



# Prevalence and role of HER2 mutations in cancer

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## ARTICLE INFO

### Keywords:

HER2 mutations  
Next-generation sequencing (NGS)  
Afatinib  
Neratinib  
TCGA

## ABSTRACT

HER2 activating mutations act as oncogenic drivers in various cancer types. In the clinic, they can be identified by next generation sequencing (NGS) in either tumor biopsies or circulating cell-free DNA (cfDNA). Preclinical data indicate that HER2 “hot spot” mutations are constitutively active, have transforming capacity *in vitro* and *in vivo* and show variable sensitivity to anti-HER2 based therapies. Recent clinical trials also revealed activity of HER2-targeted drugs against a variety of tumors harboring HER2 mutations. Here, we review the prevalence and type of HER2 mutations identified in different human cancers, their biochemical and biological characterization, and their sensitivity to anti HER2-based therapies in both preclinical and clinical settings.

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## 1. Introduction

Over the past decade, genomic profiling has enabled the identification of numerous actionable oncogenic drivers that are causative for cancer initiation and progression in multiple tumor types (Kandoth

et al., 2013; Cancer Genome Atlas Research et al., 2013; Bailey et al., 2018). The same oncogenic drivers can promote the development and the growth of tumors with different histologies (Bailey et al., 2018). This observation has provided the rationale for the design of “basket trials”, in which the efficacy of a specific targeted therapy is tested against tumors that harbor the qualifying genomic aberrations, independent of tumor histology. Basket trials have been proven to be a particularly suitable approach when the targeted genomic alterations are rare driver events and occur across different cancer types (Hainsworth et al., 2018; Hyman et al., 2015). In this setting, even if the pharmacological target is present in small percentages of patients, the studies allow the rapid enrolment of a sufficient number of patients to evaluate the efficacy of the tested drug. Responses to therapy can be either tissue agnostic or histology-dependent. As an example of the former, objective clinical responses were obtained in up to 80% of patients with adult and pediatric tumors of different origin harboring oncogenic fusions involving the neurotrophin receptors tyrosine kinase genes *NTRK1*, *NTRK2*

**Abbreviations:** NGS, Next generation sequencing; cfDNA, Circulating cell-free DNA; NTRK, Neurotrophic receptor tyrosine kinase; PFS, Progression-free survival; OS, Overall survival; ECD, Extracellular domain; JM, Juxtamembrane domain; TM, Transmembrane domain; KD, Kinase domain; TKI, Tyrosine kinase inhibitor; TCGA, The cancer genome Atlas; TDM-1, Trastuzumab emtansine; IHC, Immunohistochemistry; ER, Estrogen receptor; CBR, Clinical benefit rate; ADC, Antibody drug conjugate.

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or *NTRK3* (encoding TRKA, TRKB and TRKC, respectively) treated with the TRK inhibitor Larotrectinib (Drilon et al., 2018). By contrast, the efficacy of the irreversible pan-ERBB inhibitor Neratinib against tumors harboring activating mutations in *erbB2*, the gene encoding for the human epidermal growth factor receptor 2 (HER2), has been shown to be largely dependent on tumor histology, with clinical responses achieved in breast, cervical and biliary cancer and intrinsic refractoriness in all the other tumor types tested including colorectal, bladder and ovarian cancer (Hyman et al., 2018).

## 2. HER2 proto-oncogene

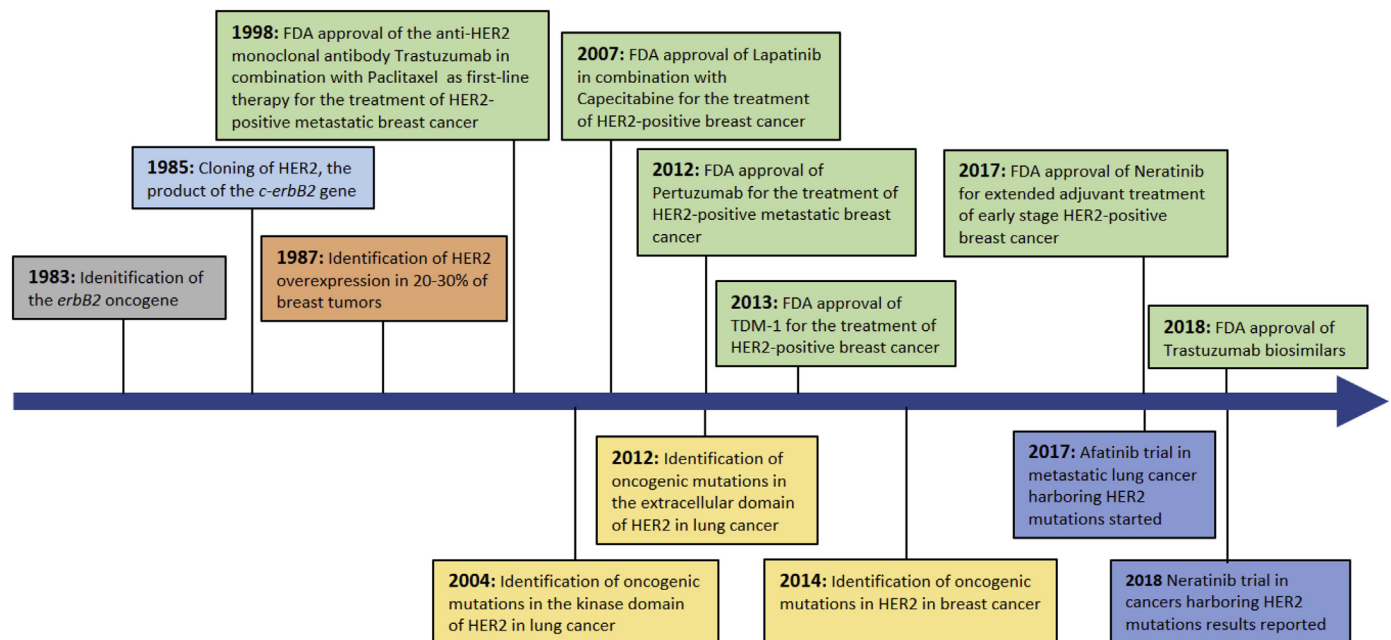
The *erbB2* gene, located on the short arm of chromosome 17, was discovered and cloned in 1983 (Fig. 1) (Sato et al., 1983). The product of this gene, HER2, is a transmembrane glycoprotein of 185 KDa that consists of three main domains: an extracellular, a transmembrane and an intracellular domain with tyrosine kinase activity (Cho et al., 2003). The epidermal growth factor receptor family of tyrosine kinases consists of 4 receptor tyrosine kinases (EGFR/*erbB1*/HER1, *erbB2*/HER2, *erbB3*/HER3, and *erbB4*/HER4) (Cho et al., 2003) that are activated by ligand-induced dimerization (Ushiro & Cohen, 1980). HER2, despite being the only receptor of the family with no known ligand, is the preferred partner for heterodimerization with other *erbB* members (Graus-Porta, Beerli, Daly, & Hynes, 1997). Receptors dimerization induces transphosphorylation of tyrosine residues within the kinase domains and consequent activation of downstream signaling cascades (Zhang, Gureasko, Shen, Cole, & Kuriyan, 2006; Wagner, Stacey, Liu, & Pawson, 2013).

