The Pretreatment Plasma Prognosis Biomarkers for Advanced NSCLC Patients Treated by Bevacizumab Plus Chemotherapy and the Correlation Between Them

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Research

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Abstract

Background

Bevacizumab combined with chemotherapy is still one of the first-line treatment of advanced non-small cell lung cancer (NSCLC). Prior studies have devoted to exploring the possible pretreatment hematological biomarkers for progression-free survival (PFS) and overall survival (OS). In this study, we provide valid predictive biomarkers for NSCLC patients before treatment, and reveal the correlation of these serum biomarkers.

Methods

Clinical characteristics and hematological indicators concentrations in NSCLC patients were collected prospectively before Bevacizumab and chemotherapy from December 2018 and December 2019. Patients were divided into high group and low group according to the means and medians. A Cox proportional hazard regression model was performed to univariate and multivariate analyze. The Pearson Correlation Coefficients were used to identify the correlation between hematological biomarkers.

Results

28 patients were enrolled in the study. The average of IL-8 was 7.32 pg/ml, and the average of VEGF-A was 65.41 pg/ml. After 12 months median follow-up time, IL-8 and VEGF-A was independent prognostic factors of OS, and VEGF-A was independent predictive factors of PFS. The death risk in high IL-8 (>7.32 pg/ml) group was 6.361 times than that in low IL-8 (≤7.32 pg/ml) group (HR=6.361, 95%CI: 2.034-19.890, p=0.001). Meanwhile, the death risk in high VEGF-A (>65.41 pg/ml) group was 9.686 times than that in low VEGF-A (≤65.41 pg/ml) group (HR=9.686, 95%CI: 1.906-49.222, p=0.006), and the disease progression or death risk in high VEGF-A group was 5.627 times than that in low VEGF-A group (HR=5.627, 95%CI: 1.322-23.951, p=0.019). There was a highly linear correlation between CTLA-4 with PD-1 and PD-L1 in peritoneal blood samples before treatment. The Pearson Correlation Coefficient was 0.984 (p=0.000) and 0.946 (p=0.000). PD-1 ($y=-13.91+0.06*x$, $R^2=0.968$) and PD-L1 ($y=-29.42+0.2*x$, $R^2=0.895$) vary with CTLA-4. Besides, VEGF-D, FGF-1 and FGF-2 also has highly linear correlation with each other.

Conclusions

IL-8 is the independently prognosis biomarker for OS of NSCLC patients treated with bevacizumab plus chemotherapy. VEGF-A is an independently prognosis biomarker of OS and PFS. There is a highly linear correlation between CTLA-4, PD-1 and PD-L1 in baseline blood. VEGF-D, FGF-1 and FGF-2 also has highly linear correlation with each other.

Background

The reason why solid tumors can growth to several cubic centimeters is that growing tumors can vascularize to provide nutrients and oxygen[1], and tumor angiogenesis is considered as one of the hallmarks of cancer[2]. There are many signal molecules expressed or released by cancers cells and stroma cells in tumors complex microenvironments, which can affect cells proliferation, migration, adhesion, invasion, angiogenesis and immune tolerance. Pro-angiogenic and anti-angiogenic factors can control the progression of angiogenesis in microenvironments, for example, vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (TSP-1) are prototypes of pro-angiogenic and anti-angiogenic factor[2]. Besides, Angiopoietin-2, some members of the fibroblast growth factor (FGF) family and interleukin (IL) family are also regulate tumor angiogenesis[3]. Bevacizumab (Avastin) as the first humanized monoclonal antibody against VEGF-A, can inhibit vascular endothelial growth factor (VEGF) signaling pathways by binding to VEGF-A, then inhibit tumors growth. And bevacizumab can decrease the interstitial pressure which is elevated by VEGF through disorganized and “leaky” vasculature[4]. So that bevacizumab can facilitate the delivery of chemotherapy to tumors, which means the combination of bevacizumab and chemotherapy is possible.

Lung cancer is the most common cancers in men, and the leading cause of cancer-related deaths in both men and women, with approximately 85% of whom are non-small cell lung cancer (NSCLC)[5]. Since the inspiring outcome of ECOG 4599 study (NCT00021060) reported[6], further review or meta-analysis studies have demonstrated that bevacizumab plus chemotherapy makes a contribution to significant objective response rate (ORR) and progression-free survival (PFS) benefit when compared with chemotherapy alone in patients with advanced NSCLC[7, 8]. FDA approves bevacizumab in combination with paclitaxel and carboplatin for first-line treatment of advanced non- squamous NSCLC without brain metastasis and no history of bleeding. Particularly, it also had been approved to be used in combination with immune checkpoint inhibitors (ICI) recently. Different from other targeted therapies, such as epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), bevacizumab is used in unselected patients[9]. Although many efforts have made to identify the predictive prognosis biomarkers, these efforts were unfortunately unsuccessful in identifying a single effective biomarker[10]. Validated biomarkers which can predict the prognosis of bevacizumab plus chemotherapy and enable personalized administration still need further investigate. Therefore, this study was designed to research the probably hematologic biomarkers and explore the relationship of those hematologic factors.

