



# Expression of cytokines in pleural effusions and corresponding cell lines of small cell lung cancer

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**Background:** Small cell lung cancer (SCLC) is a neuroendocrine aggressive tumor with a dismal prognosis due to the lack of curative therapeutic modalities. Approximately 11% of these patients show a malignant pleural effusion (MPE) that increase in frequency with progression of the disease. In MPE, fluid accumulates due to leaky vessels and mesothelial surfaces as well as impaired removal of fluid due to impaired drainage.

**Methods:** For this investigation, three SCLC MPE samples and supernatants of the corresponding isolated cell lines were analyzed for the content of 105 cytokines, chemokines, and growth factors. Overexpressed pathways including these cytokines were identified using Reactome analysis tools.

**Results:** A large range of cytokines, including vascular endothelial growth factor A (*VEGFA*), were found to be expressed in the MPEs and conditioned media of the corresponding cell line. These mediators are involved in pathways such as interleukin (*IL*) signaling, growth factor stimulation, modulation of cell adhesion molecules and proliferative cell signaling. Cytokine expression by the corresponding SCLC cell lines revealed the specific contributions of the tumor cells and included high expression of *VEGFA*, tumor-promoting factors and mediators exerting immunosuppressive and protumor effects. MPEs used here showed marked stimulation of the proliferation of four permanent SCLC cell lines.

**Conclusions:** MPEs comprise a large number of cytokines with mixed activities on tumor cells and the invading SCLC cells release a number of protumor mediators and induce an immunosuppressive pleural environment.

**Keywords:** Small cell lung cancer (SCLC); pleural effusion; cell line; cytokines; cell proliferation

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## Introduction

Approximately 10–15% of patients with lung cancer are affected by malignant pleural effusions (MPEs) at the initial diagnosis and the median survival of these patients is as short as 3–12 months (1-3). The incidence of MPE may

rise to 50% for cases at advanced stages (4). Pleural invasion was first reported in 1958 by Brewer *et al.* to be a negative predictor of survival for lung cancer (5). In detail, the median survival was 11.2, 5.9, and 4.8 months in patients with no pleural effusion, minimal pleural effusion and MPE,

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respectively. Due to the inferior overall survival (OS), MPE was reclassified to M1a (stage IV) category in the TNM system (6,7). Among malignancies, lung cancer is the most common cause of MPE and a 16% MPE rate was found in 57,685 patients with non-small cell lung cancer (NSCLC) at first presentation (8,9). It is well-known that “wet” pleural carcinomatosis has a poorer prognosis in comparison to “dry” pleural carcinomatosis (10). For SCLC, MPE was present in 7,639 (11.16%) of 68,443 patients with an estimated 1-year survival 17% *vs.* 30% without MPE (8,11). In another investigation, 1,770 primary SCLC patients showed pleural involvement (PI) in 25.4% of cases again constituting a negative prognostic factor (2). The analysis of 358 SCLC patients with extensive-stage disease and MPE revealed that 43.8% of patients died within 12 months (12). However, for most SCLC studies, the effect of PI on survival has not been considered (13).

Symptoms in most patients with MPE comprise dyspnea, cough and chest pain. In normal physiological conditions, there is 0.26 mL/kg of fluid in the pleural space and influx and exit of pleural space fluids are balanced to keep the volume constant (14). Excess fluid formation in the pleural space can be either due to malignant or benign causes (15). Fluid leaves the pleural space through the parietal pleura stomata, that are openings between mesothelial cells and fluid is drained by collecting lymphatics to the mediastinal lymph nodes (16). MPE occurs mostly because of impaired lymphatic drainage along this outward flow. Meyer *et al.* found a significant relation between mediastinal lymph

node impairment and development of MPE (17). Tumor cells infiltrate the pleural space via the hematogenous, direct or lymphatic invasion and the lymphatic drainage can be blocked by growing tumor cells (18). However, only 55–60% of patients with pleural or lymphatic metastases develop MPE (19).

A variety of cells is present in the pleural microenvironment, including mesothelial and endothelial cells as well as cells of myeloid origin and of the lymphatic system. In general, the immune system effector cells interact with tumor cells to increase angiogenesis, inflammation and vascular leakiness eventually leading to the development of MPE (15,19). The immunoregulatory mediators present include IL-2, tumor necrosis factor (*TNF*) and interferons. Vascular permeability is promoted by vascular endothelial growth factor A (*VEGFA*) and matrix metalloproteinases (MMPs) by disruption of endothelial cell integrity and cellular junctions (20–22). Likewise, angiopoietin 1/2, secreted by tumor cells, participates in increased vascular permeability (23).

For the present investigation, MPEs of three advanced SCLC patients were collected and a total of 105 cytokines analyzed by a Western blot array using the Proteome Profiler Human XL Cytokine Array Kits. Furthermore, corresponding SCLC cancer lines were established in tissue culture of the same MPEs and subjected to the same cytokine analysis to determine the fraction of cytokines secreted by the cancer cells themselves in the MPE. In addition, the effect of the MPE samples on the proliferation of four permanent SCLC cell lines were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. We present this article in accordance with the MDAR reporting checklist (available at <https://tclr.amegroups.com/article/view/10.21037/tclr-23-569/rc>).

### Highlight box

#### Key findings

- The establishment of pleural small cell lung cancer (SCLC) cell lines allowed for the determination of the fraction of cytokines secreted by the tumor cells compared to the total cytokines found in malignant pleural effusions (MPEs).

#### What is known and what is new?

- The expression of vascular endothelial growth factor A (VEGFA), angiopoietins, osteopontin and other mediators have been reported previously for MPEs but this study reports the overexpression of protumor and immunosuppressive cytokines secreted by the pleural SCLC cells.

#### What is the implication, and what should change now?

- The analysis of cytokines expressed by the SCLC cells in MPEs provides a repertoire of cell-enriched mediators as potential targets for therapeutic intervention apart from VEGFA.

