

Compound HER2 (ERBB2) Mutations in Breast Cancer: Molecular Evolution, Structural Cooperativity, and Therapeutic Implications

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Abstract

Human epidermal growth factor receptor 2 (HER2; ERBB2) has been a recognized driver of breast cancer for decades with its amplification acting as a major predictor of disease and target for treatment. However, with the advent of next-generation sequencing (NGS) technologies, it has been shown that activating mutations on the ERBB2 gene act as oncogenic events in about 2–5% of breast cancers, including cases that are HER2-negative. NGS technique identified compound mutations in the HER2 gene, which were defined as the presence of two or more mutations in a HER2 gene and found in breast cancers related to higher oncogenic potential and treatment resistance. However, compound mutations appear to be more complex than conventional mutations. Studies demonstrated that the compound mutational process can affect the function and activity of kinases, which in turn affects various signaling pathways and alters breast cancer cells' susceptibility to treatment. While the increasing number of compound mutations in HER2 has become apparent through tissue and ctDNA sequencing, the extent of their occurrence, biological diversity, and relevance to treatment remain unclear. The purpose of this review is to assess the existing evidence in relation to the genetics of compound mutations in HER2, as well as the mechanism of their emergence, their structure and functions, drug sensitivity and therapeutic implications of these mutations in breast cancers. A comprehensive list of literature-derived reported compound mutations in HER2 was compiled and future work with regard to validation of the function of HER2 mutations was also discussed.

Keywords: HER2 mutation; compound HER2 mutation; ERBB2; breast cancer; precision oncology.

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1. Introduction

The identification of the human epidermal growth factor receptor 2 (HER2; ERBB2) as one of the primary drivers for breast cancer revolutionized cancer therapy and became one of the first significant advancement in precision medicine. Around 15-20% of ERBB2 oncogene amplification was detected in all breast cancers and untreated patients receiving no targeted treatment usually have the worst prognosis. The introduction of HER2-targeted therapy, such as trastuzumab and pertuzumab, and tyrosine kinase inhibitors (TKIs), has made a remarkable impact on treatment outcomes for HER2-positive breast cancer [1–3]. Nevertheless, there are still some barriers associated with the resistance of HER2-targeted therapy.

Molecular profiling tools such as next-generation sequencing (NGS) have considerably widened the molecular scope of HER2-related breast cancer, moving beyond the amplification of the gene. Large genomic sequencing studies have revealed that activating mutations of the ERBB2 gene have been detected in approximately 2-5% of breast cancers, with the majority of those mutations being observed in the cases of breast cancer without evidence of HER2 amplification and/or overexpression [4]. In contrast to HER2-amplified tumors, HER2 mutation-positive breast cancers commonly occur in hormone receptor-positive patients and in invasive lobular carcinomas and, hence, are a molecular subtype of breast cancer distinct from those characterized by conventional HER2 testing.

Previous research shows that HER2 mutations often behave as a powerful cause of oncogenes responsible for constitutive activation of HER2 signaling through various structural mechanisms and that several of these mutations were sensitive to the irreversible HER2 inhibition with neratinib, has been a breakthrough in this area [5].

Majority of the studies done on HER2-mutated breast cancer have centered on the importance of single activating mutations in relation to biology and therapy of the disease. With increasing use of genome profiling analysis, however, it is now understood that there are some cancers where there are multiple concurrent HER2 mutations, which have been known as double or compound HER2 mutations. This can occur either at the time of diagnosis or develop during the course of HER2 targeted treatment [6]. Clinical observations from studies like SUMMIT, MutHER, and plasma MATCH shows that HER2 mutant tumors have heterogeneous reactions to HER2 targeted treatments and usually develop more genetic mutations under therapy [7–9]. This confirms that the secondary HER2 mutations do not seem to occur randomly but rather under therapeutic pressure. In certain cases, HER2 mutations occur as a result of tumor evolution or clonal diversification and contribute to adaptive resistance by restoring HER2 signaling despite kinase inhibition.

The study done by Marín et al. was the first to show how acquired secondary HER2 mutations enhancing the HER2/MAPK signaling, promote resistance to

neratinib, and have better oncogenic fitness than the primary HER2 activating mutation, making it an effective mechanism of resistance in cancer treatment [10]. The results of this study further complement previous evidence establishing that the acquisition of secondary mutations such as L755S can lead to HER2 reactivation and can also lead to drug resistance [11]. This is further supported by findings showing that the mechanism of gain-of-function mutations in HER2 and the dimerization partner HER3 enhances receptor activation and reduces sensitivity to neratinib, thereby showing that compound mutations are not limited only to HER2 [12]. In addition to HER2-targeted therapy, compound HER2 mutations have been documented as mechanisms of acquired resistance to endocrine therapy used in HER2 mutation-positive and ER-positive breast cancer [13].

