Immunohistological expression of oestrogen receptor, progesterone receptor, mammaglobin, human epidermal growth factor receptor 2 and GATA-binding protein 3 in non-small-cell lung cancer

Katharina Kriegsmann,1 Christiane Zgorzelski,2 Thomas Muley,3,4 Petros Christopoulos,5 Moritz von Winterfeld,2 Esther Herpel,2 Benjamin Goeppert,2 Gunhild Mechtersheimer,2 Peter Sinn,2 Albrecht Stenzinger,2,3 Peter Schirmacher,2 Hauke Winter,3,4,6 Monika Eichinger,7 Arne Warth8 & Mark Kriegsmann2,3,4

1 Department of Internal Medicine V, Haematology, Oncology and Rheumatology, University Hospital Heidelberg, Heidelberg, Germany, 2 Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, 3 Translational Lung Research Centre Heidelberg, Member of the German Centre for Lung Research (DZL), Thoraxklinik at Heidelberg University, Heidelberg, Germany, 4 Translational Research Unit, Thoraxklinik at Heidelberg University, Heidelberg, Germany, 5 Department of Thoracic Oncology, Thoraxklinik at Heidelberg University, Heidelberg, Germany, 6 Department of Thoracic Surgery, Thoraxklinik at Heidelberg University, Heidelberg, Germany, 7 Department of Radiology, Thoraxklinik at Heidelberg University, Heidelberg, Germany, and 8 Institute of Pathology, Cytopathology, and Molecular Pathology, UEGP MVZ Gießen/Wetzlar/Limburg, Limburg, Germany

Abstract: Aims: Non-small-cell lung cancer (NSCLC) and breast cancer are common entities. Staining for oestrogen receptor (ER), progesterone receptor (PgR), mammaglobin (MAMG) and GATA-binding protein 3 (GATA3) is frequently performed to confirm a mammary origin in the appropriate diagnostic setting. However, comprehensive data on the immunohistological expression of these markers in NSCLC are limited. Therefore, the aim of this study was to analyse a large cohort of NSCLCs and correlate the staining results with clinicopathological variables.

Methods and results: A tissue microarray was stained for ER, PgR, MAMG, human epidermal growth factor receptor 2 (HER2), and GATA3, and included 636 adenocarcinomas (ADCs), 536 squamous cell carcinomas (SqCCs), 65 large-cell carcinomas, 34 pleomorphic carcinomas, and 20 large-cell neuroendocrine carcinomas. HER2 status was determined for immunohistochemically positive cases with chromogenic in-situ hybridisation. Markers with a proportion of ≥5% positive cases in ADC and SqCC were considered for survival analysis. Among ADCs, 62 (10%), 17 (3%), one (<1%), seven (1%), and 49 (8%) cases were positive for ER, PgR, MAMG, HER2, and GATA3, respectively. Among SqCCs, 10 (2%), 14 (3%), two (<1%) and 109 (20%) cases were positive for ER, PgR, HER2, and GATA3, but none of the...
samples showed positivity for MAMG. ER positivity was associated with ADC, female sex, smaller tumour size, and lower clinical stage. None of the markers had an impact on survival.

Keywords: ER, GATA3, HER2, lung cancer, mammaglobin, NSCLC, PgR

Conclusion: We report on ER, PgR, MAMG, HER2 and GATA3 expression in a large cohort of NSCLCs. Interpretation of these markers in the differential diagnostic setting should be based on a multimarker panel.

Introduction
Non-small-cell lung cancer (NSCLC) and breast cancer are frequent entities in pathological routine diagnostics.\(^1\) Whereas the differential diagnosis is often feasible on the basis of histomorphology alone, especially in resection specimens, distinction between these entities can be challenging in small biopsies or cytology preparations. This is particularly true for adenocarcinomas (ADCs).

The lung is a frequent site of metastatic spread in patients with breast cancer, besides bone, the liver, and the brain. Specifically, 25–45% of distant breast cancer metastases are localised in the lung, depending on the intrinsic molecular subtype.\(^2\)\(^,\)\(^3\)

Distinction between primary lung and metastatic breast cancer is important, as the two entities are treated differently.\(^4\)\(^–\)\(^6\)

Common immunohistochemical (IHC) markers used in the differential diagnosis of breast cancer with or without metastases are: oestrogen receptor (ER), progesterone receptor (PgR), mammaglobin (MAMG), human epidermal growth factor receptor 2 (HER2), and GATA-binding protein 3 (GATA3).\(^7\)\(^,\)\(^8\) The expression of these markers in NSCLC has been partly, but not comprehensively, studied to date.

Therefore, we systematically analysed the expression of these five IHC markers in 1291 NSCLCs, including 636 ADCs, 536 squamous cell carcinomas (SqCCs), 65 large-cell carcinomas (LCCs), 34 pleomorphic carcinomas (PCs), and 20 large-cell neuroendocrine carcinomas (LCNECs), and correlated the results with clinicopathological variables, including survival.

Materials and methods

Cohort characteristics
Formalin-fixed paraffin-embedded NSCLC specimens resected from 2002 to 2010 in the Thoraxklinik at Heidelberg University were extracted from the archive of the Institute of Pathology, Heidelberg University, with the support of the tissue bank of the National Centre for Tumour Diseases (NCT) (no. 2591). Tissues were used in accordance with the ethical regulations of the NCT tissue bank defined by the local ethics committee (no. S315-2020). Diagnoses were made according to the recommendations of the 2015 World Health Organization classification of lung cancer.\(^9\)

A cohort of 1291 NSCLCs, including ADCs, SqCCs, LCCs, and PCs, was selected. Tissue microarray (TMA) construction was performed as described previously.\(^10\)\(^–\)\(^12\) Each patient was represented by two cores on the TMA. The staining results for the conventional NSCLC markers cytokeratin (CK) 5/6, p40, p63, napsin-A and thyroid transcription factor-1 (TTF-1) were published previously.\(^10\)\(^,\)\(^13\) A detailed description of the clinical characteristics of the NSCLC cohort is provided in Table 1.

