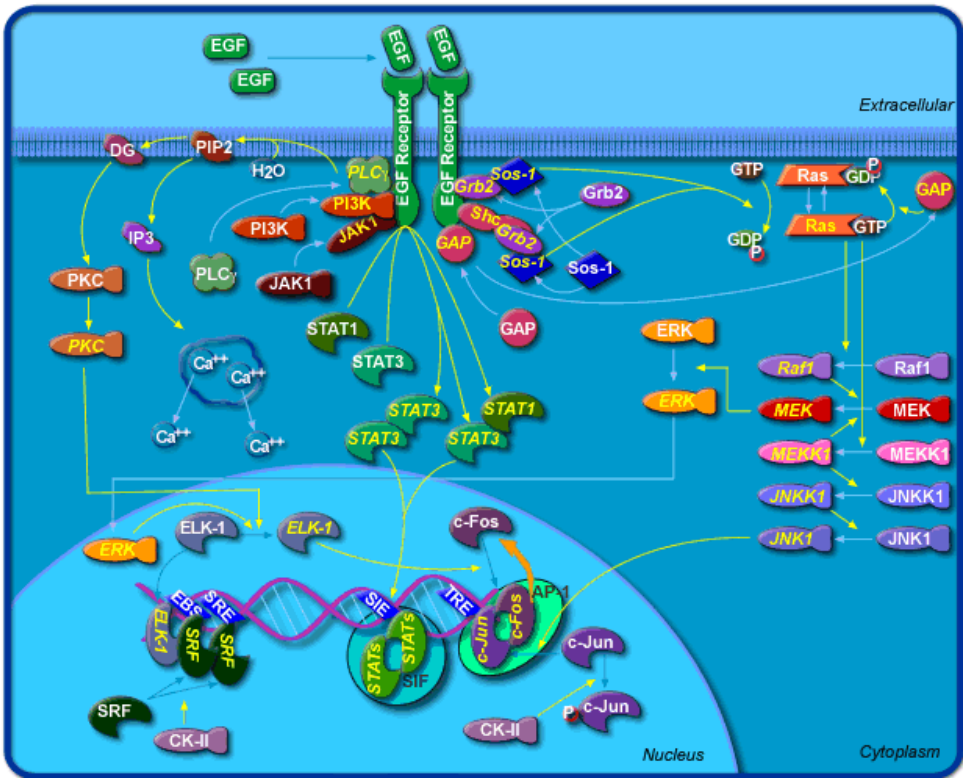

Figure 4: RTK activation in the context of EGFR and downstream pathways
Ligand binding to EGFR leads to dimerization, trans phosphorylation of tyrosine residues and activation of different downstream signaling pathways. Figure taken from http://www.genebiomarkers.com/gbm_images/content/egf_pathway.jpg

RTKs AND CANCER

RTKs as oncogenes are known to play a role in cancer and tumorigenesis as a result of their aberrant signaling[33]. Figure 3 and 4 shows mechanism of activation of RTK signaling pathway and signaling pathways associated with RTK activation.

Constitutive activation of RTK is important for malignant transformations and can occur due to a number of mechanisms such as mutations causing constitutive kinase activation, overexpression of ligand or RTK receptor and, mutations in the regular signal transduction pathways [34]. However, in most cases, gene amplification, overexpression and mutations are responsible for transformation of RTKs.[28]

Several families of RTKs are also associated with cancers. Table 1 summarizes the RTK family and the cancer associated with it[28].

Table 1: Example of some RTKs associated with different cancers

RTK family	Type of Cancers
Platelet derived growth factor receptors (PDGFR)	Glioblastoma, astrocytic brain tumors, gastrointestinal tumors
Vascular Epithelium Growth Factor recepto (VEGFR)	Non-small cell lung cancer, colorectal cancer, breast cancer
Epidermal Growth Factor Receptors (EGFR/HER)	Non-small cell lung cancer, cervical cancer, bladder cancer, ovarian cancer, kidney cancer, pancreatic cancer, squamous cell carcinomas of the head and neck,
Hepatocyte Growth Factor Receptor (HGFR/c-MET)	Colorectal carcinomas, thyroid carcinomas, breast cancer, childhood hepatocellular carcinoma, non-small lung cancer

Epidermal growth factor receptors (EGFR) or ErbB/HER family of receptors are essential for many normal morphogenic processes and are involved in various cellular

responses such as cell growth, differentiation and proliferation. Aberrant signaling and mutations in this family of receptors can lead to development of tumors in the body, primarily, epithelial based cancers[29]. An interesting finding of ErbB driven cancers is resistance to traditional chemotherapies. Over the last two decades several drugs have been developed to overcome cancer resistance induced by ErbB family of receptors. While these drugs have proven to be effective, in the long run the tumor develops resistance and thus proving anti-cancer drugs to be ineffective in the long run. This poses a great challenge in the clinic and thus there is a dire need to understand mechanisms of resistance more closely and develop drugs targeting the cancer cells' resistance to anti-cancer therapeutics.

EGFR/ERBB FAMILY OF RECEPTORS

The ErbB family of RTKs lies at the head of a complex signal transduction cascade that modulates cell proliferation, survival, adhesion and differentiation[29]. The ErbB family consists of four members: EGFR/ErbB1, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4. These transmembrane receptors all share two extracellular cysteine-rich domains and an intracellular portion with a long C-terminal tail carrying most of the autophosphorylation sites (Figure 5). It is interesting to note is that all but HER3 have intrinsic kinase activity in the cytoplasmic domain of the receptor and that HER2 has no ligands[30]. These characteristics have implications in cancer as discussed later.

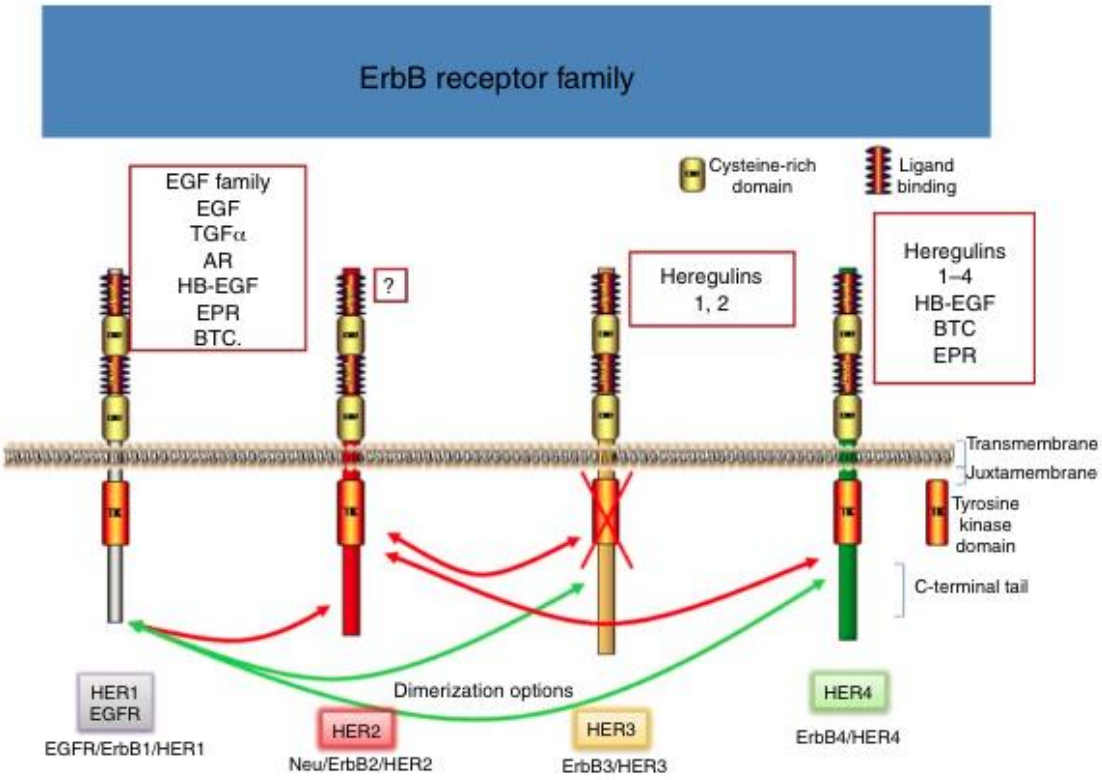

Figure 5: ErbB family of receptors and their ligands
Some of the possible heterodimers are shown above in the figure. No ligand has been identified for HER2. Also, HER3 does not have intrinsic kinase activity. Figure taken from http://www.slideshare.net/vj2510/tyrosine-kinase-inhibitors

ErbB receptors activate by forming homo and heterodimeric receptor complexes on binding of one of the 11 ligands for the family on the extracellular ligand-binding domain (Figure 5)[29]. Ligand binding leads to *trans* autophosphorylation of tyrosine residues in the cytoplasmic domain, which leads to conformational changes in the receptor structure and thus stabilize the active state of the kinase. The phosphorylated tyrosines become docking sites for signaling complexes such as enzymes and adaptor proteins. Dissociation of these complexes releases activated effector and adaptor proteins into the cytoplasm where they stimulate different signal transduction cascades such as the mitogen-activated protein kinase (MAPK), Akt and phophoinisitol kinase pathways (Figure 6)[36].

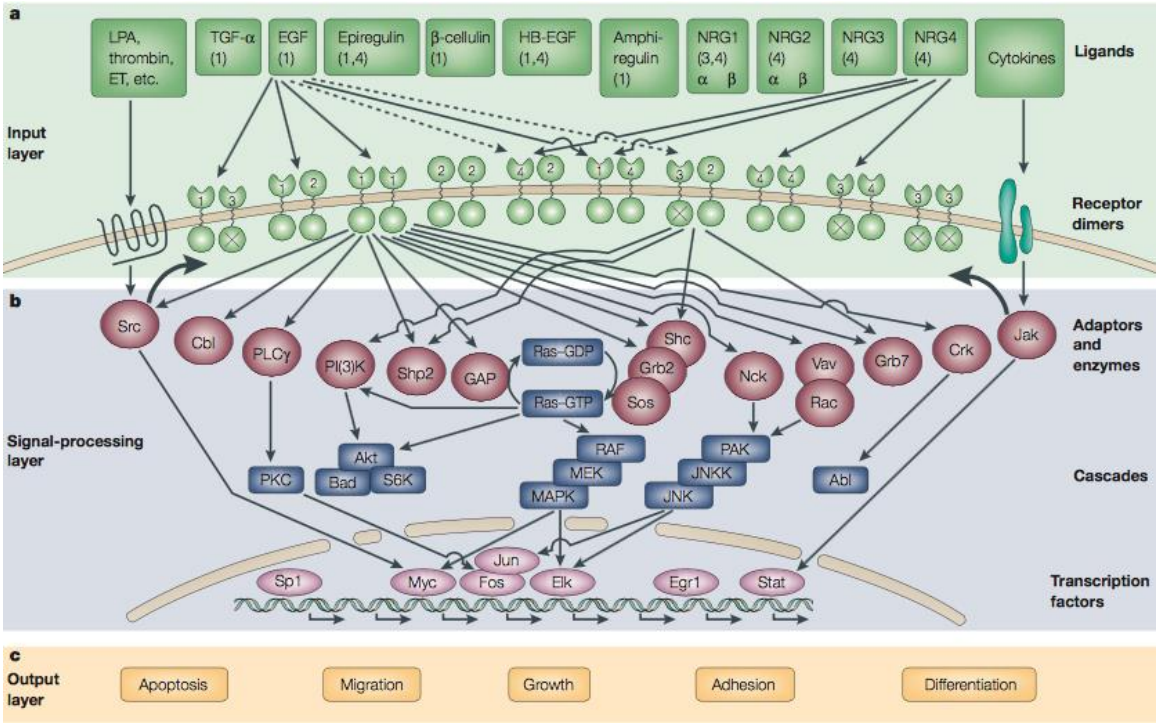

Figure 6: ErbB signaling network in response to ligands
ErbB signaling network is highly complex and consists of several layers. The input layer contains receptors and ligands, while multiple hidden layers of enzymes, adaptor proteins, second messengers and transcription factors lie beneath the cell surface. The output layer includes a variety of cellular responses. In most cases, the end result of EGFR activation is stimulation of cell growth. 1, EGFR; 2, HER2; 3, HER3; 4, HER4; LPA, lysophosphatidic acid; ET, endothelin. Figure taken from Yarden, et al. 2001(34).

