



LETTER

COX-2 inhibition synergizes with radioimmunotherapy by promoting TCF1⁺CD8⁺ T cell infiltration in NSCLC

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Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality worldwide¹⁻³. While radioimmunotherapy shows promise in boosting antitumor immunity, the clinical outcomes have been inconsistent⁴ and are often limited by the immunosuppressive tumor microenvironment (TME). Cyclooxygenase (COX)-2 and the COX-2 downstream product, prostaglandin E2 (PGE2), are increasingly implicated in immune escape^{5,6} but the impact on radioimmunotherapy efficacy has not been explored. TCF1-expressing CD8⁺ T cells demonstrate defining functional properties of progenitor-exhausted T cells, including clonal expansion through self-renewal capacity and multipotent differentiation toward terminal effector phenotypes, while maintaining long-term persistence critical for sustaining antitumor immunity⁷⁻⁹. Given the central role in anti-tumor immune maintenance, TCF1⁺CD8⁺ T cells are likely critical to radioimmunotherapy efficacy¹⁰. The role of COX-2 inhibition in enhancing the efficacy of radioimmunotherapy efficacy in NSCLC was investigated in the current study.

A mouse tumor model was used to investigate the potential of combining COX-2 inhibition with radioimmunotherapy. The results showed that the addition of the COX-2 inhibitor, celecoxib, significantly enhances the anti-tumor efficacy

of radioimmunotherapy and increased intratumoral infiltration of TCF1⁺CD8⁺ T cells. Complementary clinical data from a retrospective analysis of radioimmunotherapy-naïve NSCLC patient samples revealed an inverse correlation between COX-2 expression and TCF1⁺CD8⁺ T cell presence. Specifically, high COX-2 levels were associated with unfavorable clinical outcomes. By elucidating the interplay between COX-2 signaling, stem-like CD8⁺ T cells, and radioimmunotherapy, the current study identified a potential strategy to overcome resistance and improve treatment outcomes in lung cancer.

Upregulation of the COX-2/PGE2 axis impairs radioimmunotherapy efficacy

The combination of radiotherapy [RT] (8 Gy × 3 fractions) and anti-PD1 antibody in the CMT-167 mouse subcutaneous model resulted in a significant reduction in tumor burden compared to mice treated with RT or anti-PD1 antibody (Figure 1A–C).

Notably, radioimmunotherapy induced elevated levels of PGE2 in tumors and these levels were positively correlated with increased tumor volume in the radioimmunotherapy group, suggesting that PGE2 may impair treatment efficacy (Figure 1D, E). Given that PGE2 is a downstream metabolite of COX-2, COX-2 overexpression [COX-2 OE] (Figure S1A) in CMT-167 cells was used to investigate the functional role of the COX-2/PGE2 axis in modulating radioimmunotherapy efficacy. Subcutaneous tumor models were established by inoculating mice with COX-2 OE and control cells (NC), followed by treatment with

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Received May 26, 2025; accepted September 9, 2025;
published online October 22, 2025.

