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HER2 Mutations in Lung Adenocarcinomas: A Report from the Lung Cancer Mutation Consortium

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Abstract

Background—Human epidermal growth factor receptor 2 (HER2) mutations have been reported in lung adenocarcinomas. We describe the prevalence, clinical features and outcomes associated with HER2 mutations in 1007 patients in the Lung Cancer Mutation Consortium (LCMC).

Methods—Patients with advanced stage lung adenocarcinomas were enrolled to LCMC. Tumor specimens were assessed for diagnosis and adequacy; multiplexed genotyping was performed in CLIA certified laboratories to examine ten oncogenic drivers. The LCMC database was queried for patients with HER2 mutations to access demographic data, treatment history, and vital status. We conducted an exploratory analysis to evaluate survival of HER2 mutated patients treated with HER2 directed therapies.

Results—920 patients were tested for HER2 mutations; 24 patients (3%) harbored exon 20 insertion mutations (95% CI 2 to 4%). One patient had a concurrent MET amplification. The median age was 62 years, with a slight predominance of females (n=14) over males (n=10). The majority of patients were never-smokers (71%) and presented with advanced disease at diagnosis. The median survival for patients that received HER2 targeted therapies (n=12) was 2.1 years compared to 1.4 years in those who did not (n=12) (p=0.48). Patients with HER2 mutations had inferior survival than the rest of the LCMC cohort with other mutations: the median survival was

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3.5 years in LCMC population receiving targeted therapy and 2.4 years for patients not receiving targeted therapy.

**Conclusions**—HER2 mutations were detected in 3% of patients with lung adenocarcinoma in the LCMC. HER2 directed therapies should be investigated in this subgroup of patients.

**Keywords**

HER2; lung adenocarcinoma; LCMC; multiplexed sequencing; targeted therapy

**Introduction**

HER2 (HER2/neu or ERBB2) is a member of the human epidermal growth factor receptor (HER) family of receptor tyrosine kinases that includes HER1 (EGFR or ERBB1), HER3, and HER4. These receptors consist of a ligand-binding extracellular domain and an intracellular tyrosine kinase domain. The HER2 receptor does not have a known endogenous ligand for its extracellular domain. HER2 heterodimerizes with other HER family receptors and causes activation of downstream signaling through PI3K/AKT and RAS/MAP/MEK pathways. HER2 is a preferred dimerization partner of other HER receptors, and HER2 containing heterodimers can exert a potent oncogenic signal.

HER2 overexpression results in oncogenic transformation of cells, as demonstrated by cell culture and transgenic mouse models. The HER2 gene is amplified in 15–20% of patients with breast cancers and is associated with aggressive disease behavior. The presence of HER2 amplification correlates with shorter time to relapse (p<0.0001) and survival (p=0.0011) in node positive breast cancer. In node negative disease, HER2 protein overexpression also portends worse outcomes as an independent prognostic factor for decreased 10 year relapse free survival (odds ratio (OR) 1.71, p=0.01) and breast cancer specific survival (OR 2.03, p=0.003). The development of monoclonal antibodies that target HER2 by binding to the extracellular domain, such as trastuzumab and pertuzumab have prolonged progression-free survival and survival in patients with HER2 positive breast cancer, particularly when combined with chemotherapy. HER2 amplification also occurs in 20% of gastric and gastroesophageal junction cancers. The addition of trastuzumab to chemotherapy in patients with HER2 overexpressing gastric or gastroesophageal junction tumors decreases the risk of death (hazard ratio (HR) 0.74). As in breast cancer, the presence of HER2 gene amplification predicts benefit from the use of trastuzumab.

HER2 has been studied in lung cancers for its potential role as a target for therapy. HER2 protein overexpression is observed in 6–30% of patients with lung cancers and gene amplification is present in 2–20%. Although these rates are similar to that in breast and gastric cancers, clinical trials combining trastuzumab with chemotherapy, even selected by HER2 protein overexpression, have yielded disappointing results, although there is a hint that patients with tumors with HER2 amplification may derive benefit. The Cancer Genome Project published the first report of somatic mutations of HER2 in lung cancers in 2004. Sequencing of HER2 in 120 lung tumors revealed somatic mutations in the kinase domain of the protein, in a similar position to that observed with the exon 20 insertion mutation in EGFR. The prevalence of HER2 mutation in this series was 4% in...
lung cancers, with all mutations occurring in adenocarcinomas. Unlike patients with EGFR mutations, four of five patients with HER2 mutations were current or former smokers. The mutated tumors did not have HER2 protein overexpression by immunohistochemistry (IHC).

