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Clinical value of circulating endothelial cells and of soluble CD146 levels in patients undergoing surgery for non-small cell lung cancer

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Background: Previous studies indicate that endothelial injury, as demonstrated by the presence of circulating endothelial cells (CECs), may predict clinical outcome in cancer patients. In addition, soluble CD146 (sCD146) may reflect activation of angiogenesis. However, no study has investigated their combined clinical value in patients undergoing resection for non-small cell lung cancer (NSCLC).

Methods: Data were collected from preoperative blood samples from 74 patients who underwent resection for NSCLC. Circulating endothelial cells were defined, using the CellSearch Assay, as CD146 + CD105 + CD45 − DAPI + . In parallel, sCD146 was quantified using an ELISA immunoassay. These experiments were also performed on a group of 20 patients with small-cell lung cancer, 60 healthy individuals and 23 patients with chronic obstructive pulmonary disease.

Results: The CEC count and the plasma level of sCD146 were significantly higher in NSCLC patients than in the sub-groups of controls ($P<0.001$). Moreover, an increased CEC count was associated with higher levels of sCD146 ($P=0.010$). Both high CEC count and high sCD146 plasma level at baseline significantly correlated with shorter progression-free survival ($P<0.001$, respectively) and overall survival ($P=0.005$; $P=0.009$) of NSCLC patients.

Conclusions: The present study provides supportive evidence to show that both a high CEC count and a high sCD146 level at baseline correlate with poor prognosis and may be useful for the prediction of clinical outcome in patients undergoing surgery for NSCLC.

Despite the different therapeutic strategies developed to date, patients with non-small cell lung cancer (NSCLC) have a poor outcome. The 5-year overall survival (OS) of these patients is ~20–25% across all stages (Mountain, 2000; van Rens *et al*, 2000; Naruke *et al*, 2001; Goya *et al*, 2005; Blanchon *et al*, 2006; Pfannschmidt *et al*, 2007).

Metastasis is the primary cause of mortality in cancer patients (Siegel *et al*, 2013). In addition to the lack of effective treatment options, the absence of useful clinical indicators predicting prognosis and/or response to treatment may contribute to the poor prognosis of these patients. Tumour growth and metastasis depend on neovascularisation (Narazaki and Tosato, 2006;

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Yoneda *et al*, 2012). The process of tumour vascularisation is a potential target for new cancer therapies and can be a useful clinical marker (Ebos and Kerbel, 2011).

Circulating endothelial cells (CECs) are mature viable cells that are shed from the endothelium, circulate within the bloodstream and still exhibit proliferative capacity despite their terminal differentiation (Beerepoot *et al*, 2004). Recent studies demonstrated that CECs have an important role in tumour neovascularisation either at the primary or the metastatic site (Furstenberger *et al*, 2006; Goon *et al*, 2006; Narazaki and Tosato, 2006; Weis and Cheresh, 2011). The number of CECs and the extent of vascular damage are increased in a variety of vascular disorders including cardiovascular diseases, vasculitis and infectious diseases (Mutin *et al*, 1999; Mutunga *et al*, 2001; Woywodt *et al*, 2003; Beerepoot *et al*, 2004). It is noteworthy that the CEC count is increased in cancer patients compared with healthy subjects and may correlate with tumour progression (Mancuso *et al*, 2001; Beerepoot *et al*, 2004; Rowand *et al*, 2007). Moreover, the CEC count in cancer patients significantly decreased after surgical removal of the tumour and/or treatment with chemotherapy of the patient (Mancuso *et al*, 2001; Kawaishi *et al*, 2009). In addition, CEC kinetics and viability are promising predictors of the response to treatment with anti-angiogenic agents in patients with advanced breast cancer or colorectal cancer (Mancuso *et al*, 2006; Malka *et al*, 2012). Besides monitoring the anti-tumour activity, a marker such as CEC would reveal tumour growth and disease progression at an early stage in view of the known fragility of the tumour vasculature compared with the healthy vasculature (Strijbos *et al*, 2008). However, because of the technical difficulties and challenges associated with CEC measurement, data regarding the significance of CECs in patients with early-stage cancer are still lacking. An approach using an automated rare cell detection system has proven to be highly accurate and reliable in enumeration and isolation of CECs from peripheral blood (Rowand *et al*, 2007). However, very few studies to date have evaluated the clinical value of CECs in NSCLC patients using this methodology (Kawaishi *et al*, 2009).

