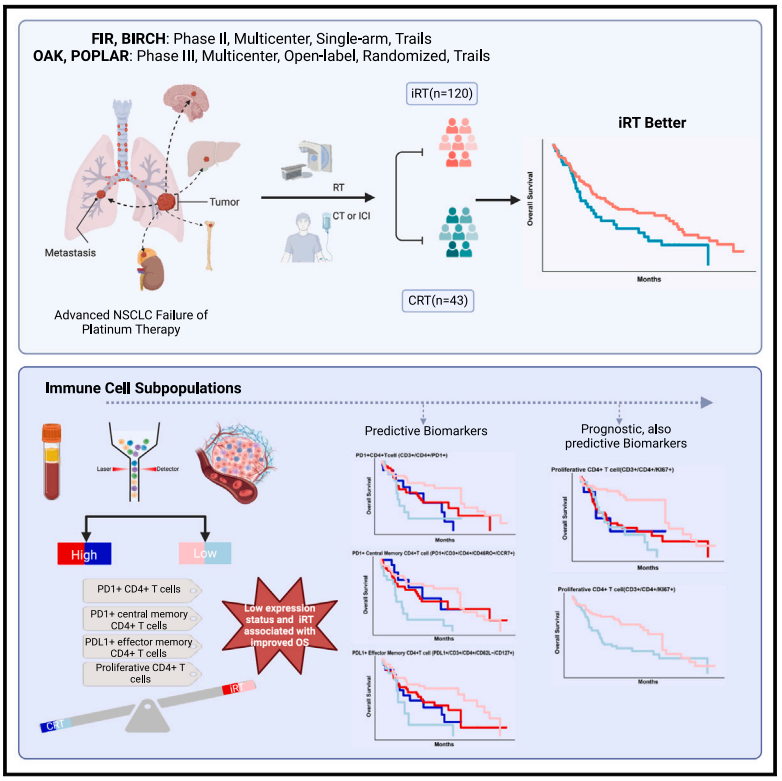
iScience

Article

# Efficacy of radiotherapy combined with atezolizumab or docetaxel in patients with previously treated NSCLC

## Graphical abstract




## Highlights

- Atezolizumab with radiotherapy (iRT) prolonged OS in advanced NSCLC after platinum failure
- PD1$^{+}$, central memory PD1$^{+}$, and effector memory PD-L1$^{+}$ CD4$^{+}$ T cells were predictive biomarkers
- Proliferative CD4$^{+}$ T cell served as prognostic and predictive biomarkers for iRT

## Authors

Junzhu Xu, Haitao Wang, Chi Zhang, ..., Udo S. Gaipl, Hu Ma, Jian-Guo Zhou

## Correspondence

udo.gaipl@uk-erlangen.de (U.S.G.), mahuab@163.com (H.M.), jianguo.zhou@zmu.edu.cn (J.-G.Z.)

## In brief

Public health; Cancer

Xu et al., 2024, iScience 27, 111363
December 20, 2024 
https://doi.org/10.1016/j.isci.2024.111363

CellPress

iScience

CellPress
OPEN ACCESS

Article

# Efficacy of radiotherapy combined with atezolizumab or docetaxel in patients with previously treated NSCLC

Junzhu Xu,[1,11] Haitao Wang,[2,11] Chi Zhang,[1] Su-Han Jin,[3] Xiaofei Chen,[4] Fangya Tan,[5] Benjamin Frey,[6,7,8] Markus Hecht,[9] Jian-Guo Sun,[10] Udo S. Gaipl,[6,7,8,*] Hu Ma,[1,*] and Jian-Guo Zhou[1,6,7,8,12,*]

[1]Department of Oncology, The Second Affiliated Hospital of Zunyi Medical University, Zunyi 563000, P.R. China
[2]Thoracic Surgery Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892, USA
[3]Department of Orthodontics, Affiliated Stomatological Hospital of Zunyi Medical University, Zunyi, China
[4]Oncology Biometrics, AstraZeneca, Gaithersburg, MD 20850, USA
[5]Harrisburg University of Science and Technology, Harrisburg, PA 17101, USA
[6]Translational Radiobiology, Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[7]Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany
[8]FAU Profile Center Immunomedicine (FAU I-MED), Friedrich-Alexander- Universität Erlangen-Nurnberg, Erlangen, Germany
[9]Department of Radiotherapy and Radiation Oncology, Saarland University Medical Center, Homburg, Germany
[10]Cancer Institute, Xinqiao Hospital, Army Medical University, Chongqing, China
[11]These authors contributed equally
[12]Lead contact
*Correspondence: udo.gaipl@uk-erlangen.de (U.S.G.), mahuab@163.com (H.M.), jianguo.zhou@zmu.edu.cn (J.-G.Z.)
https://doi.org/10.1016/j.isci.2024.111363

## SUMMARY

Radiotherapy showed synergy with immunotherapy, yet the comparative effectiveness of combining immunotherapy (iRT) or chemotherapy (CRT) after platinum therapy failure in advanced non-small cell lung cancer (NSCLC) remains unexplored. We analyzed 163 patients (iRT: $n = 120$ vs. CRT: $n = 43$) eligible for combination radiotherapy. Before matching, median overall survival (OS) was significantly longer in iRT group (7.79 vs. 4.57 months, hazard ratio [HR]: 0.62, 95% confidence interval [CI]: 0.41–0.94, $p = 0.024$). After 1:2 propensity score matching (PSM) and inverse probability of treatment weighting (IPTW), iRT group showed improved OS, consistent with unmatched analysis (PSM, $p = 0.033$ and IPTW, $p = 0.035$). Exploratory analysis suggested that PD1$^+$, central memory PD1$^+$, and effector memory PD-L1$^+$ CD4$^+$ T cells were strong predictive biomarkers for iRT-treated patients ($P_{OS} = 0.025$, $P_{OS} = 0.002$, $P_{OS} = 0.010$, respectively). Proliferative CD4$^+$ T cell$_{low}$ was a prognostic ($P_{OS} = 0.008$) and predictive biomarker for iRT ($P_{OS} < 0.001$). Our work revealed iRT was prolonged OS in previously treated advanced NSCLC patients. Additionally, proliferative CD4$^+$ T cell served as prognostic and predictive biomarkers.

## INTRODUCTION

Non-small cell lung cancer (NSCLC) constitutes 80%–85% of all lung cancer cases, with over half diagnosed at advanced stages.[1] Radiotherapy (RT) as a cornerstone of antitumor therapy plays a crucial role in the comprehensive treatment of locally advanced and metastatic NSCLC for palliation and maintaining quality of life. Notably, increasing evidence suggests that RT strengthens the immunogenicity of tumor cells and improves anti-tumor immunity.[2] As we know, immune checkpoint inhibitors (ICIs), including inhibiting programmed cell death protein 1 (PD-1) or PD ligand 1 (PD-L1), have become one of the most successful cancer therapies in the last decade, resulting in long-term remission and improved survival in patients with advanced NSCLC.[3,4] However, the objective response rate (ORR) of ICIs monotherapy in unscreened NSCLC patients is only 10%–30%.[5] Under this circumstance, the immunostimulatory effect of radiotherapy (RT) provides a theoretical basis for immunotherapy combination radiotherapy (iRT). Strategies for RT combination immunotherapy such as dosage and fractionation, immunotherapy agent and tumor type, the optimal timing and sequencing are still being explored.[6]

RT enhances the efficacy of immunotherapy by inducing adaptive and intrinsic immune responses.[7–9] Based on strong clinical evidence from the PACIFIC trial, several clinical studies have confirmed the synergistic effect of iRT in patients with locally advanced NSCLC.[10–12] However, no studies have yet compared the efficacy of iRT vs. RT combination chemotherapy (CRT) in patients with advanced NSCLC after failure of first-line platinum therapy and required local treatment. Furthermore, there remains a lack of non-invasive biomarkers to predict iRT effectiveness.