Inactivation of ErbB receptors occurs primarily by the activity of protein tyrosine phosphatases, while clathrin-mediated endocytosis of receptor-ligand complexes plays a secondary role in inactivation and a primary one in receptor internatilization[37,136]. The contents of the resulting endosomes following endocytosis can have two fates: (i) degradation in the lysosome and, (ii) recycling to the surface of the cell (Figure 7) [37].

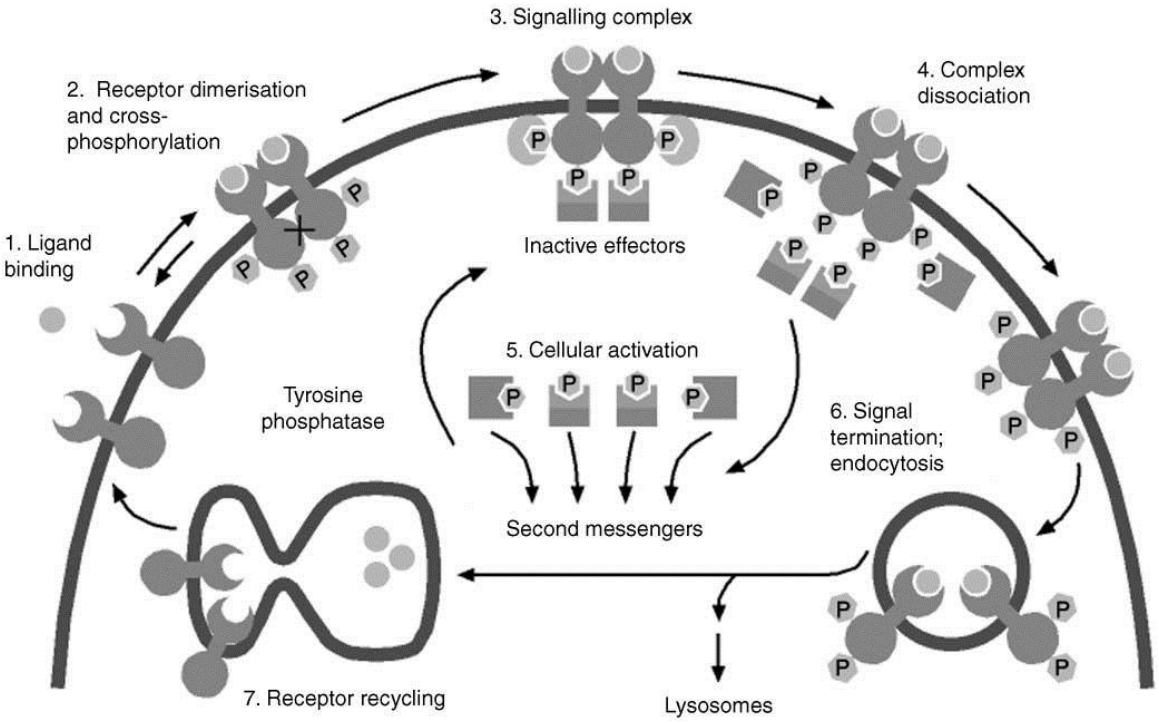

Figure 7: ErbB family signaling model
ErbB receptors are activated by ligand binding that causes dimerization of the receptor and cross phosphorylation of tyrosine residues. Inactive effector proteins bind to the phosphorylated tyrosines and dissociate as activated proteins and activate downstream signaling cascade. Signal is terminated once the receptors are endocytosed and then are either degraded in lysosomes or recycled back to the membrane. Figure taken from Yarden Y. 2001(147).

HER2 is the preferred dimerization partner for all the other receptors in the family. Several mechanisms contribute to HER2 heterodimeric signals potency [31,32]. First, activated heterodimeric complexes including HER2 as one of the monomers are found to be more stable at the cell surface than those without HER2[39]. Secondly, while HER2 does

not bind a ligand, it can decrease the rate of dissociation of ligand from the associated ErbB receptor, thereby, increasing the rate and strength of ErbB signaling network[40]. Third, ErbB heterodimers with HER2 as one of the monomers tend to stay much longer on the cell surface than those dimers without HER2. Furthermore, once activated dimers are internalized into the cell, HER2-EGFR heterodimers are targeted for recycling while other heterodimers are targeted for degradation. Thus, HER2 is recycled back to the surface for the next round of activation and downstream signaling[39]. Lastly, it is important to note that HER2-HER3 heterodimers are the most potent as they directly activate the PI3K pathway along with the MAPK pathway[137].

Implications in Cancer

Over the past couple of decades, it has become clear that members of the ErbB family play a significant role in initiation and maintenance of many solid tumors (Table 2). Several mutations and overexpression of these receptors play a major role in tumorigenesis.

<u>The EGF Receptor</u>

Originally identified as an oncogene because of its homology to v-ERBB, EGFR is mutated or overexpressed in several cancers as seen in Table 2[29]. Several single residue mutations in the EGFR extracellular region have been reported in different types of cancers, especially glioblastoma[43].

Table 2: Cancer types associated with different ErbB receptor types and alterations.*

Molecule	Alteration	Cancer Types
EGFR	Mutation	NSCLC (Adenocarcinoma)
EGFR	vIII	glioma
EGFR	Amplification	NSCLC (squamous), head and neck, glioma, esophageal colorectal
HER2	Amplification	Breast, gastric, esophageal
HER2	Mutation	Breast(lobular), lung, gastric, bladder, endometrial
HER3	Mutation	Breast, gastric, colorectal cancer and NSCLC
HER4	Mutation	Melanoma, NSCLC, medulloablastoma

*Adapted from Arteaga, C. L., et al. 2014(44).

One of the most studied EGFR mutation found in ~40% of high grade gliomas with wild type EGFR amplification is variant III (vIII), which lacks residues 6-273 in the extracellular region of the wild type receptor[28,47]. Studies have indicated that EGFRvIII has constitutive dimerization, aberrant tyrosine kinase activity and impaired downregulation and thus results in enhanced tumorigenecity[44,45,48]. Moreover, in addition to glioblastoma, EGFRvIII has also been found in a fraction of lung, breast, head and neck, ovarian and prostate cancers[49].

The second most common EGFR variant or mutant, found in ~20% of glioblastoma cases with wild type EGFR amplification, is EGFRc958. This variant lacks amino acids 521-603 and exhibits increased ligand dependent kinase activity[50.]

Apart from mutations, overexpression of EGFR is another mechanism of tumorigenecity frequently found in several cancers such as that of the cervix, bladder, ovary, kidney, pancreas and head and neck (Table 2). The primary mechanism of EGFR

overexpression was found to be amplification of the EGFR gene with more than 15 copies per cell reported in several tumors[52].

HER2 receptor

In year 1984, Schechter and colleagues found the first evidence of ErbB2/HER2 in cancer. HER-2 positive breast cancer, in which overexpression or gene amplification of HER2 occurs, accounts for 20-30% of all breast cancer subtypes and has the second-poorest prognosis among breast cancer types as well as is correlated with much lower disease free and survival rates[46].

Typically, HER2 is expressed at low levels on the surface of epithelial cells and is essential for normal development of tissues such as those of the breast, lungs, liver, kidney and nervous system[47]. However, in HER2+ breast cancer cells, immunohistochemical imaging has shown number of receptors in the upwards of 2 million per cell in contrast to HER2+ normal cells such as cardio myocytes with less than 20,000 receptors/cell[45,46,146].

As mentioned earlier in the ErbB chapter, HER2 is the preferred ErbB partner for heterodimer formation and its signaling is the most potent. Thus, with an overexpression of HER2 receptors on the cell surface, there is increased availability for heterodimer formation. This subsequently leads to prolonged activation of several downstream pathways, specifically mitogenic pathways, and leads to abnormal growth and tumorigenecity (Figure 8). In addition, HER2 amplification is also seen in gastric and esophageal cancer as well and causes tumorigenecity by a similar mechanism as that in HER2+ breast cancer discussed above[100].

HER2 has become a prognostic and predictive biomarker for a number of cancers, especially, for breast cancer. In 1987, Slamon et al. found a strong correlation between HER2 overexpression and reduced survival of breast cancer patients[44]. Furthermore, Yu and Hung extrapolated from clinical and laboratory data that overexpression of HER2 increases the metastatic potential of human lung and breast cancer thereby indicating that HER2 overexpression status can also give insight into tumor progression and its response to chemotherapies[53]. In addition, studies performed by Houston et al. found that HER2 expression leads to lack of response of breast tumor to anti-estrogen therapy and leads to resistance to tamoxifen[54].

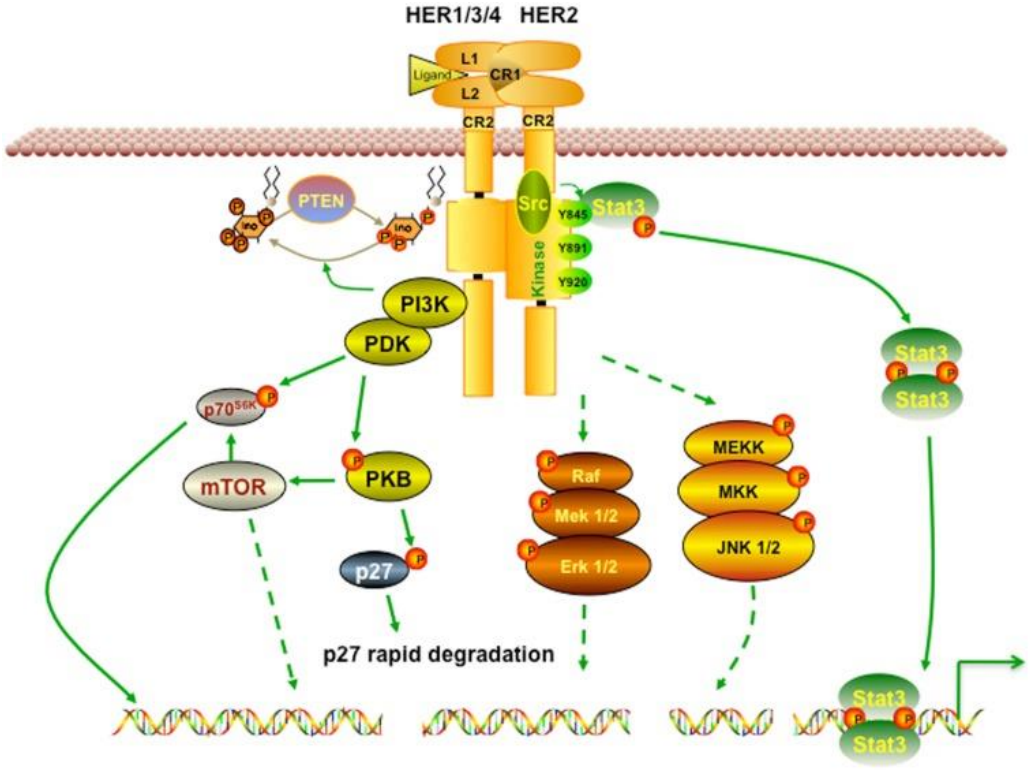

Figure 8: Signaling pathways associated with HER2 activation.
Post activation of HER2 receptors, downstream signaling cascade gets activated and can activate different pathways such as PI3K-Akt pathways, MEK/ERK pathway or MEK/JNK pathways that lead to evasion of apoptosis, cell proliferation and tumor management. Figure taken from Vu and Claret, 2012(49).

