Lung Cancer 167 (2022) 73–77

Contents lists available at ScienceDirect

Lung Cancer

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

# EpCAM-positive disseminated cancer cells in bone marrow impact on survival of early-stage NSCLC patients

Tobias Mederer [a,1], Felix Elsner [a,e,1], Tobias Robold [c], Christian Großer [d], Reiner Neu [c], Michael Ried [c], Sabine Bleicher [a], Thomas Schamberger [a], Isabell Blochberger [a], Hans-Stefan Hofmann [c,d], Christoph A. Klein [a,b,*]

[a] Chair of Experimental Medicine and Therapy Research, University Hospital of Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany
[b] Division of Personalized Tumor Therapy, Fraunhofer Institute of Experimental Medicine and Toxicology, Am Biopark 9, 93053 Regensburg, Germany
[c] Department of Thoracic Surgery, University Hospital of Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany
[d] Department of Thoracic Surgery, Hospital Barmherzige Brüder, Regensburg, Prüfeninger Str. 86, 93049 Regensburg, Germany
[e] Institute of Pathology, University Hospital, Friedrich-Alexander-University Erlangen-Nürnberg, Krankenhausstraße 8-10, 91054 Erlangen, Germany

ARTICLE INFO

*Keywords:*
Disseminated cancer cell
Metastasis
Early dissemination
Bone marrow
Lung cancer
Biomarker

ABSTRACT

*Introduction:* Detection of disseminated cancer cells (DCC) in bone marrow (BM) of patients with early-stage NSCLC has been associated with poor outcome. However, the phenotype, and hence relevant therapy targets, of DCCs in BM are unknown. We therefore compared a classical pan-Cytokeratin (CK) antibody for DCC detection with an anti-EpCAM antibody that may also detect more stem-like cells and tested whether assay positivity impacts on the survival of NSCLC patients.

*Materials and methods:* We prospectively collected BM aspirates from 104 non-metastasized NSCLC patients that underwent potentially curative tumor resection from 2011 to 2016 at the Department of Thoracic Surgery of the University Hospital and Hospital Barmherzige Brüder in Regensburg. DCCs were detected by staining with the pan anti-CK antibody A45-B/B3 and the anti-EpCAM antibody HEA-125. We analyzed the association between detection of DCCs and clinicopathological characteristic and patient outcome.

*Results:* CK + and EpCAM + DCCs were detected in 45.2% and 52.9% of patients, respectively. Correlation between the two markers was low and neither of them was associated with sex, age, histology, T or N classification, resection status, grading or smoking habit. No significant association with tumor specific survival (TSS) and progression-free survival (PFS) was observed in patients with CK + DCCs. In contrast, detection of EpCAM + DCCs significantly correlated with reduced PFS ($P = 0.017$) and TSS ($P = 0.017$) and remained an independent prognostic variable for PFS and TSS upon multivariate testing (hazard ratio: 7.506 and 3.551, respectively). Detection of EpCAM + DCCs was the only prognostic marker for PFS.

*Conclusions:* EpCAM+, but not CK + DCCs in BM predict reduced PFS and TSS. This finding suggests that EpCAM + DCCs in the BM comprise metastatic founder cells necessitating their in-depth molecular analysis for detection of novel therapy targets.

## 1. Introduction

Prognosis of NSCLC patients is still dismal [1]. Even after diagnosis at early stages of the disease followed by curatively intended tumor resection and adjuvant chemo and/or radiotherapy, up to 50% of the patients relapse within 5 years [2,3]. Available data indicate that dissemination of lung cancer cells starts years before lung primary tumors reach the size of a T1 stage cancer [4], suggesting that targeting of early disseminated cancer cells (DCCs) is mandatory for the prevention of metachronous metastasis. However, detection of DCCs and particularly their molecular characterization is hampered by the circumstantial knowledge about markers identifying disease-driving cells.