Available at www.cancerbiomed.org

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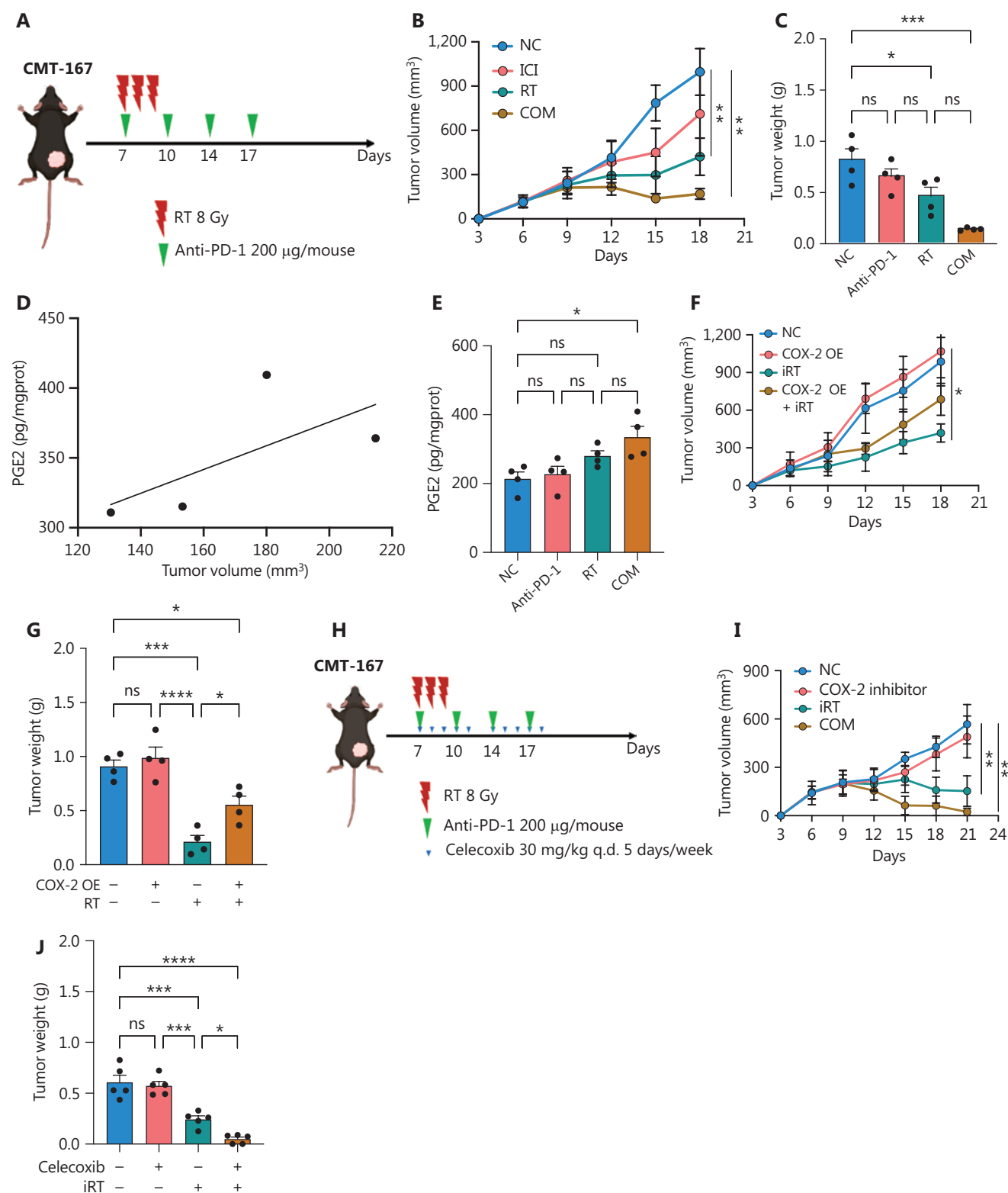