Immunohistochemistry
IHC staining was performed as previously described.\(^14\) In brief, slides were deparaffinised, pre-treated with an antigen retrieval buffer, and stained by use of a Ventana Benchmark Ultra (Roche, Rotkreuz, Switzerland). The antibody and staining characteristics are shown in Table 2. For ER, PgR, MAMG, HER2, and GATA3, any immunoreactivity of tumour cells in at least one of the two cores of the TMA was considered to be a positive staining result. For ER, PgR, and GATA3, only nuclear immunoreactivity was considered; for MAMG, only cytoplasmic immunoreactivity was considered; and for HER2, only membranous immunoreactivity was considered. HER2-positive cases with weak, moderate and strong immunoreactivity were all subjected to testing with chromogen in-situ hybridisation (CISH). Positive HER2 status was finally determined according to the recommendations of the American Society of Clinical Oncology/College of American Pathologists for CISH HER2 testing in breast cancer,\(^15\) as follows: positive—an HER2/chromosome enumeration probe 17 (CEP17) ratio of $\geq 2.0$ and an average HER2 copy number of $>4.0$ signals per cell, or an HER2/CEP17 ratio of $<2.0$ and an average HER2 copy number of $\geq 6.0$ signals per cell; equivocal—an
HER2/CEP17 ratio of $\geq 2.0$ and an average HER2 copy number of $<4.0$ signals per cell, or an HER2/CEP17 ratio of $<2.0$ and an average HER2 copy number of $\geq 4.0$ and $<6.0$ signals per cell; and negative—an HER2/CEP17 ratio of $<2.0$ and an average HER2 copy number of $<6.0$ signals per cell. The evaluation of IHC and CISH results was performed by an experienced pathologist (M.K.). Typical examples of positive and negative staining for the five markers investigated are shown in Figures 1 and 2.

**Table 1.** Patient characteristics

| Variable         | ADC   | SqCC  | LCC   | PC    | LCNEC |
|------------------|-------|-------|-------|-------|-------|
| Number           | 636   | 536   | 65    | 34    | 20    |
| Sex, n (%)       |       |       |       |       |       |
| Male             | 376 (59) | 446 (83) | 44 (68) | 29 (85) | 16 (80) |
| Female           | 260 (41) | 90 (17) | 21 (32) | 5 (15) | 4 (20) |
| Age at first diagnosis (years), median (range) | 63 (38–89) | 65 (38–83) | 60 (38–80) | 61 (34–79) | 62 (50–80) |
| T stage, n (%)   |       |       |       |       |       |
| pT1              | 127 (20) | 100 (19) | 9 (14) | 0 (0) | 4 (20) |
| pT2              | 396 (62) | 327 (61) | 40 (62) | 22 (65) | 9 (45) |
| pT3              | 97 (15) | 91 (17) | 16 (25) | 11 (32) | 6 (30) |
| pT4              | 16 (2.5) | 18 (3.4) | 0 (0) | 1 (2.9) | 1 (5.0) |
| N stage, n (%)   |       |       |       |       |       |
| pN0              | 317 (50) | 261 (49) | 27 (42) | 13 (38) | 10 (50) |
| pN1              | 96 (15) | 169 (32) | 24 (37) | 10 (29) | 5 (25) |
| pN2              | 198 (31) | 96 (18) | 14 (22) | 10 (29) | 4 (20) |
| pN3              | 4 (0.6) | 1 (0.2) | 0 (0) | 1 (2.9) | 0 (0) |
| pNX              | 21 (3.3) | 9 (1.7) | 0 (0) | 0 (0) | 1 (5.0) |
| M stage, n (%)   |       |       |       |       |       |
| pM1              | 26 (4.1) | 8 (1.5) | 2 (3.1) | 1 (2.9) | 0 (0) |
| pMX              | 610 (96) | 528 (99) | 63 (97) | 33 (97) | 20 (100) |
| Clinical stage, n (%) |       |       |       |       |       |
| I                 | 253 (40) | 186 (35) | 10 (15) | 4 (12) | 6 (30) |
| II                | 137 (22) | 207 (39) | 32 (49) | 13 (38) | 7 (35) |
| III               | 220 (35) | 135 (25) | 21 (32) | 16 (47) | 7 (35) |
| IV                | 26 (4.1) | 8 (1.5) | 2 (3.1) | 1 (2.9) | 0 (0) |

ADC, adenocarcinoma; LCC, large-cell carcinoma; LCNEC, large-cell neuroendocrine carcinoma; PC, pleomorphic carcinoma; SqCC, squamous cell carcinoma.

Statistical analysis was performed with R (R Development Core Team, 2008). For descriptive statistics, data are presented as absolute numbers and percentages, and median and range. For comparison of categorical variables, the chi-square test in cases of $2 \times 2$ contingency tables or its extension in cases of $2 \times >2$ contingency tables was used. To identify differences among groups in cases of continuous variables, a two-sided independent t-test was performed. Overall survival (OS)
rates were calculated and plotted by the use of Kaplan–Meier survival analysis. To calculate the differences between the OS curves, a log-rank test was used. Patients with ADC and SqCC with a proportion of $\geq 5\%$ positive cases for a single breast marker were considered for survival evaluation. The Cox proportional hazard model and the Breslow method were applied for multivariate analysis. A \(P\)-value of $<0.05$ was considered to be significant.

