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REVIEW ARTICLE

Role of Genomic Alterations in *HER2* Positive Breast Carcinoma: Focus on Susceptibility and Trastuzumab-therapy

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Abstract: ***Background*:** Breast cancer is the most frequently diagnosed life-threatening malignancy among women, across the globe. *HER2* positive is a distinct breast cancer subtype, on account of its unique biology and physiological behavior.

***Results*:** Amplification of *HER2* oncogene/polysomy 17 leads to *HER2* overexpression that is a significant causal implication in *HER2* positive breast cancer. *HER2* gene variants, as well as other genes/gene variants, are involved in its overexpression, disease prognosis and in predicting the susceptibility towards *HER2* positive breast cancer. Trastuzumab (Herceptin) is the most commonly used therapy for treating patients with *HER2* positive status. Genomic alterations are incriminated in the development of trastuzumab-resistance, which influences the response towards trastuzumab-therapy.

***Conclusion*:** In the current review article, we have summarized the genomic alterations that are responsible for overexpression of *HER2* and therefore, increased risk of breast cancer. In addition, the gene variants affecting response towards trastuzumab-therapy have also been discussed.

Keywords: Breast cancer, *HER2* overexpression, gene variants, genomic alterations, drug resistance, trastuzumab.

INTRODUCTION

Among the various malignancies, breast cancer is the most common malignancy found in the female population, covering about a quarter of all other malignancies [1, 2]. Worldwide, approximately 12% of women are affected by breast cancer. According to a survey, it accounts for about 23% of total cancer cases and 14% of the deaths occurring due to the disease [3]. Breast cancer has not only been found in the female population, but it has also been reported among the male population.

According to histopathological classification, based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (*HER2*), breast cancer is classified into three major clinical subtypes. These are i) the hormone receptor-positive (HR+; ER- and/or PR-positive), ii) *HER2* positive (*HER2*+), and iii) triple-negative (TN; negative expression of ER, PR, and *HER2*), all being associated with distinct biological action and sensitivity towards treatment. Another classification based on gene expression profiling describes five major subtypes of breast cancer, *i.e.,* luminal A and B (ER+/PR+ and ER+/PR–/*HER2*+ subtypes, respectively), the *HER2* (*HER2*+/HR– subtype), the basal-like (somehow overlapping with the TN subtype), and the normal breast-like subtype [4]. *HER2* positive status has been found in both *HER2* and luminal tumor classes. However, *HER2* positive breast carcinomas often lack estrogens (ERs) and progesterons (PRs) and are of high histologic grade [5]. Compared to other types of breast carcinomas, *HER2* amplified breast carcinomas possess distinct biological features such as increased potential of metastization to brain and viscera [6]. A significantly reduced disease-free survival and overall survival is seen in *HER2* positive breast cancer patients [7]. Amplification of *HER2* and many other genes is thought to be involved in tumor development and resistance to anti-*HER2* therapies [8].

So, keeping in view the influence of genetic factors, this review article has been compiled with an aim to discuss the biological function of *HER2*, genomic alterations in *HER2* as well as other genes leading to *HER2* overexpression. The role of *HER2* in the prediction of response towards *HER2*-targeted trastuzumab treatment has also been discussed.

THE NORMAL BIOLOGICAL COUNTERPART OF *HER2*: A BRIEF OVERVIEW

HER2 (also known as ErBb2/c-ErBb2/neu), is a 1255 amino acid, 185 kDa tyrosine kinase receptor protein (p185), encoded by *HER2*/ErBb2/neu gene located on the long arm of chromosome 17q (17q12) [9-13]. The discovery of the *HER2* oncogene was first made in 1984 by Weinberg and associates at Massachusetts Institute of Technology, Rockefeller, and Harvard University [14]. *HER2* belongs to the class-I receptor tyrosine kinase (TK) family of transmembrane glycoproteins *i.e.,* epidermal growth factor receptor

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(EGFR) family, comprising HER1 (EGFR/ErBb1), *HER2* (ErBb2, neu), HER3 (ErBb3) and HER4 (ErBb4), all leading to tyrosine phosphorylation activity upon dimerization [10, 15]. Three functional domains *i.e.,* an extracellular domain for ligand binding, a transmembrane domain, and an intracellular tyrosine kinase domain, are possessed by all the EGFR family members [16]. *HER2* is unique among the other EGFR family members, as it is not dependent on ligand for activation [16, 17]. *HER2* is crucial for the normal developmental processes in humans [10]. It plays a significant role in cell signal transduction pathway and hence, regulates cell proliferation, differentiation, survival, and migration. It is required for embryonic development and tissue maintenance in adults. Multiple organs express *HER2* and activation of *HER2*, is required for various physiological processes, *i.e.,* oligodendrocyte formation, Schwann cell myelination and radial glia establishment in cerebral cortex during nervous system development; cardiogenesis; the adult cardiac function; cardiomyopathy prevention in adult heart; normal mammary tissue development and muscle spindle maintenance [18, 19].

