Advances in HER2-Targeted Therapy: Novel Agents and Opportunities Beyond Breast and Gastric Cancer

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Abstract

The introduction of HER2-targeted therapy for breast and gastric patients with ERBB2(HER2) amplification/overexpression has led to dramatic improvements in oncologic outcomes. In the past 20 years, five HER2-targeted therapies have been FDA approved, four in the past 8 years. HER2-targeted therapy similarly was found to improve outcomes in HER2-positive gastric cancer. Over the past decade, with the introduction of next generation sequencing into clinical practice, our understanding of HER2 biology has dramatically improved. We have recognized that HER2 amplification is not limited to breast and gastric cancer, but is also found in a variety of tumor types such as colon cancer, bladder cancer, and biliary cancer. Further, HER2-targeted therapy has signal of activity in several tumor types. In addition to HER2 amplification and overexpression, there is also increased recognition of activating HER2 mutations and their potential therapeutic relevance. Further, there is a rapidly growing number of new therapeutics targeting HER2 including small molecule inhibitors, antibody-drug conjugates and bispecific antibodies. Taken together, an increasing number of patients are likely to benefit from approved and emerging HER2-targeted therapies.
**Introduction:**

HER2 (*ERBB2*) is emerging as a promising target for genomically-informed therapy across a variety of tumor types. For HER2, gene amplification (increased copy number) is by far the most common genomic alteration and is generally, although not always, associated with protein overexpression (1-3). HER2 overexpression drives tumorigenesis through the creation of spontaneous receptor homodimers (4,5), or heterodimers with other ERBB family members (6) resulting in activated oncogenic downstream signaling, such as PI3K/Akt/mTOR and MAPK, promoting cellular proliferation, survival and angiogenesis (6-8). In particular, HER2-HER3 heterodimers transduce PI3K signaling via direct binding between HER3 and the p85 subunit of PI3K (9). Spontaneous formation of these heterodimers increases with amplification of the HER2 gene (10).

Algorithms for HER2 classification have been evolving. For example, for breast cancer, 3+ HER2 protein overexpression by immunohistochemistry, or HER2 amplification assessed by in situ hybridization (ISH) have been considered HER2-positive, and detailed guidelines for interpretation (11) have been developed by the American Society of Clinical Oncology and College of American Pathologists and are regularly updated.

**Approved Indications for HER2 targeted Therapy**

HER2-targeted therapy has transformed outcomes for HER2-amplified/overexpressing (HER2-positive) breast and gastric/gastroesophageal cancer. Several therapies are approved for HER2-positive breast cancer in the adjuvant and metastatic setting:
trastuzumab (metastatic and adjuvant), pertuzumab (metastatic and adjuvant), lapatinib (metastatic), ado-trastuzumab emtansine (metastatic), and neratinib (adjuvant). Trastuzumab is also approved for HER2-positive metastatic gastric/gastroesophageal junction cancers, in combination with cisplatin and a fluoropyrimidine (capecitabine or 5-fluorouracil). Further, Trastuzumab-dkst (Ogivri), a trastuzumab biosimilar, was approved for all indications included in the label of trastuzumab. FDA approved indications are detailed in Supplementary Table 1.

Targeting HER2 Overexpression/Amplification Beyond Breast and Gastric Cancer with Agents Approved For Breast/Gastric Cancer

With increased genomic profiling of many types of tumor, there is growing recognition that HER2 amplification occurs in in several tumor types including salivary (3.9%), vaginal (3.6%), bladder (3.6%), endometrial (3.4%), cervical (2.2%), and colorectal cancer (CRC; 1.3%) (Figure 1A) (12).