HER3 Receptor

ErbB3 or HER3 was first discovered and isolated from human mammary tumor cell lines in 1989 by Dr. M.H. Kraus[60]. Because it has very low intrinsic kinase activity, HER3 was believed to be dependent on other HER receptors for its activation and thus its clinical relevance was considered insignificant until recently[56]. Recent studies have shown that HER3 is involved in tumorigenesis, cancer progression and chemotherapeutic resistance in several types of cancers[55]. This has been attributed to HER3's ability to directly bind to PI3K without the need of any adaptor proteins and activate the PI3K pathway[137].

To truly understand the distinctive properties of HER3 and its role in cancer, it is important to delve into its structure, mechanism of activation and signal transduction pathways associated with it.

Structure of HER3

Structurally, HER3 is very similar to the other HER receptors with an extracellular domain, transmembrane helix domain and intracellular tyrosine kinase domain (Figure 9)[56]. However several structural differences give HER3 its distinctive properties. First, the extracellular domain has 2 specific ligand-binding regions (district I and III) and 2 cysteine-rich regions (district II and IV). The interaction between the two cysteine rich regions provides a structural bias for conformational change of HER3 on ligand binding[57]. Second, while the transmembrane domain shares significant homology with other HER receptor structures, many important structural differences are found in the intracellular kinase domain that make HER3 distinctive. The intracellular domain is divided into three: the juxtamembrane region (JM), a kinase domain and the C-terminal tail. The JM region is

further divided into 2, namely, JM-A and JM-B and the kinase domain is divided into N-lobe, helix αC, activation loop and C-lobe. JM-A region interacts with other HER receptors while JM-B region binds to the C-lobe and forms a stabilizing latch[58]. C-lobe combines with and activates other HER receptors, conferring the properties of a functional activator to HER3. In addition, conformational changes in helix αC, responsible for anchoring activator kinase domain to recipient kinase domain, are important for HER3 functioning. For instance, Thr738 in helix αC of HER3 replaces Leu736 in helix αC of EGFR and stabilizes the inactivation state of HER3[59].

Some other important structural differences include 14 tyrosine residues in the HER3 C-terminal tail, which include six PI3K binding sites. These binding sites mediate interactions between three regulatory subunits of PI3K, specifically SH2 domains of p85. This interaction leads to allosteric activation of p110, the lipid kinase subunit of PI3K and causes activation of downstream signaling of the PI3K/Akt pathway[59-61]. Lastly, HER3 lacks several key residues required for catalytic activity such as the lack of Asp813, which is present in EGFR.

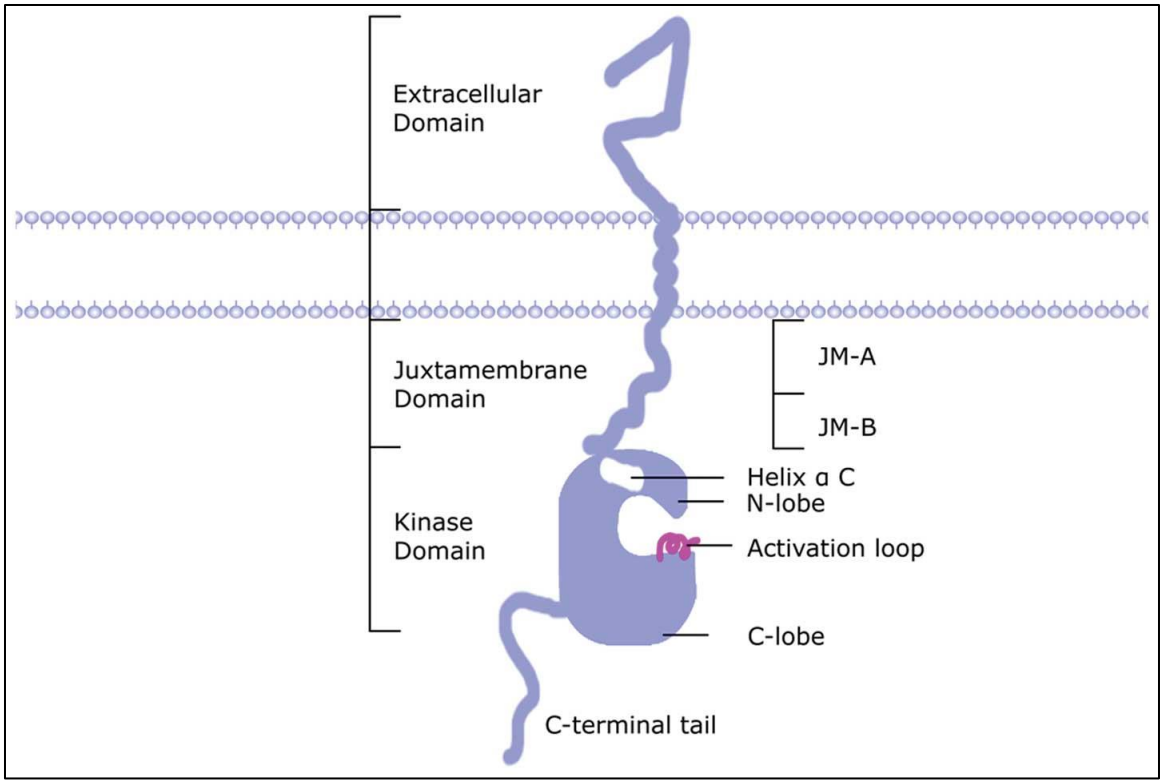

Figure 9: Structure of HER3 receptor

HER3 receptor consists of an extracellular ligand binding domain, a transmembrane helix domain and an intracellular TKD. The intracellular region is divided into a juxtamembrane region, a kinase domain and C-terminal tail. The juxtamembrane region is divided into JM-A and JM-B. The kinase domain includes N-lobe, helix αC, activation loop and C-lobe. HER-3, human epidermal growth factor receptor-3; TKD, tyrosine kinase domain. JM-A, juxtamembrane-A; JM-B, juxtamembrane-B. Adapted from Li, Q. et al. 2013(53).

Thus, as a result it was believed by the scientific community that HER3 does not have tyrosine kinase activity and catalytic activity[62,63]. However, studies by Kornev and Taylor in 2009 proved otherwise and showed that HER3 exhibits intrinsic tyrosine kinase activity, albeit very low, in comparison to other HER receptors. Further, Jura et al showed in 2009 that HER3 acted as a specific allosteric activator for recipient protein kinases from other receptors within the family.

Dimerization and Activation

HER3 functions by forming dimers just like other HER receptors. On ligand binding, HER3 changes conformation and thus forms homo or heterodimers. Dimerization of HER3 switches it from inactive state to active state and leads to tyrosine kinase activity and activation of downstream signaling pathways associated with it[66]. HER3 dimerizes with other HER family receptors, but in particular two heterodimeric combinations are significant in cancer. First, HER3 directly interacts with EGFR when EGF binds to EGFR, its ligand. EGFR, on activation with EGF, can cross activate HER3 simultaneously and lead to activation of downstream signaling pathways. This was confirmed by using tyrosine kinase inhibitors (TKI) that inhibited downstream signaling pathways as a result of blocking EGFR and HER3 interactions [67].

Secondly, HER3 interacts directly with HER2. In fact, it is the most preferred dimerization partner for HER3 and mainly interacts with HER2. Since HER3 has very low catalytic activity, it depends on HER2 for signaling, while HER2, a ligand-less receptor, functions in the context of heterodimerization with HER3. For this interaction to occur it is necessary for heregulin (HRG), ligand for HER3, to bind the extracellular domain of HER3. On binding, HRG causes a conformation change in the extracellular domain of HER3, releasing a locked conformation and extending the dimerization loop[62]. Thus the dimerization loop latches onto the predisposed dimerization loop of HER2 and forms an active heterodimer (Figure 10A). A second mechanism for HER2-HER3 dimerization has been proposed in the context of HER2 overexpression while in the absence of a ligand.

Overexpression of HER2 on the cell surface may lead to spontaneous heterodimer formation with HER3 even in the absence of a ligand (Figure 10B). This interaction leads to a ligand induced conformation and results in a weak yet prolonged receptor activation[62].

