RESEARCH ARTICLE

Meta-Analysis of Circulating Endothelial Cells and Circulating Endothelial Progenitor Cells as Prognostic Factors in Lung Cancer

Min Yu[1]*, Hai-Tao Men[1], Zhi-Min Niu[2], Yu-Xi Zhu[1], Ben-Xu Tan[1], Long-Hao Li[1], Juan Jiang[1]

Abstract

Background: The aim of this study was to analyze the prognostic implications of pretreatment circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPCs) for the survival of patients with lung cancer. Materials and Methods: Relevant literature was identified using Medline and EMBASE. Patient clinical characteristics, overall survival (OS) and progression-free survival (PFS) together with CEC and CEPC positive rates before treatment were extracted. STATA 12.0 was used for our analysis and assessment of publication bias. Results: A total of 13 articles (8 for CEC and 5 for CEPC, n=595 and n=244) were pooled for the global meta-analysis. The odds ratio (OR) for OS predicted by pretreatment CECs was 1.641 [0.967, 2.786], while the OR for PFS was 1.168 [0.649, 2.100]. The OR for OS predicted by pretreatment CEPCs was 12.673 [5.274, 30.450], while the OR for PFS was 4.930 [0.931, 26.096]. Subgroup analyses were conducted according to clinical staging. Odds ratio (OR) showed the high level of pretreatment CECs only correlated with the OS of patients with advanced lung cancer (stage III-IV). Conclusions: High counts of CECs seem to be associated only with worse 1-year OS in patients with lung cancer, while high level of pretreatment CEPCs correlate with both worse PFS and OS.

Keywords: Circulating endothelial cells - circulating endothelial progenitor cells - lung cancer - meta-analysis - prognosis

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Introduction

Lung cancer accounts for 12 % of all cancers diagnosed and its high incidence with its low survival rate leads it to be one of the leading causes of cancer death in worldwide (Jemal et al., 2011). Therefore, the studies of predictive and prognostic are in constant research, aiming to customize treatment and optimize the effectiveness of available agents (Aggarval et al., 2010). It is important to evaluate therapy effectiveness and avoid unnecessary side effects at an early stage by predictive tools. Recently, significant progress has been made in both imaging techniques and identification of serological markers which might serve to modify the therapeutic strategy of patients who do not respond.

Circulating endothelial cells (CECs) is a population of mature endothelial cells with a quite low number in healthy individuals and the increase in the circulation indicates the presence of vascular endothelium damage (Shantsila et al., 2008). The increase of CECs has been observed in various processes, such as inflammations, cardiovascular diseases, perioperative period, autoimmune diseases and cancer. Endothelial progenitor cells (EPCs) are defined as circulating precursor cells with the ability to differentiate to mature endothelial cells, form functional blood vessels, and therefore, play a role in promoting abnormal vascularization in neoplastic sites (Melero-Martin et al., 2011). These factors, such as age, male gender, smoking, blood pressure (BP) levels and cardiovascular risk factors, have been reported to reduce the number of circulating EPCs (CEPCs) (Pirro et al., 2006; Pirro et al., 2007; Fadini et al., 2008; Pirro et al., 2008; Yue et al., 2010). The contribution of these cells to the vasculature of solid tumours and prognosis of cancer patients is still controversial.

The aim of this study was to investigate whether baseline CECs and CEPCs, before the treatment of chemotherapy, radiotherapy or surgery, have prognostic or predictive roles in patients with lung cancer.

Materials and Methods

Identification and Eligibility of Relevant Studies. We identified all studies, published or not, respectively targeting all endothelial cells or endothelial progenitor cells' markers in patients with lung cancer, by an electronic

*[1]Department of Oncology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, [2]Department of Radiation Oncology, CyberKnife Center, and Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute & Hospital, Tianjin, China *For correspondence: yuminanni70@sina.com*

search using online PubMed (Medline) and EMBASE, with the search strategies based on combinations of "*circulating endothelial cells*", "*circulating endothelial progenitor cells*", and "*lung cancer*". References of retrieved articles were also screened to identify any studies missed by the search strategies.

