ESMO Open Cancer Horizons

Activating HER2 mutations as emerging targets in multiple solid cancers

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ABSTRACT

The epidermal growth factor receptor (EGFR) family of transmembrane receptor tyrosine kinases activates signalling pathways regulating cellular proliferation and survival. HER2 is a non-ligand-binding member of this family and exerts its activity through heterodimerisation with other EGFR family members. HER2 functional activation promotes oncogenesis, leading to the investigation of HER2-directed agents in cancers with HER2 alterations. This has been best characterised in the context of HER2 gene amplification in breast and gastro-oesophageal cancers, for which HER2-directed drugs form part of standard treatment regimens. More recently, somatic HER2 gene mutations have been detected in a range of human cancer types. Preclinical data suggest that functionally activating HER2 mutations may drive and maintain cancers in a manner analogous to HER2 gene amplification and that HER2 mutations may similarly confer sensitivity to HER2-directed drugs. Here, we critically review the emerging roles for HER2-directed drugs in HER2 mutant cancers. We review data from experimental models, where our knowledge of the underlying biology of HER2 mutational activation remains incomplete. We discuss clinical data from Phase I and II clinical trials which evaluate HER2-directed agents (tyrosine kinase inhibitors and antibody-based drugs) in several cancer types. We highlight the heterogeneity of HER2 mutations in human cancers, differences in the clinical efficacy of HER2-directed drugs between cancer types and possible mechanisms of primary and acquired resistance, in order to guide clinical practice and future drug development.

HER2: A RECEPTOR TYROSINE KINASE AND PROTO-ONCOGENE

HER2/ErbB2/Neu is a member of the epidermal growth factor receptor (EGFR) family of homologous transmembrane receptor tyrosine kinases (EGFR and HER2-4/ErbB1-4). Ligand binding to EGFR or HER3/4 induces a conformational change in these proteins that facilitates receptor dimerisation. Receptor dimerisation brings the two intracellular tyrosine kinase domains (TKDs) together in an asymmetrical manner and the carboxy lobe of one allosterically activates the amino lobe of the other (see Zhang *et al*[1] reviewed in Lemmon *et al*[2]). Subsequent transphosphorylation of tyrosines in the carboxy tail provides docking sites for the recruitment of downstream signalling proteins. These signalling proteins affect multiple cellular processes, including proliferation, survival and differentiation, depending on receptor subtype and cellular context (reviewed by Wagner *et al*[3]). Although HER2 has no known ligand, it is able to signal through heterodimerisation with other EGFR family members, thereby affecting the downstream signalling of these receptors.[4] Increased HER2 expression and activation of its tyrosine kinase have been associated with cell transformation and oncogenesis, establishing HER2 as an important proto-oncogene (reviewed by Moasser[5]) and paving the way for the development of HER2-targeted therapeutics.

HER2 PROTO-ONCOGENE ACTIVATION BY MUTATION IN HUMAN CANCERS

HER2 alterations and current clinical practice

The clinical impact of oncogenic HER2 activation has been exemplified by HER2 gene amplification in breast and gastro-oesophageal cancers. For patients with breast cancer, HER2 gene amplification is an independent prognostic factor associated with a shorter disease-free survival (DFS),[6] and sensitivity to HER2-directed therapy (trastuzumab, pertuzumab or ado-trastuzumab emtansine (T-DM1)) in the neoadjuvant,[7] adjuvant[8] or metastatic[9] settings. HER2 amplification may also be a negative prognostic factor for patients with gastro-oesophageal cancers[10] and HER2-directed antibody therapy with trastuzumab in combination with platinum-based and fluoropyrimidine-based chemotherapy is a standard of care for advanced disease.[11] However, HER2-directed therapy has not demonstrated success in all HER2 overexpressing/amplified cancer types. For patients with metastatic urothelial cancer overexpressing HER2, there was no survival benefit from trastuzumab[12] or the EGFR-/HER2-directed tyrosine kinase inhibitor (TKI) lapatinib.[13] For patients with HER2 overexpressing advanced non-small cell lung cancer (NSCLC), although there was no overall benefit to the entire patient cohort,