**Results**

**Patient Characteristics and Confirmation of Diagnosis**

Overall, 1291 NSCLC patient samples were analysed. Among the analysed patients, 636, 536, 65, 34 and 20 had a diagnosis of ADC, SqCC, LCC, PC, and LCNEC, respectively. In the overall cohort, 911 (71\%) of patients were male and 380 (29\%) were female. The median age was 64 years (range, 34–89 years). Clinical stage I–III disease was found in the majority of patients at first diagnosis (1254 patients, 97\%). Only 37 (3\%) of patients had stage IV disease. Patient characteristics with regard to the respective entity are summarised in Table 1. The diagnosis was confirmed by TTF-1, napsin-A, CK5/6 and p40 staining, which showed typical expression profiles. The staining characteristics for TTF-1, napsin-A, CK5/6 and p40 with regard to the respective entity are summarised in Table 3.

**Expression of Breast Markers in NSCLC Subtypes**

The proportions of NSCLCs positive for ER, PgR, MAMG, HER2 and GATA3 were determined with IHC analysis. Among ADCs, 62 (10\%), 17 (3\%), one (<1\%), seven (1\%) and 49 (8\%) cases were positive for ER, PgR, MAMG, HER2, and GATA3, respectively. Among SqCCs, 10 (2\%), 14 (3\%), two (<1\%), and 109 (20\%) cases were positive for ER, PgR, HER2, and GATA3, respectively, but none of the cases showed positivity for MAMG. Two (3\%) and 15 (23\%) LCCs were positive for PgR and GATA3, respectively, but negative for ER, MAMG, and HER2. Among PCs, only seven (21\%) cases were positive for GATA3. LCNECs were not positive for MAMG or HER2, but one (5\%), two (10\%) and two (10\%) cases were positive for ER, PgR, and GATA3, respectively.

The absolute and relative numbers of cases with breast marker positivity among the analysed NSCLC subtypes are shown in Figure 3 and Table 4.

Patients with ADC and SqCC with a proportion of $\geq 5\%$ positive cases for a single breast marker were considered for further evaluation. Thus, detailed clinical and OS analyses were performed in ADC patients regarding ER and GATA3 status, and in SqCC patients regarding GATA3 status.

**Subgroup Analysis of NSCLC Patients According to Sex**

Overall, 911 patients were male (71\%) and 380 were female (29\%). Female patients were younger ($P < 0.001$), more often had ADC than SqCC ($P < 0.001$), were more commonly node-negative ($P = 0.026$), and were diagnosed at a lower clinical stage ($P = 0.033$). Interestingly, female patients more often had ER-positive tumours than their male counterparts ($P < 0.001$). There were no differences in the T-category. PgR status, or GATA3 status. A detailed summary is provided in Table S1.

**Subgroup Analysis of ADC Patients According to TTF-1 Status**

Among the 636 ADCs, the majority were positive for TTF-1 (86\%). There was no difference in TTF-1 positivity between male and female patients ($P = 0.245$). TTF-1-positive tumours were associated with lower T-
categories \((P = 0.003)\), but there was no difference in N-categories \((P = 0.287)\). In line with this finding, TTF-1-positive tumours were more common in lower clinical stages than TTF-1-negative tumours \((P < 0.001)\). ER and PgR were more commonly expressed in TTF-1-positive tumours \((P = 0.011\) and \(P = 0.033)\), respectively, and TTF-1-negative, ER-positive or PgR-positive tumours were rare. GATA3 positivity was significantly more common in TTF-1-negative than in TTF-1-positive tumours \((P < 0.001)\). A detailed summary is provided in Table S2.

CLINICAL CHARACTERISTICS OF ADC PATIENTS ACCORDING TO ER AND GATA3 STATUS

Among the 636 ADCs, 62 of 636 (10\%) were positive for ER. Male sex was less frequent among ER-positive ADC patients than among ER-negative patients \((P = 0.009)\). Moreover, pT1 was more frequently found in ER-positive cases \((31\% \text{ versus } 19\%, P = 0.014)\).

This was represented by a higher proportion of earlier disease stages and a lower proportion of more advanced disease stages in ER-positive than in ER-negative ADC cases \(\text{e.g. stage I, 61\% \text{ versus } 37\%; stage III, 26\% \text{ versus } 36\%; } P = 0.001; \) Table 5\).

Twenty-nine of 62 (47\%) and 322 of 574 (56\%) deaths occurred in ADC patients who were positive and negative for ER, respectively, after a median follow-up of 51 months. In ER-positive patients, the median OS was reached at 121 months, and the 3-year, 5-year and 10-year OS rates were 70\%, 58\%, and 51\%, respectively. In ER-negative patients, the median OS was reached at 60 months, and the 3-year, 5-year and 10-year OS rates were 59\%, 50\%,
and 37%, respectively. As compared with ER-negative ADC patients, a trend towards improved OS was observed in ER-positive patients; however, the results did not reach statistical significance \( P = 0.091 \), hazard ratio (HR) = 0.723, 95% confidence interval (CI) 0.519–1.010; Figure 4A).

Forty-nine (8%) and 587 (92%) of ADC patients were positive and negative for GATA3, respectively. Interestingly, we observed that GATA3-positive patients were younger at first diagnosis than GATA3-negative patients (median age, 63 years versus 65 years; \( P = 0.021 \)). In GATA3-positive patients, pT2 was found more frequently, and pT1 and pT3 less frequently, than in the GATA3-negative cohort (\( P = 0.013 \)). However, this was not reflected by statistically significant differences in clinical stage at first diagnosis (\( P = 0.266 \); Table 5).

