Journal of Cancer Biomoleculars and Therapeutics

ETERNO PRESS

Review

Trastuzumab Resistance in HER2-Positive Gastric Cancer: From Molecular Mechanisms to Precision Therapeutics

Arefeh Zabeti Touchaei*

Department of Chemistry, Lahijan Branch, Islamic Azad University, Lahijan, Iran

*Corresponding author: Arefeh Zabeti Touchaei, hasti.zbt@gmail.com

Article history

Received: 17 September 2025
Revised: 30 September 2025
Accepted: 10 October 2025
Published online: 13 October 2025

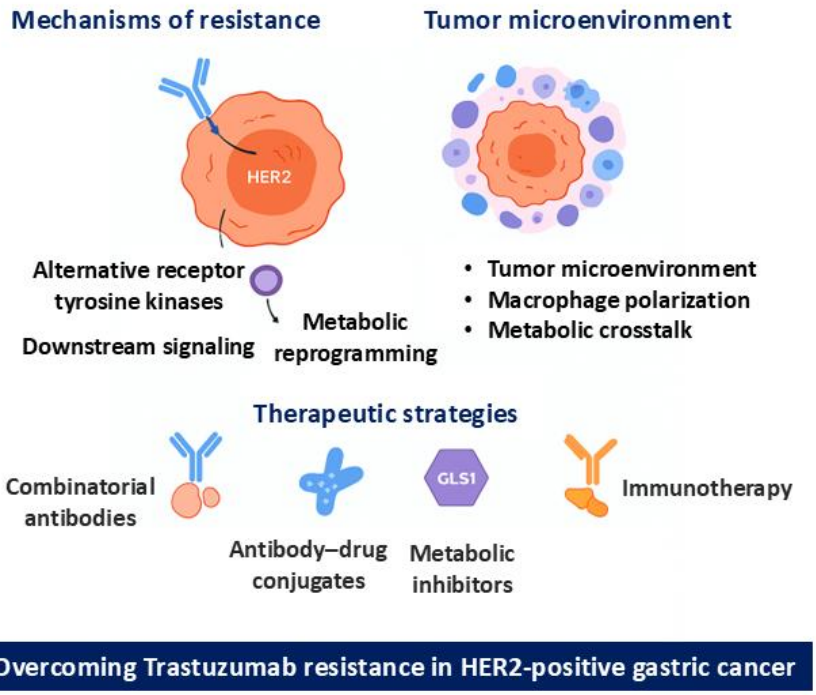
Keywords

HER2
Trastuzumab
Gastric cancer
Acquired resistance
Metabolic reprogramming
Glutaminase (GLS1)
Tumor microenvironment
Extracellular vesicles
MALDI-IMS
Combination therapy

Abstract

Trastuzumab remains the only targeted agent that has received approval as an initial therapy for HER2-amplified gastric cancer. When used alongside platinum-fluoropyrimidine chemotherapy, it has been shown to prolong overall survival compared with chemotherapy alone. However, the long-term clinical benefit is restricted because most patients develop acquired resistance within one year of starting therapy. Multiple tumor-intrinsic mechanisms (activation of alternative receptor tyrosine kinases, downstream signaling, altered glycosylation, YAP/mTOR reactivation, noncoding RNAs) and microenvironmental factors (metabolic crosstalk, macrophage polarization, extracellular vesicle transfer) have been implicated. Metabolic reprogramming, especially enhanced glycolysis, the mevalonate pathway, and glutamine metabolism, has emerged as a central mechanism that not only contributes to intrinsic trastuzumab resistance but also modulates the tumor immune microenvironment, thereby influencing therapeutic response. Spatial metabolomics (MALDI-IMS), integrated genomic/transcriptomic analyses, and functional studies have begun to identify biomarkers and drugable vulnerabilities. Promising strategies to overcome resistance include combined targeting of glutamine metabolism, angiogenesis, and macrophage polarization; dual blockade of ErbB2 with novel antibodies (H2-18) plus trastuzumab; PAM pathway inhibitors (DIACC3010) combined with trastuzumab; antibody-drug conjugates (trastuzumab deruxtecan); and immune checkpoint combinations (e.g., pembrolizumab or camrelizumab with trastuzumab-based regimens). This review integrates recent preclinical and clinical evidence on factors contributing to resistance to trastuzumab in HER2-amplified GC and highlights translational opportunities for biomarker-driven combination therapies.

Graphical Abstract



1. Introduction

Gastric cancer (GC) continues to be a major contributor to cancer-related deaths worldwide, with more than one million new diagnoses and roughly 769,000 fatalities reported in 2020 [1]. Although progress has been made in early detection and treatment approaches, GC is frequently identified at advanced stages, leading to poor patient outcomes. Key risk factors include Helicobacter pylori infection, dietary habits, genetic susceptibility, and chronic gastritis [2]. Conventional treatments, including surgery, chemotherapy, and radiotherapy, offer only limited improvements in survival for patients with advanced disease, underscoring the critical need for effective targeted therapies [3].