## Methods

### Pleural effusions and blood samples

Thoracentesis of pleural effusions has been routinely performed for SCLC patients and the samples were obtained according to the guidelines set forth in the Ethics Approval 366/2003 granted by the Ethics Committee of the Medical University of Vienna, Vienna, Austria, including informed consent of the patients. The same protocol was valid for the acquisition of blood samples and the establishment of cell lines. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

### Cell culture

Pleural effusions were centrifuged and the supernatants aliquoted and stored frozen at  $-80^{\circ}\text{C}$ . The cells were washed with tissue culture medium (RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics). All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) except indicated otherwise. Cells were kept under tissue culture conditions (5%  $\text{CO}_2$ ,  $37^{\circ}\text{C}$ ) for short-term cultures at least until two  $75\text{ cm}^2$  tissue culture flasks exhibited a complete coverage of the surface area before the conditioned media was used for the analysis of the cytokines. NCI-H69 cells were obtained from the ATCC (Manassa, VA, USA) and the SCLC lines SCLC26A, S457 and the SCLC CTC cell line BHGc40 have been established at our institution. The SCLC26A cell line was obtained from a patient prior to treatment, the S457 cell line from a patient progressing after second-line therapy with cyclophosphamide/epirubicin/vincristine (CEV) and BHGc40 from a blood sample of a patient also progressing after second-line CEV therapy.

### Cytokine western blot arrays

Cytokines of pleural effusion samples or cell line supernatants were determined using Human Profiler Arrays Cytokine XL (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The Western blot array spots were quantitated using ImageJ and Origin software. Tests were performed in duplicate and the six reference spots spotted to each membrane were used to calibrate the individual chemoluminescence intensities. The cytokines exhibiting highest expression were analyzed for overexpressed pathways using the Reactome Pathway database (reactome.org). Finally, the target effectors of these significantly altered pathways were summarized.

### MTT tests

Cells ( $1 \times 10^4$ ) were distributed to the wells of 96 microtiter plates (TPP, Trasadingen, Switzerland) in  $100\ \mu\text{L}$  medium and supplemented 1:1 with twofold dilutions of pleural effusion samples. Viability of the cells was determined after 4 days incubation in tissue culture using a modified MTT test kit (EZ4U, Biomedica, Vienna, Austria). Tests were performed in triplicate with 10 dilutions, starting at 1:1 tissue culture medium: MPE, and three independent repetitions. The resulting data were calculated with Origin software (OriginLab, Northampton, MA, USA).

### Statistical analysis

The Reactome analysis employs over-representation analysis (ORA) for checking in a dataset, whether the proportion of differentially expressed genes of a specific pathway exceeds the proportion of genes that could be randomly expected, by calculating the likelihood that the association between the sample identifiers and the found pathway is due to random chance. The statistical significance is calculated using the Binomial Test with P values ( $P < 0.05$ ) and the false discovery rate (FDR) estimated by the Benjamini-Hochberg approach, to identify false positive results.

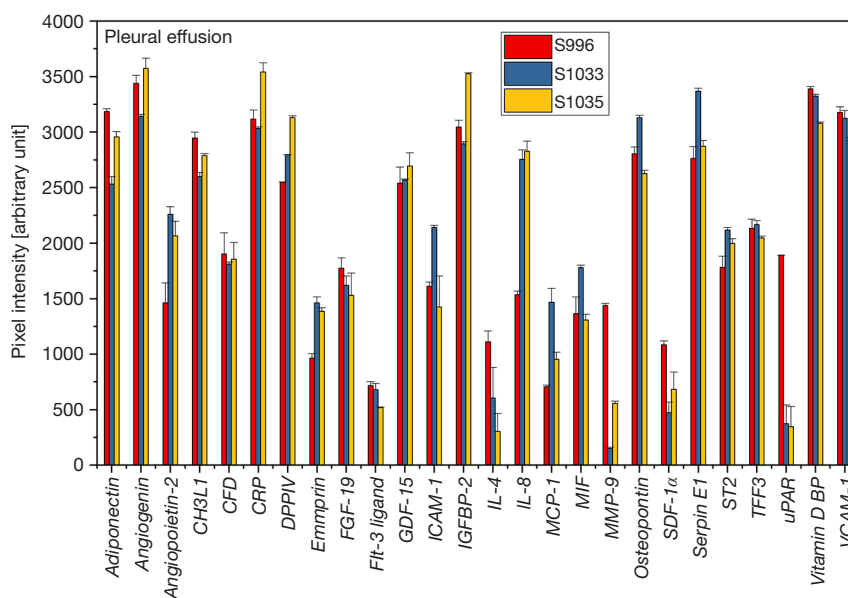
## Results

### Cytokine profile of the three MPEs

The Proteome Profiler Human XL Cytokine Array Kit detects 105 human cytokines simultaneously using Western blot technology. Pleural effusion samples of three advanced SCLC patients (S996, S1033, S1035) were collected prior to the initiation of chemotherapy and processed for determination of the cytokines by these arrays. Results were calibrated using six included reference spots. The cytokines exhibiting the highest expression for these three pleural effusions are shown in *Figure 1* and the results revealed a highly similar quantitative expression for these three independent SCLC MPE samples. Data for all cytokines measured are presented in *Table S1*. A large range of mediators are expressed in MPEs, including angiogenic effectors, cell adhesion molecules, proliferation stimulators, proteases, interleukins, and urokinase plasminogen activator surface receptor (*uPAR/CD87*) and mediators of inflammation and chemoattractants.

### Overexpressed pathways of the cytokines detected in SCLC MPE

The functional significance of the large number of cytokines detected was searched for their inclusion in overexpressed cellular pathways using the Reactome Pathway Database. All highly expressed cytokines as shown in *Figure 1* were submitted to Reactome analysis. Pathways determined for most significant overexpression include *IL-4*, *IL-13*, *IL-10*, *IL-18* and *IL-33* signaling, as well as neutrophil degranulation, extracellular matrix (ECM) organization, cell adhesion molecules, activators of *ATF4*, *IGFs*, *PK3/AKT* signaling and unfolded protein response (UPR) among others (*Table 1*).