The efficacy of pertuzumab and trastuzumab was tested for HER2-positive tumors in the MY PATHWAY basket trial (13). Thirty of 114 patients (26%; 95% CI, 19% to 35%) with HER2 amplification/overexpression had objective responses (two CR, 28 PR). Responses were seen in nine tumor types: CRC (38% [14/37] objective response rate, ORR; 95% confidence interval [CI]: 23-55%), bladder (33% ORR [3/9]; 95% CI: 8-70), biliary/gallbladder (29% ORR [2/7]; 95% CI: 4-71), salivary gland (80% ORR [4/5]; 95% CI: 28->99), NSCLC (13% ORR [2/16]; 95% CI: 2-38), pancreas (22% ORR [2/9]; 95% CI: 3-60), ovary (13% ORR [1/8]; 95% CI: 0-53), as well as one patient
each with prostate, and skin cancer (apocrine). Further data on efficacy of pertuzumab and trastuzumab is expected from the MY PATHWAY trial as well as the ASCO TAPUR and NCI-MATCH trials; all three trials are being conducted in treatment-refractory patients.

Supporting the efficacy signal with HER2-targeted therapy seen in CRC in the MY PATHWAY trial, several lines of evidence point to the importance of HER2 in CRC biology. Bertotti et al. used a patient-derived xenograft (PDX) platform to identify genotype-response correlations with cetuximab, and found HER2 amplification in cetuximab-resistant, KRAS/NRAS/BRAF/PIK3CA wild-type (WT) PDXs (14). HER2 amplification was also enriched in clinically nonresponsive KRAS WT patients. Further, Raghav et al. reported that HER2 amplification is associated with resistance to anti-EGFR therapy (cetuximab/panitumumab) and shorter progression-free survival (PFS) (15). Taken together, this data suggests that HER2 amplification may not only be a potential target, but also a resistance marker for EGFR inhibitors.

The role of HER2 as a target for metastatic CRC was also assessed in the HERACLES trial, which enrolled patients with KRAS exon 2 WT patients that were HER2-positive as defined as HER2 3+ overexpression in over 50% of tumor cells by IHC or 2+ IHC and a HER2/CEP17 ratio greater than 2 in more than 50% of cells by FISH (16). Eight (30%) of 27 patients treated with dual-targeted therapy, trastuzumab and lapatinib, achieved an objective response, (one complete response, seven partial responses). Together, the HERACLES and MY PATHWAY studies demonstrate that HER2-targeted therapy is
effective in CRC. An ongoing Phase II trial, the SWOG S1613 study (NCT03365882), is comparing the efficacy of trastuzumab and pertuzumab to cetuximab and irinotecan. The trial is accruing patients with metastatic or locally advanced, unresectable CRC who have not received prior epidermal growth factor or HER2 inhibitors and have HER2-amplified tumors that are KRAS/NRAS/BRAF WT. Further study is needed to determine the optimal therapeutic regimens and treatment sequencing.

Recently two multihistology basket trials using ado-trastuzumab emtansine(TDM1) for HER2-amplified disease were also reported. Li et al. reported a trial conducted at Memorial Sloan Kettering Cancer Center (MSKCC) that demonstrated an ORR of 28%, with responses in lung cancer(43% ORR), endometrial cancer(25% ORR), salivary cancer(100% ORR), as well as in ovarian cancer and biliary cancer, but not in CRC(17). In the NCI-MATCH trial, ORR was 8%, with responses seen in 2 of 3 salivary cancers (18). This data raises some interesting points. Although the differences in ORR may simply be due to small study size, there appear to be important differences in ORR between the two trials with similar study designs, using the same drug. It will be important to review the differences in patient populations when details are available. In NCI-MATCH, 33% of patients had received >3 lines of prior therapy, thus it is possible that NCI-MATCH patients were more heavily treated, limiting therapeutic efficacy. In contrast, it is likely that in the MSKCC series, patients were offered genomic testing, and genomically-matched therapy earlier in their disease course. If so, the differences in ORR would support earlier genomic testing to allow for greater benefit of genomically-informed therapy. Another interesting finding was that in the MKSCC series there was
efficacy in several tumor types, but there was also variability in sensitivity by tumor type, with no responses observed in CRC. This highlights that for antibody drug conjugates, in addition to expression of the marker, sensitivity to the specific conjugate needs to be taken into consideration.

