Lung Cancer 88 (2015) 34–41

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

HER2 expression and markers of phosphoinositide-3-kinase pathway activation define a favorable subgroup of metastatic pulmonary adenocarcinomas

H. Reis[a], T. Herold[a,b,c], S. Ting[a], K. Worm[a], U. Huber[d], D.C. Christoph[d], W.E. Eberhardt[d,e], K. Kostbade[d], S. Kasper[d], G. Stamatis[f], S. Welter[f], K. Darwiche[g], R. Karpf-Wissel[g], D. Theegarten[a], K.W. Schmid[a], M. Schuler[c,d,e,*], M. Wiesweg[d]

[a] Institute of Pathology, West German Cancer Center, University Hospital Essen, University Duisburg-Essen, 45122 Essen, Germany
[b] German Cancer Research Center, 69120 Heidelberg, Germany
[c] German Cancer Consortium (DKTK), 69120 Heidelberg, Germany
[d] Department of Medical Oncology, West German Cancer Center, University Hospital Essen, University Duisburg-Essen, 45122 Essen, Germany
[e] Division of Thoracic Oncology, Ruhrlandklinik, West German Lung Center, University Hospital Essen, 45239 Essen, Germany
[f] Division of Thoracic Surgery, Ruhrlandklinik, West German Lung Center, University Hospital Essen, 45239 Essen, Germany
[g] Division of Interventional Pneumology, Ruhrlandklinik, West German Lung Center, University Hospital Essen, 45239 Essen, Germany

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form 4 January 2015
Accepted 1 February 2015

Keywords:
HER2
PI3K
NSCLC
Lung adenocarcinoma
Biomarker
Prospective study

ABSTRACT

Objectives: Pulmonary adenocarcinomas (ADC) can be sub-grouped based on dominant oncogenic drivers. *EGFR* mutations define an entity of metastatic ADC with favorable prognosis and high susceptibility to EGFR tyrosine kinase inhibition. In contrast, the clinical impact of additional ERBB family members in ADC is less defined. To this end we prospectively studied HER2 expression, gene amplification, and mutation in relation to outcome of patients with advanced or metastatic ADC.
Materials and methods: Diagnostic tumor biopsies from 193 sequential patients with stage III/IV ADC were prospectively studied for HER2 expression by immunohistochemistry (IHC). Cases with IHC scores 2+ or 3+ were analyzed by *HER2* chromogenic *in situ* hybridization (CISH), and sequencing of *HER2* exons 20 and 23. Additional prospectively determined biomarkers included PTEN, cMET, pAKT, and pERK expression, *KRAS*, *EGFR*, *BRAF* and *PIK3CA* mutations, and *ALK* fluorescence ISH (FISH).
Results and conclusion: HER2–IHC was feasible in 176 (91.2%) cases. Of 53 (30%) cases with IHC scores 2+/3+, 45 (85%) could be studied by CISH and 34 (64%) by sequencing. The lower number of *HER2*-mutational analyses resulted from exhaustion of tumor tissue and DNA following mutational analysis of *KRAS*, *EGFR*, *BRAF* and *PIK3CA*. *HER2* amplification was detected in 4 cases (2.3%), while no mutation was found. HER2 expression correlated with expression of pAKT and cMET. Expression of HER2 and pAKT was associated with favorable overall survival in stage IV disease. HER2-expressing ADC more frequently harbored *KRAS* mutations, while HER2 expression was absent in all 4 cases with *BRAF* mutation.

HER2–IHC was not predictive of *HER2* gene amplification or mutation, which both were rare events in prospectively studied patients with advanced or metastatic ADC. Expression of HER2 and pAKT define a population of patients with stage IV ADC with a distinct disease course, who could benefit from specifically tailored pharmacotherapies.

