J Med Biosci Suppl 2025;7: S137 - S142 DOI: 10.58837/CHULA.JMBS.7.S1.20

Original article

The prognostic impact of various circulating cytokine levels on response to EGFR-TKIs treatment in advanced NSCLC patients

Nussara Pakvisal[1], Warissara Khuntharuks[2], Pornrat Kongkavitoon[1], Piyada Sutthideatphaiboon[1], Skunn Santisukwongchote[3], Pongsakorn Ouwongprayoon[4], Krittaya Korphaisarn[5], Chatchawit Aporntewan[5], Virote Sriurapong[1], Chanida Vinayanuwattikun[1,*]

[1]Division of Medical Oncology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[2]Medical Sciences Program, Faculty of Medicine, Chulalongkorn University and the King Chulalongkorn Memorial Hospital, Bangkok, Thailand
[3]Department of Pathology, Faculty of Medicine, Chulalongkorn University and the King Chulalongkorn Memorial Hospital, Bangkok, Thailand
[4]Department of Radiology, Faculty of Medicine, Chulalongkorn University and The King Chulalongkorn Memorial Hospital, Bangkok, Thailand
[5]Department of Medical Oncology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract

***Background**:* Non-small-cell lung cancer (NSCLC) patients were routinely treated with *EGFR* tyrosine kinase inhibitors (TKIs) and eventually acquired resistance from TME-producing intermediate factors, including various cytokines to facilitate cancer progression.

Objective: To evaluate the correlation between circulating levels of pro- and anti-inflammatory cytokines and clinical outcomes in advanced NSCLC patients with *EGFR* mutant-receiving TKI treatments.

Methods: Plasma samples were collected at pretreatment and during the 6- to 12-week follow-up in 123 patients with EGFR-mutated NSCLC. The expression of human cytokines was measured using a 27-bioplex panel by comparing the results to healthy controls (N=30). Patients were classified as durable responders (DU) based on progression-free survival (PFS): PFS ~* 104 weeks, and non-durable responders (NDU): PFS < 104 weeks.

Results: High IL-6 and IL-10 levels at baseline were significant in NDU ($P = 0.0076$ and $P = 0.0166$, respectively). Low IL-6 levels were statistically significant in NDU (median difference = -1.275 pg/ml, $P = 0.0019$) at the second time point. In contrast, IL-10 levels post-treatment were higher than baseline in DU [median difference = 0.000 pg/ml, $P = 0.0234$]. High IL-6 levels at pre-treatment were associated with poor PFS [(44.429 vs. 140.857), $P = 0.018$], overall- survival (OS) [(87 vs. 160), $P < 0.001$] and time-to-treatment failure (TTF) [(47 vs. 136), $P < 0.001$]. In contrast, high IL-10 levels were associated with longer TTF (124.895 vs. 41.14^0, $P < 0.005$).

***Conclusion**:* The pattern and levels of pro- and anti-inflammatory cytokines may be a prognostic biomarker for evaluating durable response in EGFR-mutant NSCLC patients who received targeted therapy.

Keywords: Acquired resistance, cytokine, EGFR-TKIs, NSCLC, predictive biomarkers.

***Correspondence to:** Chanida Vinayanuwattikun, Department of Medical Oncology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
E-mail: Chanida.Vi@chula.ac.th.

