Radiotherapy induces responses of lung cancer to CTLA-4 blockade

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Focal radiation therapy enhances systemic responses to anti-CTLA-4 antibodies in preclinical studies and in some patients with melanoma 8,9, but its efficacy in inducing systemic responses (abscopal responses) against tumors unresponsive to CTLA-4 blockade remained uncertain. Radiation therapy promotes the activation of anti-tumor T cells, an effect dependent on type I interferon induction in the irradiated tumor 10. The latter is essential for achieving abscopal responses in murine cancers 11. The mechanisms underlying abscopal responses in patients treated with radiation therapy and CTLA-4 blockade remain unclear. Here we report that radiation therapy and CTLA-4 blockade induced systemic anti-tumor T cells in chemo-refractory metastatic non-small-cell lung cancer (NSCLC), where anti-CTLA-4 antibodies had failed to demonstrate significant efficacy alone or in combination with chemotherapy 12. Objective responses were observed in 18% of enrolled patients, and 31% had disease control. Increased serum interferon-β after radiation and early dynamic changes of blood T cell clones were the strongest response predictors, confirming preclinical mechanistic data. Functional analysis in one responding patient showed the rapid in vivo expansion of CD8 T cells recognizing a neoantigen encoded in a gene upregulated by radiation, supporting the hypothesis that one explanation for the abscopal response is radiation-induced exposure of immunogenic mutations to the immune system.

The choice of NSCLC for testing the combination of radiation therapy with the anti-CTLA-4 antibody, ipilimumab, was supported by the case of a complete and durable abscopal response to this combination, in a patient with metastatic NSCLC 13. To prospectively evaluate radiation therapy to one metastasis (palliative dose, 6 Gy X 5 and concurrent ipilimumab, 39 patients were enrolled between June 2014 and April 2015 (NCT02221739, Supplementary Table 1 and Fig. 1a). All patients had progressed after ≥1 prior systemic treatment, and 41% had pre-existing brain metastases controlled by surgery or radiotherapy at study entry. One patient had received prior immunotherapy.

Twenty-one of 39 patients (54%) completed 4 cycles of ipilimumab and could be evaluated at day 88 by Response Criteria in Solid Tumors (RECIST). Adverse events were consistent with ipilimumab-induced side effects, and the addition of radiation therapy did not modify them (Supplementary Table 2). One additional patient received four cycles but did not undergo response evaluation. Seventeen patients received less than 4 cycles because they either died (n = 8) or progressed (n = 9) before day 88 and were taken off treatment. Patients who did not complete treatment had a more advanced disease at study entry, with significantly more organs involved by metastasis, more frequently had bone metastases, and had received more courses of prior chemotherapy (Supplementary Table 1).

Objective radiographic responses occurred in 18% of enrolled patients (7 of 39 patients) or 33% of evaluable patients (7 of 21 patients) with 2 complete (CR) and 5 partial (PR) responses (Fig. 1b,c and Supplementary Tables 3 and 4). In addition, 5 patients had stable disease (SD) at evaluation. Thus, disease control (PR + CR + SD) was achieved in 12 out of 39 (31%) patients. At median follow-up of 43 months for survivors (range: 38–47 months), the median overall survival (OS) for the entire cohort of 39 patients was 7.4 months (95% confidence interval (CI): 4.4–12.6) (Fig. 1d,e). In patients who completed treatment, the median OS was 13.0 months (95% CI: 10.6–25.2) versus 3.0 months for those who did not (95% CI: 2.5–3.5) (log-rank test P < 0.001) (Supplementary Fig. 1). In patients who achieved disease control, the median OS was 20.4 months (95% CI: 12.9 to not reached) compared to 3.5 months in patients who did not (95% CI: 3.1–7.4) (log-rank test P < 0.001) (Fig. 1f,g). Four patients who completed treatment (among which three achieved disease control) were alive at the time of last follow-up 38, 42, 44 and 47 months since study entry.

To investigate the mechanisms underlying an abscopal response to radiation therapy and ipilimumab, tumor tissue and peripheral blood were analyzed. PD-L1 expression in pretreatment tumor was not associated with response, as it was detected in 6 out of 10 non-responders, including 3 patients with progressive disease at evaluation.

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Fig. 1 | Patients survival and clinical response to radiotherapy and ipilimumab. a, Treatment, imaging, and blood sampling schema (FU, follow-up). b, Waterfall plot of aggregate tumor volume change in all non-irradiated lesions. Patient 43 was classified as PD due to a new lesion. One patient had lesions that could not be accurately measured radiographically and is not included in the graph, but was considered as PD due to new lesions. c, Best tumor volume change indicates the tumor volume change in the non-irradiated metastasis with the biggest change from baseline in each patient. d, Kaplan–Meier estimates of overall survival (d) and progression-free survival (e) for all patients (n = 39). f, g, Comparison of overall (f) and progression-free survival (g) between patients with disease control (CR + PR + SD; n = 12) and with PD (n = 27). Overall survival was 20.4 (95% CI: 12.9 to not reached) and 3.5 (95% CI: 3.1–7.4) months for CR/PR/SD and PD, respectively. Progression-free survival was 7.1 (95% CI: 5.9 to not reached) and 3.0 (95% CI: 2.4–3.8) months for CR/PR/SD and PD, respectively. Statistical significance was determined using a two-sided log-rank test.
(PD) and 3 non-evaluable patients (NE) who did not complete treatment, and in 4 to 5 patients with disease control out of the 15 patients with tissue available for testing. Likewise, CD8 T cell infiltration was not associated with response (Supplementary Table 5 and Supplementary Fig. 2).

Baseline clinical parameters, including the neutrophil to lymphocyte ratio, previously shown to be higher in patients without abscopal response to radiation therapy and granulocyte-macrophage colony-stimulating factor, were comparable in patients with or without disease control after completing treatment (Supplementary Fig. 3a). However, the proportion of patients with epidermal growth factor receptor (EGFR) mutated cancers, a feature associated with low total mutation burden and poor response to anti-PD-1/PDL-1, was significantly higher in patients with PD compared to patients with disease control (Supplementary Table 6). Neither the radiation regimen used (9GyX3 versus 6GyX5) nor the location of the irradiated lesion was significantly associated with treatment response.

