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ORIGINAL ARTICLE

Pegylated recombinant human granulocyte colony-stimulating factor regulates the immune status of patients with small cell lung cancer

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Keywords
immune status; lymphocyte subsets; pegylated recombinant human granulocyte colony-stimulating factor; small cell lung cancer; T-cell receptor.

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Abstract

Background: Small cell lung cancer (SCLC) is an aggressive disease involving immunodeficiency for which chemotherapy is the standard treatment. Pegylated recombinant human granulocyte colony-stimulating factor (PEG-rhG-CSF) is widely used for primary or secondary prophylaxis of febrile neutropenia (FN) in chemotherapy. However, whether PEG-rhG-CSF influences immune cells, such as lymphocytes, remains unclear.

Methods: A total of 17 treatment-naïve SCLC patients were prospectively enrolled and divided into the PEG-rhG-CSF and control groups according to their FN risk. Longitudinal sampling of peripheral blood was performed before, after and 4–6 days after the first cycle of chemotherapy. Flow cytometry was used to assess lymphocyte subsets, including $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, NK, and B cells. The diversity and clonality of the T-cell receptor (TCR) repertoire was analyzed by next-generation sequencing.

Results: In the PEG-rhG-CSF group, the proportions of $CD3^+$ T and $CD4^+$ T cells had increased significantly ($P = 0.002$, $P = 0.020$, respectively), whereas there was no increase in $CD8^+$ T cells. Further, TCR diversity increased ($P = 0.009$) and clonality decreased ($P = 0.004$) significantly after PEG-rhG-CSF treatment. However, these factors showed opposite trends before and after chemotherapy. Vβ and Jβ gene fragment types, which determine TCR diversity, were significantly amplified in the PEG-rhG-CSF group. The change in TCR diversity was significantly correlated with changes in the $CD3^+$ T or $CD4^+$ T cell proportions, but not the $CD8^+$ T cell proportion.

Conclusions: PEG-rhG-CSF regulates the immune status of SCLC patients; $CD4^+$ T cells may be the main effector cells involved in this process. These findings may optimize the treatment of SCLC.

Key points

- PEG-rhG-CSF regulates SCLC immunity.
- PEG-rhG-CSF increased $CD3^+$ T and $CD4^+$T cell proportions.
- PEG-rhG-CSF increased TCR diversity and decreased clonality in peripheral blood.
- Change in TCR diversity were correlated with $CD3^+$ T or $CD4^+$ T changes.

Introduction

Small cell lung cancer (SCLC) is an aggressive disease that grows rapidly and has high metastatic potential, resulting in a clinically poor prognosis. The combination of etoposide and cisplatin or carboplatin (EP/EC) remains the first-line treatment for extensive-stage SCLC, and early concurrent radiochemotherapy is the standard of care for limited-stage SCLC. Most SCLCs initially respond well to chemotherapy but nearly always recur, with a median survival of 10–12 months from diagnosis. The most important recent breakthrough in SCLC first-line treatment is immunotherapy. Programmed death ligand 1(PD-L1) inhibitor atezolizumab in combination with etoposide and carboplatin improved overall survival (OS) by 2 months over chemotherapy in the phase III IMpower 133 trial.[1] However, it appears to be less effective than in non-small cell lung cancer. Potential mechanisms may involve immunodeficiency, including marked decreases in the leukocyte count,[2] low expression of PD-L1, low numbers of tumor-infiltrating lymphocytes,[3] and downregulation of major histocompatibility complex molecules and regulatory chemokines.[4]

Granulocyte colony-stimulating factor (G-CSF) is a key regulator of granulopoiesis and differentiation.[5] Recombinant human G-CSF (rhG-CSF) and pegylated recombinant human G-CSF (PEG-rhG-CSF, a long-acting form of rhG-CSF) are recommended for primary or secondary prophylaxis of febrile neutropenia (FN) in cancer patients. In addition to myeloid cells, single-cell analyses demonstrated that $CD4^+$ T cells and $CD8^+$ T cells express G-CSF receptor after G-CSF stimulation. G-CSF directly modulates T-cell via its receptor on T cells.[6] RhG-CSF also "polarizes" T-cell subsets and reduce alloreactivity in patients receiving allogeneic transplantation.[7] It is reported that PEG-rhG-CSF enhances the antitumor activity of monoclonal antibodies such as rituximab and trastuzumab elicited via antibody-dependent cellular cytotoxicity or phagocytosis in preclinical models and clinical trials.[8,9] The regulatory effects on immune cells seems greater for PEG-rhG-CSF than rhG-CSF.[10] Based on limited evidences, the specific effects of PEG-rhG-CSF on the immune cells, such as lymphocytes, remains unclear.