Methods

Patients

Clinical characteristics and blood samples from patients with NSCLC were prospectively collected and analyzed before the treatment of bevacizumab combined with chemotherapy at Shandong Cancer Hospital and Institute between December 2018 and December 2019. The inclusion criteria were as follows: 1) 18–75 years old; 2) histological or cytological confirmed NSCLC; 3) inoperable stage III or stage IV based on the eighth edition of the American Joint Committee on Cancer (AJCC) staging

manual[11]; 4) did not receive previous systemic anticancer regimens; 5) intend to receive bevacizumab plus chemotherapy within one week; 6) had at least one measurable lesion by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[12]; 7) 0–1 performance status on the Eastern Cooperative Oncology Group (ECOG) Performance Status[13]. Patients with or without genetic mutations were permitted after the discretion of investigators. But, patients were excluded from the analysis if they had acute or chronic inflammatory disease, received any anti-inflammatory treatments.

The following clinical characteristics were reviewed and collected within one week before the administration of bevacizumab: gender, age, ECOG performance status, smoking status, histology status, mutation status and stage.

This study was in accordance with the Declaration of Helsinki, and was approved by the Ethics Committee of Shandong Cancer Hospital and Institute. Written informed consents were provided from all patients before enrolled.

Blood samples

Peritoneal blood samples which were collected into tubes within three days before the administration of bevacizumab combined with chemotherapy, were centrifuged at 1000×g for 10 minutes. Then, the serum could be frozen at -80°C until use.

Based on manufactures protocols, angiopoietin-2, fibroblast growth factor-1 (FGF-1), interleukin-8 (IL-8), placental growth factor (PLGF), vascular endothelial growth factor-C (VEGF-C), vascular endothelial growth factor-D (VEGF-D), fibroblast growth factor-2 (FGF-2) and VEGF-A were analyzed by Magnetic Circulating Cancer Kit (Millipore, USA), programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte associated protein-4 (CTLA-4), cluster of differentiation-80 (CD80/B7-1), cluster of differentiation-86 (CD86/B7-2) and programmed cell death ligand-1 (PD-L1) were assayed by Human Immuno-Oncology Checkpoint Protein Panel 1(Millipore, USA), interleukin-18 (IL-18) was measured by Human IL-18 Singleplex Magnetic Bead Kit (Millipore, USA). Serum samples were mixed with chemically dyed antibody-bound beads, and then were washed and incubated with biotinylated detection antibody and phycoerythrin-labeled streptavidin. After incubation, the fluorescent intensities were quantified by the Luminex 200 analyzer.

Follow up

Tumors responses and patients survivals were assessed by investigators every two cycles according to RECIST version 1.1, during administered bevacizumab plus chemotherapy, and then assessed every 6 to 8 weeks during follow-up. PFS was the time from the first dose of bevacizumab to disease progression according to RECIST version 1.1 or death due to any cause, whichever happened earlier. Overall survival (OS) was the time from the first administration of bevacizumab to death from any reason.

Statistical analysis

A Cox proportional hazard regression model was performed to univariate and multivariate analyze. The Kaplan-Meier survival curves and Schoenfeld residual were used to assess Proportional Hazards (PH) assumption, meanwhile, Kaplan-Meier survival curves was used to plot survival curves. The Martingale residual was performed to diagnose linear condition of continuous variable in Cox proportional hazard regression model. Before multivariate analyze, multicollinearity was analyzed. The Pearson Correlation Coefficients were used to identify the correlation between hematological biomarkers. A value of $p < 0.05$ was considered statistically significant, based on a two-sided hypothesis. SPSS Statistics 23.0 (IBM Corporation, Armonk, USA) and R (version 4.04) were used for all statistical analyses.

Results

Patient characteristics

Between December 2018 and December 2019, a total of 30 patients were enrolled in the study, but two patients lacked valid data of biomarkers concentrations, so that 28 patients were enrolled to analyzed finally. The median follow-up time was 12 months and the last follow-up time was January 27, 2021. The median age of patients was 58 years (range, 31 to 73 years), and there were 19 (68%) male and 9 (32%) female. Most patients were adenocarcinoma (26 patients, 93%), and only two patients (7%) were squamous cell carcinoma. 8 patients (29%) had gene mutations, such as EGFR mutations or ALK rearrangements, while 20 patients (71%) were gene wild type, and patients with stage III vs IV was 5 vs 23 (18% vs 82%). Demographics and patients baseline characteristics were listed in Table 1.

Prognosis biomarkers

Martingale residual showed that there was no linear trend in these continuous hematological variables before Cox proportional hazard regression model was performed, so that continuous variables were transformed into binary variables according to the means and medians (Table 2). Based on the means and medians, patients were divided into low serum biomarker groups and high serum biomarker groups respectively.