A panel of circulating soluble markers and immune cells (Supplementary Dataset 1) was analyzed in the 21 patients who completed treatment and were evaluable for response. At baseline, absolute lymphocyte count (ALC) was lower and regulatory T cells (TREG) were higher in responding compared to SD and PD patients. Inducible T cell costimulator (ICOS) expression was also higher in responders in both regulatory and conventional CD4 T cells (Supplementary Fig. 3b and Supplementary Dataset 2). Changes in circulating soluble markers and immune cells occurring during treatment were evaluated by comparing values obtained at day 22 versus baseline. We also calculated the largest change in baseline from either direction (increase or decrease) in every parameter over the entire course of treatment, to account for different kinetics of change among patients (Supplementary Dataset 2). During treatment, most patients showed an increase in ICOS expression on CD4 T cells and in proliferation of CD8 and CD4 T cells, evidenced by Ki67 expression, irrespective of response. Thus, two known pharmacodynamic biomarkers for anti-CTLA-4 activity did not distinguish responders from non-responders in this study. Expansion in ICOS+ TREG in patients with melanoma treated with interleukin-2 was associated with worse outcome but in our patients this subset was similarly expanded in responders and non-responders, suggesting a possible role in mitigating ipilimumab-induced inflammation rather than anti-tumor responses. In contrast to the results reported for a melanoma patient, no significant changes in myeloid-derived suppressor cells were detected after radiation therapy in patients with NSCLC. The percentage of PD-1+ CD8 T cells was increased during treatment in both responders and non-responders, whereas only responders showed a significant increase of PD-1+ CD4 T cells. Approximately 20% of PD-1+ T cells were Ki67+ in both responders and non-responders, suggesting an activated rather than exhausted state (Supplementary Table 7). T-effector memory cells were increased in the CD8 and CD4 compartments, irrespective of response (Supplementary Fig. 4 and Supplementary Dataset 2). CTLA-4 was expressed mainly on TREG without significant changes during treatment (Supplementary Dataset 3).

Serum levels of galectin-1, an immune suppressive tumor-secreted factor reported to be increased by radiation in NSCLC, were comparable at baseline between responders and non-responders and did not change significantly at day 22 (Supplementary Fig. 3c).

Elevated serum levels of soluble major histocompatibility complex class I chain-related protein A (sMICA) at baseline were associated with resistance to CTLA-4 blockade in patients with melanoma. In our patients baseline levels of sMICA did not differ significantly between responders and non-responders. During treatment, sMICA levels increased significantly in patients with PD while responding patients showed an increase in antibodies against sMICA and sMICB (Supplementary Dataset 2 and Supplementary Fig. 3d). These antibodies were shown to block sMICA/B-mediated downregulation of NKG2D receptor on effector CD8 T and natural killer cells, and restore tumor response to ipilimumab in some patients. An increase in NKG2D receptor expression was seen across all natural killer subsets only in patients with SD (Supplementary Dataset 2), and thus it could not be attributed to increased anti-sMICA/B antibodies seen only in patients with CR/PR. Anti-MICA/B antibodies could have different functions, including blocking MICA/B shedding from the tumor cell surface. Radiation-therapy-induced upregulation of surface MICA/B was shown to improve cancer cell killing by natural killer and CD8

**Figure 2** Increase in interferon-β levels and TCR clonal dynamics predict response to treatment. a, IFN-β serum levels at baseline (filled circles) and 22 days after treatment start (empty circles). The values for patient 4 are indicated in red. NE indicates non-evaluable patients. TCR sequencing was performed on peripheral blood at baseline and day 22. b, c, Number of expanded (b) and (c) contracted TCR clones at day 22 compared to baseline. For panels a, b and c, statistical significance was determined using the two-sided Student’s t-test and one-way analysis of variance (ANOVA), respectively (P values: *P < 0.05, **P < 0.01, and ***P < 0.001). Circles, horizontal lines, and error bars represent data points for individual patients, means, and standard deviations, respectively. d, RF classification of patients using variables with statistically significant difference between day 22 and baseline for any RECIST response group (Supplementary Dataset 2). For IFN-β, expanded and contracted clones, and anti-sMICA and sMICB antibodies (AB) measurements, n = 7, 5, and 8 patients for CR/PR, SD, and PD response groups, respectively. For all other measurements, n = 4, 5, and 8 patients for CR/PR, SD, and PD response groups, respectively. Performance of the RF classifications is shown under the heat map. ‘Correct RECIST’ indicates the actual treatment response of each patient whereas ‘Classified as’ indicates how patients were classified using RF modeling. Additionally, mean and standard deviations of variable importance scores were determined (top predictive variables: red bars with filled pattern) from 1,000 executions. NK, natural killer; MFI, geometric mean fluorescence intensity.
but no post-treatment tumor tissue was available to investigate this hypothesis.

Concordant with studies in mice, we found that radiation therapy induction of interferon-β (IFN-β) correlates with the absence of response in this patient population. Serum IFN-β showed the most significant increase from baseline shortly after completion of radiation therapy (day 22) in the seven responders (Fig. 2a and Supplementary Dataset 2). IFN-β was also significantly but more modestly increased in the 5 patients with SD, whereas no significant increase occurred in the remaining 23 patients with PD and serum available for measurement, including those who did not complete treatment with ipilimumab.

Next, we evaluated the T cell clonal representation in peripheral blood with deep sequencing of the T cell receptor (TCR) CDR3 regions (TCR-seq). A significantly larger number of T cell clones were expanded at day 22 compared to baseline in responders than in patients with SD and PD (Fig. 2b). Responders also showed a significantly larger number of contracted clones (Fig. 2c). In a
random forest (RF) analysis the levels of IFN-β at day 22 and the TCR repertoire changes showed the highest predictive value (compared to other variables that were significantly altered at day 22) in classifying correctly the patients by response status: 7 out of 7 responders, 6 out of 8 patients with PD, and 2 out of 5 patients with SD were correctly assigned, and no patient with PD was classified as a responder (Fig. 2d).

