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Molecular Pathways and Mechanisms of HER2 in Cancer Therapy

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Abstract

The oncogene *ERBB2* encoding the receptor tyrosine-protein kinase erbB-2 (HER2) is frequently overexpressed or amplified and occasionally mutated in a variety of human cancers. The early discovery of this oncogene, its established oncogenic relevance in diverse cancers, its substantial expression on surface of cancer cells, and its druggable catalytic activity have made it one of the most pursued targets in the history of cancer drug development. Initiatives targeting HER2 provided the early stimulus for several transformational pharmaceutical technologies including monoclonal antibodies, tyrosine kinase inhibitors, antibody-drug conjugates, and others. The seismic impact of these efforts has been felt in treatment of many cancers including breast, gastroesophageal, lung, colorectal and others. This impact continues to broaden with increasing indications on the horizon and a plethora of novel agents in development. However, implementation of these therapeutic strategies has been complex. The clinical translation of every one of these classes of agents has been notable for underperformance or overperformance characteristics that have informed new lines of research providing deeper insights into the mechanistic complexities and unrealized opportunities provided by this molecular target. Despite all the successes to date, the preponderance of scientific evidence indicates that the full potential of HER2 as a target for cancer therapeutics is far greater than currently realized and numerous lines of investigation are ongoing to deepen and broaden the scope of impact of HER2 as a signaling, homing, or immunologic target. In this review, we explore the existing data and evolving paradigms surrounding this remarkable target for cancer therapy.

Keywords

Amplification; Antibody drug conjugate; ERBB2; HER2; Targeted therapy

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Introduction

ERBB2, commonly referred to as Human Epidermal Growth Factor Receptor-2 (*HER2*), is a proto-oncogene (chromosome 17q21) that encodes a transmembrane receptor tyrosine kinase involved in cell growth and differentiation (Figure 1). It was one of the first genes identified in the 1980's efforts to discover oncogenes and was quickly shown to have relevance to diverse human cancers [1,2]. What followed was an explosion of scientific inquiries that validated the role of *HER2* in carcinogenesis and led to development of a variety of targeting approaches which have paved the way in transforming drug development. The oncogenic potential of *HER2* was evident in the early days of mouse genetic engineering with several models of HER2-driven mammary tumorigenesis showing an aggressive and metastatic disease biology [3]. As more elegant inducible mouse genetic models were developed, HER2 was shown to be continuously required throughout the entire disease process from initiation through advanced metastatic disease, nicely illustrating the concept of oncogene addiction and the potential of this oncogene as a target for cancer therapeutics [4]. Advances in sequencing technologies brought about the era of widespread tumor genome sequencing with findings that extended the scope of *HER2* amplification to include substantial subsets of gastric and esophageal cancers as well as smaller subsets of colon, bladder, endometrial, lung, salivary gland, and other cancers (Figure 2) [5-7]. These surveys also discovered much smaller subsets of cancers harboring activating *HER2* mutations without amplification [8]. Whether these *HER2* mutants are dominant tumor-drivers like *HER2* amplifications, remains to be determined.

The technology to generate monoclonal antibodies and to humanize them for repeated administration as therapeutic agents evolved in the late 1980s and HER2 was one of the first targets explored by this novel pharmaceutical technology [9]. Trastuzumab, the anti-HER2 monoclonal antibody (mAb), entered clinical phase in 1992, eventually leading to a pivotal phase 3 study in *HER2*-amplified metastatic breast cancer, and landmark studies in *HER2*-amplified early-stage breast cancers, paving the way and establishing the role of mAbs in treatment of human cancers [10-13]. As understanding of structure and function among the HER family receptors evolved in 1990s, these insights were applied to development of structure-guided mAbs such as pertuzumab to disrupt HER2 dimerization more effectively with additional, albeit incremental benefits demonstrated in clinical studies [14,15]. Technologies to arm these mAbs with cytotoxic agents evolved in late 1990s with improvements in linker chemistries and HER2 was among the early targets pursued leading to development of the antibody drug conjugate (ADC) aldo-trastuzumab emtansine (T-DM1) [16]. While the naked HER2 mAbs, trastuzumab or pertuzumab, showed only limited efficacies and their clinical benefits were mainly manifest in combination regimens with chemotherapeutics, T-DM1 was found to be highly efficacious in monotherapy with activities surpassing chemotherapies in treatment refractory tumors with a much more favorable toxicity profile [17]. This remarkable breakthrough fueled efforts to further explore ADCs leading to development of trastuzumab deruxtecan (DS-8201 or T-Dxd) [18]. Exploring T-Dxd revealed unforeseen findings of non-cross resistance with T-DM1 and much broader clinical activity in other *HER2*-amplified cancers and in cancers with lower expression of

HER2 [19-22]. The high efficacy of HER2 ADCs has led to development of a plethora of ADCs, now in trials.