**HER2 Mutations**

Somatic mutations can also drive HER2 signaling. While activating mutations have been best characterized within breast and lung cancers(19-21), mutations are reported in a variety of other tumor types(22). A query of the 214 tumor-based (non-cell line) studies within the cBIOPortal reveals a HER2 mutation frequency (mutations or fusions) of 2.7%(23). Figure 1B demonstrates the frequency of mutations across tumor types in the American Association for Cancer Research (AACR) GENIE dataset: tumor types with the highest frequency of mutations in HER2 include bladder(11.6%), small bowel(8.1%), and ampullary(5.9%) (12).

However, not all HER2 mutations are activating. Most of the known activating HER2 mutations are shown in Figure 2 and detailed in Supplementary Table 2. While mutations have been reported across the entire gene, they primarily localize within two regions: the extracellular domain (ECD) and the kinase domain (KD)(19,24). Within the KD, both missense mutations and in-frame insertions lead to increased kinase activity and promote tumorigenesis(19,25,26). Activating mutations have also been reported in the transmembrane domain(27,28), with enhanced protein stabilization being one proposed mechanism for gain-of-function(28).
Types of mutations vary with tumor type. In non-small cell lung cancers (NSCLC), mutations are most frequent in patients that are Asian, female, never-smokers, and in adenocarcinomas (29-31). Mutations are generally exon 20 in-frame insertions within the KD (20,21,29,32). These insertions/duplications occur at the same codons identified in EGFR, indicative of a similar mechanism of activation (29). Indeed, functional studies have shown that HER2^YVMA insertion/duplications increase auto-catalytic activity, leading to increased auto-phosphorylation and phosphorylation of its dimerization partners, and increased survival, proliferation, and tumorigenesis (25,26). In NSCLC, HER2 mutations are mutually exclusive with activating EGFR and KRAS mutations (21,29,30).

In breast cancer, primarily missense mutations are detected. A meta-analysis of 12,905 breast cancer patients published across 31 papers revealed a mutation frequency of 2.7% (33). Greater than 50% of mutations were located in the ECD (S310/Y) or the KD (33). The most frequently reported mutations in the KD include L755S, V777L, D769H/Y, and L755_T759del (33,34). While these and other HER2 mutations activate downstream signaling and/or increase colony formation (19), not all are tumor-promoting in vivo. The V777L, D769H, and G309A mutants induced tumor growth in xenograft models; however, the L755S, V842I, and R678Q mutants performed similar to WT, and the L755_T759del tumors grew slower than WT (19). Moreover, a study investigating the effects of HER2 mutants expressed at endogenous levels found none induced tumor
formation in vivo(35). However, HER2 mutations may cooperate with other oncogenic events (e.g. PIK3CA activation).

Although rare, HER2 gene fusions have also been reported. Within the TCGA PanCancer Atlas study, 1.7% of esophagus, 1.4% of breast, and 1.4% of cervical samples contained a HER2 fusion(23). Three novel fusions were reported in gastric cancer(36). Two (NOS-HER2 and ZNF207-HER2), were further characterized and found to induce auto-phosphorylation and cellular transformation(36). Thus, fusions are another potential therapeutic target.

**Targeting HER2 Mutations**

*Neratinib*

Bose et al. reported that many activating HER2 mutations are resistant to HER2 inhibitor lapatinib, but sensitive to irreversible inhibitor neratinib (19). Ma et al. evaluated efficacy of neratinib in HER2-mutant, nonamplified breast cancer (37). The clinical benefit ratio (CBR) was 31%. Upon longitudinal circulating free DNA analysis, the mutant HER2 allele decreased with treatment and increased with progression in 9 of 11 patients.