We next assessed the tumor specificity of the T cell clones that were expanded in the blood early after radiation therapy completion. For this, we performed TCR-seq on archival tumors from 4 patients representative of different response status: 1 CR (patient 4), 1 with SD (patient 32), and 2 with PD (patients 36 and 38). TCR sequences obtained from tumors were designated as TIL-TCRs (TIL, tumor infiltrating lymphocytes) and compared to the sequences in blood. In each of the four patients there were TIL-TCRs whose frequency increased from baseline over the course of treatment, but their number was markedly larger in the patient with CR (patient 4) compared to the others (Fig. 3a). Twenty of the clones present in patient 4 tumor were undetectable in the blood at baseline but showed a rapid increase during treatment and most remained elevated at follow-up. Patient 32, who had SD, showed expansion of a smaller number of clones already present at baseline that remained elevated at follow-up. In marked contrast, in the blood of patients 36 and 38, who had PD, there was an expansion of few pre-existing clones, and their increase was minimal and/or not sustained.

We next asked if the number of TIL-TCRs expanded by treatment in blood was proportional to the number of pre-existing tumor-infiltrated CD8 T cells. The highest density of CD8 T cells was found in patient 36, who also showed the highest TCR clonality, while patient 32 had very few CD8 T cells, and patients 4 and 38 had an intermediate number (Fig. 3b). Hence, the quantity and quality of the pretreatment T cell clone infiltrate was not related to the treatment-induced expansion of TIL-TCRs in peripheral blood (Fig. 3b).

We then examined the TCR CDR3 amino acid sequences relatedness24 of the clones present in the two pretreatment brain metastases of patient 4. Out of 51 TIL-TCRs increased in blood (Fig. 3a), 19 were common to both metastases and were found in multiple branches of the dendrogram, consistent with the expansion of a diverse repertoire. The largest TIL-TCR that was increased in blood had distinct features as it was found in a separate branch in each tumor (Fig. 3c). Thus, the combination of radiation therapy and CTLA-4 blockade expanded a selected and diverse set of TCR clones.

In patients with melanoma treated with ipilimumab and in patients with NSCLC treated with anti-PD-1 antibodies the mutational landscape is an independent determinant of response24–26. Thus, we assessed the number of antigenic mutations in the tumors of the four patients with different responses to radiation therapy and ipilimumab. The number of single nucleotide missense variants (SNV) in patients 4, 32, 36, and 38 ranged from 199 to 345, and the frameshift mutations ranged from 11 to 22 (Supplementary Fig. 5). The lowest number of predicted mutation-associated neoantigens was found in patients 4 (CR) and 38 (PD). There were no significant differences between the four patients in the major histocompatibility complex I (MHC-I) binding affinity, variant allele frequency (VAF), or the wild-type/mutant ratio of peptide MHC-I binding affinity of the predicted neoepitopes. Expression levels of the genes encoding the predicted neoepitopes, based on the lung adenocarcinoma The Cancer Genome Atlas (TCGA) data, did not differ among the four patients. None of the tumors had mutated TAP or B2M genes (Supplementary Dataset 4), which are required for antigen presentation and are often mutated in immunotherapy-resistant tumors27. However, loss of heterozygosity at HLA-A and -C loci was found in patient 4 (Supplementary Table 8). Finally, no somatic mutations were identified in major genes of the interferon-γ pathway (JAK1/2, STAT1, IFNG, IFN1/2) in any of the 148 genes expressed by the tumor. Selected peptides containing these neoepitopes were synthesized for functional testing.

Fig. 4 | Expansion of neoantigen-reactive CD8 T cells in a patient with NSCLC with complete response to radiotherapy and ipilimumab. a, Pipeline for neoantigen prediction. The somatic mutations identified by whole exome sequencing (WES) were filtered for expression of the mutated gene by RNA-seq, VAF (variant reads/total reads) > 0%, and binding to MHC-I alleles expressed by the tumor. Selected peptides containing these neoepitopes were synthesized for functional testing. b, c, Flow cytometry analysis of IFN-γ+ CD8 T cells after overnight stimulation of the post-treatment patient PBMCs with different peptides pools (33 peptides in 9 pools, each peptide was present in 3 separate pools) or dimethylsulfoxide (DMSO) as control. d, Reactivity to two peptides (p15 and p16) with the mutated (mt) or the germline (wt) KPNA2 amino acid sequence was tested in IFN-γ ELISPOT assay. Measurements (circles) including means (lines) are shown (n = 2 independent samples). The dotted line indicates 50 spots per well (cutoff for positive signal). e, KPNA2 mutation-reactive CD8 T cells were identified by intracellular IFN-γ expression and sorted for TCR-seq analysis. Frequency of the TCR clones from the sorted p15- and p16-reactive CD8 T cells in the patient blood at baseline (BL) and after treatment start. f, KPNA2 gene expression measured by quantitative PCR (qPCR) with reverse transcription (n = 2 for 0 and 1 x 8 Gy, and n = 4 for 3 x 8 Gy) in a NSCLC patient-derived xenograft (PDX) tumor 24 h after in vivo irradiation. Expression levels were normalized 0 Gy group average. Measurements (circles) including means (lines) are shown.
the four patients (Supplementary Dataset 4). Thus, known mechanisms of primary resistance to immune checkpoint inhibitors could not explain the lack of response in patients 36 and 38.

Although we did not find the global tumor mutation rates to correlate with response, we speculated that new tumor-specific T cell clones might be detected in patients who responded best to the combination of radiation therapy and anti-CTLA-4. To test this notion, we first tested the post-treatment peripheral blood of patient 4 for reactivity against the 33 top candidate neoepitopes identified using a neoantigen prediction pipeline (Fig. 4a). Eight candidates were selected on initial screening and two peptides (p15 and p16) were confirmed to be recognized by the patient’s CD8 T cells by enzyme-linked immunosorbent spot (ELISpot) assay (Fig. 4b–d). Both peptides contain the same mutation but p15 was predicted to bind to HLA-A2402, while p16 was predicted to bind to HLA-C1203, the two alleles expressed by the tumor at the HLA-A and -C loci (Supplementary Table 8). While the mutation did not alter the predicted binding affinity of the peptides to the respective HLA alleles, the wild-type peptides did not elicit a response in ELISpot assay, indicating T cell specificity for the mutated peptides (Fig. 4d). We next interrogated the in vitro expanded T cells against these two peptides by TCR-seq. One clone from p15-reactive CD8 T cells and one clone from p16-reactive CD8 T cells were identified in the blood of patient 4 (Fig. 4e and Supplementary Table 9). The clone reactive towards p16 was present in both pretreatment metastases and its frequency in the blood went from barely detectable at baseline to markedly expanded at day 22, remaining elevated at day 43 and at subsequent follow-up more than 6 months later (Fig. 4e).