The univariate analysis was listed in Table 3. The death risk in high IL-8 (>7.32 pg/ml) group was 6.361 times than that in low IL-8 (≤7.32 pg/ml) group (HR=6.361, 95%CI: 2.034-19.890, p=0.001) (Figure 1a). Meanwhile it showed that the death risk in high VEGF-A (>65.41 pg/ml) group was 9.686 times than that in low VEGF-A (≤65.41 pg/ml) group (HR=9.686, 95%CI: 1.906-49.222, p=0.006) (Figure 1b), and the disease progression or death risk in high VEGF-A (>65.41 pg/ml) group was 5.627 times than that in low VEGF-A (≤65.41 pg/ml) group (HR=5.627, 95%CI: 1.322-23.951, p=0.019) (Figure 1c). It seemed that patients with stage IV had a longer PFS than those with stage III (HR=0.397, 95%CI:0.137-1.148), and patients with high FGF-2 (>9.79 pg/ml) had a shorter PFS than those with low FGF-2 (≤9.79 pg/ml) (HR=2.299, 95%CI: 0.933-5.669). But the differences were not significant in these groups (p=0.088 and 0.071 respectively) in univariate analysis (Figure 1d, 1e).

Due to the small number of patients, the variables included in the multivariate analysis should not be too many, so only the variables with a p-value less than 0.05 in univariate analysis were incorporated into the multivariate analysis. Thus, the hematologic biomarkers included in multivariate analysis were as follows: IL-8 and VEGF-A in OS multivariate analysis, VEGF-A in multivariate analysis for PFS. There was no strong inter-variable correlation after multicollinearity assessed. In multivariate analysis, IL-8 (p=0.003) and VEGF-A (P=0.022) was independent prognostic factors of OS, and VEGF-A (p=0.019) was independent predictive factors of PFS. Higher IL-8 (>7.32 pg/ml) has a shorter OS (HR=5.798, 95%CI: 1.807-18.599) when patients administered bevacizumab plus chemotherapy for first or second-line therapy, higher pretreatment VEGF-A (>65.41 pg/ml) has a shorter OS (HR=7.389, 95%CI: 1.334-40.928) and PFS (HR=5.627, 95%CI: 1.322-23.951) compared with lower VEGF-A.

Correlation between hematological biomarkers

Although there were no strong inter-variable correlations between variables before incorporated into the multivariate analysis, some hematologic variables were found had highly positive correlation (Pearson Correlation Coefficient was 0.8 to 1.0). Firstly, PD-1, PD-L1, CTLA-4, CD80/B7-1 and CD86/B7-2 had highly positive correlation between each other (Table 4a). Particularly, different from the relationship of ligands and receptors, such as PD-1 with PD-L1, as well as, CTLA-4 with CD80/B7-1 and CD86/B7-2, there was a highly linear correlation between CTLA-4 with PD-1(Pearson Correlation Coefficient was 0.984, p=0.000) and PD-L1 (Pearson Correlation Coefficient was 0.946, p=0.000) in peritoneal blood samples before treatment. The higher PD-1 ($y=-13.91+0.06*x$, $R^2=0.968$) and PD-L1 ($y=-29.42+0.2*x$, $R^2=0.895$), the higher CTLA-4 (Figure 2). Secondly, as important factors of angiogenesis, VEGF-D, FGF-1 and FGF-2 had highly linear correlation between each other (Table 4b).

Discussion

Since anti-angiogenesis was widely approved in many kinds of cancers, many studies have been devoted to research probable blood biomarkers that can predict the efficacy and prognosis of bevacizumab. Some indicators have been widely reported to be important predictors of cancer patients with bevacizumab. On the one hand, IL-8 has pro-tumor functions through tumor angiogenesis, as a chemokine[14]. As the member of interleukin family, IL-8 is different with interleukin (IL-6) whose genetic variants had been demonstrated affect the prognosis of patients with metastatic colorectal cancers treated with bevacizumab-based chemotherapy[15]. IL-8 baseline level had potentially but not identical correlations with benefits of bevacizumab in some studies[16]. Surprisingly, higher pretreatment IL-8 was associated with shorter OS in this study. It probably that bevacizumab inhibit the pro-angiogenesis function of VEGF-A by blocking VEGF-A, but IL-8 mediated angiogenesis then was enhanced, leading to drug resistance. Thus, the more IL-8, the worse prognosis in patients with bevacizumab. On the other hand, as bevacizumab targeted site, VEGF-A was likely to play an important role in the treatment of bevacizumab in theory. A multicancer meta-analysis with 1816 patients confirmed that VEGF-A level was a prognostic biomarker rather than a predictive biomarker, and it revealed that higher VEGF-A was associated with

poorer survival in metastatic colorectal, lung and renal cell cancers[17]. Meanwhile, this study demonstrated that patients with higher VEGF-A has shorter OS and PFS. But, there were conflicting conclusions in some other studies, AVADO trial and AVAGST trial showed that higher baseline VEGF-A levels exhibited improved PFS and/or OS in breast cancer and gastric cancer[18, 19]. In addition of the baseline and pretreatment level of VEGF-A, the treatment-related change of VEGF-A was likely a predictive biomarker[16], but further more valid research is required. Besides, other potential indicators which were associated with bevacizumab resistance were investigated, such as other VEGF family, FGF family, PLGF and Angiopoietin-2 recent years[9]. There was a study that showed renal cell carcinoma patients with low baseline of VEGF-C and increase after treatment were more likely benefited from bevacizumab[20]. Those breast cancer or colorectal cancer patients with lower Angiopoietin-2 levels before treatment responded better to the treatment of bevacizumab[21, 22]. And the explorations are ongoing in new era