Beyond just the presence of two or more pathogenic mutations together, the biology of compound HER2 mutations can become complex due to the possibility of interactions and collaboration between these mutations. Structural and functional research has shown that these interactions could change the conformation of the receptor, boost its catalytic activity, affect the ATP binding dynamics, increase dimerization, and also strengthen the pathway of downstream signaling. This implies that the biology of compound HER2 mutations is not entirely predictable based on the properties of each single mutation. However, despite all these developments, compound HER2 mutations continue to be among the least understood aspects of HER2 biology. Majority of existing literature discusses HER2 mutations without differentiation of the specific genome arrangement, biological consequences, or therapeutic implications of compound mutations [14]. In addition, all the available evidence is spread throughout different studies including genome sequencing research, functional studies, structural analysis, basket trials, and individual case reports, which makes it hard to achieve an integrative picture of this novel molecular entity. To the best of our knowledge, there is no unified review article conducted on compound HER2 mutations in breast cancer that integrates its genomic, structural, functional, and clinical evidences. Hence, the objective of this review is to explore the formation of compound mutations due to tumor evolution under therapeutic selection, and the contribution of structural modelling to the understanding of cooperative interactions between specific pairs of mutations. It also seeks to gather the existing experimental data available on the sensitivity of compounds to drugs in case of specific compound mutations to understand the degree of their functional validation and heterogeneity of sensitivity profiles. Thus this review attempt to identify the gap in functional validation and, propose an essential step towards genotype-specific treatment of HER2-mutant breast cancer based on structural prediction.

2. Landscape of Compound HER2 Mutations

2.1 Defining Compound HER2 Mutations

The growing application of advanced genomic profiling has indicated that some cases of HER2-positive breast cancer include more than one

pathogenic ERBB2 alteration in one tumor [15]. This mutation has been denoted by different terms, such as double HER2 mutations, co-occurring HER2 mutations, multiple HER2 mutations, and compound HER2 mutations. Although they are often treated as equivalent terms, they may not always mean the same thing, leading to complications in the comparison across genomic studies due to the lack of standardized nomenclature.

In the present review, HER2 compound mutations are described as the occurrence of two or more sequence changes in the HER2 gene that have pathogenic potential, found in the same tumor sample either at the time of diagnosis or at some stage in the course of the disease progression. The concept of compound mutations includes both activating mutations that coexist in tumors not exposed to HER2-targeting therapies, as well as secondary HER2 mutations arising after HER2-targeting treatment. If the allele phasing data is known, compound mutations can be in *cis*, both mutations appearing in the same allele, or in *trans*, where each mutation affects a different allele. Despite *cis* mutation phasing being generally regarded as more probable for cooperation in the structural impact on HER2 protein, phasing data are not usually provided in regular clinical sequencing studies [5,10] which reflects a notable limitation in current genomic datasets.

From a biological standpoint, compound HER2 mutations cannot be considered merely as the simultaneous occurrence of two different genomic events. Instead, they are viewed as the cumulative result of the evolutionary process of the tumor, selective pressure and clonal diversification. Based on the combination of mutations, compound mutations may lead to an increase in receptor activity, stabilization of kinase conformations, alteration in ATP binding, or resistance to kinase inhibitors. Accordingly, the biological characteristics of compound mutations may be quite different from those of individual mutations [10].

In this review, for enhanced clarity, compound HER2 mutations are classified based on their evolutionary nature and not simply based on protein domains. Four different types are distinguished: *de novo* compound mutations, occurring before the beginning of systemic therapy [15]; therapy-selected compound mutations, occurring due to the selection pressure from therapy [6]; resistance-associated compound mutations, with experimental evidence of reduced sensitivity to HER2-targeted therapies [16]; and complex HER2 mutations, with three or more pathogenic variants [4]. This biological classification facilitates the knowledge of the processes of tumor evolution and is of greater significance when assessing the clinical relevance of compound HER2 mutations.

2.2 Genomic Landscape of Compound HER2 Mutations

Sequencing efforts have revealed that activating ERBB2 mutations exist in about 2 to 5% of breast cancer cases, with incidences being higher among hormone-receptor positive metastatic breast cancer and invasive lobular carcinoma [5,17]. Nevertheless,

the incidence rate of compound HER2 mutations is much lower and less defined due to the fact that many genomic profiling studies often report the existence of single HER2 mutations without mentioning multiple HER2 mutations existing together in a single tumor sample.

The early studies mainly concentrated on discovering the recurrent activating mutations like S310F/Y, L755S, D769H/Y, V777L, V842I, and L869R and thereby defining their importance as oncogenic drivers [5]. With an increase in the depth of sequencing and with more patients included in the study, it has been found out that a certain percentage of HER2 mutants contained two or more mutations in the ERBB2 gene. Compound mutations have thus been identified to occur in primary and metastatic breast cancer but they seem to be more common in advanced cases after HER2-targeted therapy [7,10]. Independent functional work has clearly indicated differential drug responsiveness corresponding to confirmed genotypes of particular kinase-domain compounds, whereby it appears that the L755S+V842I mutation causes resistance to trastuzumab, while the L755S+K753I mutation leads to resistance to lapatinib, with both combinations retaining susceptibility to pan-HER TKIs. [18]. Changes are not limited by the presence of coexisting point mutations; instead, HER2-mutated tumors that obtain HER2 amplification together form another kind of compound genotype, providing for a unique sensitivity to neratinib [19] (Table 1).

The molecular composition of compound HER2 mutations is heterogeneous. While some tumors carry double activating mutations in the kinase domain, others have extracellular domain mutations along with mutations in the kinase domain alterations or mutations in exon 20 along with secondary mutations [20]. Each combination represents a biologically distinct mechanism. De novo compound mutations could occur via a series of mutations occurring during cancer development [15] while therapy-selected compound mutations usually arise via an adaptive response to pharmacological inhibitors of HER2 activity [6]. Notably, all compound mutations do not have identical biological outcomes, underscoring the importance of carrying out functional analysis for each individual mutation and not generalizing that all compounds behave identically.