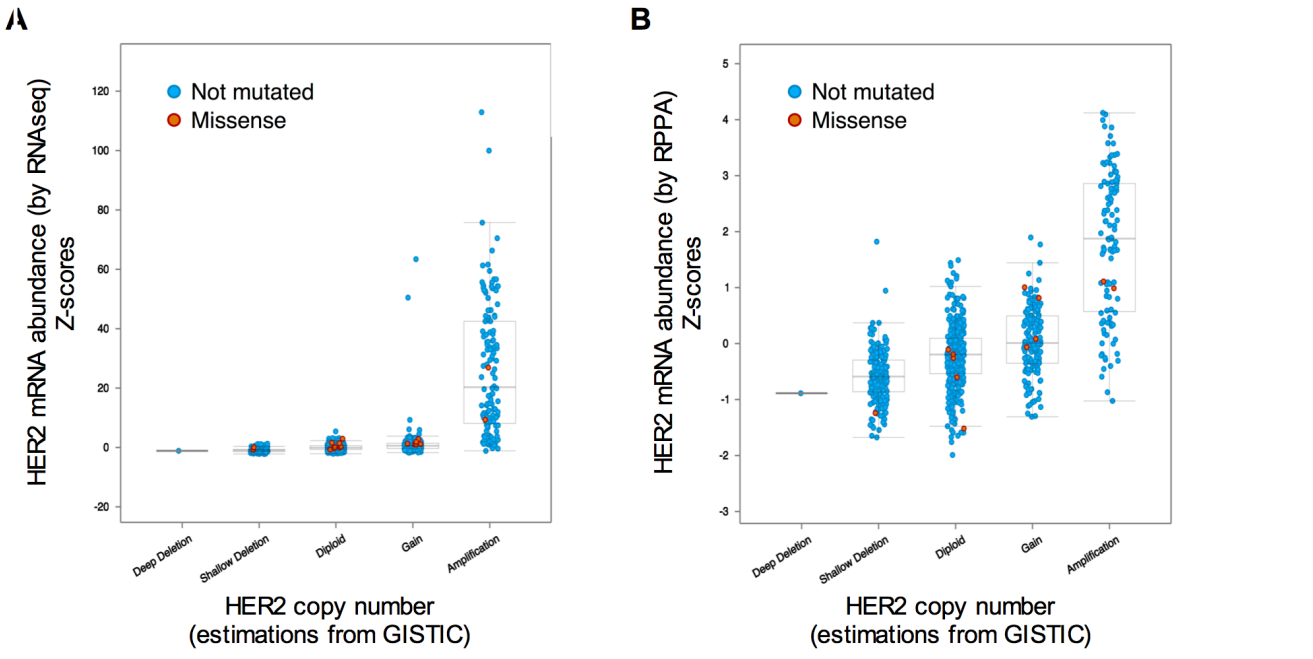

Figure 1 Plots derived from provisional TCGA data from sequencing and expression analyses of invasive breast carcinoma cases. (A) Plot demonstrating the relationship between HER2 mRNA levels (from RNA sequencing analysis) and HER2 copy number (from GISTIC analysis) for individual breast cancer cases, where sufficient data were available for analysis. Data were derived from, and plotted through, cbioportal.org. Blue dots represent individual HER2 wild-type cases, and orange dots represent individual HER2 mutant cases. (B) Plot demonstrating the relationship between HER2 protein levels (from reverse phase protein array analysis) and HER2 copy number (from GISTIC analysis) for individual breast cancer cases. Data were derived from, and plotted through, cbioportal.org. Blue dots represent individual HER2 wild-type cases, and orange dots represent individual HER2 mutant cases. GISTIC, Genomic Identification of Significant Targets in Cancer; TCGA, The Cancer Genome Atlas.

analyses have suggested potential benefit confined to the subgroup of patients strongly overexpressing HER2.[14 15] However, it is difficult to draw firm conclusions given small patient numbers. These experiences demonstrate our incomplete understanding of HER2 biology, particularly given the differences observed between tumour types.

More recently, increasing attention has been given to the emerging impact of oncogenic HER2 activation through somatic gene mutation. The majority of these HER2 mutant cancers have not been associated with concurrent HER2 gene amplification[16–18] and they thereby represent an important subgroup of HER2-activated cancers that may be missed by standard analyses of HER2 positivity based on immunohistochemistry (IHC) or fluorescent in situ hybridisation (FISH) techniques. Indeed, a recent prospective analysis of 1126 patients with advanced NSCLC found that HER2 mutations in 21 (2.9%) of EGFR wild-type cancers were mutually exclusive with HER2 IHC 3+ positivity.[19] Using data derived from the current provisional set of The Cancer Genome Atlas (TCGA) invasive breast cancer cases, we found that HER2 mutations exist across the spectrum of HER2 copy number changes as well as the wide range of measured HER2 protein and mRNA levels (figure 1) and HER2 IHC scores on tumour specimens (table 1).

Of nine HER2 mutant samples with HER2 FISH data, four were scored as positive and five were scored as negative. Taken together, these data suggest that the mutant HER2 protein (the target for HER2-targeted drugs) is expressed in the majority of HER2 mutant cancers and that, in some cases, HER2 copy number changes are found in HER2 mutant settings. Whether mutant allele frequency of the HER2 gene impacts on function remains to be seen, but this has been described for the KRAS oncogene.[20]

Prevalence of HER2 mutations in human cancer

Mutations are found across all exons of the HER2 gene, with significant heterogeneity both between and within human cancer types (figure 2). Mutations in the HER2 gene affecting the extracellular domain (ECD), transmembrane domain (TMD) or TKD of the HER2 protein are capable of activating HER2 signalling pathways, even in the presence of a normal HER2 gene copy number.[16 21 22] HER2 mutations may also be sufficient to drive lung cancers in murine models.[23 24] First detected in lung adenocarcinoma,[25] HER2 mutations

Table 1 Relationship between HER2 copy number and HER2 IHC scores for cases of invasive breast carcinoma, stratified by HER2 wild-type/mutant status

	Shallow deletion	Diploid	Low-level copy number gain	High-level amplification
IHC 0	22 (0 mutant)	26 (1 mutant)	10 (0 mutant)	2 (0 mutant)
IHC 1	65 (0 mutant)	119 (4 mutant)	53 (3 mutant)	6 (0 mutant)
IHC 2	24 (0 mutant)	75 (0 mutant)	51 (3 mutant)	25 (1 mutant)
IHC 3	4 (0 mutant)	8 (0 mutant)	7 (0 mutant)	59 (1 mutant)

As per figure 1, this was derived from provisional TCGA data from sequencing and expression analyses of invasive breast carcinoma cases, where all required data for this analysis was available. Data were derived from cbioportal.org.
IHC, immunohistochemistry; TCGA, The Cancer Genome Atlas.

have subsequently been identified in a wide variety of solid organ malignancies. The prevalence of these (from selected large scale sequencing efforts in many cancer types) is summarised in table 2.