In addition to the number of CECs in the blood, other biomarkers are thought to reflect the presence of angiogenesis and/or vascular endothelial alteration, such as the concentration of soluble factors including soluble CD146 (sCD146) in the blood. CD146 (also referred to as MUC18, MCAM, Mel-CAM, S-Endo-1 and P1H12 antigen) is a transmembrane glycoprotein that is constitutively expressed in the human endothelium, irrespectively of the anatomical localisation (Lehmann *et al*, 1989; Bardin *et al*, 1998; Bardin *et al*, 2003). CD146 is localised at the intercellular boundaries of endothelial cells and is involved in the control of cell cohesion (Bardin *et al*, 1996a; Shih, 1999; Bardin *et al*, 2001). Moreover, CD146 may have a key role in endothelial cell activity and angiogenesis (Kang *et al*, 2006). In addition to the membrane-anchored form of CD146, a soluble form of CD146 (sCD146) has been identified using an ELISA assay in the supernatant of cultured human endothelial cells and in the plasma of healthy subjects (Bardin *et al*, 1998; Bardin *et al*, 2003; Furstenberger *et al*, 2005). The plasma concentration of sCD146 is modulated in inflammatory diseases associated with endothelial alterations (Bardin *et al*, 2006). However, there is no current data concerning the level of sCD146 in lung cancer patients.

In this setting, we hypothesised that CEC detachment from the vascular wall may be associated with the release of sCD146. The purpose of this study was: (1) to quantify CEC levels in patients undergoing surgery for NSCLC, (2) to quantify the plasma level of sCD146 in the same cohort of patients, (3) to compare the absolute number of CECs and the level of sCD146 in these patients, and, finally, (4) to correlate the results with different clinicopathological parameters and the outcome of patients.

PATIENTS AND METHODS

Patients. Seventy-four patients who underwent surgery for NSCLC (including seven patients with neoadjuvant chemotherapy) between January 2009 and January 2011 at the Pasteur Hospital (Department of Thoracic Surgery, University of Nice Sophia Antipolis, France) were included in this study. In addition, 20 patients with histologically and/or cytopathologically confirmed chemotherapy-naive small-cell lung cancer (SCLC) were enrolled in the study to test for the specificity of candidate biomarkers. Two control populations were included in the study: 20 patients with chronic obstructive pulmonary disease (COPD) (16 males, 4 females; mean age 68 years, range 55–77 years; all smokers), as well as 42 age, sex and smoking status-matched healthy individuals (28 males, 14 females; mean age 57 years, range 21–74; 35 current smokers). After approval by the local Ethics Committees (Nice University Hospital Centre), written informed consent was obtained from participants after explaining the nature of the study and the study was performed according to the guidelines of the Declaration of Helsinki. Criteria for patient inclusion in the study were: NSCLC histology performed according to WHO criteria and the latest recommendations of the international association for the study of lung cancer (Travis *et al*, 2004, 2011), tumour stage I to IV (7th edition of UICC-TNM staging system, AJCC) (Goldstraw, 2009), patients treated with surgery of the primary tumour (wedge resection, lobectomy or pneumonectomy), availability of clinicopathological and outcome data, and no transbronchial and/or transparietal chest biopsies at least 15 days before surgery. The main clinical and pathological data are summarised in Table 1. In SCLC patients, blood samples were collected for analysis within 7 days before commencing treatment (baseline) chemotherapy cycle.

Methods. CellSearch technology (Veridex LLC, Raritan, NJ, USA) was carried out using previously described protocols (Strijbos *et al*, 2008; Malka *et al*, 2012). Briefly, 4 ml of peripheral blood was taken before anaesthesia and collected in the CellSave preservative tubes (Veridex LLC). The first 2 ml of peripheral blood was discarded to avoid contamination with endothelial cells from peripheral venules. Samples were maintained at room temperature and processed within 24 h after collection. The CellTracks AutoPrep System (Veridex LLC), the CellSearch Endothelial Cell Kit (Veridex LLC) and the CellSpotter Analyzer System (Veridex LLC) were used to enumerate the CECs. The CellSearch Endothelial Cell Kit was used for immunomagnetic enrichment using ferrofluids coupled to an anti-CD146 antibody. After enrichment, the following reagents were added: the nuclear dye 4,6-diamidino-2-phenylindole (DAPI), and fluorochrome-conjugated monoclonal antibodies: phycoerythrin-conjugated CD105, and allophycocyanin-conjugated CD45, a pan-leukocyte antigen was included to exclude haematopoietic cells from analysis. Analysis was done using image cytometry, in which CECs were defined as having an oval to elongated undamaged morphology and were CD146 + DAPI + CD105 + CD45 − (Supplementary Figure 1) (Rowand *et al*, 2007; Strijbos *et al*, 2008). The CEC count was expressed as the number of positive cells per 1 ml of blood. Reproducibility was tested by performing two replicates of 20 different samples (Supplementary Figure 2).