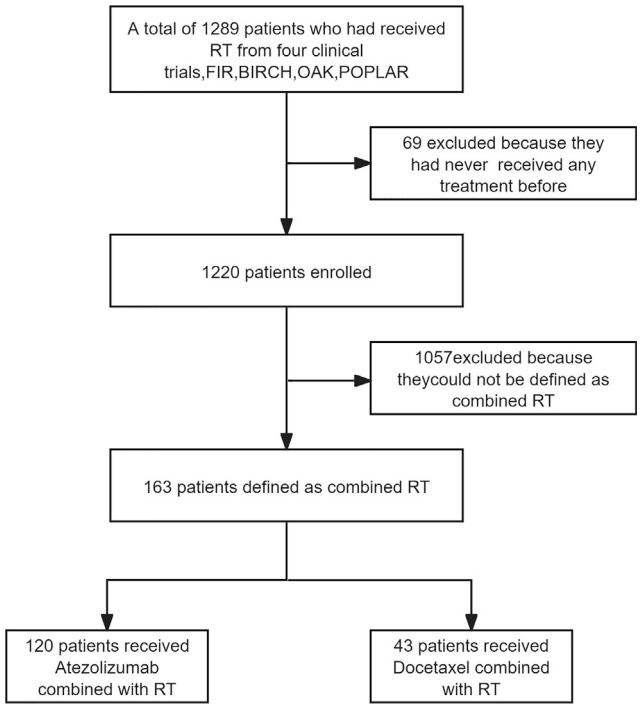



**Figure 1. Flowchart of patient selection for pooled analysis**

Therefore, the current study undertook a post hoc analysis of advanced NSCLC after failure of first-line platinum therapy across four prospective clinical trials to compare the efficacy of combining immunotherapy or chemotherapy with RT. Furthermore, exploratory biomarker analysis is used to identify immune subtypes as potential biomarkers for iRT.

## RESULTS

### Baseline characteristics in the unadjusted and adjusted data

A total of 1,289 patients treated with RT were enrolled in the four clinical trials. Sixty nine were excluded, as they had never received any treatment. Based on the combination RT criteria for iRT, 120 of iRT-group were screened, and 43 of CRT-group were selected according to same criteria, as detailed in the flowchart (Figure 1). Key baseline covariates including sex, histology, ECOG PS, and PDL1 expression were well balanced across treatments before matching; for the CRT group, more patients were Asian, older, with previous smoking history, higher than average metastatic sites, and irradiated to bone or brain (Table 1).

Adjusted after propensity score matching (PSM) and inverse probability of treatment weighting (IPTW), the differences in covariates between two groups were not statistically significant, the SMD was less than 0.2, and all critical baseline characteristics were well balanced. There was rational overlap in the distribution of PS between both groups, and non-significant weight outliers or extreme weights were observed.

### Survival analyses of iRT vs. CRT

In the unadjusted cohort, the iRT group has a longer median OS compared to the CRT group (7.79 months vs. 4.57 months, respectively, HR: 0.62, 95% CI: 0.41–0.94, $p = 0.024$; Figure 2A). After 1:2 propensity score matching (PSM), 64 people in the iRT group and 40 people in the CRT group were selected by screening, and the median OS of the two groups were 7.79 and 4.57 months, respectively, with the iRT group having a better prognosis (HR, 0.60; 95% CI, 0.37–0.96; $p = 0.033$; Figure 2B). After IPTW, the iRT group's median OS was 7.79 months longer than 5.03 months in the CRT group, which makes sense with unadjusted and PSM (HR, 0.65; 95% CI, 0.44–0.97; $p = 0.032$; Figure 2C). The median PFS was not significantly different between the iRT and CRT groups in the unadjusted cohort, at 1.64 and 2.66 months, respectively (iRT, HR: 1.03, 95% CI: 0.72–1.49, $p = 0.825$; Figure 2D), which was also observed after 2:1 PSM- and IPTW-adjusted with a median PFS of 1.64 vs. 2.66 months (iRT, HR: 1.14, 95% CI: 0.75–1.72, $p = 0.520$; Figure 2E) and 1.61 vs. 2.70 months (iRT, HR: 1.06; 95% CI, 0.77–1.47; $p = 0.709$; Figure 2F).

### Subgroup analysis

Subgroup analysis demonstrated that iRT group had the greatest benefit in patients with ECOG-PS of 1 (HR 0.55, 95% CI 0.34–0.89; $p = 0.015$), White (HR 0.59, 95% CI 0.37–0.92; $p = 0.021$), and with site of irradiation as bone (HR 0.42, 95% CI 0.18–0.99; $p = 0.048$) and were observed to have longer survival. Of note, patients with positive PD-L1 expression also had a survival benefit with iRT (HR 0.54, 95% CI 0.33–0.89; $p = 0.015$) (details in Figure 3).

Additional, among patients with liver metastasis, the iRT group had a longer median OS compared to the CRT group (4.93 months vs. 2.86 months, respectively, HR: 0.46, 95% CI: 0.23–0.92; $p = 0.028$). For patients without liver metastasis, the median OS was 13.17 months in the iRT group compared to 5.62 months in the CRT group (HR: 0.63, 95% CI: 0.37–1.08; $p = 0.092$) (Figure S1).

### Exploratory biomarker analysis

One hundred four immune cell subpopulations were analyzed in the whole population and 47 were found to be associated with OS and PFS. Subsequently, we analyzed predictive biomarkers, which means immune cell subpopulations in the iRT and CRT groups, and screened 18 biomarkers that were significantly associated in iRT ($p < 0.05$) but not in CRT ($p > 0.05$) (Table S1 and Table S2).

We analyzed the correlation between immune cell subsets and survival outcomes across treatments based on univariate Cox regression and Kaplan-Meier survival analysis (Table S3) and found that $PD1^+$ $CD4^+$ T cells, $PD1^+$ central memory $CD4^+$ T cells, and $PD\text{-}L1^+$ effector memory $CD4^+$ T cells were risk factors in the iRT group at high expression, whereas they were protective factors in the CRT group (Figure 4A). We observed that the above immune cell subsets have potential as predictive biomarkers in iRT compared with CRT. The Kaplan-Meier survival analysis curves showed an improvement of OS in iRT at low expression status ($PD1^+$ $CD4^+$ T cells $_{low}$: HR $_{iRTvsCRT}$ = 0.39, 95% CI: 0.18–0.82, $p = 0.025$; $PD1^+$ central memory $CD4^+$

**Table 1. Characteristics of patients treated with immunotherapy combination radiotherapy (iRT) and chemotherapy combination radiotherapy (CRT) before unadjusted, after propensity score matching (PSM), and after inverse probability of treatment weighting (IPTW)**