* Corresponding author at: Chair of Experimental Medicine and Therapy Research, University Hospital of Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany.
*E-mail address:* christoph.klein@ukr.de (C.A. Klein).
[1] These authors contributed equally.

https://doi.org/10.1016/j.lungcan.2022.02.008
Received 22 December 2021; Received in revised form 15 February 2022; Accepted 18 February 2022
Available online 24 February 2022

Two major detection markers for DCCs in bone marrow have been widely explored, namely antibodies directed against epithelial cytokeratins and against EpCAM, an epithelial associated surface marker. Bone marrow as surrogate detection site of hematogenous systemic cancer spread is not only attractive because of the absence of autochthonous epithelial cells, but also because recent experimental data indicate that metastatic precursor cells receive important instructive signals enabling metastasis formation elsewhere [5].

Assuming that Cytokeratin expression generally marks most epithelial cells [6], whereas EpCAM is also a marker for pluripotent (lung cancer) stem cells [7,8], we directly compared their detection in bone marrow of 104 non-metastasized and histologically verified NSCLC patients. The simultaneous analysis of the two most widely used markers for DCC detection in NSCLC should allow assessment of their prognostic significance for non-metastasized NSCLC patients.

## 2. Materials and methods

### 2.1. Patient and bone marrow samples

From 2011 to 2016, patients undergoing potentially curative surgery for presumed or histologically verified Non-Small Cell Lung Cancer (NSCLC) at the Department for Thoracic Surgery of the University Hospital Regensburg or the Hospital Barmherzige Brüder Regensburg were prospectively enrolled in this study. Bone marrow aspirates were collected as described previously [9]. All aspects of this study were approved by the local ethics committee at the University of Regensburg (protocol number 07–079) and the patients provided written, informed consent.

### 2.2. Sample processing

Bone marrow aspirates were processed as described in [9]. For details, please see Supplementary information.

### 2.3. Cytokeratin-staining

CK- staining was performed according to the consensus protocol of DCC detection [10]. For details, please see Supplementary information.

### 2.4. Immunomagnetic depletion and EpCAM-staining

To remove weakly EpCAM + erythroblastic cells [11] and other BM populations, EpCAM-high DCCs were enriched using negative immunomagnetic depletion as described in [9]. For details, please see Supplementary information.

### 2.5. Screening of bone marrow for disseminated cancer cells

Stained adhesion slides or immunofluorescently labelled cell suspensions were manually screened for CK or EpCAM + single cells using a microscope (Olympus) or inverted fluorescent microscope (Olympus or Zeiss), respectively. To minimize inter-observer variation, standardized criteria for the detection of EpCAM + DCCs were established and used throughout the study. Positivity was assigned only to cells displaying bright and homogenous surface-staining of EpCAM that remained clearly visible after photo acquisition with an exposure time of 1 sec. Furthermore, cells displaying autofluorescence in the FITC channel were excluded as possibly confounding dead cells. Screening of CK-stained slides was performed using consensus criteria for DCC detection [10] and followed validation and verification criteria according to ISO17020 rules. The total number of positively stained cells was documented as the number of DCCs per million screened MNCs (disseminated cancer cell density, DCCD).

### 2.6. Whole transcriptome amplification and gene expression analysis by PCR

Whole transcriptome amplification (WTA) and gene-specific PCR was performed as described in [12,9]. For details, please see Supplementary information.

### 2.7. Study inclusion criteria

NSCLC patients that presented with overt metastases at the time of surgery (n = 13) were excluded from this study. Furthermore, patients with positively stained cells in the isotype control were excluded from the analysis (n = 24). Lastly, only patients with results for both CK and EpCAM-staining were included, which resulted in a total of n = 104 patients.

