Lack of Prognostic Significance of Neuroendocrine Differentiation and Stem Cell Antigen Co-Expression in Resected Early-stage Non-small Cell Lung Cancer

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Abstract. *Background: Neuroendocrine (NE) carcinomas of the lung exhibit expression of various stem cell antigens, and except for carcinoid tumours, carry a poor prognosis. Despite the fact that 10%-30% of all non-small cell lung carcinomas (NSCLC) which are not classified as NE carcinomas also show expression of NE markers, data on their prognostic significance are conflicting and analyses of the expression and relevance of stem cell antigens in this subgroup are lacking. Materials and Methods: Tissue specimens of 100 resected early-stage NSCLC were analyzed by immunohistochemistry for the expression and prognostic significance of NE markers. Moreover, the subgroup of NSCLC with NE differentiation (ND) were assessed for the expression and prognostic significance of the stem cell antigens CD117, CD133 and breast cancer resistance protein-1 (ABCG2). Results: ND correlated significantly with adenocarcinoma histology (p=0.035), but not with prognosis. In the subgroup of ND-NSCLC (n=80), the stem cell antigens CD117, CD133 and ABCG2 were expressed in 51%, 14% and 33% of the cases, but likewise, showed no association with prognosis or clinicopathological characteristics. Conclusion: This study indicates that neither ND, nor co-expression of the stem cell antigens CD117, CD133 or ABCG2, have a prognostic significance in resected early-stage NSCLC.*

Lung cancer is the leading cause of cancer-related death worldwide and comprises of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) as main groups (1).

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Neuroendocrine (NE) tumours of the lung account for 20% of all lung carcinomas and include carcinoid tumours, which represent a separate class of malignancies, SCLC, and a rare subtype of NSCLC termed large cell neuroendocrine carcinoma (LCNEC) (2). However, 10%-30% of all NSCLCs which are not classified as LCNEC also exhibit expression of NE markers, including neuron-specific enolase (NSE), synaptophysin (SYP), chromogranin A (CHGA) and CD56 (3). In contrast to neuroendocrine tumours, these carcinomas are termed NSCLC with NE differentiation (ND-NSCLC) (4). Previous studies revealed conflicting results regarding the prognostic and predictive significance of NE differentiation in NSCLC: In operated patients, their expression appeared - if at all - to predict reduced survival, while patients with advanced disease tended to have an increased response to chemotherapy and an improved survival (3). Several studies demonstrated the expression of various stem cell antigens such as CD117, CD44, aldehyde dehydrogenase-1A1 (*ALDH1A1*), nestin or the transcription factor achaete-scute complex homologue-1 (*ASCL1*) in NE tumours of the lung (5-10). Particular CD117 exhibited widespread expression ranging from up to 90% in carcinoid tumours, 46%-82% in SCLC and 25%-55% in LCNEC, and an association with significantly worse survival in a study of 90 patients with NE tumours (5-8, 11). In contrast, analyses on stem cell antigens in ND-NSCLC are scant: So far only one study demonstrated expression and an unfavorable prognostic significance of *ASCL1* in adenocarcinomas (AC) with NE differentiation (12). The stem cell antigen ASCL1 represents a NE master gene which appears to be involved in the regulation of other stem cell markers such as aldehyde dehydrogenase or CD133 (13). This indicates a close relationship between NE differentiation and stem cell characteristics. CD133, together with the drug transporter breast cancer resistance protein-1 (ABCG2) represent two of the most established stem cell antigens in lung cancer, whose expression characterizes drug-resistant cancer cells with tumour-regenerating capacity

(14-16). Although the prognostic significance of these antigens for NSCLC is less consistent than for other tumour entities, the association between NE differentiation and stem cell antigens in some previous analyses indicated the potential relevance of these markers in the subgroup of neuroendocrine tumours and ND-NSCLC (15, 17-20). Since ND-NSCLC accounts for a considerable proportion of cases of NSCLC, we analyzed the prognostic significance of the stem cell antigens CD117, CD133 and ABCG2 in this subgroup.

Materials and Methods

Patients' and samples' characteristics. A total of 100 newly-diagnosed patients with early-stage NSCLC who underwent complete pulmonary resection between 2002 and 2004 at the Department of Thoracic Surgery, Thorax Clinic, University of Heidelberg were analyzed. None of the patients had received neoadjuvant or adjuvant therapy. Tissue specimens and follow-up data were obtained from the tissue bank and the Lung Cancer Registry of the Thorax Clinic and the tissue bank of the National Center for Tumour Diseases (NCT), University of Heidelberg. Patients had given informed consent following the 2000 revision of the guidelines of the Declaration of Helsinki and the local Ethics Committee of the Medical Faculty Heidelberg. Preoperative and follow-up assessments were performed according to the guidelines of the German Respiratory Society 2010 (21). Stage was determined according to the seventh edition of the TNM Classification of Malignant Tumours (22). The histological classification was based on the 2004 revision of the World Health Organization Classification of Tumours (4). Detailed patient characteristics are given in Tables I and II.