The identification of HER2 amplification/overexpression in a subset of breast cancer and the consequent development of specific anti-HER2 therapies had a tremendous impact on the clinical management of breast cancer patients. In 1998, the US Food and Drug Administration (FDA) approved Trastuzumab, a monoclonal antibody targeting the extracellular domain of HER2, in combination with paclitaxel as first-line treatment in HER2-positive metastatic breast cancer, and as a single agent for second and third-line therapy (Fig. 1). Trastuzumab approval resulted in the inclusion of HER2 testing in the guidelines of the American Society of Clinical Oncology (ASCO) as a recommended test

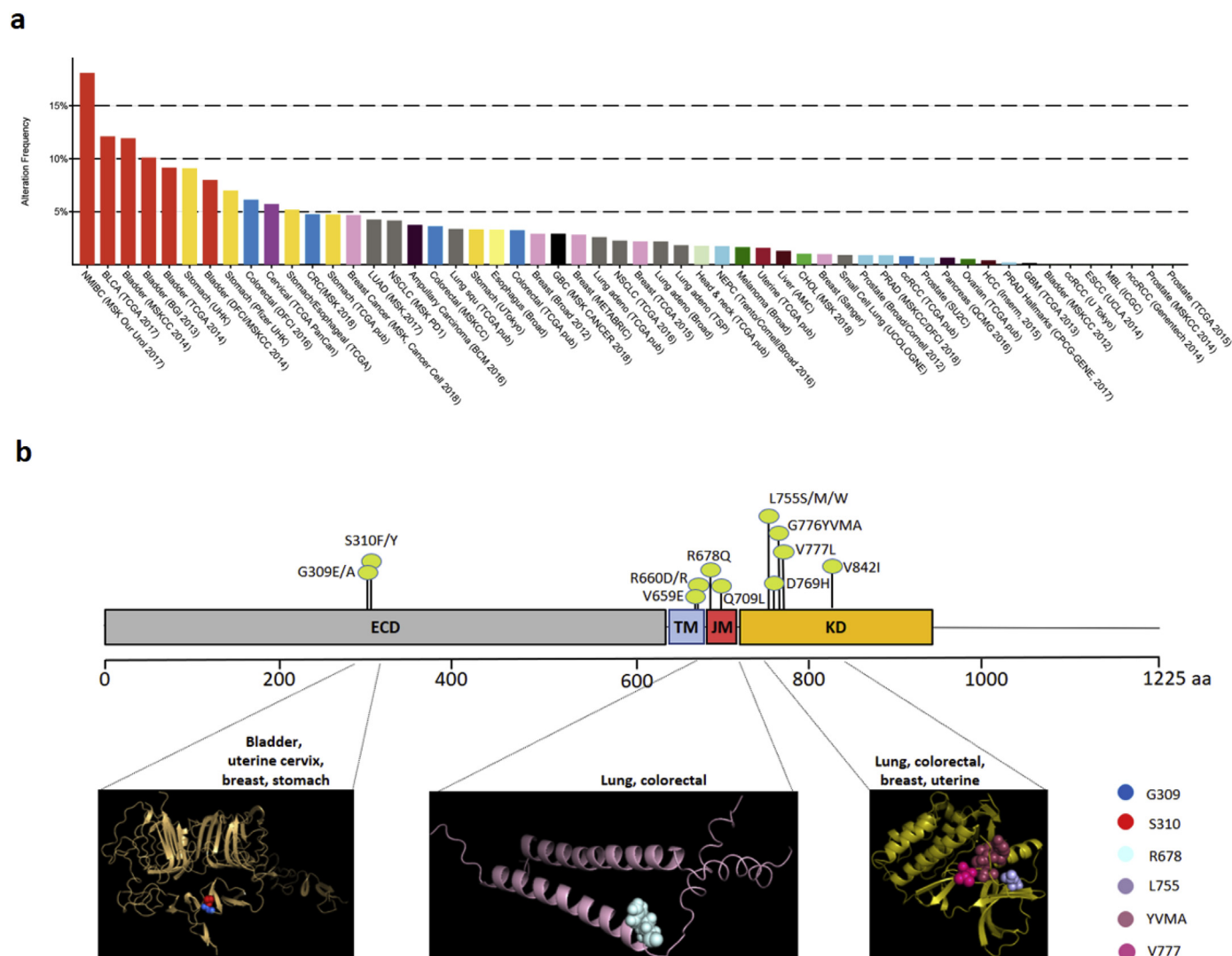
for all breast cancer one year later (Smith et al., 1999). Several studies have shown improvements in progression-free survival (PFS) and overall survival (OS) in HER2-positive breast cancer patients treated with Trastuzumab-based therapies (Vogel, et al., 2002) (Marty et al., 2005). Many other anti HER2 drugs have been further developed and nine are now FDA-approved for breast cancer treatment: Pertuzumab (a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain of the HER2), Ado-trastuzumab Emtansine (TDM-1, a HER2-targeting antibody drug conjugate (ADC)), Neratinib, Lapatinib (a reversible pan-ERBB inhibitor) and five Trastuzumab biosimilars (Fig. 1) (Amiri-Kordestani et al., 2014; Gradishar, 2012; Martin et al., 2017; Blumenthal et al., 2013; Lee et al., 2018; Voelker, 2018). More recently, HER2 amplification/overexpression has also been identified in other tumor types such as gastric, where trastuzumab-based therapy is also standard of care, colon, salivary gland, bladder and uterine serous carcinoma (Buza, English, Santin, & Hui, 2013; Grabsch, Sivakumar, Gray, Gabbert, & Muller, 2010; Bang et al., 2010; Fader et al., 2018).

