Introduction

Research into the molecular basis of lung cancer has revealed insights into various critical pathways that are deregulated, and among them, key driver genetic alterations that promote cell survival and proliferation. In the oncogene addiction model, cancer cells harbor gene amplification, rearrangement or mutations that dictate their malignant phenotype, and can thus be referred to as driver alterations (1). Among them, human epidermal growth factor 2 (HER2 erbB-2/neu) is a member of the erbB receptor tyrosine kinase family. The ERBB2 gene which encodes for HER2 is a major proliferative driver that activates downstream signaling through PI3K-AKT and MEK-ERK pathways (2). Unlike HER1/epidermal growth factor receptor (EGFR), HER2 has no known ligand, and is activated by homo-dimerization or hetero-dimerization with other members of the erbB family. Under resting conditions, these cell-surface receptors are found as monomers folded in a so-called “closed” inactive conformation that prevents dimerization (3). Upon ligand binding to the extracellular domain, conformational rearrangements lead to an “open” state that exposes the dimerization interface. This extracellular dimeric structure results in the transactivation of the intracellular tyrosine kinase portion of each receptor. Three principal mechanisms of oncogenic activation of HER2 have been described: HER2 gene amplification, gene mutation resulting in molecular alterations of the receptor or HER2 protein overexpression.

HER2 has been shown to be overexpressed in 13% to 20% of NSCLC. HER2 deregulation either by protein overexpression or gene amplification, has little clinical relevance to date, as trials investigating trastuzumab activity merely suggest a benefit in the very small minority of patients whose tumor highly overexpresses HER2, a subpopulation that amounts to 2% to 6% of mostly adenocarcinomas.

Keywords: HER2 mutations; lung cancer; afatinib; dacomitinib; irreversible pan HER-receptor inhibitor

Abstract: HER2 mutations, largely exon 20 in-frame insertions, have been described as an oncogenic driver alteration in 1% to 4% of NSCLC, exclusively in adenocarcinoma histology. The prognostic implication of these alterations is not known. Phase I and II trial data suggest that afatinib, neratinib and dacomitinib have some activity in this molecular subgroup. No comparative data, or any data regarding the activity of pertuzumab or trastuzumab-emtansine is available. HER2 deregulation either by protein overexpression or gene amplification, has little clinical relevance to date, as trials investigating trastuzumab activity merely suggest a benefit in the very small minority of patients whose tumor highly overexpresses HER2, a subpopulation that amounts to 2% to 6% of mostly adenocarcinomas.

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Targeted therapy in NSCLC driven by HER2 insertions

Solange Peters, Stefan Zimmermann

Department of Oncology, University Hospital of Vaudois (CHUV), Lausanne, Switzerland
Both authors have contributed equally to this work.
Correspondence to: Dr. Stefan Zimmermann. HFR Fribourg-Hôpital Cantonal, CH-1708 Fribourg, Switzerland. Email: Stefan.zimmermann@h-fr.ch.

Introduction

Research into the molecular basis of lung cancer has revealed insights into various critical pathways that are deregulated, and among them, key driver genetic alterations that promote cell survival and proliferation. In the oncogene addiction model, cancer cells harbor gene amplification, rearrangement or mutations that dictate their malignant phenotype, and can thus be referred to as driver alterations (1). Among them, human epidermal growth factor 2 (HER2 erbB-2/neu) is a member of the erbB receptor tyrosine kinase family. The ERBB2 gene which encodes for HER2 is a major proliferative driver that activates downstream signaling through PI3K-AKT and MEK-ERK pathways (2). Unlike HER1/epidermal growth factor receptor (EGFR), HER2 has no known ligand, and is activated by homo-dimerization or hetero-dimerization with other members of the erbB family. Under resting conditions, these cell-surface receptors are found as monomers folded in a so-called “closed” inactive conformation that prevents dimerization (3). Upon ligand binding to the extracellular domain, conformational rearrangements lead to an “open” state that exposes the dimerization interface. This extracellular dimeric structure results in the transactivation of the intracellular tyrosine kinase portion of each receptor. Three principal mechanisms of oncogenic activation of HER2 have been described: HER2 gene amplification, gene mutation resulting in molecular alterations of the receptor or HER2 protein overexpression.

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NSCLC, although 3+ expression is found in only 2% to 6% (7-9) HER2 gene amplification, as assessed by fluorescent in situ hybridization (FISH) is uncommon, found in 2% to 4% of predominantly adenocarcinoma-type NSCLCs. Similarly to breast cancer, despite the relative lack of large series, concordance between FISH and IHC 3+ has been evidenced (8).

HER2 amplifications have been described as a potential mechanism of resistance to EGFR tyrosine kinase inhibitor (TKI) therapy in mouse models of EGFR-mutant tumor cells, where FISH analysis revealed that HER2 was amplified in 12% of tumors with acquired resistance versus only 1% of untreated lung adenocarcinomas. Notably, HER2 amplification and EGFR T790M mutation, the most common mechanism of acquired resistance, were mutually exclusive (10). In a large series of 155 patients with acquired resistance to EGFR TKI that underwent rebiopsy, HER2 amplification was seen in 13%, and no ERBB2 mutation was detected (11).