HER2 SIGNALING

HER2 activation occurs by homodimerisation or heterodimerisation. All EGFR family members possess ligands for activation, except *HER2*. Homodimers of *HER2* are constitutively active and do not require ligand for activation [20]. While, in case of *HER2* heterodimerisation with HER1, HER3 or HER4, cross-activation of *HER2* by the corresponding dimerisation partner occurs [11, 12]. These constitutively active, ligand-independent homodimers or ligand-dependent heterodimers of *HER2* result into autophosphorylation of tyrosine kinase residues, hence, activating a plethora of signaling pathways *i.e.,* phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and PLCγ (phospholipase C-γ) pathways [11, 12], which, in turn, regulate cell growth, survival and differentiation [21]. *HER2* dimerization results in the activation of MAPK pathway *via* the binding of the adaptor proteins like Growth factor receptor-bound protein 2 (Grb2), Src homology-2 containing domain (Shc) and a non-receptor tyrosine kinase, Src, finally leading to increased cellular proliferation. *HER2* heterodimers activate PI3K, inducing phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP_2) to produce phosphatidylinositol-3,4,5-triphosphate (PIP_3). Conversion of PIP_2 to PIP_3 is negatively regulated by phosphatase and tensin homolog (PTEN). PIP_3 activates Akt [22]. This leads to the upregulation of various antiapoptotic proteins resulting in delayed p53-mediated apoptosis [10, 12] and cell-cycle degradation *via* cyclin-dependent kinase inhibitor 1B (CDKN1B / p27) activation. Besides, it also activates nuclear factor-κβ (NF-κβ) and mammalian target of rapamycin (mTOR) [15]. MAPK kinase also acts upon c-Myc (gene encoding for transcription factor) producing mutated p53 (tumor-suppressor gene) and hence, increased cell proliferation [10]. Apart from this nuclear transcription factor upregulation is brought about by PLC [12] and protein kinase C (PKC) activation [15]. Heterodimers of *HER2* have more signaling potency than the homodimers. *HER2* doesn't bear ligand-binding activity, but it is the most preferred dimerisation partner for all other HER-family members. On the other hand, HER3 lacks kinase activity as it is deprived of catalytic adenosine triphosphate-binding domain and requires kinase-active dimerisation partner to promote signaling. *HER2* and HER3 possess functional incapabilities, but these act as compensatory counterparts to each other forming most potent and efficient *HER2*-HER3 heterodimers. These *HER2*-HER3 heterodimers signal through PI3K pathway, making it the key regulatory pathway that stimulates *HER2* positive breast carcinogenesis [23, 24]. The signal transduction pathway of *HER2* has been shown in Fig. (**1**).

HER2 INTERACTIONS

Interactions of *HER2* with its own family members and with other related family members like Insulin-like growth factor-I receptor (IGF-1R) have been found to be implicated in multiple-pathway activation leading to mammary carcinogenesis and contributing towards drug resistance [25]. *HER2* is the preferred dimerization partner for all other HER-family members, which upon dimerization promotes processes of cellular proliferation, survival, migration, invasion, and differentiation [26]. PI3K pathway is the critical signaling pathway for *HER2* heterodimerization, and the PIK3CA mutations have been reported to promote breast carcinogenesis [27, 28]. Reactivation of PI3K pathway due to mutations in components of PI3K pathway or *via* cross talk with other receptors is the main cause of resistance towards *HER2* targeted therapy [29]. Resistance towards endocrine agents in ER+/*HER2*+ breast cancers is seen due to *HER2*/ER cross talk [30-32]. However, the exact mechanism behind the endocrine resistance is not known. But, *HER2* has been reported to lower ER expression at protein and mRNA levels. Activated members of *HER2* signaling pathway, as a result of higher expression of *HER2*, act to suppress ER levels. So, *HER2* positive tumors do not respond to endocrine therapy. Similarly, higher levels of ER suppress *HER2*. Therefore, the combined therapy of endocrine agents plus anti-*HER2* agents is employed for the treatment of HR+/*HER2*+ breast cancer [4]. *HER2* also shares homology with related family member IGF-1R and cross talk with IGF-1R confers resistance towards trastuzumab, a monoclonal antibody targeting *HER2* [33]. Co-expression of *HER2* and adipocyte-secreted factor, leptin has also been reported to contribute to enhanced *HER2* signaling and reduced sensitivity to the anti-*HER2* treatments [34]. Leptin exposure causes upregulation of heat-shock protein 90 (Hsp90) promoter activity in the breast cancer cells, leading to enhanced *HER2* levels [35]. This molecular mechanism provided insights into the associations between obesity and breast cancer progression. Androgen receptor stimulates testosterone secretion. *HER2* cross-talk with androgen receptor is responsible for driving cell proliferation in males [36]. Epithelial protein-tyrosine phosphatase 1B (PTP1B) has also been reported to contribute to tumor onset, but it is not required for the tumor maintenance [37]. Heat-shock protein 72 (Hsp 72) promotes *HER2* induced tumorigenesis *via* upregulation of p21 and downregulation of survivin (inhibitor of apoptosis, IAP) in Hsp 72 knock-out mouse models [38]. Heat-shock transcription factor 1 (HSF1) is crucial for the maintenance of HSPs and plays a significant role in promoting *HER2*-induced tumorigenesis [39]. Mucins, MUC1 and MUC4, activate *HER2* by different mechanisms, thus disrupting the cell polarity. MUC1 promotes heregulin-induced *HER2* signaling while MUC4 has EGF-like domains on which *HER2* can bind and promote