We were intrigued by the robust CD8 T cell response in patient 4 and probed the biology of the protein from which the antigen was derived. The mutation recognized by the expanded clones derives from the protein, karyopherin α2 (KPNA2, also known as importin-α). Previous work has shown that KPNA2 is upregulated by radiation therapy in human colorectal cancer cells in vitro. To evaluate whether in vivo radiation therapy might increase expression of KPNA2 in NSCLC, we implanted a patient-derived tumor into mice, and then exposed these tumors to one or three fractions of 8 Gy (the latter being a regimen similar to the one used in patients). We found that tumor-derived KPNA2 gene expression was markedly upregulated at 24 h (Fig. 4f).

Collectively these results demonstrate that radiation therapy in combination with CTLA-4 blockade induces systemic tumor responses in patients with NSCLC. The early changes in TCR clonal dynamic seen in responders are consistent with a diversification and expansion of the tumor-directed TCR repertoire induced by radiation therapy, as previously reported by us and others in preclinical tumor models. In-depth analysis in representative patients suggests that expansion of a large number of tumor-specific T cell clones in peripheral blood and their persistence over time correlate well with achievement of abscopal responses.

Ipiilimumab was similarly effective at activating the peripheral T cell compartment in responding and non-responding patients, while only responders showed a significant induction of IFN-β. Thus, the different outcome might reflect radiation therapy ability to elicit the activation of a response in the tumor that mimics a viral infection. This interpretation is consistent with preclinical data demonstrating the requirement for radiation-therapy-mediated induction of IFN-β via the cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING) pathway for the activation of anti-tumor T cells. The contribution of the cancer cells themselves to the production of IFN-β was also shown to be essential to achieve abscopal responses in combination with immune checkpoint inhibitors. Patients with PD in this study show an impaired radiation therapy induction of IFN-β. While no mutations in cGAS and STING or other components of type I IFN signaling were found in patients 36 and 38, epigenetic downregulation of these genes, as recently shown in several tumors including lung cancer, cannot be excluded. Although we did not have viable tumor tissue to test this hypothesis, it is possible that in some tumors the radiation therapy dose threshold for the induction of the exonuclease TREX1, which controls radiation therapy ability to induce the activation of cGAS by cytosolic DNA, is lower, explaining the failure to activate type I IFN.

Our most intriguing result was the identification of two T cell clones that are markedly expanded in the periphery or induced de novo in one patient with CR. Both clones recognize a mutation that was present in two separate brain metastases removed surgically before the patient entered the trial. The mutation-generated epitope is encoded in a gene, KPNA2, that is upregulated by radiation therapy. Linking all of our findings, it appears that radiation therapy exposes immunogenic mutations by enhancing their expression in the tumor, followed by antigen cross-presentation and T cell activation inducing IFN-β. Although, to date, our complete analysis has been restricted to a single patient, we believe that these results may represent a paradigm underlying the synergy of radiation therapy and CTLA-4 blockade resulting in the induction of a robust antitumor T cell response.

Recently, ipilimumab was shown to add efficacy to nivolumab (an anti-PD-1 agent) in metastatic chemotherapy-naïve NSCLC with high mutational burden. The data presented here provide evidence for an additional role of ipilimumab in combination with radiation therapy in the treatment of metastatic NSCLC.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41591-018-0232-2.

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Author contributions

S.C.F., A.C., and S.D. conceived and designed the clinical protocol. S.C.F. and S.D. designed the correlative studies. E.G., B.C., and K.F. contributed to patients enrollment and treatment and/or response evaluation. N.-P.R. developed the neoantigen prediction pipeline and analyzed the TCR repertoire. A.H. and T.Z. helped with WES and RNA-seq. E.W., C.L., N.I., and S.G. performed flow cytometry and functional T cell studies. C.V.-B. contributed to cytokine measurements and the patient-derived tumor xenograft experiment. L.E.A and K.W.W. evaluated sMICA and antibodies. R.O.E. helped with TCR repertoire analysis. X.K.Z. performed the statistical evaluations. S.D., S.C.F., and N.-P.R. wrote the manuscript. All authors had final responsibility for the decision to submit this report as written for publication.

Competing interests

Bristol Meyers Squibb did not have any role in the design, data collection and analysis, interpretation of results and preparation of the manuscript. Potential conflicts of interest: Full-time employment and equity ownership at Adaptive Biotechnologies Corporation (R.O.E.). Prior honorarium for consulting from Third Rock Ventures/Neon Therapeutics, B4CC, OncoMed, Merck, and research funding from Agenus, Bristol Meyer Squibb, Genentech, Pfizer, Janssen R&D, Immune Design (S.G.). Service on Scientific Advisory Board of Lytix Biopharma, prior honorarium for consulting/speaker from AstraZeneca, AbbVie Inc., Cytume Pharma, EMD Serono, Eisai Inc., Regeneron, Ventana Medical Systems, Inc. Research grants from Nanobiotix, and Lytix Biopharma (S.D.). Prior honorarium for consulting/speaker from Sanofi, AstraZeneca, Merck, Regeneron, Bayer, Serono/Merck, and research funding from Janssen R&D, Varian, Merck, Bristol Meyer Squibb (for a different study) (S.C.F.). Service on Scientific Advisory Board of Nextech, T-scan and TCR2, consultancy for Novartis, research funding from Astellas, Bristol-Meyers Squibb and Novartis (not related to the topic of this manuscript) (K.W.W.).

Additional information

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Methods
Study design. NCT02221739 was a single institution pilot/futility trial of local radiotherapy and ipilimumab in chemotherapy-refractory metastatic patients with NSCLC. The study was designed to test the hypothesis that radiation to a metastatic site could be converted into an in situ vaccine and synergize with blockade of the CTLA-4 receptor to induce anti-tumor immune responses.

A phase II clinical trial based on an optimum two-stage phase II Simon design was used to conduct this pilot study. Ten patients were to be treated in stage I; in the absence of abscopal responses, the trial would terminate. With one or more abscopal responses in stage I, the trial would proceed to enroll an additional 29 patients: a total of at least 4 abscopal responses (10.25%) were needed to exclude futility. A radiation regimen of 6 Gy × 3 was tested for feasibility and safety during phase I. In the absence of grade 4–5 toxicity, the study was designed to move to a radiation regimen of 9.5 Gy × 3 in phase II. No other systemic or local agent was permitted during the course of the trial.