Further studies of HER2 mutations in lung cancers demonstrate its role as an oncogene. HER2 mutations in exon 20 cause constitutive activation and downstream signaling and can induce development of lung tumors in mouse models. Cell line and mouse models of lung cancers with activating HER2 mutations can be successfully treated with dual EGFR/HER2 inhibitors such as afatinib and or pan-HER inhibitors such as dacomitinib and neratinib. Other reports have also described the clinicopathologic features of HER2 mutated lung cancers. These six studies are summarized in table 1. HER2 mutations are found exclusively in adenocarcinoma patients and the incidence of mutation is 0.84–2.6% in these cohorts. The majority of these mutations occur in exon 20, most commonly as a duplication or insertion coding for the amino acids YVMA at codon 776. Females and never smokers tend to harbor more HER2 mutations.

The Lung Cancer Mutation Consortium (LCMC) was developed as a collaboration between 14 academic centers in the United States to prospectively test specimens from patients with lung adenocarcinomas for ten oncogenic drivers. The ultimate goal, besides the feasibility of multiplexed testing, was to use these data in real-time to guide therapeutic decisions using targeted therapies directed against these mutations. Over one thousand patients were prospectively tested in this effort. We describe the analysis of HER2 mutations in the Lung Cancer Mutation Consortium. This cohort is unique among prior reports on HER2 mutations in lung adenocarcinomas since it is limited to advanced stage patients and includes treatment and survival outcomes.

Methods

The LCMC enrolled patients from 14 sites with metastatic or recurrent lung adenocarcinomas and a performance status of 0–2 that signed informed consent from 2009 to 2012. One specimen from each eligible patient was prospectively tested for specified mutations; pathologists at each site reviewed the diagnosis and adequacy of each tissue specimen. In addition, designated pathologists performed central confirmation of adenocarcinoma diagnosis. Demographic data including age, sex, smoking history, sites of metastatic disease, and treatment history were collected. Each site performed multiplex genotyping for ten predefined mutations using any of the following methods: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, Arizona Research Laboratories), multiplexed single nucleotide extension sequencing (SNAPshot, Applied Biosystems), or Sanger sequencing. Every site also performed sizing electrophoresis to find HER2 insertions and EGFR deletions. The mutation data was entered into the GeneInsight Database. Treatment data and vital status was collected annually and recorded in the database until 2012.
In the patients identified to have \textit{HER2} mutations, descriptive statistics were used to study demographic and clinical variables. Overall survival was defined as the time from the date of metastatic cancer diagnosis to the time of death or last follow-up. Survival analysis was performed by Kaplan Meier method and the cohorts were compared using log rank tests at a significance level of 0.05. All analyses were conducted using IBM SPSS Statistics (version 22.0).

\textbf{Results}

Out of 1007 patients with adequate tumor tissue for testing of at least one gene, 733 had complete genotyping with all 10 genes. Sixty-four percent (466) of the 733 completely tested patients harbored at least one oncogenic driver. A total of 920 patients were tested for \textit{HER2} mutations. The prevalence of \textit{HER2} mutations was 2.6\% (24 patients) [95\% CI 2 to 4\%]. All patients had an exon 20 insertions at codon 775. All but one \textit{HER2} mutation was mutually exclusive of other drivers (1 patient had concurrent \textit{MET} amplification). The baseline demographics are shown in table 2. The median age of the patients with \textit{HER2} mutations was 62 (range 37–73). There was a predominance of female patients (58\%). Almost all the patients were never (71\%) or former (25\%) smokers. Since the LCMC effort focused on patients with metastatic disease, over 80\% of the \textit{HER2} mutated patients presented with advanced or metastatic disease (stages IIIB–IV). The demographics of the wild-type cohort were similar except that the majority of the non-mutated patients were former smokers (60\%).

Patients with activating \textit{HER2} mutations had many different sites of metastatic disease (table 3). The most frequent site of disease involvement was in the lungs, followed by bone, brain and adrenal metastases. A similar pattern was observed in the wild-type patients, although there was a higher prevalence of brain metastases in this population (19\% versus 12\% in the \textit{HER2} mutated patients).