**Figure 1** The cytokines exhibiting the highest expression in three independent MPE samples. Data shown are mean  $\pm$  SD. This figure shows 26 cytokines of the 105 molecules determined in the Western blot array, selected by their high expression levels. CH3L1, chitinase 3 like 1; CFD, complement factor D; CRP, C-reactive protein; DPPIV, dipeptidyl peptidase 4; FGF-19, fibroblast growth factor 19; Flt-3 ligand, Fms related receptor tyrosine kinase 3 ligand; GDF-15, growth differentiation factor 15; ICAM1, intercellular adhesion molecule 1; IGFBP-2, insulin like growth factor binding protein 2; IL-4, interleukin 4; IL-8, interleukin 8; MCP-1, C-C motif chemokine ligand 2; MIF, macrophage migration inhibitory factor; MMP-9, matrix metalloproteinase 9; SDF-1 $\alpha$ , C-X-C motif chemokine ligand 12; Serpin E1, serpin family E member 1; ST2, suppression of tumorigenicity 2; TFF3, trefoil factor 3; uPAR, plasminogen activator urokinase receptor; Vitamin D BP, vitamin D-binding protein; VCAM-1, vascular cell adhesion molecule 1; MPE, malignant pleural effusion; SD, standard deviation.

The chain of mediators constituting these pathways end in effector molecules and mechanisms shown in *Table 2*. The Reactome analysis depicts the cascade of proteins comprising the respective pathways ending in specific terminal effectors.

The cytokines with the highest expression are shown and the results revealed a similar quantitative expression for these three independent cell lines (*Table 2*). Data for all cytokines measured are presented in *Table S1*. Results were calibrated using six included reference spots. A large range of cytokines are expressed by the tumor cells, including angiogenic effectors, cell adhesion molecules, proliferative effectors, proteases, interleukins, uPAR and mediators of inflammation and chemoattractants.

#### **Overexpressed pathways of the cytokines in cell line supernatants**

The functional significance of these cellular cytokines has

been searched for their inclusion in overexpressed cellular pathways using the Reactome Pathway Database. All highly expressed cytokines as shown in *Figure 2* were submitted to Reactome analysis. Pathways determined for most significant overexpression include *IL-4*, *IL-13*, *IL-10* and *IL-33* signaling, as well as platelet degranulation, growth factors, cell adhesion molecules, activation of *TPAF2*, *PK3/AKT* signaling and senescence-associated secretory phenotype (SASP) among others (*Table 3*).

The overexpressed pathways detected in supernatants of the cell lines by Reactome analysis follow a chain of effectors that terminate in specific proteins (*Table 4*).

#### **Ratios of cytokine expression in cell line supernatants/ expression in MPEs**

The cytokines determined for the MPEs and cell lines were compared and the ratios of the expression calculated (*Table 5*). In particular, cellular *DKK-1*, *ENA-78/CXCL5*,

**Table 1** The cellular pathways overexpressed involving cytokines of the MPEs

Reactome pathway	Entity/total	Ratio	P value
<i>IL-4</i> and <i>IL-13</i> signaling	17/211	0.014	1.11e-16
<i>IL-10</i> signaling	10/86	0.006	1.11e-11
Neutrophil degranulation	12/478	0.031	2.08e-06
<i>ATF4</i> activation ER	4/34	0.002	2.14e-05
Regulation <i>IGF</i> and IGFbps	6/127	0.008	3.03e-05
<i>PERK</i> regulates gene expression	4/42	0.003	4.85e-05
<i>Integrin</i> cell surface interactions	5/86	0.006	5.48e-05
<i>IL-33</i> signaling	2/42	0.63e-04	1.69e-04
Unfolded protein response (UPR)	5/156	0.01	8.37e-04
<i>IL-18</i> signaling	2/11	0.001	7.22e-04
Extracellular matrix organization	7/328	0.022	8.56e-04
Senescence SASP	4/90	0.006	8.64e-04
<i>RUNX3</i> regulation	2/10	0.001	6.57e-04
<i>PI3K/AKT</i> signaling	4/129	0.008	0.003
<i>TP53</i> regulation	2/18	0.001	0.003

Cytokines with high expression were subjected to pathway analysis and the most overexpressed pathway listed, according to the components/entities of specific cascades found (ratio of number of entities/total number of pathway members) and the P values. The FDR ranged from 1.8e-14 to 0.042 from pathways with highest to lowest P values. FDR, false discover rate; ER, endoplasmic reticulum; IGFbps, insulin-like growth factor binding proteins; SASP, senescence-associated secretory phenotype; MPE, malignant pleural effusion.

**Table 2** Terminal effectors of the overexpressed pathways of the cytokines of SCLC MPEs

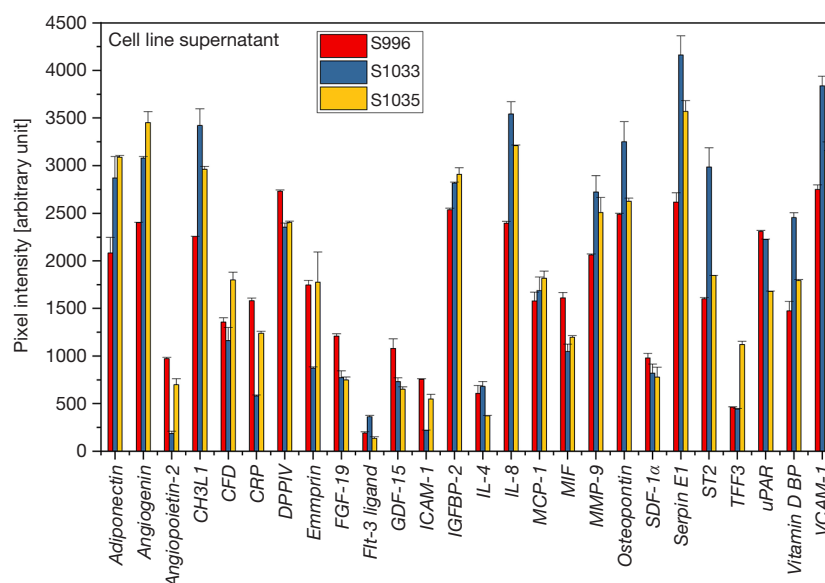
Reactome pathway targets
<i>IFN<math>\gamma</math></i> signaling
Platelet degranulation
<i>IGF1</i> regulation
<i>MET</i> <i>STAT3/5</i>
<i>Fit3</i> signaling
<i>TP53</i> repair
<i>PI3K</i> <i>AKT</i> activation
<i>Insulin receptor</i> signaling
<i>IL-4</i> and <i>IL-13</i> signaling
<i>PTEN</i> loss
<i>ErbB4</i> activation
UPR/senescence

The ultimate effectors of the pathways found overexpressed in Reactome analysis are summarized. UPR, unfolded protein response; MPE, malignant pleural effusion; SCLC, small cell lung cancer.