Lung cancer is the leading cause of cancer-related mortality and the second most common cancer worldwide, accounting for 18.9% of cases and affecting 15.9% of the Thai population in 2022. [1, 2] NSCLC constitutes 85% of lung cancers, with 70% diagnosed at an advanced stage.[3] EGFR mutations (10–35%) drive NSCLC progression, leading to the development of first- and second-generation TKIs.[4] However, resistance mutations such as EGFR T790M and L858R limit TKI efficacy.[5] Osimertinib was introduced to overcome T790 M-mediated resistance, improving outcomes over first-generation TKIs. [6] Tumor heterogeneity drives oligo-progression, necessitating continued TKI treatment, while access to third-generation TKIs remains limited. [7] Identifying prognostic markers is essential for predicting TKI responses. [8] T-helper 1 cells (TH1) were related to pro-inflammatory cytokines, including IL-2, IL-6, and TNF-a, which can stimulate the immune system through T-cell-mediated responses, whereas Th-helper (Th2) cytokines, including IL-4, IL-5, IL-10, and IL-13, can inhibit T-cell proliferation and Th1 cytokine production. [9] The earlier studies link pro-inflammatory cytokines to poor prognosis and first-generation TKI resistance. [10] Low IL-8 levels correlate with prolonged PFS (13 months) in Gefitinib-treated patients. [11] While IL-10 downregulation is limited in EGFR-mutated tumours, high IL-10 is detected post-Gefitinib and Osimertinib treatment. [12] Growth factor serum levels may also predict EGFR-TKI efficacy. [13] The prognostic role of cytokines in EGFR-mutant NSCLC is underexplored.[14]. This study aimed to evaluates cytokine patterns and outcomes to enhance insights into TKI responses in EGFR-mutated NSCLC.

Materials and Methods

Patient clinical characteristics

NSCLC patients retrospectively included in this study with EGFR-mutated advanced NSCLC were treated with first- to third-generation TKIs at King Chulalongkorn Memorial Hospital from 2021 to 2023. Eligible patients were e”18 years old with confirmed histopathology and EGFR mutation reports. Those with incomplete documentation, second primary cancer, or withdrawn consent were excluded. Tumor response was assessed by physicians. The study was approved by the Faculty of Medicine’s Ethics Committee (IRB 0425-67, which is part of IRB. No. 894-63), and written informed consent was obtained from all participants.

Sample preparation and Cytokine Bio-plex assay (ELISA)

Blood samples were collected at baseline and follow-up (6–12 weeks) of EGFR-TKI treatment into three EDTA tubes (30 ml), centrifuged at 2500 g for 10 minutes. Plasma was aliquoted and stored at -80 °C for enzyme-linked immunosorbent assay (ELISA). Cytokine levels were quantified using the 27-plex human cytokine panel (Bio-Plex Pro, Bio-Rad). Frozen plasma samples were thawed, centrifuged, and diluted (1:4), while standards were used with HB diluent. Detection antibodies and Streptavidin-PE were added after incubating with beads in a 96-well plate and washing. Fluorescence values were measured using Bio-Plex Manager software, with standards ranging from 80–120% of expected values.

Statistical analysis

Data were censored on December 31, 2023. Cytokine levels were analyzed as medians, categorized into Th1, Th2, and growth factors. High and low cytokine groups were defined based on these median values. Categorical variables were analyzed using a chi-squared test. Mann–Whitney U tests assessed the non-parametric significance of circulating cytokine levels between groups. Wilcoxon signed-rank tests examined the significance of dynamic changes in cytokine levels at 6-12 weeks in EGFR-TKI durable and non-durable responders at baseline and later points. Survival outcomes (PFS, OS, TTF) were evaluated using Kaplan-Meier analysis and Cox regression models. Data were processed with IBM SPSS and GraphPad Prism, with a significance set at $P < 0.05$.

Results

Different expression of various cytokine profiles in EGFR-mutated NSCLC patients

To assess the pattern of specific individual cytokine expression correlating with the durable response to EGFR-TKI treatments, we classified NSCLC patients into DU and NDU using a cut-off PFS at 104 weeks. At baseline levels of the 27 cytokines, high IL-6 and IL-10 levels were observed in the NDUs (**Figure 1A**), compared to the DU (83.74% vs. 16.67%) (median levels: 4.060 vs. 1.860 pg/ml, $P = 0.0076$; median levels: 0.71 vs. 0.00 pg/ml, $P = 0.0166$, respectively) (**Figure 1B**). We also found significantly higher levels of IL-1Ra (median cytokine levels: 305.6 vs. 281.7 pg/ml, $P = 0.0027$), IL-7 (median cytokine levels: 15.96 vs. 12.045 pg/ml, $P = 0.0415$), IP-10 (median cytokine levels: 405.965 vs. 220.52 pg/ml, $P = 0.0085$), and PDGF-BB (median cytokine levels: 258.37 *vs.* 159.06 pg/ml, $P = 0.0313$) in NDU compared to DU ($P < 0.05$) (data not shown) (**Figure 2A**).