T cells are the main component of the anticancer immune system, reflecting the immune status. Immune cell infiltration has been suggested as a key determinant of long-term survival in SCLC,[11] and the percentage of $CD3^+$ T cells is an independent prognostic factor for immunotherapy.[12] Moreover, biomarkers in the peripheral blood also predict clinical outcomes of immunotherapy. The distribution of peripheral lymphocyte subsets reflects the immune status and is correlated with survival in SCLC.[13] Further, the specificity of each T cell for antigens is determined by its T-cell receptors (TCRs), which together across all T cells form a repertoire of millions of unique receptors in each individual.[14] TCR diversity is predominantly derived from the highly variable complementarity-determining region 3 (CDR3) and is generated by random rearrangement and junction region mutation of the V-D-J regions, which are three fragments located in TCR-coding genes. Many studies have shown that lymphocytes recognize the patient's unique mutations in the peripheral blood[15,16]; TCR repertoire in peripheral blood could be a biomarker of clinical outcomes for chemotherapy and immunotherapy.[17–19] We predicted that the peripheral blood TCR repertoire and lymphocyte subsets reflect the immune status of patients with SCLC, and its dynamic changes can reflect immune status changes.

This study was conducted to evaluate dynamic changes in the peripheral blood TCR repertoire and lymphocyte subsets before and after the first cycle of EP/EC chemotherapy for patients with treatment-naïve SCLC. Specifically, we examined whether PEG-rhG-CSF can regulate the immune status.

Methods

The study was conducted in compliance with the Helsinki Declaration of 1975 and was approved by the Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. The study was registered with the Chinese Clinical Trial Registry under registration number ChiCTR1900022301. Written informed consent was obtained from study participants or a legally authorized representative prior to enrollment. The outline of the study pipeline is shown in Figure 1.

Patients

This study prospectively included patients with treatment-naïve SCLC who were treated with standard first-line chemotherapy regimens in the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China). Inclusion criteria included pathologically confirmed SCLC, no administration of any form of antitumor therapy, no recent surgeries, no hematological disease, baseline peripheral blood routine blood test within the normal range, and willing to participate in the experiment and signed informed consent. The exclusion criteria were as follows: patients with infections, antibiotic treatment within 72 hours before or during chemotherapy, pregnant or lactating women, women of reproductive age who were not administered contraception, allergy to biological products, and past or current autoimmune disease.

Study design

According to NCCN guidelines for myeloid growth factors, EP/EC regimens are associated with a moderate risk of FN

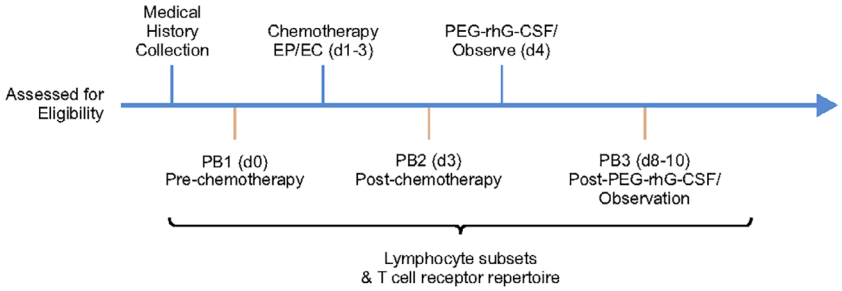

Figure 1 Outline of the study pipeline.

development. Prophylactic administration of PEG-rhG-CSF is recommended for patients who need to be treated with these regimens, concurrently considering other risk factors: prior chemotherapy or radiation therapy; persistent neutropenia; bone marrow involvement by the tumor; recent surgery and/or open wounds; liver dysfunction; renal dysfunction (creatinine clearance <50 mL/minutes*1.73 m^2); age > 65 years; and administration of full chemotherapy. Patients with SCLC with at least one risk factor above were assigned to the PEG-rhG-CSF group, and otherwise were assigned to the control group.

All patients were administered EP/EC regimens: etoposide 100 mg/m^2 on days 1–3, cisplatin 75 mg/m^2 on day 1, or carboplatin (AUC = 5) on day 1 every 21 days. In the PEG-rhG-CSF group, patients were administered 6 mg PEG-rhG-CSF (Jinyouli, CSPC) at 24 hours after the completion of chemotherapy (day 4).

Peripheral blood samples were collected from each patient at three time points: before the beginning of the first cycle of treatment (day 0), after the first cycle of treatment (day 3), and 4–6 days after PEG-rhG-CSF injection or observation (day 8–10). We selected 4–6 days after PEG-rhG-CSF injection as the observation time point with reference to the pharmacokinetic parameters of PEG-rhG-CSF.[20] A complete medical history and physical examination were performed before starting treatment, and the clinical response to treatment was evaluated by physicians every two cycles according to the Response Evaluation Criteria in Solid Tumors Version 1.1.

Peripheral blood lymphocyte subsets

Blood was sampled at three different time points from each patient by venipuncture using EDTA-coated blood collection tubes (Vacutainer, BD Biosciences, Franklin Lakes, NJ, USA). Six-color flow cytometric analysis was performed to determine the cell phenotypes using CytoFlex and CytExpert software (Beckman-Coulter, Brea, CA, USA). We evaluated the following groups of cells: $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD3^-CD56^+CD16^+$ NK cells, and $CD19^+$ B cells.

TCR sequencing

Genomic DNA was extracted from peripheral blood cells by using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Multiplex PCR amplification of CDR3 of the TCR β chain was performed using the deep resolution Immunoseq platform (Geneplus-Beijing, Beijing, China).[18] Reads from raw data with low quality and high N ratios were removed to obtain clean reads. Two-terminal reads were merged, and the reads were subjected to bioinformatics analysis. The sequences of the V, D, and J genes were compared to those in the ImMunoGeneTics database by MIXCR software, and the CDR3 sequence of TCR was identified.