### 2.1. Prevalence of HER2 mutations in human cancers

TCGA and other available databases show that HER2 mutations are present in a variety of human cancer types (Fig. 2A). Bladder cancer represents the histology with the highest prevalence of HER2 mutations (9–18%), followed by uterine cervix (6%), colorectal (5.8%), lung (4%) and breast (4%) (Robertson et al., 2017; Cancer Genome Atlas Research, 2014b; Ojesina et al., 2014; Giannakis et al., 2016; Stephens et al., 2004; Razavi et al., 2018). More recently, Pahuja et al. analyzed 111,176 tumors and found that about 3.5% of them harbored HER2 mutations (Pahuja et al., 2018). While the vast majority of these mutations mapped to the ECD and to the kinase domain (KD) of the receptor, about 3% and 7% were found in the transmembrane domain (TM) (G660D, R678Q, E693K) or in the juxtamembrane domain (JM) (Q709L), respectively. These newly identified mutations were mainly found in colorectal, breast and lung cancer and were confirmed to be oncogenic and sensitive to anti-HER2 therapies as part of this study (Pahuja et al., 2018) (Fig. 3).



**Fig. 1.** Timeline of key advances relating to the biology and therapeutic targeting of HER2 signaling. Milestone discoveries that are relevant to HER2 amplified/overexpressing (boxes above the timeline arrow) and HER2 mutant tumors (boxes below the timeline arrow). Key events relating to the following fields of study are color coded as follows: FDA approval of anti-HER2 drugs (green); identification of HER2 mutation in cancers (yellow); clinical trial enrolling HER2 mutated cancers (blue).



**Fig. 2.** Distribution and frequency of HER2 mutations in solid tumors. A: HER2 mutations identified across multiple cancer histologies. Only published studies on solid tumors with >100 samples, available on cBioPortal, were included. The frequency of HER2 mutations varies from 1% in cancer types such as melanoma and up to 18% in bladder cancer. Similar histologies are depicted with the same colors. B: Lollipop diagram depicting activating HER2 mutations identified in several cancer types. The most prevalent mutation, S310F/Y, is localized in the ECD and has been frequently reported in bladder, uterine cervix, breast and stomach cancer. HER2 mutations that mapped in the KD are also depicted. These are frequently found in lung, colorectal, breast and uterine tumors. Mutations in the TM and the JM domains recently identified in lung and colorectal cancer are also indicated. The most common mutated residues in the ECD, the JM and the KD modelled using PyMOL molecular visualization software are presented.

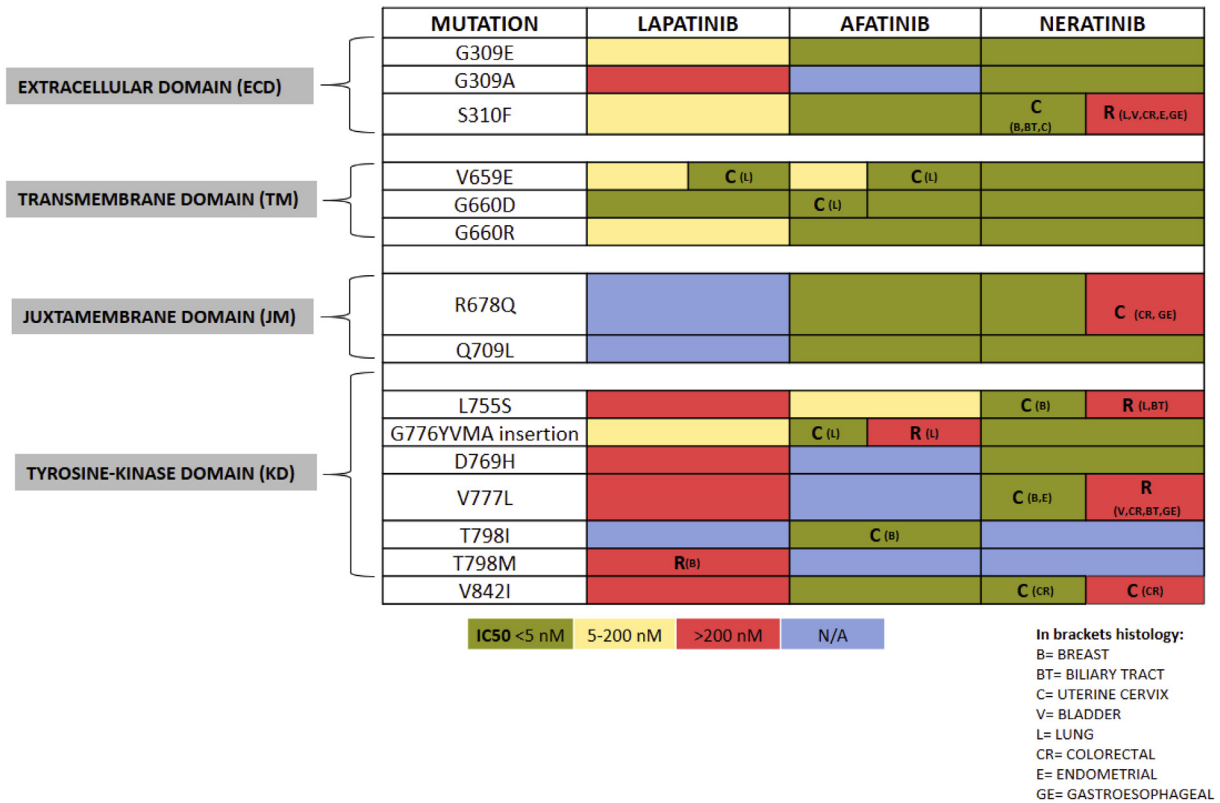
## 2.2. Lung cancers

Lung cancer was the first tumor type in which HER2 mutations were described. Stephens et al. sequenced the ERBB2 gene from 120 primary lung tumors and found that 4% of these cases harbored mutations within the kinase domain. The percentage raised up to 10% in the adenocarcinoma subtype (Stephens et al., 2004). Similar results were obtained by TCGA in a cohort of 178 lung squamous cell carcinomas (4% of cases) (Cancer Genome Atlas Research, 2012) and in 230 resected lung adenocarcinomas, where 3% of the sample carried HER2 mutations (Cancer Genome Atlas Research, 2014b). The most prevalent alteration in this cancer types is the in-frame insertion/duplication G776YVMA within the exon 20 (Fig. 2B).