Immunohistochemical analyses. Tissue microarrays (TMAs) of pulmonary resections were prepared by the tissue bank of the NCT, as previously described (23). For each patient, 1.2-mm diameter tissue cores of various tumour areas including the tumour centre, the invasion front and histologically normal lung tissue were selected. De-paraffinized 2-μm sections of the TMA were pretreated in antigen retrieval buffer (pH 6) (Dako, Glostrup, Denmark) to block non-specific binding. Sections for CD133 and ABCG2 staining were additionally blocked by avidin/biotin treatment. Subsequent steps were performed in an immunostaining device (Autostainer; Dako). The immunostaining protocol was based on the avidin-biotin peroxidase principle using 3-amino-9-ethylcarbazol as chromogen and haematoxylin for counterstaining. The following primary antibodies, clones and dilutions were used: CD117 (polyclonal, 1:50; Dako), CD133 [polyclonal (ab19898), 1:100; Abcam plc, Cambridge, MA, USA], ABCG2 (clone BXP-21, 1:100; Abcam), CD56 (clone 1B6, 1:50; Novocastra, A. Menarini Diagnostics Deutschland, Berlin, Germany), SYP (clone 27G12, 1:400; Novocastra), NSE (clone MIG-N3, 1:10,000; DCS Innovative Diagnostik-Systeme Dr. Christian Sartori GmbH & Co. KG, Hamburg, Germany) and CHGA (clone LK2H10, 1:10; Abcam). For negative control, the primary antibody was omitted. The analysis was performed by two independent observers (E.H. and S.G.). The staining intensity (score: 0-3) and the proportion of positive tumour cells (0%-100%) was determined for each spot and given as the product (H-score). For the diagnosis of NE differentiation at least one of the three specific NE markers CD56, SYP or CHGA, had to be positively-detected (4). For distinction from LCNEC and carcinoid tumours, histomorphological signs of a NE growth pattern had to be absent (24). For positive staining, the cut-off was set at ≥10% tumour cells with clear-cut staining. Specimens which were classified non-ND-NSCLC were negative for any specific NE marker. The TMAs were scanned at ×400 magnification using the Aperio ImageScope v10.1.3.2028 software (Aperio Technologies Inc., Vista, CA, USA).

Statistical analysis. The follow-up was defined as the Kaplan-Meier estimate with reversed status indicator. Death censored the true but unknown observation time, censoring was interpreted as end-point. Thus, the unobservable follow-up time of a deceased patient was interpreted as the follow-up time that potentially would have been obtained if the patient had not died. Survival time was determined from the date of first diagnosis until last follow-up or reported death. Non disease-related death was censored. The disease-free survival (DFS) was determined from the date of first diagnosis until diagnosis of relapse or disease-related death. Survival times were analyzed using the Kaplan-Meier method and the log-rank test. For multivariate analysis, the Cox proportional hazards model was used. A value of $p<0.05$ was considered statistically significant. The statistical analyses were performed using SAS® version 9.3 (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics. Tissue specimens of 100 therapy-naïve patients whith NSCLC who underwent complete pulmonary resection were analyzed. Fifty-seven patients had stage I and 43 patients stage II disease (Table I). A total of 75 patients were male. The collective comprised 42 adenocarcinomas, 42 squamous cell carcinomas, 14 pleomorphic carcinomas and two not otherwise specified NSCLC. The median follow-up was 53.3 months, the median DFS and overall survival (OS) were not reached. The relapse rate was 34%, the 1-, 2- and 5-year survival rates were 90%, 79% and 64%, respectively. 34% of the patients died within the follow-up time including 22% disease-related and 12% non-disease-related deaths. The stem cell antigens CD117, CD133 and ABCG2 were expressed in 51%, 13% and 33% of the cases, respectively (Figure 1).

Clinicopathological characteristics and prognosis of patients with ND-NSCLC. Eighty specimens exhibited expression of at least one NE-specific marker and were considered ND-NSCLC (Table I). Twenty-five cases expressed exactly one marker, 51 cases two markers and four cases all three NE markers (Figure 1). SYP and CHGA displayed predominantly diffuse staining, while CD56 showed focal expression (Figure 2, Table III). Seventy percent of the ND-NSCLC exhibited additional expression of the non-specific NE marker NSE. NE differentiation was not associated with gender, age, grading, tumour size, nodal involvement or the proportion of metastatic lymph nodes, but was associated with adenocarcinoma histology: Ninety percent of the adenocarcinomas were found in the ND group ($p=0.035$;

Table I. *Patient characteristics. Clinicopathological characteristics of the total study population and the subgroups of non-small cell lung cancer (NSCLC) with (ND-NSCLC) and without neuroendocrine differentiation. The subgroups were compared by Chi-square, log-rank and Wilcoxon rank sum test; p<0.05 was considered statistically significant.*