| | Unadjusted | | | | PSM(1: 2) | | | | IPTW-adjusted | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Atezolizumab plus radiotherapy | Docetaxel plus radiotherapy | SMD | *p* value | Atezolizumab plus radiotherapy | Docetaxel plus radiotherapy | SMD | *p* value | Atezolizumab plus radiotherapy | Docetaxel plus radiotherapy | SMD | *p* value |
| N | 120 | 43 | – | – | 64 | 40 | – | – | 120.7 | 40.4 | – | – |
| **Race, no. (%)** | – | – | 0.210 | 0.454 | – | – | 0.179 | 0.664 | – | – | 0.034 | 0.982 |
| White | 102 (85.0) | 34 (79.1) | – | – | 52 (81.2) | 31 (77.5) | – | – | 100(82.8) | 33 (81.5) | – | – |
| Asian | 9 (7.5) | 6 (14.0) | – | – | 6 (9.4) | 6 (15.0) | – | – | 11.8 (9.8) | 4.3 (10.7) | – | – |
| Other | 9 (7.5) | 3 (7.0) | – | – | 6 (9.4) | 3 (7.5) | – | – | 9.0 (7.4) | 3.1 (7.8) | – | – |
| **Age, no. (%)** | – | – | 0.294 | 0.147 | – | – | 0.125 | 0.675 | – | – | 0.037 | 0.844 |
| >62 | 58 (48.3) | 27 (62.8) | – | – | 36 (56.2) | 24(60.0) | – | – | 62.9 (52.1) | 21.8 (53.9) | – | – |
| ≤62 | 62 (51.7) | 16 (37.2) | – | – | 28 (43.8) | 16(40.0) | – | – | 37.8(47.9) | 18.6 (46.1) | – | – |
| **Sex, no. (%)** | – | – | 0.085 | 0.766 | – | – | 0.038 | 1.000 | – | – | 0.026 | 0.888 |
| Male | 72 (60.0) | 24 (55.8) | – | – | 34 (53.1) | 22 (55.0) | – | – | 70.9 (58.7) | 23.2 (57.4) | – | – |
| Female | 48 (40.0) | 19 (44.2) | – | – | 30 (46.9) | 18 (45.0) | – | – | 49.5 (41.3) | 17.2 (42.6) | – | – |
| **Histology, no. (%)** | – | – | 0.078 | 0.825 | – | – | 0.022 | 1.000 | – | – | 0.078 | 0.669 |
| Squamous | 29 (24.2) | 9 (20.9) | – | – | 15 (23.4) | 9 (22.5) | – | – | 27.9 (23.1) | 8.0 (19.9) | – | – |
| Non-squamous | 91(75.8) | 34 (79.1) | – | – | 49 (76.6) | 31 (77.5) | – | – | 92.5 (76.9) | 32.4 (70.1) | – | – |
| **Metsites, no. (%)** | – | – | 0.256 | 0.210 | – | – | 0.125 | 0.675 | – | – | 0.065 | 0.730 |
| >3 | 49 (40.8) | 23 (53.5) | – | – | 28 (43.8) | 20(50.0) | – | – | 53.7 (44.5 ) | 19.3 (47.7) | – | – |
| ≤3 | 71 (59.2) | 20 (46.5) | – | – | 36 (56.2) | 20(50.0) | – | – | 67 ( 55.5 ) | 21.1 (52.3) | – | – |
| **Smoking status, no. (%)** | – | – | 0.317 | 0.250 | – | – | 0.073 | 0.936 | – | – | 0.089 | 0.911 |
| Current | 19 (15.8) | 3 (7.0) | – | – | 4 (6.2) | 3 (7.5) | – | – | 16.1 (13.3) | 4.4 (10.8) | – | – |
| Previous | 80 (66.7) | 34 (79.1) | – | – | 49 (76.6) | 31 (77.5) | – | – | 85.0 (70.4) | 29.9 (74.0) | – | – |
| Never | 21 (17.5) | 6 (14.0) | – | – | 11 (17.2) | 6 (15.0) | – | – | 19.6 (16.3) | 6.1 (15.2) | – | – |
| **ECOG PS, no. (%)** | – | – | 0.172 | 0.457 | – | – | 0.022 | 1.000 | – | – | 0.072 | 0.707 |
| 0 | 34 (28.3) | 9 (20.9) | – | – | 15 (23.4) | 9 (22.5) | – | – | 31.1 (25.8) | 9.2 (22.7) | – | – |
| 1 | 86 (71.7) | 34 (79.1) | – | – | 49 (76.6) | 31 (77.5) | – | – | 89.6 (74.2) | 31.3 (77.3) | – | – |
| **PDL1, no. (%)** | – | – | 0.143 | 0.549 | – | – | 0.192 | 0.475 | – | – | 0.154 | 0.424 |
| Positive | 99 (82.5) | 33 (76.7) | – | – | 53 (82.8) | 30(75.0) | – | – | 96.3 (79.7) | 29.6 (73.2) | – | – |
| Negative | 21 (17.5 ) | 10 (23.3) | – | – | 11 (17.2 ) | 10(25.0) | – | – | 24.4 (20.3) | 10.8 (26.8) | – | – |
| **Site of irradiation, no. (%)** | – | – | 0.363 | 0.287 | – | – | 0.059 | 0.993 | – | – | 0.165 | 0.859 |
| Bone | 29 (24.2) – | 14 (32.6) | – | – | 18 (28.1) | 11 (27.5) | – | – | 31.6 (26.2) | 12.0 (29.6) | – | – |
| Brain | 26 (21.7) | 11 (25.6) | – | – | 17 (25.0) | 11 (27.5) | – | – | 28.5 (23.6) | 11.1 (27.4) | – | – |
| Lung | 24 (20.0) | 10 (23.3) | – | – | 16 (26.6) | 10 (25.0) | – | – | 25.1 (20.8) | 8.2 (20.2) | – | – |
| Other | 41 (34.2) | 8 (18.6) | – | – | 13 (20.3) | 8 (20.0) | – | – | 35.6 (29.5) | 9.2 (22.8) | – | – |

ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand 1.

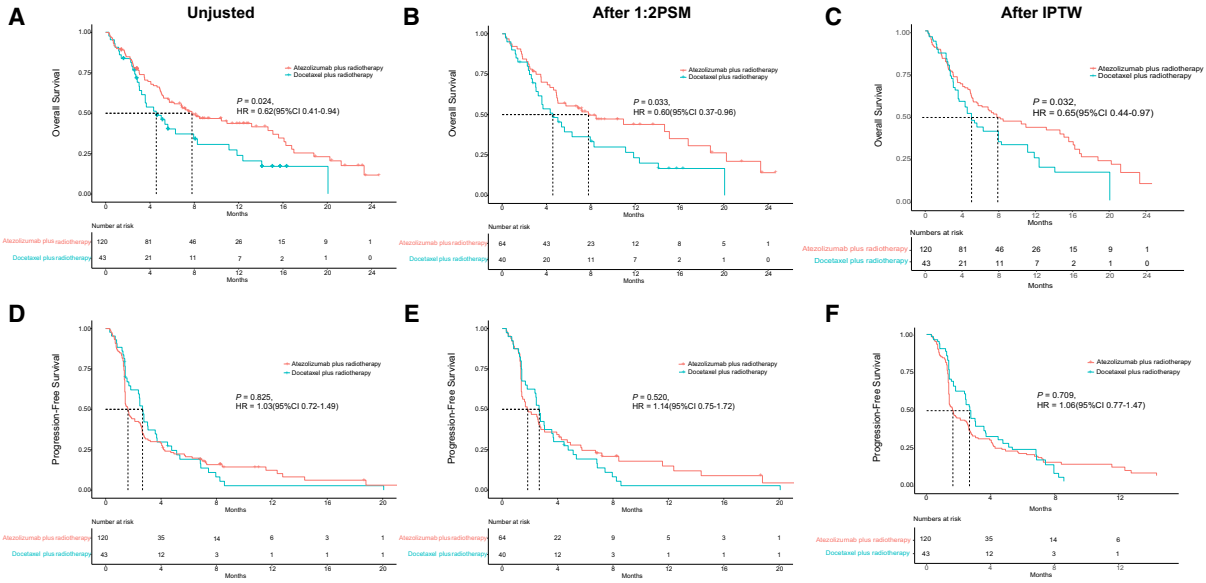



**Figure 2. Kaplan-Mayer curve comparisons of overall survival (OS) and progression-free survival (PFS) of patients treated with immunotherapy combination radiotherapy (iRT) and chemotherapy combination radiotherapy (CRT) before unadjusted, after propensity score matching (PSM), and after inverse probability of treatment weighting (IPTW)**

(A–C) Kaplan-Meier curve comparing OS between iRT and CRT patients before unadjusted ($HR_{OS}$ = 0.62, 95% CI:0.41–0.94, $p$ = 0.024, Log rank test), after PSM (1:2) ($HR_{OS}$ = 0.60, 95% CI:0.37–0.96, $p$ = 0.033, Log rank test), and after IPTW ($HR_{OS}$ = 0.65, 95% CI:0.44–0.97, $p$ = 0.032, Log rank test).

(D–F) Kaplan-Meier curve comparing PFS between iRT and CRT patients before unadjusted ($HR_{PFS}$ = 1.03, 95% CI:0.72–1.49, $p$ = 0.825, Log rank test), after PSM (1:2) ($HR_{PFS}$ = 1.14, 95% CI:0.75–1.72, $p$ = 0.520, Log rank test), and after IPTW ($HR_{PFS}$ = 1.06, 95% CI:0.77–1.47, $p$ = 0.709, Log rank test).

T cells $_{low}$: HR $_{iRTvsCRT}$ = 0.29, 95% CI: 0.14–0.57, $p$ = 0.002; PDL1$^{+}$ effector memory CD4$^{+}$ T cells $_{low}$: HR $_{iRTvsCRT}$ = 0.35, 95% CI: 0.15–0.78, $p$ = 0.010) (Figure 4B). Details were shown in the Supplementary Material (Figure S2 and Table S4). Additionally, we further found that proliferative CD4$^{+}$ T cells $_{low}$ was not only prognostic (HR = 0.53, 95% CI: 0.33–0.85, $p$ = 0.008) but also predictive biomarker (HR $_{iRTvsCRT}$ = 0.28, 95% CI: 0.14–0.56, $p$ < 0.001) (Figure 5).

## DISCUSSION

Our pooled analysis of advanced NSCLC patients after failure of first-line platinum-based chemotherapy in four prospective clinical studies revealed that iRT improved OS compared to CRT. As we know local symptoms due to disease progression or distant metastases would not be avoided during the treatment of advanced NSCLC, this was one of the first studies to provide compelling evidence for iRT in second-line or aforementioned treatments. To mitigate selection bias and ensure a comprehensive analysis, we employed two statistical methods (PSM and IPTW) to control for confounders.[13,14] Notably, IPTW offers the distinct advantage of preserving the original sample size without the reductions often associated with PSM, enhancing the robustness of our findings.