### 2.8. Statistical analysis

Statistical testing for associations of categorical variables was performed using the Chi-square ($X^2$) test. Correlation analysis was performed using the nonparametric Spearman correlation method. Survival curves were created with the Kaplan-Meier method and compared using the univariate log-rank test. When analyzing more than two groups, the results were corrected for multiple testing and considered statistically significant using the family-wise significance level of 0.05. Multivariate testing was performed using the Cox regression model. All statistical analysis was performed by using the software GraphPad Prism 6 (GraphPad Software, San Diego, California USA) or IBM SPSS Statistics (Version 25, IBM Corp., Chicago, IL). A $P$-value of $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Overview of the patient cohort

Bone marrow aspirates from 104 non-metastasized (n = 50, 22, 32 for UICC stage I, II, III, respectively) and histologically confirmed NSCLC patients met the study inclusion criteria. An overview of the patient cohort and the clinicopathological characteristics can be found in Supplementary Table 1.

### 3.2. Patient outcome and survival

The median follow-up time was 40.3 months (range 1.1–80.4 months). During the follow-up period 29 of the 104 (27,9%) patients died, while the death of 18 (17.3%) patients was directly caused by NSCLC. First relapse events within the observation period presented as local relapse (n = 7), lymph node metastasis (n = 2) or distant metastasis (n = 12) in a total of 17 patients (16.3%).

### 3.3. Associations of clinicopathological characteristics with patient outcome and survival

Statistical associations between clinicopathological characteristics and patient outcome are shown in Suppl. Fig. 1 + 2. Univariate analysis revealed no significant association between sex, age, histology, tumor size, grading or smoking habits and PFS (Suppl. Fig. 1a–d, g–h) or TSS (Suppl. Fig. 2a–d, g–h). Detection of lymph node metastasis (pN1-3) resulted in decreased TSS ($P = 0.0002$) when compared to patients without lymph node involvement (pN0) as shown in Suppl. Fig. 2e. For PFS, a separation between pN0 and pN1-3 patients could be seen, but was not significant ($P = 0.2911$; Suppl. Fig. 1e). Complete tumor resection (microscopically tumor-free resection margin; R0) resulted in significantly improved PFS ($P = 0.0252$) and TSS ($P = 0.0009$) when compared to patients with microscopic or macroscopic residual tumor (R1 and R2; Suppl. Fig. 1f, 2f).

### 3.4. Detection of DCCs in bone marrow by Cytokeratin and EpCAM staining

All 104 patients were analyzed for DCCs detected by the antibodies against Cytokeratin and EpCAM. For enrichment of CK + DCCs we used density gradient centrifugation and for EpCAM + DCC immunomagnetic depletion [9] which would result in a median 11.9-fold (interquartile range: 6.8 – 26.5) higher frequency of EpCAM cells, if no loss of DCCs occurred and both markers were fully co-expressed (Suppl. Fig. 3). However, we observed only a minor difference in prevalence. At least one CK + DCC could be found in the bone marrow of 47 (45.2%) patients after screening a median number of $2 \times 10^6$ MNCs (mean: $2.28 \times 10^6$; range: $2 \times 10^6$ –$6 \times 10^6$ MNCs). Similarly, EpCAM-staining with the PE-conjugated monoclonal antibody clone HEA-125 (Miltenyi Biotec, Germany) after immunomagnetic depletion of hematopoietic cells revealed at least one EpCAM + DCC in BM aspirates of 55 (52.9%) patients. The median number of screened cells was $2 \times 10^6$ depleted MNCs (mean: $1.61 \times 10^6$; range: $0.10 \times 10^6$ –$4 \times 10^6$ depleted MNCs). This discrepancy between the theoretical enrichment (expected 12-fold higher numbers of DCCs detected by EpCAM) and the observed data could indicate (i) either a selective loss of EpCAM + DCC during depletion or (ii) that EpCAM + DCCs are less frequent than CK + DCCs. Neither for the detection of CK + nor for EpCAM + cells in bone marrow, a significant association with analyzed clinicopathological variables could be noted ($P > 0.05$; Supplementary Table 2). In DCC-positive patients systemic or local therapies were given in similar frequency to subgroups (Suppl. Table 3). We further found no evidence for selective sensitivity of CK vs. EpCAM DCCs in patients receiving neoadjuvant therapy (n = 6; $P = 0.8074$ for CK and $P = 0.8841$ for EpCAM; $X^2$-test; Suppl. Table 4), since DCCs with either of these markers were detected after treatment. We then evaluated the frequencies of post-relapse therapy (chemotherapy and/or radiation) administered in the EpCAM + and CK + groups. The difference was not significant (69.2% vs. 44.4%, respectively; $P = 0.245$, $X^2$ test; Suppl. Table 5), however indicates that patients with EpCAM + DCCs at surgery might become in need of further treatment more frequently.