The highest prevalence of HER2 mutations is observed in prostate neuroendocrine cancer, metastatic cutaneous squamous cell carcinoma and bladder cancer (all >10% of cases). However, a significant HER2 mutation prevalence is also found in more common cancers, including lung, colorectal and breast cancers, indicating a large additional patient base that could potentially be targeted with HER2-directed therapies. HER2 mutations are enriched in certain histological subtypes, for example in 15% of invasive high grade lobular breast carcinomas[26] and in 9.8% of lung adenocarcinomas in one study,[25] although larger scale efforts suggest the prevalence in lung adenocarcinomas is nearer 3% (see table 2).[19 27]

HER2 mutations and prognosis in human cancer

Unlike HER2 gene amplification, HER2 gene mutations have not been subject to routine clinical testing and therefore data on associated prognosis and treatment responses are limited. However, a recent analysis of patients with breast cancer has identified somatic HER2 gene mutations, in the context of HER2 amplification-negative disease, to be associated with a shorter DFS than those without HER2 gene mutations.[21] In NSCLC, HER2 mutations have generally not been associated with differences in prognosis, although in the absence of stratification for HER2 gene copy number.[18 28–30] Analyses of only those patients with advanced stage lung adenocarcinomas have provided mixed results.[28 31] Our own analysis from data in cbioportal.org from TCGA projects finds that the presence of a HER2 mutation in breast adenocarcinoma correlates negatively with overall survival (OS), although this loses significance when adjusting for HER2 copy number (a known negative prognostic factor) and analysis is limited by the small number of cases (figure 2B). For other common tumour types, including colorectal and lung adenocarcinomas, no significant correlation was observed (data not shown).

HER2 MUTANT PROTEINS AS THERAPEUTIC TARGETS

Differential sensitivity of HER2 mutant models to HER2-directed agents: preclinical data

Current HER2-directed therapies include monoclonal antibodies, antibody-drug conjugates and small molecule TKIs. The monoclonal antibodies trastuzumab and pertuzumab bind to distinct regions of the ECD of HER2, at the juxtamembrane domain IV[32] and the dimerisation domain II,[33] respectively. They act to inhibit HER2 activity through both cell intrinsic effects and promotion of antibody-dependent cell-mediated cytotoxicity (reviewed in Baselga and Swain[34]). The antibody-drug conjugate T-DM1 binds to HER2 in the same manner as trastuzumab and delivers the maytansine derivative emtansine (which depolymerises microtubules) into the HER2 expressing cell.[35] The TKIs, in contrast, are small molecule inhibitors that bind to an ATP binding cleft within the TKD to block catalytic activity. These may bind reversibly, for example the EGFR/HER2 inhibitor lapatinib,[36] or irreversibly. The irreversible TKIs afatinib,[37] neratinib[38] and dacomitinib[39] bind covalently to conserved cysteine residues within the ATP binding pockets of EGFR, HER2 and HER4 and thereby block downstream signalling of EGFR family heterodimers.

The efficacy of these HER2-directed agents against various HER2 mutations has been tested in vitro. The kinase domain mutant G776insYVMA enhances downstream HER2 signalling and confers cellular sensitivity to trastuzumab, which is capable of downregulating both the wild-type and G776insYVMA mutant proteins from the plasma membrane.[23] This HER2 mutant also remains sensitive to the TKI lapatinib.[23] HER2 ECD mutations, for example G309E, which promotes HER2 dimerisation through intermolecular disulfide bonds, and S310F, which increases C-terminal tail phosphorylation without affecting dimerisation, activate similar HER2 downstream pathways and display increased sensitivity to both trastuzumab and TKIs compared with wild-type HER2.[22] This occurs despite these ECD mutants being located in proximity to the trastuzumab epitope,[22] though impact on pertuzumab binding (residues 309–310 of HER2 form part of the pertuzumab binding epitope) is yet to be determined. In contrast to the above TKD and ECD mutations, the TMD mutations at the highly conserved