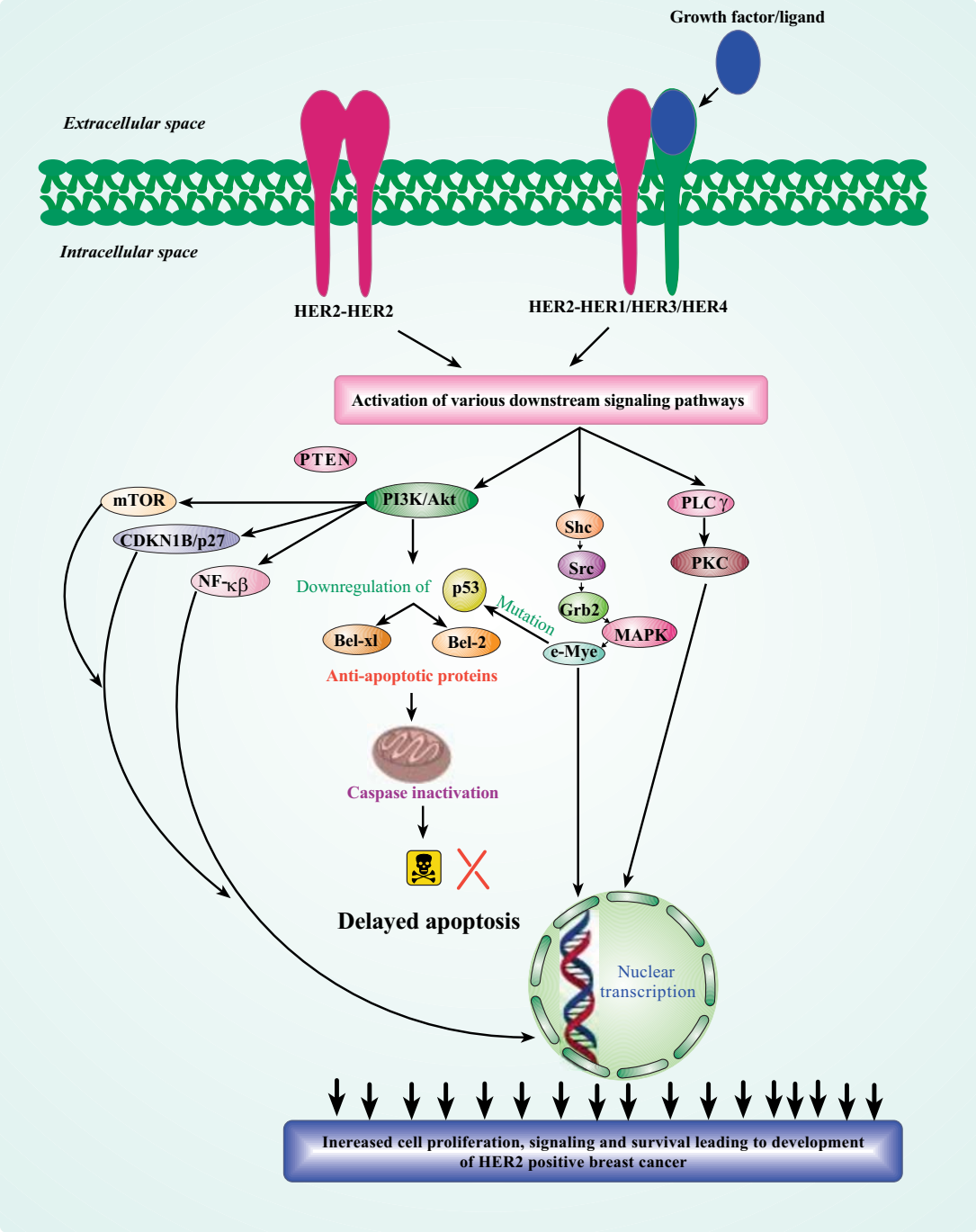

Fig. (1). *HER2* homodimerisation or heterodimerisation, activating a number of downstream signaling pathways such as PI3K pathway, MAPK pathway and PLCγ pathway that ultimately leads to enhanced cell proliferation and signaling, delayed apoptosis, hence, contributing towards *HER2* positive carcinogenesis.

tumorigenesis [40]. MUC4, a highly O-glycosylated membrane protein is a putative partner of *HER2* and is found to be overexpressed in the trastuzumab-resistant cells [41].

ROLE OF GENOMIC ALTERATIONS IN *HER2* POSITIVE BREAST CARCINOGENESIS

HER2 Overexpression

Severity in many cases of human breast carcinoma has been observed due to aberrant activation of the *HER2* receptor [42]. More aggressive tumor phenotype with lymph-node involvement and increased resistance to endocrine therapy, hence, increased chances of relapse and decreased survival have been associated with *HER2* overexpression [11]. *HER2* overexpression plays a crucial role in the initiation and progression of the breast cancer [43]. Overexpression of *HER2* protein was reported first by Van *et al.* in 1988 in *in situ* breast carcinoma [44]. *HER2* gene amplification and protein overexpression are associated, as there are rare incidences of single copy overexpression. In 90% of the *HER2* positive breast cancers, the copy number gain involving *HER2* locus *i.e.,* gene amplification is seen. However, it is not necessary

that *HER2* overexpression and *HER2* amplification will always accompany each other. Tumors deficient in *HER2* amplification may show protein overexpression and tumors deficient in protein overexpression may show *HER2* amplification, suggesting other alternative mechanisms for *HER2* overexpression [43]. Polysomy 17 (presence of extra copies of chromosome 17) is an alternative mechanism responsible for the *HER2* overexpression [45]. *HER2* overexpression has been linked to the increased formation of both homodimers and heterodimers [24], which leads to increased cell signaling and, in turn, results in the increased cell proliferation, cell motility, tumor invasiveness, progressive regional and distant metastases, accelerated angiogenesis and reduced apoptosis [5]. Amplification/overexpression of *HER2* gene is found to be associated with 15-30% of the breast cancer patients [12]. There can be up to 25-50 copies of *HER2* gene and a 40-100 fold increase in *HER2*-protein in breast carcinomas, hence, resulting in the expression of about 2 million receptors at the tumor cell surface [6]. Apart from this, *HER2* amplified breast cancers possess distinct clinical behaviour, such as increased sensitivity towards certain chemotherapeutic agents [6]. This distinct clinical behaviour of *HER2* amplified breast carcinomas is attributed to co-amplification of the topoisomerase-2-alpha (TOP2A) gene, located on chromosome 17 which is nearby *HER2* gene locus [46].