Figure 1 Continued

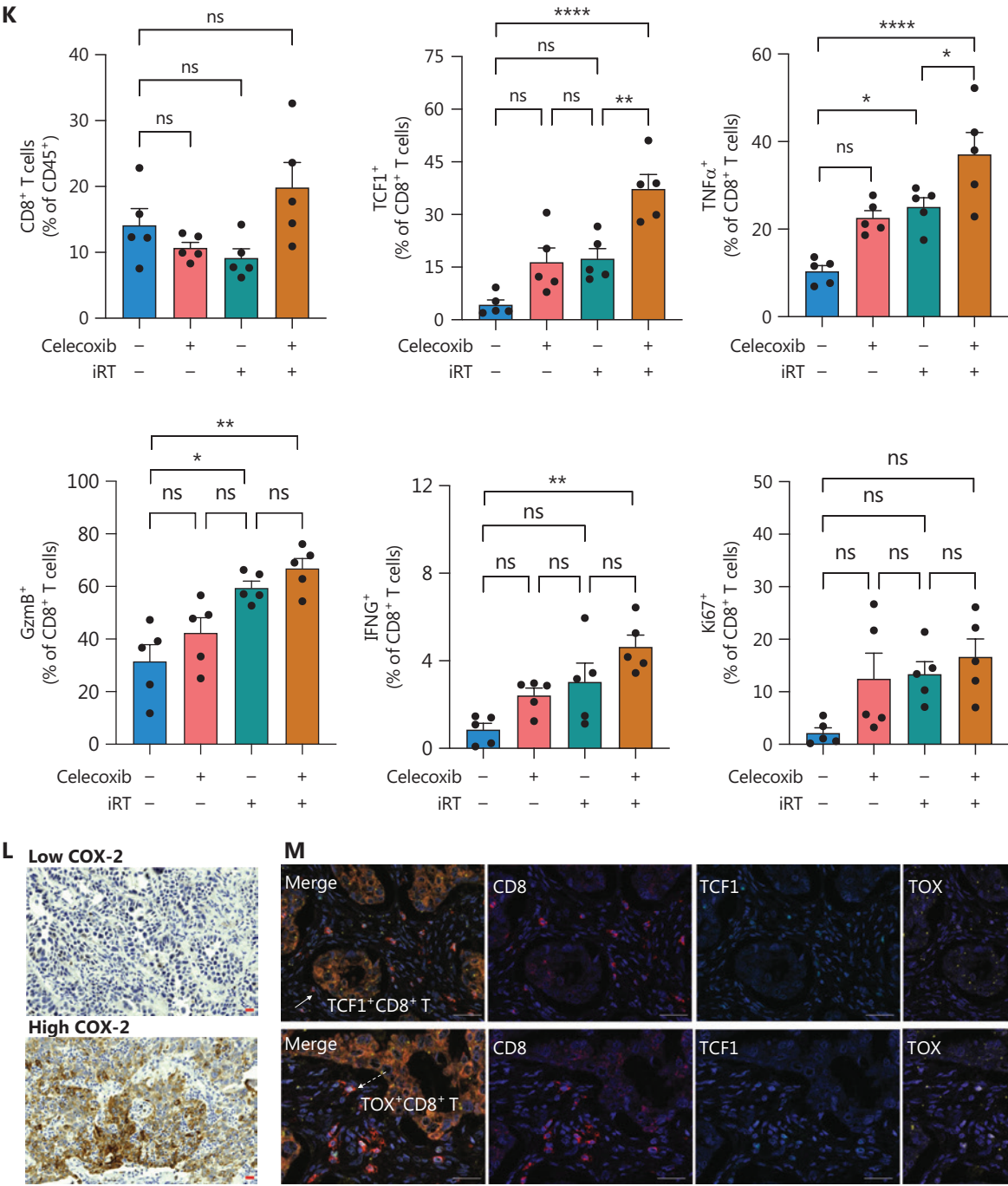


Figure 1 Continued

radioimmunotherapy. The results demonstrated that COX-2 OE significantly attenuated the therapeutic efficacy of radioimmunotherapy, as evidenced by increased tumor growth and improved tumor weight (**Figure 1E, G**). These findings indicated that elevated COX-2 levels impair the antitumor radioimmunotherapy effects, highlighting COX-2 as a potential therapeutic target to enhance treatment outcomes.

COX-2 inhibition with celecoxib enhances radioimmunotherapy efficacy

Given the identification of COX-2 as a potential therapeutic target, whether pharmacologic inhibition of COX-2 could