### 2.2.1. Preclinical data

Wang et al. showed that the G766YVMA insertion was more potent than wild-type HER2 in activating signal transducers, phosphorylating EGFR, and promoting survival, invasiveness and tumorigenicity in lung cancer models (Wang et al., 2006). Additional work by Perera et al. found that

Trastuzumab treatment in combination with Rapamycin reduced tumor growth in transgenic mice expressing the HER2 exon 20 YVMA insertion in the lung epithelium (Perera et al., 2009). Later, Greulich et al. reported that NIH 3 T3 cells overexpressing wild-type HER2 exhibited a weak anchorage-independent phenotype while the G309E, S310F, and S310Y mutants (that map to the ECD and are exclusive of the adenocarcinoma subtype) resulted in robust colony formation in soft agar (Greulich et al., 2012) (Fig. 2B). They further showed that the amino acid change from serine to phenylalanine in the 310 codon (S310F) induced hyperactivation of the kinase. These HER2 ECD mutants were activated by either elevated C-terminal tail phosphorylation or by covalent dimerization mediated by intermolecular disulfide bond formation. These distinct mechanisms of receptor activation converged to tyrosine phosphorylation of cellular effectors such as SHP-2, a phosphatase that has been shown to induce the activation of the Ras-Erk signaling, thus promoting growth and metastasis of multiple cancer types (Aceto et al., 2012; Bentires-Alj et al., 2004; Hellmuth et al., 2008; Schneeberger et al., 2014). The same authors showed that Ba/F3 cells transformed with HER2 ECD mutants were sensitive to Neratinib and Afatinib (another irreversible pan-ERBB inhibitor) with IC50s in the low nanomolar range (Fig. 3), resulting in



**Fig. 3.** Sensitivity of HER2 mutants to anti-HER2 therapy. A heat map of the half maximal inhibitory concentration (IC<sub>50</sub>) values for different pan-ERBB inhibitors is shown. These drugs have different levels of activity against mutant HER2 proteins *in vitro* (box colors indicate the range of sensitivity/resistance for each mutant). The symbols in each box indicate the clinical results obtained from case reports or clinical trials. (C: clinical response; R: clinical resistance). Of note, some mutants that have been described to be sensitive to specific inhibitors in preclinical analyses were instead found to be resistance to the same drugs in patients.

effective abrogation of cell survival. Serra et al. reported a case of a 29 years old patient with a germline TP53 mutation (Li-Fraumeni Syndrome) who developed two primary tumors in breast and lung, respectively. Adjuvant treatment with Tamoxifen completely resolved the breast tumor and the lung cancer was treated with adjuvant chemotherapy that kept the disease under control for about 4 years. Upon progression, NGS on a newly identified lung nodule found a novel HER2 mutation in the TM domain (V659E) (Serra et al., 2013). The patient was treated with Lapatinib and Paclitaxel and achieved an objective clinical response that lasted for 9 months. Additional studies by Liu et al. showed that Ba/F3 cells stably expressing the A775\_G776insYVMA insertion were sensitive to Osimertinib, a third-generation tyrosine kinase inhibitor (TKI). Osimertinib was more potent than Erlotinib in reducing pHER2 levels and inhibiting cells growth. Interestingly, they observed decreased levels of MYC after Osimertinib exposure and found that the combination of Osimertinib and JQ1 (a potent inhibitor of the BET family of bromodomain proteins which include BRD2, BRD3, BRD4) was highly effective in reducing HER2, AKT and ERK phosphorylation in these models (Liu et al., 2018). Together, their results provide the rationale to test the efficacy of Osimertinib as single agent or in combination with BRD4/MYC inhibitors in patients harboring HER2-driven (mutated or amplified) lung cancers.

### 2.2.2. Clinical responses in HER2 mutant lung cancer patients to anti HER2-based therapy

De Greve et al. reported the first evidences of clinical benefit from Afatinib treatment in HER2 mutant lung cancer patients in 2011 (De Greve et al., 2012). Three patients with advanced lung adenocarcinoma bearing HER2 mutations were treated with Afatinib single agent or in combination with Paclitaxel and all achieved significant tumor regression. Chuang et al. later reported the outcomes of nine patients with metastatic lung adenocarcinoma harboring HER2 mutations treated

with HER2-targeted therapies. Four of the nine patients experienced clinical benefits with responses that lasted up to 10 months. All four responders harbored an exon 20 insertion and they were treated with Trastuzumab, Afatinib or TDM-1 (Chuang et al., 2017). In a recent case report, a patient with the recurrent YVMA insertion treated with Afatinib first-line achieved a PR that lasted 12 months (Shi & Wang, 2018). Another group reported the case of a woman with NSCLC harboring a HER2 exon 20 mutation (c.2437A > G) who previously failed cytotoxic chemotherapy and Gefitinib treatment but responded to Afatinib (Park et al., 2018).

In 2018, Hyman et al. reported the data of a global multicenter, multi-histology basket trial to test Neratinib in HER2 and HER3 mutated cancers of any tumor type (Hyman et al., 2017). Twenty-six HER2 mutated lung cancers, mostly harboring the exon 20 insertion, were enrolled and only one objective response was observed. The median PFS was 5.5 months and the objective response rate was 3.8% (clinical benefit rate (CBR) was 42.3%). Importantly, HER2 exon 20 insertions are paralogous of EGFR exon 20 insertions, which are resistant to first- and second-generation EGFR tyrosine kinase inhibitors (Yasuda et al., 2013). More recently, in a phase II trial, Li et al. assessed the activity of TDM-1 in a cohort of heavily pretreated patients with advanced HER2-mutant lung cancers (Li et al., 2018). Eighteen patients were enrolled and responses were seen across patients with HER2 exon 20 insertions and point mutations in the kinase, transmembrane, and extracellular domains. Interestingly, mass spectrometry confirmed low or undetectable levels of HER2 protein expression among HER2 mutant responders, suggesting that HER2 mutations may promote the internalization of TDM-1 regardless of the receptor levels on the cell membrane. A plausible explanation for this phenomenon is that the constitutively active mutant receptors are more efficient than the wild type in forming homo/heterodimers, which would result in an increased internalization turnover (Li et al., 2018). Finally, it was reported a case of a lung cancer patient

who was initially treated with Carboplatin and Pemetrexed followed by Erlotinib. At progression, genomic testing revealed the presence of a germline HER2 G660D mutation and the patient experienced a quick reduction of tumor volume after 12 weeks of Afatinib (Pahuja et al., 2018).