There are many genes in addition to *HER2* that are located or not located on the *HER2* amplicon (17q12-21) which may be co-amplified with *HER2* as a result of the amplification of the region and in turn, play a crucial role in *HER2* positive carcinogenesis. Besides *HER2*, five genes namely growth factor receptor bound protein-7 (GRB7), metastatic lymph node 64 (MLN64), phenylethanolamine N-methyltransferase (PNMT), MGC9753 and MGC14832 [47, 48] have been reported to be overexpressed in *HER2* positive tumors as a result of amplification. Press *et al.* reported the *HER2*/TOP2A co-amplification in a number of breast cancer patients and concluded that it is correlated with the long-term survival in patients, when anthracycline-based chemotherapy was adopted [49]. The co-silencing of MLN64, GRB7, proteasome 26S subunit, non-ATPase 3 (PSMD3) or Post-GPI Attachment To Proteins 3 (PGAP3/ PERLD1) along with *HER2* has been reported to result in enhanced inhibition of cell viability, proliferation and induced apoptosis, indicating essential role of these genes in *HER2* positive breast carcinoma [50]. But, the functional importance of most of the co-amplified genes particularly in *HER2* positive breast cancer is not known exactly. Various mechanisms for activation of *HER2* oncogene have been proposed. Katz *et al.* indicated that migration and invasion enhancer 1 (MIEN1/C35) is an important oncogene located on *HER2* amplicon, whose mRNA expression was found to be associated with the *HER2* amplification and it is activated through downstream Syk (Spleen tyrosine kinase) signaling [51]. Li *et al.* reported that mH2A1.2 isoform of macrohistone 2A1 (H2AFY; mH2A1) activates *HER2* transcription that significantly increases the *HER2* expression and cell proliferation [52].

HER2 Allelic Variations

Single-nucleotide polymorphisms (SNPs) of *HER2* have been implicated in the pathogenesis of breast cancer. In transmembrane domain of neu oncogene of rats, a missense mutation Val655Glu that activates *HER2* oncogene, has been identified. But, in human *HER2*, no such corresponding mutation inducing *HER2* activation is known [53]. Allelic variations at the proto-oncogene *HER2* have been incriminated in the neoplastic process of breast carcinogenesis [54]. Various *HER2* allelic variations including Val655Ile (rs1136201), Ala1170Pro (rs1058808), rs4252596, rs2952155, rs1810132, Ile654Val (rs1801200), rs2517956, rs903506, dinucleotide repeat H(AC)I4 in intron 4 and haplotype [(C)-(G)-(A)-A-C-(C)] have been evaluated in association with *HER2* positive breast cancer.

The germinal polymorphism at the codon 655 [Val655Ile] (GTC/valine to ATC/isoleucine) (rs1136201), located in the transmembrane domain of the *HER2* protein has been most commonly explored [55]. Heterozygous/homozygous Val allele has been implicated in the pathogenesis of the disease in most of the cases. This variant has been found to be implicated in the increased tyrosine kinase activation and autophosphorylation [56]. This leads to increased cell-signaling causing enhanced cell-proliferation and thus, carcinogenesis [22].

HER2 Val655Ile has been found to be positively associated with breast cancer susceptibility among Asian (Chinese, Shanghainese, Arabic, Egyptian, Taiwanese, Turkish, *etc.*), Australian, African (Tunisian), European (Portuguese, Finnish, Italian-Cauacasian, German-Caucasian, Ashkenazi, , Slovak, Greek), American (African-Americans, Whites, Caucasian-Americans) ethnic groups [54, 57-79]. However, several other studies could not establish an association between *HER2* Val allele polymorphism and increased breast cancer susceptibility [37, 64, 80-92]. Hence, the role of Val655Ile genotype in breast cancer susceptibility remains to be controversial.

As already mentioned, many other variants of *HER2* have also been explored in association with the disease. However, the functional relevance of most of these variants is not known. Studies have confirmed no association between *HER2* Ala1170Pro polymorphism (rs1058808) (located at exon 27) and breast cancer risk [88-89, 93]. However, Furrer *et al.* have reported the association of Ala1170Pro polymorphism with the breast cancer prognostic factors in non-metastatic *HER2* positive breast cancer patients [94], supporting a prior haplotype analysis, that revealed a possible candidature of Ala1170Pro in inducing functional polymorphism affecting gene expression and/or breast cancer aggression [86]. Cresti *et al.* have reported the association of Ala1170Pro with the enhanced frequency of *HER2* overexpression [95]. Su *et al.* have reported the association of *HER2* Ala1170Pro and rs2517956 polymorphisms with its increased protein expression in breast cancer, thus, suggesting that *HER2* allelic variations play an indispensable role in human breast carcinogenesis [96].

In a haplotype analysis, no association with breast cancer disposition was found for common *HER2* polymorphisms *viz*. rs4252596 (START-657), rs2952155 (intron 1), rs1810132 (intron 4), Ile654Val (rs1801200) (exon 17), [88]. However, Frank *et al.* identified the association of *HER2* variant Ile654Val, with an elevated risk of familial breast cancer [89]. Further studies are required to explore the role of this rare variant as an oncogenic variant, so as to pro-

pound its relationship with the prognosis of breast cancer. Kallel *et al.* reported the association of a novel dinucleotide repeat H(AC)I4 in intron 4 of *HER2* gene with the breast cancer risk. No association with the breast cancer risk for another single-nucleotide polymorphism (SNP) rs903506 was found in a Tunisian population study [66]. Han *et al.* reported a better prognosis and lower *HER2* overexpression in subjects lacking the most common haplotype in *HER2* gene, *i.e.,* [(C)-(G)-(A)-A-C-(C)] [97].

Allelic Polymorphisms Other than *HER2*

Apart from *HER2*, variants of other genes such as, cytochrome P450 genes (CYP19A1, CYP1A1*2, CYP17), estrogen receptor 1 (ESR1), Kirsten rat sarcoma viral oncogene homolog (KRAS), vascular endothelial growth factor A (VEGFA), breast cancer 1 (BRCA1), metastasis associated in colon cancer 1 (MACC1), sulfotransferase family 1A member 1 (SULT1A1), caudal type homeobox 2 vitamin-D receptor (Cdx2 VDR) and chemokine coreceptor-2 (CCR2) have also been found to be associated with the *HER2* expression.