As technologies to develop selective tyrosine kinase inhibitors (TKIs) evolved, the HER family of kinases including EGFR and HER2 were among the first targets pursued leading to many compounds proceeding to clinical testing in *HER2*-amplified breast cancers. Of these the pan-HER reversible TKI lapatinib, pan-HER irreversible TKI neratinib, and HER2-selective reversible TKI tucatinib, have made it to clinical use with many others in development. TKIs have only modest activities in monotherapy of *HER2*-amplified cancers and their clinical use has been predominantly focused on combination therapies [23,24]. In contrast, the *HER2*-mutated subtypes of breast and other cancers appear amenable to TKI monotherapy as shown in the basket neratinib study.[25]

Mechanics

We now have a good understanding of mechanisms by which HER family of receptors function. Signals are generated when two receptors from the family come together in a homo- or hetero-dimerization configuration. The receptor extracellular domains (ECD) are generally restrained by a closed conformation that is prohibitive to dimerization and it is the binding of extracellular ligands that exposes an interface that promotes dimerization [26]. HER2 is unique in that it lacks this self-restraint or a physiologic ligand, and its ECD is always poised for dimerization [27]. As such the only physiologic restraint built into HER2 is its low expression which is overwhelmed in cancer cells through amplification and overexpression. Dimerization leads to phosphorylation of C-terminal tails and consequent initiation of second messenger signaling pathways [28]. A particularly strong relationship between HER2 and HER3 as dimerization partners is apparent from *in vitro* signaling assays [29]. Many lines of investigation also confirm that HER3 is an important HER2 partner in HER2 overexpressing tumors [30-32]. These structural insights provide ample mechanistic rationale to inactivate HER2 signaling through ECD targeting antibodies that disrupt dimerization or through TKIs that inactivate catalytic activity and eliminate phosphorylation. Yet, the story has turned out to be much more complicated.

The translational research in pursuit of mutationally activated oncogene-driven cancers such as *EGFR*- or *ALK*-mutant lung cancer among others has followed a somewhat direct path with the rational expectation that the catalytic inactivation of these disease drivers should be a highly effective treatment strategy. However, the pursuit of amplified *HER2* as a target has followed a much more tortuous and unexpected path with many mechanistic complexities and ambiguities, new insights learned, and new opportunities discovered. It is evident now that in most cases HER2 is activated through massive overexpression rather than mutation and this makes for a mechanistically more challenging target, something that was not readily anticipated in 1990s. While in the early days following development of trastuzumab and pertuzumab it was thought that these mAbs could interfere with HER2 signaling by eliminating their expression or disrupting dimerization, this was clearly not the case as was evident in simple *in vitro* studies in *HER2*-amplified cancer cells [33-37]. Pertuzumab was specifically developed based on a structure-based design to bind the HER2 dimerization interface and clearly does inhibit signaling in physiologic cell systems with low HER2

expression but fails to do the same in HER2-overexpressing cancer cells [35-38]. Similarly, a variety of rationally designed HER2 or HER3-targeting mAbs, bispecific mAbs, DARPins, and other biotherapeutics have shown only limited ability to interfere with HER2-HER2 or HER2-HER3 signaling in *HER2*-amplified cancer cells. Experimental models now more clearly demonstrate the mechanistic futility of these approaches.

The kinase domain interactions in these cancers are driven entirely by massive HER2-overexpression with no promoting or restraining functions from the ECDs essentially uncoupling intracellular signaling from the ECDs (Figure 1) [37]. Even strategies to add bulk to the ECDs and prevent receptor proximation leave kinase domain signaling intact due to conformational flexibilities across the span of these receptors and the curvatures in the plasma membrane (Figure 1) [37]. Although mechanistic studies with trastuzumab failed to show effective inhibition of HER2 signaling, preclinical and clinical studies do show measurable anti-tumor effects [11,39]. This has fueled efforts to understand the mechanistic basis for the observed *in vivo* effects and a large body of work now demonstrates this to have a substantial immunologic basis. This was initially shown through elegant mouse studies showing the critical role of host Fc receptor in mediating the *in vivo* anti-tumor activity of trastuzumab and subsequently shown to encompass a wider repertoire of immunologic activities including innate and adaptive immune functions, memory, and cytokines [40-43]. Capitalizing on these insights, enhancements in the Fc portion of trastuzumab to optimize its immunologic activities has produced margetuximab with superior clinical activity compared with trastuzumab [44].

In contrast to mAbs, HER2 targeting TKIs do effectively inactivate HER2 signaling in cell-based studies in *HER2*-amplified cancer cells, leading to apoptotic cell death [45,46]. Yet these agents fail to show substantial activities in patients as monotherapy [47,48]. Mechanistic studies highlight limitations in target inactivation, in particular with regards to inactivation of HER2-HER3 heterodimer signaling. In *HER2*-amplified cancers HER3 is tightly linked with downstream PI3K-AKT signaling in a pathway that involves robust compensatory feedback regulation. As such, inhibition of HER2 with TKIs leads to a compensatory upregulation of HER3 unleashing a 100-fold reserve in signaling capacity that overpowers an incomplete inhibition of HER2 kinase and restores HER2-HER3 signaling [49-51]. Only the complete inactivation of HER2 kinase can durably inactivate HER2-HER3 signaling in these cancers, and although this is feasible in cell culture models at higher concentrations of TKIs, it is beyond the therapeutic index of all current TKIs [50]. High dosing of oral TKIs in patients has been studied but limited by a bioavailability ceiling [52]. Irreversible TKIs, such as neratinib, that covalently bind their target have much higher molar potency, but this comes at a cost of substantial off-target activities, within and outside of the kinome, significantly limiting therapeutic index [53-56]. A rational strategy for increasing potency would be co-targeting HER2 and HER3, but HER3 is a challenging target for pharmaceutical inactivation. Its function in *HER2*-amplified cancers is engaged in a ligand and ECD-independent manner limiting the efficacy of ECD-targeting mAbs in this disease, and its kinase domain functions in allostery, not catalysis, and conventional TKIs binding within its ATP pocket have no effects on its signaling function [37,57]. Novel approaches to target HER3 through degradation are being pursued and may provide breakthroughs in this arena [58,59].