RT induces both adaptive and intrinsic immune responses, thereby augmenting the effectiveness of immunotherapy, substantiating the synergistic impact of RT and immunotherapy in combating tumors.[15–20] This was exemplified by the PACIFIC study, which provided robust evidence for iRT, demonstrating that immunotherapy combination notably extended the OS in patients with locally advanced NSCLC.[10] Further validation comes from Altorki et al., who observed that combining neoadjuvant stereotactic whole-body RT (SBRT) with durvalumab increased the major pathological response rate in patients with resectable early-stage NSCLC.[21] Most studies have focused on the efficacy of iRT in early or locally advanced lung cancer. Our study shows that iRT can be effective in patients with advanced NSCLC, which is consistent with the results of the pooled analyses of the PEMBRO-RT and MDACC studies.[22] Despite the encouraging result of our study, it is crucial to note that the current NCCN guidelines recommend ICIs as a first-line treatment for advanced lung cancer, contrasting with our study's cohort, which only received chemotherapy as their first-line therapy.[23] This discrepancy underscores the need for additional research to assess iRT's impact and potential value in treatment strategies for advanced NSCLC, particularly in the context of the prevalent use of immunotherapy as a standard care approach.

We further explored clinical characteristics, yet caution must be exercised in interpreting the results due to the small sample size. We observed that patients whose irradiation site was bone demonstrated longer OS when treated with iRT compared to CRT. Bone was a common site for NSCLC metastasis, with 35%–40% of cases developing bone metastases during the disease course, often accompanied with pain and skeletal related events.[24–26] Previous studies have documented bone metastasis as a negative prognostic factor for NSCLC and related to a poor prognosis with

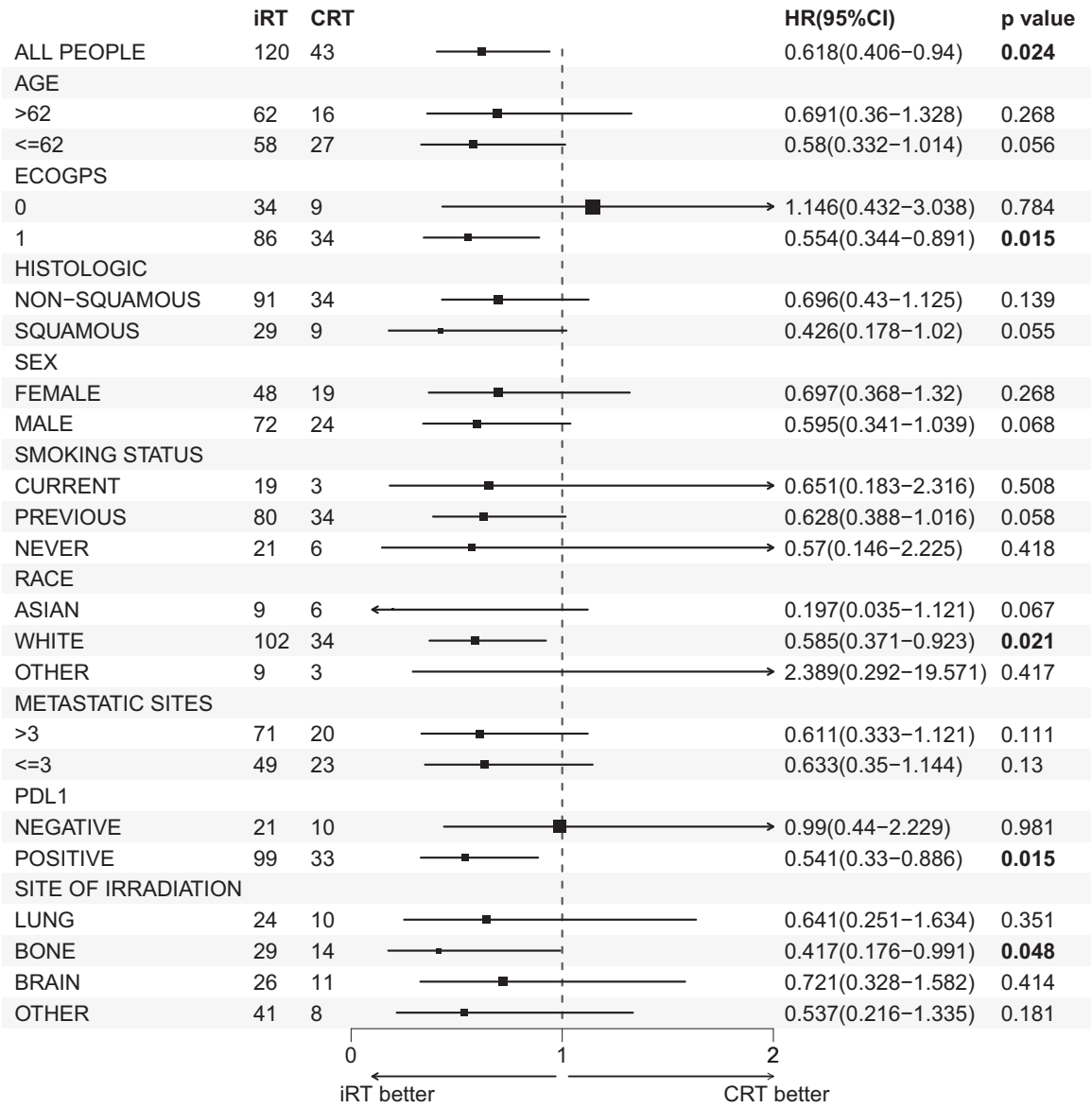



**Figure 3. Exploratory subgroup analysis of associated factors: Forest plots show factors associated with overall survival (OS)**

immunotherapy.[25] As a hematopoietic organ, the bone marrow played a crucial role in regulating the immune system and the trafficking of immune cells.[27] It might be due to these that in our study it was suggested that bone was associated with a better prognosis for receiving iRT. Additionally, we did not analyze the dosage of RT, given the ongoing debate over the optimal radiation doses and fractionation schemes for primary or metastatic lesions in advanced lung cancer patients. Further prospective studies are needed to explore the optimal RT doses and regimens.

At the moment, there is a lack of biomarkers able to identify the population that would benefit from iRT. Our exploratory study has found that several low-level expression $CD4^+$ T cell subpopulations, including $Ki67^+$, $ICOS^+$, $EMOES^+$, central memory cells ($CD45RO^+CCR7^+$, $CD45RO^+CD62L^+CCR7^+$), and effector memory cells ($CD62L^-CD127^+$, $CD45RO^+CD62L^-CCR7^-$), are associated with longer OS in patients receiving iRT. Irradiation could promote the immune infiltration of $CD8^+$ and $CD4^+$ T cell and their antigenic recognition of tumor cells by upregulating the expression of MHC-class I molecules on the surface of tumor cells to enhance the anti-tumor immune effect,[28]whereas $CD4^+$ T cells are more resistant to radiation,[29] which might explain the longer OS in iRT group patients with low levels of $CD4^+$ T cell subpopulations as more $CD4^+$ T cells may be activated, exerting antitumor effects. Similar results were observed in mismatch repair-deficient mCRC patients treated with PD-1 inhibitors, in which lower levels of $CD4^+$ T cells were associated with better