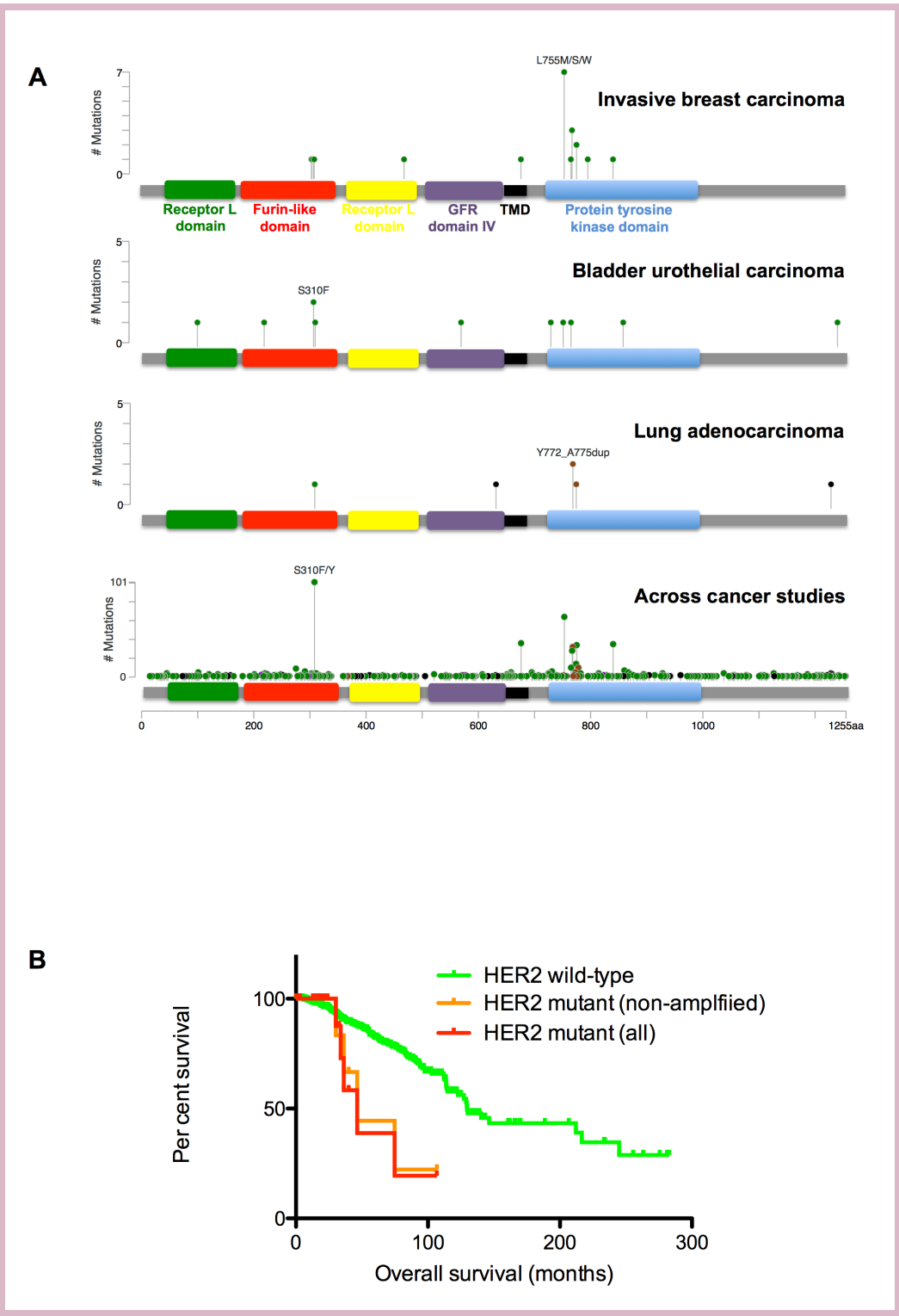

Figure 2 (A) Schematic diagrams demonstrating the HER2 mutational heterogeneity among and between cases of invasive breast cancer (n=963), bladder urothelial cancer (n=127), lung adenocarcinoma (n=230) and across cancer types (164 individual studies). Upward strokes depict the spatial location of mutations found in the HER2 gene (x axis), and the number of cases with mutations at each codon are shown by the length of the upstrokes (y axis). All data in this figure have been derived from TCGA data deposited in cbioportal.org. Green dots indicate missense mutations, brown dots indicate in-frame mutations, black dots indicate truncating mutations and other mutations are indicated by purple dots. (B) Kaplan-Meier graph showing the survival of patients in the provisional TCGA dataset of invasive breast carcinoma cases (derived from data deposited in cbioportal.org), stratified by HER2 mutational status. The median survival was 46.4 months for all HER2 mutant cases (n=20) and 46.4 months for HER2 mutant cases without copy number gains (n=11), compared with 129.6 months for HER2 wild-type cases (P=0.0056 for all HER2 mutants, and P=0.2218 for HER2 mutant cases without copy number gains, by log-rank (Mantel-Cox) test). GFR, growth factor receptor; TMD, transmembrane domain.

Table 2 Percentage of cases from studies deposited in cbioportal.org where HER2 alterations are found as well as data from US registries showing the number of deaths in the US per year from each associated cancer type

Cancer type	HER2 alterations (% cases)		Estimated annual deaths (US)	Estimated HER2 mutant deaths (US)
	Mutation	Amplification		
Colorectal (DFCI)	5.8	ND	50310	2918
Lung SCC	3.4	ND	72828	2476
Lung adenocarcinoma	2.6	2.6	72828	1894
Bladder (MSKCC)	11	1.8	15580	1714
Bladder (BGI)	10.1	ND	15580	1574
Colorectal (TCGA)	2.8	2.4	50310	1409
Breast (METABRIC)	2.8	15.1	40430	1132
Stomach	9.1	ND	10990	1000
Bladder (TCGA)	6.3	3.1	15580	982
Glioblastoma	7.7	ND	12000	924
Lung small cell	3.4	ND	24276	825
Breast (TCGA)	1.8	12.5	40430	728
Cutaneous SCC (metastatic)	17.2	ND	3270	562
Oesophagus	3.3	12	15450	510
Stomach (TCGA)	4.2–4.6	12.5–13.7	10990	462–506
Gallbladder	9.4	ND	3630	341
Head and neck SCC	3.1	ND	8390	260
Endometrial	3.3	8.3	7181	237
Cholangiocarcinoma	5.7	ND	3630	207
Cervical SCC	5.2	3.1	3417	178
Uterine carcinosarcoma	9.1	ND	1407	128
Low grade glioma	3.3	ND	2500	83
Prostate neuroendocrine	19.6	1.9	Rare	Rare
Bladder (plasmacytoid)	15.2	3	Rare	Rare

The number of HER2 mutant deaths is estimated as the total estimated deaths for each cancer, multiplied by the fractional prevalence of HER2 mutations.