Although the massive overexpression of HER2 makes it a challenging target for inactivation, its surface expression provides significant opportunities for targeted delivery of cytotoxic molecules, radioisotopes, liposomes, nucleic acids, and other moieties designed to kill cancer cells. In this regard the pursuit of HER2-targeting ADCs has proven particularly fruitful. In contrast to HER2-targeting mAbs and TKIs which underperformed expectations as oncogene inhibitors when they entered the clinical arena, HER2 ADCs have overperformed expectations, and their success has spawned many lines of study to better understand the mechanistic basis for their activities and fueled substantial investments in the pharmaceutical sector with many new agents in development. The science underlying exact mechanisms of activity of ADCs continues to evolve. The key variables appear to be the linker chemistry, the activity, cell permeability and potency of the cytotoxic payload molecule, the payload-to-antibody ratio, and the characteristics of the mAb, all of which contribute to the observed clinical activities of these agents [60,61]. Although the initial and purest vision for the development of ADCs was for the most precise and protected delivery of cytotoxic molecules intracellularly to cancer cells, it is now apparent that reducing the stringency of this vision can potentially increase, not decrease, their therapeutic index. Using cleavable linkers that more readily release their payloads or cell permeable cytotoxic agents that diffuse out of cells can lead to exposure beyond the target cells including non-targeted surrounding cells, a so-called bystander effect, and a measurable low exposure in the systemic circulation (Figure 1) [62]. Clinical exploration of the cleavable HER2-targeting ADC, T-Dxd, has revealed an unexpected range of activity. The highest efficacy is still seen in patients with classic “HER2-high” (HER2-overexpressing/amplified) subtypes of cancers, consistent with the original and simplest concept of ADCs as agents for precise tumor-targeted cytotoxic delivery [19-21]. However, pursuing an assumption that cancers with lower expression of HER2 may also afford a therapeutic index by mechanisms involving heterogenous expression and bystander effects, significant clinical efficacy was also observed in breast cancers with “HER2-low” expression (IHC 1+ or 2+) [22]. Further exploration has shown no lower cutoff for this quantitative biomarker with efficacy spanning the entire spectrum from 0 - 2+ expression and across the hormone receptor status in breast cancer [19,63]. This wide range of activity is also seen in HER2-mutant lung cancers wherein, there is no correlation of activity with tumor HER2 expression [64]. The observed broad and marker-independent clinical activities of T-Dxd have upended the mechanistic hypotheses regarding the mode of action of ADCs. Tumor-specific targeted delivery of cytotoxic payloads related to high expressing and bystander effect in lower expressing cancers can only partially account for this observed broad range of activity and the mechanistic basis remains to be determined. Traditionally, mechanism of action forms the starting point for development of rationally designed therapeutics. But clinical development of T-Dxd and similar ADCs has clearly exited this mechanistic orbit and their clinical exploration continues in an open space until a ceiling is encountered, while mechanistic studies will have to follow to fill this ever-enlarging knowledge gap.

The newly discovered broad clinical activity is not limited to T-Dxd and appears to be a class effect. Preliminary evidence from other HER2-targeting ADCs appears to show similar broad range of efficacies [65-67]. This property of ADCs is also not unique to the target HER2 and appears to apply to other targets as well, as is evident with the

experience with Trop-2 targeting ADCs. While, initially explored in triple-negative breast cancer which has the highest expression of Trop-2, its efficacy has poor correlation with Trop-2 expression levels and shows similar clinical activity in hormone receptor positive breast cancers with lower expression of Trop-2 [68-71]. A plethora of other ADCs in the investigational pipelines exploring other types of linkers, payloads, release properties, and antibodies and drug-antibody ratios will further expand the body of data available and allow us to formulate more informed hypotheses regarding potential mechanisms of action. Although initial pharmacokinetic studies of T-Dxd showed low levels of free deruxtecan in circulation, the role of systemic release products must be revisited [72,73]. Much of the mechanistic foundation for the broad clinical activities of these ADCs remains unknown and eagerly awaits new hypotheses and experimental studies.

Distinct from cancers driven by amplification and overexpression of HER2, there are also rarer cancers that harbor somatic mutations in HER2 [74,75]. Most, but not all, are within the kinase domain and generally result in increased catalytic activity of the HER2 kinase. Individual mutations appear to have different characteristics with respect to catalytic activities and partner preferences and exhibit cell-context dependent characteristics [74]. Most experimental studies have used overexpression systems and in these artificial systems many are more potent oncogenes compared with wildtype HER2. But in human cancers they are typically mutated in copy-neutral fashion without overexpression and whether they are primary disease drivers in human cancers remains speculative. The irreversible TKI neratinib has modest but short-lived activity in HER2-mutant cancers, confirming a limited biologic role for at least some of these mutants in the human disease. The diverse nature of these HER2 mutants makes for a complex arena for analysis and it remains difficult to know whether non-responsive mutants are not biologically relevant or whether they harbor intrinsic resistance to TKIs. There is much more to be learned about the biology of HER2 mutant cancer.

Distinct from HER2-amplified and HER2-mutant cancers are exceedingly rare cancers driven by ligand activation of HER2. These appear to be driven by genetic fusions of the NRG1 gene leading to neuregulin fusion proteins expressed at the plasma membrane [76,77]. Anecdotal reports of these rare cases suggest that these tumors are responsive to HER2 TKIs or HER2 or HER3 targeting mAbs that block dimerization or ligand binding [78,79].