CTLA-4 mainly express on active T cells in secondary lymphoid tissues, and dampens the antigen-presenting of antigens present cells (APC) and the activation of T cell through higher affinity of CD80/B7-1 and CD86/B7-2 compared with CD28. The anti-tumor immunity of CTLA-4 mainly happened in secondary lymphoid tissues rather than the tumor microenvironment (TME). While, PD-L1, is mainly located on the surface of tumor cells, so its receptor PD-1 is expressed on activated T cells in the TME[23]. Then, the immune cells and tumor cells can secrete some membrane immune checkpoints function parts, which are called soluble CTLA-4 (sCTLA-4), soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1)[24] (Fig. 3). There were highly linear correlations between sCTLA-4 with sPD-1 and sPD-L1 in this study, so we can hypothesize that both the CTLA-4 and PD-1/PD-L1 pathway exist in patients with cancers, meanwhile, the CTLA-4 and PD-1/PD-L1 pathway play a co-function in tumor immune escape. The highly linear correlation between sCTLA-4 with sPD-1 and sPD-L1 provides a strong theoretical support for the combination of anti-CTLA-4 therapy and anti-PD-1 or PD-L1 therapy. For example, multiple studies have prompted the approval of nivolumab plus ipilimumab combination in metastatic melanoma, advanced colorectal cancers and advanced renal cancers[25]. Checkmate 227 and Checkmate 9LA reported a better ORR, a longer duration of OS, favorable OS and risk-benefit profile in NSCLC patients[26, 27]. Although the sCTLA-4, sPD-1 and sPD-L1 could not predict the prognosis in this study, many other studies had showed its ability to be predictive biomarkers[24, 28, 29]. VEGF and FGF-2 are the strongest pro-angiogenesis factors, which can prompt endothelial cells migration and proliferation[30]. And some studies have demonstrated that FGF-2 is independent of VEGF in the function of angiogenesis[31], which possibly one of the mechanisms of bevacizumab resistance. Based on the finding that VEGF-D, FGF-1 and FGF-2 had highly linear correlation, we hypothesize that anti-FGF agents in addition to anti-VEGF agents may have a promising co-effect in anti-angiogenesis.

The study also has several limitations. Firstly, the study was conducted only at a single medical center, with a small number of patients collected. Therefore, a large sample analysis is needed. Secondly, although we adopted strict inclusion and exclusion criteria, hematological indicators of advanced NSCLC in this study would be affected by a variety of factors, and the influence of other factors on blood should

be excluded as far as possible in subsequent studies. Because of the relatively long survival after progression and complexity of subsequent therapy, some biomarkers are likely to be obscured when biomarkers of OS were researched. Thirdly, only the pretreatment concentrations were collected in the study, while some other studies have demonstrated that changes could be a prognosis biomarker during treatment[16]. Fourthly, the combination therapy of bevacizumab and erlotinib was approved in NSCLC patients with EGFR mutation after the pivotal study JO25567[32], and many other studies of targeted agents plus bevacizumab are further confirming the benefits[33–35]. Recent years, the intersection between tumor angiogenesis and immune suppression was revealed[36], VEGF signaling pathway can impair function of dendritic cells and T cells, prompt the activity of regulatory T cells, and inhibit survival of activated T cells. Targeting therapy of VEGF/VEGFR was recognized as a method to enhance antitumor immunity. The most pivotal study was Impower 150, which prompted the approval of the combination of atezolizumab, bevacizumab and chemotherapy in non-squamous NSCLC[37]. And the results of numerous bevacizumab biosimilars[38, 39] and new anti-angiogenesis agents[40] were reported recently. Nevertheless, we only focused on the most fundamental combination therapy of bevacizumab plus chemotherapy, ignored the new combination therapies and new agents in this study, so the biomarker research of new combination therapies in new era is still required. Finally, the concentrations of biomarkers were texted by Millipore panels and kits in this study, if specific hematological biomarkers are identified, standardization of assay equipment and protocols are needed internationally. These limitations can be transformed into the focus of future researches.

Conclusion

IL-8 is the independent prognosis biomarker of OS in NSCLC patients with bevacizumab plus chemotherapy. Those who with higher IL-8 levels (> 7.32 pg/ml) may have shorter OS. Pretreatment VEGF-A is an independently prognosis biomarker of PFS and OS. Patients with higher pretreatment VEGF-A levels (> 65.41 pg/ml) has shorter PFS and OS. There is a highly linear correlation between CTLA-4 with PD-1 and PD-L1 in blood before treatment, and VEGF-D, FGF-1 and FGF-2 also has highly linear correlation.

