Lung Cancer That Harbors a HER2 Mutation: Epidemiologic Characteristic...
activates downstream signaling through PI3K-AKT and MEK-ERK pathways. No ligand has been described for this receptor, which is activated by homodimerization or heterodimerization with other members of the erbB family. HER2 mutations consist of in-frame insertions in exon 20, leading to constitutive activation of the receptor and downstream AKT and MEK pathways. HER2 mutations respond to the genetic driver definition and preclinical models have proved the concept of transforming property of such a genetic alteration. Inducible expression of a HER2 mutant (HER2YVMA) in lung epithelium of mice results in the emergence of invasive adenosquamous carcinomas, with tumor maintenance requiring the continuous expression of the driver, as observed with EGFR-driven cancer.

HER2 protein overexpression and gene amplification are present in 6% to 35% and in 10% to 20%, respectively, of NSCLC. HER2 mutations were identified in approximately 2% to 4% of NSCLC. In the selected population of EGFR/KRAS/ALK-mutation–negative patients, HER2 mutations can reach up to 6%. This mutation is predominantly observed in female patients, nonsmokers, and patients with adenocarcinoma subtype, similar to EGFR–mutated NSCLC.

Among reported lung cancer biomarkers, HER2 as a target remains poorly described. HER2 overexpression or gene amplification is widely known to be associated with sensitivity to HER2-targeting drugs (trastuzumab, lapatinib, pertuzumab, and T-DM1) in breast cancer. Involvement of HER2 in lung carcinogenesis has been known for many years but clinical research was slowed down when the first clinical trials with trastuzumab were negative. Indeed, adding trastuzumab to gemcitabine-cisplatin or to docetaxel failed to show any survival benefits in patients with HER2-immunohistochemistry (IHC)–positive lung cancer. However, HER2 mutations may be more relevant in lung carcinogenesis than HER2 amplification or overexpression. Single case reports suggest that HER2 mutations may be predictive for HER2-targeting therapies in lung cancer. Some ongoing clinical trials are enrolling patients with HER2-mutated, mixed together with HER2-amplified or EGFR-mutated NSCLC patients. Large biomarker screening programs such as the French National Program or the US Lung Cancer Mutation Consortium thus propose testing for HER2 mutations.

In this article, our aim was to improve our understanding of the clinicopathologic characteristics of patients with NSCLC who carry the HER2 mutation, by performing a retrospective study of patients with HER2-positive NSCLC from three European countries, constituting a large group of this rare NSCLC subset. We also analyzed the outcome of patients treated with conventional chemotherapy and/or HER2-targeted drugs.

### Patients and Methods

This study was conducted in France, Switzerland, and Spain and represents a consecutive series of all identified patients carrying a HER2 mutation in exon 20 in the participating centers. Informed consent from patients and institutional review board approval for genetic analysis and data collection were obtained by all participating institutions. Clinical and biologic data were collected from each patient by pathologists and physicians, respectively. The data were made anonymous at the local centers and then were centralized and analyzed in Toulouse, France. Histology was assessed by a specialist lung-cancer pathologist using the WHO criteria, and adenocarcinoma described according to the new International Association for the Study of Lung Cancer classification. Patients who were diagnosed before the new classification were reclassified specifically for the obsolete bronchiolo-alveolar subtype. Histologic review was performed for every patient included in this study to exclude breast cancer. Specific markers such as TTF1, hormonal receptors, cytokeratins, and mammaglobin (if needed) were used to ensure the diagnosis of primary lung tumor. Clinicopathologic stage was assigned according to the seventh tumor-node-metastasis classification. We collected clinical data (age at diagnosis, date of diagnosis, tobacco consumption [never, current, former smoker, and packs per year], and tumor stage), outcome variables (recurrence and survival events), and therapeutics parameters (including chemotherapy and HER2-targeted treatment) for all patients. We ensured that the follow-up

![Fig 1. Diagnostic of HER2 mutation (patient No. 32). (A) Sanger sequencing read with heterozygous HER2 exon 20 insertion (p.G776_777insVC). (B) Human epidermal growth factor receptor 2 (HER2) immunohistochemistry with score 2 (antibody 4B5). (C) Fluorescent in situ hybridization with HER2 amplification (HER2 in red; centromer 17 in green). (D) Tricolor visualization of HER2 protein (in brown), HER2 gene (in black), and centromer 17 (in red).](https://example.com/fig1.png)
was performed by a computed tomography scan of the thorax and abdomen once every 6 to 8 weeks in all participating centers, concomitantly with a clinical follow-up every 2 to 3 weeks. Responses were defined as the best response from the start of treatment until disease progression according to response evaluation criteria in solid tumor (RECIST v1.1) guidelines. If needed, a strict reassessment using these criteria was repeated for every case.