Clinical Implications

Breast Cancer

The field of *HER2* amplified breast cancer has benefited greatly from decades of translational cancer research through the development of numerous targeted therapies that have made substantial impact in the clinical realm. Addition of trastuzumab and pertuzumab to chemotherapy significantly improves outcomes in patients with metastatic or early-stage breast cancer and is now the standard of care for the treatment of HER2 amplified breast cancer at all stages of disease [11-13,15,80]. T-DM1 has shown significant clinical activity in trastuzumab-resistant disease and has become a standard second-line salvage option in patients with metastatic disease or in early stage patients who have significant residual disease following neo-adjuvant therapy [17,81]. The second generation ADC, T-Dxd, has

shown clinical activity superior to T-DM1 and activity in disease that is resistant to T-DM1 [82]. As discussed previously, T-Dxd has a much broader range of clinical activity that encompasses HER2-negative breast cancers, including cancers traditionally labeled HER2-low [22,63]. This broad range of activity appears to somewhat uncouple T-Dxd from its target biomarker and it may be best considered a broadly active breast cancer agent without a biomarker association, exhibiting clinical activity in breast cancers spanning HER2 positive and negative and hormone receptor positive and negative subtypes, albeit with superior activity against HER2-amplified cancers. This has sparked substantial interest and numerous other investigational HER2 targeting ADCs are exploring this newly discovered terrain [83].

The HER2 TKIs lapatinib or tucatinib in combination with capecitabine are active regimens for the treatment of metastatic HER2 amplified breast cancers refractory to prior HER2 therapy, providing additional options for later lines of therapy [23,24]. The combination of lapatinib and trastuzumab has clinical activity that provides a non-chemo option for patients with low disease burden, indolent disease, or not suitable candidates for chemotherapy [84].

The *HER2*-amplified subtype of breast cancer has a higher predilection for brain metastases and numerous studies have attempted to define the activities of HER2-targeted therapies in the treatment of brain metastases [85]. Small molecule HER2 TKIs have better CNS penetration than mAbs and these and have been actively pursued for CNS activity. The CNS activity with lapatinib is minimal, but more significant activity is evident with neratinib in the treatment of brain metastases [86]. The highest CNS activity is seen with the brain-penetrant TKI tucatinib and the tucatinib, capecitabine, trastuzumab combination has become a standard in the management of patients with brain metastases [87]. Although the activity of ADCs in the CNS compartment were thought to be restricted by the large size of their mAb component, hints from subset analyses of their clinical studies suggested these agents may have activity in the CNS and this was followed by specific clinical studies showing modest activity with T-DM1 [88-90] and substantial intracranial activity with T-Dxd [91,92].

Gastroesophageal Cancer

HER2-overexpression/amplification, to date, remains the only clinically usable biomarker for selection of targeted therapy in metastatic gastroesophageal adenocarcinoma (GEC) and is seen in about 12%-20% of cases (Figure 2) [6,7,93]. *HER2*-amplification is seen in a greater proportion of patients with liver metastasis, GEJ adenocarcinomas and intestinal subtype tumors, although its prognostic significance is unclear [93,94]. Compared to circumferential staining seen in breast cancer, HER2 expression in GEC is predominantly basolateral/lateral and has more notable intratumoral heterogeneity, making testing (ToGA criteria) and patient selection, more challenging [93,94].

Anti-HER2 therapy is mainstay of therapy for advanced HER2-overexpressed/amplified GEC. Addition of trastuzumab to chemotherapy for first-line treatment of HER2-positive advanced GEC demonstrated a clear survival benefit over chemotherapy alone [94]. However further efforts, as in the case of breast cancer, such as addition of dual anti-HER2 therapy with pertuzumab and T-DM1, showed limited activity and failed to significantly improve overall survival in patients with HER2-positive metastatic GEC [95,96]. More recently,

addition of pembrolizumab to trastuzumab and chemotherapy significantly improved objective response rate (74% vs. 52%) highlighting the immune interactions of the HER2 pathway [97]. T-Dxd has also shown significant activity in treatment of HER2-positive metastatic GEC after progression of first line anti-HER2 therapy with a significantly higher response rate (51%) compared to physician's choice of chemotherapy (14%) and longer overall survival (median: 12.5 vs. 8.4 months) [21].

Lung Cancer

Unlike breast and gastric cancer, *HER2* amplification are rare in lung cancer (0.88% cases) and *HER2* mutations are the driver events in 3.5% cases, comprising 80% of all HER2 alterations (Figure 2) [6,7]. These *HER2* mutations, specifically exon 20 mutations are analogous to in-frame *EGFR* deletions and dysregulate the HER2 pathway due to constitutive tyrosine kinase activity, are enriched in non-smokers, adenocarcinomas, and are mutually exclusive of other oncogenic mutations in NSCLC [98]. *HER2* mutations, barring amplifications and overexpression, appear to be key determinant of response to HER2 targeted therapies. No clinical benefit was observed with addition of trastuzumab to chemotherapy in *HER2*-overexpressed/amplified non-small-cell lung cancer (NSCLC) [99]. This aspect of aberrant HER2 pathway is unique to lung cancer. Although, *HER2* kinase domain mutations are also seen in other tumor types, such as breast (4.3%), gastric (5.0%), and colorectal (2.9%) carcinomas, they are often missense mutations (not deletions/insertions) and involve exons other than exon 20, indicating tissue dependent oncogenic mechanisms [8]. Additionally, *HER2* mutation are not associated with *HER2* overexpression/amplification [100].