Human epidermal growth factor receptor 2 (HER2, or ERBB2) belongs to the ERBB family of receptor tyrosine kinases, which also comprises EGFR (ERBB1), HER3 (ERBB3), and HER4 (ERBB4) [3]. Structurally, HER2 is a membrane-bound receptor with three main parts: an extracellular segment, a transmembrane region, and a cytoplasmic kinase domain. Distinct from other ERBB family members, HER2 has no direct ligand and mainly signals by forming heterodimers with partner receptors of the ERBB family to activate downstream signaling pathways, including PI3K/AKT/mTOR and RAS/RAF/MEK/ERK, which regulate cell proliferation, survival, and differentiation [4]. HER2 overexpression or gene amplification is observed in about 15-20% of gastric cancers. This molecular change is linked to aggressive tumor characteristics, a greater propensity for metastasis, and a poorer prognosis overall [5]. The advent of trastuzumab, a humanized monoclonal antibody that targets HER2, significantly extended survival for patients with HER2-positive GC when used alongside chemotherapy, thus confirming HER2's importance as a therapeutic target [6].

While trastuzumab is effective, the majority of patients will develop acquired resistance within the first year of treatment [7,8]. To devise strategies that improve patient survival, it is essential to understand the molecular, metabolic, and microenvironmental mechanisms that contribute to this resistance. Several resistance mechanisms have been identified in preclinical studies, including activation of alternative signaling pathways, metabolic reprogramming, noncoding RNA-mediated regulation, and tumor microenvironment interactions [9,10].

Trastuzumab, a humanized monoclonal antibody targeting the HER2 protein, significantly improved survival in patients with HER2-positive gastric cancer when combined with chemotherapy, establishing it as the standard treatment and marking the beginning of targeted therapy for this disease [5]. However, despite subsequent efforts to develop additional targeted agents, including other HER2-directed drugs (lapatinib, trastuzumab emtansine) and inhibitors of mTOR or c-MET, most failed to deliver meaningful clinical benefits [11]. This review, therefore, focuses on the mechanisms underlying trastuzumab resistance, spanning molecular alterations, metabolic reprogramming, noncoding RNA regulation, and tumor microenvironmental factors-while also exploring emerging strategies such as antibody-drug conjugates, dual HER2 blockade, metabolic inhibitors, and immunotherapy combinations, to guide more effective treatments for HER2-positive gastric cancer.

2. Intrinsic Tumor Cell Resistance

Currently, research on trastuzumab resistance mainly focuses on tumor cell–intrinsic factors. Key mechanisms involve the activation of alternative signaling routes, including the insulin-like growth factor 1 receptor and HER3, along with the enhancement of downstream pathways. For a more comprehensive discussion of these resistance processes, readers are referred to existing detailed reviews [12,13].

Secondly, many molecules are associated with HER2-positive GC resistance to trastuzumab, such as PIK3CA mutation, PTEN loss, and Wnt/β-catenin activation [6].

2.1 Bypass Signaling

In HER2-positive gastric cancer GC, resistance to trastuzumab is frequently mediated by the activation of alternative signaling pathways that bypass HER2 inhibition. These compensatory mechanisms enable tumor cells to sustain proliferative and survival signals despite HER2 blockade. EGFR/HER3 Heterodimerization: A pivotal bypass mechanism involves the formation of heterodimers between HER2 and other members of the EGFR family, notably EGFR and HER3. The HER2-HER3 heterodimer, in particular, is a potent activator of downstream signaling cascades, including the PI3K/AKT and MAPK pathways, which are critical for tumor cell proliferation and survival. Studies have demonstrated that trastuzumab-resistant GC cells often exhibit increased HER3 expression and enhanced HER2-HER3 dimerization, leading to sustained signaling in the face of HER2 inhibition [14].

c-MET Activation: Activation of the c-MET receptor tyrosine kinase represents another important bypass mechanism. When stimulated by its ligand, hepatocyte growth factor (HGF), c-MET can trigger downstream signaling cascades, including PI3K/AKT and MAPK, which enhance tumor cell proliferation and invasiveness. In HER2-positive gastric cancer, abnormal c-MET activity has been linked to unfavorable outcomes and decreased responsiveness to HER2-targeted treatments [15].