*G-CSF*, *GRO $\alpha$ /CXCL1*, *MMP9*, *PF-4/CXCL4*, *RANTES/CCL5*, *TARC/CCL17* and *VEGFA* exhibited much higher concentrations compared to the levels found in the MPEs.

***Effects of the MPE samples on proliferative activity of SCLC cell lines***

In order to investigate the potential effects of the MPE samples on the growth of a number of permanent SCLC cell lines, namely NCI-H69, SCLC26A, S457 and BHGc16, cells were supplemented with samples of four MPEs in proliferation tests (Figure 3). All MPE samples were found to stimulate the proliferation of all 4 cell lines in a dose-dependent manner. Figure 3 shows the results obtained with a 25% MPE fraction in tissue culture medium. The lowest medium response was determined for the SCLC26A cell line, derived from an SCLC patient without pretreatment. The SCLC S457 and the BHGc16 SCLC CTC lines have been established from progressing and chemoresistant SCLC patients employing a MPE and a blood sample, respectively.



**Figure 2** The cytokines exhibiting the highest expression in three corresponding cell lines. Data shown are mean  $\pm$  SD. This figure shows 26 cytokines of the 105 molecules determined in the Western blot array. The proteins shown were selected for their high expression levels. CH3L1, chitinase 3 like 1; CFD, complement factor D; CRP, C-reactive protein; DPP4, dipeptidyl peptidase 4; FGF-19, fibroblast growth factor 19; Flt-3 ligand, Fms related receptor tyrosine kinase 3 ligand; GDF-15, growth differentiation factor 15; ICAM1, intercellular adhesion molecule 1; IGFBP-2, insulin like growth factor binding protein 2; IL-4, interleukin 4; IL-8, interleukin 8; MCP-1, monocyte chemoattractant protein 1; MIF, macrophage migration inhibitory factor; MMP-9, matrix metalloproteinase 9; SDF-1a, stromal cell-derived factor 1a; Serpin E1, serpin family E member 1; ST2, suppression of tumorigenicity 2; TFF3, trefoil factor 3; uPAR, plasminogen activator urokinase receptor; vitamin D BP, vitamin D-binding protein; VCAM-1, vascular cell adhesion molecule 1; SD, standard deviation.

**Table 3** The cellular pathways overrepresented involving cytokines of the three cell lines

Reactome pathway	Entity	Ratio	P value
<i>IL-10</i> signaling	22/86	0.006	1.11e-16
<i>IL-4/IL-13</i> signaling	14/211	0.014	4.27e-12
<i>TFAP2 (AP-2)</i> regulation	4/21	0.001	4.75e-06
<i>IGF and IGFBPs</i>	6/127	0.008	5.16e-05
<i>Integrin</i> interactions	5/86	0.006	8.60e-05
Platelet degranulation	6/141	0.009	9.14e-05
<i>IL-33</i> signaling	2/4	2.63e-04	2.04e-04
<i>PI3K/AKT</i> signaling	5/129	0.008	5.51e-04
Senescence SASP	4/90	0.006	0.001

Cytokines with high expression were subjected to pathway analysis and the most overexpressed pathway listed, according to the components/entities of specific cascades found (ratio of number of entities/total number of pathway members) and the P values. The FDR ranged from 1.0e10-16 to 0.003 from pathways with highest to lowest P values. SASP, senescence-associated secretory phenotype; FDR, false discovery rate.

**Table 4** Terminal entities of the overexpressed pathways of the cytokines of the cell lines

Pathway targets
Neutrophil degranulation
Platelet activation
<i>IGF1</i>
<i>TP53</i>
<i>IL-6, IL-4, IL-10, IL-13</i>
<i>AP-2</i> regulation
<i>Integrin</i> cell surface
<i>EGFR PI3K AKT</i>
<i>Pou5F1 SOX2</i> Nanog repress differentiation
<i>VENTX</i> regulation
UPR <i>PERK ATF4</i>
<i>PDGF</i>

The ultimate effectors of the pathways found in Reactome analysis for the three established SCLC cell lines are summarized. UPR, unfolded protein response; SCLC, small cell lung cancer.

## Discussion

MPEs appear in all cell types of lung cancer with highest incidence in adenocarcinoma (approximately 30%), followed by squamous cell carcinoma (SCC; approximately 9%) and SCLC (approximately 10%) (24,25). Thoracentesis of pleural effusion allows for the examination through cytology and proof of a malignant process (26). Accumulation of MPE is due to increased permeability of the pleural vessel cells as well as vasoactive and inflammatory cytokines secreted by invaded tumor cells. Even patients with minimal MPEs had a significantly increased risk of death with a hazard ratio of 1.454 (27). However, MPEs affect survival only in T2 stage but not in T3 and T4 stages. A worse prognosis associated with MPE seems to be linked to the high rate of lymph node metastasis in these patients and mediastinal lymphadenopathy is the main reason for the accumulation of pleural fluid (27,28). MPE in SCLC forms due to indirect infiltration of the lymphatic vessels (27). In general, the focus of the management MPE is palliative and without survival benefit.