CYP19A1 SNPs (rs700519 and rs4646) have been associated with a lower percentage of *HER2* positive breast tumors [98], whereas heterozygous Val allele of CYP1A1*2 (Ile462Va [99] and increased CYP17 A2 genotype frequency was reported among *HER2* positive patients [100].

ESR1 (T594T) GG genotype [101] and KRAS variant (rs61764370) have been reported to be associated with *HER2* overexpression [102]. KRAS (rs61764370) has been found to act by disrupting a let-7 mi-RNA binding, leading to increased KRAS expression and lowered let-7 levels [103, 104]. G-allele of this KRAS polymorphism (TG/GG genotype) may lead to alteration in *HER2* gene expression profile *i.e.,* G-allele may act as a potential genetic marker for the development of *HER2* negative breast cancer [105].

VEGFA genotypes (-2578CC, -634CC and -7CC) were found in association with worse prognosis in the primary *HER2* positive breast cancer patients [106]. This supported previous study that depicted a positive association between *HER2* and VEGF expression [107]. SULT1A1 SNP (Arg213His) was found to be associated particularly with *HER2* positive male breast cancer patients [108], while another polymorphism of SULT1A1 (G638A) AA genotype was reported to be associated with *HER2* positive breast cancer risk [109]. Cdx2VDR AA genotype [110] and CCR2 allelic variant (V64I) have been associated *HER2* positive breast cancer [111]. VDR (a nuclear transcription factor) and CCR2 play role in cell-cycle regulation and tumor-progression, respectively [110, 111]. Hence, these two may have plausible involvement in HER2 overexpression.

Some other allelic variations have also been found to be associated with survival among *HER2* positive breast cancer patients. BRCA1 (K1183R) AA genotype [68] MACC1 SNPs (rs1990172, rs975263, and rs3735615) were found to be associated with favourable survival in *HER2* positive breast cancer patients. Damaged MACC1 protein limited hepatocyte growth factor receptor (HGFR/ c-MET)) expression and, hence, restricting the metastatic potential of cancer cells [112]. Different genes and their variants that contribute towards *HER2* positive breast cancer susceptibility have been illustrated in Fig. (**2**).

Somatic Mutations and Copy Number Changes in *HER2* Positive Breast Cancer

Recently, geneticists have focused upon identification of somatic mutations among *HER2* positive breast cancer. These mutations may act to promote the process of carcinogenesis. These mutations have been identified in *HER2* as well as in genes that act as regulating factors for *HER2* receptor. However, the driving role *i.e.* mechanism of action of only few of these mutations is known. *HER2* mutations are one of those seven gene mutations that have been found among more than 10% cases and constitute about 58% of cancer-driving mutations [113]. Based on whole genome sequencing, *HER2* is among those ten most frequently-mutated genes that comprise 62% of driver-mutations for cancer [114]. S3F10 mutation of *HER2* is capable of activating and transforming *HER2*, as found by *in vitro* studies. These studies propose the certain activating role of S3F10 mutation in *HER2* inducible breast cancer, as the samples that were devoid of S3F10 mutation were *HER2* non-amplified [115].

Apart from *HER2*, mutations among the genes involved in the signaling pathway have also been reported in *HER2* amplified tumors. These account for 75% of p53 mutations, 42% of PI3KCA mutations, PTEN mutation/loss, 38% of cyclinD1 amplification, 24% of CDK-4 amplification and very low frequency of mutations of other signaling components. Variations in DNA copy number also occur causing aneuploidy, genomic instability and *HER2* amplification including loss/gain of chromosome loci, such as gain of 1q, 8p and loss of 8q [116]. These mutations/aberration disrupt the normal functioning of *HER2* and other signaling proteins and act as drivers to encourage carcinogenesis. According to Curtis *et al.*, the elucidation of breast cancer subtypes and their drivers at molecular level requires integrated views of the genome and transcriptome. The researchers carried out an integrated analysis of copy number and gene expression in a discovery and validation set of 997 and 995 primary breast cancer tumors, respectively from Molecular Taxonomy of Breast Cancer International Consortium (METABRIC). Inherited variants including copy number variants, SNPs and copy number aberrations (CNAs) were found to be associated with expression in about 40% of genes, with the landscape dominated by *cis* and *trans* acting CNAs. This study provides a novel molecular stratification of the breast cancer population depending on the impact of somatic CNAs on the transcriptome [117].

IMPACT OF GENOMIC ALTERATIONS ON TRASTUZUMAB-THERAPY OF *HER2* POSITIVE BREAST CARCINOMA

Cancer therapeutics mainly include traditional chemotherapeutic agents, many of which have been derived from microbes and plant products, such as anthracyclines, vinca alkaloids and taxol analogues [118]. Potent anticancer activities of plant extracts have been reported by us, previously [119-122]. But, targeted-therapeutics have now evolved as better treatment strategies, as these selectively