Recent studies highlight the role of YAP as a key mediator of resistance to HER2-targeted therapy. Even when HER2 signaling is blocked, YAP activity maintains downstream cascades, particularly the AKT-mTOR and ERK–mTOR axes, which support cell growth and survival. Tumors with high YAP expression continue to proliferate and resist apoptosis after trastuzumab treatment, whereas inhibition of YAP restores sensitivity in preclinical models. These findings indicate that YAP functions as a central regulator of adaptive resistance by sustaining survival signaling and metabolic activity, making it an attractive target for combination strategies

alongside trastuzumab [16]. These bypass signaling mechanisms underscore the complexity of trastuzumab resistance in HER2-positive GC and highlight the need for therapeutic strategies that can target these alternative pathways to overcome resistance and improve patient outcomes.

Figure 1 illustrates tumor-intrinsic mechanisms (bypass signaling via EGFR/HER3/MET, PI3K/AKT/mTOR and YAP/TEAD activation, metabolic rewiring, and noncoding RNA regulation), tumor-tumor-microenvironment interactions (macrophage polarization, exosomal ncRNA and enzyme transfer, metabolic crosstalk), and clinical predictors (HER2 heterogeneity, proteogenomic alterations, and immune infiltration). Emerging therapeutic strategies include dual HER2 blockade, antibody-drug conjugates, PAM pathway inhibitors, metabolic targeting, glycosylation and epigenetic modulators, and immunotherapy combinations.

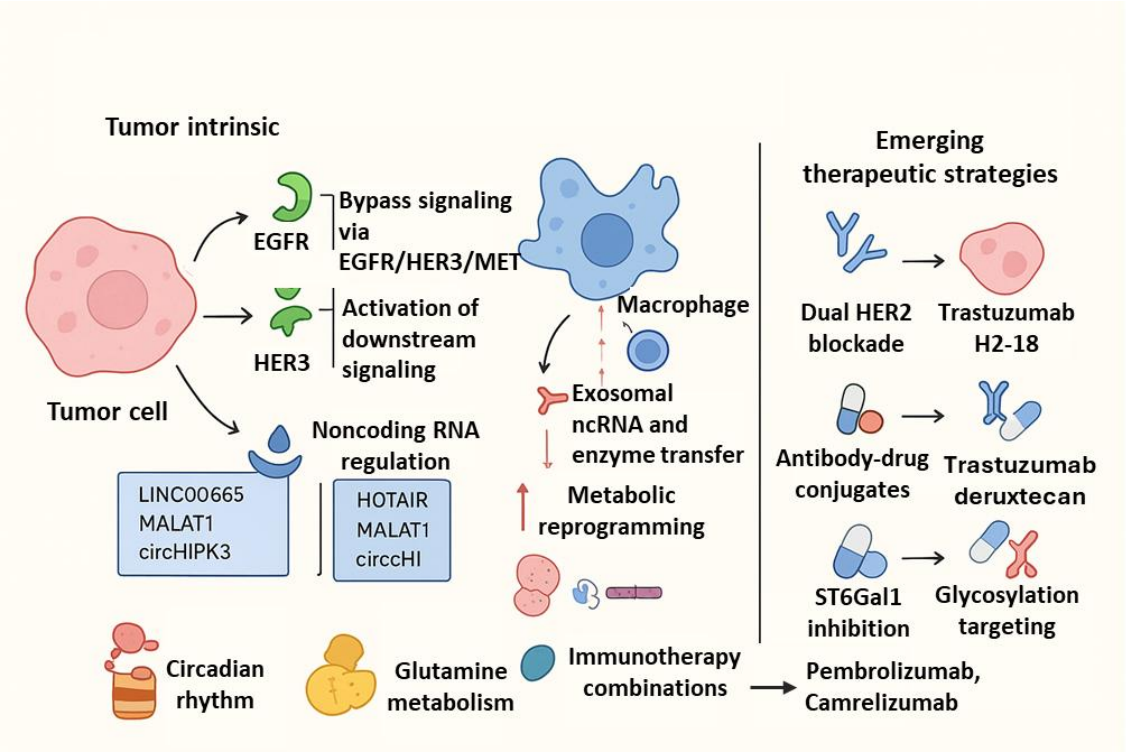


Figure 1. Integrated mechanisms of trastuzumab resistance and emerging therapeutic strategies in HER2-positive gastric cancer.

Analyses using whole-exome sequencing (WES) and single-cell RNA sequencing (scRNA-seq) in patients with HER2-positive gastric cancer demonstrated that individuals who responded to trastuzumab exhibited amplifications in cell-cycle-related genes (such as CCNE1), elevated levels of cell-cycle proteins (Cyclin A/E), and enhanced infiltration of NK cells. In contrast, non-responders frequently exhibited amplifications in EGFR, HER3, and MET, contributing to bypass signaling and resistance. These findings underscore the importance of biomarker-driven stratification in optimizing HER2-targeted therapy [17,18].