### 3.5. Cytokeratin and EpCAM positivity define subgroups of patients with different outcome

We first compared patients positive by either or both markers with patients that were negative in both assays. Patients with no DCCs displayed excellent survival, whereas DCC-positivity imposed a significant risk for PFS and TSS (PFS, $P = 0.026$; TSS, $P = 0.065$; Fig. 1a). When we analyzed the impact of CK-positivity and EpCAM-positivity separately (Fig. 1b-c), only EpCAM + patients displayed worse outcome when compared to negative patients (PFS and TSS in univariate log-rank testing $P = 0.0170$ and $P = 0.0170$, respectively), whereas detection of CK + cells in BM was not associated with PFS or TSS ($P = 0.5365$ and $P = 0.2948$, respectively; Fig. 1b).

We did not find a significant correlation between detection ($P = 0.267$, $X^2$ test) or numbers of EpCAM and CK + DCCs (Spearman r = 0.0424; $P = 0.669$; Suppl. Fig. 4), suggesting that the two antigens may define different subsets of DCCs. Therefore, we interrogated whether single-positivity (i.e. either EpCAM + or CK + assay) or double-positivity (detection of cells in both assays) had a stronger impact on outcome and compared DCC-negative, CK and EpCAM double-positive patients and CK and EpCAM single-positive patients. Using a univariate log-rank test corrected for multiple comparisons only the EpCAM single-positive group demonstrated a significantly reduced PFS ($P = 0.0132$) and TSS ($P = 0.0042$) using the family-wise significance level of 0.05 (Fig. 1d). Upon testing for associations with risk to local relapse, development of lymph node or distant metastasis, we further noted, consistent with a putative role as metastasis-initiating cells, that only EpCAM + DCCs were specifically associated with the development of distant metastasis (Suppl. Fig. 5; $P = 0.0162$).

### 3.6. Detection of EpCAM + cells is an independent prognosticator of outcome

Finally, we adjusted the analysis for confounding (clinicopathological) variables and performed a multivariate Cox regression analysis. Sex, age, histology, tumor size, presence of lymph node metastasis, resection status, grading, smoking habits, and the results of the EpCAM-staining were included in this analysis. For TSS both the N classification [hazard ratio (95% confidence interval): 3.499 (1.671–7.327)] and detection of EpCAM + DCCs [hazard ratio (95% confidence interval): 7.506 (1.476 – 38.182)] remained independent and significant prognostic variables, while the presence of EpCAM + DCCs was the only independent and significant prognostic variable for PFS in these patients (HR (95% CI): 3.551 (1.126– 11.195); Table 1).

### 3.7. Epithelial and mesenchymal gene expression in EpCAM + DCCs

For a first glimpse into the biology of EpCAM + DCCs, we isolated them, performed whole transcriptome amplification (WTA) [12] and PCR-tested for selected histogenetically informative genes (*EpCAM, KRT8, KRT18, KRT19* for epithelial differentiation and *VIM* and *CDH2* for a mesenchymal phenotype) [9]. Interestingly, while the EPCAM transcript was found in 32/38 cells (84%) co-expression with other markers was not frequent (Fig. 1e), suggesting that EpCAM-expression marks a unique cancer cell phenotype that is at least not fully congruent to the classical epithelial-mesenchymal transition (EMT)-dichotomy.