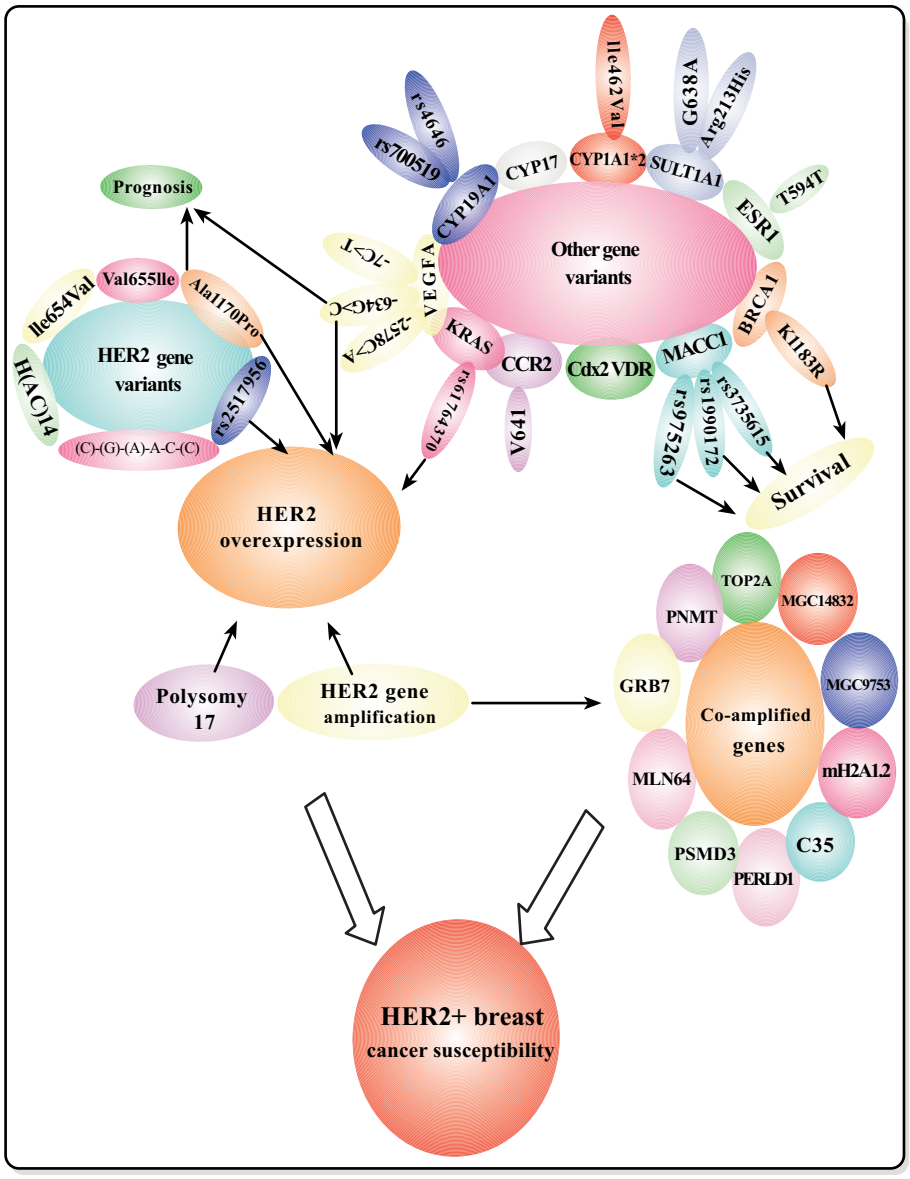

Fig. (2). Genomic alterations implicated in *HER2* positive breast carcinogenesis, which include mainly *HER2* overexpression during which many genes along with *HER2* get amplified, *HER2* and other gene variants. All these genetic alterations are either affecting prognosis or overexpression, finally leading to enhanced susceptibility towards *HER2* positive breast cancer.

target the cancer cells, without affecting normal cells [123]. Trastuzumab is the *HER2* targeted monoclonal antibody, approved by Food and Drug Administration in 1998 for the first-line treatment of metastatic breast cancer [124]. It was developed by Genentech Inc (San Francisco, CA, USA). Trastuzumab works by acting against the extracellular domain IV of *HER2* [125]. Trastuzumab is recommended for use as a monotherapeutic agent as well as a part of adjuvant therapy for the treatment of breast cancer [21]. However, trastuzumab-induced cardiotoxicity and resistance development remain the major challenges [21, 126]. Genetic variants play a critical role in predicting response to *HER2* targeted trastuzumab therapy.

HER2 Gene Variants

HER2 gene variants have been studied for their association with response to anti-*HER2* therapeutics. Specially, *HER2*-Val655Ile genotype is the most explored in association with breast cancer risk as well as the response to trastuzumab treatment. It constitutes a risk factor for trastuzumab-induced cardiotoxicity, besides contributing towards sensitivity towards trastuzumab treatment.

Beauclair *et al.* found that the cells expressing Val allele show sensitivity towards trastuzumab treatment. However, as far as cardiac dysfunction is concerned, it has been observed only in heterozygous Ile/Val subjects and not in Val/Val homozygous subjects. But, no interpretation regarding a possible role for the Val allele as a predisposing factor for cardiotoxicity can be made, as there were only 4 Val/Val homozygous subjects [55]. Roca *et al.* found an association between the *HER2*-Val655Ile and cardiac toxicity [127], the data were also confirmed by Lemieux *et al.* [128]. Han *et al.* found that the alteration of the *HER2* gene function by *HER2* Val655Ile polymorphism remains confined only to the *HER2*

positive subjects. The Val variant in *HER2* positive patients contributes to the aggressiveness and sensitivity towards trastuzumab treatment. A poor clinical outcome was observed in *HER2* positive patients with a Val variant when treated with adjuvant therapy without trastuzumab. However, treatment with adjuvant therapy in combination with trastuzumab given to these patients resulted in favourable survival [129]. Pena *et al.* confirmed the association of *HER2* 655 A>G (rs1136201) polymorphism with trastuzumab-induced cardiotoxicity in *HER2* positive breast cancer patients [130]. A recent study showed conflicting results of the association between codon 665 and cardiotoxicity. However, in the same study, Pro1170Ala polymorphism was found to be associated with an elevated risk of cardiotoxicity from trastuzumab therapy and the proline variant was found to be more prevalent than the alanine among the subjects showing trastuzumab-induced cardiotoxicity [131].