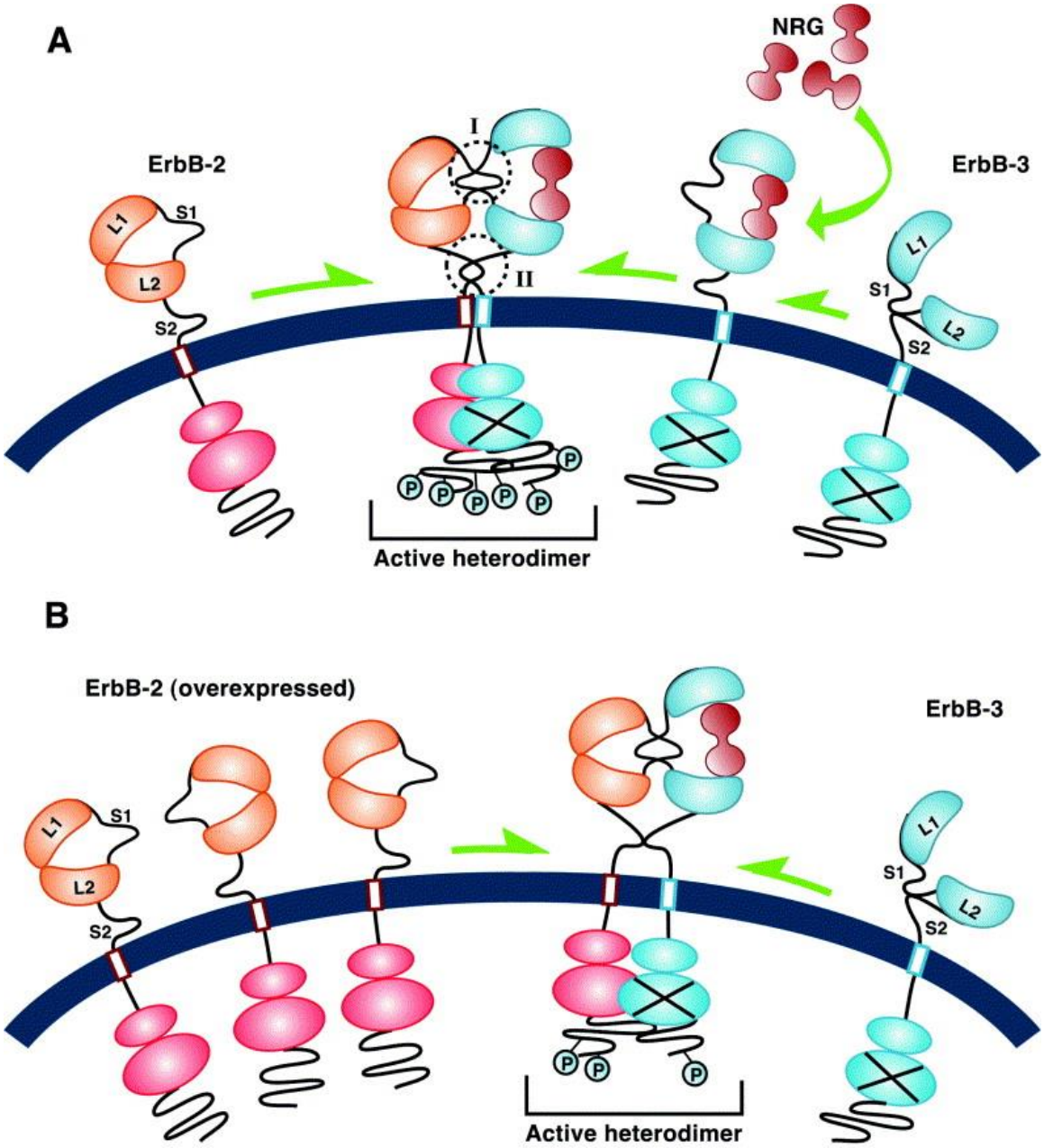

Figure 10: Mechanisms of HER3 receptor heterodimerization.
(A)The extracellular domain of HER3 binds to the dumbbell shaped HRG or NRG. On binding to the ligand, the dimerization loop is exposed and it interacts with the dimerization loop of HER2. This forms an active heterodimer.(B) HER2 overexpression leads to ligandless dimerization with HER3. The dimers may assume a ligand induced conformation and leads to weak but prolonged receptor activation. Taken from Citri A et al. 2003(60).

Downstream signaling pathway associated with HER3: MAPK and PI3K/PDK/Akt

There are two relevant signaling pathways that are associated with HER3: (i) Mitogen activated protein kinase (MAPK) pathway, and (ii) Phosphatidylinositol 3-kinase (PI3K)/3-phosphoinositide-dependant protein kinase (PDK) 1/protein kinase B (Akt) pathway[55,62].

MAPK signaling pathway, responsible for cell proliferation, is activated when there is ligand-induced activation of the HER3 heterodimer, which binds Grb2 through a phosphorylated tyrosine-based consensus site, or indirectly through interaction with SHC[65]. Grb2 associates with Sos, which in turn activates Ras, a GTPase. Ras-GTP leads to activation of a linear cascade involving activation of Raf, MEK and finally MAPK (Figure 11)[55, 62].

The PI3K pathway, on the other hand, regulates cell growth, apoptosis, tumor cell invasion, metastasis and even chemotherapy resistance in tumor cells[62]. This pathway is stimulated by formation of heterodimers between HER3 and EGFR/HER2. PI3K is a dimeric protein kinase that consists of a regulatory subunit, p85, and a catalytic subunit, p110[64]. Binding of p85 to the phosphotyrosine consensus site of the receptor leads to allosteric activation of p110. p110 then phosphorylates phosphophatidylinositol 4,5-bisphosphate (PIP2) to phosphophatidylinositol 3,4,5-triphosphate (PIP3) which can be cleaved by 5'-inositol phosphatases to phosphophatidylinositol 3,4,-triphosphate[69]. Upon production of PIP3 and phosphophatidylinositol 3,4,-triphosphate following PI3K activation, Akt is recruited to the plasma membrane by its PH domain and is phosphorylated by PDK1. Phosphorylation of Akt leads to its activation and it translocates into the nucleus

and leads to regulation of apoptosis or cell growth[68,69]. PTEN, a tumor suppressor and a lipid phosphatase is a molecule that is responsible to turn of this pathway. It basically dephosphorylates phosphophatidylinositol 3,4,-triphosphate and PIP3, thereby downregulating the activity of PDK and Akt[62,69]. Figure 11 illustrates the signaling pathways associated with HER3.

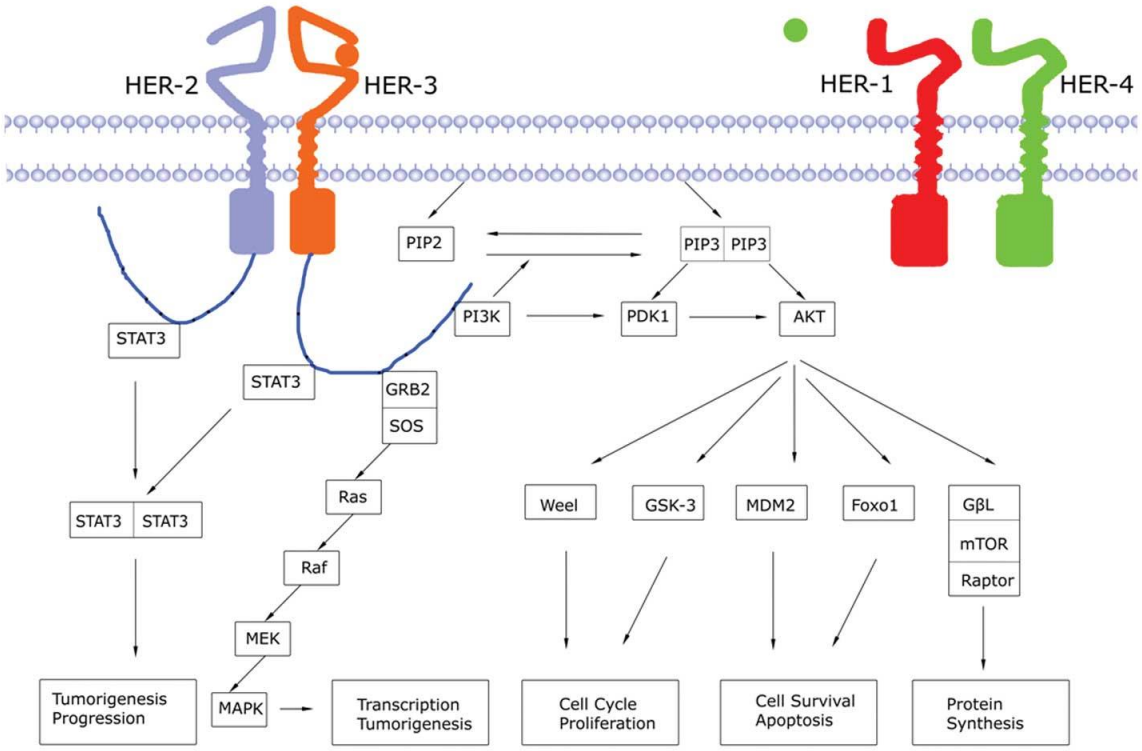

Figure 11: HER3 related signaling pathways and the physiological effects on cells
HER3 can lead to activation of both, MAPK and PI3K/PDK/Akt, pathways following heterodimer formation. Taken from Citri A et al. 2003(60).

HER3 and Cancer

The clinical significance of HER3 was not considered until recently, although, it was found to be overexpressed in several cancers such as breast cancer and colorectal

cancer. In 2009, Schoeberl et al. found, HER3 to be the most sensitive node in the network for AKT phosphorylation when stimulated with HRG or Betacellulin (BTC)[124]. This was determined by sensitivity analysis, a computational technique that was used to identify key proteins that control the ErbB receptor network in response to ligand stimulation (HRG and BTC) using Akt activation as readout[124].

Furthermore, by using a systems biology approach, Schoeberl et al. screened and developed an antibody that would effectively bind to HER3 on cells with low nanomolar affinity and inhibit downstream signaling pathways[124]. Systems biology is the computational and mathematical modeling of complex biological systems. This biology-based interdisciplinary field focuses on complex interactions using a holistic approach to biological and biomedical research. The aim of systems biology is to model and discover emergent properties of cells, tissues and organisms functioning as a system. These typically involve metabolic networks or cell signaling networks[134]. By using this approach, MM-121 (now called seribantumab) was developed and has been discussed in detail in later sections.

While overexpression, constitutive activation or mutation of HER3 alone is nonmitogenic, its expression with HER2 and HRG not only transmits potent mitogenic signals, but also leads to tumorigenesis in several types of cancers[55, 70]. Recent studies have shown that HER3 plays a pivotal role in occurrence and progression of lung cancer, breast cancer, colorectal cancer and many other cancers[70-72]. Additionally, HER3 plays a critical role in cancer cell resistance to chemotherapeutics and targeted therapies. HER3 provides an alternative path for cell proliferation, cancer progression and thereby

provides an escape mechanism from treatment for cancer cells. More on this will be discussed in the next chapter.

In NSCLC cell lines, overexpression of HER3 accelerated cell growth and metastasis of tumor cells. This was further confirmed by performing *in vivo* studies in which downregulation of HER3 inhibited migration of tumor cells, tumor growth as well as metastasis[70]. Furthermore, siRNAs to HER3 or its downstream signaling partner AKT2 led to suppression of cell growth of lung adenocarcinoma cells in culture[74]. These results indicate that lung cancer progression is dependent on HER3 expression and/or its downstream signaling pathway.

In breast cancer with HER2 gene amplification, HER3 is required for HER2 induced preneoplastic changes to breast tumor formation[55,75]. HER3 forms heterodimers with HER2 and triggers downstream signaling pathways and breast cancer cell proliferation. In fact, studies showed that deletion of HER3 in HER2+ breast cancer cell lines produces strong anti-proliferative effects[76]. This is because HER2/HER3 heterodimers are found to be the most potent of HER heterodimers.

While there is almost no expression of HER3 in the normal colon, 50-89% of colorectal cancer cases have expression of HER3 receptors[77]. Furthermore, in liver cancer metastasized from colon cancer, HRG expression is seen in the cancer cells and knockdown of HER3 by siRNA significantly reduced HRG-induced migration and metastasis of cancer cells from the colon to the liver *in vivo*. Moreover, increase phosphoAkt (pAkt) was found in these cells prior to silencing and PI3K inhibitors had a similar effect on the cells as that of knocking down HER3. These findings not only

confirm the role of HER3 in progression and metastasis of colorectal cancer, but also the involvement of HRG/HER3/PI3K/Akt pathway[78].

Because of the significant involvement of HER3 in tumor dynamics, it has become a new therapeutic target with the hopes of developing effective targeted drugs to not only treat HER3 driven cancers, but also overcome HER3-induced tumor resistance.

ERBB3 INHIBITORS AS CANCER THERAPEUTICS

While today the importance of HER3 as a critical node in cancer is understood and targeted therapies have been developed, the first ever cancer therapeutic, mustard gas, was discovered serendipitously in the 1940s during WWII[143]. Ever since, hundreds of chemotherapies are available today for treating cancers. These chemotherapies can be categorized in different classes of drugs based on their mode of action as seen in Table 3[79]. While there are a myriad of chemotherapeutic agents, only some of these have become standard of care (SoC) chemotherapies based on their efficacy at treating cancers. Some SoC chemotherapies today are cisplatin, doxorubicin, gemcitabine, 5-FU and are used either as a monotherapy or a combination therapy with other anti-cancer drugs. It is important to note that not only do different types of cancer have various SoC treatments, but also different stages within the same cancer type can have different SoC treatment. This is based on extensive clinical evidence proving the safety, efficacy and potency of these chemotherapies. For example, the SoC for stage IV gastric cancer are eight chemotherapies therapies, one of which is a combination therapy with cisplatin and 5-FU[80]. Whereas for NSCLC lung cancer, one of the SoC treatment is a combination of cisplatin and gemcitabine [135].