Despite the fact that genomic evidence shows that compound HER2 mutations are of clinical significance, there are still many questions that remain unanswered. It is still uncertain how prevalent they are among the different intrinsic subtypes of breast cancer. The effects of other alterations that occur along with these mutations, such as mutations of PIK3CA or TP53, have yet to be adequately studied. Finally, the physiological and biological meaning of many rare mutation combinations is still unknown. Advancing knowledge in these areas require a standardized approach to reporting the presence of HER2 mutations as well as prospective genomic studies and methods for serial molecular monitoring.

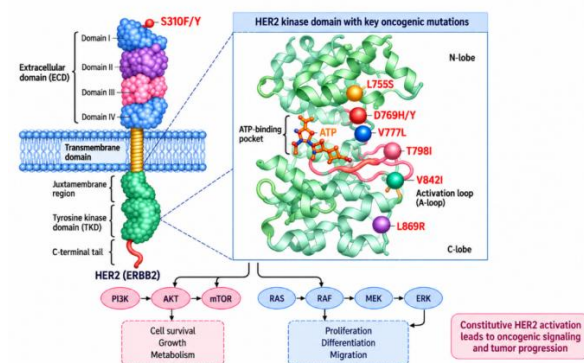


Figure 1. **Structural organization of HER2 (ERBB2) and major oncogenic mutation hotspots.**

Schematic of HER2 highlighting its extracellular, transmembrane, juxtamembrane, and tyrosine kinase domains, with major oncogenic mutations mapped to the receptor. The enlarged kinase-domain view depicts the ATP-binding pocket and activation loop. HER2 activation engages the PI3K–AKT–mTOR and RAS–RAF–MEK–ERK pathways, promoting cell survival, proliferation, and tumor progression.

2.3 Origin-Based Classification of Compound HER2 Mutations

2.3.1 De novo compound HER2 mutations

De novo compound mutations occur prior to systemic therapy and are expected to occur during the spontaneous evolution of tumor formation. Despite being relatively rare, de novo mutations demonstrate that acquiring several activating ERBB2 mutations may be an early oncogenic event and not merely an acquired form of resistance. The most common combinations of de novo mutations involve either two activating kinase-domain mutations or an extracellular domain mutation coupled with a kinase-domain mutation. Functionally, these mutations are shown to work cooperatively and increase the transforming potential of HER2 signaling compared to individual mutations [5,10].

The biological importance of these mutations is still unclear due to a limited number of prospective studies and the fact that the majority of evidence comes from retrospective analysis of the genetic sequences. Nevertheless, it is proven that de novo compound mutations exist and cannot be treated only as therapy-induced mutations. Rather, they are one way for a tumor to refine HER2 signaling pathways during malignant transformation.

2.3.2 Therapy-selected compound HER2 mutations

The highest number of identified HER2 compound mutations occurs after the patients have undergone therapy, especially when treated with irreversible tyrosine kinase inhibitors such as neratinib. Research has shown that patients who had one single activating HER2 mutation at first, usually develop additional mutations during therapeutic treatments, thus forming compound mutated clones, which predominate hereafter during tumor resistance [10,21].

Moreover, in many cases, secondary mutations

affect the sites responsible for kinase activation, ATP binding, or receptor conformation, which indicates that resistance to therapy, does not depend only on activation of signaling pathways, but also on the structural reorganization of the kinase domain of HER2. These kind of mutational changes are not limited to two co-occurring mutations rather in specific instances they may expand further under sustained selective pressure.

Studies on genomic alterations have also shown the existence of cancers with three or more pathogenic variants, which is termed as HER2 complex alterations. Such instances are still rare and now represented mostly by individual case reports or through comprehensive sequencing studies. However, these cases demonstrate great genomic plasticity of HER2-mutant breast cancer [22].

A key consideration is that the biological aspects of complex HER2 mutations are underexplored. It is unclear whether successive mutations function in an additive way or act synergistically against the receptor activation. It is also unclear that HER2 mutations accumulation indicates progressive optimization of the kinase signaling or the increased genomic instability during the tumor development. Therefore, functional validation of rare genomic combinations becomes an important aim for future research.

With the emergence of ultra-deep sequencing and the use of longitudinal ctDNA monitoring, there will most likely be an increase in the cases of reported complex HER2 mutations. Therefore, in order to classify these new compounds, future classifications should include compound HER2 mutations as part of a continuum and not limit analyses to tumors with only two pathogenic variants.

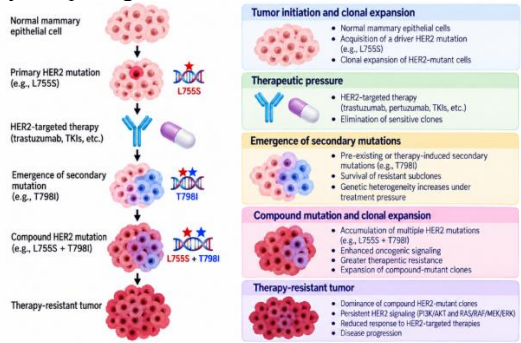


Figure 2. Evolutionary emergence of compound HER2 mutations under therapeutic pressure. Schematic illustrating the progression from a primary HER2 mutation through therapy-associated secondary mutation acquisition and clonal expansion to compound HER2-mutant, therapy-resistant disease, highlighting persistent HER2 signaling and increased tumor heterogeneity during treatment.