### 2.3. Breast cancer

HER2 somatic mutations are present in about 4% of breast cancer patients (Banerji et al., 2012; Cancer Genome Atlas Research, et al., 2013; Ellis et al., 2012) (Fig. 2 panel A). In invasive lobular breast cancer, which composes about 15% of the ER-positive subtype, the prevalence of HER2 mutations is higher (about 10% including the three largest studies available on cBioPortal).

Approximately 70% of HER2 mutations occur in the kinase domain, between amino acids 755 and 781 (exons 19 and 20) (Fig. 2 panel B). Another 20% are found in the extracellular domain (ECD) at either amino acid 309 or 310 (exon 8) (Fig. 2B).

#### 2.3.1. Preclinical data

In 2006, Wang et al. showed that the HER2 exon 20 YVMA insertion mutation was also a “driver” mutation in breast tumors (Wang, et al., 2006). They found that, despite this alteration mediated resistance to EGFR TKIs, both Trastuzumab and Lapatinib remained active against MCF10A cells transduced with this mutant. Bose et al. later investigated the impact of 13 additional HER2 mutations using the same model. They found that three mutations (R678Q, I767M, and Y835F) exerted no functional effects, while the others, including HER2 G309A, L755S, D769H/Y, V777L, P780\_781insGSP, V842I, and R896Q, resulted in increased HER2 signaling and increased tumor growth when compared to HER2 wild type transduced controls. The HER2 L755S mutation, the most frequent in breast cancer, induced a weak oncogenic phenotype. Computational modeling suggests that Lapatinib cannot bind to the L755S substitution due to steric hindrance. It is worth noting that Neratinib was effective against all the HER2 mutants (Bose et al., 2013) (Fig. 3).

Trastuzumab activity was also investigated against HER2 mutants. Trastuzumab treatment was able to reduce the formation of colonies in Matrigel-based assays but did not show a cytotoxic effect (Bose et al., 2013). More recently, a study conducted on both preclinical models and patients by our group showed that the presence of HER2 mutations can limit the sensitivity to Trastuzumab and Lapatinib in HER2-amplified breast cancer patients. However, in this context, tumors with concomitant mutation of HER2 and amplification of the ERBB2 gene remain sensitive to Neratinib monotherapy (Cocco et al., 2018). Together, these data suggest that HER2 mutated breast cancers are sensitive to Neratinib regardless the mutant allele and the copy number status of the ERBB2 gene.

Interestingly, a recent study reported that the vast majority of HER2 mutations identified in breast cancer (about 70%) is found in metastatic ER+ tumors, suggesting that the emergence of HER2 mutations may represent a mechanism of acquired resistance to endocrine therapy (Croessmann et al., 2019). Therefore, the use of anti-HER2 agents may partially restore the sensitivity of these tumors to endocrine therapy. In support of this hypothesis, HER2 mutations (L755S and V777 L) were found to over-activate the PIK/AKT/mTOR pathway inducing endocrine resistance, while addition of Neratinib or Alpelisib or Everolimus restored the sensitivity to Fulvestrant in HER2 mutant breast cancer cells. Interestingly, triple therapy with Neratinib, Everolimus and Fulvestrant induced a more potent tumor growth inhibition achieving a complete tumor regression in a preclinical model of breast cancer xenograft harboring the V777 L HER2 mutation (Croessmann et al., 2019). Although the authors did not investigate the molecular mechanisms that link HER2 inhibition and ER activity in these models, a previous study showed that PI3K inhibition in PIK3CA mutated ER+ breast cancer cells induced a robust ER-dependent transcriptional program mediated by the activation of KMT2D, a histone H3 lysine 4 methyltransferase (Toska et al., 2017). Perhaps a similar phenomenon is taking place in this setting, partially explain why HER2 inhibition combined with Fulvestrant is effective in ER

+ HER2 mutated cancer. Further studies are needed to elucidate this mechanism and evaluate which agent (Neratinib, TDM-1 or other potent ADCs conjugated to a derivative of the camptothecin analog exatecan targeting HER2 such as DS-8201a) in combination with Fulvestrant may be more effective against HER2 mutated, ER+ breast tumors.

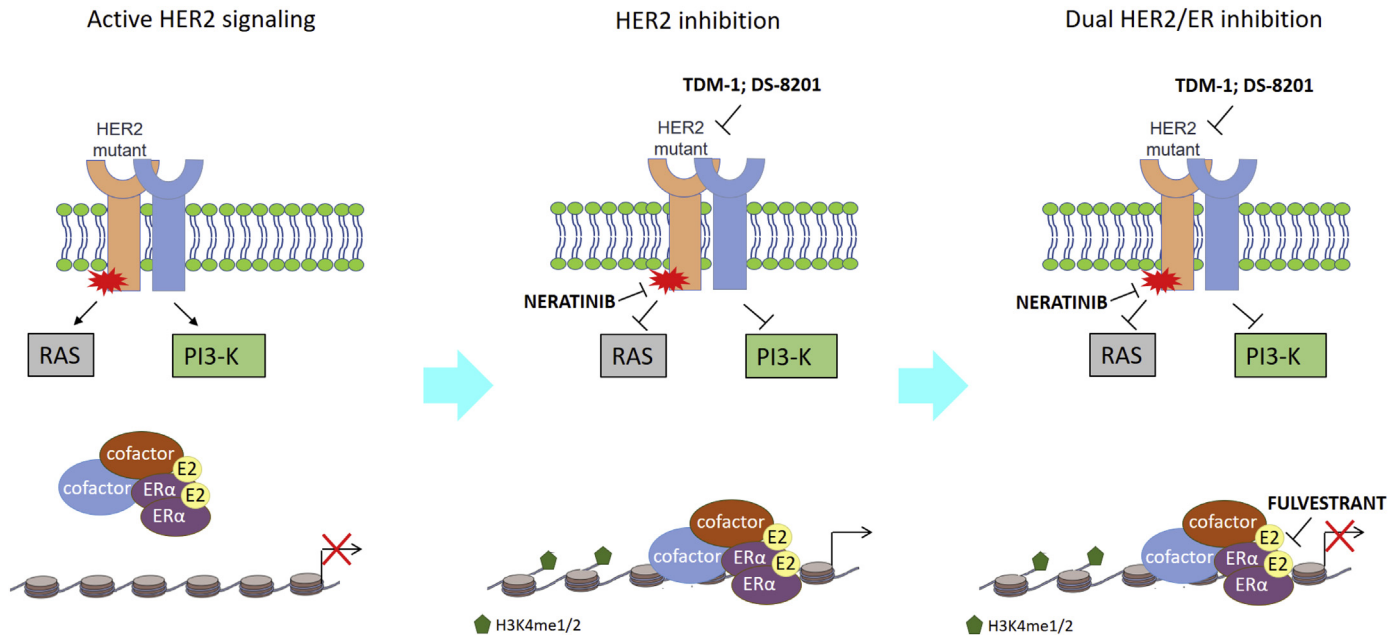
#### 2.3.2. Clinical responses of HER2 mutant breast tumors to anti HER2-based therapies