Table 3: Categories of different types of chemotherapies.*

Category of Chemotherapy	Examples
Alkylating Agents	Nitrogen mustards: chlorambucil, mechlorethamine, cyclophosphamide Nitrosurea: streptozocin, carmustine (BCNU), and lomustine Alkyl sulfonates: busulfan Triazines: dacarbazine (DTIC) and temozolomide (Temodar®) Ethyleinmines: thiotepa and altretamine (hexamethylmelamine) Platinum drugs: cisplatin, carboplatin and oxoplatin
Antimetabolites	5-fluorouracil (5-FU),6-mercaptopurine (6-MP), Capecitabine (Xeloda®)Cladribine, Clofarabine, Cytarabine (Ara-C®)Floxuridine, Fludarabine, Gemcitabine (Gemzar®), Hydroxyurea, Methotrexate, Pemetrexed (Alimta®), Pentostatin, Thioguanine
Anti-tumor Metabolites	Anthracyclines: Daunorubicin, Doxorubicin (Adriamycin®), Epirubicin ,Idarubicin Non anthracyclines: Actinomycin-D, Bleomycin, Mitomycin-C
Topoisomerase inhibitors	Topoisomerase I inhibitors: topotecan and irinotecan (CPT-11). Topoisomerase II inhibitors: etoposide (VP-16), teniposide and Mitoxantrone
Mitotic inhibitors	Taxanes: paclitaxel (Taxol®) and docetaxel (Taxotere®) Epothilones: ixabepilone (Ixempra®) Vinca alkaloids: vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®) Estramustine (Emcyt®)
Corticosteroids	prednisone, methylprednisolone (Solumedrol®), and dexamethasone (Decadron®).

*Adapted from www.cancer.org[77]

Traditional chemotherapies have been effective at killing cancer cells by interfering with the cell division process[79]. However, while these drugs specifically target rapidly dividing cells, they are non-specific in that they don't distinguish between cancer cells and rapidly diving cells of the body such as GI epithelium, skin and bone marrow[79]. As a result, they cause undesirable adverse effects to patients in the clinic. Moreover, over the course of the treatment, some cancer cells in the tumor develop resistance to these drugs and become unresponsive to chemotherapies[51]. This poses a major concern in the clinic and limits the use of these drugs. Thus, researchers are constantly investigating new drugs and therapeutics to overcome resistance and minimize toxicity.

One way to overcome the problems mentioned above and effectively treat cancer is by the development and utilization of targeted therapies. Many signaling pathways are deregulated in cancer cells leading to their malignant phenotypes[82]. Targeted therapies are designed to selectively target molecular agents involved in cancer pathways.

Several EGFR driven cancers such as that of the breast, lung, head and neck, and colon cancer have targeted therapies developed against different ErbB receptors and are used to treat associated cancers. These can be divided into different classes such as small molecule inhibitors and monocloncal antibodies (mAB)[83].

For instance, gefitinib and erlotinib are EGFR inhibitors and are used in first line treatment of advanced NSCLC with EFGR mutation that leads to activation of anti-apoptotic Ras[83]. Gefitinib and erlotinib, both, have a similar mode of action and inhibit the tyrosine kinase domain of EGFR by binding to the ATP binding domain of the enzyme thereby inhibiting phosphorylation of the tyrosine domains of the receptor

(Figure 12). This in turn inhibits the anti-apoptotic signaling from the mutant receptor and causes cell death[83]. These two inhibitors were significant more effective than treatment with traditional chemotherapies alone. For gefitinib, median progression free survival (PFS) of patients who received gefitinib versus those who received carboplatin plus paclitaxel was 10.8 months versus 5.4 months, respectively, which is a two-fold increase in PFS[84]. Similarly, erlotinib also showed 2x increase in the median PFS of patients versus patients given platinum based chemotherapy only. The median PFS was 10.4 months in the erlotinib arm and 5.2 months in the platinum-based chemotherapy arm[85]. For HER2 driven cancers, especially, HER+ breast cancer, lapatinib, a small molecule inhibitor that inhibits the kinase activity of EGFR and HER2 simultaneously, has already been approved for treatment. Its mode of action is similar to that of erlotinib and gefitinib except it has dual action and blocks the ATP binding sites in EGFR and HER2 and inhibits receptor signaling[86]. In clinical setting, while lapatinib was found to be effective as a monotherapy, it has been found to be most effective when used in a combination therapy. In a phase III clinical trial, median PFS in the lapatinib plus capecitabine arm was 36.9 weeks as compared with 17.9 weeks in the capecitabine monotherapy arm[87]. Today, many other small molecules are under investigation in lab and the clinic.

Cetuximab, a chimeric mAb against the extracellular ligand binding domain of EGFR (Figure 12), is used first line for treating metastatic colorectal cancer, metastatic NSCLC and head and neck cancer[88,89]. In clinical trials with two arms: one with Cetuximab in combination of FOLFIRI (irinotecan, 5-FU and leucovorin) and the other

with just FOLFIRI, PFS was significantly better in the former compared to the latter[90]. A second mAb in the clinic, Panitumumab, is a fully humanized monoclonal antibody against the extracellular ligand-binding domain of EGFR. This is used to treat metastatic colorectal cancer with disease progression on or following fluoropyrimidine-, oxaliplatin, and irinotecan-containing regimens and was found to benefit such patients in phase III trials[91].

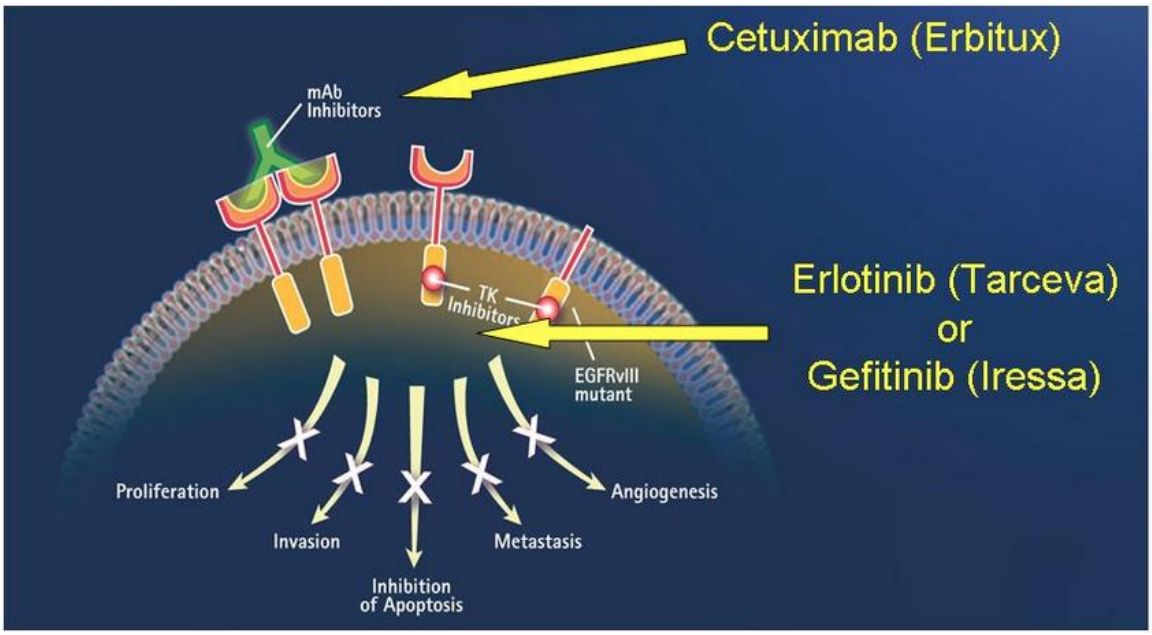

Figure 12: EGFR inhibitors and their mode of action
Cetuximab, mAb, binds to the extracellular domain of the EGFR receptors, while Erlotinib and Gefinitib, TKIs, bind to the tyrosine kinase domain.
Taken from http://cancergrace.org/cancer-treatments/2008/03/21/egfr-combo-in-adv-nsclc/[90].

For treating metastatic HER2+ breast cancer, Herceptin, a fully humanized monocloncal antibody that targets the extracellular domain of HER2 was developed (Figure 13)[93]. It acts by blocking the HER2 signaling cascade as well as recruiting antibody-dependent cellular cytotoxicity[94,100]. Original studies by Hudis et al. showed

that trastuzamab improved the overall survival in metastatic HER2+ breast cancer patients from 20.3 to 25.1 months. In the trastuzumab for gastric cancer (ToGA) trial, patients were randomized into either a control arm with standard chemotherapy or in the treatment arm with standard chemotherapy plus trastuzumab. The results from this study found that the overall survival was much longer in the case of the treatment arm than in the control arm with a median overall survival of 13.8 month versus 11.1 months in the trastuzumab arm versus control arm respectively. As a result of these studies, trastuzumab is used as first-line treatment for HER+ breast cancer, as an adjuvant therapy in late stages of breast cancer[95] and first line combination therapy for gastric cancer[100]. Another monoclonal antibody, Pertuzamab, is used to treat HER2+ breast cancer, however, in combination with trastuzamab and docetaxel[96]. This antibody is a HER2 dimerization inhibitor. It binds to HER2 such that it inhibits dimerization of HER2 with other receptors and slows tumor growth (Figure 13)[97]. In the CLEOPATRA phase III clinical trial, the efficacy of combination of pertuzumab, trastuzamab and docetaxel compared to placebo, trastuzumab, and docetaxel in patients with HER2-positive first-line metastatic breast cancer was evaluated. The former arm had a significantly prolonged PFS as compared with placebo, trastuzumab and docetaxel arm, when used as first-line treatment for HER2-positive metastatic breast cancer, with a PFS of 18.7 versus 12.4 months, respectively[99].

A new class of drugs, antibody-drug conjugates, is being used to target specific cancers such as HER2+ breast cancer. TDM-1 is an antibody-drug conjugate comprising of trastuzumab conjugated to a cytotoxic agent called DM-1[138]. Trastuzumab inhibits the

growth of tumor cells by directly binding to HER2, while DM1 enters the cells and bind to tubulin, thereby, killing the cells[139]. Furthermore, in the EMILIA phase III clinical trial, TDM-1 proved to be superior in treating advanced stage metastatic breast cancer patients already resistant to trastuzumab. TDM-1 increased the mean PFS by 5.8 months when compared to the combination of lapatinib and capecitabine[139].