Twenty-seven of 49 (55%) and 324 of 587 (55%) deaths occurred in ADC patients who were positive and negative for GATA3, respectively, after a median follow-up of 51 months. In GATA3-positive patients, the median OS was reached at 62 months, and the 3-year and 5-year OS rates were 58% and 53%, respectively. In GATA3-negative patients, the median OS was reached at 62 months, and the 3-year, 5-year and 10-year OS rates were 61%, 51%, and 42%, respectively. No statistically significant differences in OS were found between GATA3-positive and GATA3-negative patients (\( P = 0.999 \), HR = 1.000, 95% CI 0.675–1.481; Figure 4B).

In multivariate analysis, sex, age, and stage at first diagnosis reached high prognostic significance with regard to OS. However, neither ER status nor GATA3 status had a significant influence on OS (Table 5).

Figure 2. Examples of pulmonary squamous cell carcinoma cases with positivity and negativity for oestrogen receptor (ER), progesterone receptor (PgR), mammaglobin (MAMG), human epidermal growth factor receptor 2 (HER2), and GATA-binding protein 3 (GATA3). Haematoxylin and eosin-stained (A,E,K,O,C,G,I,M,Q) positive (B,F,L,O) and negative (D,H,J,N,R) examples of pulmonary squamous cell carcinoma are shown for ER (first row), PgR (second row), MAMG (third row), HER2 (fourth row), and GATA3 (fifth row).
CLINICAL CHARACTERISTICS OF SQCC PATIENTS WITH REGARD TO GATA3 STATUS

Overall, 109 (20%) and 427 (80%) of SqCC patients were positive and negative for GATA3, respectively. We could not identify any statistically significant differences in sex, age, TNM status or stage between GATA3-positive and GATA3-negative patients (Table 6).

Fifty-seven of 109 (52%) and 223 of 427 (52%) deaths occurred in SqCC patients who were positive and negative for GATA3, respectively, after a median follow-up of 55 months. In GATA3-positive patients, the median OS was reached at 98 months, and the 3-year, 5-year and 10-year OS rates were 66%, 56%, and 33%, respectively. In GATA3-negative patients, the median OS was reached at 75 months, and the 3-year, 5-year and 10-year OS rates were 67%, 55%, and 39%, respectively. No statistically significant differences in OS were found between GATA3-positive and GATA3-negative patients (P = 0.976, HR = 0.996, 95% CI 0.775–1.331; Figure 5).

In multivariate analysis, sex, age and stage at first diagnosis reached high prognostic significance with regard to OS. However, GATA3 status had no significant influence on OS (Table 6).

Discussion

In the present study, we investigated the expression of ER, PgR, MAMG, HER2 and GATA3 in a large NSCLC tissue cohort comprising 1291 cases. We found that all five markers may be positive in divergent constellations in NSCLC, which is important for differential diagnostic considerations.

Our cohort is representative of NSCLC in developed countries, as the distribution of age, sex, TNM categories and stage is very similar to that previously reported.1

ER is a steroid hormone receptor that is divided into two subtypes: ER-α and ER-β. ER-α is well known as a nuclear prognostic and predictive marker in breast cancer.16 In lung cancer, oestrogens have been shown to stimulate growth of cell lines.17
Studies on NSCLC tissue specimens found ER positivity in 0–73%. Some of this great variability may be explained by the use of different clones, and the definition of positivity in tumours with cytoplasmic reactivity in some studies. In primary breast cancer, ER positivity is currently defined as ≥1% positive tumour cell nuclei according to the American Society of Clinical Oncology/College of American Pathologists guideline recommendations, based on the lack of benefit from hormonal therapy in patients with <1% positivity. In lung tissue, however, hormonal therapy does not play a role, and ER expression is normally absent, so there is no such threshold. In addition, breast cancer can undergo a switch in biomarker status, so it seems reasonable to count any positivity in lung specimens as a positive result for differential diagnostic purposes. We detected ER expression in 10% of ADCs, 2% of SqCCs, and 5% of LCNECs, which is within the reported range from most investigations that focused on nuclear reactivity by using the SP1 clone against ER-α. Specifically, in ADCs, ER was more often expressed in specimens from female patients, and in tumours of smaller size and lower clinical stage. This finding is well in line with the current literature. On the basis of the fact that the majority of breast cancers express ER and a low proportion of lung cancer specimens are positive for ER, it has been suggested that ER should be included in a differential diagnostic panel. However, caution is warranted, as interpretation of ER positivity alone may lead to the false assumption of a pulmonary breast cancer.

Table 4. Staining characteristics for oestrogen receptor (ER), progesterone receptor (PgR), mammaglobin (MAMG), human epidermal growth factor receptor 2 (HER2), and GATA-binding protein 3 (GATA3)

| Entity     | Positive cases*, n (%) | ER     | PgR    | MAMG   | HER2†   | GATA3  |
|------------|------------------------|--------|--------|--------|---------|--------|
| ADC, N = 636 | 62 (9.7)               | 17 (2.7)| 1 (0.2)| 7 (1.1)| 49 (7.7) |
| SqCC, N = 536 | 10 (1.9)              | 14 (2.6)| 0 (0) | 2 (0.4)| 109 (20) |
| LCC, N = 65 | 0 (0)                  | 2 (3.1)| 0 (0) | 0 (0) | 15 (23)  |
| PC, N = 34  | 0 (0)                  | 0 (0) | 0 (0) | 0 (0) | 7 (21)   |
| LCNEC, N = 20 | 1 (5.0)              | 2 (10)| 0 (0) | 0 (0) | 2 (10)   |

ADC, adenocarcinoma; LCC, large cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; PC, pleomorphic carcinoma; SqCC, squamous cell carcinoma.