Last query was updated on June 20, 2014. Candidate articles were identified for the meta-analysis studies based on title and abstract after reading by two independent reviewers (Yu M and Men HT). When cannot be categorized, full-text review was retrieved. Abstract review was restricted to English. Reported data required for meta-analysis were then identified and extracted. Prespecified quality-related inclusion or exclusion criteria were not used and each study had not been weight by a quality score because no such score has received general agreement for meta-analyses of observational studies (Altman et al., 2001). We made an effort to contact investigators by e-mail to get unpublished data regarding CECs, CEPCs and survival.

Definitions and Standardizations. The studies included defined pretreatment CECs and CEPCs using flow cytometry, CellSearch Assay or fluorescent microscopy. The main outcome of our meta-analysis was 1-year PFS and OS. All of these patients were followed up until death or for at least 24 months. We also listed age, sex, clinical stage and different makers to identify CECs and CEPCs.

Data Extraction. Two reviewers (Yu M and Niu ZM) independently extracted data from all primary studies. The primary data were the *p* value, the Kaplan-Meier survival curves or Odds Ratio (OR) and 95% confidence interval (CI) of survival outcomes. Additional data obtained from the studies included the first author, publication year, origination country, number of patients, types for CECs and CEPCs assessment, the marker(s) used for staining, cut-off value, number of events in each category, and 1-year OS and PFS. Disagreements were resolved by consensus between the two readers. All studies included were retrospective.

Statistical Analyses. Using the median or mean CECs and CEPCs as a cut-off, patients were classified into the "*high CECs/CEPCs*" group and "*low CECs/CEPCs*" group. A study was considered significant when the *p* value was less than 0.05 in univariate analysis. OR with 95% confidence intervals (CI) synthesized were used to assess the strength of association. For the quantitative aggregation of survival results, we measured the impact of CECs and CEPCs on survival by estimating the OR between the "*high CECs/CEPCs*" and "*low CECs/CEPCs*" group. The simplest method to get OR and their 95% CI is to find the exacted value from the original article. When the values were not available, the methods described by Parmer et al. (1998) were used to estimate OR. The published data including total number of patients and number of events in each group from articles were extracted, and the calculations were done, presuming that the rate of censored patients was constant during the study follow-up. We read Kaplan-Meier curves (Men HT) by Engauge Digitizer version 4.1 (free software downloaded from http//sourceforge.net). Considering the many sources of heterogeneity between studies and consequently between their individual OR estimates, we calculated the overall OR according to the Der Simonian and Laird's method (DerSimonian et al., 1986). A fixed effect model was used for secondary analysis, when homogeneity was fine ($p \leq 0.10$, $I^2 \leq 50\%$), and a random effect model was used if not. An observed OR>1 indicated worse outcome for the "*high CECs/CEPCs*" group relative to "*low CECs/CEPCs*" group and would be considered statistically significant if the 95%CI did not overlap 1, with $p<0.05$. Forrest plots were used to estimate the effect of high CECs or CEPCs counts on survival outcome, and STATA 12.0 (STATA Corporation, College Station, TX) was used for our analysis (Yu M and Zhu YX). Potential publication bias was evaluated using the Begg's funnel plot. It was considered that there is no publication bias when the *p* value was more than 0.05 (Begg et al., 1994).

Results

Eligible Studies. Our electronic search algorithm retrieved a total of 340 references (221 from PubMed and 24 from Embase) for CECs/CEPCs (245 for CECs and 95 for CEPCs)and NSCLC. 14 reports were finally identified, while one of which was excluded for lacking informative clinical data (Nowak et al., 2010). For all the patients, CECs and CEPCs measurements had been done before any treatment.