Abbreviations

VEGF-A, vascular endothelial growth factor-A; TSP-1, thrombospondin-1; FGF, fibroblast growth factor; IL, interleukin; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; ICI, immune checkpoint inhibitors; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; AJCC, American Joint Committee on Cancer; RECIST, Response Evaluation Criteria in Solid Tumors; ECOG, Eastern Cooperative Oncology Group; FGF-1, fibroblast growth factor-1; IL-8, interleukin-8; PLGF, placental growth factor; VEGF-C, vascular endothelial growth factor-C; VEGF-D, vascular endothelial growth factor-D; FGF-2, fibroblast growth factor-2; PD-1, programmed cell death protein-1; CTLA-4, cytotoxic T-lymphocyte associated Protein-4; CD80/B7-1, cluster of differentiation-80; CD86/B7-2, cluster of differentiation-86; PD-L1, programmed cell death

ligand-1; IL-18, interleukin-18; OS, overall survival; PH, Proportional Hazards; IL-6, interleukin; APC, antigens present cells; TME, tumor microenvironment; sCTLA-4, soluble CTLA-4; sPD-1, soluble PD-1; sPD-L1, soluble PD-L1.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Shandong Cancer Hospital and Institute.

Consent for publication

Not applicable.

Availability of data and materials

The data in the study are available from the corresponding author after reasonable request.

Competing interests

The authors had no conflicts of interest.

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Authors' contributions

Jingru Liu: Contributed to study design, research performing, writing and revising the article. ZG, SW, MW and Jianing Li: Contributed to writing and revising the article. XM: Contributed to supervising, writing and revising the article and funding acquisition. Jie Liu: Contributed to study design, research performing, supervising, writing and revising the article. All authors read and approved the final manuscript.

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Tables

Table 1

Demographics and patient baseline characteristics

	No. (%)
Gender	
Male	19 (68)
Female	9 (32)
Age (years)	
≤65	19 (68)
>65	9 (32)
ECOG performance status	
0	11 (39)
1	17 (61)
Smoking status	
Never	13 (46)
Current or former	15 (54)
Histology status	
Squamous	2 (7)
Adenocarcinoma	26 (93)
Mutation status	
Negative	20 (71)
Positive	8 (29)
Stage of disease	
III	5 (18)
IV	23 (82)

Abbreviations: No., number; ECOG, Eastern Cooperative Oncology Group.

Table 2

The means and medians of hematological variables.

	Means (pg/ml)	Medians (pg/ml)
Angiopoietin-2	1417.11	1226.50
FGF-1	5.09	4.81
IL-8	7.32	5.97
PLGF	17.85	14.29
VEGF-C	1104.67	851.04
VEGF-D	7.71	6.48
FGF-2	104.12	9.79
VEGF-A	65.41	17.12
PD-1	2799.66	1933.00
CTLA-4	148.98	75.41
CD80/B7-1	750.95	562.72
CD86/B7-2	2080.65	1234.00
PD-L1	902.49	633.76
IL-18	349.42	354.87

Abbreviations: FGF-1, fibroblast growth factor-1; IL-8, interleukin-8; PLGF, placental growth factor; VEGF-C, vascular endothelial growth factor-C; VEGF-D, vascular endothelial growth factor-D; FGF-2, fibroblast growth factor-2; VEGF-A, vascular endothelial growth factor-A; PD-1, programmed cell death protein-1; CTLA-4, cytotoxic T-lymphocyte associated protein-4; CD80/B7-1, cluster of differentiation-80; CD86/B7-2, cluster of differentiation-86; PD-L1, programmed cell death ligand-1; IL-18, interleukin-18.

Table 3

The univariate analysis of indicators for PFS and OS.

	PFS Univariate analysis			OS Univariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Gender (male/female)	1.598	0.678-3.764	0.284	1.232	0.472-3.213	0.670
Age (≤65/>65)	1.369	0.573-3.272	0.480	1.663	0.652-4.242	0.287
ECOG performance status (0/1)	1.703	0.729-3.979	0.219	1.082	0.417-2.807	0.871
Smoking status (never/current or former)	0.901	0.406-1.998	0.797	1.377	0.542-3.497	0.502
Histology status (squamous/adenocarcinoma)	0.520	0.117-2.310	0.390	0.778	0.176-3.448	0.741
Mutation status (negative/positive)	0.491	0.193-1.249	0.135	0.515	0.169-1.571	0.243
Stage of disease (III/IV)	0.397	0.137-1.148	***0.088***	1.484	0.420-5.240	0.539
Angiopoietin-2						
≤1417.11/>1417.11	0.788	0.344-1.806	0.573	0.865	0.317-2.357	0.776
≤1226.50/>1226.50	0.966	0.437-2.133	0.932	0.534	0.196-1.452	0.219
FGF-1						
≤5.09/>5.09	1.345	0594-3.044	0.477	0.740	0.280-1.950	0.542
≤4.81/>4.81	1.298	0.571-2.910	0.540	0.999	0.385-2.593	0.998
IL-8						
≤7.32/>7.32	1.418	0.611-3.290	0.416	6.361	2.034-19.890	***0.001***
≤5.97/>5.97	1.391	0.610-3.167	0.432	2.578	0.982-6.768	0.055
PLGF						
≤17.85/>17.85	0.576	0.236-1.404	0.225	0.649	0.231-1.826	0.412
≤14.29/>14.29	0.600	0.267-1.347	0.216	0.948	0.373-2.414	0.911