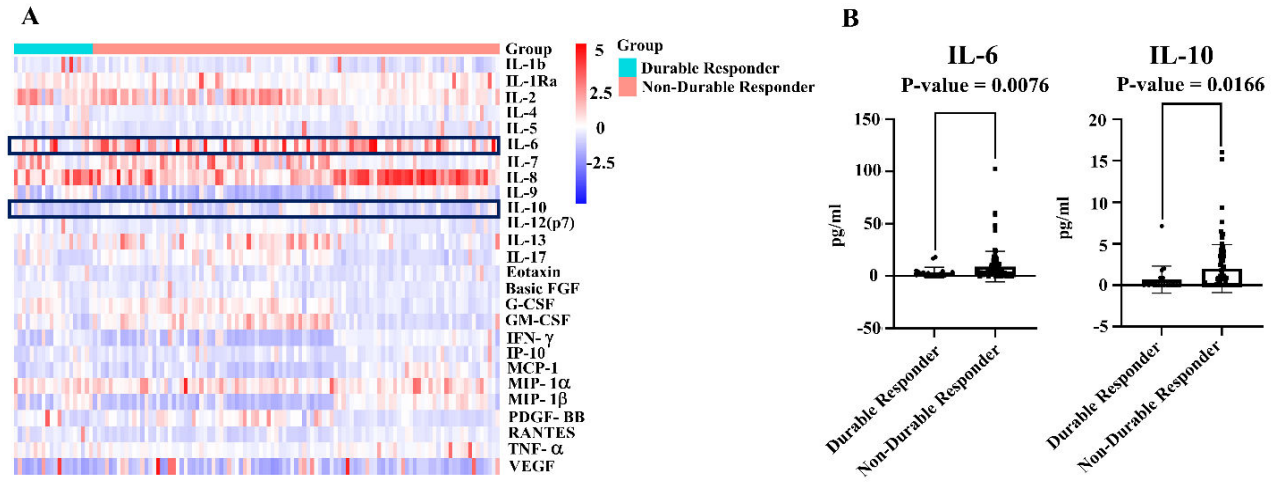

Figure 1. (A) heatmap of 27 pre-treatment cytokine levels in NSCLC patients (N = 123) categorized them into DU (N = 20) and NDU (N=103) at a PFS cut-off of 104 weeks with significant cytokines outlined in blue, mainly in NDU. **(B)** A scatter plot visualizes Th1 and Th2 cytokine levels via ELISA (Multiplex assay), with significance assessed using the Mann-Whitney test ($P < 0.05$).