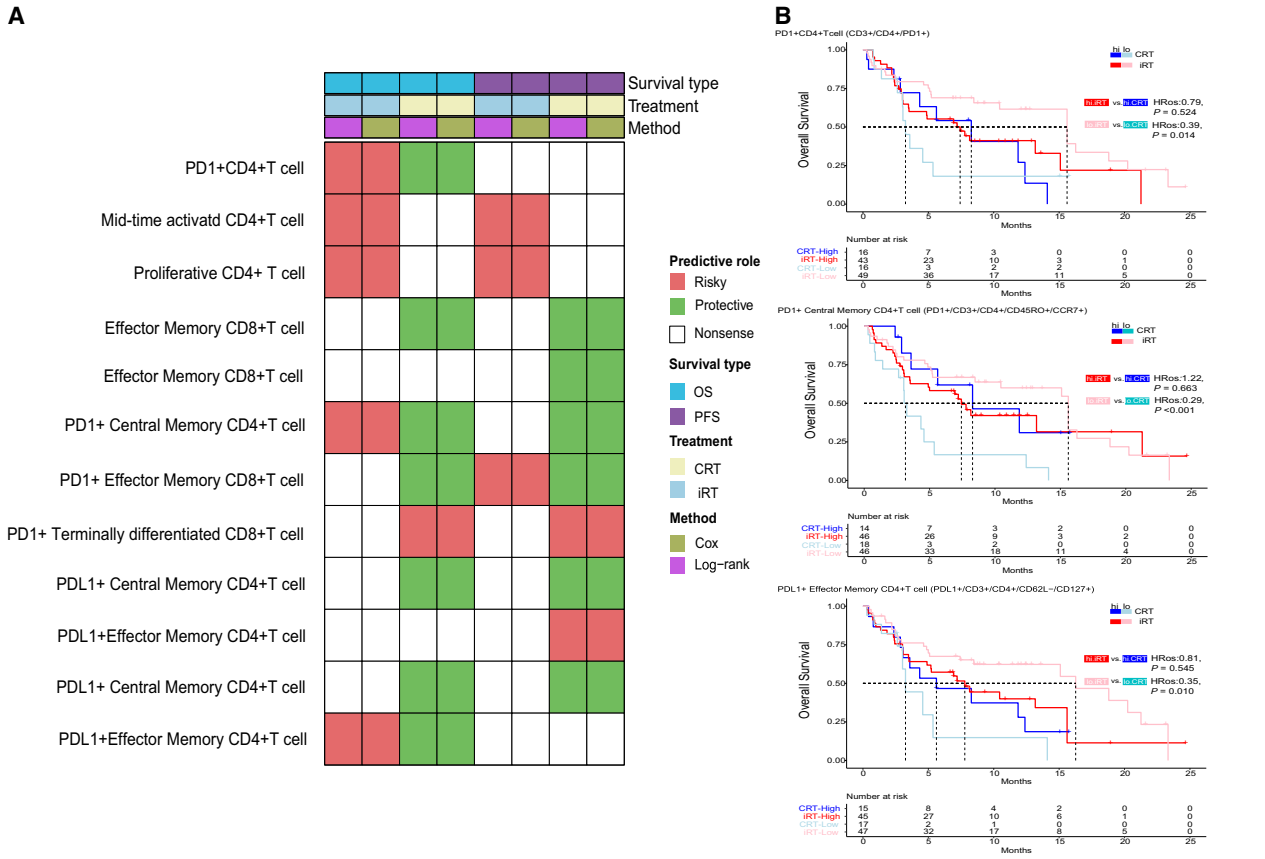



**Figure 4. Correlation of immune cell subpopulations with overall survival (OS) and progression-free survival (PFS), and Kaplan-Meier survival analysis of iRT vs. CRT patients**

(A) Summary of the correlation between expression of immune cell subpopulations with overall survival (OS) and progression-free survival (PFS) in iRT and CRT patients based on univariate Cox regression and Kaplan-Meier model. Red indicates that high expression of this immune cell subpopulations as a risk factor for the survival, and green indicates a protective factor. Only *p* values less than 0.05 are shown.

(B) Kaplan-Meier survival curves based on different treatments (iRT, CRT) with high and low expression of, comparing overall survival of high and low expression receiving iRT vs. CRT. (1) PD1+ $CD4^+$ T cells ($p = 0.025$, Log rank test), Low.iRT vs. Low.CRT ($HR_{OS} = 0.39$ [95% CI: 0.18–0.82], $p = 0.014$), High.iRT vs. High.CRT ($HR_{OS} = 0.79$ [95% CI: 0.38–1.64], $p = 0.524$); (2) PD1+ central memory $CD4^+$ T cells ($p = 0.002$, Log rank test), Low.iRT vs. Low.CRT ($HR_{OS} = 0.29$ [95% CI: 0.14–0.57], $p < 0.001$), High.iRT vs. High.CRT ($HR_{OS} = 1.22$ [95% CI: 0.50–2.98], $p = 0.663$); (3) PDL1+ effector memory $CD4^+$ T cells ($p = 0.033$, Log rank test), Low.iRT vs. Low.CRT ($HR_{OS} = 0.35$ [95% CI: 0.15–0.78], $p = 0.010$), High.iRT vs. High.CRT ($HR_{OS} = 0.81$ [95% CI: 0.40–1.62], $p = 0.545$).

ORR and survival.[30] Central memory T cells (TCM), characterized by self-renewal and replication, could adopt anti-tumor properties and be reactivated by tumor antigens to act as direct tumor killers.[31] The survival-related $CD4^+$ TCM and effector memory T cells (TEM) in our study were almost all PD-$1^+$/PD-$L1^+$. However, the high expression of PD-$1^+$/PD-$L1^+$ in T cells usually indicated the activate T cells; it might also imply the depletion of T cell function, leading to poor immunotherapy outcomes. Studies by Takeuchi et al.[32] and the analysis of oral squamous carcinoma patients by Wu et al.[33] further supported our finding that an increased peripheral blood $CD4^+$TCM subpopulation was predicted, underscoring the potential role of $CD4^+$ TCM in forecasting iRT and immunotherapy prognosis. Additionally, ICOS, a T-cell-specific molecule expressed after activation,[34] was associated with clinical benefits in melanoma patients undergoing anti-CTLA-4 therapy.[35] In our study, patients with ICOS+ $CD4^+$ T cells who received iRT demonstrated longer OS. $CD8^+$ T cells drove anti-tumor responses by recognizing and killing tumor cells, playing a crucial role in anti-tumor immunity and the efficacy of immunotherapy.[36] Studies showed that PD-$1^+$ intratumoral $CD8^+$ T cells predicted response and survival following immunotherapy.[37] Similarly, our findings suggested that a high expression of PD-$1^+$/$CD8^+$ T cells was associated with extended OS in iRT group. Furthermore, EOMES, a key transcription factor, regulated T cell development and function,[38] promoting T cell expansion and proliferation in both $CD4^+$ and $CD8^+$ subsets.[39] Our study found that EOMES$^+$ $CD4^+$ and $CD8^+$ T cells correlated with longer survival in iRT, indicating EOMES as a potential target for enhancing immunotherapy. Notably, proliferative $CD4^+$ T cells were found to be both prognostic and also a predictive biomarker for iRT. Similar to our result, Song et al. reported hepatocellular carcinoma receiving tremelimumab plus durvalumab that patients

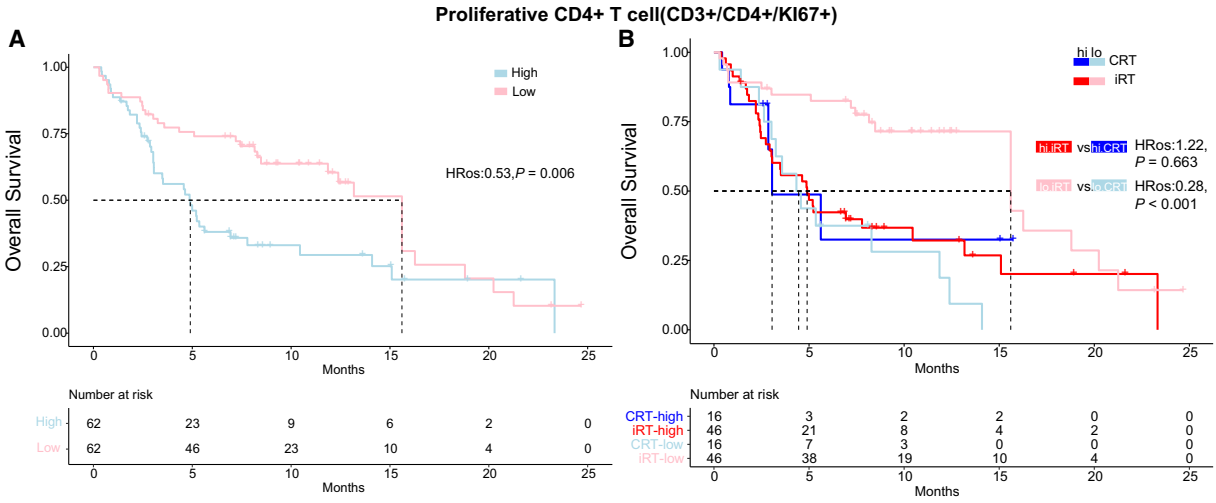



**Figure 5. Kaplan-Meier survival curves of proliferating CD4⁺ T cells based on high and low expression vs. OS**

(A) In overall patients: Low vs. High ($HR_{OS}$ = 0.53 [95% CI: 0.14–0.57], $p$ = 0.006).

(B) In iRT and CRT patients ($p$ < 0.001, Log rank test), Low.iRT vs. Low.CRT ($HR_{OS}$ = 0.28 [95% CI: 0.14–0.56], $p$ < 0.001), High.iRT vs. High.CRT ($HR_{OS}$ = 1.22 [95% CI: 0.50–2.98], $p$ = 0.663).

with lower baseline CD4⁺ and CD8⁺ Ki67+ T cell counts were associated with better outcomes.[40] They also suggested that CTLA-4 inhibitors played an essential role in activating proliferation, which was maintained by the anti-PD-L1 monotherapy. Although CTLA-4 inhibitors were not part of our study, whether irradiation would have comparable effects and be associated with longer survival after combining anti-PD-L1 has to be further explored.