Clinicopathological characteristics	Total	ND-NSCLC	Non ND-NSCLC	*p*-Value
	n=100	n=80	n=20	
Gender				
Male	75 (75%)	62 (77%)	13 (65%)	0.248
Female	25 (25%)	18 (23%)	7 (35%)	
Age (years)				
Mean±SEM	63.4±8.3	62.7±8.7	66.4±5.8	0.070
Stage (UICC)				
IA	22 (22%)	20 (25%)	2 (10%)	0.304
IB	35 (35%)	28 (35%)	7 (35%)	
IIA	25 (25%)	20 (25%)	5 (25%)	
IIB	18 (18%)	12 (15%)	6 (30%)	
PT				
T1	16 (16%)	15 (19%)	1 (5%)	0.134
T2	84 (84%)	65 (81%)	19 (95%)	
pN				
N0	66 (66%)	55 (69%)	11 (55%)	0.246
N1	34 (34%)	25 (31%)	9 (45%)	
Metastatic lymph nodes (%)				
N1	n=34	n=25	n=9	0.172
Mean±SEM (n=34)	12.9±15.6	12.6±17.3	13.6±10.5	
Histology				
AC	42 (42%)	38 (48%)	4 (20%)	0.035
SCC	42 (42%)	33 (41%)	9 (45%)	
NSCLC, other	14 (14%)	8 (10%)	6 (30%)	
NOS	2 (2%)	1 (1%)	1 (5%)	
Grading				
≤G2	33 (34%)	28 (36%)	5 (25%)	0.358
>G2	65 (66%)	50 (64%)	15 (75%)	
Missing data	n=2	2	0	
Smoking status				
Ever	69 (69%)	54 (67%)	15 (75%)	0.599
Never	1 (1%)	1 (1%)	0 (0%)	
Missing	n=30	25	5	
Surgery				
Lobectomy	82 (82%)	67 (84%)	15 (75%)	0.307
Bi-lobectomy	4 (4%)	4 (5%)	0 (0%)	
Pneumonectomy	12 (12%)	8 (10%)	4 (20%)	
Wedge resection	2 (2%)	1 (1%)	1 (5%)	
Relapse				
Relapse rate	34 (34%)	27 (34%)	7 (35%)	0.916
Median DFS (months)	n.r	n.r.	n.r.	n.a.
Mortality				
Death total	34 (34%)	28 (35%)	6 (30%)	0.673
Disease-related death	22 (22%)	17 (21%)	5 (25%)	0.717
Non-disease-related death	12 (12%)	11 (14%)	1 (5%)	0.281
Missing data	n=1	1	0	n.a.
Survival				
1-year rate	88 (90%)	70 (90%)	18 (90%)	0.495
2-year rate	75 (79%)	61 (79%)	16 (80%)	
5-year rate	30 (64%)	21 (61%)	9 (74%)	
Median OS months	n.r.	n.r.	n.r	n.a.
Follow-up (months)				
Median	53.3	51.6	61.1	0.225
Stem cell antigens				
CD117				
Positive	49 (49%)	41 (51%)	8 (40%)	0.363
negative	51 (51%)	39 (49%)	12 (60%)	
CD133				
Positive	11 (11%)	11 (14%)	0 (0%)	0.079
Negative	89 (89%)	69 (86%)	20 (100%)	
ABCG2				
Positive	30 (30%)	26 (33%)	4 (20%)	0.275
Negative	70 (70%)	54 (67%)	16 (80%)	

SEM: Standard error of the mean, UICC: Union internationale contre le cancer, AC: adenocarcinoma, SCC: squamous cell carcinoma, NOS: not otherwise specified NSCLC, DFS: disease-free survival, OS: overall survival, ABCG2: breast cancer resistance protein-1, n.r.: not reached, n.a.: not applicable.

Table I). There were no significant differences in relapse, mortality and the 1-, 2- and 5-year survival rates between ND-NSCLC and non-ND-NSCLC. Moreover, Cox proportional-hazards regression analysis showed no association between NE differentiation, DFS [hazard ratio (HR)=1.1, 95% confidence interval (CI)=0.4-2.6, p=0.906] or OS (HR=1.4, 95% CI=0.6-3.3, p=0.497). Division of the ND-NSCLC according to the number of positive NE markers (1 *vs*. >1 NE marker) revealed also no differences in DFS (HR=1.1, 95% CI=0.7-1.7, p=0.754) or OS (HR=1.0, 95% CI=0.7-1.6, p=0.839) (Table II).

Expression and prognostic significance of stem cell antigens in ND-NSCLC. The stem cell antigens CD117, CD133 and ABCG2 were expressed in 51%, 14% and 33% of the ND-NSCLC, respectively, but showed no association with NE differentiation (Table I). However, there was a trend towards more frequent expression of CD117 (58% *vs*. 36%, p=0.066) and CD133 (18% *vs*. 4%, p=0.088) in samples positive for more than one NE marker (Table II). CD117 exhibited mostly diffuse staining, while CD133 and ABCG2 were mainly arranged in clusters (Table III, Figure 3). Univariate Cox proportional-hazards regression analysis revealed no association between the expression of stem cell antigens and DFS or OS in ND-NSCLC (Table IV). In the subgroup of patients with expression of only one specific NE marker, multivariate analysis suggested that co-expression of CD117 might be associated with a 5.2-fold increased risk of disease-

Table II. *Patients' characteristics of non-small cell lung cancer with neuroendocrine differentiation (ND-NSCLC). Clinicopathological characteristics of ND-NSCLC subdivided into ND-NSCLC with expression of exactly one specific neuroendocrine marker [ND-NSCLC (1)] and ND-NSCLC with expression of more than one specific neuroendocrine marker [ND-NSCLC (>1)]. ND-NSCLC (1) and ND-NSCLC (>1) were compared by Chi-square, log-rank and Wilcoxon rank sum test; p<0.05 was considered statistically significant.*