8 studies (n=595 patients) were finally eligible for the CECs meta-analysis and 5 of them found an inverse relationship between survival and CECs, that is positive studies (Kawaishi et al., 2009; Chu et al., 2012; Fleitas et al., 2012; Wang et al., 2013; Ilie et al., 2014), leaving 3 studies negative (Najjar et al., 2015, Sanchez Hernandez et al., 2015, Yuan et al., 2015). 5 studies (n=244 patients) were included for the CEPCs meta-analysis. The relationships were positive for 4 studies (Dome et al., 2006; Bogos et al., 2009; Morita et al., 2011; Pirro et al., 2013) and negative for only one (Sakamori et al., 2012).

Baseline characteristics of the 13 eligible studies are listed in Table 1. Seven reports originated from Asian, five from Europe and one from Oceania. All of the eligible studies were observational retrospective studies. All the patients included for CECs and CEPCs meta-analysis are diagnosed with non-small cell lung cancer (NSCLC) except the patients with small lung cancer (SCLC) from

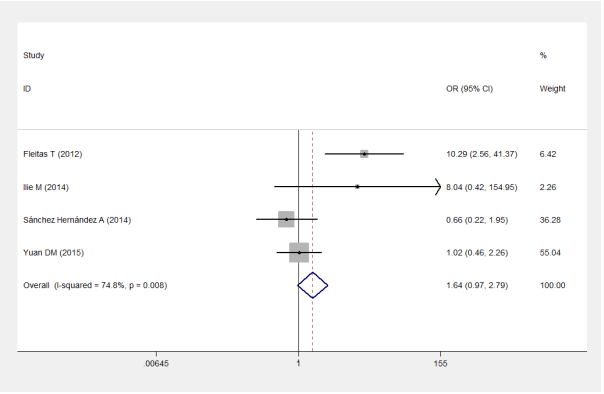

Figure 1. Meta-analysis of the Association Between CECs and OS at 1 Years. Each study is shown by the name of the lead author and the OR with95% CIs

Table 1. Characteristics of the Eligible Studies

Author	Country	Histologic cell type	N.of patients	Clini calstage	Methods	Positive definition	Cut-off value	Sampling time	Outcomes	Attitude
Circulating Endothelial Cells (CEC)										
Kawaishi M 2009	Japan	NSCLC	31	III-IV	Cell Tracks	CD146+ DAPI+ CD105+ CD45-	400/4ml	before TM	PFS	positive
Chu TQ 2012	China	NSCLC	107	IIIb-IV	FCM	P1H12+ CD133- CD45-	0.58/ìl	before and after TM	PFS/OS	positive
Fleitas T 2012	Spain	NSCLC	60	IIIb-IV	IMT/FM	CD146+	152/mL	before TM	OS	positive
Wang J 2013	China	NSCLC	63	IIIb-IV	FCM	CD146+ CD105+ CD45-	NR	before and after TM	PFS	positive
Ilie M 2014	France	NSCLC	74	I-IV	Cell Search	CD146+ CD105+ CD45- DAPI+	114/mL	before TM	PFS/OS	positive
Najjar F 2014	Syria	NSCLC	89	III-IV	IMT/FM	CD146+	362/mL	before TM	PFS	negative
S Cnchez Hern Cndez A 2014	Spain	NSCLC	69	IV	Cell Search	CD146+ CD105+ DAPI+ CD45-	153/4 mL	before TM	PFS/OS	negative
Yuan DM 2015	China	NSCLC	102	IIIB-IV	FCM	CD45? CD31+ CD146+	210 cells/10 5	before TM	PFS/OS	negative
Circulating Endothelial Progenitor Cells (CEPC)										
Dome B 2006	Hungary	NSCLC	53	I-IV	FCM	CD34+ VEGFR2+ CD133+	1,000/ mL	before TM	OS	positive
Bogos K 2009	Austria	SCLC	88	LS	FCM	CD34+ VEGFR3+ CD133+	1,625/ mL	before TM	OS	positive
Morita R 2011	Japan	NSCLC	31	I-IV	FCM	CD34+ VEGFR3+ CD133+	1,000/ mL	before TM	PFS	positive
Sakamori Y 2012	Japan	NSCLC	38	III-IV	FCM	CD45- CD34+ CD31+ CD133+	168.70%	before and after TM	PFS	negative
Pirro M 2013	Italy	NSCLC	34	I-CII	FCM	CD34+ KDR+	320/mL	before and after TM	EFS	positive