**RESULTS**

### Genetic Characteristics of Lung Cancer With HER2 Mutations

We identified 65 patients carrying a HER2 mutation. HER2 mutation testing was performed in 3,800 patients, leading to an incidence of 1.7%. All tumors displayed an exon-20 mutation within the HER2 gene coding sequence, as analyzed by validated procedures (see Patients and Methods for details and Fig 1 for examples). All mutations were in-frame insertions of exon 20 without progression were censored. Follow-up was updated as of July 2012. We estimated and compared PFS in the subpopulation of stage IV patients, according to the administration of anti-HER2–targeted drugs. Statistical analyses were performed using STATA SE v11.2 (STATA, College Station, TX).

### Table 1. Clinical and Biologic Characteristics of Patients With HER2-Mutated Disease (n = 65)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>%</th>
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<tr>
<td>Age at diagnosis, years</td>
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<td>100</td>
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<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
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<tr>
<td>Median</td>
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<tr>
<td>Women</td>
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<td>69</td>
</tr>
<tr>
<td>Men</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>Tobacco</td>
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<tr>
<td>Never</td>
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<td>52.3</td>
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<tr>
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<td>12.3</td>
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<tr>
<td>Tumor stage</td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>11</td>
<td>16.9</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
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</tr>
<tr>
<td>IV</td>
<td>33</td>
<td>50.8</td>
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<tr>
<td>Unknown</td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td>Metastasis sites for stage IV</td>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td>8</td>
<td>24.2</td>
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<tr>
<td>Brain</td>
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<tr>
<td>Other or unknown</td>
<td>7</td>
<td>21.3</td>
</tr>
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</table>

Abbreviation: SD, standard deviation.

Figure 2. Survival curves of patients carrying HER2 mutations. (A) Overall survival in the whole population (n = 65). (B) Overall survival of stage IV patients (n = 33) versus early-stage patients (stages I to III; n = 32).
All patients were previously tested for *EGFR*, and a vast majority were tested for *KRAS* mutations (93%) and *ALK* rearrangement (91%). If tumor material was still available, *BRAF* and *PI3KCA* mutation tests were also performed. All *HER2*-mutated tumors were found negative for *EGFR*-activating mutation in exon 18 to 21 and *ALK* rearrangement, as well as for *BRAF* and *PI3KCA* mutations. Most of the mutations were exclusive, except for one patient with a tumor carrying a *HER2* mutation plus a classical *KRAS* exon 2 mutation. Only *HER2* mutations were tested in the vast majority of centers. Nevertheless, some platforms added *HER2* FISH testing on request on *HER2*-mutated NSCLC (Fig 1C). We collected 34 tests. Among them, we found eight samples with *HER2* increased gene copy number in the context of polysomy (23%) and only three with *HER2* amplification (9%).

**Clinicopathologic Characteristics of Lung Cancer With *HER2* Mutations**

Clinical features of patients carrying *HER2* mutations were analyzed (Table 1). Patients were diagnosed with *HER2* mutation at a median age of 60.4 years (range, 31 to 86 years; standard deviation, 11.6). Higher proportions of women (45 women vs 20 men; 69%) and of never-smokers (34 never-smokers vs 11 former-smokers and 12 current-smokers; 52.3%) were observed, with a median of 20 pack-years for the smokers. All tumors were adenocarcinomas, including two with a lepidic component. All stages were represented: 11 patients with stage I, three patients with stage II, 15 patients with stage III, and 33 patients with stage IV disease. Sites of metastases were lungs (n = 8), brain (n = 3), and bone (n = 2), and most patients had metastases in several organs concomitantly (n = 13). Of interest, we observed a high frequency of patients with disseminated lung nodules and tumor excavation patterns (Appendix Fig A1, online only). Median overall survival was 40 months for all stages. More specifically, overall survival was 89.6 months and 22.9 months for patients with stages I to III disease and stage IV disease, respectively (P = .01; Fig 2).