In parallel, peripheral blood was drawn into EDTA tubes (Becton Dickinson, Le Pont-De-Claix, France). Within 1 h, the tubes were subjected to centrifugation at 820 *g* for 10 min. Transferred 1-ml aliquots of the plasma to 1.5-ml tubes was done followed by centrifugation at 16 000 *g* for 10 min to pellet any remaining cellular debris. The supernatant was transferred to fresh tubes and stored at −80 °C until use.

Soluble CD146 levels were determined with a commercial enzyme-linked immunosorbent assay (CY-QUANT ELISA,

Table 1. Clinical and pathological variables of the 53 patients with operable non-small cell lung carcinoma enrolled in the study

		CEC count (per ml)			sCD146 levels (ng ml^{-1})		
Variables	**Overall *n* (%)**	**Low**	**High**	***P*-value**	**Low**	**High**	***P*-value**
Age (years)[a]							
Mean ± s.d.	65.2 ± 8.6	65.4 ± 8.2	65 ± 9	0.710	63.9 ± 9.1	66.5 ± 8	0.205
Gender[b]							
Male	54 (73%)	24 (44%)	30 (56%)	0.585	25 (46%)	29 (54%)	0.216
Female	20 (27%)	9 (45%)	11 (55%)		12 (60%)	8 (40%)	
ECOG PS							
0	44 (59%)	24 (55%)	20 (45%)	0.451	26 (59%)	18 (41%)	0.937
1	30 (41%)	19 (63%)	11 (27%)		18 (60%)	12 (40%)	
Baseline FEV_1							
< 2 L	35 (47%)	23 (66%)	12 (24%)	0.931	25 (71%)	10 (29%)	0.262
⩾ 2 L	39 (53%)	26 (67%)	13 (23%)		23 (59%)	16 (41%)	
Hb level (g dl^{-1})							
< 12	27 (37%)	18 (67%)	9 (23%)	0.900	16 (59%)	11 (41%)	0.173
⩾ 12	47 (63%)	32 (68%)	15 (22%)		35 (74%)	12 (26%)	
Smoking history[b]							
Never	9 (12%)	3 (33%)	6 (67%)	0.361	4 (44%)	5 (56%)	0.500
Current and former	65 (88%)	30 (46%)	35 (54%)		33 (51%)	32 (49%)	
Neoadjuvant chemotherapy							
No	67 (91%)	36 (54%)	31 (46%)	0.370	38 (57%)	29 (23%)	0.982
Yes	7 (9%)	5 (71%)	2 (29%)		4 (57%)	3 (23%)	
Tumour size[a]							
Mean ± s.d.	3.8 ± 2	3.5 ± 2.3	4.1 ± 1.8	0.226	3.5 ± 1.8	3.9 ± 2.1	0.544
Histological subtypes[b]							
Adenocarcinoma	50 (68%)	23 (46%)	27 (54%)	0.461	25 (50%)	25 (50%)	0.598
Squamous cell carcinoma	24 (32%)	10 (42%)	14 (58%)		12 (50%)	12 (50%)	
Histological grade[b]							
Well	32 (43%)	16 (50%)	16 (50%)	0.441	14 (44%)	18 (56%)	0.636
Moderate	26 (35%)	9 (35%)	17 (65%)		14 (54%)	12 (46%)	
Poor	16 (22%)	8 (50%)	8 (50%)		9 (56%)	7 (44%)	
pTNM stage[b]							
I	25 (34%)	12 (48%)	13 (52%)	0.342	15 (60%)	10 (40%)	0.129
II	32 (43%)	16 (50%)	16 (50%)		17 (53%)	15 (47%)	
III + IV	17 (23%)	5 (29%)	12 (71%)		5 (29%)	12 (71%)	

Abbreviations: CEC = circulating endothelial cells; ECOG = Eastern Cooperative Oncology Group; FEV_1 = forced expiratory volume in one second; PS = performance status; TNM = tumour-node-metastasis. The baseline CEC count, sCD146 level according to the patient characteristics are shown.
[a] *T*-test.
[b] χ^2-test.