The efficacy of neratinib in HER2-mutant tumors was also tested in the SUMMIT trial(38). Neratinib demonstrated the greatest activity in breast cancer patients (ORR at 8 weeks 32%), all with HER2-nonamplified tumors. Responses were observed in both ER+ and ER- tumors and in patients with mutations in the ECD, KD as well as KD insertions.
In lung cancer, only one objective response (in a patient with L755S mutation) was observed among the 26 patients enrolled; but progression free-survival was 5.5 months. Responses were also observed in biliary tumors and cervical cancer, while none were seen among the 16 bladder cancer and 12 CRC patients enrolled, suggesting that histology may impact neratinib's efficacy.

**Pertuzumab/trastuzumab**

Although preclinical data have suggested that monoclonal antibodies may not be as effective as irreversible kinase inhibitors for HER2 mutations, there have been anecdotal reports of responses with trastuzumab-based therapy. In the MYPATHWAY trial, among the initial 36 patients who received trastuzumab plus pertuzumab for tumors with HER2 mutations without amplification/overexpression; four patients (11%) had objective responses: three patients (out of 14) with NSCLC and one with biliary cancer (13). The efficacy of pertuzumab/trastuzumab for HER2 mutations is also being explored in the ASCO TAPUR trial.

**TDM1**

Li et al. tested TDM1’s efficacy in HER2 mutant lung tumors (39). In patients with HER2-nonamplified lung cancer with HER2 mutations, the ORR was 33%. Scientifically this result is somewhat surprising, but the efficacy, at least in part, is being attributed to HER2 internalization with ADC treatment.
Mechanisms of Intrinsic and Acquired Resistance to HER2-targeted therapy and Rational Combinations to Overcome Resistance

There is increasing understanding of mechanisms of intrinsic and acquired resistance to HER2-targeted therapy (Table 1). Much of this data has emerged from breast cancer studies so more study is needed to determine if they are extrapolatable to other tumor types and whether additional mechanisms of resistance are identified in other histologies. Intratumoral HER2 heterogeneity (some cells having HER2 overexpression/amplification and others not) as well as genomic evolution with loss of HER2 amplification are concerns that support the use of pre-treatment biopsies for confirmation of HER2 status, and exploration of the role of liquid biopsies and functional imaging with HER2-PET for assessment of HER2 status.

In spite of years of study of mechanisms of sensitivity and resistance, HER2 aberrations remain the primary biomarker for patient selection. HER2 copy number, including HER2 copy number in cfDNA and HER2-enriched subtype by RNA-seq analysis(40) are associated with sensitivity to HER2-targeted therapy. However, there is evidence for intrinsic mechanisms of resistance including PI3K pathway alterations (mainly PTEN loss and PIK3CA mutations), and emerging data that activating receptor tyrosine kinases/RAS/RAF may limit the efficacy of anti-HER2 therapy(38), making these survival pathways appealing targets for combination therapy. Small molecule inhibitors of HER2, have been association with the emergence of gatekeeper HER2 mutations (L755S, T862A, T798M for lapatinib; HER2 T798I for neratinib) that may still be sensitive to alternate small molecule inhibitors (e.g. afatinib) (41,42).
Combination therapy trials have focused on several approaches: a) combinations of HER2-targeted therapies (doublets and triplets), b) combination with chemotherapy, c) overcoming ER and HER2 cross-talk via combination with endocrine therapy, d) combination with CDK4/6 inhibitors, and e) targeting intrinsic and acquired resistance mechanisms with cell signaling inhibitors such as PI3K/Akt/mTOR pathway inhibitors. A summary of targeted therapies combined with HER2-targeted agents are listed in Supplementary Table 3.