Patients. Eligible patients had to be aged 18 or older, have metastatic NSCLC and an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less, a life expectancy of >3 months, with adequate organ and marrow functional initial laboratory tests. Patients needed to have at least two distinct measurable metastatic sites. Patients with a previous history of brain metastasis were eligible if the brain metastases were controlled. A history of autoimmune disease excluded patients from eligibility to this trial. Similarly, any immunotherapy within four weeks of entering the study was a criterion for exclusion.

Endpoints. The primary endpoint of the study was the induction of immunity-mediated tumor response outside the radiation field (abscopal effect), as evidence of synergy of radiotherapy with ipilimumab. Per protocol these measurements excluded the irradiated lesion and were conducted by both immune-related response criteria (irRC) and RECIST1.1 criteria to facilitate comparison with other studies, and are reported consistently. Secondary endpoints were (1) To determine if clinical response was associated with an anti-tumor immune response and (2) to assess the toxicity of immunotherapy with radiotherapy in the tested regimens of dose and fractionation (6 Gy × 5 and 9.5 Gy × 3).

Study oversight and ethical compliance. The study protocol was approved by New York University and by Weill Cornell Medicine Institutional Review Boards, and the investigators complied with all ethical regulations pertaining to clinical research. All patients provided written informed consent before study entry. The study was deemed exempt from Investigational New Drug (IND) approval by the Food and Drugs Administration (FDA), exemption 122404. The Principal Investigator (S.C.F.) was responsible for oversight of the clinical study.

Bristol Meyers Squibb provided free ipilimumab for use in this study but did not have any role in the design, data collection and analysis, interpretation of results, and preparation of the manuscript.

Procedures. Patients underwent baseline radiographic assessment by position emission tomography (PET)/computed tomography (CT) within a week before first treatment to establish two measurable lesions and select one for radiotherapy. The radiotherapy regimen consisted of 6 Gy × 5 daily fractions in phase I of the study, and 9.5 Gy × 3 in phase II. Radiation was delivered by an external beam with linear accelerator with image-guided radiotherapy or intensity-modulated radiotherapy technique.

All patients received intravenous ipilimumab after the first radiation treatment, at a dose of 3 mg kg⁻¹. Ipilimumab was repeated every three weeks for four cycles, unless disease progression or death occurred, there was unacceptable toxicity, or a patient decided to withdraw from the study. With the exception of radiation-associated mucositis and/or skin toxicity, the protocol required the return to baseline of all grade 3–4 toxicities prior to the start of the next immunotherapy treatment. Dose modifications for hematologic or gastrointestinal toxicity were based on the worst toxicity observed during the prior dose. Specifically for >grade 3 diarrhea, immunotherapy was held until recovery to <grade 1. Ipilimumab-induced gastrointestinal toxicity was managed following established common guidelines (https://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM249435.pdf).

Assessment of response by PET/CT occurred at day 88 from first dose of ipilimumab. Patients were evaluated for abscopal responses (tumor regression of non-irradiated lesions) with baseline and post-treatment (day 88) PET/CT. Assessment excluded the irradiated site, both in baseline measurement and at day 88. Metastases were measured on axial CT in two perpendicular dimensions, per protocol, following irRC. Specifically, the products of the measurements (length × width) in all measurable lesions outside the radiation field were summed at baseline and compared to the sum of the measurements of the same lesions and potential new lesion post-treatment. The difference representing such a potential new lesion post-treatment. The difference representing such an abscopal response. Abscopal responses were assessed by PET/CT at day 88 and reported as: CR, complete resolution; PR, decrease in size ≥50% (irRC) or ≥30% (RECIST); PD, increase in size ≥25% (irRC) or >20% of the appearance of new lesions (RECIST); and SD, insufficient shrinkage or growth to qualify for PR/CR or PD. Toxicities were reported according to the common terminology criteria for adverse events (CTCAE) version 4.0.

Serial blood samples were collected for serum, plasma, and peripheral blood mononuclear cells (PBMCs) at baseline, on days 22, 43, 64, and 88, and when indicated, at follow-up visit. Viable PBMCs were stored in liquid nitrogen. Serum and plasma samples were stored frozen in aliquots.

Immunohistochemistry. Tumor tissue was analysed for programmed cell death ligand 1 (PD-L1) expression by immunohistochemistry, performed on formalin-fixed, paraffin-embedded, 4-µm tissue sections using unconjugated, rabbit anti-human PD-L1 (CD274) clone SP142 (PD-L1, Spring Biosciences catalog number M44220). Staining was performed on a Ventana Medical Systems Discovery XT instrument with online deparaffinization and using Ventana’s reagents and detection kits. PD-L1 was antigen retrieved in Ventana Cell Conditioner 1 (Tris-borate-EDTA) for 20 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 4 min. PD-L1 was diluted 1:50 in phosphate buffered saline and incubated for 60 min at 37°C. Primary antibody was detected with hapten linked anti-rabbit multimer incubated for 20 min followed by anti-hapten horseradish peroxidase conjugate for 20 min. The complex was visualized with 3,3-diaminobenzidine and enhanced with copper sulfate. A tissue microarray containing placential tissue was used as positive control. PD-L1 expression was quantified as the percentage of tumor cells with positive membrane staining and intensity classified on a scale of 1 to 3. In some samples, CD8 T cell infiltration was assessed by staining with rabbit anti-human CD8 clone SP57 (Ventana Medical Systems catalog number 790–4460). The number of CD8⁺ intratumor T cells was counted in three to five representative fields (magnification ×200). CD8 T cell infiltration was considered marked (average >50 cells per ×200 field), moderate (average 10–49 cells per ×200 field), and minimal (average <10 cells per ×200 field).

Galectin, IFN-β, and sCD25 ELISA measurements. We used enzyme-linked immunosorbent assay (ELISA) to measure serum levels of each marker that has been shown to have prognostic value in patients with melanoma treated with ipilimumab, (b) IFN-β that is mechanistically relevant to the immunogenicity of radiotherapy, and (c) galectin-1, an immune suppressive tumor-secreted factor that can be increased by radiation. Serum samples were stored at −80°C and thawed once prior to analysis. Commercially available ELISA kits were used to determine serum levels of galectin-1 (Cat D5G10, R&D Systems), IFN-β (Cat 41415-1, PBL assay Science), and soluble CD25 (Cat BMS212INST, ebioscience) according to manufacturer’s instructions with each sample run in triplicate wells. The mean minimum detectable concentrations for Gal-1, IFN-β, and sCD25 are 0.022 ng ml⁻¹, 1.29 pg ml⁻¹, and 0.21 ng ml⁻¹, respectively.