The first case report of a HER2 mutated breast cancer was published in 2014 (Ali et al., 2014). A 58 years old woman was diagnosed with triple negative, inflammatory breast cancer and, after progressing to multiple standard chemotherapies, two HER2 mutations (V777L and S310F) were detected by NGS in the absence of ERBB2 gene amplification. The patient experienced quick symptoms improvements upon Lapatinib and Trastuzumab-based therapies that lasted 6 months (Ali et al., 2014). A second case reported a 51 years old woman with a ER+, HER2 negative breast cancer who developed metastatic disease refractory to many chemotherapy regimens (Chumsri et al., 2015). NGS on a progressing lesion identified a HER2 S310F mutation and the patient was treated with the combination of Trastuzumab, Pertuzumab, and Fulvestrant. Her liver metastases and malignant ascites markedly improved and she remained on this therapy for 12 months (Chumsri et al., 2015). An additional case reported a 43 years old patient with ER+, HER2 negative, metastatic breast cancer with a HER2 L755S mutation. She was treated with Neratinib monotherapy and experienced dramatic improvement in symptoms and tumor markers that lasted 11 months (Ben-Baruch, Bose, Kavuri, Ma, & Ellis, 2015). At progression, the addition of Capecitabine to Neratinib resulted in a second response that lasted >6 months (Ben-Baruch et al., 2015). Similarly, another report described a HER2 (D769H) mutant patient with metastatic HER2 negative breast cancer who achieved a partial response upon Trastuzumab, Pertuzumab, and chemotherapy (Shih et al., 2015).

In the phase II MutHER trial, they evaluated the activity of Neratinib in HER2 mutant non-amplified metastatic breast cancer. Clinical endpoints included CBR, PFS, toxicity, and HER2 mutations detection in circulating tumor DNA (ctDNA) (Ma et al., 2017). CBR was observed in 31% of patients. This included one CR, one PR, and three SD > 24 weeks. Median PFS was 16 weeks and the on-target effect of Neratinib was supported by early decreases of ctDNA HER2mut variant allele frequency, which then increased at progression. In the SUMMIT trial conducted by Hyman and colleagues, breast cancer patients were the ones who experienced the greatest antitumor activity with a median PFS of 3.5 months and an overall response of 32% (Hyman, Piha-Paul, Rodon, et al., 2017). Given that the vast majority of HER2-mutant tumors are also ER+, and that dual inhibition of HER2 and ER+ has been shown to be more effective than each of the single agent in inhibiting tumor growth in preclinical models (Croessmann et al., 2019), the SUMMIT trial has subsequently tested the combination of Neratinib plus Fulvestrant in HER2 mutated, ER+ metastatic breast cancer patients. Initial results on the first 12 patients treated with the combination showed a CBR of 58%, OOR of 42%, and median PFS of 3.7 months (Hyman et al., 2017). These findings are in line with the preliminary results obtained by the SOLAR-1 trial that is currently evaluating the efficacy and safety of Alpelisib (α-specific PI3K inhibitor) plus Fulvestrant in patients with HR+, HER2-negative advanced breast cancer (Fabrice, 2018) and confirm the preclinical results obtained by our group in this setting (Toska et al., 2017). Specifically, the addition of Fulvestrant to Neratinib or to Alpelisib may prevent the increase of ER activity thus inhibiting tumor growth (Fig. 4). Given these premises, it would be of interest to evaluate the feasibility of a triple combination with Neratinib, Alpelisib and Fulvestrant in patients harboring ER+, PIK3CA mutated or HER2 mutated breast cancer in the near future.

### 2.4. Bladder cancers, gastrointestinal and cervical cancer

Bladder cancer represents the tumor type with the highest prevalence of HER2 mutations ranging from 9 to 18% (Fig. 2 panel A). The



**Fig. 4.** Model of HER2 dependent ER regulation. Proposed model of regulation of ER activity by HER2 signaling in HER2 mutant ER+ breast tumors. In the absence of HER2 inhibition, active HER2 signaling results in suppression of ER dependent transcription. Inhibition of HER2 (with Neratinib or antibody-drug-conjugates such as TDM-1 or DS-8201) promotes increased ER activity as a result of an adaptive mechanism of cellular defense. The activation of ER-dependent transcription may be prevented through dual HER2/ER inhibition (Neratinib plus Fulvestrant or TDM-1/DS-8201 plus Fulvestrant).

S310F hotspot in the ECD is the most common substitution in this tumor type, and the same mutation is also the most prevalent in stomach/esophageal and cervical cancer, malignancies in which HER2 mutations are found in about 5% and 6% of cases, respectively (Ojesina et al., 2014) (Cancer Genome Atlas Research et al., 2017) (Fig. 2 panel B). TCGA data showed that about 4% of colorectal cancer patients harbor HER2 somatic mutations, with the substitution V842I (Cancer Genome Atlas, 2012).

#### 2.4.1. Preclinical data

De Martino et al. showed that bladder cancer cells with the mutations S310F, S653C, R678Q, or the double D277H/S310F mutation had enhanced phosphorylation of HER2 and were sensitive to Lapatinib (de Martino et al., 2014). In another study, a HER2 mutated (S310F) bladder cancer cell line was also found to be sensitive to Afatinib and Dacomitinib (an irreversible pan-ERBB inhibitor) (Tamura et al., 2018). In colorectal cancer, Kavouri et al. showed that the HER2 mutations V842I, V777L, and L755S, as well as the ECD mutation S310F, were oncogenic in this malignancy (Kavuri et al., 2015). They also demonstrated that, although the V842I substitution induced resistance to Cetuximab, Panitumumab and Trastuzumab, cells expressing this mutant retained sensitivity to Neratinib and Afatinib. A more recent work evaluated the efficacy of pan-HER2 inhibitors in Lynch and Lynch-like HER2 mutated colorectal cancer cell lines (Kloth et al., 2016). The authors found that both the L755S and the V842I substitutions were recurrent events in this setting and sensitive to Afatinib, Neratinib and Dacomitinib. While Lapatinib and Trastuzumab were active against the V842I, both agents failed to target the L755S, thus confirming the results previously obtained in breast cancer (Bose et al., 2013).