BioCytex, Marseille, France) according to the manufacturer's instructions. The ELISA assay was validated previously (Bardin *et al*, 1996b; Bardin *et al*, 1998; Bardin *et al*, 2003). Reproducibility was tested by performing two replicates of 20 different samples.

Statistical analysis. The χ^2-test, the Fisher's exact test and the Mann–Whitney *U*-test were used to compare CECs and sCD146 levels according to the baseline clinicopathological characteristics. A nonparametric correlation method (Spearman's Rank test) was used to evaluate the correlation between the CEC count and sCD146 plasma levels. The median time of follow-up was calculated according to the method of Schemper and Smith (1996). The survival time was calculated using Kaplan–Meier estimates and the differences between the progression-free (PFS) and OS curves, which were analysed with the log rank test. A Cox proportional hazard model was created to identify independent

predictors of survival. Variables that were associated with survival with a *P*-value <0.20 in the univariate analysis were included in the multivariate regression. Differences were considered significant when a *P*-value was <0.05. All statistical evaluations were performed using SPSS for Windows software system, version 16.0.0 (SPSS, Chicago, IL, USA).

RESULTS

The baseline CEC count, the sCD146 level and correlation with clinicopathological parameters of patients. The detection and enumeration of CECs in the current cohort demonstrated a significantly higher baseline CEC count in the blood of NSCLC patients (median 114 CEC per ml; range, 22–661 per ml) than in SCLC patients (median 46 CEC per ml; range, 26–94 per ml), COPD patients (median 22 CEC per ml; range, 7–37 per ml) or in healthy controls (median 3 CEC per ml; range, 1–13 per ml) ($P<0.001$, Figure 1). Higher CEC count was observed in SCLC patients when compared with COPD patients or the healthy individuals (Figure 1). There was a tendency towards higher CEC count in COPD patients when compared with healthy subjects ($P=0.068$). No significant difference was observed in the control group according to gender, age and tobacco status (data not shown).

Baseline sCD146 levels were significantly higher in NSCLC patients (median 275 ng ml^{-1}, range, 89–905.4 ng ml^{-1}) as compared with SCLC patients (median 133.5 ng ml^{-1}; range, 66–193 ng ml^{-1}), COPD patients (median 107 ng ml^{-1}; range, 60–180 ng ml^{-1}) and healthy subjects (median 78 ng ml^{-1}; range, 20–349 ng ml^{-1}) ($P<0.001$, Figure 1). Significant variations were observed between SCLC patients and COPD patients or healthy individuals (Figure 1). No significant difference was observed in the control group according to gender, age and tobacco status (data not shown).

A significant positive correlation was observed between the CEC count and sCD146 levels in NSCLC patients ($\rho=0.9$, $P=0.010$; Figure 2). Correlation of the coefficient values for variation of duplicate CECs and sCD146 samples from the same subject were $R^2=0.90$ and 0.92, respectively.

Furthermore, we categorised the CEC count per ml and sCD146 levels as 'low' and 'high' using the 75th percentile of the interquartile range of the median value as the cutoff, as previously described (Malka *et al*, 2012). Accordingly, the range of low sCD146 levels was between 89 and 219.1 ng ml^{-1}, and the range of high sCD146 levels was between 220.7 and 905.4 ng ml^{-1}.

Among the NSCLC patients, there was no significant difference in the CEC count and sCD146 level according to age, gender, performance status, FEV_1, Hb level, smoking history, tumour size, histological subtypes, tumour grade or pTNM stage ($P>0.05$, Table 1).

The CEC count, sCD146 levels and clinical outcome. At the last follow-up, 30 out of 74 (41%) patients had local (55%), regional (27%) or distant (18%) tumour recurrence and 22 out of 74 (30%) patients died specifically from cancer progression.