Recent multi-omics studies have expanded our understanding of patient heterogeneity beyond WES and scRNA-seq. Spatial transcriptomics has revealed that HER2 expression is highly heterogeneous within individual tumors, with resistant subclones often co-expressing MET or FGFR2, creating bypass signaling hubs. Proteogenomic analyses have identified phosphorylated RPS6 and altered glycosylation of HER2 as markers of reduced trastuzumab efficacy [19]. Moreover, immune profiling demonstrates that durable responders frequently show inflamed tumor microenvironments, characterized by cytotoxic T cell and NK cell infiltration, whereas non-responders exhibit immunosuppressive myeloid signatures [18]. Importantly, emerging data indicate that such biomarkers may also predict benefit from novel HER2-directed antibody-drug conjugates (e.g., T-DXd), highlighting the need for integrative biomarker-driven patient stratification [20,21] (Table 1).

Table 1. Genomic, transcriptomic, and immune features associated with trastuzumab response in HER2-positive gastric cancer.

Patient Subgroup	Genomic/Transcriptomic Features	Immune Microenvironment	Clinical Implication	Ref
Responders	CCNE1 amplification; ↑ Cyclin A/E	High NK and CD8+ T cell infiltration	Enhanced trastuzumab sensitivity	[18]
Non-responders	EGFR, HER3, MET amplifications; phosphorylated RPS6	Immunosuppressive myeloid infiltration	Alternative bypass signaling, resistance	[10]
Heterogeneous tumors	Spatial HER2 variability; co-expression of FGFR2	Mixed immune infiltration	Predicts poor durability of response	[22]
Biomarker-driven prediction	Altered glycosylation (ST6Gal1-mediated HER2 dimer stabilization)	Not immune-specific	Reduced trastuzumab efficacy, a potential biomarker for antibody-drug conjugates benefit	[23]

2.2 Downstream Pathway Activation

The PI3K/AKT/mTOR (PAM) pathway represents one of the most common dysregulated signaling cascades found in gastric cancer. As a downstream mediator of HER2, this pathway is central to regulating cell proliferation, growth, and survival [24]. Critically, the sustained activation of PI3K/AKT/mTOR signaling is a major factor in the development of trastuzumab resistance [24].

DIACC3010 is a novel compound that selectively inhibits p7056 and Akt1/3 kinases, providing effective targeting of the PAM pathway. When used as a monotherapy, DIACC3010 was able to stop the proliferation of trastuzumab-refractory HER2-expressing gastric tumor cells by blocking PAM signaling and significantly decreased tumor growth in murine models. Moreover, combining DIACC3010 with trastuzumab showed synergistic antitumor activity, significantly reducing the survival rate of resistant cells. Mechanistically, DIACC3010 enhanced the efficacy of trastuzumab by inhibiting downstream proteins of the HER2 pathway. Biomarker analysis revealed that PIK3CA mutations, HER2/3 amplifications, and lower basal levels of phosphorylated ERK may predict response to DIACC3010 in HER2-positive gastric cancer [25].

YAP, a key effector of the Hippo signaling pathway, is reactivated in trastuzumab-resistant gastric cancer. Chromatin immunoprecipitation sequencing (ChIP-seq) revealed the enrichment of histone marks H3K27ac and H3K4me1 on YAP target gene loci, indicating epigenetic activation. RNA-seq analysis further confirmed the transcriptional reactivation of YAP, enhancing cell proliferation and drug resistance [25,26].

Inhibition of YAP using verteporfin combined with trastuzumab showed significant antitumor synergy both in vitro and in vivo. This combination suppressed tumor cell proliferation, clonogenicity, and tumor growth by jointly inhibiting AKT/mTOR and ERK/mTOR pathways [16,27].

2.3 Noncoding RNA Regulation

Long noncoding RNAs (lncRNAs) have been identified as important modulators of drug resistance. One such lncRNA, LINC00665, is found at elevated levels in both gastric cancer tissues and trastuzumab-resistant gastric cancer cells. Functioning as a competing endogenous RNA (ceRNA), LINC00665 sequesters miR-199b-5p, which in turn increases SERPINE1 expression. This upregulation activates the PI3K/AKT signaling pathway, facilitating tumor cell proliferation, migration, invasion, and resistance to trastuzumab. Importantly, silencing LINC00665 was shown to restore sensitivity to trastuzumab, suggesting its potential as a therapeutic target to counteract resistance [28,29].