The choice of systemic therapy was varied in the 24 patients harboring \textit{HER2} mutations. Of these patients, 12 (50\%) received a targeted therapy directed against \textit{HER2} (Table 4). Many of these patients were enrolled in clinical trials with tyrosine kinase inhibitors, including dacomitinib\textsuperscript{25}. The patient with the concurrent \textit{MET} amplification received dacomitinib with crizotinib. Only three patients received trastuzumab, as monotherapy or in combination with laptatinib and bevacizumab. The patients treated with systemic therapy alone received platinum doublets, bevacizumab, pemetrexed, and vinorelbine. A small number of patients received radiation to thorax (n=4), brain (n=2), or bone (n=1).

The median overall survival of the patients that received \textit{HER2} directed therapy was 2.09 years compared to 1.37 years without targeted therapy (p=0.48). In comparison, the survival of the \textit{HER2} wild-type patients was 2.62 years. At the last assessment of vital status, 50\% of the patients treated with targeted therapy were alive compared to only 33\% who received systemic chemotherapy alone. Data were not collected on response rates associated with treatment.
Discussion

Lung adenocarcinomas are a heterogeneous group of illnesses and are characterized by a variety of molecular abnormalities. Besides EGFR and KRAS mutations, each of the other driver events is observed in less than 5% of patients. ALK, RET, and ROS1 fusion abnormalities are noted in approximately 5%, 1%, and 1% of patients respectively\(^35\). These patients are candidates for treatment with crizotinib and other ALK inhibitors. Recently, an exon splicing mutation in the MET gene has been found in nearly 4% of patients, causing prolonged stability of the MET protein due to loss of the ubiquitin binding domain needed for protein degradation\(^36\). This group appears to benefit from MET inhibitors, as seen in 3 patients who responded to MET inhibition with crizotinib or capmatinib\(^36\); in another recent report, 3 out of 8 patients responded to crizotinib or cabozaatinib\(^37\). In larger cohorts of NSCLC patients, MET exon 14 skip mutations have been reported in 3% of patients and are being investigated as a new target for MET inhibitors\(^38\),\(^39\). Consequently, the rationale to screen for the less common molecular events beyond EGFR, KRAS and ALK is growing as additional oncogenic drivers are identified that can be effectively treated with agents targeted against these drivers.

HER2 mutations in lung cancers occur in a subset of patients with adenocarcinomas and result in a unique clinical phenotype compared to other oncogenic drivers in lung cancers. Our analysis of HER2 mutations in the LCMC adds to the current understanding of the impact of HER2 mutations in lung cancers.

The incidence of HER2 mutation found in LCMC (2.6%) is similar to previous reports in the literature. Also this population exclusively had the YVMA amino acid repeat at codon 775. Although HER2 mutations tend to be mutually exclusive of other oncogenic drivers, we found one patient with concurrent MET amplification. This analysis includes a more robust genetic evaluation of the tumors than any previous report\(^30\),\(^31\). The Cancer Genome Atlas (TCGA) study of lung adenocarcinomas (n=230) included 5 patients with HER2 mutations; none had coincident amplification of MET\(^40\). Unfortunately, data on HER2 protein expression by IHC and gene amplification by copy number by next generation sequencing (NGS) or fluorescence in-situ hybridization (FISH) was not obtained in the LCMC platform.

Unlike, EGFR mutations, the prevalence of HER2 mutations appear to be similar between Asian and Caucasian populations. In common with patients with EGFR mutant lung cancers, those harboring HER2 mutations tend to be light or never smokers and are more likely to be women. Patients with tumors with HER2 mutations are younger, with a median age around 60.