Clinicopathological characteristics	Total	ND-NSCLC (1)	ND-NSCLC (>1)	*p*-Value
	n=80	n=25	n=55	
Gender				
Male	62 (77%)	20 (80%)	42 (76%)	0.718
Female	18 (23%)	5 (20%)	13 (24%)	
Aqe (years)				
Mean±SEM	62.7±8.7	61.7±8.7	63.2±8.7	0.460
Stage (UICC)				
IA	20 (25%)	5 (20%)	15 (27%)	0.119
1B	28 (35%)	13 (52%)	15 (27%)	
IIA	20 (25%)	3 (12%)	17 (31%)	
IIB	12 (15%)	4 (16%)	8 (15%)	
pT				
T1	15 (19%)	4 (16%)	11 (20%)	0.671
T2	65 (81%)	21 (84%)	44 (80%)	
pN				
N0	55 (69%)	19 (76%)	36 (66%)	0.346
N1	25 (31%)	6 (24%)	19 (34%)	
Metastatic lymph nodes (%)				
N1	n=25	n=6	n=19	0.265
Mean±SEM (n=34)	12.6±17.3	19.1±25.5	10.5±14.1	
Histology				
AC	38 (48%)	8 (32%)	30 (55%)	0.225
SCC	33 (41%)	14 (56%)	19 (34%)	
NSCLC, other	8 (10%)	3 (12%)	5 (9%)	
NOS	1 (1%)	0 (0%)	1 (2%)	
Grading				
≤G2	28 (36%)	8 (33%)	20 (37%)	0.753
>G2	50 (64%)	16 (67%)	34 (63%)	
Missing	2	1	1	
Smoking status				
Ever	54 (67%)	20 (80%)	34 (62%)	0.446
Never	1 (1%)	0 (0%)	1 (2%)	
Missing	n=25	5	20	
Surgery				
Lobectomy	67 (84%)	22 (88%)	45 (82%)	0.476
Bilobectomy	4 (5%)	0 (0%)	4 (7%)	
Pneumonectomy	8 (10%)	3 (12%)	5 (9%)	
Wedge resection	1 (1%)	0 (0%)	1 (2%)	
Relapse				
Relapse rate	27 (34%)	6 (24%)	21 (38%)	0.214
Median DFS (months)	n.r.	n.r.	n.r.	n.a.
Mortality				
Death total	28 (35%)	9 (36%)	19 (35%)	0.899
Disease-related death	17 (21%)	3 (12%)	14 (25%)	0.173
Non-disease-related death	11 (14%)	6 (24%)	5 (2%)	0.073
Missing data	n=1	1	0	n.a.
Survival				
1-year rate	70 (90%)	20 (87%)	50 (91%)	0.411
2-year rate	61 (79%)	15 (69%)	46 (84%)	
5-year rate	21 (61%)	8 (56%)	17 (63%)	
Median OS (months)	n.r.	n.r.	n.r.	n.a
Follow-up (months)				
Median	51.6	34.8	54.5	0.016
Stem cell antigens				
CD117				
Positive	41 (51%)	9 (36%)	32 (58%)	0.066
Negative	39 (49%)	16 (64%)	23 (42%)	
CD133				
Positive	11 (14%)	1 (4%)	10 (18%)	0.088
Negative	69 (86%)	24 (96%)	45 (82%)	
ABCG2				
Positive	26 (33%)	8 (32%)	18 (33%)	0.949
Negative	54 (67%)	17 (68%)	37 (67%)	

SEM: Standard error of the mean, UICC: Union internationale contre le cancer, AC: adenocarcinoma, SCC: squamous cell carcinoma, NOS: not otherwise specified NSCLC, DFS: disease-free survival, OS: overall survival, ABCG2: breast cancer resistance protein-1, n.r.: not reached, n.a.: not applicable.

related death (HR=5.2, 95% CI=1.1-23.9, p=0.033), while co-expression of ABCG2 might tentatively be associated with a 8.1-fold reduced risk of disease-related death (HR=0.1, 95% CI=0.0-1.1, p=0.063) (Table V, Figure 4A and B).

Discussion

In this study, we analyzed the prognostic significance of NE differentiation in 100 patients with completely resected early-stage NSCLC. Moreover, we analyzed the prognostic significance of the stem cell antigens CD117, CD133 and ABCG2 in the subgroup of ND-NSCLC. We found no association between the expression of the specific NE markers SYP, CHGA, CD56 and prognosis. This is in line with most previous studies on the relevance of NE differentiation in resected NSCLC, albeit those studies used other criteria for NE differentiation ranging from weak staining for any NE marker in 1% of the tumour cells to expression of two or more NE markers in >5% of the tumour cells (25-30). Due to these differences in assessment and the results of Harada *et al*. and Schleusner *et al*., who reported a significantly better overall survival in patients expressing two or more NE markers, we also analyzed, whether the number of expressed NE markers might play a prognostic role (3, 31). In the current collective, we found no correlation between the number of expressed NE markers and relapse or survival. However, NE differentiation showed a significant correlation with AC histology: 90% of