HER2-mutant lung cancer responds to HER2 inhibitors, especially small-molecule TKIs and ADCs [101]. Although, selective HER2 TKIs have yielded response rates of 20-35% in single-arm phase 2 studies, many of these have shown limited overall clinical benefit. Recent evidence has brought HER2 ADCs to the forefront in treatment of *HER2*-mutant NSCLC. T-DM1 demonstrated a response rate of 44% (N = 18) in treatment refractory *HER2*-mutant lung cancer, across a variety of *HER2* exon 20 insertions and point mutations (in kinase, transmembrane, and extracellular domains) and regardless of HER2 expression or amplification status [102]. Similarly, T-Dxd showed a confirmed objective response in 55% patients (N = 91) with refractory metastatic *HER2*-mutant NSCLC, again notwithstanding HER2 expression or amplification [103]. Currently there is no mechanistic paradigm to account for this activity of ADCs and this is an active area of pursuit as discussed previously.

Colorectal Cancer

HER2 overexpression/amplification is seen in 2-3% of all colorectal cancers (CRC) and represents a very distinct subset of CRC (Figure 2) [6,7]. Pre-clinical and retrospective clinical evidence shows that *HER2* amplification is enriched in *RAS/BRAF* wild-type tumors (5-6%) compared to *RAS*-mutant CRC (1-2%) [104,105]. In *RAS//BRAF* wild-type mCRC, *HER2* amplification appears to be a negative predictive biomarker of response to the anti-EGFR monoclonal antibody based therapy, which is the current standard of care in these patients with metastatic CRC [105]. Although a diverse array of *HER2* somatic mutations are seen in a very small subset of CRC, only a small number appear to have oncogenic potential

and show response to irreversible TKIs (such as neratinib and afatinib), and as such they have not been exploited clinically [106].

As opposed to gastric cancer, single agent anti-HER2 therapy shows limited efficacy in *HER2*-amplified metastatic CRC [104,107]. However, dual-HER2 targeting with trastuzumab combined with lapatinib, pertuzumab and tucatinib has shown robust activity in treatment refractory metastatic CRC with response rates ranging from 28-40% in *RAS* wild-type tumors [107-110]. Notably, dual anti-HER2 therapy benefits only patients with *RAS* wild-type tumors (response rate of 40% vs. 8% for *RAS*-mutant cases) [109]. Along similar lines although the HER2 ADC, T-DM1 showed very limited activity (response rate 9.7% combined with pertuzumab) in *HER2*-amplified metastatic CRC, trastuzumab deruxtecan (DS-8201) showed very promising and durable activity in *HER2*-amplified metastatic CRC refractory to standard treatment with response rate of 45%, including those who had received prior anti-HER2 therapies [20,111].

Other Solid Tumors and HER2-mutant cancers

In addition to tumor types mentioned above, HER2 alterations can be seen in a variety of tumor types such as salivary gland tumors (particularly non-adenoid cystic carcinoma, non-secretory type), hepatobiliary tumors (principally gallbladder cancer) and others (Figure 2). Dual HER2 targeting with trastuzumab and pertuzumab showed a response rate of 23% (N = 39) in *HER2*-amplified metastatic biliary tract cancer [112]. A phase 2 trial of trastuzumab and docetaxel in *HER2*-amplified salivary duct carcinoma (N = 57) showed an overall response rate of 70.2% and is the preferred treatment option for these rare cancers [113]. Basket trials of HER2 targeted therapies to engage tumor types with lower prevalence of HER2 alterations are ongoing.

Numerous basket studies and disease-specific phase 2 studies of TKIs have been conducted in cancers harboring somatic mutation of HER2 (Figure 2) [6,7]. As a class, TKIs do have activity, albeit modest, in these cancers although there is variation in activity according to disease type, specific mutations, and specific drug. The irreversible class of TKIs have clinically meaningful activity and neratinib has been studied the most with modest activity in breast and lung cancers but evidence of activity in other cancers [25,101,114-117]. Pyrotinib and poziotinib also have similar efficacies while afatinib appears to lack efficacy [118-120].

Future Directions

The field of HER2 targeting has been an archetype for pan-cancer developmental therapeutics with promising clinical results, continuously evolving new mechanistic insights, profound translational efforts, and tremendous hope for patients suffering with cancer. While we have achieved unprecedented success, a substantial subset of patients derive limited benefits from current approaches and resistance develops eventually. Through a barrage of ongoing clinical trials (Table 1), three major themes are evolving: 1) therapeutics strategies involving combining anti-HER2 therapies with immunotherapy, inhibitors of DNA damage pathway, receptor cycling modulators among others which may work synergistically to increase the responses, 2) targeting a large subset of patients who have tumors that were traditionally considered HER2-low and not amenable to past generation anti-HER2

therapies, and 3) immune targeting of HER2 using novel immunotherapy approaches, such as CAR-Tcells, and vaccines. No matter the strategy, one thing is for sure, HER2 targeting remains the epitome of precision cancer medicine in oncology.