Our analysis of HER2 mutations in the LCMC shows shorter survival outcomes in these patients compared to individuals whose tumor harbor EGFR mutations and ALK fusions. The median overall survival of the HER2 mutated patients who received HER2 directed therapies was no different from those who did not (2.09 years versus 1.37 years, p=0.48) and was similar to the HER2 wild-type patients (2.62 years). In the overall LCMC population, patients that received targeted therapy for a driver mutation achieved a median survival of 3.5 years while those not treated with genotype-directed therapy lived an average of 2.4
years for the overall LCMC patients. The survival of the HER2 patients here is worse but not very different from the median survival reported by Arcila et al (19 months) and Mazieres et al (23 months) in metastatic patients. The outcomes of the HER2 patients in LCMC were also similar to the recent report of the EUHER2 cohort, which had a median survival of 24 months. The median overall survival of HER2 patients in the French study reported by Barlesi et al was only 11.5 months; however, this study included patients with performance status of 2 or higher, which would explain the inferior survival outcomes. This suggests that exon 20 mutations in HER2 lead to an aggressive phenotype and may be a marker of poor prognosis. HER2 amplification in breast cancer and gastric cancer similarly has been associated with shorter survival.

There is a great need to improve outcomes for HER2 mutant lung cancers. There have been case reports on the benefits of trastuzumab with taxanes, pan HER inhibitors such as dacomitinib, and a combination of lapatinib and bevacizumab in patients with HER2 mutations. In the analysis of the European HER2 mutated cohort, some patients were successfully treated with trastuzumab plus chemotherapy combinations and afatinib. More recently a phase II study of dacomitinib including 26 patients with HER2 mutations achieved a response rate of 12% (95% CI 2–30%). In a phase I study of neratinib, an irreversible pan-HER inhibitor, in combination with temsirolimus, two patients with HER2 mutant lung cancers responded. This formed the basis for a phase II randomized study of neratinib with or with temsirolimus; in 27 patients treated, the overall response rate was 21% with combination therapy while no responses were seen with neratinib alone. There are several ongoing clinical trials investigating HER2 targeted therapies in HER2 mutated and amplified NSCLC. These include trastuzumab emtansine (NCT02675829 and NCT02314448), afatinib (NCT02183883, NCT02795156, and NCT02597946), and lapatinib (NCT01306045). Newer agents targeting the HER2 pathway, including pyrotinib, an oral tyrosine kinase dual inhibitor of HER2 and HER2 (NCT02834936), poziotinib a pan HER inhibitor (NCT02979821), and bispecific monoclonal antibodies against HER2 such as MCLA-128 (NCT02912949) and ZW25 (NCT02892123) are in early phases of clinical development. Strategies utilizing the immune system, such as chimeric antigen receptor (CAR) T cells (NCT02713984) and vaccines targeting HER2 (NCT02713984) are also being investigated. Our study is the first to our knowledge that compares overall survival outcomes for patients treated with HER2 directed therapy compared to those who did not receive targeted therapy. While there was no significant difference survival between HER2 mutated patients who received targeted therapy and those who did not, this was a small sample size and included different HER2 therapies. We feel further development of therapies that more effectively target HER2 mutations still has the potential to improve outcomes for this poor prognosis group of patients.

In summary, HER2 mutations are present in a subset of lung adenocarcinomas. Patients with activating exon 20 HER2 mutations have worse survival outcomes compared to other patients with lung adenocarcinomas with oncogenic drivers. Further research into new targeted therapies targeting HER2 in lung cancers has the potential to improve outcomes in this molecular subset of patients.
Acknowledgments

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References


Figure 1. Overall Survival of HER2 Mutated Patients Stratified by Receipt of HER2 Directed Therapy

Patients with HER2 mutations who received HER2 targeted therapy (yellow) had improved median OS of 2.09 years compared to 1.37 years in HER2 mutated patients (blue) who did not receive targeted therapy p=0.48. The wild-type patients (green) had median OS of 2.62 years. Vertical tick marks represent censoring events.
Table 1