DFCI, Dana Farber Cancer Institute; METABRIC, Molecular Taxonomy of Breast CancerInternational Consortium; MSKCC, Memorial Sloan Kettering Cancer Center; ND, no data available; SCC, squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

amino acid residues V659 and G660 have been associated with resistance to trastuzumab.[40] These mutations reduce HER2 degradation[41] and stabilise homo-dimerisation and hetero-dimerisation[40 42] in a manner refractory to trastuzumab binding to the ECD, but maintaining sensitivity to the irreversible TKI afatinib.[40 43]

Differential sensitivity of HER2 mutations to TKI therapy has also been observed, notably among TKD mutants. For example, the TKD mutant H878Y, found in 11% of hepatocellular carcinomas,[44] sensitises to lapatinib therapy in vitro.[45] In contrast, the kinase domain mutants L755S,[16 45 46] P780-Y781insertionGSP (P780ins),[16] L755P and T798M (a gatekeeper mutation; see below)[45] confer primary resistance to lapatinib in vitro. The L755S mutant, located adjacent to the TKI binding site,[16] is associated with both primary and secondary resistance in vitro,[45 47] possibly through stabilising HER2 in an active conformation.[45] These lapatinib-resistant mutants retain sensitivity to irreversible TKI inhibitors.[45–47]

The HER2 'gatekeeper' mutations T798M[45] and T798I,[48] located within the ATP binding site, are analogous to the T790M mutation in EGFR that is resistant to first-generation and second-generation EGFR inhibitors. Recently, T798I has been associated with secondary resistance to neratinib in a patient with breast cancer harbouring the otherwise activating L869R mutation (HER2 L869R is analogous to the gain of function EGFR L861R/Q and BRAF V600E mutations). T798I confers steric hindrance to neratinib binding, while maintaining sensitivity to afatinib.[48] Interestingly, even low-level amplification of the T798M allele in a HER2 wild-type-amplified setting is sufficient to confer neratinib (but not afatinib) resistance.[48] Another TKD mutant capable of conferring secondary resistance to neratinib is C805S, identified in a N-ethyl-N-nitrosourea screen of HER2 InsYVMA expressing Ba/F3 cells; in this case, the mutation is also associated with resistance

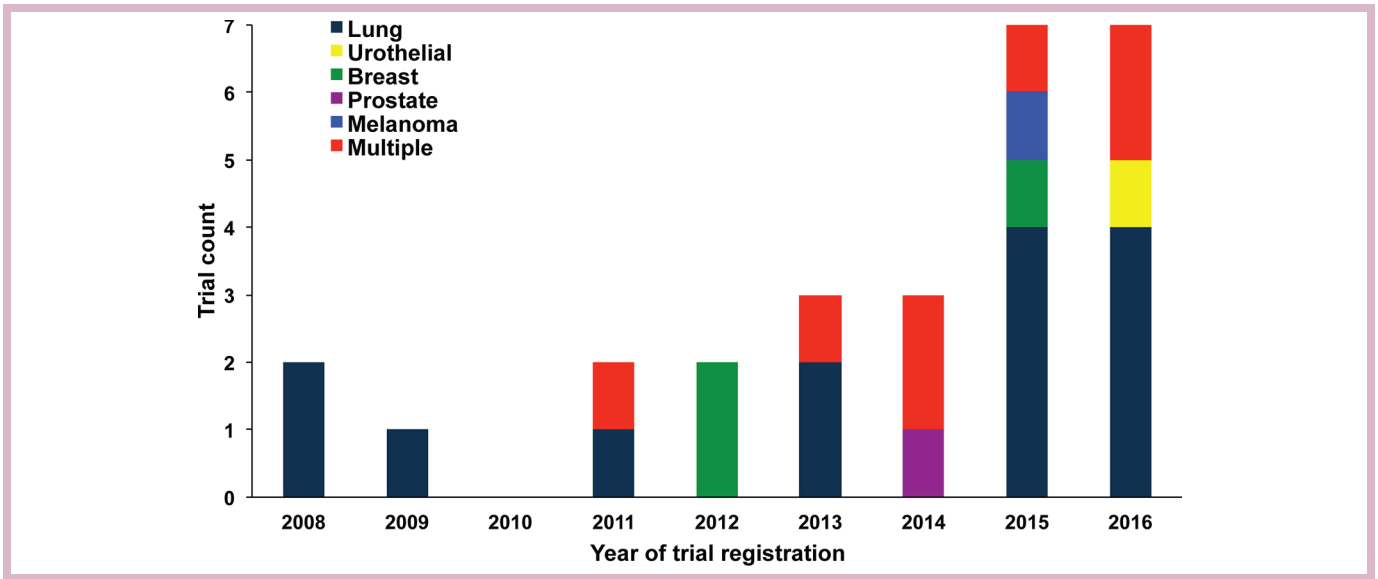

Figure 3 Graph showing the annual registration count of trials where HER2-directed agents have been, or are being, tested in HER2 mutant cancers. Trials registered by 13 August 2017 on the US National Institutes of Health (NIH) trial registry were categorised according to year of registration and targeted cancer type. All trials have been included, including one trial withdrawn prior to enrolment and one trial terminated due to insufficient accrual. GFR, growth factor receptor; TMD, transmembrane domain.

to afatinib and dacomitinib.[49] However, the contribution of C805S to clinical TKI resistance remains to be determined.

The diversity of HER2 mutations thereby appears to confer differential sensitivity to different modalities of HER2-directed therapies.[16 45 46] However, a degree of caution should be taken when interpreting functional and/or drug sensitivity assays in the context of HER2 mutant overexpression since, as discussed above, human cancers often do not have increased levels of HER2 mutant mRNA/protein. The effects of HER2 mutant proteins expressed at endogenous levels has been studied by editing single alleles of the HER2 gene within non-amplified HER2 cell lines.[50] Although increased HER2 signalling was observed with the V777L mutation, it required the presence of a PIK3CA E545K mutation to translate into increased migratory capacity. Furthermore, the majority of mutations previously categorised as activating failed to promote downstream pathway activation or a transformed phenotype, and no single mutation was found to promote tumour formation in vivo. The studied HER2 mutations did not affect sensitivity to lapatinib, and this included the L755S mutant associated with lapatinib resistance when overexpressed.[16 45 46] Therefore, the impact of HER2 mutations is likely to be dependent on both coexistent mutations and levels of HER2 mutant expression (see above, table 1 and figure 1), and studies should consider context-appropriate levels of expression.