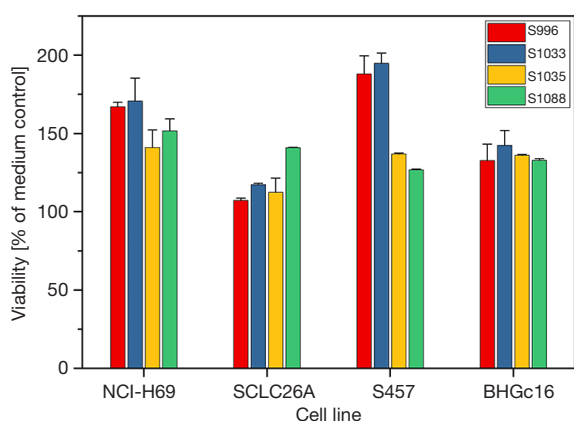
The pleural mesothelial cell is a common cell in the pleural space and initiates responses to stimuli such as tumor cells (4,29). Malignant cells invade the parietal pleura and proliferate to form pleural metastases that secrete mediators such as *VEGFA*, chemokines, *IL-6*, osteopontin (*OPN*) and

**Table 5** Ratio of the expression of cytokines of cell lines/MPE

Gene	Ratio
<i>BDNF</i>	1.93
<i>DKK-1</i>	8.03
<i>ENA-78 CXCL5</i>	18.80
<i>G-CSF</i>	5.28
<i>GM-CSF</i>	2.43
<i>GROa CXCL1</i>	6.10
<i>IL-16</i>	1.68
<i>IL-33</i>	3.07
<i>IP-10 CXCL10</i>	3.71
<i>I-TAC CXCL11</i>	7.27
<i>LIF</i>	8.30
<i>MCP-1</i>	1.63
<i>MCP-3 CCL7</i>	9.77
<i>M-CSF</i>	1.98
<i>MIG CXCL9</i>	4.00
<i>MIP 3a</i>	8.54
<i>MMP-9</i>	3.40
<i>PF4 CXCL4</i>	13.42
<i>RANTES CCL5</i>	5.10
<i>TARC CCL17</i>	6.46
<i>Thrombospondin-1</i>	2.30
<i>TNF-a</i>	3.32
<i>uPAR</i>	2.38
<i>VEGFA</i>	31.23

MPE, malignant pleural effusion.

*TNF* provoking the accumulation of inflammatory cells (30,31). *VEGFA* stimulates proliferation and migration of endothelial cells and proved necessary for the accumulation of pleural fluid through increase of vascular permeability (32,33). Additionally, these cytokines have been reported to trigger in turn proinflammatory and proangiogenic mediators controlled by *NF-κB* and *STAT3* (31,34). *OPN* seems to promote MPE by enhancing *VEGFA* release from vascular endothelial or mesothelial cells (35). Increases of the expression of *ICAM-1* and *VCAM-1* promote release of metalloproteinases by the cancer cells (36,37). In summary, different cell populations and molecules are involved in the formation of MPE either stimulating pleural inflammation (e.g., *IL-2*, *TNF* and *IFN*) or tumor angiogenesis [e.g.,



**Figure 3** Stimulation of the proliferation of the four SCLC cell lines by MPE samples. Data shown are mean  $\pm$  SD. The medium control has been set to 100% and all results for the MPE samples are significantly elevated compared to the medium controls. An additional MPE sample, S1088, has been included for the proliferation assay. MPE, malignant pleural effusion; SD, standard deviation; SCLC, small cell lung cancer.

angiopoietin 1 (*ANG-1*), angiopoietin 2 (*ANG-2*)] and vascular hyperpermeability (e.g., *VEGFA*, *MMP*, *MCP-1/CCL2*, *OPN*, and others) (18,21). Our analysis of the cytokines expressed in MPEs and corresponding SCLC cell lines document presence of the cytokines cited above and a wide range of other mediators. The net effect of this host of cytokines seems to be the high stimulation of tumor growth in a suitable modified pleural environment.

The establishment of corresponding SCLC cell cultures from the same MPEs used for the measurement of cytokines allows to identify the fraction of mediators provided by the tumor cell populations for the first time. A large range of cytokines of the SCLC cell lines exhibit a relatively higher production compared to the matching MPE. Of these, high expression of *MIP-3 $\alpha$ /CCL20* recruits inflammatory cells and promotes lung cancer cell migration and proliferation through the activation of *ERK* and *PI3K* signaling pathways (38). The receptor for advanced glycation end (RAGE) products is known to induce the accumulation of tumor-associated macrophages (TAMs) in lung cancer tissue and to further accelerate the tumor progression (39). The higher concentrations of the epithelial neutrophil-activating peptide 78 (*ENA-78*) in MPEs can stimulate a progressive influx of neutrophils into the pleural space (40). Furthermore, *CXCL9*, also known as monokine-induced by interferon- $\gamma$  (*MIG*), is secreted during inflammatory conditions and may act directly on tumor cells via their

*CXCR3* receptor to promote cell migration and epithelial mesenchymal transition (EMT) (41).

*TARC/CCL17* is a ligand of *CCR4* that induces chemotaxis of *Th2* and inhibitory regulatory T (Treg) cells and, additionally, recruit eosinophils into the tumor (42). Tumor-derived *GRO $\alpha$ /CXCL1* contributes to tumor-associated neutrophil (TAN) infiltration in lung cancer which accelerates tumor growth (43). A correlation exists between the *IL-16* concentrations and the number of *CD4<sup>+</sup>* T-cells, and furthermore, *IL-16* produces a significant influx of *CD4<sup>+</sup>* T-cells into the pleural space (44). *TNF* binds to its receptor *TNFR2* of tumor cells and enhances the suppressive activity of Tregs by up-regulating *CTLA-4* and *PD-L1* expression (45). Besides regulating angiogenesis and tumor vasculature, thrombospondin-1 limits antitumor immunity by *CD47*-mediated inhibition of innate and adaptive immune cells (46). Additionally, *IP-10/CXCL10* is highly expressed by cancer cells and correlates with infiltration by Tregs and poor survival (47). Moreover, *IP-10* signaling via its cognate receptor *CXCR3* was shown to increase tumor growth, migration and invasion of cancer cells in various tumor types (48). The high concentrations of *PF4* in MPE is linked with a poor prognosis, most likely by the suppression of T lymphocyte response that promotes tumor progression (49). The *G-CSF*- or *GM-CSF*-secreting cancers exhibit rapid progression due to a cytokine-mediated immune suppression and angiogenesis (50). These range of cytokines derived from cancer cells seem to promote tumor growth and development of MPE by the generation of a markedly immunosuppressive milieu in the pleural effusion (51).