## 4. Discussion

Here we show that detection of DCCs in bone marrow of non-metastasized NSCLC patients correlates with reduced survival. Surprisingly, the prognostic impact apparently depends on the DCC detection marker used. While the presence of CK + DCCs was not associated with patient outcome, detection of EpCAM + DCCs resulted in reduced PFS and TSS. This effect remained significant upon multivariate testing with EpCAM + DCCs being the only independent prognostic marker for PFS.

Our detection rates are consistent with published data [13–16], however clinical impact varied in the different studies [13–17]. Comparability between studies is limited due to the heterogeneity of patient inclusion criteria, sample origin, processing methods and antibody clones used for detection antibodies of DCCs. In our study, the use of different enrichment methods limits the direct comparison of EpCAM and CK + DCCs as well. However, quantitative and molecular data suggest that EpCAM + BM-derived DCCs indeed represent a less frequent and unique phenotype that is associated not only with poor outcome but also specifically with distant metastasis. Dynamic EpCAM expression, reflecting histogenetic plasticity has been reported before and apparently does not follow the classical marker expression of mesenchymal vs. epithelial EMT phenotypes [18,19,20]. Since EpCAM is expressed in embryonic cells [21], tissue-specific stem cells [22,23] and NSCLC stem cells [6], it is tempting to speculate that it directly marks cells with higher metastatic potential than cytokeratin expression. Consistently, an important role for tumor-initiation, proliferation, migration, resistance to chemotherapeutic drugs and survival for NSCLC has been noted [6–8,24], whereas CK with and without EpCAM co-expression might mark a more differentiated DCC population. However, we could not observe a differential impact of systemic therapy on the two cell populations. Finally, the association with distant metastasis formation might indicate that BM-educated DCCs expressing EpCAM, are linked to the establishment of secondary growths elsewhere, consistent with recent experimental data [5].

The limited molecular profiling of EpCAM + DCCs performed here, underscores the need for more comprehensive phenotypic, molecular/genetic and functional characterization. As BM-derived EpCAM + DCCs seem to be clinically relevant, they could be prime targets for therapy