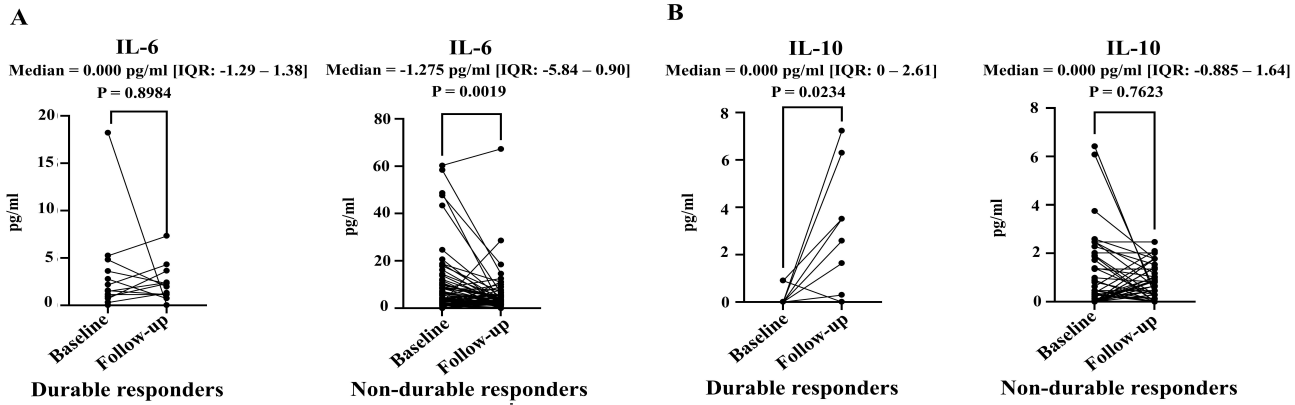

Figure 2. The scatter plot presents the dynamic changes of circulating cytokine levels, which were separated into DU and NDUs patients. The cytokine levels were compared to pre- and post-treatment at second-time points between 6 to 12 weeks. The levels of two cytokines were divided into two groups, such as Th1 cytokines (IL-6) (**A**) and Th2 cytokines (IL-10) (**B**). The Wilcoxon matched-pairs signed-rank test evaluates the significance ($P < 0.05$).

Association of cytokine levels as a prognostic biomarker for long-term durable response

The six cytokines differed significantly between DU and NDU, highlighting their potential as predictive markers. This study analyzed cytokine levels correlations with clinical outcomes. Independent prognostic biomarkers included IL-1Ra, IL-6, IL-7, IL-10, IP-10, and PDGF-BB, assessed via univariate analysis and Cox regression for PFS, OS, and TTF. Cytokine levels were categorized as high or low based on median values. The baseline plasma of NSCLC patients showed a PFS of 48 weeks [95% CI: 35.641-75.787]. Kaplan-Meier analysis revealed high IL-6 levels [3.6 pg/ml, range: 0-102.82 pg/ml] linked to shorter PFS (44.429 vs. 140.857, $P = 0.018$), OS (87 vs. 160 weeks; $P = 0.011$), and TTF (47.000 vs. 136.000, $P = 0.001$). Conversely, high IL-10 levels [0.5 pg/ml, range: 0-16.06 pg/ml] correlated with longer PFS (114.160 vs. 35, $P = 0.001$), OS (186.720 vs. 82.000, $P = 0.001$), and TTF (124.895 vs. 41.43, $P = 0.001$). Univariate analysis identified a common EGFR mutation (exon 19 deletion and L585R) as the sole independent prognostic factor for PFS ($P = 0.01$), OS ($P < 0.001$), and TTF ($P = 0.015$). High levels of IL-6, IL-10, IP-10, and PDGF-BB demonstrated significant independent prognostic value for PFS (**Table 2**), OS (**Table 3**), and TTF (**Table 4**) with $P < 0.05$. Multivariate analysis indicated that sensitizing common EGFR, high IL-6, and high IL-10 levels were significant independent prognostic factors (HR = 4.500; 95% CI: 1.823-11.111, HR = 1.713; 95% CI: 1.002-2.928, and HR = 0.394; 95% CI: 0.177-0.876, respectively). These findings suggest that EGFR status and pre-treatment cytokine levels in plasma may be prognostic indicators for durable TKI response in NSCLC patients.