The mean PFS was 36.9 months (95% CI, 32.6–41.3). In the univariate analysis, the clinical factor that significantly associated with PFS was the pTNM stage ($P=0.012$). The unadjusted survival analysis showed that patients with a high baseline CEC count and a high sCD146 level had poor PFS ($P<0.001$, $P<0.001$, respectively; Figure 3A and B) and worse OS ($P=0.005$, $P=0.009$, respectively; Figure 3D and E). This significance was even higher when adjusted for both endothelial biomarkers ($P<0.001$, respectively; Figure 3C and F). Patients with only one increased endothelial marker harboured intermediate outcomes.

The risk of progression was higher for patients with stage III + IV ($P=0.030$), high CEC count ($P=0.001$) and high sCD146 plasma level ($P=0.002$; Table 2). The multivariate Cox analysis revealed that the pTNM stage ($P=0.086$), the CEC count ($P=0.006$) and sCD146 level ($P=0.020$) were the prognostic factors that influence the OS of NSCLC patients (Table 2).

DISCUSSION

This study demonstrated that the baseline CEC count was markedly higher for NSCLC patients than for healthy individuals

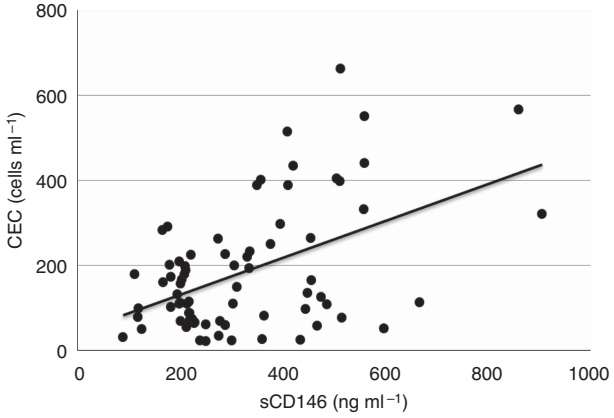

Figure 2. Correlation between the CEC count and the plasma level of sCD146 in 74 patients with operable NSCLC.

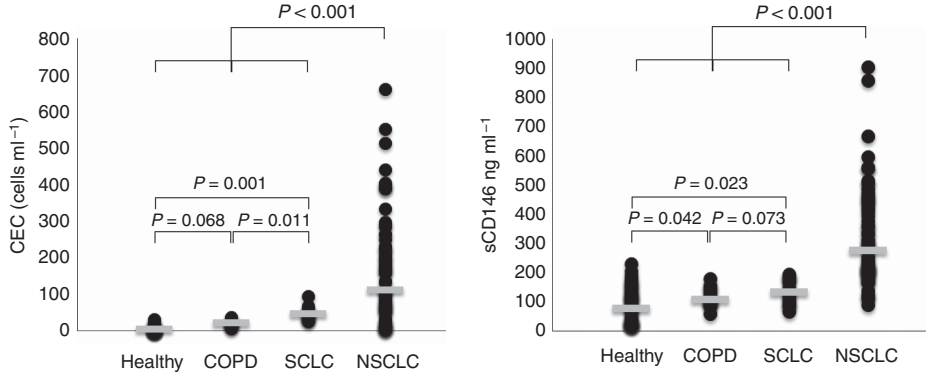

Figure 1. Distribution of the absolute CEC count per ml (left) and the sCD146 level (ng ml^{-1}) (right) in 74 patients with operable NSCLC, 20 patients with SCLC and in controls (60 healthy individuals and 23 patients with COPD). The Mann–Whitney *U*-test was used for testing the significance.