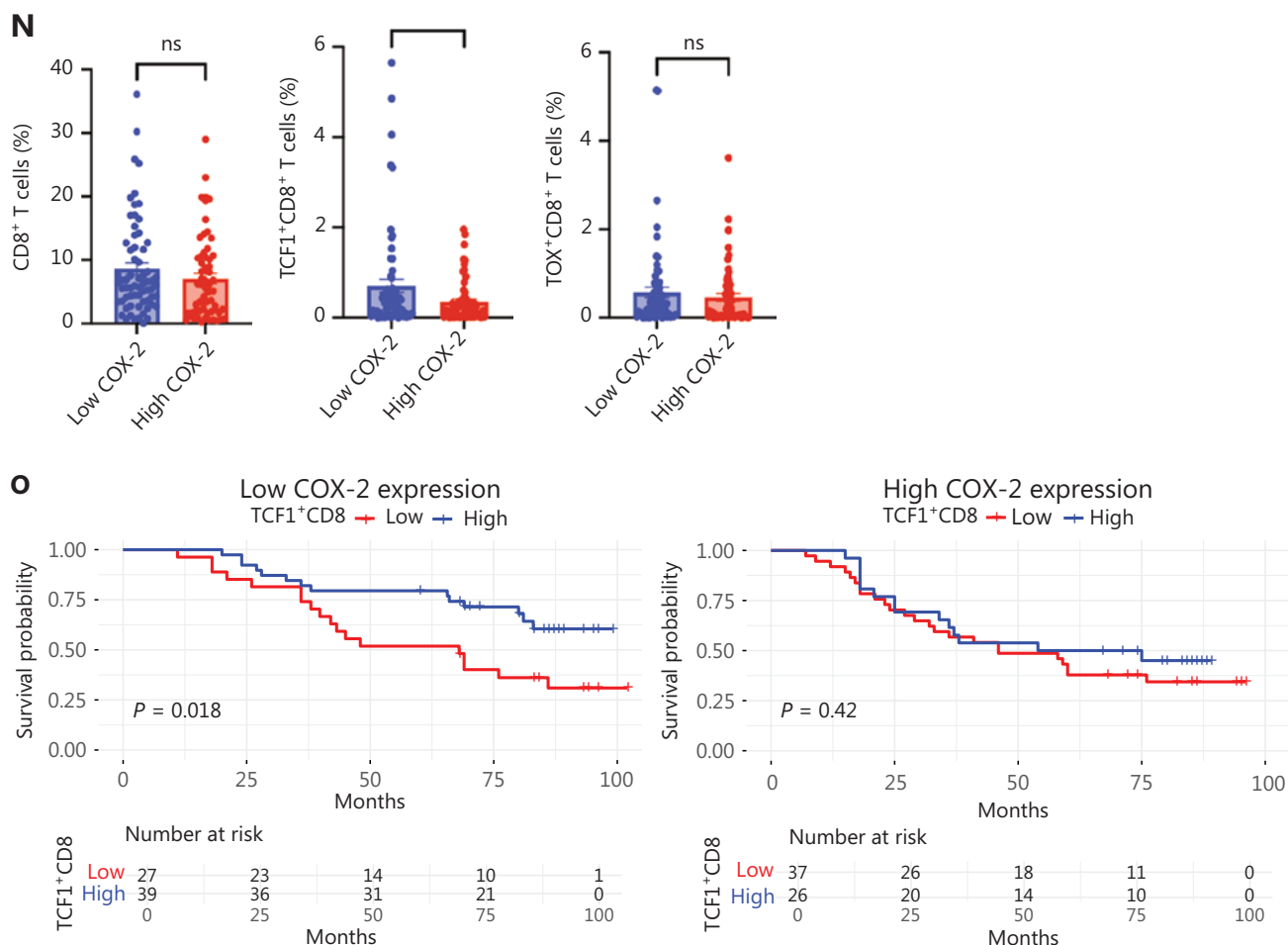


Figure 1 Upregulation of the COX-2/PGE2 axis impairs the therapeutic efficacy of radioimmunotherapy in mice. (A) CMT-167 cells were inoculated into the right hind leg of the mice, then radiotherapy, anti-PD-1, and radioimmunotherapy were given at the indicated times. (B) The tumor burden of mice was significantly reduced after radioimmunotherapy ($n = 4$). (C) The tumor weight was significantly reduced after radioimmunotherapy ($n = 4$). (D) The relationship between the PGE2 level in the tumor microenvironment after radioimmunotherapy ($n = 4$). (E) The PGE2 level in tumor tissue increased significantly after radioimmunotherapy ($n = 4$). (F, G) COX-2 overexpression significantly attenuated the efficacy of radioimmunotherapy in mice. (H) CMT-167 cells were inoculated into the right hind leg of the mice, then celecoxib, radioimmunotherapy, and celecoxib combined with radioimmunotherapy were given at the indicated times. (I) The tumor burden of mice was significantly reduced after radioimmunotherapy combined with celecoxib ($n = 5$). (J) The tumor weight was significantly reduced after radioimmunotherapy combined with celecoxib ($n = 5$). (K) Changes in the immune microenvironment of tumor-bearing mice after radioimmunotherapy combined with celecoxib ($n = 5$). (L) The images are representative of low and high intratumoral COX-2 expression. Scale bars: 20 μ m. (M) Multiple immunofluorescence (mIF) images representative of T cell infiltration. Fused and single-channel mIF images of tumor tissues. Single staining images of CD8, TOX, TCF-1, and double-staining images of TCF1⁺CD8⁺ and TOX⁺CD8⁺ were included. Scale bars: 50 μ m. Orange represents CK+ used to mark tumor cells. CK: orange, CD8: red, TOX: yellow, TCF-1: green. (N) Correlation between intratumoral COX-2 expression and TCF1⁺CD8⁺ cells. (O) TCF1⁺CD8⁺ T cells were associated with better OS under low COX-2 conditions; TCF1⁺CD8⁺ T cells were not significantly associated with OS. ns, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; iRT, radioimmunotherapy.

enhance radioimmunotherapy efficacy was investigated. This approach was designed to evaluate the translational potential of targeting the COX-2 pathway in improving cancer treatment. The pharmacologic inhibition of COX-2 using celecoxib synergistically improved the antitumor response to

radioimmunotherapy. Mice treated with celecoxib in combination with radioimmunotherapy had a significant reduction in tumor volume and weight (**Figure 1H–J**), indicating that celecoxib potentiated radioimmunotherapy efficacy in lung cancer.