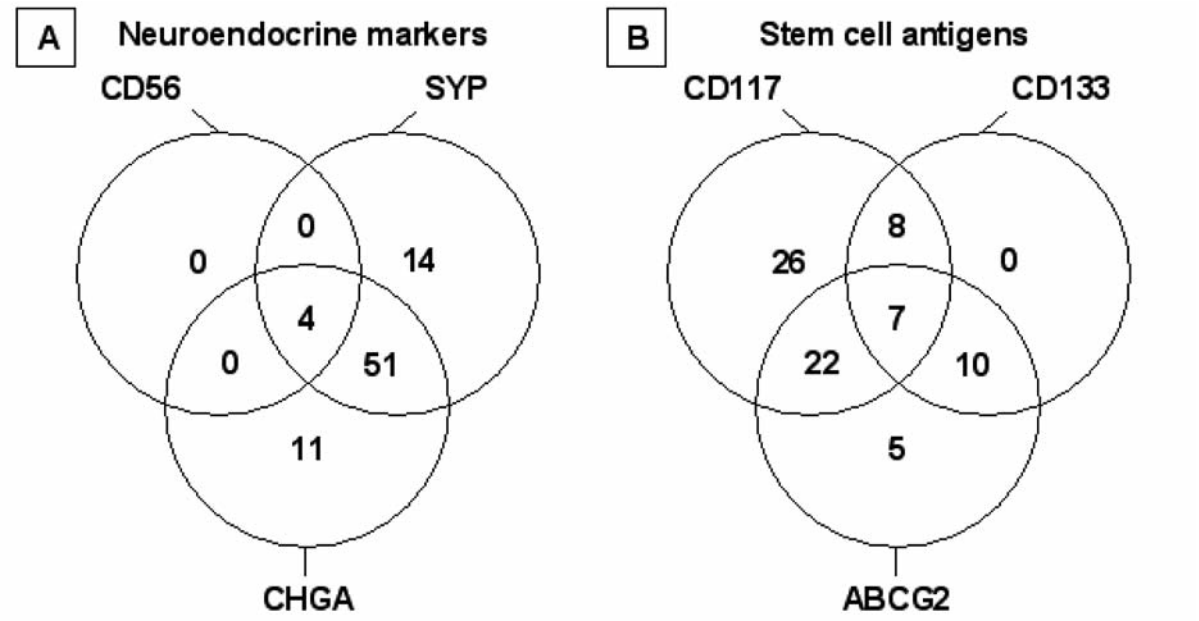

Figure 1. *Expression of neuroendocrine markers and stem cell antigens. Numbers of samples that express the respective neuroendocrine marker (A) or stem cell antigens (B) are shown. Tissue from a total of 100 patients with resected early-stage non-small cell lung cancer were analyzed. SYP: synaptophysin, CHGA: chromogranin A, ABCG2: breast cancer resistance protein-1.*

the AC were comprised in the subgroup of ND-NSCLC (p=0.035), while other clinicopathological characteristics were not associated. Except for Sundaresan *et al*. (25) and Slodkowska *et al*. (32), who found a correlation between NE differentiation and disease stage in 359 and 85 cases of resected NSCLC, respectively, a previous study reported similarly a higher rate of NE differentiation in AC, but no correlation with other clinicopathological features (29). In line with this correlation between AC and NE differentiation, a recent analysis in mice tracing the early events of pulmonary NE cell (PNEC) transformation, demonstrated that PNEC might contribute to Clara and ciliated cells, which represent putative progenitor cells for AC (33).

Although several studies demonstrated expression of stem cell antigens in pulmonary NE carcinomas, analyses of stem cell antigens in ND-NSCLC are virtually lacking (5, 9, 12). In this study, the stem cell antigens CD117, CD133 and ABCG2 were expressed in 51%, 14% and 33% of the ND-NSCLCs, respectively. Compared to the expression rates reported for NSCLC overall, the staining frequencies in ND-NSCLC did not differ significantly (7, 17, 20, 34, 35). However, while CD117 and ABCG2 showed no significant correlation with NE differentiation, the expression of CD133 was exclusively, albeit not significantly, restricted to ND-NSCLC. When ND-NSCLC were sub-classified according to the number of positive NE markers, there was a strong trend towards an increased frequency of stem cell antigens in NSCLC which were positive for more than one specific NE marker: While only 9%-31% of the cases with expression of exactly one NE marker were positive for CD117, CD133 or ABCG2, 69%-91% of the cases with expression of more than one marker were positive for at least one of the stem cell antigens. This suggests that stem cell characteristics might increase with increasing grade of NE differentiation.

Table III. *Immunohistochemical results showing the proportion of positive samples and staining pattern for stem cell antigens and neuroendocrine markers.*

	Immunohistochemical results			
	Total positive	Single-cell	Cluster	Diffuse
Stem cell antigens				
CD117	n=49	0 (0%)	8 (16%)	41 (84%)
CD133	n=11	0 (0%)	8 (73%)	3 (27%)
ABCG2	n=30	0 (0%)	19 (63%)	11 (37%)
Neuroendocrine markers				
SYP	n=69	0 (0%)	11 (16%)	58 (84%)
CHGA	n=66	0 (0%)	6 (9%)	60 (91%)
CD56	n=4	0 (0%)	4 (100%)	0 (0%)

ABCG2: Breast cancer resistance protein-1, SYP: synaptophysin, CHGA: chromogranin A.