Beyond LINC00665, multiple lncRNAs and miRNAs have been implicated in trastuzumab resistance in gastric cancer. For instance, HOTAIR enhances epithelial–mesenchymal transition (EMT) and activates HER2/PI3K signaling, while MALAT1 promotes immune evasion by modulating PD-L1 expression through miR-200c sponging [30,31]. Emerging evidence also highlights the role of circular RNAs (circRNAs), such as circHIPK3, which sequesters miR-29b to sustain PI3K/AKT activity. Importantly, exosomal transfer of ncRNAs between tumor cells and stromal components contributes to spreading resistance phenotypes across the tumor microenvironment. These findings suggest that ncRNAs not only serve as biomarkers of trastuzumab response but may also represent actionable therapeutic targets through RNA interference, antisense oligonucleotides, or CRISPR-based strategies [32,33].

Table 2 summarizes selected lncRNAs, miRNAs, and circRNAs linked experimentally or by high-quality review evidence to trastuzumab (or HER2-targeted) resistance mechanisms in GC and related HER2 contexts.

Table 2. Representative noncoding RNAs implicated in trastuzumab resistance in HER2-positive gastric cancer.

ncRNA (gene)	Type	Reported mechanism(s)	Key downstream target(s) / pathway(s)	Evidence (model)	Ref
LINC00665	lncRNA	Acts as a competing endogenous RNA (ceRNA) that sponges **miR-199b-5p**, leading to upregulation of **SERPINE1** and activation of **PI3K/AKT**; knockdown restores trastuzumab sensitivity in GC models.	SERPINE1 → PI3K/AKT; proliferation, migration, invasion	patient tissues, trastuzumab-resistant GC cell lines, functional knockdown	[29]
HOTAIR	lncRNA	Promotes EMT/stemness and activates PI3K/AKT axis via miRNA networks; implicated in chemoresistance and suggested to modulate resistance phenotypes relevant to HER2 signaling.	PI3K/AKT, MRP1, EMT transcription factors	GC studies (chemoresistance) and comprehensive reviews; mechanistic links to PI3K/AKT	[34]
circHIPK3	circRNA	Functions as miRNA sponge (e.g., miR-29 family) to sustain PI3K/AKT signaling and modulate autophagy/ferroptosis—mechanisms that can underlie therapy resistance.	PI3K/AKT; autophagy/ferroptosis regulators	GC functional studies and recent reviews on circRNAs in therapy resistance	[35]
miR-199b-5p	miRNA	Tumour-suppressive miRNA sequestered by LINC00665; loss leads to de-repression of SERPINE1 and PI3K/AKT activation.	SERPINE1 → PI3K/AKT	expression analyses and functional rescue experiments in GC cell models	[29]
miR-200c	miRNA	Regulates EMT and PD-L1; downregulation correlates with EMT-driven resistance and immune-evasive phenotypes that reduce HER2 therapy efficacy.	ZEB1, PD-L1, EMT program	immune profiling and mechanistic literature spanning GC/HER2 contexts	[36]
H19	lncRNA (exosomal)	Exosome-mediated transfer can promote angiogenesis, EMT and treatment resistance; implicated in GI cancer exosomal ncRNA literature as mediator of microenvironmental crosstalk.	Angiogenesis pathways, EMT regulators	exosome profiling and reviews in GI cancers (including GC)	[37]
ROR (lncRNA-ROR)	lncRNA	Modulates autophagy and stress responses; linked to multidrug resistance phenotypes in GC (plausible contributor to HER2 therapy resistance via survival/autophagy pathways).	ATG5/ATG12 autophagy axis	GC chemoresistance studies and review summaries	[38]
NORAD	lncRNA	Oxidative-stress induced; promotes resistance to DNA-damaging agents via miRNA/ATG-mediated autophagy pathways — highlights role of stress-responsive lncRNAs in drug resistance programs.	miR-433-3p → ATG5/ATG12 autophagy	GC chemoresistance research synthesized in recent reviews	[38]
Various exosomal ncRNAs (general)	lncRNAs / miRNAs / circRNAs	Tumour→stroma/extracellular vesicle transfer of ncRNAs reprograms macrophages and other microenvironmental cells to a pro-resistance state (e.g., M2 polarization), enabling spread of resistance.	Immune polarization (M2), metabolic crosstalk	exosome profiling studies and GI cancer exosomal reviews	[37]

3. Tumor Microenvironment-Driven Resistance

3.1 Metabolic Crosstalk

Beyond their intrinsic properties, tumor cells reside within a dynamic and complex microenvironment that significantly affects their behavior and response to treatments. The metabolic activities within this environment are continuously adapting, which in turn shapes tumor progression and therapeutic outcomes. Variations in metabolic states can influence the development and function of different immune cell populations, driving changes in the microenvironment that favor tumor growth. Consequently, metabolic reprogramming is recognized as a crucial aspect of cancer biology and plays a central role in the development of acquired resistance to trastuzumab [10].