*Positivity for ER, PgR, MAMG, HER2 or GATA3 was defined as any staining in tumour cells.
†Positivity confirmed by chromogen in-situ hybridisation, in cases of low, intermediate and strong immunohistochemical staining intensity.
metastasis, specifically because of the high sensitivity of SP1, the ER clone used in this study. Controversial observations have been made regarding survival, as some authors have reported a favourable outcome in patients with ER-positive tumours, whereas others could not find a statistically significant difference. In our study, there was a trend towards improved OS in patients with ER-positive ADC, but statistical significance was not reached ($P = 0.091$).

PgR is a steroid hormone receptor that has been linked to a favourable prognosis and the prediction of
response to anti-hormone therapy in breast cancer.\textsuperscript{15,16} Only positivity in the nucleus is regarded a positive result. Among lung cancer specimens, PgR expression has been reported in 0–47%\textsuperscript{19,21,37,38} Again, some of this variability may be attributable to the use of different antibody clones.\textsuperscript{30} Unlike in breast cancer, where PgR and ER are often coexpressed, PgR-positive NSCLCs were reported to be rarely ER-positive, suggesting an ER-independent mechanism of PgR expression in NSCLC.\textsuperscript{38} In our study, we found PgR expression in 3% each of ADCs, SqCCs, and LCCs, as well as in 10% of LCNECs. Thus, we could not confirm an association with ADC, as previously reported.\textsuperscript{18} The relatively low number of PgR-positive tumours in our cohort prevented a statistically sound survival analysis. The clinical significance of PgR expression in NSCLC remains controversial. In the differential diagnostic setting, one has to be aware of the (low-frequency) expression of PgR in several different subtypes of NSCLC.

MAMG is a member of the uteroglobin protein family, and has been described as a specific marker for breast cancer, but suffers from a lack of sensitivity. Among primary mammary carcinomas, MAMG is expressed in >80% of cases, but this rate can decrease to 50% in metastases.\textsuperscript{39} Among NSCLCs, MAMG expression has been reported in 0–1% of cases.\textsuperscript{40} Little is known about the characteristics of MAMG-positive NSCLC. Only one TTF-1-positive and napsin-A-positive ADC was weakly positive for MAMG in our cohort. We therefore confirm that MAMG expression is very rare in NSCLC.

**Table 5. (Continued)**

| ADC, N = 636 | ER Positive | ER Negative | p-value | GATA3 Positive | GATA3 Negative | p-value | OS HR (95% CI) | p-value |
|-------------|-------------|-------------|---------|----------------|----------------|---------|----------------|---------|
| Clinical stage, n (%) | 0.001\textsuperscript{1} | 0.266\textsuperscript{3} | 1.851 (1.650–2.076) | <0.001 |
| I | 38 (61) | 215 (37) | 0.001\textsuperscript{1} | 18 (37) | 235 (40) | 0.266\textsuperscript{3} | 1.851 (1.650–2.076) | <0.001 |
| II | 8 (13) | 129 (22) | 0.001\textsuperscript{1} | 15 (31) | 122 (21) | 0.266\textsuperscript{3} | 1.851 (1.650–2.076) | <0.001 |
| III | 16 (26) | 204 (36) | 0.001\textsuperscript{1} | 15 (31) | 205 (35) | 0.266\textsuperscript{3} | 1.851 (1.650–2.076) | <0.001 |
| IV | 0 (0) | 26 (4.5) | 0.001\textsuperscript{1} | 1 (2.0) | 25 (4.3) | 0.266\textsuperscript{3} | 1.851 (1.650–2.076) | <0.001 |

CI, confidence interval; HR, hazard ratio; TTF-1, thyroid transcription factor-1.

*Without pNX cases.

\textsuperscript{1}Without pNX and pN3 cases.

\textsuperscript{2}Stage III and stage IV grouped.

\textcopyright 2020 The Authors. Histopathology published by John Wiley & Sons Ltd, Histopathology, 77, 900–914.

**Figure 4.** Overall survival (OS) analysis of pulmonary adenocarcinoma (ADC) cases with positivity and negativity for oestrogen receptor (ER) and GATA-binding protein 3 (GATA3); OS analysis of ADC patients with regard to ER (A) and GATA3 (B) status. CI, confidence interval; HR, hazard ratio.
marker panel, MAMG positivity favours a breast cancer metastasis.

HER2 is a tyrosine kinase receptor encoded by \( \text{HER2} \), and belongs to the epidermal growth factor receptor (EGFR) family. Approximately 15–20% of breast cancer cases show HER2 overexpression as an indicator of \( \text{HER2} \) amplification. Among lung cancers, both \( \text{HER2} \) mutations (mostly in exons 18–21 of the tyrosine kinase domain) and \( \text{HER2} \) amplifications have been identified in approximately 2–4% and

### Table 6. Clinical characteristics and multivariate overall (OS) analysis of squamous cell carcinoma (SqCC) cases with positivity for GATA-binding protein 3 (GATA3)