NSCLC,non-small cell lung cancer; SCLC,small cell lung cancer;FCM,flow cytometry;IMT/FM,immunomagnetic technique /fluorescent microscopy,DAPI, 4¡¯,6-diamidino-2-phenylindole;NR,not repoeted TM,treatment; OS, overall survival; PFS, progression free survival;EFS,Event-free survival

Bogos K's study. The markers used for CECs staining were commonly factor CD146, CD105 or CD45, whereas only one study stained for CD31 (Yuan et al., 2015). The markers for CEPCs staining were CD34, VEGFR2, VEGFR3, CD133 or KDR in all of our eligible studies. The methods to count the cells include CellTracks, immunomagnetic technique / fluorescent microscopy (IMT/FM) and flow cytometry (FCM).

Survival at 1 years. As between-study heterogeneity was significant (I^2>50.0%) for both CECs and CEPCs group, random model was used. High baseline CECs levels were associated with worse prognosis of OS regarding to the result within 1 years (OR 1.641, 95% CI 0.967-2.786, p=0.008) (Figure 1), but not PFS (OR 1.168, 95% CI 0.649-2.100, p=0.062) (Figure2). Therefore, the overall mortality was 1.641-fold higher for patients whose baseline CECs levels were above the cut-off, compared with those below it. Begg's test and funnel was used to evaluate publication bias. No significant publication biases were found in results of meta-analyses of CECs

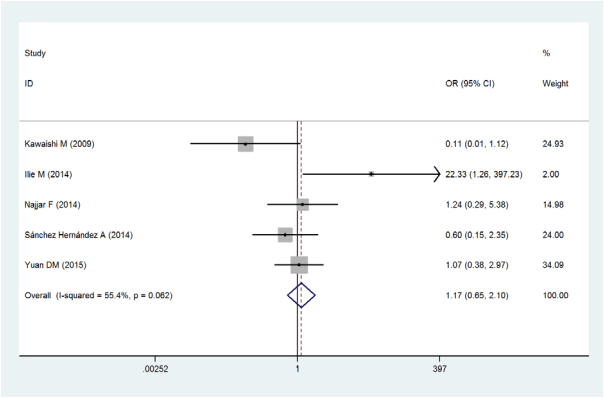

Figure 2. Meta-analysis of the Association Between CECs and PFS at 1 Years. Each study is shown by the name of the lead author and the OR with 95% CIs

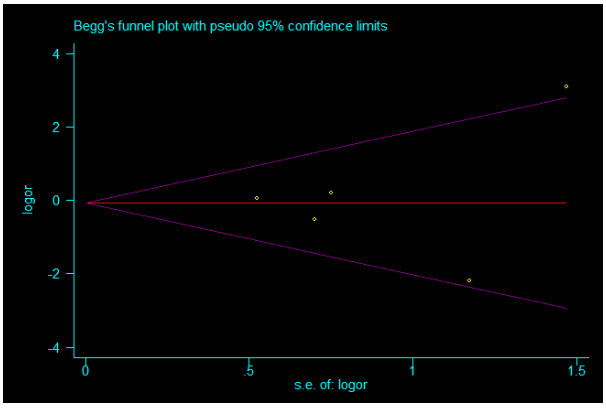

Figure 3. Begg's Funnel Plot Showing the Relation Between OR and 1/SE for the analysis of CECs and OS. Larger Studies (Those with Larger 1/SE) Show Generally Smaller ORs