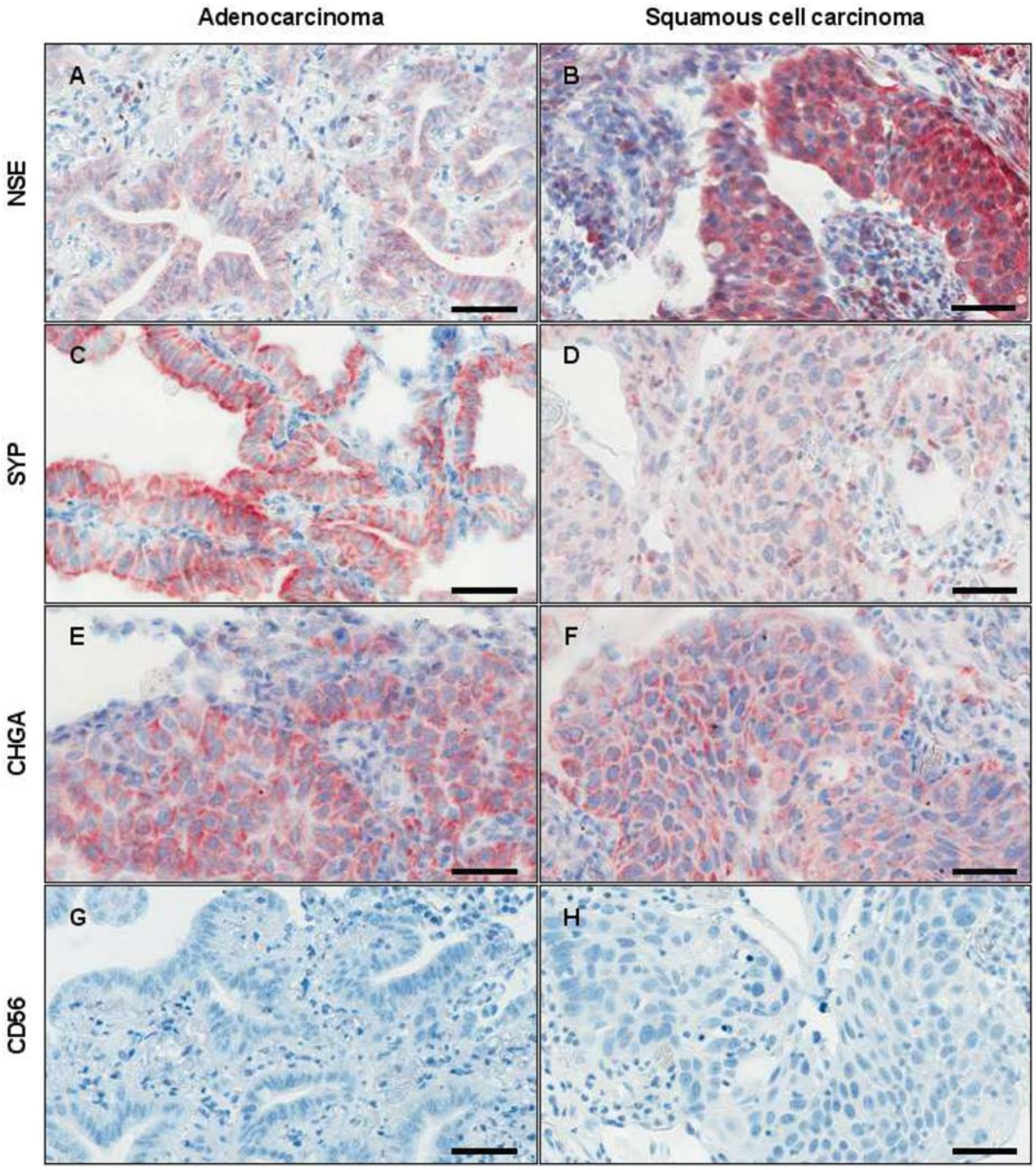

Figure 2. *Representative immunostaining for neuroendocrine markers. The microphotographs show an adenocarcinoma with lepidic growth pattern and a squamous cell carcinoma. Both were considered as non-small cell lung cancer with neuroendocrine differentiation. The specimens display diffuse expression of neuron-specific enolase (NSE) and the specific neuroendocrine markers synaptophysin (SYP) and chromogranin A (CHGA), while CD56 is negative. Basic stain: haematoxylin, magnification: ×200, scale bar=50 μm.*

In the entire population of ND-NSCLCs, expression of stem cell antigens had no prognostic significance. In the subgroup of ND-NSCLC with expression of exactly one specific NE marker, multivariate analysis suggested that co-expression of CD117 might be associated with a 5.2-fold increased and co-expression of ABCG2 with an 8.1-fold reduced risk of disease-related death. However, the significance of these results is strongly limited and requires