Patient-derived HER2 mutant cancer cell lines provide an opportunity to study the role of HER2 signalling in a genetic background better representative of human cancers than the aforementioned highly engineered models. The large-scale Catalogue of Somatic Mutations In Cancer (COSMIC) cell lines project (V.82[51]) has identified 63 cell lines derived from solid cancers that contain HER2 mutations, Multiple cancer types are represented, including 17 lung and 11 colorectal cell lines; other cancer types are less well represented, including breast cancer for which there are only two. Analysing data from in vitro drug screening of these cell lines, through the Genomics of Drug Sensitivity in Cancer project (GDSC; release 6.1[52]), we found afatinib IC50 values to be widely distributed among HER2 mutant cell lines, with a trend towards significance with afatinib sensitivity when compared with HER2 wild-type cancer cell lines (P=0.075; 9 HER2 mutant cell lines tested). Although these analyses are restricted by the limited availability of HER2 mutant cell lines, they do demonstrate that HER2 mutation alone is not necessarily predictive of HER2 signalling dependence.

The GDSC (release 6.1[52]) database may also be used to identify novel putative treatments for patients with HER2 mutant cancers. Analyses of drugs tested on nine or more HER2 mutant cell lines identified the following to be associated with increased sensitivity in HER2 mutant cell lines (with a P value<0.05): TAK-715 (p38α/β inhibitor), EHT-1864 (Rac1-3 inhibitor), enzastaurin (PKCβ inhibitor), WHI-P97 (JAK3 inhibitor) and TL-2–105 (mechanism of action unclear). Surprisingly, of the six drugs associated with significant resistance (with a P value<0.05), three were MEK1/2 (typically considered to act downstream of HER2) inhibitors. While these results are interesting, they will require validation.

In conclusion, in vitro analysis has provided mechanistic insight into the sensitivity of HER2 mutant proteins to HER2-directed agents. However, conclusions from highly

engineered systems studying overexpression of the HER2 mutant protein have not necessarily been supported by models of endogenous HER2 mutant expression or patient-derived HER2 mutant cell lines. These differences highlight our incomplete understanding of the determinants of HER2 signalling dependence and suggest caution when translating their findings into the clinic. To better investigate these crucial determinants, it will be important to perform comparative genetic, transcriptional and proteomic analysis of tumour samples from patients whose tumours are sensitive or resistant to HER2-directed agents.

Clinical data from the use of HER2-directed therapy against HER2 mutant cancers

Interest in the potential for targeting HER2 mutant cancers with HER2-directed therapy has been translated into a progressive increase in the number of registered prospective trials evaluating these therapies across a range of cancer types (figure 3). Out of 30 registered trials, 26 (87%) examine the use of HER2 targeting TKIs as monotherapy (n=18) or in combination with cytotoxic, hormonal, targeted or antibody-based therapy (n=8). Three (10%) trials examine the use of HER2-directed antibody monotherapy and one trial examines T-DM1 monotherapy.

HER2-directed antibody-based drugs

Retrospective analyses have suggested a benefit from the use of trastuzumab in patients with pretreated HER2 mutant advanced NSCLC.[17 53] Initially, 16 patients who were administered a total of 22 HER2-directed treatments achieved a disease control rate (DCR) of 93% for the 15 patients treated with trastuzumab-based therapies, 100% for the 3 patients treated with afatinib and no response for the 3 patients who received either lapatinib (n=2) or masatinib (n=1). The median progression-free survival (PFS) with HER2-directed treatment was 5.1 months.[17] Subsequently, a study of 58 patients with pretreated advanced lung adenocarcinoma, harbouring an in-frame insertion within exon 20 of HER2, treated with HER2-directed antibody-based therapies (including trastuzumab for 57 patients (in combination with chemotherapy for 55 of these patients) and T-DM1 for one patient) demonstrated an overall response rate (ORR) of 50.9%, a DCR of 75.5% and a median PFS of 4.8 months (95% CI 3.4 to 6.5). In the same study, an additional 11 patients treated with afatinib had an ORR of 18.2%, a DCR of 63.7%, and a PFS of 3.9 months, while all five patients treated with lapatinib had progressive disease at their initial response assessment. In comparison, patients who received treatment with conventional therapy and EGFR-targeted TKIs had an ORR of 43.5% and a median PFS of 6 months (95% CI 5 to 7.1) in the context of first-line therapy and a ORR of 10% with a median PFS of 4.3 months (95% CI 3.1 to 5) in the second-line setting.[53]

Potential efficacy has been demonstrated for trastuzumab in other combination regimes. These smaller case series and case reports include combinations with chemotherapy,[54 55] hormonal therapy in the context of oestrogen receptor positive (ER+) metastatic breast cancer with a HER2 S310F mutation[56 57] and a further HER2-targeted agent (lapatinib) with bevacizumab.[58] Trastuzumab combination therapy, with lapatinib and albumin-bound paclitaxel, has demonstrated clinical efficacy in a patient with heavily pretreated inflammatory breast cancer containing V777L and S310F HER2 mutations.[59]

T-DM1 has also demonstrated possible benefit in HER2 mutant cancers,[60 61] with an ORR of 33% (95% CI 12% to 62%); the median duration of response was not reached (range 3 to 7+ months) at an interim review in 15 patients with HER2 mutant lung adenocarcinoma. Responders were observed across multiple HER2 mutation types.[61]

HER2-directed tyrosine kinase inhibitors

TKIs have generated mixed clinical results in patients with HER2 mutant cancers. Response rates from published prospective trials have been summarised in table 3.