The Shannon index and clonality were calculated to characterize the features of the T cell repertoire, and Morisita–Horn similarity index (MH overlap) was used to detect similarities between samples, as described previously.[21–24]

Statistical analysis

Differences between groups were compared using the two-tailed Student *t*-test, Mann-Whitney test, or Wilcoxon matched-pairs signed rank tests. Correlations between variables were analyzed by Spearman's rank test. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad, Inc., La Jolla, CA, USA) or SPSS 24.0 software (SPSS, Inc., Chicago, IL, USA). A two-sided *P*-value <0.05 was considered as statistically significant.

Results

Patient characteristics and clinical outcomes

A total of 17 patients with SCLC were enrolled prospectively between February 2019 and August 2019, with 10 patients in the PEG-rhG-CSF group and seven patients in the control group based on FN risk factor stratification. The baseline patient characteristics are shown in Table 1.

Table 1 Baseline patient characteristics

Characteristics	All patients (*N* = 17)	PEG-rhG-CSF group (*N* = 10)	Control group (*N* = 7)
Median age - years old (range)	63 (50–72)	65.5 (52–72)	62 (50–64)
Sex - No. (%)			
Male	14 (82.4)	8 (80.0)	6 (85.7)
Female	3 (17.6)	2 (20.0)	1 (14.3)
Stage - No. (%)			
Limited	12 (70.6)	5 (50.0)	6 (85.7)
Extensive	5 (29.4)	5 (50.0)	1 (14.3)
Smoking history - No. (%)	13 (76.5)	7 (70.0)	6 (85.7)

In these patients, the median age was 63 years (50–72 years old), most patients were male (82.4%), patients showed a limited stage (70.6%), and had a history of heavy smoking (76.5%). All patients had ECOG scores of 0–2 and could tolerate chemotherapy. Because patients ≥65 years old have a higher risk of FN, these patients were enrolled in the PEG-rhG-CSF group based on ethical considerations. Other clinical features were comparable between the two groups.

Only one (1/10) patient in the PEG-rhG-CSF group experienced transient leukopenia (grade 2) and neutropenia (grade 3) during the interchemotherapy period; this patient recovered spontaneously. In contrast, although the leukopenia risk was theoretically lower, six (6/7) patients in the control group experienced ≥3 grade hematologic toxicity. All occurred after the third blood sampling timepoint and were administered therapeutic G-CSF on at least three days after all blood sampling finished. After two cycles of EP/EC chemotherapy, 12 patients achieved a partial response, two achieved a complete response, and three showed stable disease, with an objective response rate of 82.4%.

PEG-rhG-CSF regulates peripheral blood lymphocyte subsets

At baseline, the average percentages of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells were within normal ranges of 64.1% (61.1%–77%), 40.1% (25.8%–41.6%), 22.9% (18.1%–29.6%), 11.7% (7.3%–18.2%), and 24.0% (8.1%–25.6%), respectively. The average $CD4^+$ T cell/$CD8^+$ T cell ratio was 2.01 (0.98–1.94). Additionally, 35.3% (6/17) of patients had a low $CD3^+$ T cell proportion and 29.4% (5/17) had a low $CD8^+$ T cell proportion. The proportion of baseline $CD3^+$ T cells was lower ($P = 0.009$) and that of NK cells was higher ($P = 0.005$) in the PEG-rhG-CSF group (Fig 2a).

In the PEG-rhG-CSF group, the proportion of $CD3^+$ T cells and $CD4^+$ T cells increased significantly on day 8–10 compared to day 3 ($P = 0.002$, $P = 0.020$, respectively), whereas there was no significant change in the control group (Fig 3). No significant change was observed before and after chemotherapy (day 0 vs. day 3) both in the PEG-rhG-CSF and control group. There were no significant changes in $CD8^+$ T cells. Dynamic changes in NK cells and B cells were also not significant.

These results indicated that PEG-rhG-CSF can increase the proportion of $CD3^+$ and $CD4^+$ T cells but not that of $CD8^+$ T cells, whereas short-term chemotherapy has a minimal influence on the peripheral blood lymphocyte distribution.

PEG-rhG-CSF regulates peripheral blood TCR repertoire

To further explore the immune status, we evaluated changes in the peripheral blood TCR repertoire. In this study, we analyzed four metrics of the TCR repertoire:

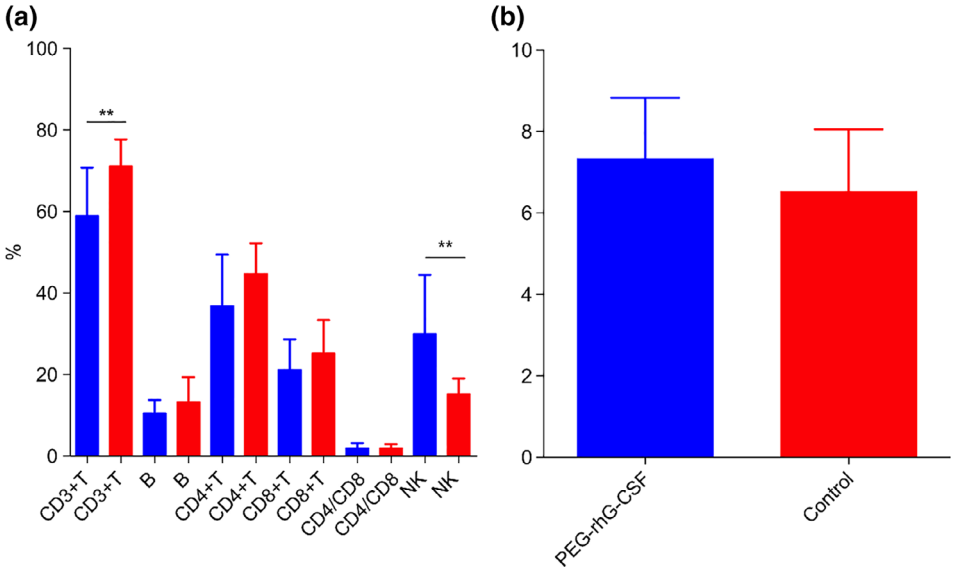

Figure 2 Baseline lymphocyte subsets and TCR diversity. (**a**) Baseline lymphocyte distributions. (■) PEG-rhG-CSF; (■) Control. (**b**) Baseline TCR diversity.

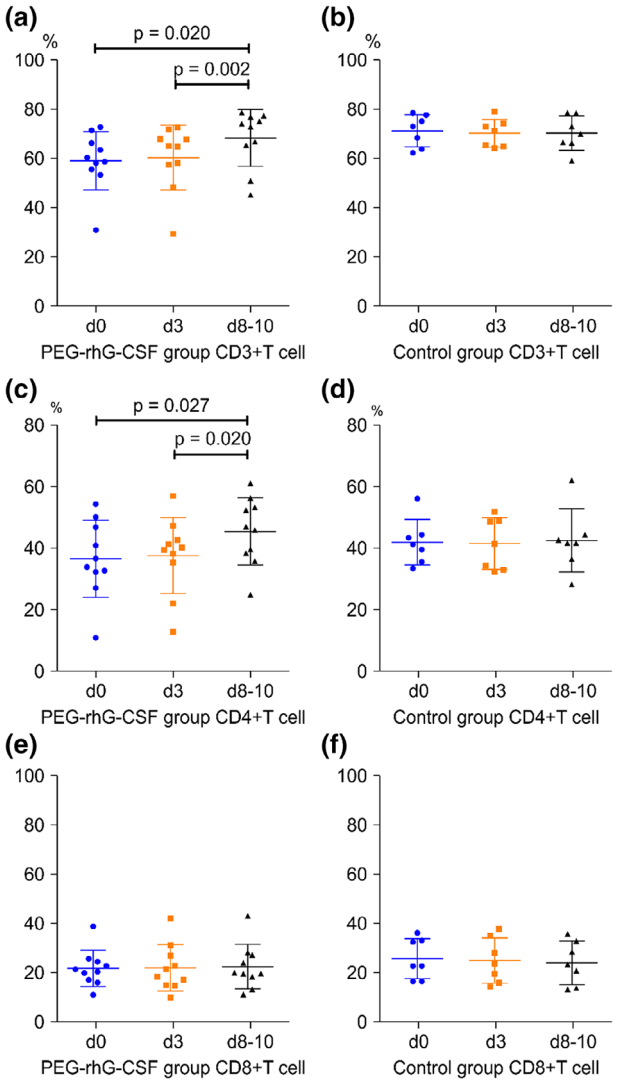

Figure 3 Changes in lymphocyte subsets in PEG-rhG-CSF group and control group. (**a**) $CD3^+$ T cell proportion in PEG-rhG-CSF group. (**b**) $CD3^+$ T cell proportion in control group. (**c**) $CD4^+$ T cell proportion in PEG-rhG-CSF group. (**d**) $CD4^+$ T cell proportion in control group. (**e**) $CD8^+$ T cell proportion in PEG-rhG-CSF group. (**f**) $CD8^+$ T cell proportion in control group.

diversity,[22] clonality,[23] MH overlap,[24] and Vβ and Jβ gene fragment types. The stability of these factors has been previously reported.[18,25] The TCR repertoire was not significantly different between the groups at baseline (Fig 2b).

First, peripheral blood TCR diversity according to the Shannon index was used to measure the diversity of the clonotype population at each time point. Following chemotherapy, we found that TCR diversity was significantly decreased in the PEG-rhG-CSF group (P = 0.046) but not significantly decreased in the control group (P = 0.645). There was an outlier in the statistics; when the patient showing a sharp increase after chemotherapy was excluded, a significant decrease in TCR diversity was observed in the control group (P = 0.036). For PEG-rhG-CSF, we found that TCR diversity was significantly increased after PEG-rhG-CSF injection (P = 0.009), while no significant increase was detected in the control group (P = 0.113). Based on these results, the variation trend in TCR diversity was generally decreased after chemotherapy and increased after PEG-rhG-CSF therapy (Fig 4a,b).

In contrast to TCR diversity, a clonality increase represents the expansion of a mono- or oligo-clonal population of T cells. PEG-rhG-CSF significantly reduced clonality in the peripheral blood (P = 0.004), reflecting reduced oligoclonality in the peripheral blood (Fig 4c,d).