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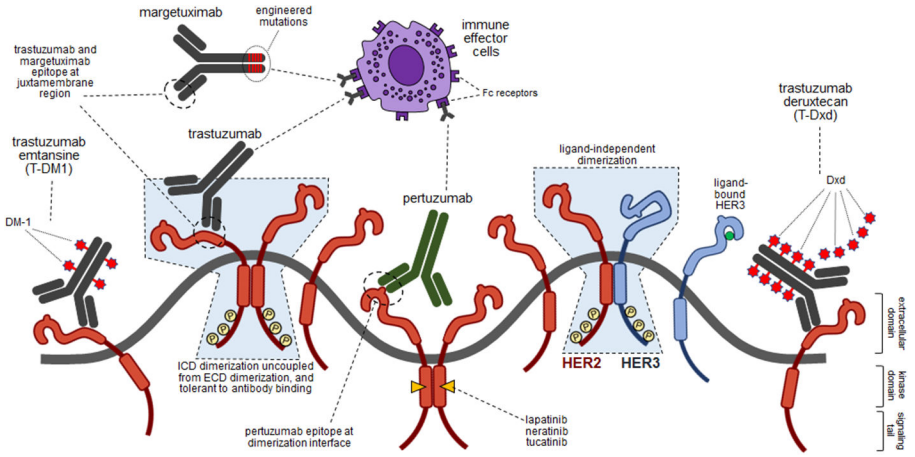

Figure 1. HER2 pathway and pharmacologic strategies

Schematic showing the structures and events occurring in *HER2*-amplified cancers and the site of binding of various HER2-targeted therapies. HER2 is shown in red and HER3 is shown in blue. HER3 is shown in the activated ligand-bound state as well as the inactive unbound state. HER2 only has one conformation always poised for dimerization. The shaded light blue enclosures highlight aspects of dimerization that are non-physiologic but occur in cancers propelled by the massive expression of HER2, reflecting the uncoupling of kinase domain dimerization and signaling from the ECDs and tolerance to antibody binding.

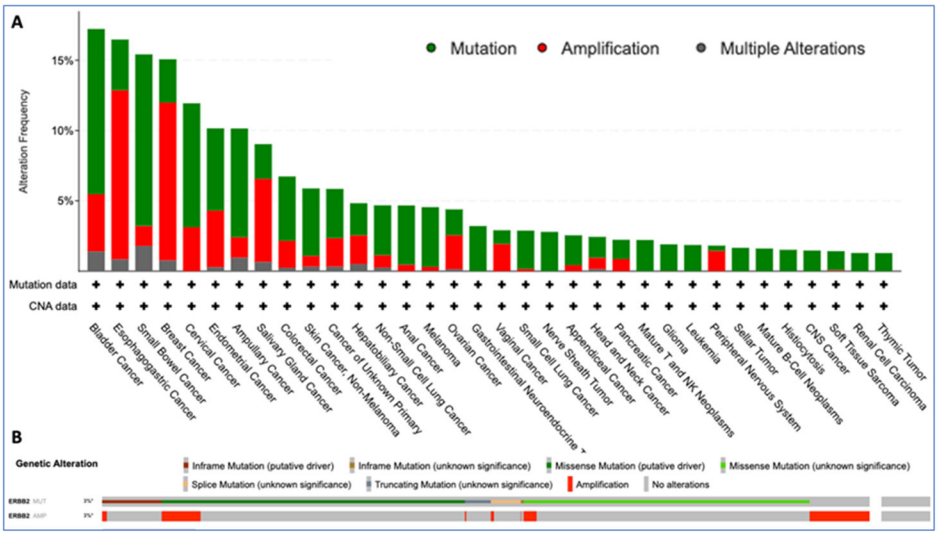

Figure 2. Pan-cancer prevalence of HER2 alterations (mutations and amplifications)

Panel A shows *ERBB2* alterations seen in 6% of 85,575 patients with diverse cancers as per the cancer genomic data aggregated through AACR Project Genomics Evidence Neoplasia Information Exchange (GENIE) effort (AACR Genie v11.0-public). Only tumor types with ≥ 10 cases and at least 1% prevalence were included. **Panel B** shows the oncoprint in the same cohort and highlights the type of mutations seen in these patients with limited overlap of mutations and amplifications.

Table 1.

Key contemporary clinical trials exemplifying the landscape of HER2 targeting strategies in solid tumors