| SqCC, \( N = 536 \) | GATA3 | OS |
|------------------|------|----|
|                  |      |    | \( P \)-value | \( P \)-value |
| \( n \) (% of all cases) | 109 (20) | 427 (80) | – | – |
| GATA3 status (positive versus negative) | – | – | – | 0.931 (0.694–1.249) | 0.634 |
| Sex, \( n \) (%) | | | 0.439 | 0.679 (0.471–0.978) | 0.038 |
| Male | 88 (81) | 358 (84) | | |
| Female | 21 (19) | 69 (16) | | |
| Age at first diagnosis (years), median (range) | | | | |
| \( \leq 60 \) versus \( >60 \) | 67 (44–81) | 65 (38–83) | 0.105 | 1.306 (1.003–1.699) | 0.047 |
| T stage, \( n \) (%) | | | 0.324 | – | – |
| \( pT1 \) | 14 (13) | 86 (20) | | |
| \( pT2 \) | 70 (64) | 257 (60) | | |
| \( pT3 \) | 20 (18) | 71 (17) | | |
| \( pT4 \) | 5 (4.6) | 13 (3.0) | | |
| N stage, \( n \) (%) | | | 0.167* | – | – |
| \( pN0 \) | 47 (43) | 214 (40) | | |
| \( pN1 \) | 34 (31) | 135 (32) | | |
| \( pN2 \) | 26 (24) | 70 (16) | | |
| \( pN3 \) | 0 (0) | 1 (0.2) | | |
| \( pNX \) | 2 (1.8) | 7 (1.6) | | |
| M stage, \( n \) (%) | | | – | – | – |
| \( pM1 \) | 1 (0.9) | 7 (1.6) | | |
| \( pMX \) | 108 (99) | 420 (98) | | |
| Clinical stage, \( n \) (%) | | | 0.152† | 1.503 (1.298–1.741) | <0.001 |
| I | 33 (30) | 153 (36) | | |
| II | 39 (36) | 168 (39) | | |
| III | 36 (33) | 99 (23) | | |
| IV | 1 (0.9) | 7 (1.6) | | |

CI, confidence interval; HR, hazard ratio.

*Without \( pN0 \) and \( pN3 \) cases.

†Stage III and stage IV grouped.
10–20% of cases, respectively, whereas HER2 overexpression has been described in 6–35% of cases. HER2 mutations have been associated with ADC, female sex, and never smoking. Although the incidence of HER2 alterations in NSCLC is low, they are of particular importance, as novel agents (particularly those targeting both EGFR and HER2) have shown promising preliminary results, and are currently being investigated in clinical trials. It is of note that, whereas in breast cancer a high correlation between protein expression and gene amplification is observed, the correlation in NSCLC seems to be only moderate. This is particularly true for cases with moderate and strong IHC HER2 expression. Thus, it remains a matter of debate which assay should be used to predict treatment response to HER2-targeted therapy in NSCLC patients. In our study, IHC HER2 overexpression (moderate or strong membranous staining intensity) was observed in 1% of ADCs and in only two SqCCs, all of which had been confirmed to have HER2 amplification with CISH. The reason why we observed fewer HER2-positive cases in our cohort than have other investigators is not entirely clear. However, the low number of patients identified prevented further statistical analysis. A poor prognostic role of HER2 alterations has been suggested but remains controversial.

GATA3 is a zinc finger transcription factor, and has been reported to be expressed in 32–92% of breast cancer samples. Most primary mammary tumours show nuclear positivity (80–90%), but triple-negative breast cancer may have a lower frequency of positivity. GATA3 has been reported to be more specific than MAMG for breast cancer. An important differential diagnosis of a GATA3-positive tumour is urothelial carcinoma, which may be GATA3-positive in 70% of cases, but other carcinomas, including, for example, SqCC from different sites, pancreatic ADC, mesothelioma, skin adnexal tumours, and salivary gland tumours, also fall into the differential diagnostic spectrum. Among NSCLCs, GATA3 expression has been reported in 0–12% of cases. We found GATA3 positivity in 8%, 20%, 23%, 21% and 10% of ADCs, SqCCs, LCCs, PCs, and LCNECs, respectively. These data suggest that GATA3 may be more frequently expressed than previously reported. Interestingly, only 59% of GATA3-positive cases in our study expressed TTF-1, which may cause a differential diagnostic problem, especially in female patients (six of 20 cases). However, none of the GATA3-positive TTF-1-negative cases showed ER, PgR or HER2 expression, which may be an indication of a pulmonary primary tumour. In any case, a clinical and radiological correlation is warranted. No association of GATA3 positivity with survival was observed in our large cohort, in contrast to a previous report on 95 ADCs.

There are some limitations inherent to the study design. As the investigations were conducted on a TMA, and all investigated stains may be heterogeneous, we cannot fully exclude the possibility of a false-negative result in a subset of cases. On the other hand, it has been shown that TMAs are suitable for investigating biomarkers, and the unprecedented degree of standardisation often gives them an advantage over large-section studies. Moreover, our cut-
off for staining positivity may be a critical issue, as the distinction between background staining and unequivocal positivity may sometimes be arbitrary. However, we wanted to stay as close as possible to the real-life scenario, where we must tackle the same challenge, and another cut-off in small biopsies is rarely helpful in this regard.

In summary, we stained 1291 NSCLCs for ER, PgR, MAMG, HER2, and GATA3, and found expression of all five markers in at least a proportion of cases. Thus, interpretation of these IHC markers in the differential diagnostic setting should always be made in the context of other markers. None of these stains had prognostic value.

Acknowledgements
Open access funding enabled and organized by Projekt DEAL.

Conflicts of interest
The authors state that they have no conflicts of interest.

Funding
No funding was received for this work.

Author contributions
M. Kriegsmann and A. Warth designed the study. M. Kriegsmann evaluated IHC staining results. K. Kriegsmann performed statistical analyses. M. Kriegsmann and K. Kriegsmann drafted the manuscript. E. Herpel, C. Zgorzelski, T. Muley and A. Warth constructed the TMA. T. Muley, P. Christopoulos and H. Winter contributed tissue samples and clinical data. M. Kriegsmann, M. von Winterfeld, E. Herpel, B. Goeppert, G. Mechtersheimer, P. Sinn, M. Eichinger, A. Stenzinger and A. Warth were involved in diagnosis of the analysed cases. P. Schirmacher financed the study. All authors reviewed the manuscript for important intellectual content.