#### 2.4.2. Sensitivity to anti HER2-based therapies

Among the 16 bladder cancer patients enrolled in the SUMMIT trial, no responses were observed (median PFS of 1.8 months) (Hyman et al., 2018). Genomic characterization showed the presence of a higher number of TP53 and PIK3CA mutations, as well as Cyclin E amplification when compared to tumors that experienced clinical benefits in the same cohort, thus suggesting that these genomic alterations may have contributed to the intrinsic resistance of these tumors to Neratinib therapy. Interestingly, TCGA data showed that about 52% and 22% of HER2

mutated bladder cancers harbored a TP53 or a PIK3CA mutation, respectively, and up to 10% had Cyclin E amplification (Robertson et al., 2017). The SUMMIT trial also included 12 colorectal cancer and 5 gastro-esophageal cancer patients with no responses observed (PFS of 1.8 and 1.7 months, respectively) (Hyman et al., 2018). Better outcomes were observed in cervical cancer and biliary tract cancer patients, with objective responses of 20% and 22.2% and a PFS of 20.1 and 2.8 months, respectively (Hyman et al., 2018).

### 3. Ongoing trials

Sixteen clinical trials investigating the efficacy of HER2 targeted therapies in HER2 mutant cancers are currently ongoing (Table 1). Two phase II studies are investigating the efficacy of Neratinib plus Trastuzumab or plus Cetuximab (NCT03457896) and the activity of the ADC DS-8201a (NCT03505710) in non-small lung cancers. Multiple trials investigating the efficacy of Afatinib in HER2 mutant tumors of various histologies are currently enrolling (NCT03810872, NCT02183883, NCT02780687, NCT02597946). In addition, the NCI MATCH trial subprotocol B (NCT02465060) is testing Afatinib in HER2 mutated cancers of any type and is currently enrolling patients nationwide. More trials are investigating the role of Neratinib in combination with other agents such as Fulvestrant (NCT01670877, NCT01953926) in breast cancer or Everolimus, Palbociclib or Trametinib in patients with advanced solid tumors harboring EGFR or HER2 mutation/amplification or HER3/4 mutations (NCT03065387). Additionally, new TKIs are now under clinical investigation: Pyrotinib (an oral irreversible pan-ERBB receptor TKI with activity against HER1, HER2 and HER4) in non-small cell lung cancer and in breast cancer (NCT02834936 and NCT03412383) and Pozotinib (a pan-ERBB inhibitor) in metastatic breast cancer and in patients with advanced NSCLC (NCT02544997, NCT03066206) harboring HER2 mutations.

### 4. Resistance to targeted therapy

The idea of treating HER2 mutant tumors with specific therapies is relatively new and, as a consequence, little is known about the potential mechanisms of resistance to anti-HER2 agents in this setting. Primary

**Table 1**  
Active clinical trials enrolling HER2 mutated cancers.

| NCT #       | Title                                                                                                                                                                                                                                 | Status     | Conditions                                                             | Interventions                                                                                                                                                                                                                                  | Phases              |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| NCT02834936 | A Clinical Study of Pyrotinib in Patients of Advanced Non-Small Cell Lung Cancer With HER2 Mutation                                                                                                                                   | Recruiting | NSCLC                                                                  | Pyrotinib                                                                                                                                                                                                                                      | Phase 2             |
| NCT03065387 | Study of the Pan-ERBB Inhibitor Neratinib Given in Combination With Everolimus, Palbociclib or Trametinib in Advanced Cancer Subjects With EGFR Mutation/Amplification, HER2 Mutation/Amplification, HER3/4 Mutation or KRAS Mutation | Recruiting | Solid Tumors                                                           | Neratinib Everolimus Palbociclib Trametinib                                                                                                                                                                                                    | Phase 1             |
| NCT01670877 | Neratinib +/- Fulvestrant in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer                                                                                                                                              | Recruiting | Breast Neoplasms                                                       | Neratinib Fulvestrant Trastuzumab                                                                                                                                                                                                              | Phase 2             |
| NCT03810872 | An Explorative Study of Afatinib in the Treatment of Advanced Cancer Carrying an EGFR, a HER2 or a HER3 Mutation                                                                                                                      | Recruiting | Cancers Harboring an EGFR Mutation, a HER2 Mutation or a HER3 Mutation | Afatinib Paclitaxel                                                                                                                                                                                                                            | Phase 2             |
| NCT02716116 | A Trial of TAK-788 (AP32788) in Non-small Cell Lung Cancer (NSCLC)                                                                                                                                                                    | Recruiting | NSCLC                                                                  | TAK-788                                                                                                                                                                                                                                        | Phase1 <br>Phase 2  |
| NCT02183883 | Deciphering Afatinib Response and Resistance With INratumour Heterogeneity                                                                                                                                                            | Recruiting | NSCLC                                                                  | Afatinib                                                                                                                                                                                                                                       | Phase 2             |
| NCT02544997 | A Phase II, Single-Arm Trial of Pozitotinib as Salvage Treatment in Patients With Metastatic Breast Cancer Who Has HER2 or EGFR Mutation or Activated AR or EGFR Pathway                                                              | Recruiting | Metastatic Breast Cancer                                               | Pozitotinib                                                                                                                                                                                                                                    | Phase 2             |
| NCT03410927 | A Study of TAS0728 in Patients With Solid Tumors With HER2 or HER3 Abnormalities                                                                                                                                                      | Recruiting | Advanced Solid Tumors                                                  | TAS0728                                                                                                                                                                                                                                        | Phase 1 <br>Phase 2 |
| NCT03505710 | DS-8201a in Human Epidermal Growth Factor Receptor 2 (HER2)-Expressing or -Mutated Non-Small Cell Lung Cancer                                                                                                                         | Recruiting | NSCLC                                                                  | Trastuzumab deruxtecan (DS-8201a)                                                                                                                                                                                                              | Phase 2             |
| NCT03318939 | Phase 2 Study of Pozitotinib in Patients With NSCLC Having EGFR or HER2 Exon 20 Insertion Mutation                                                                                                                                    | Recruiting | NSCLC                                                                  | Pozitotinib                                                                                                                                                                                                                                    | Phase 2             |
| NCT01953926 | Neratinib HER Mutation Basket Study (SUMMIT)                                                                                                                                                                                          | Recruiting | Malignant Solid Tumor Fibrolamellar Carcinoma                          | Neratinib Paclitaxel Fulvestrant Trastuzumab                                                                                                                                                                                                   | Phase 2             |
| NCT03066206 | Pozitotinib in EGFR Exon 20 Mutant Advanced Non-Small Cell Lung Cancer (NSCLC) and HER2 Exon 20 Mutant NSCLC                                                                                                                          | Recruiting | Malignant Neoplasm of Respiratory and Intrathoracic Organ  NSCLC       | Pozitotinib                                                                                                                                                                                                                                    | Phase 2             |
| NCT03457896 | Study of Neratinib +Trastuzumab or Neratinib + Cetuximab in Patients With KRAS/NRAS/BRAF/PIK3CA Wild-Type Metastatic Colorectal Cancer by HER2 Status                                                                                 | Recruiting | Metastatic Colorectal Cancer                                           | Trastuzumab Cetuximab Neratinib                                                                                                                                                                                                                | Phase 2             |
| NCT03412383 | Pyrotinib in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer                                                                                                                                                              | Recruiting | Breast Cancer                                                          | Pyrotinib                                                                                                                                                                                                                                      | Phase 2             |
| NCT02780687 | Afatinib Monotherapy in Patients With ERBB-deregulated Metastatic Urothelial Tract Carcinoma After Failure of Platinum Based Chemotherapy                                                                                             | Recruiting | Urologic Neoplasms                                                     | Afatinib                                                                                                                                                                                                                                       | Phase 2             |
| NCT02465060 | Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)                                                                   | Recruiting | Solid Tumors                                                           | Adavosertib Afatinib Binimetinib Capivasertib Crizotinib Dabrafenib Dasatinib Defactinib Larotrectinib Nivolumab Osimertinib Palbociclib Pertuzumab GSK2636771 Sapanisertib Sunitinib Malate Taselisib Trametinib Trastuzumab TDM-1 Vismodegib | Phase 2             |