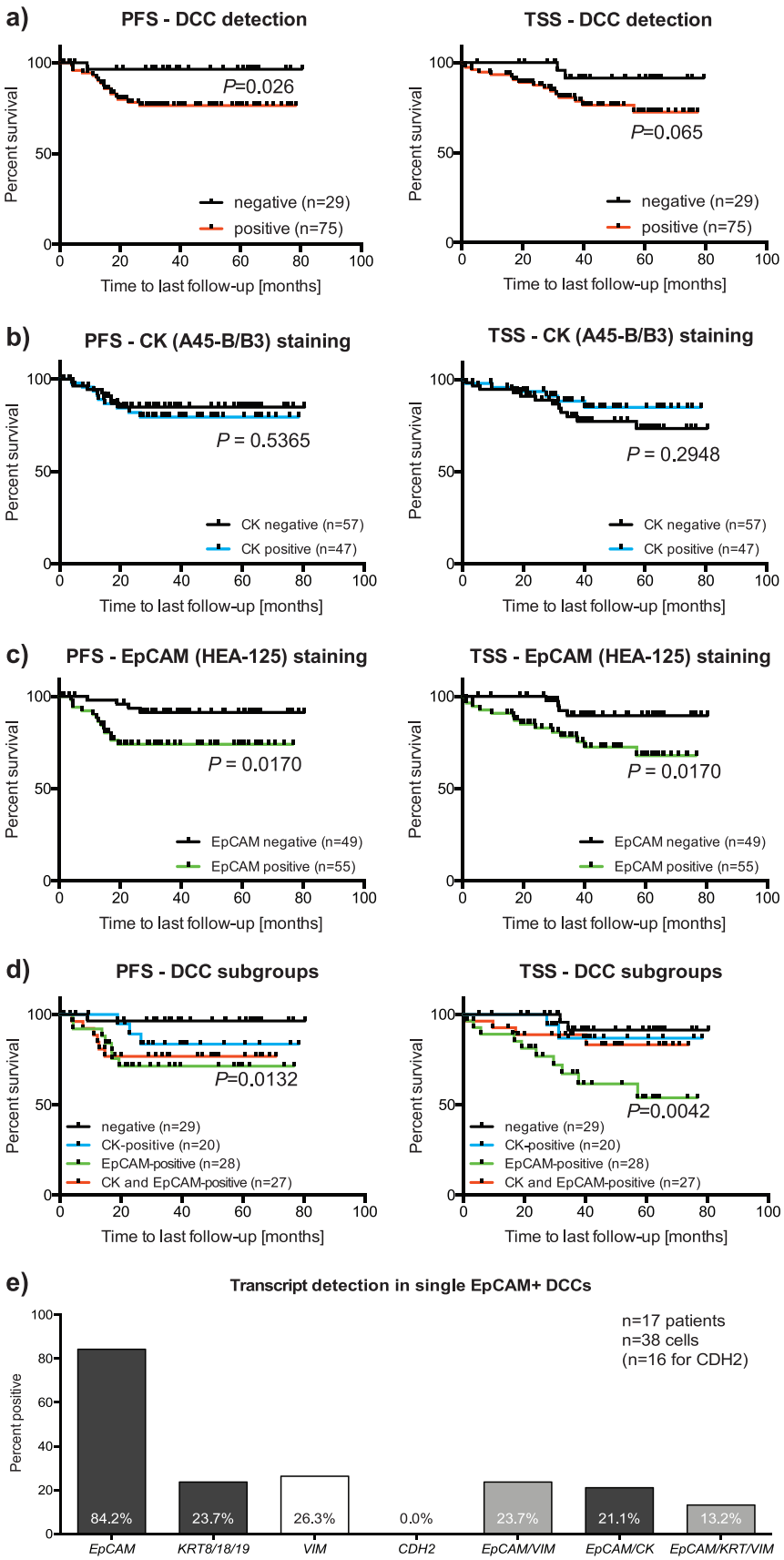


**Fig. 1.** Kaplan-Meier plots of the progression-free survival (PFS, left) and tumor-specific survival (TSS, right). **a)** shows PFS and TSS for DCC detection by EpCAM and/or CK staining, while **b)** and **c)** show survival analysis for CK + and EpCAM + groups versus negative patients, respectively. **d)** displays the survival of the different subgroups of EpCAM+, CK + and EpCAM and CK + groups versus negative patients. *P*-values (univariate log-rank tests) and number of patients in each group (n) are provided. For d) only the statistically significant *P*-value for the EpCAM + group is shown. **e)** Detection rates for *EPCAM*, *KRT8/18/19* (cytokeratins 8/18/19)), *VIM* (vimentin), and *CDH2* (N-cadherin) transcripts in EpCAM + DCCs assessed by endpoint PCR. Co-expression of selected transcripts is depicted as percentage of positive cells.

**Table 1**
Multivariate Cox regression analysis (n = 100) of selected variables. Shown are the *P*-values and the hazard ratio with the 95% confidence interval for progression-free and tumor-specific survival.

| Variable | (categories) | Progression-free survival | | Tumor-specific survival | |
|---|---|---|---|---|---|
| | | *P*-Value | Hazard Ratio (95% CI) | *P*-Value | Hazard Ratio (95% CI) |
| EpCAM DCC | (negative; positive) | **0.031** | **3.551 (1.126–11.195)** | **0.015** | **7.506 (1.476–38.182)** |
| N classification | (N0-3) | 0.279 | 1.423 (0.751–2.695) | **0.001** | **3.499 (1.671–7.327)** |
| Sex | (female; male) | 0.985 | 1.01 (0.35–2.915) | 0.249 | 2.223 (0.572–8.634) |
| Histology | (ADC; SCC; other) | 0.661 | 0.818 (0.334–2.006) | 0.435 | 1.506 (0.538–4.218) |
| Grading | (G1-3) | 0.551 | 0.751 (0.293–1.925) | 0.732 | 1.219 (0.393–3.777) |
| Age | (in years) | 0.34 | 0.972 (0.917–1.03) | 0.718 | 1.013 (0.945–1.086) |
| R status | (R0-2) | 0.489 | 1.635 (0.406–6.579) | 0.904 | 0.917 (0.224–3.749) |
| T status | (T1-4) | 0.593 | 0.849 (0.466–1.546) | 0.566 | 0.799 (0.371–1.719) |
| Smoking habit | (yes; no) | 0.749 | 1.227 (0.349–4.314) | 0.712 | 0.76 (0.177–3.269) |