Whether CD8⁺ T cells in the TME were modulated by combination therapy was determined to further elucidate the mechanisms underlying the celecoxib effect. Flow cytometry analysis (**Figure 1K**) revealed that celecoxib increased the infiltration of TCF1⁺CD8⁺ T cells, a stem-like subset of CD8⁺ T cells critical for long-term immune responses. Although CD8⁺ T cell infiltration was not significantly altered by celecoxib treatment, combination therapy increased the proportion of functional CD8⁺ T cells expressing granzyme B, IFN- γ , and TNF- α , suggesting enhanced cytotoxic activity and proliferation. These findings suggested that COX-2 inhibition augments radioimmunotherapy by promoting the infiltration of stem-like CD8⁺ T cells and bolstering the cytotoxic activity and proliferative capacity. This synergistic interaction highlights the potential of targeting the COX-2/PGE2 axis to optimize the therapeutic outcomes of radioimmunotherapy in lung cancer. Furthermore, given the crucial role of PD-L1 in regulating the tumor immune microenvironment, the results indicated that COX-2 inhibition did not alter the level of PD-L1 expression in tumor cells (**Figure S1B**). This finding suggested that the therapeutic effects of COX-2 inhibitors in the context of radioimmunotherapy are not directly mediated through changes in PD-L1 expression.

COX-2 expression and TCF1⁺CD8⁺ T cell infiltration as prognostic markers in radioimmunotherapy-naïve NSCLC

To assess the clinical relevance of these findings, tumor samples were analyzed from 129 radioimmunotherapy-naïve NSCLC patients. The selection of this specific cohort was intentional and aimed at minimizing potential confounding factors that prior radioimmunotherapy interventions might exert on the composition and distribution of CD8⁺ T cell subpopulations. Immunohistochemistry (IHC) was used to evaluate COX-2 expression in tumor specimens from 129 NSCLC patients (**Figure 1L**). Notably, individuals with low COX-2-expressing tumors achieved a substantially longer median overall survival [mOS] [not reached (NR) vs. 54 months, $P = 0.042$; **Figure S1C**]. The findings indicated that elevated intratumoral COX-2 levels are associated with diminished long-term antitumor responses. Multivariate Cox regression analysis revealed that higher COX-2 expression is significantly associated with poorer OS (HR, 1.880; $P = 0.009$, **Table 1**).

This finding suggested that COX-2 expression is an independent prognostic factor for OS, indicating COX-2 expression potential as a therapeutic target in lung cancer. Consequently, upregulation of COX-2 following radioimmunotherapy may potentially compromise the induction of comprehensive esystemic antitumor effects.

TCF1 regulates the stem-like properties of CD8⁺ T cell subpopulations, which enables the persistence and differentiation into effector T cells. Conversely, TOX supports the epigenetic and transcriptional programs of exhausted T cells¹¹. Multiplex immunofluorescence staining of CK, CD8, TCF1, and TOX (**Figure 1M**) enabled quantification of total CD8⁺ T cells, TCF1⁺CD8⁺ T cells, and TOX⁺CD8⁺ T cells. Elevated intratumoral COX-2 expression was inversely correlated with the infiltration of TCF1⁺CD8⁺ T cells (**Figure 1N**, **Table S1**). A stratified analysis was performed to assess the impact of COX-2 expression on the prognosis associated with TCF1⁺CD8⁺ T cell infiltration. Patients in the cohort with low COX-2 expression exhibiting high levels of TCF1⁺CD8⁺ T cell infiltration had significantly improved OS (**Figure 1O**). Conversely, the degree of TCF1⁺CD8⁺ T cell infiltration within the cohort characterized by high COX-2 expression did not show a significant correlation with OS, suggesting that COX-2 expression may influence the prognostic role of TCF1⁺CD8⁺ T cells in this patient population (**Figure 1O**). These results indicated that COX-2 expression may have an immunosuppressive role in NSCLC, limiting the infiltration of stem-like T cells and compromising treatment efficacy.