Previous research has shown that increased expression of enzymes such as hexokinase 2 and lactate dehydrogenase A promotes trastuzumab resistance by boosting glycolytic activity [39]. The mevalonate pathway has also been implicated in resistance through the activation of mTOR signaling [40]. Although the relationship between trastuzumab resistance and amino acid metabolism, particularly glutamine metabolism, remains

less explored [41], existing studies suggest its significance. Glutamine metabolism in tumor cells can activate the AMPK-AKT signaling cascade, contributing to resistance against agents like metformin [42]. Key metabolic enzymes, including GLS1 and glutamate dehydrogenase (GDH), mediate the effects of glutamine on drug resistance. Additionally, studies in breast cancer indicate that tumor-associated fibroblasts can produce and release glutamine into the tumor microenvironment, thereby enhancing tumor cell metabolism and promoting resistance to therapies such as tamoxifen. HER2-positive breast cancers display elevated glutamine metabolic activity, supporting the hypothesis that glutamine metabolism may play a critical role in trastuzumab resistance [43].

The initial generation of glutamine metabolism inhibitors was developed in the 1980s, and more recently, compounds such as telaglenastat (CB-839) have progressed to multiple clinical trials. Consequently, modulating glutamine metabolism represents a promising strategy for counteracting trastuzumab resistance [44,45].

3.2 Immune Cell Polarization (M2 Macrophages)

In the tumor milieu, macrophages can shift between an M1 pro-inflammatory state and an M2 anti-inflammatory profile. The dominance of M2-polarized macrophages promotes immune suppression, facilitates tumor advancement, and has been linked to poor response to treatments such as trastuzumab in HER2-driven gastric cancer [46,47].

Recent studies have elucidated that tumor cells can influence macrophage polarization through metabolic reprogramming and intercellular communication. Specifically, glutamine metabolism within tumor cells has been shown to drive M2 macrophage polarization, thereby mediating trastuzumab resistance. Hu et al. demonstrated that HER2-positive gastric cancer cells with high glutaminase 1 (GLS1) expression secrete GLS1-containing microvesicles, which are internalized by macrophages, promoting their M2 polarization and enhancing angiogenesis. This metabolic crosstalk between tumor cells and macrophages contributes to the development of trastuzumab resistance [10]. Furthermore, tumor-derived succinic acid has been identified as a metabolic byproduct that can activate succinate receptors on macrophages, leading to M2 polarization. This process is mediated through the activation of the NF-κB signaling pathway, underscoring the role of metabolic intermediates in modulating immune cell function and therapeutic response [48,49].

3.3 Extracellular Vesicle-Mediated Transfer

Extracellular vesicles, such as exosomes and microvesicles, play a vital role in facilitating communication between cells within the tumor microenvironment. These vesicles can transfer a variety of bioactive molecules, such as proteins, lipids, and nucleic acids, to recipient cells, thereby influencing their phenotype and function [50]. In the context of trastuzumab resistance, tumor-derived EVs have been implicated in the modulation of immune responses and therapy resistance. For instance, exosomal microRNAs (miRNAs) can be transferred to macrophages, where they may induce M2 polarization and contribute to an immunosuppressive microenvironment. Additionally, EVs can carry metabolic enzymes, such as GLS1, which, upon uptake by macrophages, promote their M2 polarization and facilitate resistance to trastuzumab. Moreover, EVs can influence the expression of immune checkpoint molecules and growth factor receptors on macrophages, further enhancing their immunosuppressive functions and supporting tumor progression. The ability of EVs to modulate macrophage polarization and function highlights their potential as therapeutic targets to overcome trastuzumab resistance [10,32].

Understanding the roles of macrophages and extracellular vesicles in trastuzumab resistance opens new avenues for therapeutic intervention. Strategies aimed at reprogramming M2 macrophages to a pro-inflammatory M1 phenotype, inhibiting EV-mediated intercellular communication, or targeting specific metabolic pathways involved in macrophage polarization may enhance the efficacy of trastuzumab therapy.

In summary, both macrophages and extracellular vesicles are central contributors to trastuzumab resistance in HER2-positive gastric cancer, primarily by modulating metabolic pathways and facilitating intercellular signaling. Interventions aimed at disrupting these processes could offer innovative therapeutic approaches to counteract resistance and enhance clinical outcomes for patients.