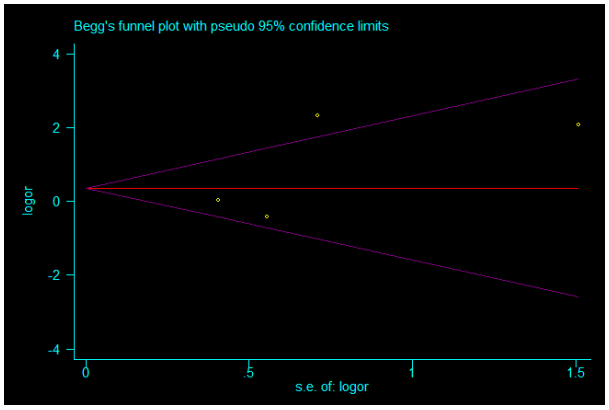

Figure 4. Begg's Funnel Plot Showing the Relation Between OR and 1/SE for the analysis of CECs and PFS. Larger Studies (Those with Larger 1/SE) Show Generally Smaller ORs

levels for 1-year PFS or OS (Figure 3). Consequently, for CEPCs, the patients with high baseline CEPCs levels were associated both with worse prognosis of OS (OR 12.673, 95% CI 5.274-30.450, $p<0.0001$) and PFS (OR 4.930, 95% CI 0.931-26.096, $p=0.006$).

Subgroup analyses were conducted according to clinical stage. We analyzed the effect of high CECs counting before treatment on patients with advanced non-small-cell lung cancer (NSCLC). Negative effects of CECs on 1-year PFS were shown still (n=521, RR 0.735, 95% CI 0.377-1.433, $p=0.302$) and positive effects on OS were displayed (n=521, RR 1.493, 95% CI 0.866-2.576, $p=0.005$).

Discussion

It has been reported that CECs and CEPCs levels might either increase or decrease after treatment (Schillaci et al., 2009, Stein et al., 2008). In patients achieving complete remission or complete response, the level of CECs and CEPCs can be subsequently reduced (Mancuso et al., 2001), while in patients with incomplete surgery or nonresponding patients (patients with local recurrence or stable/progressive disease) tended to have higher CECs and CEPCs levels (Dome et al., 2006). But the current result of the predictive role of pretreatment CECs level is ambiguous, even within the 7 studies we included. Sanchez Hernandez et al. (2015), Najjar et al. (2015), Wang et al. (2013) reported no correlation between response to treatment and pretreatment CECs levels. While the increase of CECs numbers after the first cycle could be a negative predictive factor. Ilie et al. (2014), Fleitas et al. (2012) demonstrated high baseline level of CECs correlated with poor prognosis. However, Chu' study implies that higher pretreatment CECs level indicates higher response rate and improved PFS (Chu et al., 2012).

According to our meta-analysis, it seems that the pretreatment CECs level is a promising predictor of clinical response and survival in advanced NSCLC. Because CECs is a marker of angiogenesis, it is likely that a high CECs value is associated with a poor prognosis and lower effectiveness of antiangiogenic therapy, which will lead to poor PFS/OS. This also indicates an anti- angiogenic regimen might be more effective against tumors with high CECs values. But it still needs more clinical trials before trying to make any certain conclusions.

Circulating endothelial cells (CECs) have been recognized as a useful biomarker for vascular damage (Kawaishi et al., 2009). It is increased in many benign diseases and various cancers. In our meta-analysis, high counts of CECs seem to be associated only with worse 1-year OS in patients with lung cancer. This might partly due to the lack of survival data and small sample size. Many related studies have shown controversial results.

The two main methods for the quantification of CECs and CEPCs are based on flow cytometry or inmunomagnetic separation (Woywodt et al., 2006; Mancuso et al., 2009). Twelve studies in our analysis have been using flow cytometry for the quantification of CECs and CEPCs, while 1 study has been using inmunomagnetic separation. Those two different methods have both been proved to be effective.

Not long ago, malignant tumors were thought to acquire vasculature only through local vessel angiogenesis, the mechanism by which new capillaries can only arise from pre-existing ones (Sakamori et al., 2012). However, recent evidence suggests that tumor vasculature can also arise through vasculogenesis, a process in which bone marrow derived endothelial

progenitor cells contribute to neovascularization (Davidoff et al., 2001; Bolontrade et al., 2002). It is postulated that circulating endothelial progenitor cells (CEPs) are mobilized from the bone marrow into the circulation by tumor- or ischemia-induced signals (Sakamori et al., 2012). CEPs subsequently migrate through blood flow to sites of tumor neovascularization, where they differentiate into endothelial cells and contribute to angiogenesis (Asahara et al., 1997; Rafii et al., 2002; Gao et al., 2008). The angiogenic cytokine released from CEPCs might be a supportive mechanism to improve neovascularization (Urbich et al., 2005;Yoon et al., 2005).