The other cytokines overexpressed in the SCLC lines are involved in promotion of increased cellular signaling, tumor progression and invasion. High expression of *VEGFA* in conjunction with *MMP-9* has been reported in pleural effusions of patients due to malignant diseases (52-54). In MPEs, *VEGFA* is the most important angiogenic factor and increases vascular permeability as well as migration of endothelial cells together with MMPs and other factors (20,21,52). Drugs targeting *VEGFA* are effective and safe for the clinical management of MPE (21). Among the interleukins, the alarmin *IL-33* induces several factors that activate *NF- $\kappa$ B*, *JNK*, *ERK*, and *p38*, eventually leading to the expression of cytokines, chemokines, and growth factors (55). Overexpression of leukemia inhibitory factor (*LIF*) is linked to the characteristics of aggressive tumors including lymph node metastasis and progression and increases *STAT3* phosphorylation (56). Brain-derived neurotrophic factor (*BDNF*) can increase cancer cell growth, survival, migration



and anoikis, via *TrkB* and the *p75NTR* death receptor (57). The *BDNF/TrkB* pathways trigger downstream signaling, including *PI3K/Akt*, *Jak/STAT*, *ERK*, *NF-κB*, and activation of *EGFR*. CC chemokine ligand 7 (*CCL7*) and its receptors *CCR1*, *CCR2* and *CCR3* were found highly expressed during lung cancer bone metastasis (58). The expression of *uPAR* is increased during inflammation, especially in invasive tumors (59). *uPAR* expression plays a key role in tumorigenicity, tumor proliferation, invasion and glycolytic tumor metabolism. Monocyte chemoattractant protein-1 (*MCP-1*)/*CCL2* is secreted from tumor cells and tumor stroma. The blockade of *MCP-1*, by neutralizing antibodies, has been demonstrated to suppress tumorigenesis in solid tumors (60). Dysregulated expression of *CXCL5/ENA-78* has been shown to be involved in tumor metastasis and angiogenesis (61). Neutralizing *CXCL5/ENA-78* with antibodies impairs cancer progression and increases the inhibitory effects of tyrosine kinase inhibitors (62). Furthermore, *CXCL5* inhibition showed low side effects in experimental animals. In regard to *PF4*, antiplatelet factors with great potential for clinical application are studied but it proved difficult to reduce the side effects of such drugs (63). In conclusion, SCLC cells in MPEs produce cytokines that promote tumor growth and protect the malignant cells by impairing antitumor immune system responses.

## Conclusions

We report for the first time a comparison of the cytokines expressed in MPEs with the same mediators released into supernatants of the corresponding SCLC cell lines. Thus, tumor-promoting factors and immunosuppressive mediators of the tumor cells could be separated from effectors supplied by nonmalignant cell populations of the pleural space.

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## References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. *CA Cancer J Clin* 2022;72:7-33.
2. Yang J, Yin H, Liu M, et al. Effect of pleural invasion on survival of patients with small cell lung cancer: Propensity score analysis and nomogram establishment based on the SEER database. *Front Surg* 2023;10:1108732.
3. Bibby AC, Dorn P, Psallidas I, et al. ERS/EACTS statement on the management of malignant pleural effusions. *Eur Respir J* 2018;52:1800349.
4. Feller-Kopman DJ, Reddy CB, DeCamp MM, et al. Management of Malignant Pleural Effusions. An Official ATS/STS/STR Clinical Practice Guideline. *Am J Respir Crit Care Med* 2018;198:839-49.
5. Brewer LA, Bai AF, Little JN, et al. Carcinoma of the