4. Multi-Omics Technologies in Resistance Studies

4.1 Genomic/Transcriptomic Profiling

A combined analysis of the TCGA-STAD (stomach adenocarcinoma) and GEO datasets identified 310 shared differentially expressed genes (DEGs). Subsequent evaluations using Gene Set Enrichment Analysis (GSEA), Gene Ontology (GO), and KEGG pathway enrichment revealed several key oncogenic signatures and signaling pathways associated with trastuzumab resistance. From the protein-protein interaction (PPI) network, ten hub genes were highlighted, and ROC curve analysis demonstrated that five of these genes GNGT1, KRT7, KRT16, SOX9, and TIMP1 exhibited strong diagnostic potential for gastric cancer. Additionally, log-rank and Kaplan-Meier survival analyses indicated that elevated expression of KRT16 correlates with overall survival outcomes in gastric cancer patients [51]. One bioinformatics study identified hub genes in gastric adenocarcinoma in general, providing potential molecular candidates for future validation in trastuzumab-resistant settings [52].

In a separate study, Wang et al. examined trastuzumab-resistant gastric cancer cells and identified the PER1-HK2 signaling axis as a key contributor to resistance. These resistant cells exhibited increased glycolytic activity that is regulated by the circadian

rhythm, with hexokinase 2 (HK2) serving as a central mediator and the PPARγ-PER1 transcriptional complex acting as the driver. Silencing PER1 not only disrupted this circadian-controlled glycolysis but also reversed trastuzumab resistance. Moreover, combining trastuzumab with metformin, which inhibits both glycolysis and PER1 activity, significantly enhanced the anti-tumor efficacy in gastric cancer models [53].

4.2 Spatial Metabolomics

Recent studies indicate that spatial metabolomics offers a promising approach to uncover the mechanisms underlying trastuzumab resistance in HER2-positive gastric cancer. While the direct use of MALDI–IMS in trastuzumab-resistant gastric cancer is still limited, research in both gastric and breast cancers has shown that metabolic heterogeneity within and between tumors plays a crucial role in determining therapeutic outcomes [54,55]. For instance, spatially resolved metabolomic profiling has revealed glycolysis- and glutamine-enriched tumor niches, which may support survival of resistant HER2-positive subclones and promote immune evasion. These findings indicate that tumor metabolic heterogeneity could underlie differential sensitivity to trastuzumab. Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) allows linking molecular signatures to histological regions, thereby detecting subpopulations not identifiable by conventional pathology. When integrated with spatial transcriptomics and single-cell proteogenomics, this approach has uncovered resistance-associated features such as phosphorylated RPS6, abnormal HER2 glycosylation, and immunosuppressive metabolic microenvironments [23,56,57].

Therefore, while spatial metabolomics has not yet been directly applied to clinical trastuzumab resistance studies in gastric cancer, it represents a promising future direction. By revealing metabolic and microenvironmental heterogeneity, these technologies may facilitate biomarker discovery and guide personalized therapeutic strategies. In this sense, spatial metabolomics should be regarded as an emerging platform offering mechanistic insights and future perspectives in overcoming trastuzumab resistance.

Figure 2 highlights how multi-omics platforms (genomics, transcriptomics, proteomics, and metabolomics) and spatial technologies (MALDI-IMS, spatial transcriptomics, single-cell RNA sequencing) provide insight into trastuzumab resistance in HER2-positive gastric cancer. These approaches reveal intratumoral heterogeneity, metabolic niches (e.g., glycolysis- and glutamine-enriched subclones), immune microenvironment features, and proteogenomic signatures (e.g., phosphorylated RPS6, HER2 glycosylation). Integration of these data enables biomarker discovery and supports personalized therapeutic strategies.

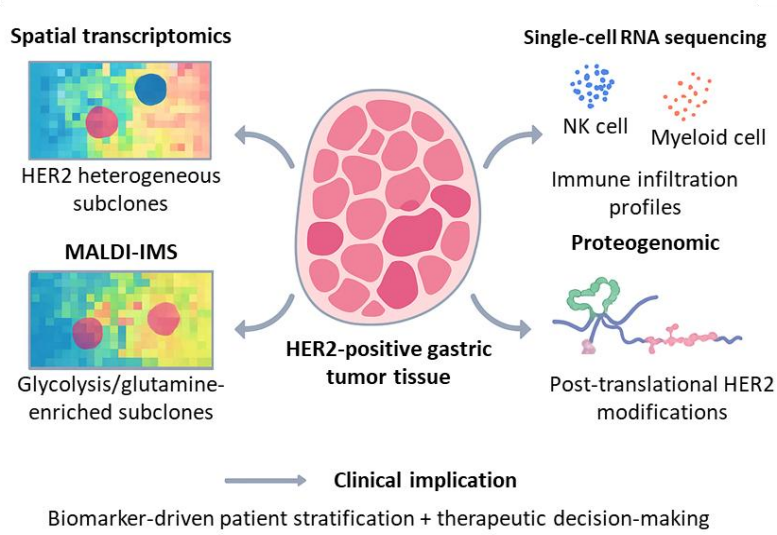


Figure 2. Spatial metabolomics and multi-omics technologies in unraveling trastuzumab resistance.