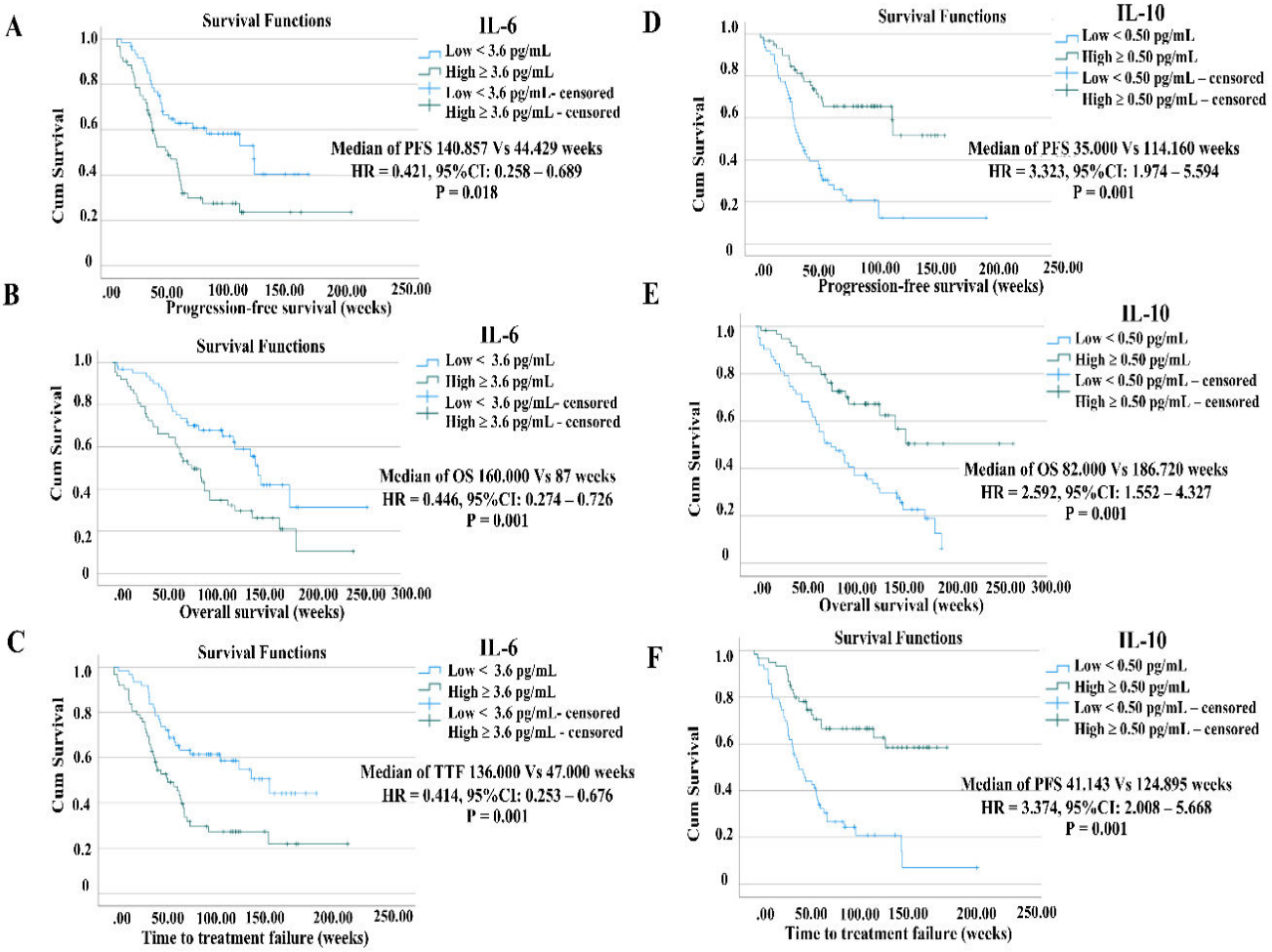

Figure 3. Kaplan-Meier curves for progression-free survival (PFS), overall survival (OS), and time to treatment failure (TTF) show according to IL-6 **(A-C)** and IL-10 **(D-F)** levels in overall mutant-EGFR NSCLC patients (N=123).