VEGF-C						
≤1104.67/>1104.67	0.771	0.337-1.766	0.539	0.932	0.359-2.419	0.885
≤851.04/>851.04	1.099	0.494-2.446	0.818	1.326	0.523-3.365	0.552
VEGF-D						
≤7.71/>7.71	1.337	0.588-3.041	0.488	1.477	0.579-3.769	0.415
≤6.48/>6.48	1.167	0.530-2.571	0.702	1.210	0.473-3.094	0.690
FGF-2						
≤104.12/>104.12	1.580	0.664-3.758	0.301	0.525	0.169-1.628	0.265
≤9.79/>9.79	2.299	0.933-5.669	***0.071***	2.241	0.728-6.896	0.160
VEGF-A						
≤65.41/>65.41	5.627	1.322-23.951	***0.019***	9.686	1.906-49.222	***0.006***
≤17.12/>17.12	0.902	0.409-1.988	0.797	0.533	0.196-1.449	0.218
PD-1						
≤2799.66/>2799.66	1.281	0.557-2.944	0.560	1.003	0.373-2.694	0.996
≤1933.00/>1933.00	1.746	0.781-3.899	0.174	1.278	0.503-3.248	0.607
CTLA-4						
≤148.98/>148.98	1.080	0.459-2.540	0.859	0.783	0.277-2.214	0.644
≤75.41/>75.41	1.643	0.735-3.673	0.227	1.256	0.493-3.195	0.633
CD80/B7-1						
≤750.95/>750.95	1.185	0.526-2.670	0.682	0.542	0.190-1.545	0.252
≤562.72/>562.72	1.465	0.661-3.248	0.347	0.640	0.237-1.724	0.377
CD86/B7-2						
≤2080.65/>2080.65	1.281	0.557-	0.560	1.003	0.373-	0.996

		2.944			2.694	
≤1234.00/>1234.00	1.643	0.735-3.673	0.227	1.256	0.493-3.195	0.633
PD-L1						
≤902.49/>902.49	1.281	0.557-2.944	0.560	1.003	0.373-2.694	0.996
≤633.76/>633.76	1.552	0.694-3.472	0.284	0.993	0.391-2.522	0.988
IL-18						
≤349.42/>349.42	0.608	0.271-1.363	0.227	0.473	0.184-1.214	0.119
≤354.87/>354.87	0.608	0.271-1.363	0.227	0.473	0.184-1.214	0.119

Abbreviations: PFS, progression-free survival; OS, overall survival; NSCLC, non-small cell lung cancer; HR, Hazard Ratio; CI, Confidence Interval; ECOG, Eastern Cooperative Oncology Group; FGF-1, fibroblast growth factor-1; IL-8, interleukin-8; PLGF, placental growth factor; VEGF-C, vascular endothelial growth factor-C; VEGF-D, vascular endothelial growth factor-D; FGF-2, fibroblast growth factor-2; VEGF-A, vascular endothelial growth factor-A; PD-1, programmed cell death protein-1; CTLA-4, cytotoxic T-lymphocyte associated protein-4; CD80/B7-1, cluster of differentiation-80; CD86/B7-2, cluster of differentiation-86; PD-L1, programmed cell death ligand-1; IL-18, interleukin-18.

Table 4 Pearson Correlation Coefficients between hematologic factors.

a. Pearson Correlation Coefficients between PD-1, PD-L1, CTLA-4, CD80/B7-1 and CD86/B7-2

	PD-L1	CTLA-4	CD80/B7-1	CD86/B7-2
PD-1	0.972	0.984	0.924	0.988
PD-L1		0.946	0.930	0.948
CTLA-4			0.891	0.971
CD80/B7-1				0.916

P=0.000

b. Pearson Correlation Coefficients between VEGF-D, FGF-1 and FGF-2

	FGF-1	FGF-2
VEGF-D	0.849	0.950
FGF-1		0.861

P<0.05

Abbreviations: PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand-1; CTLA-4, cytotoxic T-lymphocyte associated protein-4; CD80/B7-1, cluster of differentiation-80; CD86/B7-2, cluster of differentiation-86; VEGF-D, vascular endothelial growth factor-D; FGF-1, fibroblast growth factor-1; FGF-2, fibroblast growth factor-2.