We next analyzed MH overlap at different time points to identify similarities between samples. There was no significant difference in TCR overlap between day 3 and day 8–10 in the two groups (Fig 4e).

Finally, we analyzed the amplified-frequencies of the Vβ and Jβ gene fragment types (V-J types) in each patient. Each V-J type represented a type of TCR, and Wilcoxon rank sum test was used to determine if the V-J type was significantly increased from day 3 to day 8–10. Significantly increased V-J types were illustrated in Figure 5. The number of increased V-J types was much greater in the PEG-rhG-CSF group than in the control group (29 vs. 10).

These data demonstrate that PEG-rhG-CSF increased diversity and decreased clonality in the PEG-rhG-CSF group and that the significantly increased V-J types were more diverse after PEG-rhG-CSF injection. These changes illustrate that an altered immune status is associated with PEG-rhG-CSF.

Correlation between TCR repertoire and lymphocyte subsets

At the cellular level, the type, count, and function of lymphocytes affect the immune status. At the effector structure level, the type and frequency of TCRs on the surface of lymphocyte affect the immune response. Lymphocyte subsets reflect the distribution of different functional cells, while TCR diversity comprehensively reflects the TCRs. Taken together, we determined the relationship between the TCR repertoire and lymphocyte subsets.

At baseline, the $CD3^+$, $CD4^+$, and $CD8^+$ T cell proportions were not correlated with TCR diversity (Fig 6a). The change in TCR diversity between day 0 and day 3 was significantly correlated with changes in the $CD3^+$ and $CD4^+$ T cell proportion (P = 0.015, P = 0.046, respectively) but was not correlated with the $CD8^+$ T cells proportion (P = 0.978) (Fig 6b). The change in TCR diversity between day 3 and day 8–10 was also significantly correlated with changes in the $CD3^+$ and $CD4^+$ T cell proportions

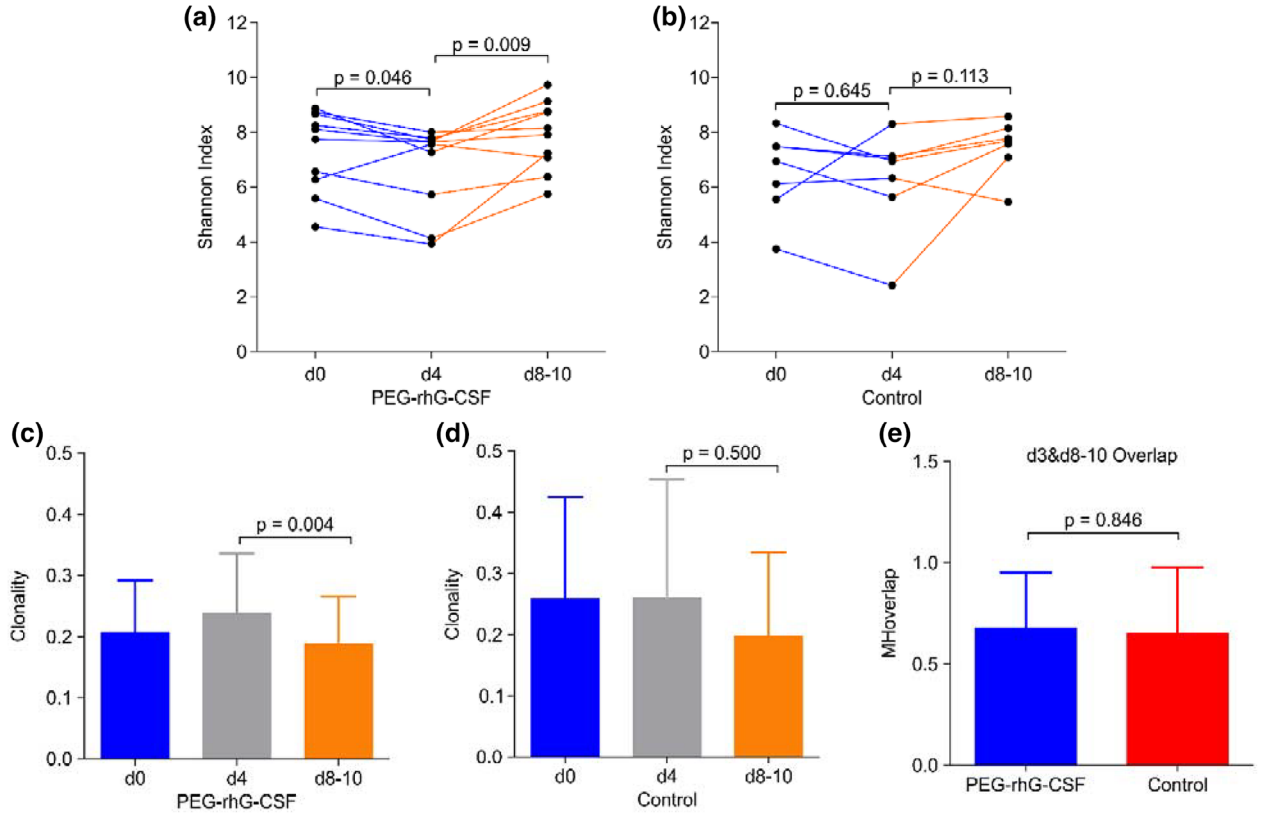

Figure 4 Changes in peripheral blood TCR repertoire. (**a**) TCR diversity dynamic changes in PEG-rhG-CSF group. (**b**) TCR diversity dynamic changes in control group. (**c**) Dynamic changes in clonality in the PEG-rhG-CSF group. (**d**) Dynamic changes in clonality in the control group. (**e**) MH overlap at days 8–10 and day 3 in the two groups.