2.4 Evolutionary Dynamics of Compound HER2 Mutations

Compound HER2 mutations should be viewed in the context of tumour evolution. Tumour development involves accumulation of genetic alterations, where selective pressures exerted by the tumor microenvironment and systemic treatment affect the clonal structure over time [23]. In HER2-positive breast cancer, HER2-targeted treatment is regarded as one of the strongest selective forces influencing clonal evolution.

Current evidence supports the stepwise theory of tumor evolution. Initially, the tumor acquires the activating HER2 mutation which acts as the key oncogenic driver. The application of HER2-targeted therapy represses the dominant clone but creates the conditions for survival of rare subclones with secondary HER2 alterations. Ultimately, the further growth of these resistant subclones leads to generation of compound HER2 mutations which can sustain downstream signaling even with continued therapeutic inhibition [7,10,21].

Notably, clonal evolution is not always linear. Multiple ctDNA studies have demonstrated the existence of several resistant subclones at the same time, each with its own combinations of HER2 mutations. As a result, breast cancer is often characterized by significant temporal and spatial heterogeneity, and the staging of metastases in different locations can show specific genetic pattern. The resulting variability is of significant clinical importance as the therapeutic choice based on one single biopsy cannot fully reflect all mechanisms of resistance observed in an individual patient [7,21].

The evolutionary theory also gives a conceptual framework that helps explain the remarkable diversity of compound HER2 mutations reported in the last few years. These mutations have been seen to develop through an adaptive process rather than following some predetermined pattern of mutational events; therefore, different combinations of mutations can be viewed as different solutions to a similar adaptive goal of maintaining HER2 signaling. This view brings the focus from the individual mutations to the larger evolutionary processes responsible for therapeutic resistance as well as emphasizes the significance of longitudinal molecular monitoring in the course of therapy.

While there is substantial data regarding therapy-driven clonal evolution, there are still some significant questions that are yet to be answered. These include the sequence of events leading to the development of the secondary HER2 mutations, the extent to which the resistant clones already present contribute to resistance compared to new mutations that could arise, and how other genomic changes may affect the evolutionary pattern.

Table 1. Reported combinations of primary and secondary HER2 (ERBB2) mutations in breast cancer

Category	Founder (primary) mutation	Co-occurring / acquired (secondary) mutation	Reported biological significance	Structural Location	Reference
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Extracellular domain (ECD) + Kinase domain (KD)	S310F/S310Y	L755S, V777L, L755S, D769Y, G776V, T798I, L841V, L869R	Enhanced receptor activation; potential cooperative signaling; increased transforming activity	ECD + Tyrosine kinase domain	[5,10]
Kinase domain + Kinase domain	L755S, L755P, V777L, D769Y, L869R, G778_P789dup	S310Y, S310F, R678Q, V697L, I767M, T798I, T862A,	Increased kinase activation; altered ATP-binding; reduced sensitivity to specific HER2 TKIs	Tyrosine kinase domain	[7,10,18]
Extracellular domain (ECD) + Exon 20 insertion	S310F/S310Y	Exon 20 insertion	Rare combinations; functional significance remains largely unknown	ECD + Kinase domain	[5]
Kinase domain + Exon 20 insertion	L755S, L869R, D769Y	Exon 20 insertion	Predicted enhancement of kinase signaling; limited functional validation	Kinase domain	[10]
HER2 alterations	G778_P780dup, P761del	S310Y, S310F, R678Q, V697L, I767M, T798I, D808H,	Exon-20 insertion; extends the α C- β 4 loop.	Kinase domain	[10]
Therapy-acquired secondary mutation	Pre-existing primary HER2 mutation	New mutation emerging after neratinib therapy	Mechanism of acquired resistance; restoration of HER2/MAPK signaling	Usually kinase domain	[7,8,10]

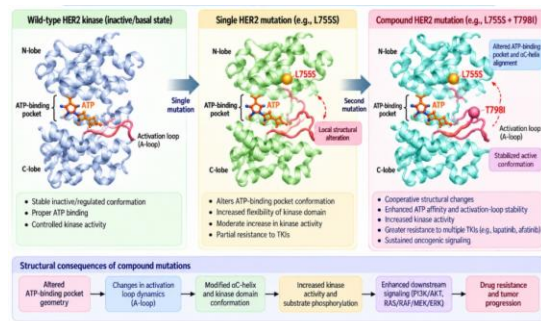


Figure 3. Structural cooperativity of compound HER2 mutations and its implications for kinase activation and drug resistance.

Comparison of wild-type HER2, a single L755S mutation, and the compound L755S/T798I mutation illustrating alterations in the ATP-binding pocket, activation-loop dynamics, and kinase-domain conformation that may enhance kinase activity, sustain downstream signaling, and contribute to therapeutic resistance.