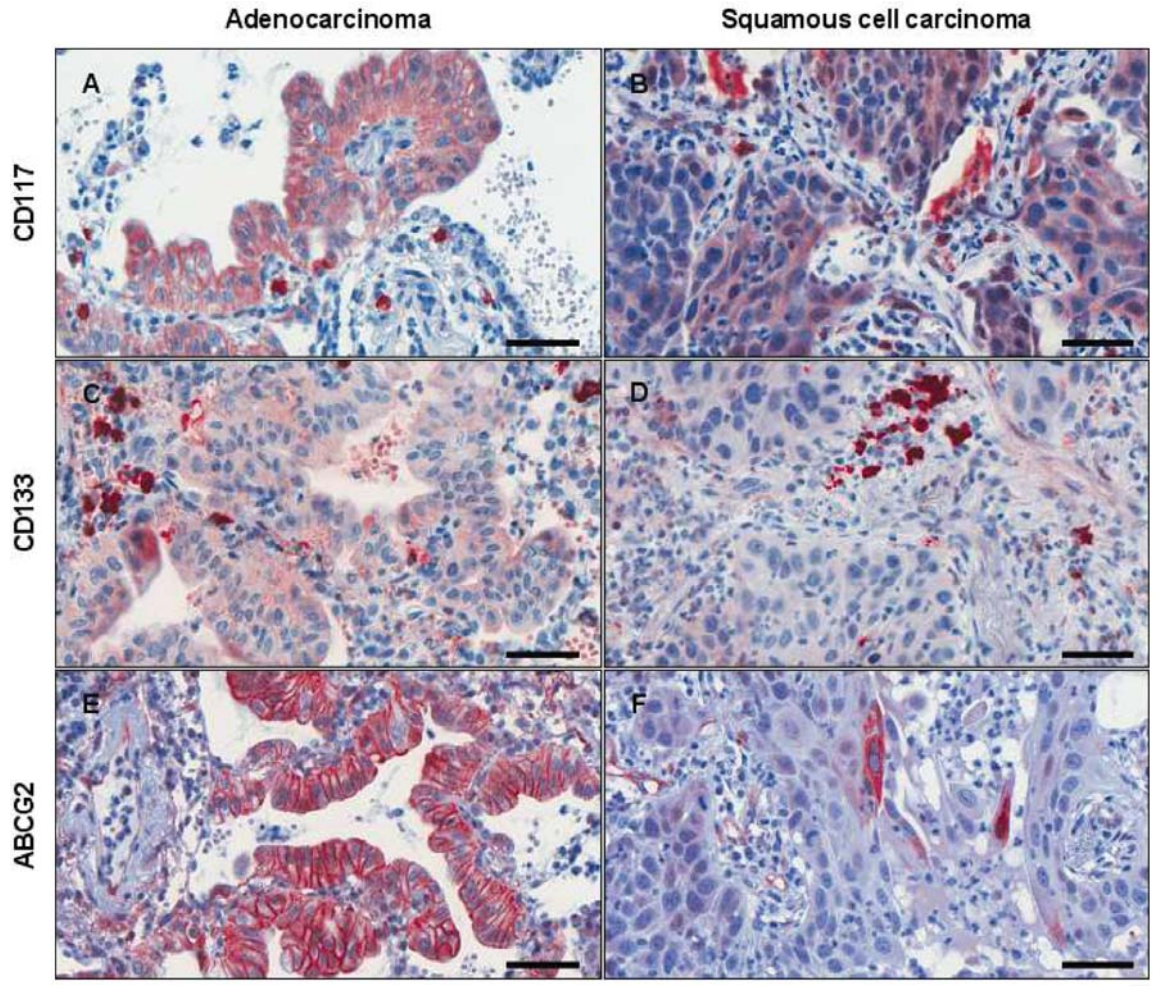

Figure 3. *Representative immunostaining for stem cell antigens. The microphotographs show an adenocarcinoma with lepidic growth pattern and a squamous cell carcinoma. While the adenocarcinoma is positive for CD117, CD133 and breast cancer resistance protein-1 (ABCG2), the squamous cell carcinoma exclusively displays expression of CD117. Basic stain: haematoxylin, magnification: ×200, scale bar=50 μm.*

confirmation in larger collectives since the subgroup of ND-NSCLC with expression of exactly one NE marker comprised of only 23 evaluable individuals, and moreover, 50% of the CD117-negative and 25% of the ABCG2-negative cases in this subgroup were censored within the first three years after surgery, resulting in a significantly shorter follow-up than the comparison group.

In summary, this study on resected early-stage NSCLC revealed that neither NE differentiation, nor the number of positive NE markers, nor co-expression of the stem cell antigens CD117, CD133 or ABCG2 in ND-NSCLC has any prognostic relevance. However, the significant correlation between ND and AC histology and the higher frequency of CD117 and CD133 staining in NSCLC with expression of more than one specific NE marker might indicate a pathogenetic link between stem cells, NE differentiation and AC.

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Conflicts of Interest

None declared.

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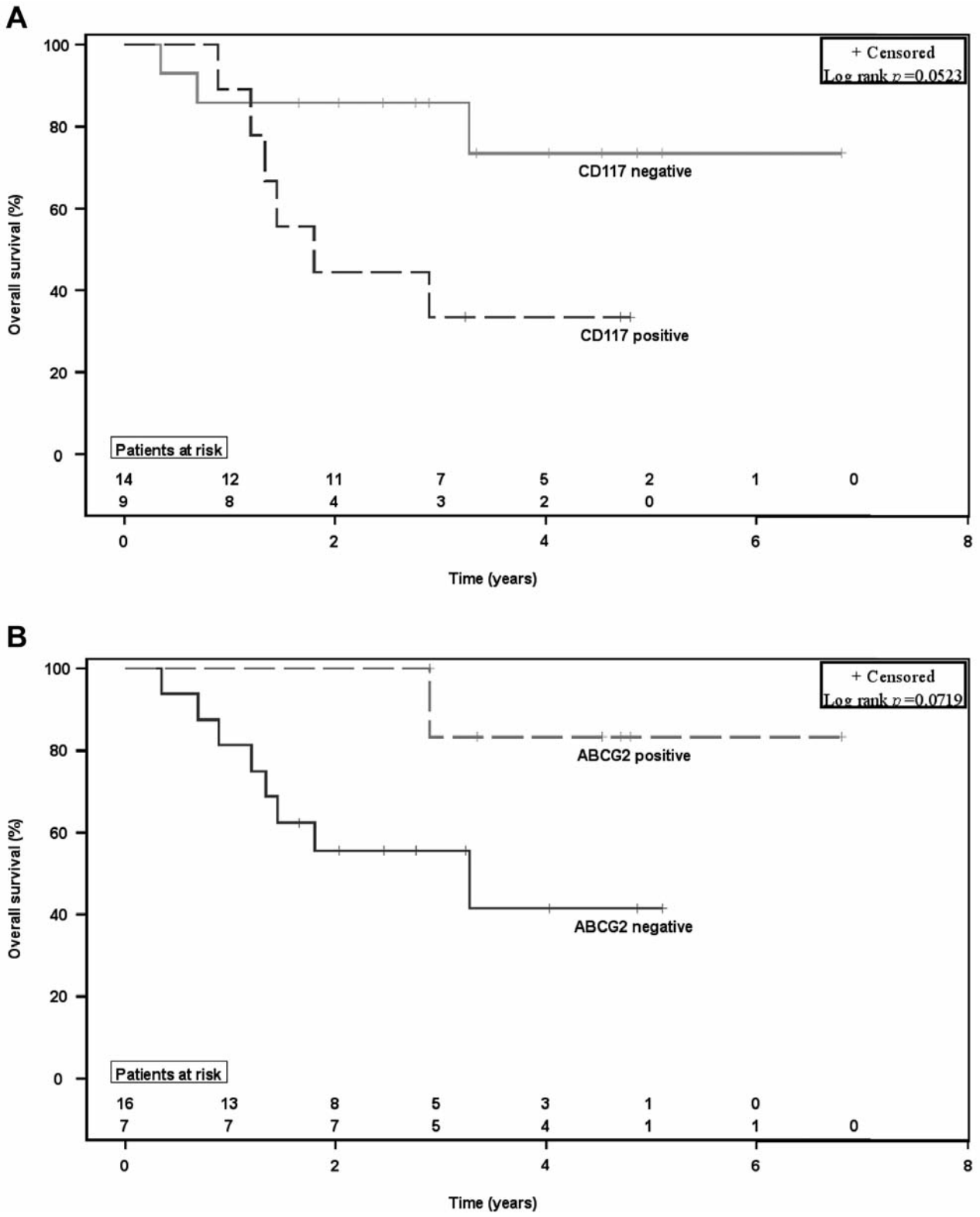