Abbreviations: ADCa, denocarcinoma of the lung; ALKa, naplastic lymphoma kinase; (p)AKT, (phospho-)protein kinase B; bpb, ase pair; BRAF, V-Raf murine sarcoma viral oncogene homolog B; (C/F/D)ISH, (chromogenic/fluorescence/dual color) *in-situ* hybridization; cMETh, epatocyte growth factor receptor; EGFRe, pidermal growth factor receptor; (p)ERK, (phospho-)extracellular-signal-regulated kinase; FDA, Food and Drug Administration; FFPEf, ormalin fixed, paraffin embedded; HER2h, uman epidermal growth factor receptor 2; HER4r, eceptor tyrosine–protein kinase erbB-4; HRh, azard ratio; IHCi, mmunohistochemistry; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAPKm, itogen-activated protein kinase; mTORm, echanistic target of rapamycin; NGSn, ext generation sequencing; NSCLCn, on-small cell lung cancer; OSo, verall survival; PCRp, olymerase chain reaction; PFSp, rogression-free survival; PI3K (CA)p, hosphoinositide-3 kinase (catalytic subunit alpha); PTENp, hosphatase and tensin homolog; RECISTr, esponse evaluation criteria in solid tumors; TKIt, yrosine kinase inhibitor; TTFt, ime to treatment failure after first treatment line; UICC, Union internationale contre le cancer; WHO, World Health Organization.

* Corresponding author at: Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Hufelandstrasse 55, 45147 Essen, Germany. Tel.: +49 201 723 2000; fax: +49 201 723 5924.
E-mail address: martin.schuler@uk-essen.de (M. Schuler).

http://dx.doi.org/10.1016/j.lungcan.2015.02.002
0169-5002/

1. Introduction

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer related mortality worldwide [1]. The discovery of molecular pathways driving lung cancer has led to new therapeutic options. This particularly applies to patients with pulmonary adenocarcinomas (ADC) harboring somatic epidermal growth factor receptor (*EGFR*) mutations [2]. This patient subgroup shows exquisite sensitivity to small-molecule tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib and afatinib [2]. Screening for *EGFR* mutations has therefore become standard-of-care in clinical management of advanced ADC [3].

Next to EGFR, additional members of the ERBB family such as the human epidermal growth factor receptor 2 (HER2) and the receptor tyrosine–protein kinase erbB-4 (HER4) are frequently expressed in ADC. HER2 is a membrane-bound receptor tyrosine kinase with structural homology to EGFR. HER2 is considered an orphan receptor as no specific ligand has been identified [2]. By forming homo- and heterodimers with other ERBB family members HER2 primarily signals via the phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT) pathway and the mitogen-activated protein kinase (MAPK) pathway [4].

In breast and gastric cancer, HER2 overexpression as detected by immunohistochemistry (IHC) or *HER2* gene amplification detected by *in-situ* hybridization (ISH) techniques define sub-entities with distinct biologies. With the availability of HER2-targeting treatments patients with HER2-positive cancers now-a-days have a superior prognosis [5]. This suggests a HER2-phenotype existing across different cancer entities. Driven by this concept, trastuzumab was clinically studied in NSCLC, albeit with negative outcomes. However, signals were obtained that patients with HER2 overexpression might benefit from HER2-directed therapy [6,7]. In contrast to breast and gastric cancer, a universal definition of criteria for HER2 positivity is still missing in NSCLC [8,9]. For example, HER2 protein expression has been reported in 11% to >50% of ADC cases with cutoff values between 10 and 65% [10]. HER2 positivity rates in NSCLC ranged from 2% to 43% if breast cancer criteria (so called 'Dako-score' 2+ or 3+) were applied [10]. However, "true positivity" as defined by strong (3+) protein expression and/or gene amplification seems less prevalent [10–12]. Most of these findings were obtained from retrospective analyses of surgical cases, which do not reflect the patient population with advanced or metastatic NSCLC that is usually targeted by innovative pharmacotherapies.

More recently, *HER2* mutations were nominated to define a sub-entity of ADC with oncogenic dependency [13,14]. These *HER2* mutations usually comprise small insertions in exon 20 of the *HER2* gene. The majority represents a 12 base pair in-frame insertion leading to amino acid duplication and constitutive activation of the HER2 receptor [2]. The reported prevalence of such *HER2* mutations ranges from 1% to 6% in highly selected NSCLC cohorts [12,14–17]. *HER2* mutations were not correlated with different survival outcomes [2,10,14].