Our analysis showed a high level of pro-treatment CEPCs correlated with both with worse PFS and OS. Further predictive role of CEPCs can't be decided due to the lack of clinical data. It is noteworthy, that the CEPCs level was found to be a sensitive surrogate marker of the angiogenic activity in murine model (Shaked et al., 2005). A study has also showed a tendency that responders tended to have lower pretreatment CEPCs numbers than those who did not respond (Treat et al., 2005). Jeanine (Roodhart et al., 2010) has also demonstrated CEPCs number can increase after chemotherapy and predicted worse PFS/OS, regardless of tumor type or chemotherapy regime. Adjuvant chemotherapy showed similar kinetics indicated the increase in CEPCs is seemingly unrelated to the presence of a tumor. Dome et al. (2006) has reported a significantly higher incidence of death from NSCLC in patients with high pretreatment CEPCs levels compared with patients with low CEPCs levels, which suggesting that the pretreatment levels of CEPCs correlate with the clinical behavior of human NSCLC. It has also been reported the numbers of CEPCs rapidly decline in rectal cancer patients receiving antiangiogenic treatment (Willett et al., 2005). This assumption corresponds to the "*vessel normalization*" hypothesis described by Jain et al. (2005) (Jain et al., 2005; Stetler-Stevenson et al., 2014) regarding the clinical effects of anti-VEGF therapy. Our meta-analysis along with those studies suggest that those patients with higher pretreatment CEPCs numbers (more tortuous intratumoral capillaries), presumably having less normalized tumor vessels, respond worse, whereas those with lower level of CEPCs respond better. Consequently, patients with high pretreatment CEPCs numbers could be treated with anti-VEGF therapy to lower CEPCs number (normalizing the vasculature) before/with chemotherapy, thus potentially improving therapeutic response, which also indicates that pretreatment CEPCs might be a potential target biomarker for the effectiveness of anti-angiogenic therapy.

Between-study heterogeneity was significant in our study (I^2>50%). Different cut-off value of CECs and CEPCs levels were used in different studies. We tried to reduce the variability by screening the literature using the same standard and dividing studies into subgroups, such as the same sex,same clinical stage, and cut-off values in prognostic meta-analysis. Although the heterogeneity could not be eliminated totally, the heterogeneity had decreased in some subgroups such as patients with advanced lung cancer in clinical stage III-IV (17.7%), which revealed that all the factors have effects on the generation of heterogeneity and cannot be eliminated at the same time. In addition, the limitations still exist in the present detection method. We attempted to minimize publication bias by searching completely, but it was unavoidable that some data was missed for various reasons such as unpublished or ignored studies (Nowak et al., 2010).

In conclusion, this meta-analysis suggested that High counts of CECs seem to be associated with worse 1-year OS in patients with lung cancer, while high level of pretreatment CEPCs correlated with both with worse PFS and OS. These results need be confirmed by more clinical trials.

References

Aggarval C, Somaiah N, Simon GR (2010). Biomarkers with predictive and prognostic function in non-small cell lung cancer, ready for prime time? *J NCCN*, **8**, 822-32.

Altman DG (2001). Systematic reviews of evaluations of prognostic variables. *BMJ*, **323**, 224- 8.

Asahara T, Murohara T, Sullivan A, et al (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Sci*, **275**, 964-7.

Begg CB (1994). Publication bias. **25**, 299-409.

Bogos K, Renyi-Vamos F, Dobos J, et al (2009). High VEGFR-3-positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. *Clin Cancer Res*, **15**, 1741-6.