Analysis of antibodies specific for MICA/B. MICA and MICB antibodies in human sera were analyzed with a fluorescence-based sandwich ELISA. Recombinant soluble proteins representing the MICA*008 or MICB*005 extracellular domains were incubated separately at 1 µg ml⁻¹ in DELFIA Yellow 96-well plates for 12–16 h at 4°C. The wells were then washed with TBS/0.05% Tween-20 and blocked with DELFIA Assay Buffer (PerkinElmer, catalog 1244-111) for 2 h at room temperature. Human serum samples were diluted 1:1,000 in DELFIA Assay Buffer, added to the wells, and incubated for 2 h at room temperature. The wells were subsequently washed with TBS/0.05% Tween-20 and incubated for 1 h at room temperature with DELFIA Eu-N1 Anti-Human IgG (PerkinElmer, catalog 1244-330) diluted 1:500 in DELFIA Assay Buffer. Wells were then washed with TBS/0.05% Tween-20 followed by addition of DELFIA Enhancement Solution (PerkinElmer, catalog 1244-105). Time-resolved europium fluorescence was analyzed using an EnVision Plate Reader (PerkinElmer). Analyses were done in triplicate for each sample.

Quantification of serum-shed MICA and MICB. Shed MICA and MICB were measured in human sera were quantified using Human MICA ELISA Kit (AbCam, ab59569) and Duoset Human MICA Kit (R&D Systems, DY1599), respectively. The procedures were performed according to recommendations in the data sheets for both kits. All replicates were run for each sample. Human sera were quantified using Human MICA ELISA Kit (AbCam, ab59569) and Duoset Human MICA Kit (R&D Systems, DY1599), respectively. The procedures were performed according to recommendations in the data sheets for both kits. All replicates were run for each sample. Quantification of serum-shed MICA and MICB.

Flow cytometry analysis of PBMCs. Cryopreserved PBMCs were thawed and stained with fixable viiability dye eFlour 430 (eBioscience) for 30 min at 4°C. FeR-binding inhibitor (ebioscience) was added to all cells followed by incubation with antibodies (see the Nature Research Reporting Summary for a list of antibodies and dilutions used).

Prior to addition of the antibodies targeting intracellular targets, cells were permeabilized and fixed using Fixation/Permabization Solution Kit (ebioscience). Samples were acquired on a MACSquant Analyser 10 (Miltenyi.
Biotec) and data were analyzed using the FlowJo software (Treestar). The gating strategy is illustrated in Supplementary Fig. 6.

TCRB CDR3 sequencing and repertoire analysis. DNA was isolated from PBMCs using the DNeasy Blood & Tissue Kit (Qiagen). Amplification and sequencing of TCRβ CDR3 regions was performed at Adaptive Biotechnologies (Seattle, WA) using their developed ImmunoSEQ platform49. The TCRβ CDR3 regions in the sequencing reads were defined according to the ImMunoGeneTics collaboration. A standard algorithm was used to identify which V-, D-, and J-segments contributed to each TCRβ CDR3 sequence49, and only in-frame TCR rearrangements were included in the analysis. For all samples, a standardized amount of DNA was used to amplify and sequence TCRβ CDR3 regions. Several molecular and bioinformatic methods are used to control for PCR bias and ensure that the ImmunoSEQ assay is quantitative49. For every sample investigated, synthetic TCR genes are added and act as controls for bioinformatic normalization of amplification bias for every V and J gene segment in the genome. These synthetic molecules also permit measurement of the absolute cellular abundance of T cell clones in a sample50. TCRβ CDR3 repertoire completeness was calculated as 1 – Piolo’s evenness51. Normalization ensures that estimation of completeness is robust to variations in sample size and enables assessment of the relative degree of clonality between samples.

Expansion and contraction of T cell clones was determined using the Differential Abundance tool in the Adaptive Biotechnologies immunoSEQ analyzer. Analysis settings: count = nucleotide; minimum total filter = 10, productive only = TRUE; method = binomial; alternative = two-sided; multiple test correction = Bonferroni; alpha = 0.01. Distance matrix and normalization were calculated using UPGMA algorithm. Secondly, we performed an in-depth analysis of the TIL-TCR dynamics for patients 4, 32, 36, and 38, for which we were able to perform TCR sequencing on pretreatment tumor tissue. Clones with statistically significant expansion in PBMCs from baseline during treatment were determined and classified as TILs if they were also present in ≥1 tumor biopsy (patient 4 had two tumor tissue samples available). When plotting TCR frequency against time, frequencies were log10 transformed, and not detected clones were given a frequency of 10⁻⁶ (annotated as n.d. in figures).

The Needleman–Wunsch (NW) global alignment algorithm was used to calculate the similarity between TIL-TCR CDR3 regions for patient 4 (previously described in refs. 22, 23). All work related to sequence similarity analysis was performed in R 3.4.3. A distance matrix based on NW similarity score was calculated between all TIL-TCRs >0.1% in the tumor and not increased in blood, together with the TIL-TCRs detected in blood during treatment and present in one or both tumor samples. The distance matrix was calculated using the score function within the Biostrings package (version 2.46.0; settings: type = global, substitutionMatrix = PAM10, gapOpening = 30, gapExtension = 8). Then, the NJ function within the phangorn package (version 2.4.0) was used to construct a sequence similarity dendrogram52. The result was visualized as weighted unrooted dendrograms using the ggtree function within the ggtree package (version 1.1.0.153). The areas of circles represent frequency of a given TIL-TCR clone.

RF classification and variable importance analysis. RF classification was performed using the randomForestSRC package (version 2.5.1) in R 3.3.254. Patients’ response was classified using variables with statistically significant difference when comparing day 22 and baseline for any response group. The rsrc function was executed in R with default parameters except for ntree = 2000, tree = 1, importance = T, n.a.action = na.impute. Variable importance scores, classification error rates, and maximal subtree data were determined from 1,000 executions of rsrc. Each variable, importance score average and standard deviation were calculated. All RF classification analysis, patient 33 was removed due to missing data for all variables except ALCs. Additionally, missing flow cytometry for patients 17, 23, and 44 were imputed using the default missing data algorithm within the R randomForestSRC rsrc function154,155.