3. Molecular and Structural Basis of Compound

HER2 Mutations

3.1 Structural Organization of the HER2 Receptor and Activation Mechanisms

While HER2 has traditionally been considered an orphan receptor in the epidermal growth factor receptor (EGFR/ERBB) family, a large number of structural studies have shown that it is found in a permanently open state, which allows HER2 to heterodimerize with other receptors of the ERBB

family. The special structural characteristic makes HER2 different from EGFR and HER3, thus making HER2 a highly oncogenic molecule under gene amplification or activating mutation conditions [24,25].

The HER2 protein has a structure that comprises an extracellular domain (ECD), a single-pass transmembrane helix, intracellular juxtamembrane region, tyrosine kinase domain, and a C-terminal regulatory tail. Mutations that activate the receptor can occur anywhere within the receptor, although the most common mutations occur in the extracellular domain (e.g., S310F/Y) and in the kinase domain (e.g., L755S, D769Y, V777L, V842I, L869R). These mutations induce receptor activation through different structural mechanisms such as stabilization of active kinase conformation; improved interaction with ATP; inactivation of auto inhibitory contacts, or increased receptor dimerization [5,10].

Notably, mutations in individual proteins use different methods for promoting HER2 signaling. Mutations in the extracellular domain mainly facilitate receptor dimerization [26], while mutations in the kinase domain lead to an increase in the catalytic activity [27]. As a consequence combinations of mutations on different structural domains can lead to synergistic biological activities that transcend what each mutation alone can accomplish [6]. This concept supports the structural premise of interpreting compound HER2 mutations in terms of unique molecular entities instead of mere combinations of isolated mutations.

3.2 Structural Cooperation between Co-occurring HER2 Mutations

One of the most important emerging concepts in HER2 biology is that the functional consequences of compound mutations cannot be predicted solely based on the effects of their components. In fact, many mutations may interact with each other and contribute to a new receptor conformation and changes in the kinase activity. Experimental studies that combine structural modeling and biochemical analysis have shown that secondary HER2 mutations may potentially stabilize an active form of the kinase or modify an ATP binding site, thus enhancing downstream signaling in the presence of HER2 inhibition [10,28].

For instance, extracellular domain mutations like S310F lead to receptor dimerization due to their interference with intramolecular interactions that maintain the inactive state of the receptor. In combination with mutations in the kinase domain, the receptors such as L755S or V777L are able to achieve both high levels of dimerization and higher catalytic activity at once. These dual mechanism can be attributed to the greater transforming capacity of some compound mutations compared to constituent mutation alone [6,26,27].

Similarly, combinations of two mutations in the kinase domain can together give rise to degradation of the ATP-binding dynamics, modification of the activation loop flexibility or stabilization of the α C-helix in the active position. Structural simulations executed by Ishiyama and colleagues showed changes in several hydrogen-bonding networks due

to activating mutations at HER2, resulting in conformational changes in the kinase and affecting the binding of inhibitors as well as their catalytic efficiency [28]. Despite the absence of comprehensive structural analysis for all known compound mutations, the available data clearly confirms the key role of cooperative structural interactions in their biological function. The cooperative structural interactions are not restricted only to the alterations happening with HER2, as computational structural simulations supported by biochemical studies have suggested that a gain-of-function HER3 kinase domain mutation can increase HER2/HER3 heterodimer affinity and decrease the binding of neratinib, exemplifying a type of cross-gene structural cooperation that was previously shown to occur in terms of intermolecular cooperation with the involvement of compound HER2 mutation [12].

3.3 Functional Consequences of Compound HER2 Mutations

Structural cooperativity ultimately leads to better oncogenic signaling. In comparison with single activating mutations, compound HER2 mutations are known for increased receptor phosphorylation, endured activity of the MAPK and PI3K/AKT pathways, induction of faster cellular proliferation, and making the cells less sensitive to the targeted therapy against HER2 [7,10]. Preclinical studies have demonstrated that cells expressing compound HER2 mutations are characterized by an increased ability to transform, anchorage-independent growth, and better tumor formation efficiency in xenografts models as compared to cells carrying only the corresponding single mutation. At the same time, it should be noted that not all mutant pairs are equally functional. Some are associated with a slight additive effect, while others show a synergistic activity, suggesting that the biological behavior depends not only on the number of mutations but on their structural interaction as well [26,27]. These findings highlight the importance of performing an experimental study on HER2 mutations in compounds rather than relying on information from single mutations. It also explains biologically the variable treatment reactions seen among patients carrying various compound mutations. Although significant progress has been achieved, significant mechanistic questions remain unsolved. It is not known whether different combinations will lead to the common signaling mechanisms or different downstream pathways.

4. Therapeutic Landscape and Drug Sensitivity of Compound HER2 Mutations

4.1 Current HER2-targeted therapies in HER2-mutant Breast Cancer

In the past decade, the therapeutic landscape of HER2-mutant breast cancer has changed significantly due to advancement in the molecular profiling and the introduction of HER2-directed agents that are able to specifically inhibit the activity of ERBB2 mutants independent of HER2 amplification. While monoclonal antibodies continue to dominate the treatment of HER2-positive breast cancer, HER2-mutant tumors depend

on kinase activity in treating patients, thus making small-molecule tyrosine kinase inhibitors (TKIs) offer a promising avenue for research [5,7]. Out of all existing drugs, neratinib is considered to be the most reliable clinically active drug against HER2 mutant breast cancer (Table 2). Neratinib is described as irreversible pan-HER tyrosine kinase inhibitor that forms covalent binding of ATP pocket of HER2 receptor and leads to persistent phosphorylation inhibition. Clinical trials, especially SUMMIT basket study, proved neratinib to be a reliable agent in the treatment of HER2 mutant metastatic breast cancer, especially in combination with fulvestrant and trastuzumab [21].