Figure 4. *Overall survival of patients with non-small cell lung cancer with neuroendocrine differentiation (ND-NSCLC) stratified for CD117 (A) and breast cancer resistance protein-1 (ABCG2) expression. The survival curves (Kaplan-Meier estimates) of patients positive for exactly one specific neuroendocrine marker show a strong trend towards reduced overall survival upon co-expression of CD117 (A) and a trend towards increased overall survival upon co-expression of ABCG2. The numbers above the x-axis represent the patients at risk at the respective time point.*

Table IV. *Univariate analysis of stem cell antigens in non-small cell lung cancer with neuroendocrine differentiation (ND-NSCLC). Univariate Cox proportional-hazards regression analysis revealed no association between the expression of stem cell antigens and disease-free or overall survival in ND-NSCLC expressing one [ND-NSCLC (1)] or more than one [ND-NSCLC (>1)] specific neuroendocrine marker; p<0.05 was considered statistically significant.*

	Univariate analysis	
Stem cell antigen	HR (95% CI)	*p*-Value
Disease-free survival		
ND-NSCLC (1)		
CD117	1.2 [0.2-7.1]	0.850
CD133	n.d.	n.d.
ABCG2	0.5 [0.1-4.3]	0.511
ND-NSCLC (>1)		
CD117	1.5 [0.5-4.2]	0.436
CD133	0.9 [0.3-3.2]	0.869
ABCG2	1.9 [0.7-5.0]	0.212
Overall survival		
ND-NSCLC (1)		
CD117	3.7 [0.9-14.8]	0.069
CD133	n.d.	n.d.
ABCG2	0.2 [0.0-1.5]	0.108
ND-NSCLC (>1)		
CD117	1.6 [0.6-4.3]	0.311
CD133	1.1 [0.4-3.3]	0.853
ABCG2	1.0 [0.4-2.5]	0.994

ABCG2: Breast cancer resistance protein-1, HR: adjusted hazard ratio, 95% CI: 95% confidence interval, n.d.: not done due to insufficient number of cases.

Table V. *Multivariate analysis of stem cell antigens in non-small cell lung cancer with neuroendocrine differentiation (ND-NSCLC). Multivariate Cox proportional-hazards regression analysis revealed no association between the expression of stem cell antigens and disease-free survival in ND-NSCLC expressing one [ND-NSCLC (1)] or more than one [ND-NSCLC (>1)] specific neuroendocrine marker. In ND-NSCLC (1), co-expression of CD117 appears to predict a 5.2-fold better overall survival and co-expression of ABCG by trend a 8.1-fold worse overall survival; p<0.05 was considered statistically significant.*

	Multivariate analysis	
Stem cell antigen	Adjusted HR (95% CI)	*p*-Value
Disease-free survival		
ND-NSCLC (1)		
CD117	1.1 [0.2-6.8]	0.892
CD133	n.d.	n.d.
ABCG2	0.6 [0.1-5.1]	0.608
ND-NSCLC (>1)		
CD117	1.2 [0.4-3.7]	0.785
CD133	0.5 [0.1-2.0]	0.331
ABCG2	2.3 [0.7-7.8]	0.172
Overall survival		
ND-NSCLC (1)		
CD117	5.2 [1.1-23.9]	0.033
CD133	n.d.	n.d.
ABCG2	0.1 [0.0-1.1]	0.063
ND-NSCLC (>1)		
CD117	1.7 [0.6-4.9]	0.281
CD133	1.1 [0.3-4.3]	0.853
ABCG2	0.8 [0.2-2.5]	0.672

ABCG2: Breast cancer resistance protein-1, HR: adjusted hazard ratio, 95% CI: 95% confidence interval, n.d.: not done due to insufficient number of cases.

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