Whole exome sequencing. DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor blocks using the QIAGEN QIAamp DNA FFPE Tissue kit (catalog no. 56404). Then, 250 ng of DNA from each sample were sheared on a Covaris instrument for 360’s (duty cycle, 10%; intensity, 5; cycles per burst, 200). Bar-coded libraries were prepared using the Kapa Low-Throughput Library Preparation Kit Standard (Kapa Biosystems), amplified using the KAPA HiFi Library Amplification kit (Kapa Biosystems), and subjected to quality control and Bioanalyzer analysis using Qubit Fluorimetric Quantitation (Invitrogen) and Agilent Bioanalyzer. Two equimolar pools of the 6 and 5 bar-coded libraries, respectively, were used as input to capture the exome using one reaction tube each of the NimbleGen SeqCap EZ Human Exome Library v3.0 (Roche, catalog no. 00646586001), according to the manufacturer’s protocol. The pooled capture libraries were quantified by Qubit (Invitrogen) and Bioanalyzer (Agilent) and sequenced on an Illumina HiSeq 2500 using a paired end, 100 nucleotides in length run mode, across three lanes. The sequencing reads were cleaned by trimming adapter sequences and low-quality bases. Clones were aligned to the human reference genome (GRCh37) using Burrows-Wheeler Aligner (BWA) software55. The aligned bam file was further refined by duplicate removal with the Picard toolkit, and indel realignment and base quality recalibration with the Genome Analysis Toolkit56.

Baseline tumor and PBMC WES data were used in VarScan2 to detect somatic variants57. Variants with somatic P < 0.05 were considered statistically significant.

RNA sequencing. RNA was isolated from FFPE tumor blocks using the RNeasy FFPE kit (catalog no. 73504). Libraries were prepared using the Illumina TrueSeq Stranded Total RNA library preparation, after ribodepletion with Ribozero Gold kit (catalog no. 20020597) starting from 800 ng of DNAs treated total RNA, following the Biostris’s protocol, with the exception that 10 cycles of PCR were performed to amplify the libraries, to keep the duplication rate lower than with the recommended 15 cycles. The amplified libraries were purified using AMPure beads, quantified by Qubit and qPCR, and visualized in an Agilent Bioanalyzer. The libraries were pooled equimolarly, and sequenced on an Illumina HiSeq 2500 instrument, running v4 chemistry with 50 base pair-end reads. The sequencing reads were cleaned by trimming adapter sequences and low-quality bases, and then aligned to the human reference genome (GRCh37) using STAR58. Cufflinks was used to measure transcript abundances in fragments per kilobase of the exon model per million mapped reads (FPKM)159.

Neotagen predictions. To predict patient-specific neoantigens, HLA typing was performed from WES data using Optitype, a HLA genotyping algorithm (Supplementary Table 8). Before predicting, variant call format (VCF) files containing somatic mutations were subjected to standard variant effect predictor (VEP), downstream, and wild-type annotations, and filtered for frameshift and missense mutations. Finally, VAF data was generated using bamreadcount (https://github.com/genome/bam-readcount). The NetMHC and NetMHCpan algorithms were used to predict patient-specific MHC-I binding for all possible 8–11 amino acid long variant and corresponding wild-type peptide chains spanning the mutation using the pVAC-seq pipeline57.

When comparing neotagen load between patients 4, 32, 36, and 38, a neotagen was defined as a variant peptide binding MHC-I with an affinity <500 nM passing the following filters: normal tissue WES site coverage >5, normal tissue VAF <3, tumor tissue WES site coverage >5, and tumor tissue VAF >10. Additionally, a lung adenocarcinoma gene expression dataset was used to determine median RNA gene expression for each gene encoding a predicted neoepitope57.

For patient 4 (CR), baseline tumor tissue was sufficient to perform RNA-seq in addition to WES. Gene expression levels were calculated using cufflinks and RNA-seq VAF data were determined using bamreadcount. Then, variant peptides for this patient were further filtered on baseline tumor gene expression (>1 FPKM) and RNA-seq VAF (>0%).

T cell response to neoantigens. The 33 peptides that qualified as potential neoantigens were synthesized at a purity exceeding 85% (Genscript), dissolved in DMSO (5 mg/ml) and tested in vitro for recognition by the patient 4 T cells from peripheral blood samples. PBMCs were thawed and activated with the peptides (final concentration: 2 μg/ml) in RPMI medium ( Gibco) containing 5% human AB serum (Sigma-Aldrich, H3667), 30 μl/ml IL-2 (Miltenyi Biotec, 130-097-745), 20 μl/ml IL-7 (Miltenyi Biotec, 130-093-937), 10 μl/ml IL-15 (Miltenyi Biotec, 130-093-955), and 1x non-essential amino acids (Sigma-Aldrich, M7145) in a 96-well round-bottom plate. Peptides were first tested as pools (33 peptides in 9 pools) in comparison with DMSO treatment alone. Brefeldin A (Thermo Fisher Scientific, 00-4506-51) was added 1 h after adding the peptides to the culture. After overnight incubation, cells were washed and stained with the viability dye eFlour 450 (Thermo Fisher Scientific, 65-0863-14) and with CD3-APC and CD8-PerCP-eFluor780 antibodies (Miltenyi Biotec, 130-091-373 and 130-091-525, respectively). Subsequently, all RF were fixed and permeabilized (Thermo Fisher Scientific, 88-8824-00) before intracellular staining of IFN-γ FITC (Miltenyi Biotec, 130-091-641). Samples were analyzed on a MACSQuant flow cytometer (Miltenyi Biotec) and data were analyzed using FlowJo software. Peptides selected from the positive pools were then tested individually using the same procedure.

In this experiment, the reactivity of healthy donor CD8+ PBMCs against the same peptides was assessed, demonstrating a lack of background/non-specific staining.