## Conclusion

In conclusion, advanced NSCLC patients after platinum-based chemotherapy failure treated with iRT were associated with longer OS. Low expression of PD1⁺, PD1⁺ CD4⁺ TCM cells, and PDL1⁺ CD4⁺ TEM cells was correlated with better OS in iRT-group. Proliferative CD4⁺ T cells were found as both prognostic and predictive biomarker for iRT.

## Limitations of the study

There were several limitations to this study. Firstly, this was a post hoc analysis based on four clinical trials, and although we have minimized bias through PSM and IPTW, there could still be unidentified confounders. Nonetheless, our study only explored the correlation of immune cell subsets at baseline and could not examine in detail the dynamic changes of these cell subsets before and after treatment. Accordingly, we suggest that future studies should explore in greater depth the changes in specific immune cell subsets during iRT treatment and how these changes may affect treatment outcomes.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jian-Guo Zhou (jianguo.zhou@zmu.edu.cn).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- The patient's data reported in this study could not be deposited in a public repository because of third-party restrictions on the availability of these data. Qualified researchers may request access to individual patient-level data through the clinical study data request platform (Vivli, Inc., https://vivli.org/). For further details please refer to Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents; see https://www.roche.com/innovation/process/clinical-trials/data-sharing/and https://vivli.org/ourmember/roche/. This publication is based on research using data from data contributors, Roche, that has been made available through Vivli, Inc. (Data Request ID: 6762; Lead Investigator: Dr. Jian-Guo Zhou). Vivli has not contributed to or approved and is not in any way responsible for the contents of this publication.
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## ACKNOWLEDGMENTS

We would like to thank all of the patients, investigators, and staff involved in the FIR, BIRCH, POPLAR, and OAK studies who released and shared their data.

Funding: this work was supported by the National Science and Technology Major Project of National Health Commission of China (2023ZD0502105), National Natural Science Foundation of China (Grant No. 82060475), Chunhui program of the MOE (Ministry of Education of China) (Grant No. HZKY20220231), MOE Liberal arts and Social Sciences Foundation (Grant No. 24YJCZH462), the Natural Science Foundation of Guizhou Province (Grant No. ZK2022-YB632), Youth Talent Project of Guizhou Provincial Department of Education (Grant No. QJJ2022-224), Future Science and Technology Elite Talent Cultivation Project of Zunyi Medical University (ZYSE 2023-02), the Key Program of the Education Sciences Planning of Guizhou Province (Grant No.7), and Collaborative Innovation Center of Chinese Ministry of Education (Grant No. 2020-39).

## AUTHOR CONTRIBUTIONS

Study design and developed concept: J.G.Z., H.T.W., and U.S.G.; data collection: X.F.C., J.G.Z., C.Z., and S.H.J.; data analysis: J.Z.X., J.G.Z., F.Y.T., X.F.C., and H.T.W.; study coordination: B.F., M.H., J.G.S., H.M., U.S.G., and J.G.Z.; writing the manuscript: J.Z.X., H.T.W., J.G.Z., and M.H.; obtained funding: J.G.Z; accessed and verified the data: J.G.Z; decided to submit the manuscript: H.M., U.S.G., and J.G.Z. All authors read and approved the final version of the manuscript.

## DECLARATION OF INTERESTS

M.H. reports collaborations with Merck Serono (advisory role, speakers' bureau, honoraria, travel expenses, research funding); MSD (advisory role, speakers' bureau, honoraria, travel expenses, research funding); AstraZeneca (research funding); Novartis (research funding); BMS (advisory role, honoraria, speakers' bureau); Teva (travel expenses). U.S.G. and P.R.F. received support for presentation activities for Dr Sennewald Medizintechnik GmbH, have received support for investigator initiated clinical studies (IITs) from MSD and AstraZeneca, and contributed at Advisory Boards Meetings of AstraZeneca and Bristol-Myers Squibb. No other declaration of interests were reported.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Data source and patients
- METHOD DETAILS
  - Procedures
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Clinical outcome measures
  - Statistical analysis
- ADDITIONAL RESOURCES

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.111363.

Received: August 20, 2024
Revised: October 1, 2024
Accepted: October 15, 2024
Published: November 8, 2024

## REFERENCES

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. *71*, 209–249. https://doi.org/10.3322/caac.21660.
2. O'Donnell, J.S., Teng, M.W.L., and Smyth, M.J. (2019). Cancer immunoediting and resistance to T cell-based immunotherapy. Nat. Rev. Clin. Oncol. *16*, 151–167. https://doi.org/10.1038/s41571-018-0142-8.
3. Pasello, G., Pavan, A., Attili, I., Bortolami, A., Bonanno, L., Menis, J., Conte, P., and Guarneri, V. (2020). Real world data in the era of Immune Checkpoint Inhibitors (ICIs): Increasing evidence and future applications in lung cancer. Cancer Treat Rev. *87*, 102031. https://doi.org/10.1016/j.ctrv.2020.102031.
4. Leonetti, A., Wever, B., Mazzaschi, G., Assaraf, Y.G., Rolfo, C., Quaini, F., Tiseo, M., and Giovannetti, E. (2019). Molecular basis and rationale for combining immune checkpoint inhibitors with chemotherapy in non-small cell lung cancer. Drug Resist. Updates *46*, 100644. https://doi.org/10.1016/j.drup.2019.100644.
5. Memon, D., Schoenfeld, A.J., Ye, D., Fromm, G., Rizvi, H., Zhang, X., Keddar, M.R., Mathew, D., Yoo, K.J., Qiu, J., et al. (2024). Clinical and molecular features of acquired resistance to immunotherapy in non-small cell lung cancer. Cancer Cell *42*, 209–224.e9. https://doi.org/10.1016/j.ccell.2023.12.013.
6. Bayless, N.L., Bluestone, J.A., Bucktrout, S., Butterfield, L.H., Jaffee, E.M., Koch, C.A., Roep, B.O., Sharpe, A.H., Murphy, W.J., Villani, A.C., and Walunas, T.L. (2021). Development of preclinical and clinical models for immune-related adverse events following checkpoint immunotherapy: a perspective from SITC and AACR. J. Immunother. Cancer *9*, e002627. https://doi.org/10.1136/jitc-2021-002627.
7. David, S., Tan, J., Siva, S., Karroum, L., Savas, P., and Loi, S. (2022). Combining Radiotherapy and Immunotherapy in Metastatic Breast Cancer: Current Status and Future Directions. Biomedicines *10*, 821. https://doi.org/10.3390/biomedicines10040821.
8. Mondini, M., Levy, A., Meziani, L., Milliat, F., and Deutsch, E. (2020). Radiotherapy-immunotherapy combinations - perspectives and challenges. Mol. Oncol. *14*, 1529–1537. https://doi.org/10.1002/1878-0261.12658.
9. Rückert, M., Flohr, A.S., Hecht, M., and Gaipl, U.S. (2021). Radiotherapy and the immune system: More than just immune suppression. Stem Cells *39*, 1155–1165. https://doi.org/10.1002/stem.3391.
10. Hui, R., Özgüroğlu, M., Villegas, A., Daniel, D., Vicente, D., Murakami, S., Yokoi, T., Chiappori, A., Lee, K.H., de Wit, M., et al. (2019). Patient-reported outcomes with durvalumab after chemoradiotherapy in stage III, unresectable non-small-cell lung cancer (PACIFIC): a randomised, controlled, phase 3 study. Lancet Oncol. *20*, 1670–1680. https://doi.org/10.1016/s1470-2045(19)30519-4.
11. Faivre-Finn, C., Vicente, D., Kurata, T., Planchard, D., Paz-Ares, L., Vansteenkiste, J.F., Spigel, D.R., Garassino, M.C., Reck, M., Senan, S., et al. (2021). Four-Year Survival With Durvalumab After Chemoradiotherapy in Stage III NSCLC-an Update From the PACIFIC Trial. J. Thorac. Oncol. *16*, 860–867. https://doi.org/10.1016/j.jtho.2020.12.015.
12. Jabbour, S.K., Lee, K.H., Frost, N., Breder, V., Kowalski, D.M., Pollock, T., Levchenko, E., Reguart, N., Martinez-Marti, A., Houghton, B., et al. (2021). Pembrolizumab Plus Concurrent Chemoradiation Therapy in Patients With Unresectable, Locally Advanced, Stage III Non-Small Cell Lung Cancer: The Phase 2 KEYNOTE-799 Nonrandomized Trial. JAMA Oncol. *7*, 1–9. https://doi.org/10.1001/jamaoncol.2021.2301.
13. Desai, R.J., and Franklin, J.M. (2019). Alternative approaches for confounding adjustment in observational studies using weighting based on the propensity score: a primer for practitioners. BMJ *367*, l5657. https://doi.org/10.1136/bmj.l5657.
14. Austin, P.C. (2011). An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav. Res. *46*, 399–424. https://doi.org/10.1080/00273171.2011.568786.
15. Chen, D., Song, X., Wang, H., Gao, Z., Meng, W., Chen, S., Ma, Y., Wang, Y., Li, K., Yu, J., and Yue, J. (2018). Previous Radiotherapy Increases the Efficacy of IL-2 in Malignant Pleural Effusion: Potential Evidence of a Radio-Memory Effect? Front. Immunol. *9*, 2916. https://doi.org/10.3389/fimmu.2018.02916.
16. Herrera, F.G., Bourhis, J., and Coukos, G. (2017). Radiotherapy combination opportunities leveraging immunity for the next oncology practice. CA Cancer J. Clin. *67*, 65–85. https://doi.org/10.3322/caac.21358.
17. Formenti, S.C., and Demaria, S. (2013). Combining radiotherapy and cancer immunotherapy: a paradigm shift. J. Natl. Cancer Inst. *105*, 256–265. https://doi.org/10.1093/jnci/djs629.