Bolontrade MF, Zhou RR and Kleinerman ES (2002). Vasculogenesis Plays a Role in the Growth of Ewing's Sarcoma *in Vivo*. *Clin Cancer Res*, **8**, 3622-7.

Chu TQ, Ding H, and Garfield DH (2012). Can determination of circulating endothelial cells and serum caspase-cleaved CK18 predict for response andsurvival in patients with advanced non-small-cell lung cancer receiving endostatin and paclitaxel-carboplatinchemotherapy? a retrospective study. *J Thorac Oncol*, **7**, 1781-9.

Davidoff AM, Ng CY, Brown P, et al (2001). Bone marrow-derived cells contribute to tumor neovasculature and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. *Clin Cancer Res*, **7**, 2870-9.

DerSimonian R, Laird NM (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials*, **7**, 177-88

Dome B, Timar J, Dobos J, et al (2006). Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lungcancer. *Cancer Res*, **66**, 7341-7.

Fleitas T, Martinez-Sales V, Vila V, et al (2012). Circulating endothelial cells and microparticles as prognostic markers in advanced non-small cell lung cancer. *PLoS One*, **7**, 47365.

Fadini GP, Kreutzenberg S, Albiero M, et al (2008). Gender differences in endothelial progenitor cells and cardiovascular risk profile, the role of female estrogens. *Arterioscler Thromb Vasc Biolb*, **28**, 997-1004.

Gao D, Nolan DJ, Mellick AS, et al (2008). Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science*, **319**, 195-8.

Ilie M, Long E, Hofman V, et al (2014). Clinical value of circulating endothelial cells and of soluble CD146 levels in patients undergoing surgery for non-small cell lung cancer. *Br J Cancer*, **110**, 1236-43

Jain RK (2005). Normalization of tumor vasculature, an emerging concept in antiangiogenic therapy. *Sci*, **307**, 58-62.

Jemal A, Bray F, Center MM, et al (2009). Global cancer statistics. *CA Cancer J Clin*, **61**, 69.

Kawaishi M, Fujiwara Y, Fukui T, et al (2009). Circulating

endothelial cells in non-small cell lung cancer patients treated with carboplatin and paclitaxel. *J Thorac Oncol*, **4**, 208-13

Mancuso P, Burlini A, Pruneri G, et al (2001). Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood*, **97**, 3658-61.

Mancuso P, Antoniotti P, Quarna J, et al (2009). Validation of a standardized method for enumerating circulating endothelial cells and progenitors, flow cytometry and molecular and ultrastructural analyses. *Clin Cancer Res*, **15**, 267-73.

Melero-Martin JM, Dudley AC (2011). Concise review,vascular stem cells and tumor angiogenesis. *Stem Cells*, **29**, 163-8.

Morita R, Sato K, Nakano M,et al (2011). Endothelial progenitor cells are associated with response to chemotherapy in human non-small-cell lung cancer. *J Cancer Res Clin Oncol*, **137**, 1849-57.

Najjar F, Alammar M, Bachour M, et al (2015). Predictive and prognostic value of circulating endothelial cells in non-small cell lung cancer patients treated withstandard chemotherapy. *J Cancer Res Clin Oncol*, **141**, 119-25.

Nowak K, Rafat N, Belle S, et al (1998). Circulating endothelial progenitor cells are increased in human lung cancer and correlate with stage of disease. *Eur J Cardiothorac Surg*, **37**, 758-63.

Parmar MK, Torri V, Stewart L, et al (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Pirro M, Cagini L, Paciullo F, et al (2013). Baseline and post-surgery endothelial progenitor cell levels in patients with early-stage non-small-cell lungcarcinoma, impact on cancer recurrence and survival. *Eur J Cardiothorac Surg*, **44**, 245-52.

Pirro M, Schillaci G, Bagaglia F, et al (2008). Microparticles derived from endothelial progenitor cells in patients at different cardiovascular risk. *Atherosclerosis*, **197**, 757-67.