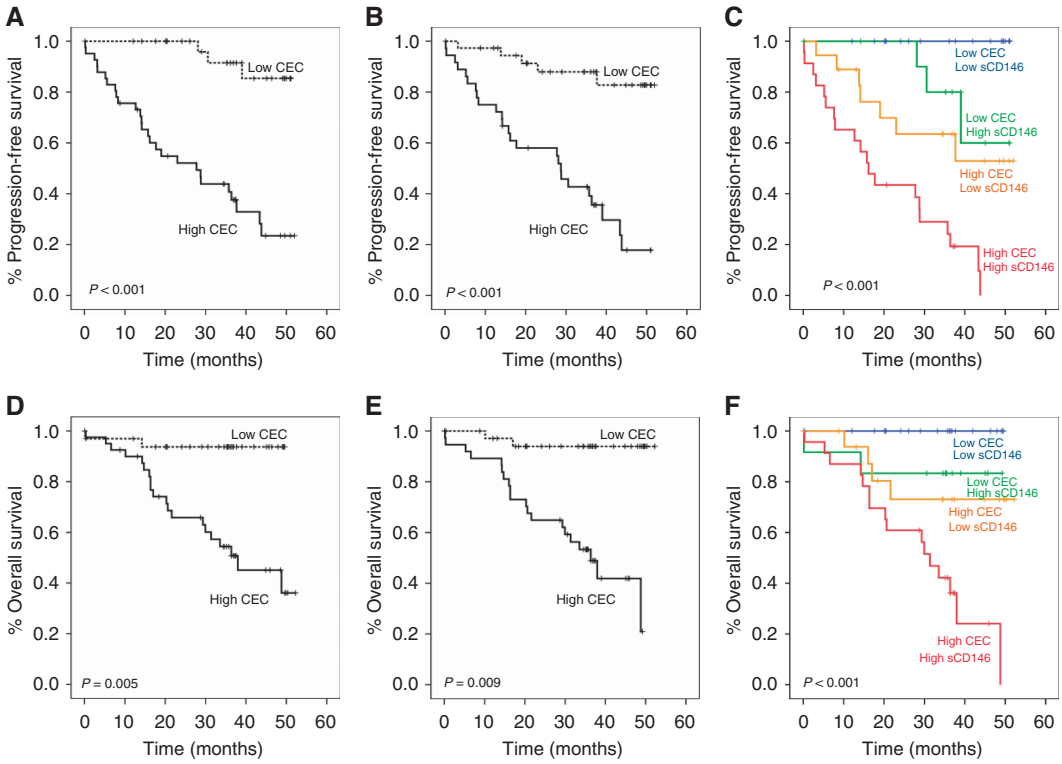

Figure 3. Kaplan–Meier curves of PFS (**A**, **B** and **C**) and overall survival (**D**, **E** and **F**) stratified according to the CEC count (left panels) and the sCD146 level (middle panels) or combined parameters (right panels).

Table 2. Multivariate analysis of prognostic factors identified in our study with PFS and OS as the end point in patients with operable NSCLC

Prognostic factor	HR	95% CI	*P*-value[a]
PFS			
pTNM stage			
I + II *vs* III + IV	0.172	0.035–0.844	0.030
CEC count (per ml)			
High *vs* low	9.958	2.885–34.374	0.001
sCD146 levels (ng ml^{-1})			
High *vs* low	6.018	1.954–18.537	0.002
Overall survival			
pTNM stage			
I + II *vs* III + IV	0.430	0.164–1.126	0.086
CEC count (per ml)			
High *vs* low	8.478	1.846–38.931	0.006
sCD146 levels (ng ml^{-1})			
High *vs* low	6.138	1.334–28.251	0.020

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]*P*-value < 0.05 statistically significant.

and COPD patients. Circulating endothelial cells are mature cells from the intima that are released into the circulation after vascular injury (D'Souza-Schorey and Clancy, 2012). This has been well documented in many human diseases, including different types of cancers. In cancer patients, the level of CECs is persistently higher over time than that of healthy individuals, and this increased level has been identified as a surrogate marker of neovascularisation and anti-angiogenic drug activity (Goon *et al*, 2006; Mancuso *et al*, 2006; Malka *et al*, 2012). We also observed an increased number of CECs in SCLC patients compared with healthy individuals and COPD patients. The CEC count was significantly lower than in NSCLC patients. As our SCLC population was limited in size, the value of CEC in SCLC patients should be further evaluated in larger series. Most importantly, our results show that the CEC count is associated with clinical outcome in NSCLC patients undergoing surgery, and thus may be a prognostic factor for this disease. In our study, patients with a high CEC count at baseline had a significantly worse PFS and OS. A majority of reports showed that high baseline CEC levels above the 75th percentile significantly correlated with prognosis, and could be useful as prognostic markers in patients with advanced NSCLC (Fleitas *et al*, 2010; Morita *et al*, 2011; Wang *et al*, 2013). In contrast, a study of 31 patients with advanced NSCLC treated with first-line carboplatin and paclitaxel reported that a CEC count of $>400/4$ ml at baseline showed a significantly improved PFS. However, no statistical data were presented in this study, which was limited in size and follow-up, to justify the choice of the cutoff (Kawaishi *et al*, 2009).