18. Takahashi, J., and Nagasawa, S. (2020). Immunostimulatory Effects of Radiotherapy for Local and Systemic Control of Melanoma: A Review. Int. J. Mol. Sci. *21*, 9324. https://doi.org/10.3390/ijms21239324.

19. Balazs, K., Kis, E., Badie, C., Bogdandi, E.N., Candeias, S., Garcia, L.C., Dominczyk, I., Frey, B., Gaipl, U., Juranyi, Z., et al. (2019). Radiotherapy-Induced Changes in the Systemic Immune and Inflammation Parameters of Head and Neck Cancer Patients. Cancers *11*, 1324. https://doi.org/10.3390/cancers11091324.

20. Wu, M., Liu, J., Wu, S., Liu, J., Wu, H., Yu, J., and Meng, X. (2021). Systemic Immune Activation and Responses of Irradiation to Different Metastatic Sites Combined With Immunotherapy in Advanced Non-Small Cell Lung Cancer. Front. Immunol. *12*, 803247. https://doi.org/10.3389/fimmu.2021.803247.

21. Altorki, N.K., McGraw, T.E., Borczuk, A.C., Saxena, A., Port, J.L., Stiles, B.M., Lee, B.E., Sanfilippo, N.J., Scheff, R.J., Pua, B.B., et al. (2021). Neoadjuvant durvalumab with or without stereotactic body radiotherapy in patients with early-stage non-small-cell lung cancer: a single-centre, randomised phase 2 trial. Lancet Oncol. *22*, 824–835. https://doi.org/10.1016/S1470-2045(21)00149-2.

22. Theelen, W.S.M.E., Chen, D., Verma, V., Hobbs, B.P., Peulen, H.M.U., Aerts, J.G.J.V., Bahce, I., Niemeijer, A.L.N., Chang, J.Y., de Groot, P.M., et al. (2021). Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials. Lancet Respir. Med. *9*, 467–475. https://doi.org/10.1016/S2213-2600(20)30391-X.

23. Ettinger, D.S., Wood, D.E., Aisner, D.L., Akerley, W., Bauman, J.R., Bharat, A., Bruno, D.S., Chang, J.Y., Chirieac, L.R., DeCamp, M., et al. (2023). NCCN Guidelines® Insights: Non-Small Cell Lung Cancer, Version 2.2023. J. Natl. Compr. Cancer Netw. *21*, 340–350. https://doi.org/10.6004/jnccn.2023.0020.

24. Roodman, G.D. (2004). Mechanisms of bone metastasis. N. Engl. J. Med. *350*, 1655–1664. https://doi.org/10.1056/NEJMra030831.

25. Kuchuk, M., Addison, C.L., Clemons, M., Kuchuk, I., and Wheatley-Price, P. (2013). Incidence and consequences of bone metastases in lung cancer patients. J. Bone Oncol. *2*, 22–29. https://doi.org/10.1016/j.jbo.2012.12.004.

26. Santini, D., Barni, S., Intagliata, S., Falcone, A., Ferraù, F., Galetta, D., Moscetti, L., La Verde, N., Ibrahim, T., Petrelli, F., et al. (2016). Corrigendum: Natural History of Non-Small-Cell Lung Cancer with Bone Metastases. Sci. Rep. *6*, 22205. https://doi.org/10.1038/srep22205.

27. Zhao, E., Xu, H., Wang, L., Kryczek, I., Wu, K., Hu, Y., Wang, G., and Zou, W. (2012). Bone marrow and the control of immunity. Cell. Mol. Immunol. *9*, 11–19. https://doi.org/10.1038/cmi.2011.47.

28. Jin, W.J., Zangl, L.M., Hyun, M., Massoud, E., Schroeder, K., Alexandridis, R.A., and Morris, Z.S. (2023). ATM inhibition augments type I interferon response and antitumor T-cell immunity when combined with radiation therapy in murine tumor models. J. Immunother. Cancer *11*, e007474. https://doi.org/10.1136/jitc-2023-007474.

29. Heylmann, D., Rödel, F., Kindler, T., and Kaina, B. (2014). Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. Biochim. Biophys. Acta *1846*, 121–129. https://doi.org/10.1016/j.bbcan.2014.04.009.

30. Cheng, Y.K., Chen, D.W., Chen, P., He, X., Li, P.S., Lin, Z.S., Chen, S.X., Ye, S.B., and Lan, P. (2022). Association of Peripheral Blood Biomarkers With Response to Anti-PD-1 Immunotherapy for Patients With Deficient Mismatch Repair Metastatic Colorectal Cancer: A Multicenter Cohort Study. Front. Immunol. *13*, 809971. https://doi.org/10.3389/fimmu.2022.809971.

31. Sallusto, F., Lenig, D., Förster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708–712. https://doi.org/10.1038/44385.

32. Takeuchi, Y., Tanemura, A., Tada, Y., Katayama, I., Kumanogoh, A., and Nishikawa, H. (2018). Clinical response to PD-1 blockade correlates with a sub-fraction of peripheral central memory CD4+ T cells in patients with malignant melanoma. Int. Immunol. *30*, 13–22. https://doi.org/10.1093/intimm/dxx073.

33. Wu, J., Zhang, T., Xiong, H., Zeng, L., Wang, Z., Peng, Y., Chen, W., Hu, X., and Su, T. (2022). Tumor-Infiltrating CD4(+) Central Memory T Cells Correlated with Favorable Prognosis in Oral Squamous Cell Carcinoma. J. Inflamm. Res. *15*, 141–152. https://doi.org/10.2147/JIR.S343432.

34. Dong, C., Juedes, A.E., Temann, U.A., Shresta, S., Allison, J.P., Ruddle, N.H., and Flavell, R.A. (2001). ICOS co-stimulatory receptor is essential for T-cell activation and function. Nature *409*, 97–101. https://doi.org/10.1038/35051100.

35. Ng Tang, D., Shen, Y., Sun, J., Wen, S., Wolchok, J.D., Yuan, J., Allison, J.P., and Sharma, P. (2013). Increased frequency of ICOS+ CD4 T cells as a pharmacodynamic biomarker for anti-CTLA-4 therapy. Cancer Immunol. Res. *1*, 229–234. https://doi.org/10.1158/2326-6066.Cir-13-0020.

36. Philip, M., and Schietinger, A. (2022). CD8(+) T cell differentiation and dysfunction in cancer. Nat. Rev. Immunol. *22*, 209–223. https://doi.org/10.1038/s41577-021-00574-3.

37. Siddiqui, I., Schaeuble, K., Chennupati, V., Fuertes Marraco, S.A., Calderon-Copete, S., Pais Ferreira, D., Carmona, S.J., Scarpellino, L., Gfeller, D., Pradervand, S., et al. (2019). Intratumoral Tcf1(+)PD-1(+) CD8(+) T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. Immunity *50*, 195–211.e10. https://doi.org/10.1016/j.immuni.2018.12.021.

38. Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell *100*, 655–669. https://doi.org/10.1016/s0092-8674(00)80702-3.

39. Llaó-Cid, L., Roessner, P.M., Chapaprieta, V., Öztürk, S., Roider, T., Bordas, M., Izcue, A., Colomer, D., Dietrich, S., Stilgenbauer, S., et al. (2021). EOMES is essential for antitumor activity of CD8(+) T cells in chronic lymphocytic leukemia. Leukemia *35*, 3152–3162. https://doi.org/10.1038/s41375-021-01198-1.