Dynamic changes in cytokine expression levels and potential biomarkers for predicting durable responses

Cytokine levels assessed NSCLC patients' responses to targeted therapy. Plasma samples were collected 6 to 12 weeks post-TKI treatment. Significant pretreatment cytokine levels in NDUs included IL-1Ra, IL-6, IL-7, IL-10, IP-10, and PDGF-BB. A notable decrease in IL-1Ra levels was observed in DU and NDU: median difference = -42.29 pg/ml (P = 0.0083) and -96.04 pg/ml (P = 0.0049), respectively (not shown). IL-6 was found to have a significant decrease in NDUS (median difference = -1.275 pg/ml, P = 0.0019), while DU exhibited a substantial increase in IL-10 (median difference = 0.000 pg/ml, P = 0.0234). The analysis reveals that changes in cytokine levels offer insights into NSCLC patients. A scatter plot indicated circulating cytokine levels in DU (PFS ~* 104 weeks, N = 15) and NDU (PFS < 104 weeks, N = 72), with a significant decrease in IL-6, specifically in NDU, implying a link to poor treatment outcomes. High IL-10 levels in DU suggest its potential as a biomarker for positive outcomes, emphasizing cytokine profiling's importance in tracking patient responses to TKI therapy.

Discussion

This study evaluated cytokine patterns as prognostic biomarkers for durable TKI responses in *EGFR*-mutated NSCLC patients. T-helper 1 cells (Th1) cytokines (IL-2, IFN-γ, TNF-α) stimulate T-cell responses, while T-helper 2 cells (Th2) cytokines (IL-4, IL-5, IL-10, IL-13) inhibit them. [15,16] Th1/Th2 imbalance contributes to NSCLC progression, with Th1 downregulation promoting resistance. [17] Elevated IL-6 and IL-8 were linked to tumor progression and immune dysregulation. [18] IL-6 correlated with poor

PFS, OS, and TTF, while IL-10 was associated with prolonged TTF. IL-6 promotes tumor growth via the JAK/STAT3 pathway and an immunosuppressive TME. [19, 20] High IL-10 levels in NDUs suggest immune modulation. [21] A 104-week PFS threshold defined durable responses, with TTF providing a broader treatment durability measure. These findings highlight IL-6 and IL-10 as key prognostic biomarkers, supporting immune profiling for personalized NSCLC treatment strategies. Further research on IL-10 may optimize TKI efficacy.

Conclusion

This study underscores the importance of Th1/Th2 cytokine patterns and levels in forecasting the response to EGFR-TKI treatment in patients with NSCLC. The findings illustrate that pro-inflammatory IL-6 and anti-inflammatory IL-10 are potential prognostic biomarkers for long-term TKI response. Elevated levels of IL-6 were correlated with unfavourable survival outcomes. In contrast, increased IL-10 levels were associated with prolonged time to treatment failure, thereby highlighting their roles in tumour progression and immune modulation. The results indicate that integrating molecular and immune profiling may enhance personalized treatment strategies for NSCLC patients, emphasizing cytokine-driven immune interactions as critical factors in optimizing EGFR-TKI therapy.

Acknowledgements

The authors thank the Medical Oncology team, the Department of Medicine, Chulalongkorn University, for writing this manuscript. Biospecimen collection was supported by Biobank, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Conflict of interest statement

The authors declare no commercial or financial relationships that could be perceived as potential conflict of interest.

Data sharing statement

All data produced or analyzed in this study are included in this published article. Additional information can be obtained from the corresponding author for noncommercial purposes upon reasonable request.