Figures

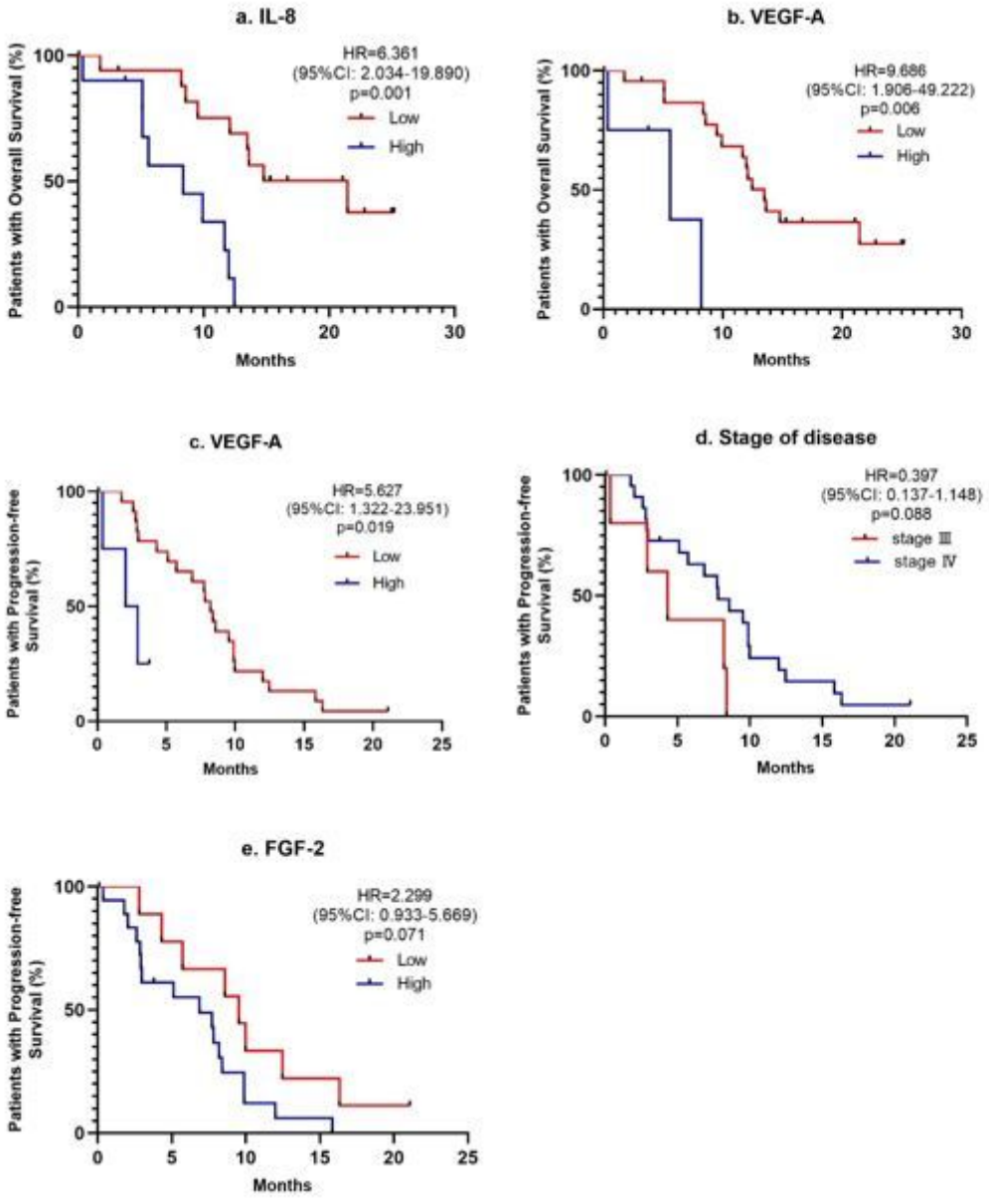

Figure 1

The Kaplan-Meier survival curves of IL-8, VEGF-A, stage of disease and FGF-2 for OS and PFS. (a) The death risk in high IL-8 (>7.32 pg/ml) group was 6.361 times than that in low IL-8 (≤7.32 pg/ml) group (HR=6.361, 95%CI: 2.034-19.890, p=0.001). (b) The death risk in high VEGF-A (>65.41 pg/ml) group was 9.686 times than that in low VEGF-A (≤65.41 pg/ml) group (HR=9.686, 95%CI: 1.906-49.222, p=0.006). (c) The disease progression or death risk in high VEGF-A (>65.41 pg/ml) group was 5.627 times than that in low VEGF-A (≤65.41 pg/ml) group (HR=5.627, 95%CI: 1.322-23.951, p=0.019). (d) Patients with stage IV seemed to have a longer PFS than those with stage III (HR=0.397, 95%CI:0.137-1.148, p=0.088). (e) Patients with high FGF-2 (>9.79 pg/ml) seemed to have a shorter PFS than those with low FGF-2 (≤9.79 pg/ml) (HR=2.299, 95%CI: 0.933-5.669, p=0.071). Abbreviations: HR, Hazard Ratio; CI, Confidence Interval; IL-8, interleukin-8; VEGF-A, vascular endothelial growth factor-A; FGF-2, fibroblast growth factor-2.