(P = 0.031, P = 0.013, respectively) but was not correlated with the $CD8^+$ T cell proportion (P = 0.866) (Fig 6c). This result demonstrates an intrinsic correlation between lymphocyte subsets and TCR diversity, and increased TCR diversity occurs mainly because of $CD4^+$ T cells rather than $CD8^+$ T cells.

Discussion

The poor outcomes for patients with SCLC remain a challenge for oncologists. Immune deficiency may contribute to the limited efficacy of routine chemotherapy and immunotherapy. Regulation of the immune microenvironment may be important for improving the treatment efficacy for SCLC. PEG-rhG-CSF is a long-acting rhG-CSF widely used to prevent leukopenia, neutropenia, and FN caused by chemotherapy in patients with cancer. In addition, it may have immune-modulatory effects; however, the evidence is very limited.[8,10] This is the first prospective study to explore the influence of PEG-rhG-CSF on the immune status of patients with SCLC. We found that PEG-rhG-CSF has a regulatory effect on the immune system by monitoring dynamic changes in the TCR repertoire and lymphocyte subsets before and after routine chemotherapy.

At baseline, although patients in the PEG-rhG-CSF group tended to have a lower proportion of $CD3^+$ T cells, differences in functional $CD4^+$ T cell or $CD8^+$ T cell proportions and TCR diversity were not significant between groups, indicating that the baseline immune status was approximately equivalent. We did self-comparisons to avoid differences between groups. To exclude confounding factors of chemotherapy, we conducted dynamic monitoring of the blood samples in both groups at the same time before and after chemotherapy. Despite the higher risk of leukopenia or neutropenia in the PEG-rhG-CSF group, this study confirmed that PEG-rhG-CSF contributes to the prevention of leukopenia, neutropenia, and FN. Additionally, the proportion of $CD4^+$ T cell and TCR diversity were more obviously improved in the PEG-rhG-CSF group whose immune status was slightly worse than those in the control group at baseline.

From the perspective of circulating lymphocyte subsets, we found increases in the $CD3^+$ T cell and $CD4^+$ T cell proportions after treatment with PEG-rhG-CSF. $CD3^+$ T cells include αβT cells and γδT cells, among which αβT