There have been different activities observed with other HER2-specific TKIs such as tucatinib, poziotinib, and pyrotinib. Their effectiveness seems to be influenced by the particular mutation involved since kinase domain mutations tend to be selectively sensitive depending on the structural consequences brought by a particular amino acid substitution. This variability suggests the potential for compound HER2 mutations to have a mutation-specific response to therapy rather than drug sensitivity [8,10]. Antibody drug conjugates (ADCs), specifically Trastuzumab deruxtecan (T-DXd), have proved to be a highly active form of treatment against HER2 mutated breast cancers. Unlike TKIs, ADCs do not function by inhibition of kinases but instead use receptor expression and internalization for treatment. Thus, even the presence of multiple HER2 mutations does not necessarily affect the activity of ADCs, although there are limited research on this subject matter [29].

4.2 Co-occurring Alterations and Resistance Monitoring

Resistance to HER2 targeted therapy is an evolving process that entails both HER2-dependent as well as HER2 independent mechanisms. Compound mutations of HER2 are involved in developing resistance mainly because of the restored receptor signaling regardless of drug mediated inhibition. In addition to the secondary mutations of HER2, resistant tumors often acquire additional genomic alterations affecting PIK3CA, TP53, NF1, as well as cell-cycle regulatory genes. These other alterations reduce dependency on HER2 signaling and limit the degree of effectiveness of HER2 therapies. Thus, the resistance mechanism involving HER2 compound mutations should be considered as the part of a broader adaptive evolution process instead of individual molecular event [10,17].

The rising utilization of serial circulating tumor DNA (ctDNA) analysis has considerably enhanced the comprehension of resistance mechanisms. In contrast to the conventional biopsies of tissues, ctDNA allows for the observation of clonal development in real-time and identification of new mutations of HER2 before their clinical emergence becomes apparent. This method presents an effective possibility of early detection of resistant HER2 mutants and optimizing treatment accordingly.

4.3 Mechanistic Basis of Altered Drug Sensitivity

In the context of therapeutic choices, not every

mutation is effective in imparting resistance to the treatment. All available experimental proof shows that certain combinations of mutations play a substantial role in reducing the sensitivity to HER2 inhibition. Marín and co-authors established the fact that a number of acquired secondary mutations of HER2 can enhance MAPK signaling even though it is inhibited and lead to obtaining much higher resistance to the application of neratinib compared to the original activating mutation only [10]. These results show that the compound mutations associated with resistance are a biologically unique group of mutations due to their role in cooperatively enhancing cancer cell signaling processes rather than simple mutation accumulation. From a mechanistic point of view, resistance-associated compound mutations seem to act via various complementary pathways. Structural modelling has revealed that some secondary mutations help to maintain an active kinase conformation while others may change ATP-binding or interactions between HER2 and inhibitors. Functional analyses have additionally reported that there is enhanced phosphorylation of HER2 and activation of downstream ERK signaling even in the presence of high concentrations of irreversible TKIs. Overall, these mechanisms allow maintaining proliferative signaling under drug pressure and promotes the growth of the tumor [10,28].

4.4 Mutation-specific Evidence of Differential Drug Sensitivity

The L755S+V842I compound mutation leads to trastuzumab resistance, whereas L755S+K753I is linked to lapatinib resistance. Nevertheless, both variants remain sensitive to pan-HER tyrosine kinase inhibitors (TKIs) including neratinib [18]. Such data differ from those reported by Marín et al., where a different compound HER2 mutation exhibited lower sensitivity to neratinib. Thus, it can be concluded that the therapeutic outcomes of compound HER2 mutations strongly depends on the clinical context. In other words, compound mutations might lead to resistance to one class of HER2-targeted treatment while remaining sensitive to another type, depending on the changes made in kinase conformation, ATP-binding site geometry or drug-binding pocket accessibility. Moreover, another compound genotype, which consists of HER2 mutation with HER2 gene amplification, reveals increased sensitivity to neratinib [19]. Thus, all of these data indicate that compound HER2 mutations are not an identical group of drug-resistant mutations rather demonstrate varied biological and therapeutic responses based on its molecular conditions. The rest of the HER2 mutation combinations listed in Table 1, such as those involving ECD in combination with exon 20 insertions, kinase domain (KD) in combination with exon 20 insertions, and triple or complex HER2 mutations, are only justified by their structure and mechanism, without any supporting experimental data about their treatment response. As such, the majority of the combinations of HER2 mutations described in this review remain unstudied using either preclinical or clinical studies against HER2-targeted therapies mentioned above. This

demonstrates a considerable gap in knowledge, pointing to the necessity for systematic functional characterization in order to know how these various combinations of mutations affect drug sensitivity and resistance.