References
1. Howlader N, Noone AM, Krapcho M et al. SEER cancer statistics review. 1975–2013. Bethesda, MD: National Cancer Institute, 2016. Available at: http://seer.cancer.gov/csr/1975_2013/
2. Lester SH, David GH. Diagnostic pathology: Breast. 2nd ed. Altona, Canada: Amirsys, 2017:600.
3. Wu Q, Li J, Zhu S et al. Breast cancer subtypes predict the preferential site of distant metastases: a seer based study. Oncotarget 2017; 8: 27990–27996.
4. Reck M, Rabe KE. Precision diagnosis and treatment for advanced non-small-cell lung cancer. N. Engl. J. Med. 2017; 377: 849–861.
5. Hortobagyi GN, Stemmer SM, Burris HA et al. Ribociclib as first-line therapy for hr-positive, advanced breast cancer. N. Engl. J. Med. 2016; 375: 1738–1748.
6. Finn RS, Martin M, Rugo HS et al. Palbociclib and letrozole in advanced breast cancer. N. Engl. J. Med. 2016; 375: 1925–1936.
7. Gowen AM, Fulton RS, Kandalaft PL. Markers of metastatic carcinoma of breast origin. Histopathology 2016; 68: 86–95.
8. O’Brien N, O’Donovan N, Foley D et al. Use of a panel of novel genes for differentiating breast cancer from non-breast tissues. Tumour Biol. 2007; 28: 312–317.
9. Travis WDB, Burke AP, Marx A, Nicholson AG. Who classification of tumours of the lung, pleura, thymus and heart. 4th ed. Lyon, France: IARC Publication, 2015.
10. Warth A, Muley T, Herpel E et al. Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. Histopathology 2012; 61: 1017–1025.
11. Lisenko K, Leichenring J, Zgorzelski C et al. Qualitative comparison between carrier-based and classical tissue microarrays. Appl. Immunohistochem. Mol. Morphol. 2017; 25: e74–e79.
12. Kriegsmann M, Harms A, Longuespee R et al. Role of conventional immunomarkers, hnf4-a, and satb2 in the differential diagnosis of pulmonary and colorectal adenocarcinomas. Histopathology 2018; 72: 997–1006.
13. Kriegsmann K, Cremer M, Zgorzelski C et al. Agreement of ck5/6, p40, and p63 immunoreactivity in non-small-cell lung cancer. Pathology 2019; 51: 240–245.
14. Kriegsmann M, Muley T, Harms A et al. Differential diagnostic value of cd5 and cd117 expression in thoracic tumors: a large scale study of 1465 non-small cell lung cancer cases. Diagn. Pathol. 2015; 10: 210.
15. Wolff AC, Hammond MEH, Allison KH et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of american pathologists clinical practice guideline focused update. J. Clin. Oncol. 2018; 36: 2105–2122.
16. Pan H, Gray R, Braybrooke J et al. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N. Engl. J. Med. 2017; 377: 1836–1846.
17. Stabile LP, Davis AL, Gabish CT et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. Can. Res. 2002; 62: 2141–2150.
18. Wei S, Said-Al-Naief N, Hameed O. Estrogen and progesterone receptor expression is not always specific for mammary and gynecologic carcinomas: a tissue microarray and pooled literature review study. Appl. Immunohistochem. Mol. Morphol. 2009; 17: 393–402.
19. Sun HB, Zheng Y, Ou W et al. Association between hormone receptor expression and epidermal growth factor receptor mutation in patients operated on for non-small cell lung cancer. Ann. Thorac. Surg. 2011; 91: 1562–1567.
20. Aleric I, Razumovic JJ, Koprivica B. Her-2/neu oncogene and estrogen receptor expression in non small cell lung cancer patients. Med. Pregl. 2012; 65: 210–215.
21. Provenzano E, Byrne DJ, Russell PA, Wright GM, Generali D, Fox SB. Differential expression of immunohistochemical...
markers in primary lung and breast cancers enriched for triple-negative tumours. Histopathology 2016; 68: 167–377.

22. Cheng TD, Darke AK, Redman MW et al. Smoking, sex, and non-small cell lung cancer: steroid hormone receptors in tumor tissue. (60244). J. Natl. Cancer Inst. 2018; 110: 734–742.

23. Lund-Iversen M, Scott H, Strom EH, Theiss N, Brustugun OT, Gronberg BH. Expression of estrogen receptor-alpha and survival in advanced-stage non-small cell lung cancer. Anticancer Res. 2018; 38: 2261–2269.

24. Gome-Campos R, Meijias A, Walker G, Nadji M. Immunohistochemical expression of estrogen receptor in adenocarcinomas of the lung: the antibody factor. Appl. Immunohistochem. Mol. Morphol. 2010; 18: 137–141.

25. Kawai H, Ishii A, Washiya K et al. Estrogen receptor alpha and beta are prognostic factors in non-small cell lung cancer. Clin. Cancer Res. 2005; 11: 5084–5089.

26. Hammond ME, Hayes DF, Dowsett M et al. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch. Pathol. Lab. Med. 2010; 134: 907–922.

27. Allison KH, Hammond MEH, Dowsett M et al. Tumour estrogen and progesterone receptor testing in breast cancer: Ansoicap guideline update. J. Clin. Oncol. 2020; 38: 1346–1366.

28. Thompson AM, Jordan LB, Quinlan P et al. Prognostic and predictive value of estrogen receptor expression in patients with endometrial adenocarcinoma: a validation study. Anticancer Res. 2010; 30: R92.

29. Schrijver W, Suijkerbuijk KPM, van Gils CH, van der Wall E, Moelans CB, van Diest PJ. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J. Natl. Cancer Inst. 2018; 110: 568–580.