The identification of EGFR mutations, another member of the ERBB-family kinases, in a distinct subset of non-squamous NSCLCs was followed by the identification of HER2 mutations, which mainly consist of in-frame insertions in exon 20, leading to constitutive activation of the receptor and downstream AKT and MEK pathways. HER2 mutations fit the definition of genetic driver, and preclinical models have proved the transforming property of this alteration. Transgenic mice expressing the Her-2 Tyr-Val- Met-Ala mutation develop lung adenocarcinomas. In these models, substantial tumor shrinkage was observed when BIBW2992, a tyrosine kinase inhibitor that inhibits EGFR and Her-2, was combined with temsirolimus, an inhibitor of the downstream effector protein mTOR (12,13). HER2 mutations have been identified in approximately 1% to 4% of NSCLC. In the initial report, mutations in the HER2 kinase domain were identified in 4.2% of 120 primary NSCLC overall and 9.8% in adenocarcinomas (14). A subsequent study of 671 primary resected NSCLC, HER2 mutations were found in 1.6% of samples overall, but in 3.9% of adenocarcinoma samples, and more frequently in Asian ethnicity (15-17). The largest retrospective series published to date, comprising 65 patients with NSCLC and HER2 mutations, provides important insights into the clinic-pathological features and correlates: mutations were found exclusively in patients with adenocarcinoma subtype, and predominantly in female patients and non-smokers, a population similar to the EGFR-mutated NSCLC (18). Nevertheless, mutations were found in some men and heavy smokers, suggesting that HER2 testing could be guided by tumor subtype (adenocarcinoma), but should not be restricted to clinically defined subgroups. All mutations were in-frame insertions of exon 20 within the HER2 gene coding sequence, with duplication of amino-acids YVMA at codon 775. All HER2-mutated tumors were found negative for EGFR-activating mutation in exon 18 to 21, as well as ALK rearrangement and BRAF and PI3KCA mutations. Of interest, a high frequency of patients with disseminated lung nodules and tumor excavation patterns was observed. Of note, using stringent definition of gene amplification (as opposed to gene copy number gain), HER2 mutations were not found associated with concurrent HER2 gene amplification in this series and a previous report (15).

Although oncogenic tyrosine kinase mutations most frequently alter the ATP-binding pocket, as EGFR exon 19 and 21 as well as in HER2 exon 19 or 20 mutations, mutations affecting the extracellular domain have recently been described, resulting in constitutively dimerized and activated HER2 (19). Mutations in the transmembrane domain of HER2 have also been described in familial lung adenocarcinomas (20).

There is scarce data regarding the prognostic impact of HER2 mutations. In a series of 504 Japanese patients with resected NSCLC, 2.6% were found to harbor a HER2 mutation. There was no difference in overall survival of patients with HER2 mutations compared with patients harboring EGFR mutations and patients harboring wild types for both EGFR and HER2 (17).

**HER2 as a target**

In the landscape of lung cancer biomarkers-based precision medicine, HER2 as a target remains poorly described. While in breast cancer HER2 overexpression or gene amplification is widely known to be associated with sensitivity to HER2-targeting drugs like trastuzumab, lapatinib, pertuzumab, and trastuzumab-emtansine, clinical research in lung cancer has been slowed down after the first negative clinical trials of trastuzumab added to chemotherapy in advanced NSCLC. In a phase II trial performed by the Cancer and Leukemia Group B, single-agent trastuzumab did not exhibit significant clinical activity against HER2 2+ or 3+ non-small cell lung carcinoma (21). A randomized phase II trial investigated the addition of trastuzumab to gemcitabine and cisplatin, in 103 previously untreated HER2-positive NSCLC patients. Trastuzumab
was given both concomitantly to chemotherapy and as a maintenance. Although the combination was well tolerated, it failed to show a survival benefit in all HER2 IHC-positive lung cancer overall. However, 80% of patients with IHC 3+ disease on study treatment were still alive after a follow up of 6 months, compared with 64% of the overall population, and a response rate of 83% and median progression free survival (PFS) of 8.5 months was observed in the six trastuzumab-treated patients with HER2 3+ or FISH-positive NSCLC (22). In a phase II trial comprising only 13 patients with HER2-positive tumors (2+ or 3+), the addition of trastuzumab to weekly docetaxel after failure of platinum based-chemotherapy showed limited clinical activity, with a PR rate of 8% (23). The Eastern Cooperative Oncology Group launched a phase II study evaluating the combination of carboplatin, paclitaxel and trastuzumab in patients with HER2-positive (1+ to 3+) NSCLC. Of 139 screened patients, 36% were indeterminate, 5% inconclusive, 27% scored 1+, 22% score 2+, and 13% were 3+. Overall survival was found to be similar to historical data using carboplatin and paclitaxel alone, while patients with 3+ HER2 expression did well in contrast to historical data (24).