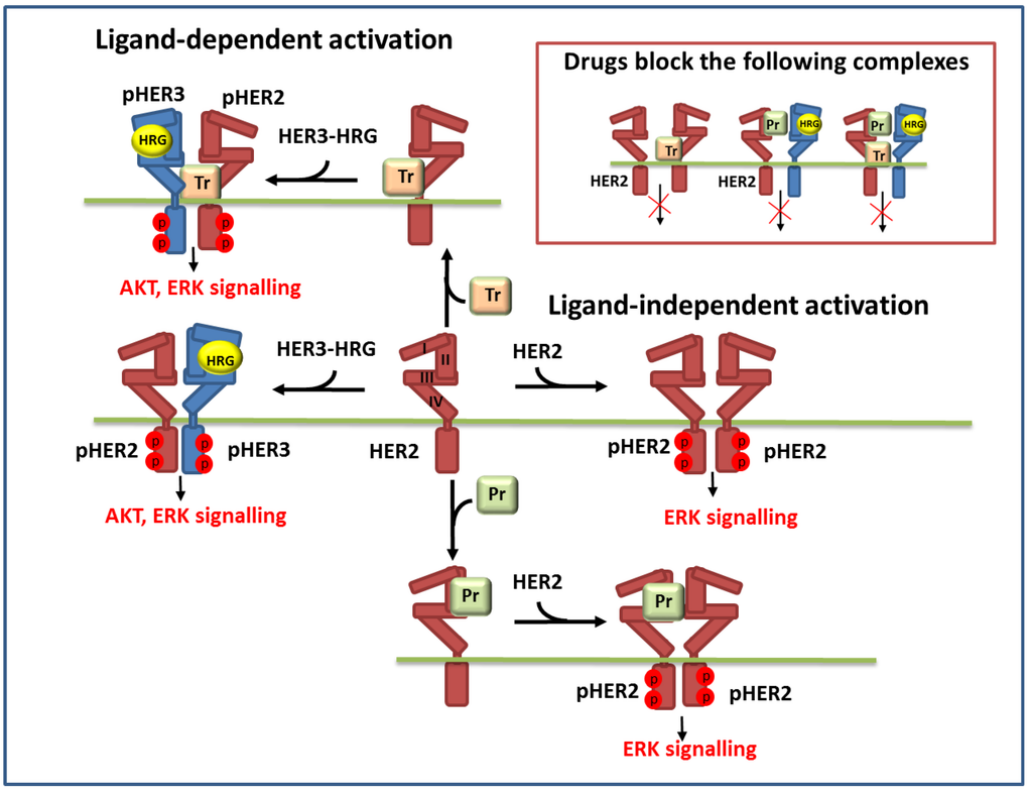

Figure 13: HER2 inhibitors and their mode of action
Trastuzumab and pertuzumab both bind differently to the extracellular domain of HER2 and act under different conditions. Pertuzumab is effective at blocking ligand-dependent heterodimerization of HER2, while Trastuzumab is effective at blocking ligand-independent heterodimerization of HER2. Taken from Goltsov, A. et al 2014(113).

RESISTANCE TO TARGETED THERAPIES

Although targeted therapies have exhibited significant therapeutic benefit over traditional chemotherapies, tumors eventually become resistant to these drugs as well. One case by Kobayashi et al. in 2005 reported an additional mutation in the EGFR receptor in specimens from patients with NSCLC who were responsive to gefitinib. This mutation was discovered after these patients relapsed after two years of complete remission post gefitinib treatment. The mutant EGFR, later called EGFR 790M, was found to cause resistance to gefitnib[101]. However, alternate mechanisms of resistance to gefitinib and erlotinib have also been found. One of them involves amplification of MET oncogene, which causes resistance by HER3-dependant activation of PI3K pathway[102]. This was confirmed in the same study by examining MET copy number in lung cancer specimens of patients that developed resistance to gefitinib or erlotinib[102]. Studies have proposed that lateral signaling occurs between ErbB receptors and that HER2 overexpression due to EGFR TKI treatment may lead to resistance by lateral signaling and formation of HER2-HER3 heterodimers. As a result, these heterodimers would activate the PI3K signaling pathway and lead to resistance to TKIs[103]. Other *in* vitro and *in vivo* studies have implicated EGFR and HER2 in activating HER3 signaling and induced resistance in tumor cells post ligand activation of HER3[104,105,111].

TKIs against EGFR and HER2 have found to block the MAPK pathway, but very transiently block the PI3K pathway. This was attributed to continual activation of HER3, which potently activates PI3K. However, HER2 as a heterodimeric partner was found to be necessary for HER3 activation. Furthermore, the desensitization of HER3 signaling to

TKI was caused due to a forward shift in the HER3 phosphorylation-dephosphorylation equilibrium reactions and establishes a new steady stage for HER3 phosphorylation in spite of inhibition by TKIs. This forward shift leads to superphosphorylation of the HER3 and Akt, which constantly activates the Akt pathways. This was confirmed by adding an irreversible HER family TKI that led to desensitization of HER3 activity even in the presence of the other drugs.

Similarly, studies have found that trastuzumab associated resistance in HER2+ breast cancer can also be driven by HER3 upregulation and activation[106]. Trastuzumab blocks HER2 and HER3 when they are not dimerized. Since trastuzumab does not inhibit ligand induced HER2-HER3 heterodimer formation, resistance occurs via upregulation of HER3, which overcomes the effects of trastuzumab and activates the PI3K pathway[107,108]. HER3 upregulation has also been shown to provide tumors an escape mechanism from lapatinib therapy in not only preclinical models, but also in patients[114].

Additionally, studies have found HRG induced heterodimerization in NSCLC as an important resistance mechanism as well[109]. Finally another important consequence of HER3 overexpression and driven resistance is poor outcomes in a number of cancers. A meta-analysis performed by Ocana et al. showed that in a number of solid tumor studies, HER3 expression was shown to have worse survival outcomes in patients. One of the proposed mechanisms was attributed to HER3 induced resistance to therapeutics[110].

Since HER3 is a focal point for both, initial effectiveness of EGFR and HER2 therapies as well as for tumor resistance, it has been of great interest as a pharmacological

target[112]. Several molecules against HER3 are currently in clinical development. However, since HER3 insignificant kinase activity, only mAb can be developed HER3.

Some of the HER3 inhibitors that are currently under investigation can be found in Table 4.

Table 4: ErbB3 inhibitors currently in development.*

DRUG	TYPE	TARGETS	DEVELOPMENT PHASE	SPONSOR
MM-111	Bispecific Antibody	HER2,HER3	Phase II**	Merrimack Pharmaceuticals
MM-121 (seribantumab)	Humanized mAb	HER3	Phase II	Merrimack Pharmaceuticals
MM-141	Bispecific Antibody	IGFR-1, HER3	Phase I	Merrimack Pharmaceuticals
U3-1287 (patritumab)	Humanized mAb	HER3	Phase III	Daiichi Sankyo
MEHD7945A	mAb	EGFR, HER3	Phase II	Genentech
AZD8931	Pan inhibitor	HER1/2/3	Phase I/II	AstraZeneca

*Table adapted from Jiang et al. 2012(119)
**Phase II trial was discontinued in February 2015

In preclinical as well as early clinical studies, HER3 inhibitors listed above have shown promising effects on tumors. For instance, MM-111 is a bispecific antibody with specificity and avidity to HER2 and HER3 expressing tumor cells (Figure 14). The HER2 –binding arm of MM-111 acts as the docking arm and the therapeutic HER3 arm blocks HRG induced HER3 activity, thereby, inhibiting cell growth, proliferation and progression of cancer[115].

MM-121 is a fully humanized monoclonal antibody targeted against the ligand-binding domain of HER3. Figure 15 below gives the mode of action of this antibody. MM-121 binds to the ligand binding domain of HER3 and (i) inhibits downstream

signaling of HER3 i.e. PI3K/Akt and MAPK pathway, (ii) receptor degradation by receptor internalization[120].

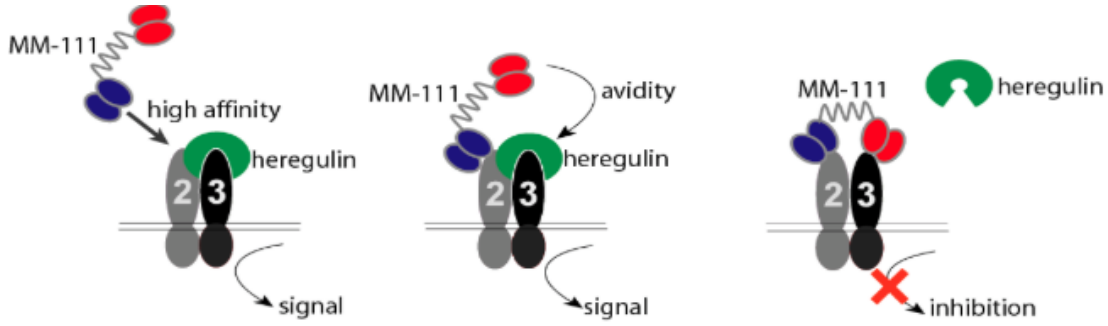

Figure 14: Mode of action of MM-111
MM-111's two arms, the docking HER2 arm is a high affinity arm and binds first, followed by the avidity due to which the therapeutic HER3 arm binds to HER3. This leads to inhibition of downstream signaling by preventing the binding of heregulin. Taken from Onsum, M et al. 2012(118).

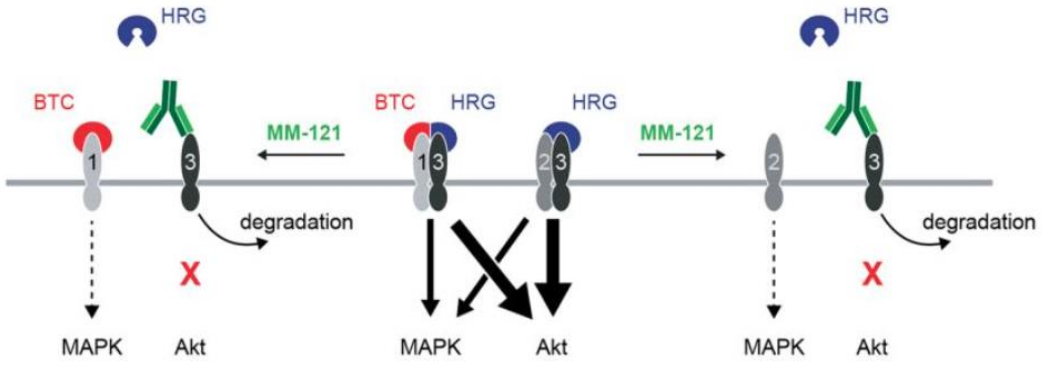

Figure 15: Mode of action of MM-121
MM-121 binds to the ligand-binding domain of HER3 and prevents heterodimerization. This leads to (i) inhibition of signaling cascade and, (ii) degradation of the receptor by endocytosis. Taken from Onsum, M et al. 2012(118).

Studies from MM-111 and MM-121 have led to a deeper understanding of their therapeutic potential and actions in humans. As mentioned earlier, mathematical modeling of the ErbB signaling pathway and sensitivity analysis of this model reveals HER3 as the most sensitive node for inhibiting the PI3K pathway. Further simulation analysis demonstrated two important findings: (1) an anti-HER3 antibody would be

potent in a low HER2 setting and, (ii) an HER2/HER3 bispecific molecule would be effective in a high HER2 setting. Figure 16 shows the results of computational simulations that led to these results. MM-121 and MM-111 were two molecules designed and developed that fit in the criteria determined by computational models. The results from the simulations were further confirmed by examining the effects of MM-111 and MM-121 *in vitro* in different cancer cell lines with a variation in their HER2 receptor levels as seen in Figure 17. It has been hypothesized that the potency of these inhibitors is affected by HER2 expression levels because of the fact that pre-formed HER2/HER3 dimers have a much higher affinity for HRG and thus high HER2 levels decreases the potency of MM-121 due to large numbers of HER2/HER3 dimers. However, in order to counter the effects of pre-formed HER2/HER3 dimers in high HER2 setting, a bispecific molecule such as MM-111 would be much more potent than MM-121 due to avid binding of this molecule [118,140].