As mentioned above, lapatinib has yet to demonstrate efficacy in retrospective case series,[17 53] and no responses were observed in seven patients with lung cancers treated with lapatinib as part of a phase II basket trial.[62] However, a marked response in one patient harbouring a L869R mutation has been noted.[63] For afatinib, an early exploratory phase II study which treated three patients with HER2 mutant advanced lung adenocarcinoma demonstrated clinical activity of afatinib alone/afatinib plus paclitaxel, with survivals of between 12 and 32 months from study entry. For one of these patients, this was despite resistance to previous EGFR family-targeted treatments including erlotinib, trastuzumab and lapatinib.[64] More recently, in a retrospective study of 27 patients with pretreated metastatic HER2 mutant lung adenocarcinoma, afatinib was associated with an ORR of 15% (n=4) and an OS (from date of diagnosis of metastatic disease) of 23 months (95% CI 18 to 62 months),[65] while a recent phase II single arm trial (NICHE) of afatinib in 13 pretreated patients with advanced NSCLC with HER2 exon 20 mutations was closed early as the stopping boundary was crossed. However, some potential clinical utility was suggested, with a DCR at 12 weeks of 53.8% and a median PFS of 13 weeks.[66] An analysis of results from 28 patients with HER2 mutant advanced NSCLC treated with afatinib (on a named patient use programme) had an overall time to treatment failure (TTF; duration between start to discontinuation of treatment) of 2.9 months, with 9 (32%) patients achieving a TTF of greater than 1 year. The DCR for the four evaluable patients with the exon 20 2325/YVMA insertion was 100%, with a TTF of 9.9 months (vs 1.9 months for other mutations). For the 16 patients with response assessments, the DCR was 69% (11/16) and the ORR was 19% (3/16). This was despite 16 (57%) patients receiving afatinib as fourth-line treatment and 7 (25%) patients previously receiving HER2-directed therapy.[67]

Clinical responses to neratinib have also been variable, but promising. In a phase II basket trial (SUMMIT),

Table 3 Clinical data that has been reported from prospective trials of HER2-targeted agents in HER2 mutant cancers

Trial registration ID	Tumour site	Arm(s)	Number of patients with evaluable responses	ORR (PR/CR)	Reference
NCT01306045	NSCLC/SCLC*	Lapatinib	6 NSCLC, 1 SCLC	0/7 (0%)	Lopez-Chavez *et al*[62]
NCT00730925	Lung adenocarcinoma	Afatinib	3	3/3 (100%)	De Grève *et al*[64]
NCT00818441	Lung adenocarcinoma	Dacomitinib	26	3/26 (12%)	Kris *et al*[71]
NCT01670877	Breast cancer	Neratinib	16	2/16 (13%)	Ma *et al*[74]
NCT01827267	NSCLC	Neratinib	17	0 (0%)	Gandhi *et al*[70]
		Neratinib with temsirolimus	43	8/43 (19%)	
NCT01953926	Multiple	Neratinib	110	Variable; from 24% (6/25; breast), 20% (1/5; cervical) and 3.8% (1/26; lung), to 0% (bladder n=16 and colorectal n=12)	Hyman *et al*[68]
	ER+ metastatic breast cancer	Neratinib	24	5/24 (21%)	Hyman *et al*[69]
		Neratinib with fulvestrant	11	2/11 (18%)	
NCT02535507	Lung adenocarcinoma	Pyrotinib	11	6/11 (55%)	Ren *et al*[72]
NCT02675829	Lung adenocarcinoma	Ado-trastuzumab emtansine	15	5/15 (33%)	Li *et al*[61]

*Signifies where patients with HER2 mutations and amplifications are included.
CR, complete response; NSCLC, non-small cell lung cancer; ORR, overall response rate; PR, partial response; SCLC, small cell lung cancer.

125 patients with HER2- and 16 patients with HER3 mutant cancers received neratinib therapy (in combination with hormonal therapy for those patients with ER+ breast cancer and with paclitaxel for those patients with bladder cancer). Responses were observed in patients with breast, cervical, non-small cell lung, biliary and salivary gland cancers. More patients had stable disease than objective response, and responses were seen across a range of mutational subtypes. No objective responses were observed in bladder or colorectal cancers or in the HER3 mutant cohort.[68] Promising results were observed in the subgroup analysis of 11 patients with ER+ HER2 mutant metastatic breast cancer treated with neratinib in combination with fulvestrant, with an ORR of 18.2% (2/11, with four patients still on treatment at the time of reporting; 95% CI 2.3 to 51.8) and a clinical benefit rate of 54.5% (6/11; 95% CI 23.4 to 83.3).[69] Clinical benefit has also been observed with neratinib in combination therapy in a phase II study in which patients with stage IIIb/IV NSCLC received neratinib or neratinib with temsirolimus. In the single agent neratinib group, 6 out of 17 patients had stable disease, but none had a clinical response. In contrast, in the neratinib and temsirolimus combination arm, 14 out of 43 patients (33%) had stable disease, with 8 patients (19%) achieving responses (which lasted 2–18 months).[70]

Other TKIs have also been evaluated in the phase II setting. Dacomitinib (an irreversible inhibitor of EGFR, HER2 and HER4) demonstrated potential benefit for patients with stage IIIb/IV lung adenocarcinoma, with an ORR of 12% (95% CI 2% to 30%) for 26 patients with HER2 mutant disease, compared with 0% (95% CI 0% to 60%) in 4 patients with HER2 amplified disease.[71] Pyrotinib (an irreversible dual EGFR and HER2 inhibitor), in a trial of 11 patients with HER2 mutant advanced lung adenocarcinoma, resulted in partial responses in 54.5% and stable disease in 27.3% of patients; one patient responded despite previous resistance to afatinib.[72]