40. Song, X., Kelley, R.K., Green, M., Standifer, N., Lim, K., Zhou, D., Dunyak, J., Negro, A., Kurland, J.F., Ren, S., et al. (2023). Modeling of Proliferating CD4 and CD8 T-Cell Changes to Tremelimumab Exposure in Patients with Unresectable Hepatocellular Carcinoma. Clin. Pharmacol. Ther. *114*, 874–882. https://doi.org/10.1002/cpt.2992.

41. Peters, S., Gettinger, S., Johnson, M.L., Jänne, P.A., Garassino, M.C., Christoph, D., Toh, C.K., Rizvi, N.A., Chaft, J.E., Carcereny Costa, E., et al. (2017). Phase II Trial of Atezolizumab As First-Line or Subsequent Therapy for Patients With Programmed Death-Ligand 1-Selected Advanced Non-Small-Cell Lung Cancer (BIRCH). J. Clin. Oncol. *35*, 2781–2789. https://doi.org/10.1200/jco.2016.71.9476.

42. Spigel, D.R., Chaft, J.E., Gettinger, S., Chao, B.H., Dirix, L., Schmid, P., Chow, L.Q.M., Hicks, R.J., Leon, L., Fredrickson, J., et al. (2018). FIR: Efficacy, Safety, and Biomarker Analysis of a Phase II Open-Label Study of Atezolizumab in PD-L1-Selected Patients With NSCLC. J. Thorac. Oncol. *13*, 1733–1742. https://doi.org/10.1016/j.jtho.2018.05.004.

43. Fehrenbacher, L., Spira, A., Ballinger, M., Kowanetz, M., Vansteenkiste, J., Mazieres, J., Park, K., Smith, D., Artal-Cortes, A., Lewanski, C., et al. (2016). Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet *387*, 1837–1846. https://doi.org/10.1016/s0140-6736(16)00587-0.

44. Rittmeyer, A., Barlesi, F., Waterkamp, D., Park, K., Ciardiello, F., von Pawel, J., Gadgeel, S.M., Hida, T., Kowalski, D.M., Dols, M.C., et al. (2017). Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre

randomised controlled trial. Lancet *389*, 255–265. https://doi.org/10.1016/S0140-6736(16)32517-X.

45. Anscher, M.S., Arora, S., Weinstock, C., Amatya, A., Bandaru, P., Tang, C., Girvin, A.T., Fiero, M.H., Tang, S., Lubitz, R., et al. (2022). Association of Radiation Therapy With Risk of Adverse Events in Patients Receiving Immunotherapy: A Pooled Analysis of Trials in the US Food and Drug Administration Database. JAMA Oncol. *8*, 232–240. https://doi.org/10.1001/jamaoncol.2021.6439.

46. Bauml, J.M., Mick, R., Ciunci, C., Aggarwal, C., Davis, C., Evans, T., Deshpande, C., Miller, L., Patel, P., Alley, E., et al. (2019). Pembrolizumab After Completion of Locally Ablative Therapy for Oligometastatic Non-Small Cell Lung Cancer: A Phase 2 Trial. JAMA Oncol. *5*, 1283–1290. https://doi.org/10.1001/jamaoncol.2019.1449.

47. Rubin, D.B. (1997). Estimating causal effects from large data sets using propensity scores. Ann. Intern. Med. *127*, 757–763. https://doi.org/10.7326/0003-4819-127-8_part_2-199710151-00064.

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Software and algorithms | | |
| Vivli platform | Vivli, Inc | https://vivli.org/ |
| R version v.4.2.2 | R Core Team | https://www.r-project.org/; https://www.cell.com/iscience/fulltext/_blank |
| survminer version 0.4.9 | Kassambara A | https://CRAN.R-project.org/package=survminer |
| jskm version 0.4.9 | Kim J | https://CRAN.R-project.org/package=jskm |
| VGAM version 1.1–12 | Yee T | https://cran.r-project.org/web/packages/VGAM/index.html |
| Matching version 4.10–15 | Sekhon JS | https://cran.r-project.org/package=Matching |
| MatchIt version 4.5.5 | Ho D | https://www.rdocumentation.org/packages/MatchIt/versions/4.5.5/topics/matchit |

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

#### Data source and patients

Data were derived from four clinical trials, including the Phase II BIRCH (NCT02031458),[41] Phase II FIR (NCT01846416),[42] Phase II POPLAR (NCT01903993),[43] and Phase III OAK (NCT02008227) trials.[44] BIRCH and FIR constituted multicenter, single-arm studies, where patients were administered atezolizumab (1200 mg intravenously every 3 weeks). Conversely, POPLAR and OAK were multicenter, open-label, randomized studies, where patients were randomly assigned to receive either atezolizumab (1200 mg intravenously every 3 weeks) or docetaxel (75 mg/m$^2$ intravenously every 3 weeks). However, it must be noted that our analysis specifically included patients with locally advanced or metastatic NSCLC who had previously failed platinum therapy and had undergone prior radiotherapy. Only this specific subset of advanced NSCLC patients was included in the extraction of valid data for the secondary analysis. Table 1 provided demographic and clinical characteristics of patients and race. Details of inclusion and exclusion criteria can be found elsewhere.[41–44]

### METHOD DETAILS

#### Procedures

Based on the design of previous clinical studies and considering the safety of therapy, combination radiotherapy was defined in our analysis as treatment with atezolizumab or docetaxel within 90 days of the end of radiotherapy or receiving RT during atezolizumab or docetaxel, referred to as iRT and CRT, respectively.[45,46] The clinicopathologic characteristics in NSCLC patients treated with iRT and CRT was shown in Table 1. Pertinent characteristics include mean age (>62 vs . $\leq$ 62 years), gender, Eastern Cooperative Oncology Group-physical status (ECOG- PS) (1 vs. 0), smoking status (current smoker, pre-smoker vs. never smoker), histology (squamous vs. non-squamous), number of metastatic sites (>3 vs . $\leq$ 3), PDL-1 expression status (The definition of programmed cell death ligand 1 (PD-L1) positive in OAK and POPLAR was Combined Positive Score (CPS) $\geq$1%, but in BIRCH and FIR was CPS $\geq$5%), and sites of irradiation (bone, brain, lung, and others).

### QUANTIFICATION AND STATISTICAL ANALYSIS

#### Clinical outcome measures

The primary endpoint is overall survival (OS), and progression-free survival (PFS) serves as secondary endpoint. To further explore relevant biomarkers, we assessed the prognostic role of immune cell populations in the two groups of patients, classifying the abundance of immune cell subpopulations into high and low according to the median value between iRT and CRT.

#### Statistical analysis

To correct data imbalances and reduce bias between the iRT and CRT groups, we used two propensity score (PS) -based methods: propensity score matching (PSM) and inverse probability of treatment weighting (IPTW).[13,47] PS for each participant was calculated with logistic regression from baseline covariates including age, gender, smoking status, histology, number of metastatic sites, and site of radiotherapy. PSM used nearest-neighbor matching at a ratio of 1:2, with a caliper width of 0.2. IPTW adjusted the differences in patient characteristics between treatment groups based on PS. For this, the PS, which was calculated as the probability that patient would receive specific treatments, was estimated from the covariates. Subsequently, IPTW was converted from the PS, where patients receiving specific treatment were given a weight of 1. At last, weighted survival analyses were performed using these weights

to account for possible differences in patient characteristics between treatment groups. The standardized mean difference (SMD) was most used to assess the balance of parameter distributions after PS matching, and SMD $\geq$0.2 was considered to show a significant difference between treatment groups. Therefore, in addition to using $p$ values $\geq$ 0.05, SMD <0.2 was used to assess IPTW or PSM-adjusted balance of characteristics between groups.

Continuous variables are expressed as mean ± standard deviation (SD) and median (interquartile range, IQR). Categorical variables were reported as absolute numbers and percentages. The OS and PFS were analyzed using Kaplan-Meier, and log rank tests were used to compare survival curves. In exploratory subgroup analyses, risk factors associated with OS were assessed by univariate Cox proportional risk regression analyses, and risk ratios (HR) with 95% confidence intervals (CIs) were calculated. Subgroup results are presented as forest plots. All statistical tests were two-sided and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using R in Vivli platform (v.4.2.2, R Core Team 2022).

## ADDITIONAL RESOURCES

The BIRCH, FIR, POPLAR and OAK trials were registered at ClinicalTrials.gov: https://clinicaltrials.gov/study/NCT02031458?term=NCT02031458&rank=1, https://clinicaltrials.gov/study/NCT01846416?term=NCT01846416&rank=1, https://clinicaltrials.gov/study/NCT01903993?term=NCT01903993&rank=1, and https://clinicaltrials.gov/study/NCT02008227?term=NCT02008227&rank=1.