Pirro M, Schillaci G, Menecali C, et al (2007). Reduced number of circulating endothelial progenitors and HOXA9 expression in CD34+ cells of hypertensive patients. *J Hypertens*, **25**, 2093-9.

Pirro M, Schillaci G, Paltriccia R, et al (2006). Increased ratio of CD31+/CD42– microparticles to endothelial progenitors as a novel marker of atherosclerosis in hypercholesterolemia. *Arterioscler Thromb Vasc Biol*, **26**, 2530-5.

Rafii S, Lyden D, Benezra R, et al (2002). Vascular and haematopoietic stem cells, novel targets for anti-angiogenesis therapy? *Nat Rev Cancer*, **2**, 826-35.

Roodhart JM, Langenberg MH, Vermaat JS, et al (2010). Late release of circulating endothelial cells and endothelial progenitor cells after chemotherapy predicts response and survival in cancer patients. *Neoplasia*, **12**, 87-94.

Sakamori Y, Masago K, Ohmori K, et al (2012). Increase in circulating endothelial progenitor cells predicts response in patients with advanced non-small-celllung cancer. *Cancer Sci*, **103**, 1065-70.

Sanchez Hernandez A, Jose Juan O, et al (2015). Vidal Martinez J,Quantification of circulating endothelial cells as a predictor of response to chemotherapy with platinum andpemetrexed in patients with advanced non-squamous non-small cell lung carcinoma. *Clin Transl Oncol*, **17**, 281-8.

Schillaci G, Rondelli F, Pirro M, et al (2009). Endothelial progenitor cells are mobilized after major laparotomic surgery in patients with cancer. *Int J Immunopathol Pharmacol*, **22**, 1035-41.

Shaked Y, Murohara T, Sullivan A, et al (2005). Optimal biologic dose of metronomic chemotherapy regimens is associated with maximum antiangiogenic activity. *Blood*, **106**, 3058-61.

Shantsila E, Blann AD, Lip GY (2008). Circulating endothelial cells, from bench to clinical practice. *J Thromb Haemost*, **6**, 865-8.

Shantsila E, Lip GY (2008). Circulating endothelial cell in health and disease, how do we best quantify them? *J Thromb Haemost*, **6**, 1021-4.

Stein A, Montens HP, Steppich B, et al (2008). Circulating endothelial progenitor cells decrease in patients after endarterectomy. *J Vasc Surg*, **48**, 1217-22.

Stetler-Stevenson WG and Gavil NV (2014). Normalization of the tumor microenvironment, evidence for tissue inhibitor of metalloproteinase-2 as a cancer therapeutic. *Connect Tissue Res*, **55**, 13-9.

Treat J (2005). Incorporating novel agents with gemcitabine-based treatment of NSCLC. *Lung Cancer*, **50**, 8-9.

Urbich C, Aicher A, Heeschen C, et al (2005). Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol*, **39**, 733-42.

Wang J, Xiao J, Wei X, et al (2013). Circulating endothelial cells and tumor blood volume as predictors in lung cancer. *Cancer Sci*, **104**, 445-52.

Willett CG, Boucher Y, Duda DG, et al (2005). Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy, continued experience of a phase I trial in rectal cancer patients. *J Clin Oncol*, **23**, 8136-9.

Woywodt A, Blann AD, Kirsch T, et al (2006). Isolation and enumeration of circulating endothelial cells by immunomagnetic isolation, proposal of a definition and a consensus protocol. *J Thromb Haemost*, **4**, 671-7.

Yoon CH, Hur J, Park KW, et al (2005). Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells, the role of angiogenic cytokines and matrix metalloproteinases. *Circulat*, **112**, 1618-27.

Yuan DM, Zhang Q, Lv YL, et al (2015). Predictive and prognostic significance of circulating endothelial cells in advanced non-small cell lung cancerpatients.*Tumour Biol*.

Yue WS, Wang M, Yan GH, et al (2010). Smoking is associated with depletion of circulating endothelial progenitor cells and elevated pulmonary artery systolic pressure in patients with coronary artery disease. *Am J Cardiol*, **106**, 1248-54.