**Novel Her2-targeted Therapies**

**Antibody Drug Conjugates (ADC)**

DS-8201a is a novel anti-HER2 ADC, with a derivative of DX-8951(DXd), a topoisomerase I inhibitor(43) with a drug to antibody ratio (DAR) of approximately 8. Pre-clinically, DS-8201a was effective in a T-DM1-insensitive HER2-positive PDX model. DS-8201a, but not T-DM1, also demonstrated efficacy against HER2-low breast cancer models. In 2017, FDA granted DS-8201 breakthrough designation for HER2-positive breast cancer. DS-8201a demonstrated remarkable activity in heavily pretreated patients, with a confirmed ORR of 54.5%(54/99) and disease control rate(DCR) of 93.9% (93/99) and with preliminary signal of activity in HER2-low tumors: ORR 50%(17/34) and DCR 85.3% (29/34)(44). However, cases of fatal pneumonitis have also been reported(44), highlighting that greater efficacy in HER2-low cells may have a safety trade-off. Studies are ongoing to better define the efficacy as well as safety of DS-8201a.
Additional HER2 ADCs are in development, varying in antibody and linker-payload. The use of antibodies with greater affinity for HER2 or ADCs with higher DAR may overcome resistance due to decreased HER2 expression. Use of toxins with greater bystander effect may help overcome resistance due to tumor heterogeneity and may prove to be effective even in HER2-low cancers, but therapeutic window will need to be assessed.

*Bispecific Antibodies*

ZW25 is biparatropic antibody that simultaneously binds two HER2 epitopes, extracellular domain 4 (the trastuzumab binding domain) and extracellular domain 2 (the pertuzumab binding domain). Pre-clinically, its unique binding facilitates increased tumor cell binding, ZW25-HER2 clustering and enhanced internalization (including in the setting of lower HER2 concentrations). In a Phase I trial, ZW25 led to objective responses in heavily pretreated patients with HER2-amplified/overexpressing breast cancer (33% ORR), gastroesophageal cancer (44% ORR) as well as other HER2-amplified/overexpressing tumor types (33% ORR), including CRC and gallbladder cancer (45). MCLA-128 targets both HER2 and HER3, enhancing antibody-dependent cell mediated cytotoxicity, with clinical benefit rate of 70% in heavily pretreated patients (46). Other bispecific antibodies of special interest include antibodies that also directly engage immune mediators discussed below.

*Small molecule inhibitors*
Several new HER2 tyrosine kinase inhibitors are in development, including tucatinib, poziotinib and pyrotinib. Tyrosine kinase inhibitors are being explored in multiple tumor types and for tumors with HER2-amplified/overexpression as well as activating mutations. Tucatinib was granted fast track designation by the FDA in 2016 for treatment of HER2-positive metastatic breast cancer and orphan drug status in 2017 for treatment of HER2-positive CNS metastasis. Tucatinib as a single agent, as well as in combination with standard-of-care therapies, demonstrated significant growth inhibition in HER2-positive xenografts, including breast cancer models (47,48). Tucatinib also significantly enhanced survival in intracranial tumor xenograft models (49). In the Phase I trial, tucatinib monotherapy had activity in heavily pretreated patients: ORR was 14% and the clinical benefit rate (CBR, PR + stable disease ≥ 24 weeks) was 27% (50). Efficacy of tucatinib has also been tested in combination studies, for example, in combination with TDM1, 68% patients treated with the maximum tolerated dosage had ORR of 47% and CBR was 58% (51). Further, it has been explored in combination with capecitabine (ORR 83%), trastuzumab (ORR 40%), and with trastuzumab and capecitabine (ORR 61%) (52). In both phase 1b combination studies (NCT01983501 and NCT02025192), clinical benefit has been noted in patients with CNS disease at similar rates compared to those without CNS disease and patients were allowed to continue on study with isolated CNS progression following local directed therapy which allowed patients to remain on study longer (52). Validation of overall efficacy and further evaluation of the latter approach will be determined in the ongoing pivotal double-blinded randomized registration study, HER2CLIMB (NCT02614794), which is enrolling...
patients with and without CNS metastases. Tucatinib is also being evaluated in combination with trastuzumab in HER2-positive, RAS WT CRC.