These trials are a reminder of the definition of an oncogenic driver alteration, as HER2 overexpression and probably amplification per se are probably only modulators of cancer biology. In addition, as in breast cancer, the need to define specifically for every cancer type a threshold of significance for HER2 overexpression becomes obvious. In particular, the biological role of HER2 expression in the absence of gene amplification remains to be defined, potentially explaining the negative results of clinical trials relying on an inaccurate selection of patients.

HER2 mutations may be much more relevant in lung cancer carcinogenesis than HER2 amplification or overexpression, and several kinase inhibitors are being evaluated for the treatment of HER2-dependent lung adenocarcinoma. Lapatinib, an oral reversible dual TKI of EGFR and HER2, has been tested in a phase II trial that included 75 patients with recurrent or metastatic NSCLC; no responses were seen in the 3 patients with EGFR mutations. No mutations in HER2 were found in this population, leaving the question of lapatinib activity in HER2-mutant tumors unanswered (25). In the European retrospective study (18), 2 patients were treated with lapatinib, all experiencing progressive disease. The most promising data to date have been obtained using irreversible TKIs targeting HER2/3 and EGFR, such as afatinib, neratinib, and dacomitinib. Afatinib is a potent irreversible ErbB receptor family blocker. In an exploratory phase II study, 5 patients with HER2 mutated advanced adenocarcinoma were treated with afatinib, 3 out of which were evaluable for response. Objective response was observed in all three, even after failure of other EGFR- and/or HER2-targeted treatments (26). This series was completed with the treatment of 7 additional HER2 mutated patients, all 5 evaluable with a stable disease (27).

Neratinib, another irreversible pan-ErbB-receptor family blocker, has been evaluated in a phase I trial in combination with temsirolius on the basis of preclinical data suggesting synergy of HER2 inhibition and mTOR inhibition on lung cancer models. Partial response was observed in 2 out of 6 patients with HER2-mutant NSCLC (28). Dacomitinib is an irreversible pan-HER TKI. Tested in a phase II cohort of patients with HER2-mutant or amplified lung cancers, dacomitinib demonstrated an overall 13% response rate in the 26 HER2-mutant patients, and no response in the 4 patients with HER2 amplification or the 2 with HER2 point mutations (29).

Pertuzumab, a first-in-class HER2 dimerization inhibitor, is a humanized monoclonal anti-HER2 antibody that prevents HER2 dimerization and inhibits HER2 signaling. A phase II trial of pertuzumab monotherapy in patients with recurrent NSCLC showed no response in 43 patients, but information on the mutational status of HER2 in these patients is lacking (30).

**Ongoing trials**

Surprisingly, neither pertuzumab nor trastuzumab-emtansine is presently being studied in HER2-mutant lung cancer. A phase II exploratory trial is evaluating neratinib monotherapy and in combination with temsirolius in patients with HER2-mutant NSCLC (NCT1827267). Dacomitinib is being tested in a variety of settings, but its present development remained to date mainly focused on EGFR-mutant NSCLC. Its phase I trials in combination with pemetrexed (NCT01918761), or c-MET inhibitor PF-02341066 (NCT01121575) will not improve our understanding of its activity in HER2-mutant NSCLC. No late-phase trial targeting this particular subgroup of patients in presently ongoing.

**Conclusions**

The identification of oncogenic driver mutations in NSCLC has triggered the development of multiple drugs interfering...
with intracellular signaling pathways. HER2 deregulation by overexpression or amplification has been demonstrated to represent an important therapeutic target in breast and gastric cancer, but has to date little clinical relevance in NSCLC, potentially because due to the lack of definition of HER2 positivity in that particular disease. Phase II trial data merely suggests a benefit of trastuzumab therapy in patients with 3+ HER2-positive NSCLC. On the other hand, HER2 mutations, largely exon 20 in-frame insertions, have been described as an oncogenic driver alteration in 1% to 4% of NSCLC, exclusively in adenocarcinoma histology. The prognostic implication of these alterations is not known. Phase I and II trial data suggest that afatinib, neratinib and dacomitinib have some activity in this molecular subgroup. No comparative data, or any data regarding the activity of pertuzumab or trastuzumab-emtansine is available. In order to improve our understanding of such alterations and aiming at offering new treatment options to our patients, given the high prevalence of lung cancer worldwide and the availability of investigational therapies targeting HER2, routine genotyping of lung adenocarcinoma should include HER2. Patient selection should be based on histology but should not discriminate for other clinic-pathologic features. The few currently ongoing trials are unlikely to foster our understanding of the role of HER2 TKIs in the treatment of this particular subgroup of patients. The sharp contrast between the wealth of investigational activity in other subgroups of NSCLC like ALK-rearranged NSCLC, which shares a similar prevalence, and the dearth of clinical research ongoing in HER2-mutant NSCLC is striking. Further development of afatinib and possibly of dacomitinib in this setting will be pursued. In addition, assessing the activity of pertuzumab in combination with trastuzumab, as well as trastuzumab-emtansine in patients presenting with NSCLC with 3+ HER2-overexpression would be of great interest.

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