**Treatment Response to HER2-Targeted Drugs in Patients With NSCLC Who Carried a HER2 Mutation**

Thirty-three patients with stage IV or recurrent NSCLC received conventional chemotherapy (platinum-based doublet with or without bevacizumab). Of these, 16 patients also received *HER2*-targeted therapies in additional lines of treatment (Table 2). Because some patients received two (n = 3) or four (n = 1) different *HER2*-targeting drugs, a total of 22 individual anti-*HER2* treatments were evaluable. Overall, we observed four patients with progressive disease, seven with disease stabilization, and 11 with partial responses according to RECIST v1.1 (overall response rate, 50%; disease control rate, 82%).

![Fig 3. Progression-free survival of stage IV patients treated with anti–human epidermal growth factor receptor 2 (HER2) targeted drugs (n = 15). Only first-line HER2-targeted treatments were analyzed.](image-url)
Specifically, we observed a disease control rate of 96% for trastuzumab-based therapies \((n = 15)\) and 100% for afatinib \((n = 4)\), but no response to lapatinib \((n = 2)\) or to masatinib \((n = 1)\). It should be noticed that trastuzumab was always used in combination with chemotherapy (vinorelbine, docetaxel, or carboplatin-paclitaxel). In contrast, afatinib and lapatinib were used as monotherapy. We also analyzed PFS from the start of the first HER2-specific treatment until documented disease progression by RECIST v1.1 \((n = 15)\). Median PFS was 5.1 months in patients treated with HER2-targeting drugs (Fig 3). The clinical course of a patient receiving several HER2-targeting drugs is shown in Figure 4, including lapatinib as the first drug, trastuzumab as the second, and afatinib as the third.

**DISCUSSION**

In this article, we report on the largest series to date \((n = 65)\) of patients with NSCLC and HER2 mutations. Despite the limitations of this retrospective study, it provides important insights into HER2-driven NSCLC.

Data about the real incidence of HER2 mutations occurring in patients with lung cancer are heterogeneous, ranging from 1% to 6% in highly selected patients. In this article, we reported an incidence of 1.7%, which is consistent with recent publications.\(^9\),\(^11\)-\(^12\) Nevertheless, we cannot conclude the real incidence of HER2 mutations, because we cannot exclude a selection bias in some participating centers.

First, we confirm the suggested profile of patients presenting with HER2-mutated NSCLC, as suggested in smaller series.\(^11\),\(^21\) Our NSCLC patients with mutated HER2 were mainly female, nonsmokers, and exclusively suffering from adenocarcinoma subtype disease. Nevertheless, we identified some men and heavy smokers (up to 60 packs-year) suggesting that HER2 testing could be guided by tumor subtype (adenocarcinoma), but should not be restricted to clinically defined subgroups. Looking at the natural history of HER2-mutated NSCLC, irrespective of the treatment delivered, which was highly variable in our study, we found that overall survival (89 months for early-stage disease and 23 months for stage IV disease) seemed to be better than reported in large, unselected NSCLC cohorts. In a recent series, Arcila et al\(^11\) reported a median overall survival of 19 months for patients with HER2-mutated NSCLC in advanced stages (stages IIIb

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**Fig 4.** Example of tumor response (patient No. 32). (A) Positron emission tomography–computed tomography scan at initial diagnosis. (B) No response to chemotherapy (platinum, gemcitabine, and bevacizumab, followed by pemetrexed). (C) No response to lapatinib. (D and E) Good partial remission with trastuzumab and vinorelbine. (F) Local progression with trastuzumab maintenance therapy. (G) No response to afatinib. (H) Mixed response to trastuzumab and carboplatin. (I) Disseminated progression, switch to nab (nanoparticle albumin-bound) –paclitaxel and trastuzumab.
or IV), compared with a survival rate of 30 months for patients with EGFR mutation.