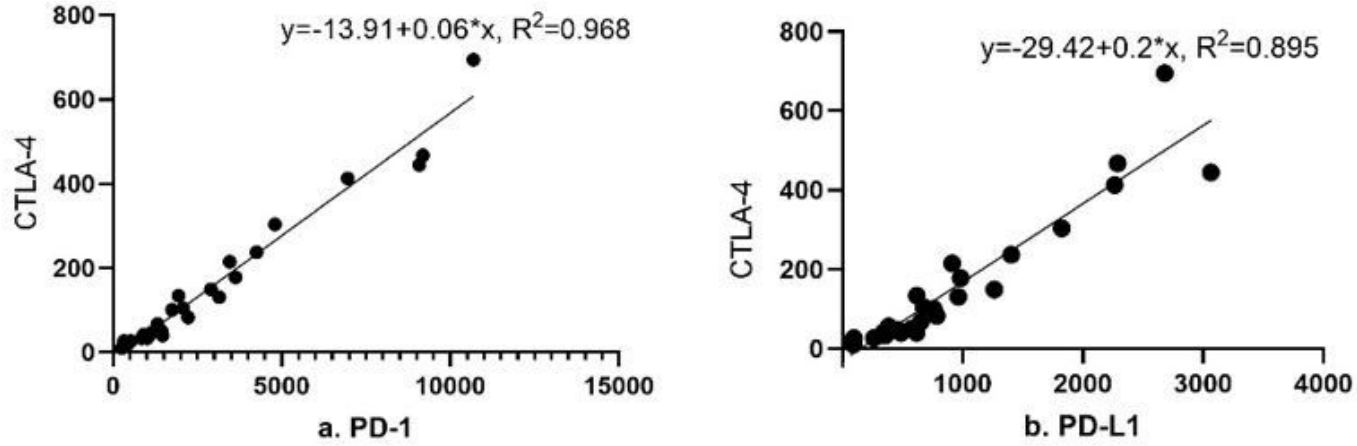

Figure 2

The scatter diagrams of CTLA-4 with PD-1 and PD-L1. (a) The higher PD-1 (y=-13.91+0.06*x, R2=0.968), the higher CTLA-4. (b) The higher PD-L1 (y=-29.42+0.2*x, R2=0.895), the higher CTLA-4. Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein-4; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand-1.

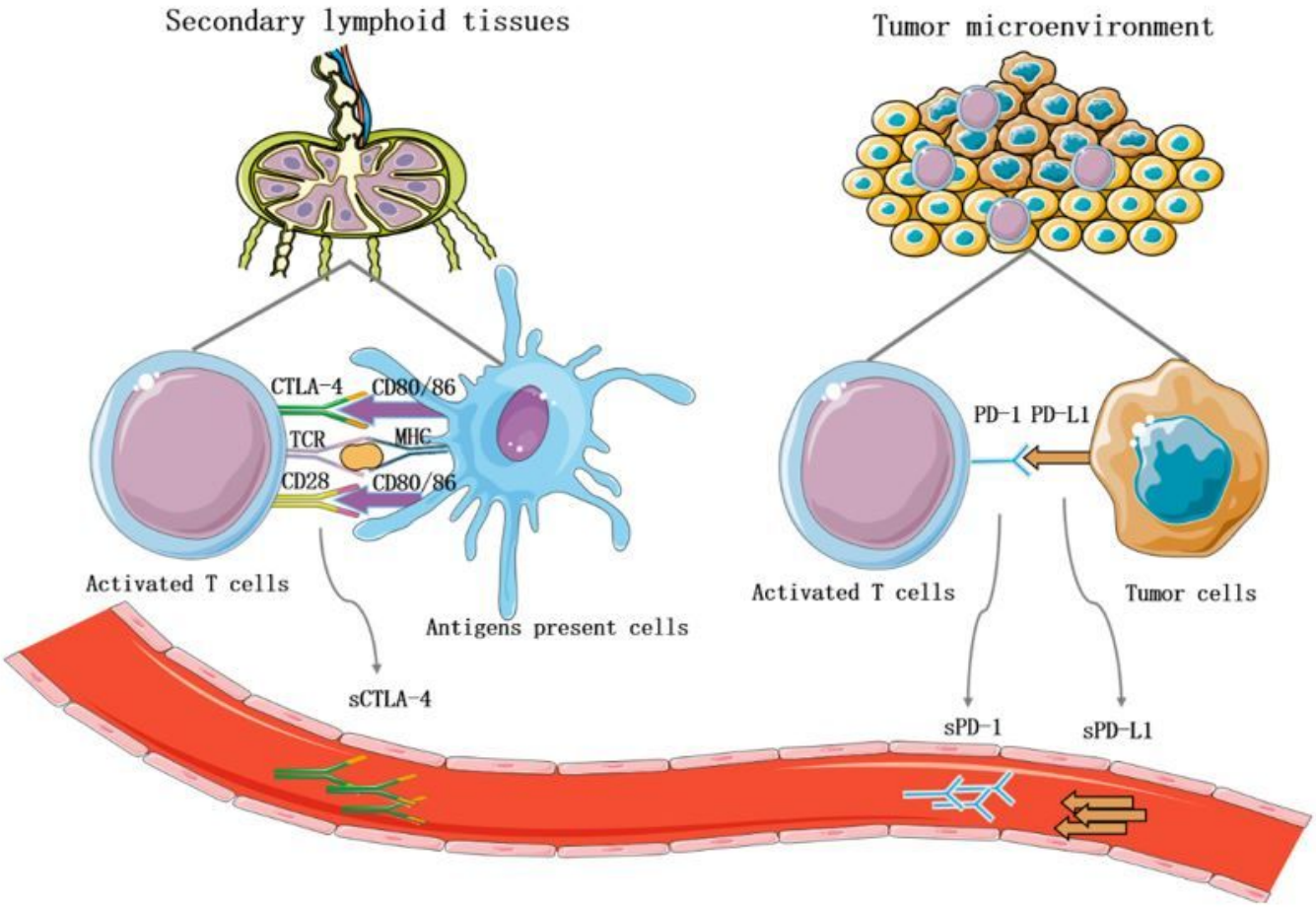

Figure 3

The distribution of CTLA-4, PD-1 and PD-L1, and the procession of sCTLA-4, sPD-1 and sPD-L1.