Poziotinib is a small molecule irreversible inhibitor of EGFR, HER2 and HER4. Poziotinib has shown clinical activity in HER2-positive breast cancer and is being developed in combination therapy. Robichaux et al. reported that poziotinib is a potent inhibitor of exon 20 mutations in EGFR and HER2, preclinically. This is notable as exon 20 mutations have been intrinsically resistant to approved targeted therapies(53). Preliminary data from a Phase II trial demonstrated ORR of 64% in lung cancer patients with exon 20 EGFR mutations. Clinical activity was also reported in a lung cancer patient with exon 20 HER2 mutation, and this efficacy for HER2 mutations is being better defined in ongoing trials. Several other small molecule inhibitors are in development, some with proposed greater selectivity for HER2, or for selected HER2 mutations (e.g., exon 20) (Figure 3).

DARPins

MP0274 is a proprietary, designed ankyrin repeat protein (DARPin)-based agent targeting HER2. MP0274 binds to two distinct non-overlapping epitopes on HER2, inhibiting the activity of HER2 and promoting internalization. MP0274 is now in Phase I clinical trials.

HER2-targeted immunotherapy
PANACEA, the first Phase Ib/II trial evaluating the antitumor efficacy of immunotherapy in combination with HER2-targeted therapy (pembrolizumab and trastuzumab), reported an ORR of 15% and DCR of 25% in PDL1-positive patients, and no responses in PDL1-negative patients (54). Although there was modest benefit in the PDL1+ cohort, the disease control in those who responded was durable for one year without chemotherapy, which is notable. Heavily pretreated metastatic breast tumors are thought to be poorly immunogenic and there is much interest in assessing the association between immune function and benefit from trastuzumab in earlier settings. Many other HER2-immunotherapy combinations are ongoing (Supplementary Table 3). There is also great interest in combinations of HER2 ADCs and immunotherapy, as ADCs elicit immune responses and have enhanced efficacy in combination with checkpoint inhibitors pre-clinically (44). In addition, bispecifics are being explored targeting HER2 and immune components. For example, GBR1302 is proposed to direct HER2 and CD3 redirecting cytotoxic T cells onto HER2+ cells, while PRS-343 increases tumor lymphocyte infiltration via bispecific targeting of 41BB(CD137) and HER2. In addition, HER2 vaccines are in clinical trials and CAR-T approaches are still being explored (55).

**Conclusions**

Outside of breast and gastric cancer, in what diseases, and in what clinical setting HER2 testing for amplification/overexpression should be initiated remains controversial. However emerging data suggests efficacy of HER2-targeted therapy for HER2 amplified/overexpressing tumors across a variety of tumor types. Thus, genomic testing...
or specifically HER2 testing by IHC and/or ISH should be considered for advanced/metastatic disease for tumor types where HER2 is known to be amplified (Figure 1A). HER2 also represents an important opportunity for seeking histology-agnostic approvals. It should be noted that most NGS platforms call HER2 amplification at 6-7 copies. Additional studies are needed to validate the efficacy of HER2-targeted therapy across tumor types and to determine whether efficacy can be extended to patients with lower levels of amplification detectable by ISH, and patients with overexpression of RNA or protein in the absence of amplification.

With increasing number of HER2-targeted therapies, we will likely be able to truly personalize therapy selection, offering targeted therapies with greatest expected efficacy based on mutation type and expression status, as well as expected adverse events. Newer therapies may allow us to offer HER2 targeted therapies to patients with lower HER2 expression, leading to a redefinition of “HER2-negative”. For HER2 mutations, evolving data will likely allow us to select optimal therapies individualizing therapy based on variant type.