resistance may be mediated by the presence of on-target mutations that hamper the binding of first generation TKIs such as Lapatinib. Studies have shown that three point mutations (L755S, L755P and T798 M) confer primary as well as acquired resistance to this agent (Bose et al., 2013; Kancha et al., 2011). Acquired resistance can also be mediated by the emergence of *de novo* mutations in HER2. Hanker et al. reported a case in which the emergence of the gatekeeper HER2 T798I was identified in the cfDNA of a HER2 L869R-mutant breast cancer patient who initially responded and then progressed to Neratinib. Structural modeling of this acquired mutation suggested that the increased bulk of isoleucine in HER2 T798I reduces Neratinib binding by generating steric hindrance. Interestingly, while this mutation induced Neratinib resistance, Afatinib and the Osimertinib metabolite AZ5104 were still able to suppress cell growth in transduced preclinical models (Hanker et al., 2017).

In addition to on-target mechanisms, it has been described that TP53, PIK3CA and K-RAS mutations may contribute to intrinsic resistance to anti-HER2 therapy in HER2 overexpressing or EGFR mutated

tumors (Black et al., 2015; Canale et al., 2017; Linardou et al., 2008; Ma et al., 2017). Cyclin E gene amplification also has been reported as mechanism of resistance to Trastuzumab in several cancer models (Cocco et al., 2016; Scaltriti et al., 2011). These genomic alterations may therefore also contribute to confer resistance to anti-HER2 therapies in the context of HER2 mutant tumors. As mentioned before, TP53, PIK3CA and CCNE are among the most altered genes in HER2 mutated bladder cancers, thus potentially explaining why these tumors are refractory to anti HER2 therapies. Moreover, among the 66 total patients of the SUMMIT trial that did not respond to Neratinib monotherapy, HER3 mutations were found in 14% of their tumors. Although additional studies are needed to evaluate whether these mutations can confer resistance to Neratinib, it has recently been demonstrated that breast cancer cell lines harboring HER3 mutations (F94 L, G284R, D297Y, T355I, and E1261A) have enhanced HER2-HER3 heterodimerization that induces a gain-of-function phenotype and results in Lapatinib resistance (Mishra et al., 2018). Furthermore, it has been recently demonstrated that the FDA-approved pan-ERBB inhibitors Afatinib and Neratinib

effectively reduced the expression of mutant K-RAS proteins in several tumor types (Booth et al., 2018; Booth et al., 2019; Moll et al., 2018), thus supporting the use of these agents to treat K-RAS mutated cancers.

## 5. Conclusions

Despite their low frequency, activating HER2 mutations have recently emerged as novel therapeutic targets for a variety of human tumors. Although anti-HER2 therapies have shown promising clinical results in a subset of HER2-mutated breast, lung and cervical cancer patients, the same therapies were largely ineffective in other tumor types such as bladder and colorectal cancers. Whether the lack of responses is related to concurrent presence of genomic alterations that can obliterate the dependency to HER2-mutations remains to be elucidated, but the available data from basket trials suggest that *ad hoc* registrational clinical studies may be successfully designed for specific histologies. In this context, the use of novel emerging anti-HER2 agents or combinatorial treatments may represent meaningful advancements for the treatment of HER2 mutated cancer patients.

## Conflict of interest statement

M.S. is in the Advisory Board of Bioscience Institute and Menarini Ricerche, received research funds from Puma Biotechnology, Daiichi-Sankio, Targimmune, Immunomedics and Menarini Ricerche, is a co-founder of Medendi Medical Travel and in the past two years he received honoraria from Menarini Ricerche and ADC Pharma. ADS has received research funds from Merck, Genentech/Roche, Puma Biotechnology, Gilead Science and Immunomedics Inc.

## Acknowledgments

This work was founded by the NIH grants R03CA187094-01 and P30CA008748 and the Breast Cancer Research Foundation. EC is a recipient of a MSK Society Scholar Prize. This work was also founded by the NIH grant U01 CA176067-01A1 to ADS.

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