Alterations in the PI3K Pathway

PI3K plays an essential role in diverse cellular signaling pathways such as proliferation, metabolism, migration, translation, apoptosis avoidance, and angiogenesis [132]. *HER2*-PI3K-AKT pathway alterations such as mutation and amplification of PIK3CA, tumor suppressor gene, PTEN loss, AKT1 mutations, extracellular domain-truncated *HER2* (p95*HER2*) expression, are associated with the poor response towards the trastuzumab treatment in breast cancer patients [133]. PIK3CA encodes the catalytic subunit of class 1A, a lipid phosphokinase, *i.e.,* p110α. PIK3CA mutations result in increased lipid kinase activity and Akt phosphorylation. 8% to 40% PIK3CA mutation frequency was reported in human breast cancers [132]. High frequency of PIK3CA mutations have been found in *HER2*+ breast tumors [134]. Most of the PIK3CA mutations occur at three hotspots: two in exon 9 and one in exon 20 and His1047Arg is the most common mutation, which is located in exon 20 [135]. PTEN negatively regulates the PI3K pathway resulting in decreased Akt activity. Thus, PIK3CA mutations or PTEN loss lead to increased downstream signaling, conferring resistance to trastuzumab [136]. Esteva *et al.* analyzed *HER2*-overexpressing metastatic breast cancer patients undergoing trastuzumab-based therapy, for four biomarkers *viz.,* PTEN, p-AKTSer473, p-p70S6K-Thr389, and PIK3CA. Out of these, only PTEN alone significantly correlated with the shorter survival times and PTEN loss combined with each of other three biomarkers, *i.e.,* p-AKTSer473, p-p70S6K-Thr389, and PIK3CA, accounted for an increased correlation. PTEN loss and/or PIK3CA mutations accounted for shorter survival time among the subjects under investigation, hence, depicting a pivotal role of PI3K pathway activation in trastuzumab resistance [137]. Jensen *et al.* supported the association of PIK3CA mutations or increased PI3K pathway activity with significantly poorer survival among patients receiving trastuzumab-based therapy [136]. Takada *et al.* concluded that the gene alterations in the PI3K and ER (estrogen receptor) pathway were associated with the poor survival among *HER2* positive patients along with a positive status for hormone receptor; receiving neoadjuvant chemotherapy with trastuzumab. Pathological complete response is less likely to be achieved among *HER2* positive breast carcinoma patients with a PIK3CA mutation to whom neoadjuvant anthracycline-taxane–based chemotherapy plus anti-*HER2* treatment was given [138]. Adamczyk *et al.* suggested the involvement of androgen receptor along with PTEN in conferring sensitivity towards trastuzumab-based treatment [139].

Apart from the mutations as mentioned earlier, the truncation of the *HER2* molecule itself to produce p95*HER2* or carboxy-terminal fragments (CTFs) of *HER2* is one of the most potential mechanisms contributing to trastuzumab resistance. The p95*HER2* fragments confer resistance to trastuzumab, as these are lacking the trastuzumab-binding portion of the extracellular domain of *HER2* [140].

Fc-gamma Receptor Polymorphisms

Trastuzumab exerts its action through antibody-dependent cell-mediated cytotoxicity (ADCC), and the notion is supported by several studies [141-144]. Fc-gamma receptor (FcgR) is involved in the ADCC effect of trastuzumab. Thus, the FcgR polymorphisms affect the response of the patients towards trastuzumab.

Musolino *et al.* evaluated *HER2* positive breast cancer patients for the FcgR IIIa-158 valine(V)/phenylalanine(F), FcgR IIa-131 histidine(H)/arginine(R), and FcgRIIb-232 isoleucine(I)/threonine(T)polymorphisms and found an association of FcgR IIIa-158 V/V and FcgR IIa-131 H/H genotype with objective response rate (ORR) and progression-free survival (PFS). Peripheral blood mononuclear cells (PBMCs) harboring V/V and/or H/H genotype had a significantly higher trastuzumab-mediated cytotoxicity than the PBMCs harboring different genotypes [145].

Tamura *et al.* found an association between FcgRIIA-131 H/H polymorphism with the pathological response to trastuzumab-based neoadjuvant chemotherapy in the early-stage breast cancer and the objective response to trastuzumab in metastatic breast cancer. However, the FcgRIIIA-158 V/V genotype did not correlate with the pathological response, but exhibited a tendency to be associated with the objective response [146]. Hurvitz *et al.* reported no correlation between FcgRIIIA-V/F and FcgRIIA-H/R SNPs and disease-free survival (DFS) in *HER2* positive patients treated with trastuzumab. The discrepancies from previous studies may be due to differences in the intrinsic population factors or chemotherapy regimens [147]. Roca *et al.* reported a significant correlation of FcgRIIA-131 H/H genotype with a shorter survival rate and no correlation of FcgRIIIA-158 V/V genotype with survival in trastuzumab-treated patients [127]. Norton *et al.* identified no genetic association of FcgRIIA and FcgRIIIA polymorphisms with superior DFS in patients treated with trastuzumab [148].

Other Genes Involved in Prediction of Response Towards Trastuzumab-therapy

MET oncogene, localised on chromosome 7 encodes for the dimeric tyrosine kinase receptor for hepatocyte growth factor (HGF) and is involved in the cell proliferation, survival, and angiogenesis. Minuti *et al.* found a correlation between the MET and HGF overexpression with short relapse-free and overall survival (OS) in *HER2* positive metastatic breast cancer patients undergoing trastuzumab-based treatment [149]. MYC is a commonly amplified oncogene with diverse functions in the cell signaling and is associated