For advanced disease, more efficacious therapy could improve outcomes. Additionally, transitioning new agents to early stage disease may allow us to offer targeted therapies alone, sparing patients the toxicity of chemotherapy. Finally, increasing the efficacy of neoadjuvant therapy in breast cancer may increase breast-conserving surgery and spare patients axillary lymph nodal dissections, and further strengthen the evolving paradigm of avoiding surgery altogether in exceptional responders. In conclusion,
greater awareness of the HER2 status of patients can enhance incorporation of HER2-
targeted therapy into the multidisciplinary care across tumor types.

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Figure Legends

**Figure 1. HER2 alterations across tumor types A.** Prevalence of HER2 amplifications across diverse cancer types in a cohort of 37,436 sequenced cases extracted from AACR Project GENIE (version 3.0.0, accessed on 16th July 2018) (12). B. Prevalence of HER2 mutations across diverse cancer types (version 3.0.0, accessed on 16th July 2018).

**Figure 2. Activating HER2 mutations.** Mutations defined as ‘activating’ based on published data demonstrating that the alteration increases the activity, expression, or stability of the encoded protein. Additionally, alterations shown to enhance downstream signaling or increase tumorigenic properties when expressed are shown (56). Mutation Mapper from cBIO portal was used to visualize mutations (57,58).

**Figure 3. Approved and emerging HER2 targeted therapies in clinical development.** HER2 targeted therapies approved by the United States Food and Drug Administration (FDA) for HER2-positive cancer or currently in clinical trials (from www.clinicaltrials.gov, last accessed July 11th 2018). “FDA” = the drug is approved by the FDA.
Table 1. Mechanisms of intrinsic and acquired resistance for HER2 targeted therapy