and prevention of metachronous metastasis.

## 5. Conclusions

DCC detection in BM of stage I-III NSCLC patients predicts outcome, with EpCAM + DCCs being candidate driver cells for metastasis initiation. Future studies should include a detailed characterization of DCCs to provide novel rationales for the development of (neo)adjuvant therapies.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

We thank Irene Nebeja and Anthea Povall for excellent technical assistance. This work was supported by the ERC-2012-ADG-322602 and the Deutsche Forschungsgemeinschaft (DFG; KL 1233/10-2). Felix Elsner has received a Mildred-Scheel scholarship of the German Cancer Aid (Deutsche Krebshilfe, grant number 70110339).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2022.02.008.

## References

[1] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer Statistics, 2021, CA. Cancer J. Clin. 71 (1) (Jan. 2021) 7–33.
[2] H. Uramoto, F. Tanaka, Recurrence after surgery in patients with NSCLC, Transl. Lung Cancer Res. 3 (4) (Aug. 2014) 242–249.
[3] M.D. Taylor, et al., Tumor recurrence after complete resection for non-small cell lung cancer, Ann. Thorac. Surg. 93 (6) (Jun. 2012) 1813–1821.
[4] C.A. Klein, Cancer progression and the invisible phase of metastatic colonization, Nat. Rev. Cancer 20 (11) (Nov. 2020) 681–694.
[5] W. Zhang, et al., The bone microenvironment invigorates metastatic seeds for further dissemination, Cell 184 (9) (Apr. 2021) 2471–2486.e20.
[6] A. Eramo, F. Lotti, G. Sette, E. Pilozzi, M. Biffoni, A. Di Virgilio, C. Conticello, L. Ruco, C. Peschle, R. De Maria, Identification and expansion of the tumorigenic lung cancer stem cell population, Cell Death Differ. 15 (3) (2008) 504–514.
[7] N. Satar *et al.*, "Novel triple-positive markers identified in human non-small cell lung cancer cell line with chemotherapy-resistant and putative cancer stem cell characteristics," *Oncol. Rep.*, May 2018.
[8] N. Zakaria, N.M. Yusoff, Z. Zakaria, M.N. Lim, P.J.N. Baharuddin, K.S. Fakiruddin, B. Yahaya, Human non-small cell lung cancer expresses putative cancer stem cell markers and exhibits the transcriptomic profile of multipotent cells, BMC Cancer 15 (1) (2015).
[9] M. Guzvic, et al., Combined genome and transcriptome analysis of single disseminated cancer cells from bone marrow of prostate cancer patients reveals unexpected transcriptomes, Cancer Res. 74 (24) (2014) 7383–7394.
[10] T. Fehm, S. Braun, V. Muller, W. Janni, G. Gebauer, C. Marth, C. Schindlbeck, D. Wallwiener, E. Borgen, B. Naume, K. Pantel, E. Solomayer, A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation, Cancer 107 (5) (2006) 885–892.
[11] R. Lammers, C. Giesert, F. Grünebach, A. Marxer, W. Vogel, H.J. Bühring, Monoclonal antibody 9C4 recognizes epithelial cellular adhesion molecule, a cell surface antigen expressed in early steps of erythropoiesis, Exp. Hematol. 30 (6) (2002) 537–545.