Upregulation of COX-2 expression in the TME leads to increased production of PGE2, a key mediator in various immunosuppressive mechanisms. PGE2 has been shown to negatively affect the functionality of TCF1⁺CD8⁺ T cells, a critical subset responsible for sustaining long-term antitumor immunity. Specifically, PGE2 impairs the proliferative expansion and effector differentiation of these stem-like T cells by disrupting interleukin-2 (IL-2) signaling pathways. In addition, PGE2 can mediate TCF1⁺CD8⁺ T cell inhibition *via* its receptors, particularly EP2 and EP4, which are expressed on these T cells. Genetic knockout of these receptors in mouse models has been shown to rescue TCF1⁺CD8⁺ T cell function, leading to enhanced expansion, differentiation, and improved tumor control. These findings suggested that the COX-2/PGE2 signaling axis contributes to tumor immune evasion by impairing TCF1⁺CD8⁺ T cell function, making the COX-2/PGE2 signaling axis a potential target for enhancing

Table 1 Univariate analyses and multivariate analysis of prognostic markers for OS

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age				
< 60 vs. ≥ 60	1.816 (1.135–2.906)	0.013	2.067 (1.286–3.321)	0.003
Gender				
Male vs. female	0.598 (0.372–0.962)	0.034		
Smoking history				
Yes vs. No	0.707 (0.441–1.132)	0.148		
TNM stage		0.002		< 0.001
I	1			
II	3.336 (1.154–9.641)	0.026		0.017
III	5.394 (1.929–15.082)	0.001	0.410 (0.254–0.662)	< 0.001
CD8 ⁺ TILs				
Low vs. high	0.600 (0.370–0.971)	0.037		
TCF1 ⁺ CD8 ⁺ TILs				
Low vs. high	0.550 (0.341–0.888)	0.014		
COX2				
Low vs. high	1.606 (1.002–2.572)	0.049	1.880 (1.168–3.028)	0.009

immunotherapy efficacy by restoring the functionality of this critical T cell subset within the TME.

The current study showed that activation of the COX-2/PGE2 pathway has a pivotal role in diminishing the effectiveness of radioimmunotherapy, primarily by inhibiting the stem-like TCF1⁺CD8⁺ T cell population, which is essential for maintaining robust antitumor immunity (**Figure S2**). Inhibition of COX-2 activity was shown to enhance recruitment of TCF1⁺CD8⁺ T cells into the tumor, thereby improving therapeutic responses to radioimmunotherapy. A strong negative correlation between intratumoral COX-2 levels and TCF1⁺CD8⁺ T cell infiltration was detected with no significant association noted between the COX-2 level and TOX⁺CD8⁺ T cells. This finding suggested that the COX-2/PGE2 signaling pathway selectively impairs the renewal and infiltration of stem-like T cells without affecting the exhausted TOX⁺CD8⁺ T cell subset. The findings herein are consistent with earlier reports by Lacher et al.¹², who demonstrated that the COX-2/PGE2 axis can limit the expansion and efficacy of TCF1⁺ tumor-infiltrating lymphocytes (TILs) and for the first time revealed a negative correlation between intratumoral COX-2

expression and the presence of stem-like CD8⁺ T cells in NSCLC patients.

While current immunotherapeutic strategies focus on reversing T-cell exhaustion, such as targeting TIGIT and TIM-3, the current study highlights the importance of preserving stem-like T cells in the TME through COX-2 inhibition. This strategy offers a novel and potentially synergistic approach for optimizing the therapeutic response in lung cancer patients. However, we acknowledge the limitations of our study, particularly the retrospective nature of the patient cohort analysis. Prospective studies are warranted to confirm these findings. In addition, further research is warranted to elucidate the molecular mechanisms by which elevated COX-2 disrupts stem-like T cell functionality and identify new therapeutic targets.

In conclusion, the current study provides novel insights into the role of COX-2 in regulating the TME and its impact on radioimmunotherapy efficacy. The findings provide a theoretical basis for targeting the COX-2/PGE2 axis to optimize the outcomes of radioimmunotherapy in lung cancer. Future clinical trials evaluating the combination of COX-2 inhibitors and radioimmunotherapy have the potential to improve patient prognosis.

Grant support

This work was funded by the National Natural Science Foundation of China (Grant Nos. 82030082 and 82272845) and the Natural Science Foundation of Shandong Province (Grant No. ZR2023LZL001).

Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Xiangjiao Meng, Jinming Yu.

Collected the data: Lin Ma, Yao Wang, Xueying Zhai.

Performed the analysis: Lin Ma, Menglin Bai.

Wrote the paper: Lin Ma, Menglin Bai.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

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Cite this article as: Ma L, Bai M, Wang Y, Zhai X, Yu J, Meng X. COX-2 inhibition synergizes with radioimmunotherapy by promoting TCF1⁺CD8⁺ T cell infiltration in NSCLC. *Cancer Biol Med*. 2025; 22: 1537-1543. doi: 10.20892/j.issn.2095-3941.2025.0205