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<tr>
<th>HER2 Targeted Therapy</th>
<th>Mechanisms of Resistance</th>
<th>Potential Strategy to Overcome Resistance</th>
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<td><strong>Intrinsic/Acquired:</strong> Co-expression of EGFR or HER3</td>
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<td>Dual inhibition of EGFR and HER2</td>
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<td><strong>Intrinsic/Acquired:</strong> Overexpression of IGF1R</td>
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<td>Treatment with mTOR/IGF1R inhibitors, inhibition of HER2 kinase activity</td>
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<tr>
<td><strong>Intrinsic/Acquired:</strong> Overexpression of MET or HGF</td>
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<td>Treatment with an Epha2 neutralizing antibody</td>
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<td><strong>Intrinsic/Acquired:</strong> PIK3CA activation</td>
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<td>Inhibition of PI3K or mTOR pathways</td>
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<tr>
<td><strong>Intrinsic/Acquired:</strong> PTEN loss</td>
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<td>Inhibition of PI3K or mTOR pathways</td>
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<tr>
<td><strong>Intrinsic:</strong> High levels of cathecolamines in TM</td>
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<td>Use of β-blockers</td>
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<tr>
<td><strong>Intrinsic:</strong> Expression of estrogen receptor</td>
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<td>Blockade of ER signaling</td>
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<tr>
<td><strong>Intrinsic:</strong> High p95 HER2 expression or high p95/HER2 ratio</td>
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<td>Inhibition of HER2 kinase activity</td>
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<tr>
<td><strong>Intrinsic:</strong> D16 HER2 expression</td>
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<td>Inhibition of Src kinase</td>
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<tr>
<td><strong>Intrinsic:</strong> HER2 exon 20 insertion</td>
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<td>HER2 exon 20 inhibitors</td>
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<tr>
<td><strong>Intrinsic:</strong> Higher expression of PD-L1</td>
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<td>Combination with a checkpoint inhibitor</td>
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<tr>
<td><strong>Intrinsic:</strong> FcgRIII deficiency/polymorphism (deficiency in NK cells and macrophages capable of binding to Fc region of trastuzumab)</td>
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<td>Inhibition of HER2 kinase activity</td>
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<tr>
<td><strong>Acquired:</strong> Overexpression of AXL</td>
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<td>AXL inhibitor or AXL ADC</td>
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<tr>
<td><strong>Acquired:</strong> constitutive activation of Src kinase</td>
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<td>Src kinase inhibitors</td>
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<td><strong>Acquired:</strong> upregulation of survivin and Mcl-1</td>
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<td>Broad spectrum kinase inhibitors or Mcl1 inhibitors</td>
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<td><strong>Acquired:</strong> upregulation of cyclin E2</td>
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<td>Treatment with CDK2 inhibitors</td>
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<td><strong>Acquired:</strong> downregulation of p27kip1</td>
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<td>Treatment with CDK2 inhibitors</td>
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<td><strong>Acquired:</strong> downregulation of HER2 expression</td>
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<td>Treatment HER2-targeted therapies with efficacy in lower HER2-expressing tumors</td>
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<td><strong>Intrinsic:</strong> poor internalization of HER2-T-DM1 complexes/increased recycling/defective trafficking to lysosomes</td>
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<td>Modification of linker</td>
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<tr>
<td><strong>Acquired:</strong> upregulation of ABCC1 (MRP1)</td>
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<td><strong>Acquired:</strong> Increase in HER3 transcription and phosphorylation</td>
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<td>Inhibition of HER3, and HER3 ADC</td>
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<tr>
<td><strong>Lapatinib/neratinib</strong> (59,60), (41) (42)</td>
<td><strong>Acquired:</strong> Overexpression of AXL</td>
<td>Combination with an AXL inhibitor or AXL ADC</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td><strong>Acquired:</strong> increased activation of Src family kinase activity</td>
<td>Combination with Src inhibitors</td>
<td></td>
</tr>
<tr>
<td><strong>Acquired:</strong> Increased signaling through PI3K and AKT/mTOR pathways</td>
<td>Inhibition of PI3K or mTOR pathways</td>
<td></td>
</tr>
<tr>
<td><strong>Intrinsic/Acquired:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low/downregulation of BIM levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acquired:</strong> upregulation of ERα, leading to FoxO3a-mediated transcription of survivin</td>
<td>Treatment with ER antagonists</td>
<td></td>
</tr>
<tr>
<td><strong>Acquired:</strong> HER2 gatekeeper mutations (L755S, T862A, T798M for lapatinib; HER2 T798I for neratinib)</td>
<td>Treatment with Afatinib</td>
<td></td>
</tr>
</tbody>
</table>
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Figure 1:

A

Amplification frequency

B

Mutation frequency

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Figure 2:
Antibodies:
- Trastuzumab (FDA)
- Trastuzumab-dkst (FDA)
- Pertuzumab (FDA)
- ZW25

Designed ankyrin repeat proteins (DARPins):
- MP0274

Other drugs:
- Bispecific antibodies targeting HER2 and immune cells:
  - Margetuximab
  - BTRC4017A
  - GBR-1502
  - PRS-343

HER2 peptide vaccines:
- AVX901
- E75
- E7BX-021

CAR T-cell therapy:
- HER2Bi-armed activated T cells

Small molecules (tyrosine kinase inhibitors):
- Lapatinib (FDA)
- Neratinib (FDA)
- Afatinib
- Domitinib
- Brutininib
- Poxotinib
- Pydonitbin
- Sapitinib
- Tarxotinib
- Tesematinib
- Tucatinib
- AP32788
- TAS0728

Antibody–drug conjugates:
- Ado–trastuzumab emtansine (TDM1) (FDA)
- ARX788
- ALT–P7
- DS8201a
- MEDI4276
- MM302
- PF–06804103
- SYD985
- XMT–1522

Designed ankyrin repeat proteins (DARPins):
- MP0274

Figure 3:
Clinical Cancer Research

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Funda Meric-Bernstam, Amber Johnson, Ecaterina E Ileana Dumbrava, et al.

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