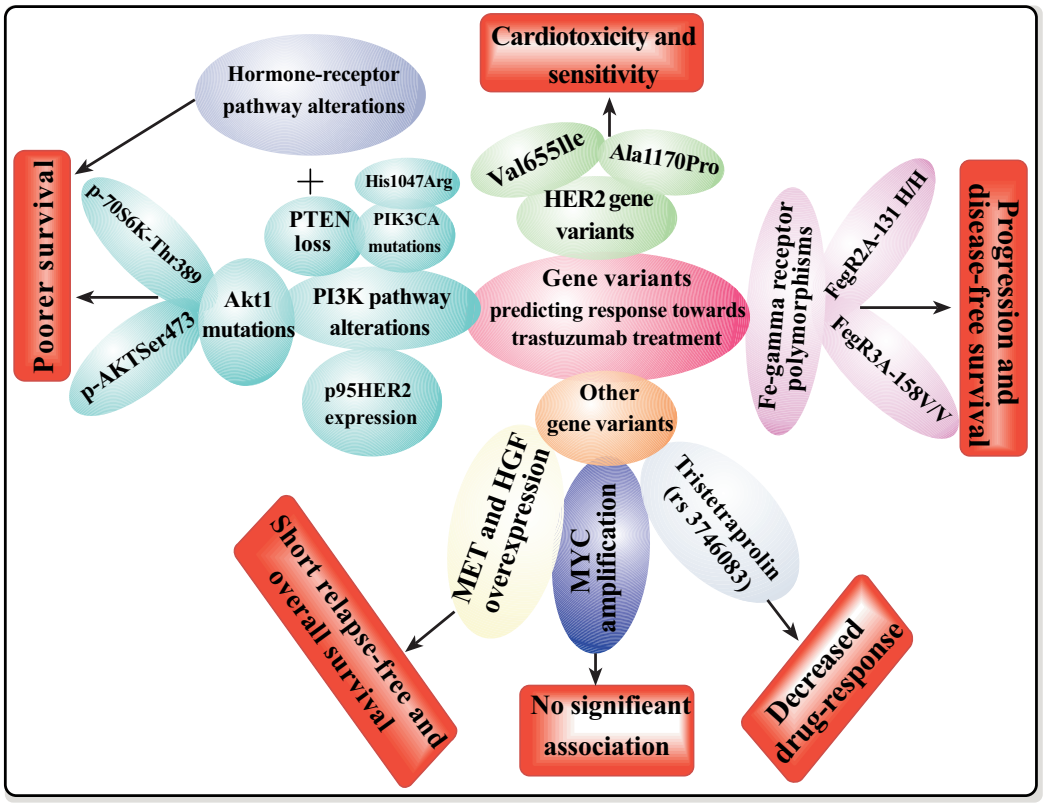

Fig. (3). Gene variants in association with response elucidation towards trastuzumab-treatment. The major gene alterations among them include PI3K pathway alterations, Fc-gamma receptor polymorphisms and *HER2* gene variants. Apart from them, other gene variants that have been shown in the figure may also make a contribution towards the process.

with both poor and good prognoses [150, 151]. National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 trial suggested the association of MYC/*HER2* co-amplification with additional benefit from adjuvant trastuzumab in patients with early-stage breast cancer. Perez *et al.* investigated an association between the MYC amplification and disease-free survival (DFS) in a similar adjuvant trastuzumab *HER2* positive breast cancer trial North Central Cancer Treatment Group (NCCTG) N9831, finding no association between the MYC amplification and additional trastuzumab benefit [152].

Tristetraprolin (TTP), also known as zinc finger protein 36 (ZFP36), is the member of a family of three human genes (ZFP36, ZFP36L1, and ZFP36L2), characterized by two tandemly repeated zinc finger motifs. TTP plays a key role in the inflammation and tumor development. Griseri *et al.* identified a synonymous polymorphism (rs3746083) in TTP showing a statistically significant association with decreased response towards trastuzumab in *HER2* positive-breast cancer patients [153]. Various genomic alterations predicting response towards trastuzumab treatment have been summarized in Fig. (**3**).

Besides, there are several other biomarkers that are implicated in development of resistance against trastuzumab, which have been reviewed by Menyhart *et al.* [154].

CONCLUSION

Breast cancer is the most prevalent cancer among females, leading to morbidity and mortality. *HER2* positive breast cancer possesses distinct biological features relative to other malignancies and constitutes 15-30% of breast cancer cases. *HER2* overexpression is the major factor implicated in *HER2* positive breast cancer. Causal mechanism of *HER2* overexpression is *HER2* gene amplification/polysomy 17. During *HER2* amplification, many other genes such as TOP2A, PNMT, GRB7, PSMD3, PERLD1, MGC9753, MGC14832, C35 get co-amplified. C35 and mH2A1.2 have been found to activate *HER2* oncogene. Besides, various genomic variants of *HER2* like Val655Ile, Ala1170Pro, haplotypes and many other rare variants are reported to be positively associated with *HER2* positive breast cancer susceptibility. However, some variants have shown a negative association with breast cancer risk that may be attributed to variation in ethnicity. Besides, variants of many other genes such as KRAS, CYP1A1*2, CYP19A1, CYP17, VEGFA, SULT1A1, ESR1, MACC1, Cdx2VDR, and CCR2 play a crucial role in predicting susceptibility towards breast cancer. Trastuzumab is the FDA-approved targeted-therapy for first-line treatment of *HER2* positive breast cancer. However, this therapy is associated with the adverse drug reactions such as cardiomyopathy and patients eventually develop resistance to it after sometime. Genetic variants such as *HER2* SNPs (Val655Ile, Pro1170Ala), alterations in PI3K pathway (PIK3CA mutations, PTEN loss, Akt1mutations and p95*HER2* expression), FcgR polymorphisms (FcgRIIIa-158V/V, FcgRIIa-131H/H), MET and HGF overexpression; and TTP polymorphism (rs3746083) are incriminated in elucidating response towards trastuzumab therapy. Hence, the information regarding genomic variants must be included to have a holistic approach towards finding out cause and novel treatment-strategies for *HER2* positive breast cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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