[12] C.A. Klein, S. Seidl, K. Petat-Dutter, S. Offner, J.B. Geigl, O. Schmidt-Kittler, N. Wendler, B. Passlick, R.M. Huber, G. Schlimok, P.A. Baeuerle, G. Riethmüller, Combined transcriptome and genome analysis of single micrometastatic cells, Nat. Biotechnol. 20 (4) (2002) 387–392.
[13] C.P. Hsu, C.Y. Chen, P.C. Kwang, J. Miao, J.Y. Hsia, S.E. Shai, Bone-marrow microinvolvement in non-small cell lung cancer is not a reliable indicator of tumour recurrence and prognosis, Eur. J. Surg. Oncol. 26 (7) (2000) 691–695.
[14] A.K. Rud, E. Borgen, G.M. Mælandsmo, K. Flatmark, H. Le, D. Josefsen, I. Solvoll, C.B. Schirmer, Å. Helland, L. Jørgensen, O.T. Brustugun, Ø. Fodstad, K. Boye, Clinical significance of disseminated tumour cells in non-small cell lung cancer, Br. J. Cancer 109 (5) (2013) 1264–1270.
[15] K. Pantel, M. Angstwurm, G. Riethmüller, B. Passlick, J. Izbicki, O. Thetter, K. Häussinger, Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases, Lancet 347 (9002) (1996) 649–653.
[16] V.W. Rusch, et al., Occult metastases in lymph nodes predict survival in resectable non-small-cell lung cancer: Report of the ACOSOG Z0040 trial, J. Clin. Oncol. 29 (32) (2011) 4313–4319.
[17] R.J. Cote, E.J. Beattie, B. Chaiwun, S.-R. Shi, J. Harvey, S.-C. Chen, A.E. Sherrod, S. Groshen, C.R. Taylor, Detection of occult bone marrow micrometastases in patients with operable lung carcinoma, Ann. Surg. 222 (4) (1995) 415–425.
[18] K. Bartkowiak, et al., Discovery of a Novel Unfolded Protein Response Phenotype of Cancer Stem/Progenitor Cells from the Bone Marrow of Breast Cancer Patients, J. Proteome Res. 9 (6) (Jun. 2010) 3158–3168.
[19] B. Bao, et al., Over-expression of FoxM1 leads to epithelial-mesenchymal transition and cancer stem cell phenotype in pancreatic cancer cells, J. Cell. Biochem. 112 (9) (Sep. 2011) 2296–2306.
[20] S. Imrich, M. Hachmeister, O. Gires, EpCAM and its potential role in tumor-initiating cells, Cell Adh. Migr. 6 (1) (Jan. 2012) 30–38.
[21] V.Y. Ng, S.N. Ang, J.X. Chan, A.B.H. Choo, Characterization of Epithelial Cell Adhesion Molecule as a Surface Marker on Undifferentiated Human Embryonic Stem Cells, Stem Cells 28 (1) (Jan. 2010) 29–35.
[22] S.-M. Yoon, D. Gerasimidou, R. Kuwahara, P. Hytiroglou, J.E. Yoo, Y.N. Park, N. D. Theise, Epithelial cell adhesion molecule (EpCAM) marks hepatocytes newly derived from stem/progenitor cells in humans, Hepatology 53 (3) (2011) 964–973.
[23] D. Sarrio, C.K. Franklin, A. Mackay, J.S. Reis-Filho, C.M. Isacke, Epithelial and Mesenchymal Subpopulations Within Normal Basal Breast Cell Lines Exhibit Distinct Stem Cell/Progenitor Properties, Stem Cells 30 (2) (Feb. 2012) 292–303.
[24] W. Sterlacci, S. Savic, M. Fiegl, E. Obermann, A. Tzankov, Putative Stem Cell Markers in Non-Small-Cell Lung Cancer: A Clinicopathologic Characterization, J. Thorac. Oncol. 9 (1) (Jan. 2014) 41–49.