- lung; practical classification for early diagnosis and surgical treatment. *J Am Med Assoc* 1958;166:1149-55.
6. Morgensztern D, Waqar S, Subramanian J, et al. Prognostic impact of malignant pleural effusion at presentation in patients with metastatic non-small-cell lung cancer. *J Thorac Oncol* 2012;7:1485-9.
  7. Mountain CF. Revisions in the International System for Staging Lung Cancer. *Chest* 1997;111:1710-7.
  8. Shojae S, Singh I, Solsky I, et al. Malignant Pleural Effusion at Presentation in Patients with Small-Cell Lung Cancer. *Respiration* 2019;98:198-202.
  9. Skok K, Hladnik G, Grm A, et al. Malignant Pleural Effusion and Its Current Management: A Review. *Medicina (Kaunas)* 2019;55:490.
  10. Agalioiti T, Giannou AD, Krontira AC, et al. Mutant KRAS promotes malignant pleural effusion formation. *Nat Commun* 2017;8:15205.
  11. Keidan N, Aujayeb A. Small Cell Lung Cancer and Pleural Effusion: An Analysis from a District General Hospital. *Pulm Ther* 2023;9:359-65.
  12. Penz E, Watt KN, Hergott CA, et al. Management of malignant pleural effusion: challenges and solutions. *Cancer Manag Res* 2017;9:229-41.
  13. Wang S, Yang L, Ci B, et al. Development and Validation of a Nomogram Prognostic Model for SCLC Patients. *J Thorac Oncol* 2018;13:1338-48.
  14. Feller-Kopman D, Light R. Pleural Disease. *N Engl J Med* 2018;378:740-51.
  15. Beaudoin S, Gonzalez AV. Evaluation of the patient with pleural effusion. *CMAJ* 2018;190:E291-5.
  16. Sahn SA. The pathophysiology of pleural effusions. *Annu Rev Med* 1990;41:7-13.
  17. Meyer PC. Metastatic carcinoma of the pleura. *Thorax* 1966;21:437-43.
  18. Psallidas I, Kalomenidis I, Porcel JM, et al. Malignant pleural effusion: from bench to bedside. *Eur Respir Rev* 2016;25:189-98.
  19. Stathopoulos GT, Kalomenidis I. Malignant pleural effusion: tumor-host interactions unleashed. *Am J Respir Crit Care Med* 2012;186:487-92.
  20. Hamed EA, El-Noweih AM, Mohamed AZ, et al. Vasoactive mediators (VEGF and TNF-alpha) in patients with malignant and tuberculous pleural effusions. *Respirology* 2004;9:81-6.
  21. Chen Y, Mathy NW, Lu H. The role of VEGF in the diagnosis and treatment of malignant pleural effusion in patients with non-small cell lung cancer (Review). *Mol Med Rep* 2018;17:8019-30.
  22. Damianovich M, Hout Siloni G, Barshack I, et al. Structural basis for hyperpermeability of tumor vessels in advanced lung adenocarcinoma complicated by pleural effusion. *Clin Lung Cancer* 2013;14:688-98.
  23. Fang SC, Zhang HT, Hu HD, et al. Effect of Endostar combined with angiopoietin-2 inhibitor on malignant pleural effusion in mice. *Med Oncol* 2015;32:410.
  24. Medenica M, Medenica M, Cosovic D. Pleural Effusions in Lung Cancer: Detection and Treatment. In: Torres AFC, editor. *Lung Cancer - Strategies for Diagnosis and Treatment*. London: IntechOpen, 2018. DOI: 10.5772/intechopen.78307.
  25. Hsu LH, Hsu PC, Liao TL, et al. Pleural fluid osteopontin, vascular endothelial growth factor, and urokinase-type plasminogen activator levels as predictors of pleurodesis outcome and prognosticators in patients with malignant pleural effusion: a prospective cohort study. *BMC Cancer* 2016;16:463.
  26. Shahini L, Hoxha M, Marku F, et al. Role of cytoblock on pleural effusion for diagnosis of malignant disease. *Diagn Cytopathol* 2023;51:684-8.
  27. Ryu JS, Lim JH, Lee JM, et al. Minimal Pleural Effusion in Small Cell Lung Cancer: Proportion, Mechanisms, and Prognostic Effect. *Radiology* 2016;278:593-600.
  28. Wang F, Li P, Li F. Nomogram for Predicting the Relationship between the Extent of Visceral Pleural Invasion and Survival in Non-Small-Cell Lung Cancer. *Can Respir J* 2021;2021:8816860.
  29. Tsavlis D, Katopodi T, Anastakis D, et al. Molecular and Immune Phenotypic Modifications during Metastatic Dissemination in Lung Carcinogenesis. *Cancers (Basel)* 2022;14:3626.
  30. Sahn SA. Pleural diseases related to metastatic malignancies. *Eur Respir J* 1997;10:1907-13.
  31. Stathopoulos GT, Zhu Z, Everhart MB, et al. Nuclear factor- $\kappa$ B affects tumor progression in a mouse model of malignant pleural effusion. *Am J Respir Cell Mol Biol* 2006;34:142-50.
  32. Yano S, Herbst SH, Shinohara H, et al. Treatment for malignant pleural effusion of human lung adenocarcinoma by inhibition of vascular endothelial growth factor receptor tyrosine kinase phosphorylation. *Clin Cancer Res* 2000;6:957-65.
  33. Thickett DR, Armstrong L, Millar AB. Vascular endothelial growth factor (VEGF) in inflammatory and malignant pleural effusions. *Thorax* 1999;54:707-10.
  34. Giannou AD, Marazioti A, Spella M, et al. Mast cells mediate malignant pleural effusion formation. *J Clin Invest* 2015;125:2317-34.
  35. Cui R, Takahashi F, Ohashi R, et al. Osteopontin is

- involved in the formation of malignant pleural effusion in lung cancer. *Lung Cancer* 2009;63:368-74.
36. Heino J. Biology of tumor cell invasion: interplay of cell adhesion and matrix degradation. *Int J Cancer* 1996;65:717-22.
  37. Cheng D, Rodriguez RM, Perkett EA, et al. Vascular endothelial growth factor in pleural fluid. *Chest* 1999;116:760-5.
  38. Wang B, Shi L, Sun X, et al. Production of CCL20 from lung cancer cells induces the cell migration and proliferation through PI3K pathway. *J Cell Mol Med* 2016;20:920-9.
  39. Chen MC, Chen KC, Chang GC, et al. RAGE acts as an oncogenic role and promotes the metastasis of human lung cancer. *Cell Death Dis* 2020;11:265.
  40. Liu GN, Shi HZ, Xie ZH, et al. Epithelial neutrophil-activating peptide-78 recruits neutrophils into pleural effusion. *Eur Respir J* 2009;34:184-90.
  41. Neo SY, Lundqvist A. The Multifaceted Roles of CXCL9 Within the Tumor Microenvironment. *Adv Exp Med Biol* 2020;1231:45-51.
  42. Thielen C, Radermacher V, Trimeche M, et al. TARC and IL-5 expression correlates with tissue eosinophilia in peripheral T-cell lymphomas. *Leuk Res* 2008;32:1431-8.
  43. Yuan M, Zhu H, Xu J, et al. Tumor-Derived CXCL1 Promotes Lung Cancer Growth via Recruitment of Tumor-Associated Neutrophils. *J Immunol Res* 2016;2016:6530410.
  44. Qin XJ, Shi HZ, Huang ZX, et al. Interleukin-16 in tuberculous and malignant pleural effusions. *Eur Respir J* 2005;25:605-11.
  45. Ye LL, Peng WB, Niu YR, et al. Accumulation of TNFR2-expressing regulatory T cells in malignant pleural effusion of lung cancer patients is associated with poor prognosis. *Ann Transl Med* 2020;8:1647.
  46. Kaur S, Bronson SM, Pal-Nath D, et al. Functions of Thrombospondin-1 in the Tumor Microenvironment. *Int J Mol Sci* 2021;22:4570.
  47. Lunardi S, Lim SY, Muschel RJ, et al. IP-10/CXCL10 attracts regulatory T cells: Implication for pancreatic cancer. *Oncoimmunology* 2015;4:e1027473.
  48. Billottet C, Quemener C, Bikfalvi A. CXCR3, a double-edged sword in tumor progression and angiogenesis. *Biochim Biophys Acta* 2013;1836:287-95.
  49. Mulet M, Zamora C, Porcel JM, et al. Platelet factor 4 regulates T cell effector functions in malignant pleural effusions. *Cancer Lett* 2020;491:78-86.
  50. Aliper AM, Frieden-Korovkina VP, Buzdin A, et al. A role for G-CSF and GM-CSF in nonmyeloid cancers. *Cancer Med* 2014;3:737-46.
  51. Atanackovic D, Cao Y, Kim JW, et al. The local cytokine and chemokine milieu within malignant effusions. *Tumour Biol* 2008;29:93-104.
  52. Bradshaw M, Mansfield A, Peikert T. The role of vascular endothelial growth factor in the pathogenesis, diagnosis and treatment of malignant pleural effusion. *Curr Oncol Rep* 2013;15:207-16.
  53. Popper HH. Progression and metastasis of lung cancer. *Cancer Metastasis Rev* 2016;35:75-91.
  54. Jin HY, Lee KS, Jin SM, et al. Vascular endothelial growth factor correlates with matrix metalloproteinase-9 in the pleural effusion. *Respir Med* 2004;98:115-22.
  55. Yang K, Tian C, Zhang C, et al. The Controversial Role of IL-33 in Lung Cancer. *Front Immunol* 2022;13:897356.
  56. Wang H, Si S, Jiang M, et al. Leukemia inhibitory factor is involved in the pathogenesis of NSCLC through activation of the STAT3 signaling pathway. *Oncol Lett* 2021;22:663.
  57. Malekan M, Nezamabadi SS, Samami E, et al. BDNF and its signaling in cancer. *J Cancer Res Clin Oncol* 2023;149:2621-36.
  58. Han S, Wang T, Chen Y, et al. High CCL7 expression is associated with migration, invasion and bone metastasis of non-small cell lung cancer cells. *Am J Transl Res* 2019;11:442-52.
  59. Lv T, Zhao Y, Jiang X, et al. uPAR: An Essential Factor for Tumor Development. *J Cancer* 2021;12:7026-40.
  60. Fridlender ZG, Kapoor V, Buchlis G, et al. Monocyte chemoattractant protein-1 blockade inhibits lung cancer tumor growth by altering macrophage phenotype and activating CD8+ cells. *Am J Respir Cell Mol Biol* 2011;44:230-7.
  61. Deng J, Jiang R, Meng E, et al. CXCL5: A coachman to drive cancer progression. *Front Oncol* 2022;12:944494.
  62. Kuo PL, Huang MS, Hung JY, et al. Synergistic effect of lung tumor-associated dendritic cell-derived HB-EGF and CXCL5 on cancer progression. *Int J Cancer* 2014;135:96-108.
  63. Yu L, Guo Y, Chang Z, et al. Bidirectional Interaction Between Cancer Cells and Platelets Provides Potential Strategies for Cancer Therapies. *Front Oncol* 2021;11:764119.