NCT Number	Title of Study	Cancer Type	Drug	Mechanism of Action	Ph.	Sample Size
NCT04941339	A Study of MRG002 in the Treatment of Patients with HER2-positive Advanced Solid Tumors	HER2+ Advanced Solid Tumor	MRG002	ADC	1	74
NCT03944499	Phase 1 Study of FS-1502 in Patients with HER2 Expressed Advanced Solid Tumors and Breast Cancer.	HER2+ Advanced Solid Tumor	FS-1502	ADC	1	92
NCT04513223	A Phase 1 Study of SHR-A1811 in Patients With Selected HER2 Expressing Tumors	HER2+ Advanced Solid Tumor	SHR-A1811	ADC	1	114
NCT05311397	A Study of A166 in Patients With Advanced Solid Malignant Tumors	HER2+ Advanced Solid Tumor	A166	ADC	1	120
NCT03821233	A Dose Finding Study of ZW49 in Patients With HER2-Positive Cancers	HER2+ Advanced Solid Tumor	ZW49	ADC	1	174
NCT03255070	A Dose-escalation, Expansion Study of ARX788, in Advanced Solid Tumors Subjects With HER2 Expression (ACE-Pan Tumor 01)	HER2+ Advanced Solid Tumor	ARX788	ADC	1	190
NCT04257110	A First-in-human Study of Multiple Doses of BB-1701 in Subjects With Locally Advanced/Metastatic HER2 Expressing Solid Tumors	HER2+ Advanced Solid Tumor	BB-1701	ADC	1	208
NCT05018676	ARX788 in Breast Cancer With Low Expression of HER2	HER2-Low Breast Cancer	ARX788	ADC	2	54
NCT02675829	Trial of Ado-Trastuzumab Emtansine for Patients With HER2 Amplified or Mutant Cancers	HER2+/MUT Advanced Solid Tumors	Trastuzumab emtansine	ADC	2	135
NCT04829604	ARX788 in HER2-positive, Metastatic Breast Cancer Subjects (ACE-Breast-03)	HER2+ Breast Cancer	ARX788	ADC	2	210
NCT04714190	A Study of RC48-ADC in Local Advanced or Metastatic Gastric Cancer With the HER2-Overexpression	HER2+ GEC	RC-48 ADC	ADC	3	351
NCT04400695	A Study of RC48-ADC for the Treatment of Locally Advanced or Metastatic Breast Cancer With Low Expression of HER2	HER2-Low Breast Cancer	RC-48 ADC	ADC	3	366
NCT04704934	Trastuzumab Deruxtecan for Subjects With HER2-Positive Gastric Cancer or Gastro-Esophageal Junction Adenocarcinoma After Progression on or After a Trastuzumab-Containing Regimen (DESTINY-Gastric04)	HER2+ GEC	Trastuzumab deruxtecan	ADC	3	490
NCT04494425	Study of Trastuzumab Deruxtecan (T-DXd) vs Investigator's Choice Chemotherapy in HER2-low, Hormone Receptor Positive, Metastatic Breast Cancer	HER2-Low Breast Cancer	Trastuzumab deruxtecan	ADC	3	850
NCT04622319	A Study of Trastuzumab Deruxtecan (T-DXd) Versus Trastuzumab Emtansine (T-DM1) in High-risk HER2-positive Participants With Residual Invasive Breast Cancer Following Neoadjuvant Therapy (DESTINY-Breast05)	HER2+ Breast Cancer	Trastuzumab deruxtecan	ADC	3	1600
NCT04704661	Testing the Combination of Two Anti-cancer Drugs, DS-8201a and AZD6738, for The Treatment of Patients With Advanced Solid Tumors Expressing the HER2 Protein or Gene, The DASH Trial	HER2+ Advanced Solid Tumor	DS8201 + AZD6738	ADC + ATRi	1	39

NCT Number	Title of Study	Cancer Type	Drug	Mechanism of Action	Ph.	Sample Size
NCT04042701	DS8201a and Pembrolizumab in Participants With Locally Advanced/Metastatic Breast or Non-Small Cell Lung Cancer	HER2+ Breast Cancer/ NSCLC	DS8201 + Pembrolizumab	ADC + IO	1	115
NCT04585958	Testing the Combination of DS-8201a and Olaparib in HER2-Expressing Cancers With Expansion in Patients With Endometrial Cancer	HER2+ Advanced Solid Tumor	DS8201 + Olaparib	ADC + PARPi	1	36
NCT03272334	Her2-BATS and Pembrolizumab in Metastatic Breast Cancer	HER2+ Breast Cancer	Her2-BATS	BATS (IO)	1	33
NCT03842085	Phase I Clinical Study of MBS301 in Treatment of HER2 Positive Recurrent or Metastatic Malignant Solid Tumor	HER2+ Advanced Solid Tumor	MBS301	Bispecific Ab	1	34
NCT04162327	A Phase Ia/Ib Study of IBI315 in Patients With HER2-expressing Advanced Solid Tumor	HER2+ Advanced Solid Tumor	IBI315	Bispecific Ab	1	191
NCT03448042	A Study of Runimotamab in Participants With Locally Advanced or Metastatic HER2-Expressing Cancers	HER2+ Advanced Solid Tumor	Runimotamab	Bispecific Ab	1	521
NCT05152147	A Study of Zanidatamab in Combination With Chemotherapy Plus or Minus Tislelizumab in Patients With HER2-positive Advanced or Metastatic Gastric and Esophageal Cancers	HER2+ GEC	Zanidatamab (ZW25)	Bispecific Ab	3	714
NCT04224272	A Study of ZW25 (Zanidatamab) With Palbociclib Plus Fulvestrant in Patients With HER2+/HR+ Advanced Breast Cancer	HER2+/HR+ Breast Cancer	Zanidatamab (ZW25) + Palbociclib	Bispecific Ab + CDKi	2	86
NCT03929666	A Safety and Efficacy Study of ZW25 (Zanidatamab) Plus Combination Chemotherapy in HER2-expressing Gastrointestinal Cancers, Including Gastroesophageal Adenocarcinoma, Biliary Tract Cancer, and Colorectal Cancer	HER2+ GI Cancers	Zanidatamab (ZW25) + Chemo	Bispecific Ab + Chemo	2	362
NCT04040699	KN026 Combined With KN046 in Subjects With HER2 Positive Solid Tumor	HER2+ Advanced Solid Tumor	KN026 + KN046	Bispecific Ab + IO	1	24
NCT04660929	CAR-macrophages for the Treatment of HER2 Overexpressing Solid Tumors	HER2+ Advanced Solid Tumor	CAR-Macrophage	CAR-Macrophage	1	18
NCT04511871	A Phase I Trial of CCT303-406 in Patients With Relapsed or Refractory HER2 Positive Solid Tumors	HER2+ Advanced Solid Tumor	CCT303-406	CAR-Tcells	1	15
NCT05325632	Study of HER2 Directed Dendritic Cell (DC1) Vaccine + Weekly Paclitaxel, Trastuzumab & Pertuzumab	HER2+ Breast Cancer	DC1	DCV	2	34
NCT04029922	Study of MT-5111 in HER2-positive Solid Tumors	HER2+ Advanced Solid Tumor	MT-5111	ETB	1	178
NCT04278144	A First-in-human Study Using BDC-1001 as a Single Agent and in Combination With Nivolumab in Advanced HER2-Expressing Solid Tumors	HER2+ Advanced Solid Tumor	BDC-1001	ISAC	1	390
NCT04908813	Study of HLX22 in Combination With Trastuzumab and Chemotherapy Versus Placebo in Combination With Trastuzumab and Chemotherapy for Treatment of Locally Advanced or Metastatic Gastric Cancer	HER2+ GEC	HLX22	ISAC	2	150
NCT04319757	ACE1702 in Subjects With Advanced or Metastatic HER2-expressing Solid Tumors	HER2+ Advanced Solid Tumor	ACE1702	NK-cell	1	36
NCT04147819	A First in Human Study of BAY2701439 to Look at Safety, How the Body Absorbs, Distributes and Excretes the Drug, and How Well the Drug Works in Participants With Advanced Cancer Expressing the HER2 Protein	HER2+ Advanced Solid Tumor	BAY2701439	Radionuclide	1	213