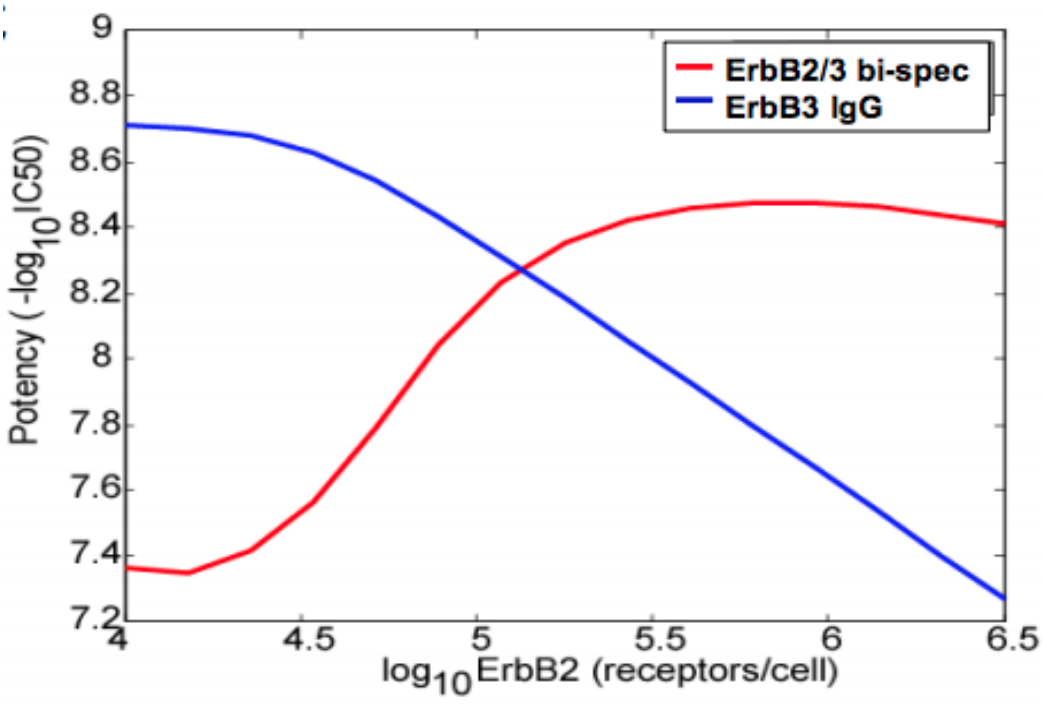

Figure 16: Stimulation of ErbB signaling network
The curve above indicates that HER3 specific IgG is more effective in low HER2 settings while HER2/3 bi-specific IgG is most effective in high HER2 settings. Taken from Onsum, M et al. 2012(118)

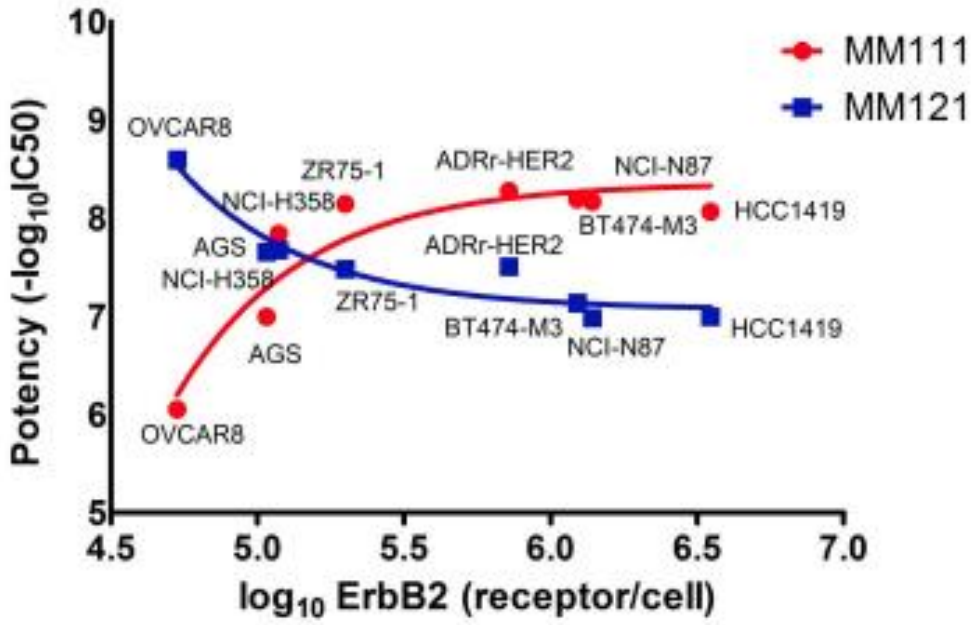

Figure 17: Potency of MM-111 and MM-121 is determined by levels of HER2
Potency of MM-111 and MM-121 related to HER2 expression on cells. Different cancerous cell lines with variable HER2 levels are shown above. MM-111 is most potent in a high HER2 setting while MM-121 is most potent in low HER2 setting. It is important to note that exogenous ligand, HRG stimulation was present in all experiments above as per computational stimulations. Taken from Onsum, M et al. 2012(118)

Consequently, another important finding from the results discussed above was the identification of biomarkers in cancer cells to determine what molecule would be most effective in treating patients. For MM-111, after *in vitro* and *in vivo* validation, we now know that biomarkers positive patients are those that have high HER2, high HRG and HER3[141]. On the other hand, for MM-121 biomarker positive patients are those that have high HRG and low HER2[141].

In preclinical studies, MM-111 has proven to be a superior inhibitor of heregulin-induced HER3 activation to lapatinib, pertuzumab and trastuzumab, which was at inhibiting ligand induced HER3 and AKT phosphorylation. Moreover, MM-111

combines favorably with trastuzumab or lapatinib to inhibit growth of HER2-overexpressing tumors *in vivo*. Additionally, MM-111 has been shown to re-sensitize HRG-HER3 induced paclitaxel resistant gastric cancer spheroid to paclitaxel as well as xenograft tumors *in vivo*. In addition to overcoming resistance in HER2+ gastric cancer models, MM-111 was shown to be synergistic with trastuzumab and paclitaxel in treating HER2+ xenograft tumors *in vivo*. These findings led to further clinical investigation of MM-111 in patients. Although MM-111 was proven to be safe and tolerable by patients in phase I studies, phase II trials investigating combination of MM-111 with trastuzumab and paclitaxel versus trastuzumab and paclitaxel in second line treatment of HER2+ gastric/gastroesophageal cancer, were discontinued due to shortage of PFS in the experimental arm compared to the control arm of the study[116, 117]. However, preliminary analysis of the study reveals that the patients enrolled in the study had HRG levels well below the threshold necessary for MM-111 to be therapeutic[117].

In preclinical studies, MM-121 potently prevented resistance to anti-EGFR therapies *in vitro* and *in vivo*[120]. Furthermore, the activity of MM-121 was assessed in genetically engineered mouse models of lung cancers driven by EGFR T790M-L858R. T790M mutation in EGFR causes cancer cells to be resistant to EGFR TKIs, gefitinib and erlotinib, and is seen in this animal model as well[101,122]. Furthermore, this animal model exhibited resistance to cetuximab as a result of reactivation of HER3 and increase HRG expression. Adding MM-121 along with cetuximab inhibited the resistance and made the cancer cells sensitive to cetuximab[120]. Other preclinical studies have reported that combination of MM-121 with erlotinib completely inhibited Akt activation in pancreatic

cancer cells[123]. MM-121 also inhibited growth of ovarian cancer cells both *in vitro* and *in vivo*[124]. Gefitinib-resistant lung cancer cell lines were resensitized to gefitinib after being treated with MM-121[122].

As a result of these findings, currently there are 7 active clinical trials in phase I/II for MM-121 in combination with chemotherapies and other HER inhibitors for different cancer indications. While several phase II studies for MM-121 have been completed, some of them include two trials in breast cancer in combination with either paclitaxel or exemestane; one trial in ovarian cancer in combination with paclitaxel; and one trial in lung cancer in combination with erlotinib. In all of these studies, MM-121 showed a favorable effect in treating the cancer types in combination with other chemotherapies in patients with high heregulin.

MM-141 is a bispecific tetravalent antibody that binds to IGR1-R and HER3 and behaves as a tetravalent inhibitor of the PI3K pathways associated with IGF-1R and HER3. This novel molecule was designed and developed using a systems biology approach and was proven to be superior to monoclonal antibody combinations to IGF-R1 and HER3 *in silico*, *in vitro* and *in vivo*[142]. The rationale underlying the design of this molecule lies in the fact that many cancer cells respond to multiple growth factors and redundantly activate Akt. Thus in the case of MM-141, this molecule blocks PI3K signaling associated with IGF1, IGF2 and HRG by blocking their binding to IGF-R1 and HER3 receptively and induces receptor degradation[142]. Currently, the clinical activity and safety of MM-141 is under investigation in a phase I trial on patients with advanced solid tumors[125].

Another HER3-targeted drug is patritumab(U3-1287), the first fully humanized HER3 monoclonal antibody. Patritumab functions by inhibiting proximal and distal HER signaling and inducing rapid internalization of the receptor. In preclinical studies, patritumab showed inhibition of cancer growth in several *in vivo* cell lines such as those of the breast, lungs and colorectal. Furthermore, similar effects were seen *in vivo* in pancreatic, NSCLC and colorectal cancer xenograft models[127]. Currently, three phase I trials have been completed and they have shown that patritumab is well tolerated in NSCLC patients when combined with erlotinib; it was well tolerated in patients with advanced solid tumors and even showed evidence of disease stabilization in some cases[128]. A phase Ib/II trial using patritumab in combination with trastuzumab and paclitaxel is currently active with newly diagnosed metastatic breast cancer while a phase III trial is currently recruiting NSCLC patients to be treated with patritumab plus erlotinib or placebo plus erlotinib[129].

Aside from mAb, there are also antibodies developed that inhibits the dimerization of HER family receptors. Pertuzumab, discussed above, is one of those antibodies, which has proven to provide clinical benefit in HER2+ breast cancer patients. There are clinical trials going on to see the effects of pertuzumab in other cancer indications such as gastric cancer[130,131].

Another class of drugs called, pan inhibitors, are under development. One of the most advanced candidates is AZD8931, which is an equipotent, reversible inhibitor of EFGR, HER2, HER3. *In vitro* studies showed that AZD8931 is more potent than gefitinib or lapatinib in NSCLC and squamous cell carcinoma cell lines of the head and neck.

Furthermore, *in vivo* studies showed that ZD8931 inhibited xenograft growth in a range of models while significantly affecting EGFR, HER2, and HER3 phosphorylation and downstream signaling pathways, apoptosis, and proliferation[132]. Currently, a phase I trial is recruiting patients with esophageal cancer and gastro-esophageal cancer for combination treat of AZD8931 with oxaliplatin and capecitabine[133].

With these new and upcoming targeted therapies for HER3, researchers and clinicians are hopeful that more effective therapies for ErbB driven cancers will enter the clinic soon.

DISCUSSION

For decades, research around the ErbB family of receptors has been limited to dysregulation of EGFR and HER2 in cancer. Preclinical and clinical evidence indicates that HER3 reactivation or HER3 induced resistance to therapeutics in cancer is a major concern. Only recently have scientists have understood the clinical significance of HER3 and its role in cancer. Thus, while there are many potentially promising HER3 targeted drugs currently in clinical development, there is still an urgency and need to strategically develop new drugs in order to eliminate HER3 driven resistance and progression of cancer.