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin 2024;74:12-49.
2. Raksasri N, Kamsa-Ard S, Musika W, Santong C, Phunmanee A. Outcomes of treatment, survival rates, and factors related to survival of stage III lung cancer patients treated at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. Asian Pac J Cancer Prev 2023;24:1743-9.
3. Supinda Petchjorm M, Wisut Lamlertthon M. Frequency of EGFR mutations among Thai non-small cell lung cancer [NSCLC] patients. J Med Assoc Thai 2018;101:S123-S8.
4. Diniz G, ÜnlÜ Ý, Kömürcüoðlu B. Histopathological and molecular features of lung cancer. Tepecik Eðit Hast Derg 2017;27:77-87.
5. Nassar AH, Kim SY, Aredo JV, Feng J, Shepherd F, Xu C, et al. Consolidation osimertinib versus durvalumab versus observation after concurrent chemoradiation in unresectable EGFR-Mutant NSCLC: a multicenter retrospective cohort study. J Thorac Oncol 2024; 19:928-40.
6. Chung JH, Rho JK, Xu X, Lee JS, Yoon HI, Lee CT, et al. Clinical and molecular evidences of epithelial to mesenchymal transition in acquired resistance to EGFR-TKIs. Lung Cancer 2011;73:176-82.
7. Zubair T, Bandyopadhyay D. Small molecule EGFR Inhibitors as anti-cancer agents: discovery, mechanisms of action, and opportunities. Int J Mol Sci 2023 31;24:2651.
8. Takeyasu Y, Yoshida T, Masuda K, Matsumoto Y, Shinno Y, Okuma Y, et al. Distinct progression and efficacy of first-line osimertinib treatment according to mutation subtypes in Metastatic NSCLC harboring EGFR mutations. JTO Clin Res Rep 2024;5:100636.
9. Lee HL, Jang JW, Lee SW, Yoo SH, Kwon JH, Nam SW, et al. Inflammatory cytokines and change of Th1/Th2 balance as prognostic indicators for hepatocellular carcinoma in patients treated with transarterial chemoembolization. Sci Rep 2019;9:3260.
10. Gong K, Guo G, Gerber DE, Gao B, Peyton M, Huang C, et al. TNF-driven adaptive response mediates resistance to EGFR inhibition in lung cancer. J Clin Invest 2018;128:2500-2518.
11. Qiao M, Zhou F, Liu X, Jiang T, Wang H, Jia Y, et al. Interleukin-10 induces expression of CD39 on CD8+T cells to potentiate anti-PD1 efficacy in EGFR-mutated non-small cell lung cancer. J Immunother Cancer 2022;10:e005436.
12. Muhsin M, Graham J, Kirkpatrick P. Gefitinib. Nat Rev Drug Discov 2003;2:515-6.

13. Jia Y, Li X, Zhao C, Jiang T, Zhao S, Zhang L, et al. Impact of serum vascular endothelial growth factor and interleukin-6 on treatment response to epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small-cell lung cancer. Lung Cancer 2018;125:22-8.
14. Choi YW, Jeon SY, Jeong GS, Lee HW, Jeong SH, Kang SY, et al. EGFR Exon 19 deletion is associated with favorable overall survival after first-line gefitinib therapy in advanced non-small cell lung cancer Patients. Am J Clin Oncol 2018;41:385-390.
15. De Vita F, Orditura M, Galizia G, Romano C, Roscigno A, Lieto E, et al. Serum interleukin-10 levels as a prognostic factor in advanced non-small cell lung cancer patients. Chest 2000;117:365-73.
16. Ghayumi MA, Mojtahedi Z, Fattahi MJ. Th1 and Th2 cytokine profiles in malignant pleural effusion. Iran J Immunol 2011;8:195-200.
17. Frafjord A, Buer L, Hammarström C, Aamodt H, Woldbæk PR, Brustugun OT, et al. The Immune landscape of human primary lung tumors is Th2 skewed. Front Immunol 2021;12:764596.
18. Jacenik D, Karagiannidis I, Beswick EJ. Th2 cells inhibit growth of colon and pancreas cancers by promoting anti-tumorigenic responses from macrophages and eosinophils. Br J Cancer 2023;128:387-97.
19. Tamura T, Kato Y, Ohashi K, Ninomiya K, Makimoto G, Gotoda H, et al. Potential influence of interleukin-6 on the therapeutic effect of gefitinib in patients with advanced non-small cell lung cancer harbouring EGFR mutations. Biochem Biophys Res Commun 2018; 495:360-7.
20. He ZN, Zhang CY, Zhao YW, He SL, Li Y, Shi BL, et al. Regulation of T cells by myeloid-derived suppressor cells: emerging immunosuppressor in lung cancer. Discov Oncol 2023;14:185.
21. Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The tumor microenvironment: A Milieu hindering and obstructing antitumor immune responses. Front Immunol 2020;11:940.