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**Table S1** ARY022B RAW data for cytokines of MPE and cell lines (mean  $\pm$  SD)

Cytokine	Pleura PixelIntens	SD	Cell line PixelIntens	SD
Adiponectin	2891.125396	332.2623779	2679.238955	529.5095347
Apolipoprotein A-I	2957.067277	408.9183233	2668.426003	492.4892186
Angiogenin	3383.983321	221.2665112	2978.322933	532.971435
Angiopoietin-1	63.17075623	30.21151349	155.0339784	127.3422327
Angiopoietin-2	1928.083242	415.5552746	618.8911895	398.8596763
BAFF	3300.946172	222.2611577	3119.606339	427.1228226
BDNF	274.156331	29.54280752	528.9652824	382.9273803
Complement component C5/C5a	2712.224011	183.6085345	2435.847513	249.9042063
CD14	809.2141805	124.5859854	623.139756	174.7221675
CD30	291.252873	133.9241357	255.9266989	110.8637682
CD40 ligand	415.0085384	101.6128178	401.6400204	143.1952442
Chitinase 3-like1	2778.730889	173.7143732	2879.373392	587.147477
Complement factor D	1855.173738	46.48794213	1438.371767	327.078371
C-reactive protein	3230.041836	273.1674472	1132.734375	508.9944166
Cripto-1	171.0250959	42.86151892	74.05185023	65.06010218
Cystatin C	2866.476568	310.4254574	821.6573012	147.7060177
Dkk-1	316.4407915	162.5185242	2539.762426	122.3780422
DPPIV	2824.720339	292.4563842	2496.487596	205.0392539
EGF	62.27427268	25.11774491	79.46782137	54.11887669
Emmprin	1269.824758	267.604248	1465.716385	512.0356557
ENA-78	154.1102237	20.65018077	2894.44748	277.9386908
Endoglin	2358.366968	190.6612874	1957.133022	578.8925568
Fas ligand	75.89633042	14.43727039	85.88479912	46.19232707
FGF basic	354.3063412	54.55936847	202.7487141	128.4642475
FGF-7	46.27582304	8.709628563	59.22161382	54.85457018
FGF-19	1640.626329	124.2856266	910.0472509	258.9073763
Flt-3 ligand	637.6079085	106.597281	229.0726149	118.9112425
G-CSF	113.2707501	60.08748856	597.7099982	811.9344439
GDF-15	2600.593303	80.94082752	821.2530036	227.7991159
GM-CSF	116.2789936	30.4809818	282.8410051	337.8362112
GROa	373.8372483	126.572485	2275.765744	327.0582248
Growth hormone	444.6095632	130.4346571	501.2833891	307.7735132
HGF	601.7801403	196.2253397	276.3695784	140.6824735
ICAM-1	1725.936086	371.2653194	507.2550851	272.3926083
IFN-gamma	274.5609374	38.71252843	293.6773764	114.6315355
IGFBP-2	3155.359773	330.4358583	2753.893039	193.9673828
IGFBP-3	1716.541284	139.4889196	881.0849909	96.63513276
IL-1a	239.0417351	43.10588357	281.3321149	135.4667113
IL-1b	38.19845885	11.25199021	49.6065697	49.19589461
IL-1ra	144.8381078	41.98041374	420.109655	169.360194
IL-2	45.92456963	5.490830762	94.75541356	71.23502674
IL-3	25.40394451	8.977128238	44.65058898	20.95608408
IL-4	672.4071533	408.1366789	552.7730912	161.3791934
IL-5	63.48360593	44.1909409	109.529742	10.6902645
IL-6	1613.432102	381.1367826	498.7336552	153.8380156
IL-8	2372.27905	726.1871274	3049.518139	590.1698309
IL-10	102.3055919	31.08358637	139.9074411	115.5962593
IL-11	184.583774	33.06453695	185.1155428	78.81686841
IL-12 p70	48.86399442	9.927387432	63.28546698	63.49028403

MPE, malignant pleural effusion; SD, standard deviation.