Such advancements can be made by two ways: (i) Using the systems biology to determine the most effective molecule designs by understanding ErbB network biology (ii) Making strategic drug combinations to supplement standard chemotherapies regimens and targeted agents.

Systems biology, as discussed earlier, is the computational and mathematical modeling of complex biological systems. By utilizing this holistic approach to biomedical research, Schoeberl et al. developed and trained a computational model to explore optimal ways to therapeutically inhibit combinatorial ligands that induce activation of ErbB/PI3K axis[120,124]. Further analysis found that HER3 was a critical node in PI3K/Akt pathway activation in cancer. The model further suggested that therapeutic intervention of this target might help cure cancer in patients. Based on this knowledge, MM-121 was developed and its action was consistent with that described by the computational model of binding to HER3, blocking HRG binding to HER3 and block BTC induced

phosphorylation of HER3[120,124]. Furthermore, these models helped determine the positive biomarkers that these drugs would be most therapeutically responsive.

Similarly, McDonagh et al. extended the computational model of ErbB signaling previously developed by Schoeberl et al. to design an optimal molecule to inhibit ligand activated HER2/HER3 heterodimers in HER2 overexpressing tumors[115]. Simulations from the model predicted a bispecific antibody that would bind to HER2 and subsequently to HER3 and would block ligand induced receptor activation[115]. This proved to be highly effective and superior in treating HER2-amplified tumors over monospecific HER2/HER3 inhibitors, which have been discussed in the previous section[115].

The studies discussed above confirm systems biology as a valid approach in designing and developing molecules. By understanding the network biology of ErbB receptors using systems biology, one can get a deeper insight into the interaction of how different ErbB monomers interact with one another and form all possible heterodimers. From this knowledge, potential molecules could be designed using systems biology that could block all the possible heterodimers of HER3 while simultaneously leading to receptor internalization as well as recruiting the immune system to attack targeted tumor cells.

Merrimack Pharmaceutical is a development stage pharmaceutical company that develops drugs using a systems biology approach. In addition to MM-111 and MM-121, Merrimack has several other antibodies in its pipeline aimed towards treating ErbB resistance cancers. Using predictive models and computational analysis, Merrimack has

developed MM-141, a bispecific antibody that blocks HER3 and IGF-1R; MM-151 which is a trimeric oligoclonal antibody that binds to three different epitopes on the EGFR receptor and MM-131 which is a bivalent anti-c-Met antibody that has a c-Met arm that competitively binds to c-Met receptor while the targeting arm binds to EpCAM, an tumor antigen[125]. In fact, Merrimack is using computational modeling to determine the optimal drug combinations in addition to its own drugs as well as the optimal dosage to effectively treat tumors.

Researchers and pharmaceutical companies should start adopting systems biology as a valid approach for finding and developing novel therapeutic targets for not only fighting tumor resistance, but also inhibiting cancer progression driven by HER3. Utilization of this strategic *in silico* approach would not only provide information for developing novel and effective molecules, but also expedite drugs to the clinic by overcoming the need of performing large chemical screens in the laboratory.

A second way to make advancements in therapies for HER3 driven and HER3 resistant tumors is by combining targeted therapies with traditional chemotherapies. While combination therapies have been around since the 1960s, when it was used first by James Holland, Emil Freireich and Emil Frei, strategic and rationally justified combination therapies should be developed to conquer drug resistance and enhance anti-tumor activity of ErbB directed therapies[145]. Due to the relationship of HER3 signaling with sensitivity or resistance to HER-targeted therapy, HER3 may be considered a valuable biomarker to monitor the efficacy of HER-targeted therapy. Thus, for example, in HER2+ breast cancer treated with trastuzumab, the potential HER3-driven resistance

should be targeted with HER3 inhibitors in combination to trastuzumab. In other words, combination of HER3 and EGFR/HER2-targeted agents might be an effective way to overcome HER3 induced resistance and deliberate the sensitivity to HER-2 targeted drugs. This approach has already been validated and is currently used in the clinic for treating HER2+ breast cancer by combining trastuzumab and pertuzumab[144]. On the contrary, the phase III MARIANNE study in which combination of TDM-1 and pertuzumab was compared the standard of care combination of trastuzumab and taxanes in treating previously untreated HER2+ breast cancer, did not prove the TDM-1 plus pertuzumab arm to be superior over the trastuzumab plus taxane arm in spite of the combination of two HER2 targeted therapies in the experimental arm[148]. This study indicates that while in theory targeted combination therapies maybe seem effective, it is much more complicated than that. Another approach could be the combination of inhibitor of molecular targets downstream of HER3, such as PI3K/Akt inhibitors, in combination to other targeted drugs and chemotherapies. This might prove to enhance antitumor effects by blocking PI3K/Akt pathway which plays an important role in tumor progression and drug resistance. Moreover, reducing or blocking the release of ligands such as heregulin can also lead to HER3 inhibition and thus could be added to a combination therapy.

Furthermore, systems biology can play a major role in determining the most effective and optimal combination of targeted drugs and chemotherapies based on predictive models and outcomes. Using computational models one would be able to pick

the most optimal combinations, dosage and any temporal order of drug delivery to the tumor.

Thus, systems biology as a field could prove to be a very effective approach for developing novel drugs for HER3 resistant and driven cancers as well as determining the most optimal combination therapies. Such combination therapies would include TKIs HER1/2, mAb against HER1/2/3, ligand blockers or antagonist and PI3K/Akt inhibitor in addition to standard chemotherapies that would hinder cell division. Finally, systems biology and computational modeling can be used to optimize the ideal dosage and temporal order of drug delivery, if needed. While in theory this may seem very idealistic, it is difficult to determine the toxicity of drugs without performing *in vivo* studies and clinical trials. Thus, further clinical evidence would be needed to validate the efficacy of systems biology in the clinic.

FINAL CONCLUSION

HER3 is a focal point in the ErbB family of receptors and plays a pivotal role in HER-related cancer cell resistance as well as tumor progression. Thus, it is important to target HER3 to regain sensitivity to SoC drugs and treat cancer. Over the past decade, many HER3 inhibitors have been under investigation and have proven to be promising in preclinical studies. As more therapies against HER3 develop, adoption of strategic approaches, such as systems biology, are necessary to discover novel and effective molecules as well as to develop and optimize these molecules and use them in different combination therapies. One should also consider the synergistic potential of these drugs

in treating HER3 resistance cancers in combination to those already out there in the clinic.

Thus, further research is needed to develop new or extend current computation models for ErbB networks in order to develop effective targeted drugs against HER3 with the aid of systems biology.

LIST OF ABBREVIATED JOURNAL TITLES

ESMO J.	European Society for Medical Oncology Journal
JAMA	Journal of the American Medical Association
PLoS Medicine	Public Library of Science Medicine

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CURRICULUM VITAE

Ankush Chandra
91 Bay State Road, #7098
Boston, MA 02215
(516)244-6810
achandra@bu.edu

PERSONAL DATA

Year of Birth: 1989
Place of Birth: Hicksville, New York
Marital Status: Single
Sex: Male

EDUCATION

Boston University School of Medicine, Boston, MA *Expected May 2015*
Masters of Science in Medical Sciences

Boston University, Boston, MA *May 2011*
***Bachelor of Arts* in Biology and Neuroscience (with Distinction)**

RESEARCH EXPERIENCE

Merrimack Pharmaceuticals, Cambridge, MA
Research Intern, June 2013-Present
Principle Investigator: Dr. Johanna Lahdenranta

Massachusetts General Hospital, Department of Neurology, Boston, MA
Research Technician, June 2011- July 2013
Principal Investigator: Dr. Florian Eichler

Boston University, Department of Biology, Boston, MA
Undergraduate Researcher, September 2009- June 2011
Principal Investigator: Dr. Hengye Man

Boston University, Center for Memory and Brain, Boston, MA
Undergraduate Research Assistant, Summer 2009
Principal Investigator: Dr. Howard Eichenbaum

Boston Medical Center, Department of Endocrinology, Boston, MA
Undergraduate Research Assistant, December 2007- December 2008
Principal Investigator: Dr. Shalender Bhasin

Weill Cornell Medical College, Department of Obstetrics and Gynecology, New York, NY
Research Assistant, June 2007- August 2007
Principal Investigator: Dr. Brij Saxena

POSTER PRESENTATIONS

October 2012 — Presented poster at *Society for Neuroscience 2012* meeting in New Orleans, LA

Ankush Chandra, Brian Schmidt, Tracey Suter, Florian Eichler. *L-Serine and L-Alanine have divergent SPT dependent effects upon Dorsal Root Ganglion Morphology: Implications for the Neuropathy in HSAN1*

March 2012 — Presented Poster at American Society for Neurochemistry, Baltimore, MD

Ankush Chandra, Brian Schmidt, Tracey Suter, Florian Eichler. *L-Serine and L-Alanine have divergent SPT dependent effects upon Dorsal Root Ganglion Morphology: Implications for the Neuropathy in HSAN1*

PUBLICATIONS

Chandra A, Schmidt BP, Suter TA, Eichler F. *SPT dependent effects upon growth of DRG neurons: Implications for HSAN1*.(2013) Manuscript in preparation

Singh H, Singh P, Kumari K, **Chandra A**, Dass SK, Chandra R. *A Review on Noscapine, and its impact on Heme Metabolism*. Curr Drug Metab. 2013 Mar;14(3):351-60

Chandra R, Madan J, Singh P, **Chandra A**, Kumar P, Tomar V, Dass SK. *Implications of Nanoscale Based Drug Delivery Systems in Delivery and Targeting Tubulin Binding Agent, Noscapine in Cancer Cells*. Curr Drug Metab. 2012 Dec;13(10):1476-83.

AWARDS/HONORS

Robert F. Troxley Award in Biochemistry, Boston University School of Medicin
2013

- Recipients of this award are graduate students in the Masters of Medical Science program that obtain the highest overall grade in Biochemistry among the 180 students in the program.

Scarlet Key Award, Boston University
2011

- Recipients of the Scarlet Key Award are students in their senior year who have exhibited exceptional leadership during their years at Boston University.
- Selection to Scarlet Key is based on excellence in University student activities and organizations, commitment to the individual's School or College, and scholarship.

Senior Independent Work for Distinction in Neuroscience
2011

- Completed senior thesis under the mentorship of Dr. Hengye Man

Commitment to Residence Life Award, Office of Residence Life, Boston University
2011

- Awarded for commitment and dedication to the mission and goals of residence life at Boston University

Summer Undergraduate Research Grant, Undergraduate Research Opportunity Program
2010

- Awarded by Boston University to conduct research in the Man laboratory

LEADERSHIP AND VOLUNTEER EXPERIENCE

SquashBusters- Boston, MA
Academic Coach, February 2013- July 2013

United Innoworks Academy - Boston University (www.innoworks.org)
Chief Curriculum Officer, Spring 2009- May 2011

- Founding members of InnoWorks at BU.
- The purpose of the group is to organize a summer science camp for underprivileged youth.

Boston University, Office of Residence Life
Resident Assistant, August 2009- May 2011 and August 2012-August 2013
Graduate Resident Assistant, August 2013- Present

Boston University Cricket Club
President and Captain, Fall 2009- May 2011

ADDITIONAL EXPERIENCE

Boston University, Information Services and Technology
Student Customer Service Support Consultant,
Summer 2010- May 2011 and July 2013- Present

Boston University, Educational Resource Center
Peer Tutor, Spring 2009- May 2011