NCT Number	Title of Study	Cancer Type	Drug	Mechanism of Action	Ph.	Sample Size
NCT04982926	A Study of TAS2940 in Participants With Locally Advanced or Metastatic Solid Tumor Cancer	HER2+ Advanced Solid Tumor	TAS2940	RTKI	1	42
NCT05315700	Study of ORIC-114 in Patients With Advanced Solid Tumors Harboring an EGFR or HER2 Alteration	HER2+ Advanced Solid Tumor	ORIC-114	RTKI	1	42
NCT04487236	Trial of ZN-A-1041 Enteric Capsules in Patients With HER2-Positive Advanced Solid Tumors	HER2+ Advanced Solid Tumor	ZN-A-1041	RTKI	1	84
NCT04886804	A Study to Test Different Doses of BI 1810631 in People With Different Types of Advanced Cancer (Solid Tumours With Changes in the HER2 Gene)	HER2+ Advanced Solid Tumor	BI 1810631	RTKI	1	96
NCT05245058	SPH5030 Tablets in Subjects With Advanced Her2-positive Solid Tumors	HER2+ Advanced Solid Tumor	SPH5030	RTKI	1	105
NCT04447118	Phase 3 Study of Pyrotinib Versus Docetaxel in Patients With Advanced Non-squamous NSCLC Harboring a HER2 Exon 20 Mutation Who Failed Platinum Based Chemotherapy	HER2-MUT NSCLC	Pyrotinib	RTKI	3	150
NCT04539938	A Study of Tucatinib Plus Trastuzumab Deruxtecan in HER2+ Breast Cancer	HER2+ Breast Cancer	Tucatinib + Trastuzumab deruxtecan	RTKI + ADC	2	70
NCT03975647	A Study of Tucatinib vs. Placebo in Combination With Ado-trastuzumab Emtansine (T-DM1) for Patients With Advanced or Metastatic HER2+ Breast Cancer	HER2+ Breast Cancer	Tucatinib + T-DM1	RTKI + ADC	3	460
NCT05132582	A Study of Tucatinib or Placebo With Trastuzumab and Pertuzumab for Metastatic HER2+ Breast Cancer	HER2+ Breast Cancer	Tucatinib + Trastuzumab	RTKI + mAb	3	650
NCT05253651	A Study of Tucatinib With Trastuzumab and mFOLFOX6 Versus Standard of Care Treatment in First-line HER2+ Metastatic Colorectal Cancer	HER2+ CRC	Tucatinib + Trastuzumab + Chemo	RTKI + mAb + Chemo	3	400
NCT04430738	Tucatinib Plus Trastuzumab and Oxaliplatin-based Chemotherapy or Pembrolizumab-containing Combinations for HER2+ Gastrointestinal Cancers	HER2+ GI Cancers	Tucatinib + Chemo	RTKI+ Chemo	1	120
NCT05356741	To Access the Safety and Effects of Intravenous Administration of AMX 818 Alone and in Combination With Pembrolizumab in Adult Participants With Locally Advanced or Metastatic HER2-Expressing Cancers	HER2+ Advanced Solid Tumor	AMX 818 + Pembrolizumab	T cell engager	1	560
NCT04727151	TAC T-cells for the Treatment of HER2-positive Solid Tumors	HER2+ Advanced Solid Tumor	TAC-Tcells	TAC-Tcells	1	70
NCT05013554	Dose Escalation and Expansion Study of SAR443216 in Participants With Relapsed/ Refractory HER2 Expressing Solid Tumors	HER2+ Advanced Solid Tumor	SAR443216	Trispecific Ab	1	184
NCT04246671	TAEK-VAC-HerBy Vaccine for Brachyury and HER2 Expressing Cancer	HER2+ Advanced Solid Tumor	TAEK-VAC-HerBy	Vaccine	1	55
NCT03632941	A Study to Evaluate Concurrent VRP-HER2 Vaccination and Pembrolizumab for Patients With Breast Cancer	HER2+ Breast Cancer	VRP-HER2	Vaccine	2	39

Abbreviations: Ab, antibody; ADC, antibody drug conjugate; Chemo, chemotherapy; ETB, engineered toxin body; GEC, gastroesophageal cancer; GI, gastrointestinal; i, inhibitor; IO, immunotherapy; ISAC, immune stimulating antibody conjugate; mAb, monoclonal antibody; MUT, mutated; Ph., study phase; RTKI, receptor tyrosine kinase inhibitor.