Endothelial progenitor cells (EPCs), which are circulating cells that originate from bone marrow, have the ability to form endothelial colonies *in vitro*, and to contribute to neovascularisation (Mancuso *et al*, 2003; Strijbos *et al*, 2008). In theory, cEPCs may be a more appropriate surrogate of tumour vascularisation. Subsequently, the pre-treatment level of cEPCs was proposed as a promising candidate biomarker for disease progression and prediction of clinical outcome in NSCLC (Dome *et al*, 2006; Fleitas *et al*, 2010; Nowak *et al*, 2010; Roodhart *et al*, 2010; Vroling *et al*, 2010). However, the clinical evaluation of cEPCs has been hampered by the extreme rarity of these cells and the lack of

consensus on the surface marker phenotype and on the techniques used for cEPC enumeration (Peters *et al*, 2005; Wang *et al*, 2013). Nevertheless, a decrease in both CECs and cEPCs correlated with a longer PFS or OS in the majority of studies, before and after effective anti-angiogenic therapy and/or chemotherapy (Chu *et al*, 2012; Wang *et al*, 2013).

The present study showed that sCD146 levels were significantly higher in NSCLC patients than in healthy subjects. Previous studies have shown that sCD146 is detectable in the human blood and its level is modulated in different pathologies, such as inflammatory bowel diseases, vasculitis, pathologic pregnancies and chronic renal failure (Bardin *et al*, 2003; Pasquier *et al*, 2005; Bardin *et al*, 2006).

Furthermore, we showed a positive correlation between the CEC count and the sCD146 level, indicating that both biomarkers can be indirect surrogates of tumour vascularisation, as previously suggested for these individual markers (Monestiroli *et al*, 2001; Harhouri *et al*, 2010). For instance, sCD146 is involved in inflammation by specifically binding to monocytes and thereby stimulating transendothelial migration, as well as in angiogenesis (Bardin *et al*, 2009; Harhouri *et al*, 2010; Stalin *et al*, 2013). Thus, sCD146 exhibits chemotactic activity on different cell types involved in vessel formation, including endothelial cells. In addition, treatment of EPCs with sCD146 led to increased migration, proliferation and the capacity to form capillary-like structures. Finally, local treatment with sCD146 led to the recruitment of EPCs and to an increase in blood flow and neovessel formation (Harhouri *et al*, 2010). Interestingly, recent evidence suggested that sCD146, through inhibition of endothelial cell adhesion, may have a role in CEC migration and homing (Jiang *et al*, 2012; Wang and Yan, 2013). Moreover, the binding of CD146 to an anti-CD146 monoclonal antibody inhibited p38 mitogen-activated protein kinase phosphorylation, suppressed NF-kB activation and downregulated matrix metalloproteinase 9 and intercellular adhesion molecule 1 expression, suggesting that the CD146/NF-kB axis is critical in endothelial cell migration, angiogenesis and tumour metastasis (Ouhtit *et al*, 2009).

We further investigated whether sCD146 can assist in determining the outcome of NSCLC surgical patients at baseline. Our results support the hypothesis that CEC counts together with sCD146 levels are associated with clinical outcome in NSCLC patients undergoing surgery. We showed that patients with a high CEC count and an elevated sCD146 level at baseline had a significantly worse PFS and OS, whereas patients with a single high endothelial marker harboured intermediate outcomes, suggesting a possible synergistic effect on cancer progression.

CD146 is a member of the immunoglobulin superfamily that was originally identified as a melanoma marker and has a role in tumour metastasis (Shih, 1999). Recent data suggest that CD146 may be involved in tumour development and may influence tumour prognosis in a number of solid tumours (Liu *et al*, 2012; Zeng *et al*, 2012b; Kapoor, 2013). It is expressed in a high proportion of NSCLC and might be predictive of shortened patient survival in lung adenocarcinomas (Kristiansen *et al*, 2003; Oka *et al*, 2012). Moreover, CD146 promotes cancer progression by induction of epithelial–mesenchymal transition, a critical step in tumour metastasis, through modulating the remodelling of cytoskeleton (Luo *et al*, 2012; Zeng *et al*, 2012a).

In conclusion, our findings demonstrate that the CEC count and the sCD146 plasma level might be suitable biomarkers at baseline for predicting outcome of patients with NSCLC following surgical resection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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