4.5 Clinical Confirmation: Compound Mutation Status and Outcomes in Prospective Trials

Clinical evidence that supports the idea of compound HER2 mutations being a biologically separate subgroup comes from exploratory studies such as the SUMMIT and MutHER trials, two key trials assessing neratinib for HER2-mutant breast cancer. In the SUMMIT trial, almost all patients with tumors containing compound HER2-activating mutations had poor or lack of clinical response to neratinib treatment. A similar trend was observed in the small number of patients having tumors with dual HER2 mutations included in the MutHER trial [9]. Despite the low number of patients, consistency between both of the mentioned clinical trials serves as good evidence for the possibility of compound HER2 mutations reducing sensitivity to neratinib. It is important to note that this clinical evidence is consistent with the preclinical mechanism explained previously where compound mutations restore HER2/MAPK signaling pathway and thus reduce effectiveness of inhibitors compared to activating mutations.

These results need to be interpreted in context of the wider world of clinical trials, which has been largely focused on HER2-mutant disease in general rather than compound HER2 mutations in particular. SUMMIT, MutHER, and plasma MATCH trials have all independently confirmed that the neratinib-based treatments are capable of providing clinically meaningful response in HER2-mutant metastatic breast cancer patients, where response is determined

by both treatment regimen and drug combination strategy. Importantly, plasma MATCH enrolled patients based on ctDNA testing instead of tumor tissue sequencing, and found that ctDNA-based identification is capable of identifying patients who have similar response to those identified by traditional methods (Table 2) [8,9]. None of these studies were actually designed to evaluate compound HER2 mutations as a predetermined stratification factor. The insights from SUMMIT and MutHER trials also came from exploratory analyses of small subgroups of patients rather than pre-specified endpoints of the respective studies. Thus, though these results present interesting preliminary information on the potential role of compound HER2 mutations, they should be viewed as hypothesis-generating and require further confirmation with a large sample size with prospectively designed studies.

Evidence supporting the use of treatments apart from neratinib in relation to compound HER2 mutations is quite limited. Tucatinib and the first-generation trastuzumab deruxtecan (T-DXd) have proven their efficacy primarily in patients with HER2 amplified, but not HER2 mutant, breast cancer [29,30]. Therefore, studies do not provide information on the effect of compound HER2 mutations on treatment outcomes (Table 2). An exception to this is DESTINY-PanTumor01, the mutation-selected trial, investigating the efficacy of trastuzumab deruxtecan in multiple solid tumors with activating HER2 mutations. Yet, the efficacy of these therapies was not described separately for patients with single and compound HER2 mutations [31]. Therefore, the effect of compound mutation status on the efficacy of trastuzumab deruxtecan remains unknown. This indicates another significant gap in the contemporary clinical evidence.

Table 2. Therapeutic landscape of HER2-targeted therapies in HER2-mutant breast cancer and current evidence for compound HER2 mutations

Therapy class	Regimen / Trial	Mechanism of action	Population	Key efficacy	Evidence in compound HER2 mutations	References
Irreversible pan-HER TKI	SUMMIT — neratinib monotherapy	Covalent inhibition of HER family kinases	HER2-mutant MBC	ORR 17.4%; PFS 3.6 mo	Not reported for this arm	[9]
	SUMMIT — neratinib + fulvestrant		HR+, HER2-mutant MBC	ORR 29.8%; PFS 5.4 mo; DOR 9.2 mo	Not reported for this arm	[9]
	SUMMIT — neratinib + fulvestrant + trastuzumab		HR+, HER2-mutant MBC	ORR 39%; PFS 8.3 mo	6 of 7 patients with >1 baseline HER2-activating	[8,9]

Compound HER2 (ERBB2) Mutations in Breast Cancer: Molecular Evolution, Structural Cooperativity, and Therapeutic Implications

					event did not benefit	
	MutHER — neratinib + fulvestrant		HER2-mutated, non-amplified MBC	CBR 30–38%; PFS 5.0–6.0 mo	2 of 3 dual-mutation patients did not benefit	[9,32]
	plasma MATCH — neratinib ± fulvestrant (ctDNA-selected)		ctDNA - detected HER2-mutant MBC	Comparable activity to tissue-selected cohorts	Not reported	[9,33]
Selective HER2 TKI	HER2CLIMB — tucatinib + trastuzumab + capecitabine	Selective HER2 kinase inhibition	HER2-amplified MBC	Established clinical activity	Not applicable — amplified, not mutation-selected population	[29]
Reversible HER2/E GFR TKI	Lapatinib-based regimens	Reversible HER2/E GFR inhibition	HER2-mutant MBC (mixed)	Variable; efficacy influenced by mutation type	L755S+K753I resistant to lapatinib, retains pan-HER TKI sensitivity	[5,18]
Antibody-drug conjugate	DESTINY-Breast01 — T-DXd	HER2-directed cytotoxic payload delivery	HER2-amplified, previously treated MBC	Established clinical activity	Not applicable — amplified population	[30]
	DESTINY-PanTumor01 — T-DXd		Solid tumors, incl. breast, with activating HER2 mutations	Mutation-selected trial	Not reported by single vs. compound mutation status	[31]
Monoclonal antibody	Standard trastuzumab/pertuzumab regimens	Extracellular HER2 blockade	HER2-amplified disease	Standard of care; limited data in isolated HER2 mutations	Unknown	[2,3]

Note: MBC = metastatic breast cancer; ORR = objective response rate; PFS = progression-free survival; DOR = duration of response; CBR = clinical benefit rate.