Against this background we have prospectively studied biomarker profiles and clinical outcome of patients with advanced or metastatic pulmonary adenocarcinomas presenting over a period of 18 months at the West German Cancer Center of the University Hospital Essen.

2. Materials and methods

2.1. Patient cohort

Diagnostic tumor samples from 193 successive patients with ADC presenting to the West German Cancer Center at University Hospital Essen, Essen, Germany, from January 1, 2012 to July 1, 2013 were prospectively subjected to extended biomarker analyses. All biomarkers reported in this study were determined following a pre-specified, multiplexed algorithm, with the exception of *HER2* mutational analysis. The latter was retrospectively added at a later point of time upon availability of a HER2-directed clinical trial [18]. All patients had histological confirmation of ADC according to the current WHO criteria [19]. Only patients with stage III or IV disease according to the 7th edition of the UICC staging system were included [20]. Patient characteristics, overall survival (OS), and time to treatment failure after first line of treatment (TTF) were collected from the electronic patient records of the Department of Medical Oncology, where all included patients had been treated and followed.

All biomarker assays were conducted at the Institute of Pathology, University Hospital Essen, using formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from diagnostic biopsies. In patients that had been primarily diagnosed at a community hospital or practice, FFPE tissue blocks were obtained from external pathology laboratories if the patient consented. Histopathological classification was confirmed in all referral cases.

2.2. Immunohistochemistry (IHC)

For IHC analysis, 1–3 μm thick whole-mount sections were cut from FFPE tissue blocks. From January 1, 2012 to November 30, 2012, HER2 IHC was performed using the HercepTest™ test kit for the Dako Autostainer (K5207; Dako, Glostrup, Denmark) following the manufacturer's instructions. Since December 1, 2012 the Institute of Pathology changed to the PATHWAY anti-HER-2/neu (4B5) antibody in combination with a Benchmark Ultra System (both Ventana Medical Systems, Tucson, AZ, USA), which were applied per manufacturer's instructions. This change of platform derived from an institutional decision to establish the complete diagnostic immunohistochemical workup on a single platform. Both HER2-test systems applied during the period of this prospective study hold FDA-approval for breast cancer and exhibit excellent rates of concordance in breast cancer [21].

To achieve the highest possible rate of comparability with published data and applicability to ongoing clinical trials, we applied the so called 'Dako-score' for HER2–IHC evaluation and the H-score for cMET–IHC analysis. A recent retrospective study by Yoshizawa and colleagues on resected early stage ADC applied the same approach to HER2–IHC analysis [12]. For the remaining primary antibodies no 'standard' evaluation method is established. Accordingly, we selected an evaluation based on a modified immunoreactive score. In addition to the itemized analysis, all IHC results (including HER2) were also dichotomized into IHC-negative (no immunoreactivity) and IHC-positive (any immunoreactivity) groups. Additional information is available in the Supplemental Methods.

2.3. Chromogenic and fluorescence in situ hybridization (CISH/FISH)

HER2–CISH analyses were conducted in case of equivocal (2+) or positive (3+) HER2 IHC results. In each case, 4 μm thick tissue slides were cut and de-paraffinized according to institutional standards. *HER2*–CISH slides were created using a commercially available kit (ZytoDot® 2C SPEC HER2/CEN17 Probe kit, Zytomed Systems, Berlin, Germany) following the manufacturer's protocols. A *HER2/CEN17* ratio cutoff level of ≥2.0 was defined positive, considering criteria for trastuzumab admission [22]. Polysomy was defined as a mean number > 2 of HER2 and CEN17 in the evaluated tumor nuclei [23]. For detection of ALK-rearrangements the ZytoLight® SPEC ALK/EML4 TriChekTM Probe system (Zytomed Systems, Berlin, Germany) was used according to manufacturer's