In a meta-analysis of 40 published studies, HER2 overexpression assessed by IHC was associated with poor prognosis in NSCLC, specifically in adenocarcinomas, with no prognostic value in squamous cell carcinomas. Other reports confirmed the prognostic impact of HER2 overexpression, which has been found in up to 35% of patients with NSCLC. Conversely, HER2 amplification determined by FISH was not prognostic. In our series, because only a subset of patients received HER2-targeted agents in variable lines of treatment, sometimes in the final course of the disease, survival is reported from the time of diagnosis for the whole HER2-mutated population. Obviously, the retrospective nature of the report precludes a definitive statement on whether HER2 mutations in patients with NSCLC are prognostic or predictive. Based on the encouraging responses and the long median survival of our patients, we can speculate that HER2 mutations are equally predictive and prognostic but this warrants prospective validation.

Most HER2 mutations described to date are insertions within a small stretch of exon 20 with A775_G776insYVMA insertion/duplication on the COOH-terminal side of the α-helix. In our series, although many centers sequenced exons 18 to 20 of the HER2 gene, all patients presented with an exon 20 insertion and no mutation in exons 18 and 19 were found. HER2 amplification (or polysomy) by FISH was not tested routinely. Nevertheless, we asked some platforms to perform additional FISH on available tissues. As already published, we found only a minority of patients with mutated gene with HER2 real amplification, suggesting that the two molecular alterations are not associated. In addition, until this point, data are lacking to address the possible interest of FISH testing in a general NSCLC population.

We aimed to analyze the potential interest of HER2-targeted drugs. To our knowledge, our report is the largest series to date reporting on HER2-mutated NSCLC treated with HER2-targeted drugs. In our study, 17 patients with advanced NSCLC did not receive any HER2-targeting drugs, owing to the absence of standard at the time of diagnosis, the lack of dedicated clinical trials, and the difficulties to access some unregistered drugs. Some patients were therefore treated following conventional guidelines without taking into account their HER2 mutation status. Available data from the literature concerning HER2-targeted agents in NSCLC are still scarce and are somewhat anecdotal. The addition of trastuzumab to chemotherapy has clearly improved survival in breast cancer patients with HER2 protein expression or gene amplification. Trastuzumab in combination with cisplatin and gemcitabine in advanced NSCLC patients failed to show a benefit, although a trend toward better outcome with trastuzumab was observed in patients with strongly positive (3+) HER2-IHC or positive HER2-FISH. In HER2-amplified NSCLC, there seems to be no clear benefit from lapatinib. A single-arm trial with afatinib used as a monotherapy showed a response in three of three evaluable patients with HER2-mutated adenocarcinoma, even in the context of resistance to other EGFR- or HER2-targeted compounds. In addition, there are single reports of patients with HER2-mutated NSCLC who responded to trastuzumab in combination with paclitaxel or vinorelbine. Trastuzumab is currently being tested as a single-agent in patients with HER2-IHC–positive, HER2-mutated, or HER2-amplified NSCLC (trials NCT00004883 and NCT00758134), as well as in combination with carboplatin and paclitaxel. Pertuzumab is currently being tested in a phase II trial in patients with advanced, pretreated NSCLC (trial NCT00063154). Our study indicates that anti-HER2 therapies are associated with encouraging response rates (50%), disease control rates (80%), and PFS (5.1 months) in patients with heavily pretreated HER2-mutated NSCLC. Three different HER2-targeting drugs were used in our study. Trastuzumab and afatinib seemed to be associated with satisfactory disease control, whereas lapatinib was not, which is consistent with a prior case report. In our study, trastuzumab was mostly used in combination with chemotherapy as the first anti-HER2 therapy, whereas lapatinib and afatinib were mostly used at later stages, except in one patient. The relative efficacy of these molecules clearly deserves prospective evaluation in larger international clinical trials.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Although all authors completed the disclosure declaration, the following author(s) and/or an author’s immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a “U” are those for which no compensation was received; those relationships marked with a “C” were compensated. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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**Manuscript writing:** All authors

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Appendix

Fig A1. Tumor presentation with a high frequency of disseminated nodules and excavation (computed tomography scan for three patients).