5. Current Challenges and Future Directions

5.1 Challenges in the Study of Compound HER2 Mutations

Although the significance of compound mutations in HER2 (ERBB2) is becoming more widely accepted as a relevant biological and clinical genomic change, there remain some difficulties that prevent thorough analysis of this type of mutation. This low incidence along with their great molecular diversity has led to an absence of substantial clinical data. Most published evidences are basket trials, retrospective sequencing cohorts, case reports, and preclinical studies with population sizes that are relatively low [5,7,21].

Another difficulty is that the majority of research does not indicate whether the co-occurring mutation happens in cis on the same allele or in trans on two different alleles. This difference is expected to affect receptor conformation, kinase activation, and therapy sensitivity, however it has been minimally studied due to the fact that the standard short-read next-generation sequencing (NGS) technologies unable to ascertain allelic configuration [10].

The other key limitation is the absence of standardization of functional characterization. Despite the extensive research on recurrent mutations like S310F, L755S, and V777L, only a few combinations of compound mutations have been studied to confirm their biochemical or structural validation. As a result, the biological significance of many of the mutation pairs is unclear since they have not been clinically validated either.

5.2 Unresolved Biological Questions

Certain essential inquiries about compound HER2 mutations have not yet been resolved. It is not clear if all compound mutations have an equal ability to induce oncogenic effects, or if the different combination creates a different type of signaling activation. In addition, a systematic comparison has not been done between the impact of mutation pairs in the extracellular domain with the mutation pairs in the kinase domain on signaling activation.

The evolutionary origin of compound HER2 mutations is still considered ambiguous. Longitudinal sequencing studies indicate that the majority of secondary HER2 mutations are the result of therapeutic selection; nevertheless, it is plausible that the presence of low-frequency resistant subclones existed before treatment and subsequently proliferated due to targeted HER2 therapy. Prospective studies that implement both serial ctDNA analysis and tissue-based genomic analysis will be essential for investigating the alternatives and understanding the dynamics of clonal evolution [17]. These interactions are yet to be thoroughly studied. Thus, future research should evaluate compound HER2 mutations in relation to other mutations rather than isolated molecular events.

5.3 Future Research Priorities

Future research should focus on the need for global collaborative registries that will create databases of patients with compound HER2 mutations. These registries will provide organized data on the frequency of mutations, alongside their clinical and pathological features, treatment results, and survival over time from the disease among diverse populations.

Functional characterization should not be limited

only to recurrent hotspot mutations. The use of high-throughput saturation mutagenesis, CRISPR-based genome engineering, patient-derived organoids, and animal models provides useful tools for probing the function of newly identified compound mutation combinations. The integration of cryo-electron microscopy and molecular dynamics simulation may provide more insight into the effects of the combination of mutations on receptor structure and influence on inhibitor binding.

Lastly, it is essential to make sure that serial ctDNA monitoring becomes a regular part of clinical practice as this could help to identify the compound HER2 mutation in real time and lead to the therapy adjustments before the radiographic progression of disease.

Altogether, these innovations may play a key role in the shift from phenomenological genomics research to mechanism-driven precision oncology of patients with complex HER2-mutated breast cancer.

5.4 Future Therapeutic Strategies

The advent of compound HER2 mutations holds significant consequences for future drug development. Instead of focusing on the mutation of individual HER2, next-generation therapies may have to target the issue of adaptive kinase evolution. Several strategies are currently being studied, including:

- Creation of new types of irreversible inhibitors of HER2 with extended effectiveness against mutation classes;
- Rational drug combinations that inhibit not only HER2 but other downstream signaling pathways as well;
- Early use of antibody-drug conjugates in the treatment paradigm;
- Structural design of drugs based on compound mutation-specific conformity.

Structural modelling and molecular dynamics simulations assisted by artificial intelligence can help in the prediction of sensitivity of drugs for novel mutations of HER2 compounds, thus helping formulate individual treatment strategies even prior to functional validation.

Even though there is insufficient evidence at the moment, the future approach to managing HER2 mutations in breast cancer would move mutation-driven therapy to the use of precision oncology, taking into account the primary activating mutation along with evolutionary alterations that arise during the course of treatment.

6. Conclusions

Compound HER2 mutations are a new and biologically unique group of ERBB2 mutations that go beyond the classical concept of single activating mutations. Increasing evidence from genomics, structural, and functional studies demonstrates the interactions between coexisting HER2 mutations can enhance receptor activation, maintenance of downstream signaling, and sustained resistance to therapies, in particular under selective therapeutic pressure of HER2-targeted therapy.

While the understanding of the molecular background of HER2 mutations in breast cancer has improved greatly, data about the specific

combination of mutations is still limited. The majority of information stems from retrospective sequencing studies and preclinical studies, highlighting the need for prospective clinical trials and functional studies to understand the different pathogenic features of the mutations. Technological advances in structural biology, molecular dynamics, and longitudinal ctDNA profiling have the potential to increase the understanding of the specific roles of mutations and allow for better detection of the mutational process that causes resistance.

Importantly, compound HER2 mutations should no longer be regarded as incidental co-occurring genomic alterations. Instead, they signify molecular entities with distinct structural, biological, and therapeutic implications, thus necessitating independent consideration in both research and clinical practice. Future approaches to precision oncology will likely involve the integration of comprehensive genomic profiling with functional annotation of specific mutation combinations to optimize the selection of treatment and enhance patient outcome.

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