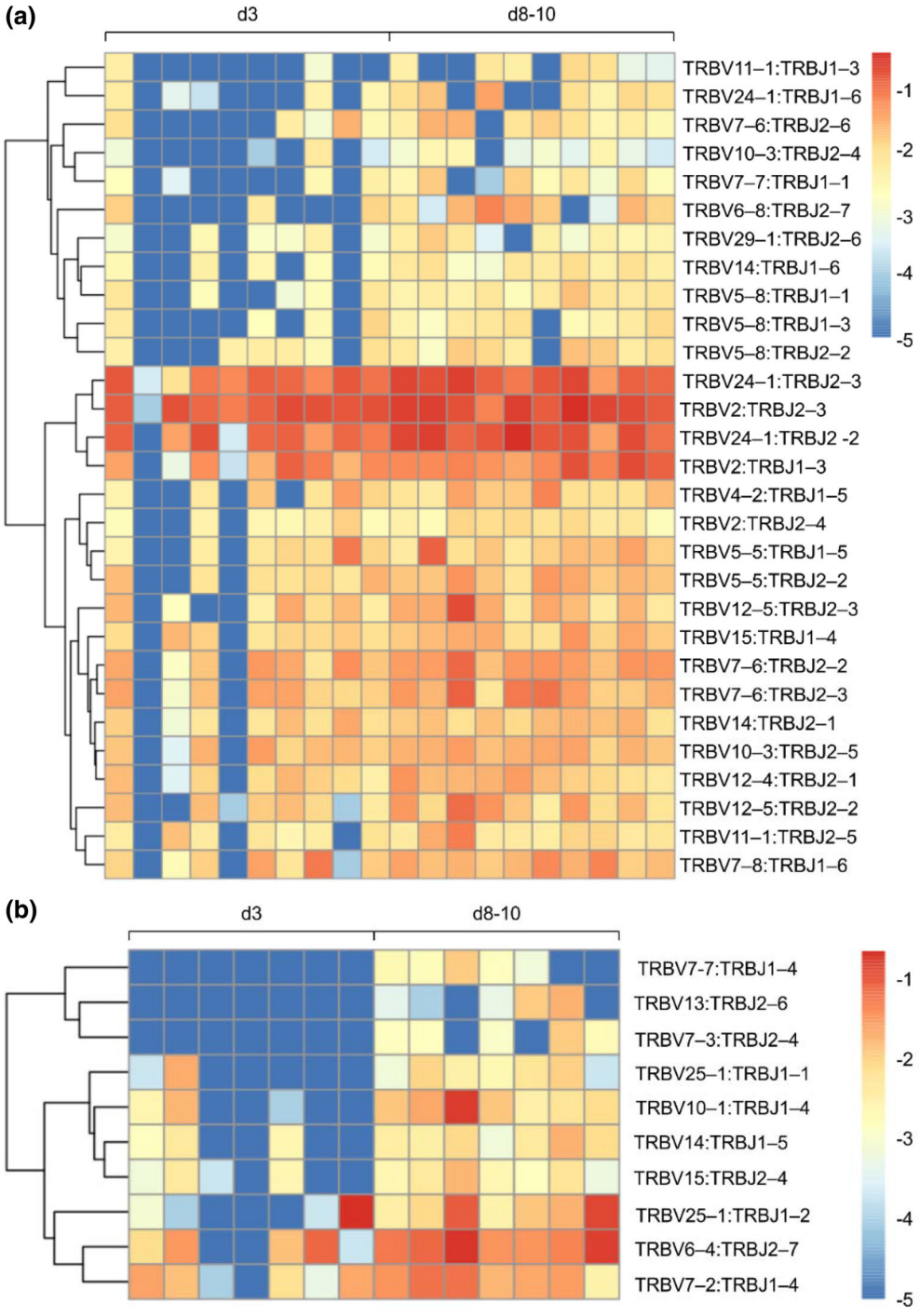

Figure 5 Comparison of frequency-amplified V-J gene types between groups. Frequency-amplified V-J gene types on day 8–10 compared to day 3. Each column represents one patient and each row represents a TCR type. (**a**) PEG-rhG-CSF group; (**b**) Control group.

cells account for 90%–95% of mature T cells in the peripheral blood and are the main cell subgroups involved in the adaptive immune response. $CD4^+$ T cells, as helper T cells, play a major role in cellular immunity and assist in humoral immunity, which can be used to evaluate the immune system status. Interestingly, $CD4^+$ T cells appeared to be more sensitive than $CD8^+$ T cells to immune status changes. Previous studies emphasized the central role of MHC-II presentation in tumor evolution.[26] $CD4^+$ T cells recognize most of the immunogenic

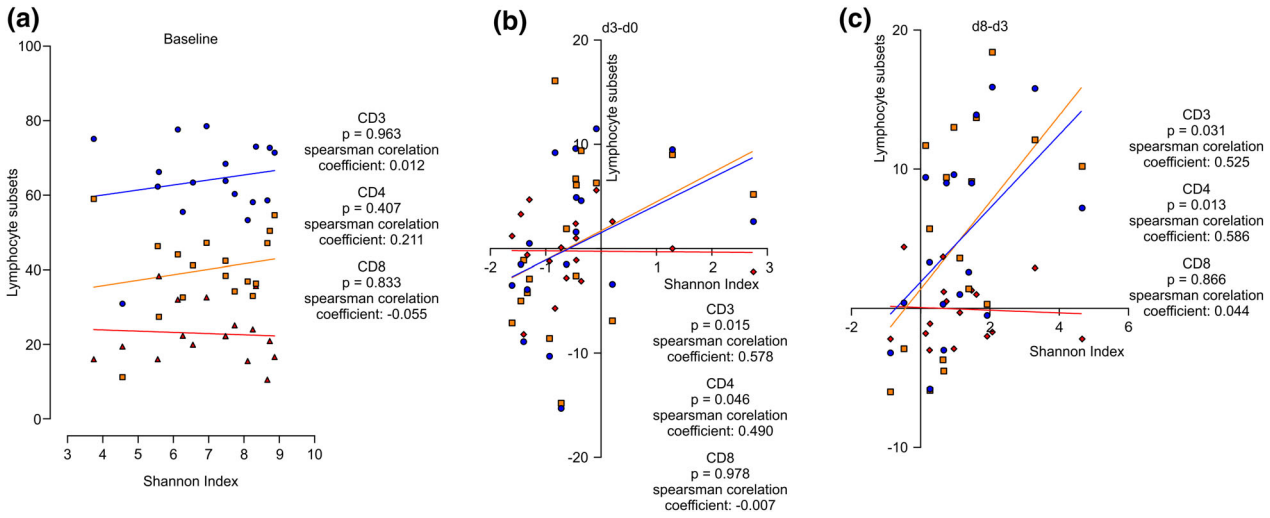

Figure 6 Correlation between TCR diversity and lymphocyte subsets. (**a**) Correlation between TCR diversity and $CD3^+$, $CD4^+$, and $CD8^+$ T cell proportions at baseline. (—●—) CD3+T; (—■—) CD4+T; (—◆—) CD8+T. (**b**) Correlation between TCR diversity changes and $CD3^+$, $CD4^+$, and $CD8^+$ T cell proportion changes before and after chemotherapy. (—●—) CD3+T; (—■—) CD4+T; (—◆—) CD8+T. (**c**) Correlation between TCR diversity changes and $CD3^+$, $CD4^+$, and $CD8^+$ T cell proportion changes before and after PEG-rhG-CSF. Statistical analysis was performed using the Spearman's rank test. (—●—) CD3+T; (—■—) CD4+T; (—◆—) CD8+T.

mutanome; in neoantigen vaccination murine models, $CD4^+$ T cell responses to neoantigens are more prevalent and potentially more effective in antitumor immunity than $CD8^+$ T cell responses.[27] This may be because of the less stringent length and sequence requirement for peptides binding to MHC class II molecules as compared to those binding to MHC class I epitopes, increasing the likelihood that a given mutation is found within a presented peptide.[28] Significantly expanded peripheral blood $CD4^+$ T cells were also found in patients who responded to immunotherapy.[29] Our results also support that $CD4^+$ T cells respond to the immune status earlier and may play a more important role in the anti-tumor immune response. Further studies are needed to identify how the specific $CD4^+$ T cell subsets are changed when PEG-rhG-CSF is administered.