30. Raso MG, Behrens C, Herynk MH et al. Immunohistochemical expression of estrogen and progesterone receptors identifies a subset of in situ and correlates with egfr mutation. Clin. Cancer Res. 2009; 15: 5359–5368.

31. Lau SK, Chu PG, Weiss LM. Immunohistochemical expression of estrogen receptor in pulmonary adenocarcinoma. Appl. Immunohistochem. Mol. Morphol. 2006; 14: 83–87.

32. Bogina G, Zamboni G, Sapino A et al. Comparison of anti-estrogen receptor antibodies sp1, 6f11, and 1d5 in breast cancer: Lower 1d5 sensitivity but questionable clinical implications. Am. J. Clin. Pathol. 2012; 138: 697–702.

33. Chen QX, Zheng LX, Li ZY, Lin TY. Clinicopathological significance of oestrogen receptor expression in non-small cell lung cancer. J. Int. Med. Res. 2017; 45: 51–58.

34. Berardi R, Morgese F, Santinelli A et al. Hormonal receptors in lung adenocarcinoma: expression and difference in outcome by sex. Oncotarget 2016; 7: 82648–82657.

35. Mohsin SK, Weiss H, Harighurst T et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. Mod. Pathol. 2004; 17: 1545–1544.

36. Nordenskjold A, Fohlman H, Fernander T, Loflahal B, Skog L, Stal O. Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast Cancer Res. Treat. 2016; 160: 313–322.

37. Di Nunno L, Larsson LG, Rinehart JH, Beissner RS. Estrogen and progesterone receptors in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection. Arch. Pathol. Lab. Med. 2000; 124: 1467–1470.

38. Iwashita H, Suzuki T, Suzuki S et al. Progesterone receptor in non-small cell lung cancer–a potent prognostic factor and possible target for endocrine therapy. Can. Res. 2005; 65: 6450–6458.

39. Takeda Y, Tsuta K, Shibuki Y et al. Analysis of expression patterns of breast cancer-specific markers (mammaglobin and gross cystic disease fluid protein 15) in lung and pleural tumors. Arch. Pathol. Lab. Med. 2008; 132: 239–243.

40. Wells JM, Ginter PS, Liu Y, Chen Z, Narula N, Shin SJ. Evaluating the utility of trefoil factor 3 as a mammary-specific immunostain compared and in conjunction with gata-3 and mammaglobin in the distinction between carcinoma of breast and lung. J. Clin. Pathol. 2015; 144: 444–451.

41. Hirsch FR, Varella-Garcia M, Franklin WA et al. Evaluation of her-2/neu gene amplification and protein expression in non-small cell lung carcinomas. Br. J. Cancer 2002; 86: 1449–1456.

42. Mazieres J, Peters S, Lepage B et al. Lung cancer that harbors an her2 mutation: epidemiologic characteristics and therapeutic perspectives. J. Clin. Oncol. 2013; 31: 1997–2003.

43. Hirsch FR, Suda K, Wiens J, Bunn PA Jr. New and emerging targeted treatments in advanced non-small-cell lung cancer. Lancet 2016; 388: 1012–1024.

44. Kim EK, Kim KA, Lee CY, Siim HS. The frequency and clinical impact of her2 alterations in lung adenocarcinoma. PloS One 2017; 12: e0171280.

45. Kobayakoske DK, Avidalyan AM, Klimachvez VV, Lazarev AF, Lavshnikova EL, Nepomnyaschikh LM. Non-small cell lung cancer: Her2 oncogene status. Arch. Pathol. 2015; 77: 3–9.

46. Reis H, Herold T, Ting S et al. Her2 expression and markers of phosphoinositide-3-kinase pathway activation define a favorable subgroup of metastatic pulmonary adenocarcinomas. Lung Cancer 2015; 88: 34–41.

47. Ko YS, Kim NY, Pyo JS. Concordance analysis between her2 immunohistochemistry and in situ hybridization in non-small cell lung cancer. Int. J. Biol. Markers 2018; 33: 49–54.

48. Tan D, Deeb G, Wang J et al. Her-2/neu protein expression and gene alteration in stage i-iii non-small-cell lung cancer: A study of 140 cases using a combination of high throughput tissue microarray, immunohistochemistry, and fluorescent in situ hybridization. Diagn. Mol. Pathol. 2003; 12: 201–211.

49. Hirsch FR, Varella-Garcia M, Bunn PA Jr et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J. Clin. Oncol. 2003; 21: 3798–3807.

50. Liu L, Shao X, Gao W et al. The role of human epidermal growth factor receptor 2 as a prognostic factor in lung cancer: a meta-analysis of published data. J. Thorac. Oncol. 2010; 5: 1922–1932.

51. Cimino-Mathews A, Subhawong AP, Illei PB et al. Gata3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. Hum. Pathol. 2013; 44: 1341–1349.
Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. NSCLC subgroup analysis according to sex.

Table S2. ADC subgroup analysis according to TTF-1 status.

55. Mohammed KH, Siddiqui MT, Cohen C. Gata3 immunohistochemical expression in invasive urothelial carcinoma. *Urol. Oncol.* 2016; 34: e432 e439–e432 e413.

56. Miettinen M, McCue PA, Sarlomo-Rikala M et al. Gata3: A multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. *Am. J. Surg. Pathol.* 2014; 38: 13–22.

57. Hashiguchi T, Miyoshi H, Nakashima K et al. Prognostic impact of gata binding protein-3 expression in primary lung adenocarcinoma. *Hum. Pathol.* 2017; 63: 157–164.

58. Sauter G. Representativity of tma studies. *Methods Mol. Biol.* 2010; 664: 27–35.