5. Therapeutic Strategies

5.1 Dual HER2 Blockade

Novel antibody combinations have shown promise in overcoming trastuzumab resistance. For example, the addition of H2-18, an antibody targeting domain I of HER2, to trastuzumab significantly enhanced cell death and reactive oxygen species (ROS) generation compared with trastuzumab plus pertuzumab. This dual blockade of HER2 offered superior efficacy in preclinical gastric cancer models [22].

The antibody drug conjugate trastuzumab deruxtecan (T-DXd) has recently proven effective in patients whose HER2-positive gastric tumors no longer respond to trastuzumab. This therapy couples trastuzumab with a potent cytotoxic agent, ensuring targeted delivery to HER2-positive cells, thereby bypassing several resistance pathways. It is now clinically approved for both gastric and breast cancers [21].

5.2 Metabolic Targeting

Metformin, an anti-diabetic drug, has been shown to inhibit glycolysis and exert anti-tumor effects within

HER2-expressing gastric tumors. Investigations demonstrated that metformin synergizes with trastuzumab to overcome resistance. For instance, Wang et al. reported that metformin enhances trastuzumab sensitivity by modulating circadian rhythm–regulated glycolysis through the PER1–HK2 axis, leading to improved antitumor efficacy in gastric cancer models [53]. Similarly, Kim et al. explored that the combination of metformin and trastuzumab significantly reduced cell proliferation and colony formation in in gastric cancers exhibiting HER2 overexpression, as well as inhibited tumor growth in xenograft models, by suppressing HER2, AKT, and ERK phosphorylation [54].

In addition to glucose metabolism, glutamine metabolism has emerged as another therapeutic target. Inhibitors such as telaglenastat (CB-839) have been shown to disrupt tumor–immune cell metabolic crosstalk, thereby potentially restoring trastuzumab sensitivity and enhancing immune responses [58,59]. These findings suggest that targeting tumor metabolic plasticity with agents such as metformin and glutamine inhibitors may represent promising strategies to improve trastuzumab efficacy in gastric tumors positive for HER2.

Gastric cancer has been found to have a high incidence of HER2 heterogeneity, which is linked to trastuzumab usefulness and chemotherapy response. Proteomic subpopulations associated with patient survival in GC have been identified in several investigations [19]. One important characteristic that has been found to assist in avoiding treatment resistance is metabolic reprogramming [60]. Metabolomics has been identified as a predictor of therapy response in malignancies, integrating information on differences in major metabolic processes and providing metabolite profiles. These mechanisms include immunosuppressive metabolism, the formation of treatment-resistant cancer stem cells, modifications in lipid and amino acid metabolism, and the Warburg effect. Along with HER2 status, the metabolite profile has been regarded as a major determinant in assessing the early response to trastuzumab treatment in patients with GC [60,61].

5.3 Epigenetic and Glycosylation Modulators

Aberrant N-glycosylation of HER2, particularly the sialylation mediated by ST6Gal1, has been shown to stabilize HER2 dimers and thereby attenuate trastuzumab sensitivity [23]. Functional inhibition or knockdown of ST6Gal1 restores trastuzumab responsiveness in preclinical models. Beyond glycosylation, epigenetic regulators such as the Hippo pathway effector YAP also contribute to trastuzumab resistance by promoting tumor cell survival and transcriptional reprogramming; therefore, pharmacological inhibition of YAP and other chromatin-associated regulators has been proposed as a complementary strategy to enhance trastuzumab efficacy [23,62].

6. Conclusion

Loss of trastuzumab efficacy in HER2-driven GC reflects a complex interplay of tumor-intrinsic plasticity, metabolic adaptation, and microenvironmental influences that collectively undermine sustained therapeutic efficacy. Advances in single-cell and spatial multi-omics have uncovered dynamic subclonal evolution, ncRNA-mediated signaling rewiring, and metabolic crosstalk with immune and stromal compartments as key drivers of resistance. Importantly, these insights redefine trastuzumab resistance not as a singular pathway defect but as a systems-level adaptation requiring multipronged intervention. Next-generation strategies, including combinatorial antibody approaches, antibody–drug conjugates, metabolic inhibitors, and immunotherapy, offer tangible opportunities to re-sensitize resistant tumors. Moving forward, the integration of predictive biomarkers derived from transcriptomic, proteogenomic, and metabolomic platforms will be essential to rational patient stratification and the design of precision combination regimens. Ultimately, overcoming trastuzumab resistance will depend on embracing the molecular and ecological complexity of gastric cancer and leveraging it to develop adaptive, biomarker-driven therapeutic strategies.

Competing Interests

There is no conflict of interest.

Authors' Contributions

AZT wrote the manuscript comprehensively in all parts.

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