Furthermore, we sequenced the peripheral blood TCR repertoire. We found that TCR diversity decreased immediately after chemotherapy and significantly increased after PEG-rhG-CSF injection. Another indicator, clonality, was decreased after PEG-rhG-CSF treatment, with no significant change observed in the control group. These results support activation of the immune status for PEG-rhG-CSF. The relationship between the peripheral blood TCR and tumor immune microenvironment has been investigated. Previous studies showed that T cell clones circulating in the blood may correspond to tumor-infiltrating lymphocytes in melanoma, some of which may have expanded in response to tumor antigens.[19,30–32] The TCR clonality of both $CD4^+$ and $CD8^+$ T cells is more restricted than in healthy people, and broader T cell responses with greater diversity of $CD4^+$ blood T cell clones may predict the longer survival of patients with melanoma treated with anti-CTLA4 or PD-1 antibodies.[19] Many studies have shown that peripheral blood TCR diversity can be used to predict the efficacy of immunotherapy.[17,21,33,34] Therefore, PEG-rhG-CSF may improve the efficacy of immunotherapy by increasing peripheral blood TCR diversity; however, further clinical studies are needed to confirm this. We found no MH overlap on day 3, and day 8–10 showed a significant difference between groups. This may be because there were many identical clones in series samples, but the significantly expanded clones contributed to the diversity change. TCR diversity was determined by the diversity of V-D-J gene types. We selected V-J gene types showing significantly increased frequencies in the two groups. The PEG-rhG-CSF group showed a greater number of increased types compared to the control group.

We also explored the intrinsic correlation of lymphocyte subsets and TCR diversity. As expected, there was no correlation between these factors at baseline, as the TCR is a microscopic structure on lymphocytes and its diversity is not limited by the number of lymphocytes. In dynamic monitoring, we observed a correlation between changes in $CD3^+$ or $CD4^+$ T cells and changes in TCR diversity. However, the change in $CD8^+$ T cells was not correlated with TCR diversity. This suggests that the dynamic changes in immune status are impacted more by $CD4^+$ T cells than by $CD8^+$ T cells and that $CD4^+$ T cells contribute to increased TCR diversity.

Numerous studies of immunotherapy have been explored for cancer treatment, with many patients showing benefits and even long-term survival. How to transform the immune microenvironment from ineffective to effective

for immunotherapy may be a key issue in improving prognosis. This study shows that PEG-rhG-CSF improves the proportion of peripheral blood immune cells and TCR repertoire in patients after chemotherapy, suggesting that it may potentially influence the immune status and has potential applicability in the field of immunotherapy. PEG-rhG-CSF is commonly used in clinical practice; its role with regard to the combination of chemotherapy and immunotherapy requires further analysis.

We observed the influence of chemotherapy on the immune status of patients with SCLC. The decreased TCR diversity and increased clonality after chemotherapy indicates a downregulated immune status when a routine and adequate dose of chemotherapy has just been administered. However, previous studies showed that chemotherapy combined with immunotherapy can further improve the clinical benefits to patients, possibly by promoting the release of neoantigens. The timing of immunotherapy may be critical in the response. Based on our results, the immune status in vivo was decreased within a short period after chemotherapy, and it is possible that immunotherapy should not be started immediately after chemotherapy. However, approximately 4–6 days after the end of chemotherapy, particularly in the PEG-rhG-CSF group, the immune status was improved, which may be a better time for immunotherapy intervention. Further studies are needed to explore this.

There were some limitations to this study. Because of the exploratory prospective design and dynamic monitoring of the peripheral blood, the sample size was small, but PEG-rhG-CSF was shown to affect the immune status. Further studies are ongoing in this area at our institute; the $CD4^+$ T cell proportion but not the $CD8^+$ T cell proportion was significantly changed in this study, but the specific subtypes were not clear, the distribution of CD4 positive cell subtypes may determine whether immune tolerance or activation, further studies are needed. Whether the regulatory effect of PEG-rhG-CSF on the immune status can be translated into improvements in immunotherapy in patients must be explored in clinical studies.

In conclusion, by longitudinal sampling of the peripheral blood, we found that PEG-rhG-CSF regulates circulating lymphocyte subsets and the TCR repertoire, which reflect changes in the immune status of SCLC patients treated with chemotherapy. $CD4^+$ T cells may be the main effector cells involved in this process. These findings may optimize the treatment of SCLC.

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Disclosure

Ruimin Guo is employee of China Shijiazhuang Pharmaceutical Group Co., Ltd. No other conflicts were reported.

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