Published studies analyzing HER2 mutations in NSCLC patients.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients</th>
<th>Origin of Patients</th>
<th>Stage of Disease</th>
<th>Rate of HER2 mutation</th>
<th>Female vs. Male Rate of HER2 Mutation</th>
<th>Never Smoker vs. Smoker Rate of HER2 mutation</th>
<th>Median Age (years)</th>
<th>Concurrent Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buttitta F, et al.</td>
<td>403</td>
<td>Italy</td>
<td>I–III</td>
<td>2.2%</td>
<td>4.1%/1.8%</td>
<td>3.1%/1.9%</td>
<td>60.8</td>
<td>None (EGFR or KRAS)</td>
</tr>
<tr>
<td>Shigematsu H, et al.</td>
<td>671</td>
<td>Japan, Taiwan, USA, Australia</td>
<td>Surgically resectable</td>
<td>1.6%</td>
<td>2.7%/1%</td>
<td>3.2%/0.7%</td>
<td>NR</td>
<td>None (EGFR or KRAS)</td>
</tr>
<tr>
<td>Tomizawa K, et al.</td>
<td>504</td>
<td>Japan</td>
<td>I–IV</td>
<td>2.6%</td>
<td>2.0%/0.59%</td>
<td>2.2%/0.39%</td>
<td>60</td>
<td>NR</td>
</tr>
<tr>
<td>Arcila ME, et al.</td>
<td>560</td>
<td>USA</td>
<td>I–IV</td>
<td>2.0%</td>
<td>3.0%/1.4%</td>
<td>3.0%/1.4%</td>
<td>64</td>
<td>None (EGFR, KRAS, BRAF, NRAS, PIK3CA, MEKI, AKT, ALK)</td>
</tr>
<tr>
<td>Mazieres J, et al.</td>
<td>3800</td>
<td>France, Switzerland, Spain</td>
<td>I–IV</td>
<td>1.7%</td>
<td>1.2%/0.52%</td>
<td>0.89%/0.61%</td>
<td>60.4</td>
<td>1 with KRAS mutation</td>
</tr>
<tr>
<td>Barlesi F, et al.</td>
<td>11723</td>
<td>France</td>
<td>I–IV</td>
<td>0.84%</td>
<td>0.49%/0.34%</td>
<td>0.36%/0.20%</td>
<td>66.2</td>
<td>1 with BRAF, 1 with ALK</td>
</tr>
</tbody>
</table>
Table 2
Baseline characteristics of HER2 mutated patients compared to HER2 wild-type patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HER2 Cohort (n=24)</th>
<th>Non-HER2 Cohort (n=896)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (percent)</td>
<td>Number of patients (percent)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14 (58.3%)</td>
<td>532 (59.4%)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (41.7%)</td>
<td>364 (40.6%)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>62 (37–73)</td>
<td>61 (18–88)</td>
</tr>
<tr>
<td>Stage at Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–IIIA</td>
<td>4 (16.7%)</td>
<td>230 (25.7%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>3 (12.5%)</td>
<td>66 (7.4%)</td>
</tr>
<tr>
<td>IV</td>
<td>17 (70.8%)</td>
<td>578 (64.5%)</td>
</tr>
<tr>
<td>Unknown stage</td>
<td></td>
<td>21 (2.3%)</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>17 (70.8%)</td>
<td>288 (32.1%)</td>
</tr>
<tr>
<td>Former</td>
<td>6 (25%)</td>
<td>539 (60.2%)</td>
</tr>
<tr>
<td>Current</td>
<td>1 (4.2%)</td>
<td>65 (7.3%)</td>
</tr>
<tr>
<td>Smoking status unknown</td>
<td></td>
<td>4 (0.4%)</td>
</tr>
</tbody>
</table>
Table 3

Location of metastatic disease in *HER2* mutated patients compared to *HER2* wild-type patients

<table>
<thead>
<tr>
<th>Metastatic Site</th>
<th>HER2 Cohort (n=24)</th>
<th>Non-HER2 Cohort (n=896)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (Percentage)</td>
<td>Number of patients (Percentage)</td>
</tr>
<tr>
<td>Lung</td>
<td>12 (50%)</td>
<td>430 (48%)</td>
</tr>
<tr>
<td>Bone</td>
<td>8 (33.3%)</td>
<td>199 (22.2%)</td>
</tr>
<tr>
<td>Brain</td>
<td>3 (12.5%)</td>
<td>174 (19.4%)</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3 (12.5%)</td>
<td>53 (6%)</td>
</tr>
<tr>
<td>Liver</td>
<td>3 (12.5%)</td>
<td>80 (9%)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (20.8%)</td>
<td>204 (22.7%)</td>
</tr>
</tbody>
</table>
Table 4

Administration of HER2 Targeted Therapies in *HER2* Mutated Patients

<table>
<thead>
<tr>
<th>Targeted Therapy</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacomitinib</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>Neratinib/temsirolimus</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>Dacomitinib/crizotinib</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>Lapatinib/trastuzumab/bevacizumab</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>STA-9090</td>
<td>1 (8.3%)</td>
</tr>
</tbody>
</table>