Ongoing trials

Active trials are further investigating HER2-directed therapies in HER2 mutant cancers, and results from these are eagerly anticipated. Those examining the efficacy of HER2-directed antibodies include the My Pathway Trial (NCT02091141), which is investigating combination therapy with pertuzumab and trastuzumab in HER2 altered cancers (amplified/overexpressed/mutant) and only permitting patients with breast, gastric

or gastro-oesophageal cancers if they have HER2 mutations. A phase II study in Japan will trial T-DM1 monotherapy in 30 patients with recurrent NSCLC harbouring HER2 alterations (defined as IHC/FISH positive or an insertion mutation in exon 20) that have been previously treated with platinum-based chemotherapy.[73] It is unclear how many of these patients will have HER2 mutations. There are multiple trials investigating the role of afatinib in patients with HER2 mutant cancers, including those with NSCLC previously treated with platinum-based chemotherapy (NCT02597946) and those with refractory advanced solid tumours (excluding NSCLC; subprotocol B in the NCI-MATCH trial: NCT02465060). Other TKIs under investigation include pyrotinib for patients with pretreated advanced NSCLC (NCT02834936) and poziotinib (an irreversible inhibitor of EGFR, HER2 and HER4) for patients with stage IV lung adenocarcinoma (NCT02979821) or metastatic breast cancer refractory to conventional treatments (NCT02544997; in addition to HER2 mutations, this trial is also recruiting patients activating EGFR or androgen receptor pathway mutations). A further trial is comparing neratinib to combination therapy with neratinib and fulvestrant (NCT01670877), after a protocol amendment following promising early results[74] (table 3). Neratinib is being trialled in the phase I setting in combination with everolimus, palbociclib or trametinib with patients who have advanced cancer with EGFR or HER2 mutation/amplification or HER3/4 mutations (NCT03065387).

Resistance to HER2-directed therapies: mechanisms and implications

As detailed above, preclinical and preliminary clinical evidence support a potential benefit from the use of HER2-directed therapy in patients with HER2 mutant cancers. However, response rates to HER2-directed therapy are modest, highlighting our incomplete understanding of the biology and targetability of HER2 mutations in individual patients.

As discussed above, primary or secondary resistance to HER2-directed therapy may be a consequence of HER2 mutation(s), for example affecting TKI binding (see above and eg, Kancha *et al* and Hanker *et al*[45 48]). However, the factors governing response to treatment remain largely obscure. Changes to downstream or parallel oncogenic pathways may contribute to primary and secondary resistance. For example, HER2 inhibition with TKI therapy has been associated with compensatory increases in the level of HER3 phosphorylation and downstream PI3K/AKT prosurvival signalling due to residual HER2 activity.[75] Similar pathway activation has been observed in response to HER3 engagement by the ligand heregulin.[76] HER3 mutations are found in ~2% and ~8% of HER2 wild-type and mutant cancers, respectively, and coexpression of HER2 L869R and HER3 E928G leads to enhanced downstream signalling compared with either one alone.[48] Importantly, the increased signalling in comutated cells retains sensitivity to neratinib in vitro. More efficient HER2/HER3 blockade[75 76] or combination with PI3K/mTOR inhibitors[76] are among possible strategies that may help overcome resistance.

In the HER2-amplified setting, multiple mechanisms of resistance to trastuzumab have been suggested, including PI3K/mTOR and insulin-like growth factor 1 receptor pathways (reviewed by[77]) and steric hindrance of trastuzumab binding by the membrane glycoprotein Mucin-4.[78] Resistance to T-DM1 has also been associated with heregulin, and in this case is reversible through coadministration with pertuzumab,[79] known to inhibit HER2/HER3 heterodimerisation.[33] Whether these resistance mechanisms are also found in the HER2 mutant setting remains unclear. As discussed above, HER2-directed combination therapies are reaching clinical trials in HER2 mutant cancers, and efficacy with coadministration of neratinib and temsirolimus has recently been reported.[70] Likewise, the recent data from the use of neratinib in combination with fulvestrant[69] supports the use of combination therapy to target HER2 mutant cancers known to be dependent on multiple oncogenic pathways. Given the efficacy demonstrated in HER2-amplified breast cancer,[7–9] and the ability to overcome compensatory feedback mechanisms by more complete suppression of HER2 signalling,[75 76] the potential benefit of multiple HER2-directed therapies should be investigated.

SUMMARY AND FUTURE OUTLOOK

The evidence base supporting HER2-directed drugs in HER2 mutant cancers is promising and rapidly growing. In order to ensure a smooth transition into routine clinical care for patients with HER2 mutant cancers, attention must be directed at refining predictive markers of response to HER2-directed therapy, particularly given the complex preclinical data that we have described. At a cellular level, this should include elucidating the effects of individual HER2 mutations and the impact of coexisting HER2 copy number alterations and comutated genes on the phenotypes and resistance of HER2 mutations to HER2-directed drugs. This will help identify signatures predictive of individual HER2-directed drug sensitivity and facilitate the rational design of combination strategies. Variations between tumour types should also be determined and analysed. At a patient and trial level, we must use these data to aid patient stratification/selection for HER2-directed therapy. The high level of diversity of HER2 mutations complicates patient selection strategies, given an incomplete knowledge of the functional consequences of individual mutations. Structural approaches, such as those taken with the HER2 T798I mutation,[48] may aid in the rational selection of patients likely to benefit from specific agents. Attention must also be given to identifying and counteracting acquired resistance mechanisms, including molecular analysis of postprogression biopsies. These measures will help use HER2-directed therapies to improve survival across the population of patients with HER2 mutant cancers.

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