Patients’ PBMC reactivity towards selected peptides was then confirmed by measuring IFN-γ production using a standard overnight ELISPOT assay. Briefly, 96-well nitrocellulose plates (Milltipore Sigma, MSHA54510) were coated with anti-IFN-γ monoclonal antibody (4 μg/ml) and incubated overnight at 4 °C. Plates were washed and blocked with 0.5% BSA (BioRad) and supplemented with 10% human serum for 2 h at 37 °C. T cells stimulated for 20 days with the test peptide were added to wells in duplicate at 25,000 or 5,000 CD8+ T cell per well and were stimulated overnight with autologous B-APCs loaded with mutated peptides or their wild-type counterpart (2 μg/ml). Stimulation with phosphol 12- and 13-acetate (PMA)/ionomycin was used as positive control for T cell reactivity. After 18 h,
the ELISpot plate was washed and processed for spot development following manufacturer's instructions. Spots were counted using an automated ELISpot plate reader (CTL Immunospot, Cellular Technology Limited). Empirically, we consider that four times the spot number in the well with 5,000 CD8+ T cells is equivalent to the spot number in the well with 25,000 CD8+ T cells. This spot number correction was used when calculating spot number means. Peptide-stimulated responses were considered positive if the mean >50 spots (positive cutoff).

**Gene expression analysis using qRT–PCR.** Patient-derived tumor xenographs (PDXT) from freshly resected primary lung tumor were implanted in NOD/SCID/gamma (NOG) female mice (CIEA NOG mouse; NOD.Cg-Prkdcscid (PDTX) from freshly resected primary lung tumor were implanted in NOD/SCID/B6 mice. Patient-derived tumor xenografts were analyzed using gene expression analysis using qRT‒PCR. Five hundred spots (positive cutoff) were analyzed by the ELISpot reader (CTL ImmunoSpot, Cellular Technology Limited). Empirically, we consider that four times the spot number in the well with 5,000 CD8+ T cells is equivalent to the spot number in the well with 20,000 CD8+ T cells. This spot number correction was used when calculating spot number means. Peptide-stimulated responses were considered positive if the mean >50 spots (positive cutoff).

**Statistical analyses.** All time- and event endpoints were summarized using the Kaplan–Meier method. Differences in these endpoints between groups of interest were examined using a log-rank test. These analyses were carried out among the intention-to-treat population. A two-sided P < 0.05 was considered statistically significant.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data reported are tabulated in the manuscript and supplementary figures and tables, and Supplementary Dataset 2 and 3. Raw data for soluble markers and flow cytometry are available in Supplementary Dataset 1. The raw TCR sequence data tables, and Supplementary Dataset 2 and 3. Raw data for soluble markers and flow cytometry are available in Supplementary Dataset 1. The raw TCR sequence data tables include in every run. The reaction was conducted in the Applied Biosystems plate (Applied Biosystems), and a negative control with no cDNA template was included in every run. The reaction was conducted in the Applied Biosystems 7500 real-time PCR cycler (Thermo Fisher Scientific) with amplification under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A melting curve analysis was also done to ensure the specificity of the corresponding RT–PCR reactions. The relative expression of the gene was determined with the 2−ΔΔCT method, with RPL13a as a housekeeping gene.

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Reporting Summary

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| Clearly defined error bars | Yes |
| State explicitly what error bars represent (e.g. SD, SE, CI) | Yes |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | No software was used to collect data. |
|---|---|
| Data analysis | Random forest classification: randomForestSRC package (version 2.5.1) in R 3.3.2. Whole exome sequencing and variant calling: BWA, GATK, Picard, Varscan2. RNA-sequencing: STAR, Cufflinks HLA-typing and neoantigen predictions: Optitype, VEP, NetMHC, pVAC-seq. Needleman-Wunsch TCRb CDR3 region sequence similarity: Biostrings, NJ, and ggtree packages in R 3.4.3. Flow Cytometry: FlowJo version 10.1. Graphical display: GraphPad Prism version 7.0a Statistical analysis: R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data reported are tabulated in the main text and supplemental materials. The raw TCR sequence data have been deposited into the ImmuneACCESS project repository of the Adaptive Biotech database (doi:10.21417/B7BW6X). WES and RNAseq data have been deposited into the Sequence Read Archive (Study SRP136187).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences    ☐ Behavioural & social sciences    ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
A Phase II clinical trial based on an optimum two-stage Phase II Simon design was used to conduct this pilot study. Ten patients were to be treated in Stage 1; in the absence of abscopal responses, the trial would terminate. With one or more abscopal responses in Stage 1, the trial would proceed to enroll an additional 29 patients: a total of at least 4 abscopal responses (10.25%) were needed to exclude futility.

Data exclusions
No data were excluded.

Replication
In vitro measurements (e.g., measurements of soluble factors in the serum) were performed in duplicate or triplicate with similar results.

Randomization
Randomization is not relevant to our study because the study was not designed to compare two treatment arms. A radiation regimen of 6 Gy X 5 was tested for feasibility and safety during Phase I of the two-stage Simon design. In the absence of grade 4-5 toxicity, the study continued to a radiation regimen of 9.5 Gy X 3 in Phase II.

Blinding
Blinding was not relevant to this study since all patients received treatment and the study was not meant to compare outcome of different treatments.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Unique biological materials |
| ☒   | Antibodies |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology |
| ☒   | Animals and other organisms |
| ☒   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq |
| ☒   | Flow cytometry |
| ☒   | MRI-based neuroimaging |

Antibodies

Antibodies used
For immunohistochemistry:
Rabbit anti-human PDL-1 (CD274) clone SP142 (PDL1, Spring Biosciences catalog number M4420) was used at 1:50 dilution
Rabbit anti-human CD8 clone SP57 (Ventana Medical Systems catalog number 790-4460) was used at 1:100 dilution

For flow cytometry:
Antibody/dye Fluorochrome - Clone - Manufacturer - Concentration
Viability dye eFluor 450 - NA - eBioscience - 1:500
CD4 APC-vio770 M-T466 Miltenyi Biotec 1:50
Validation

The antibodies are commercially available and validated by the manufacturer. We used healthy donors peripheral blood cells to confirm the staining specificity and dilutions for flow cytometry. For immunohistochemistry a tissue microarray containing placental tissue was used as positive control for PDL-1 staining, and tissue microarray containing lymph node and tonsil tissue was used as positive control for CD8 staining.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals
Patient-derived tumor xenografts (PDTX) from freshly resected primary lung tumor were implanted in NOD/SCID/gamma (NOG) female mice (CIEA NOG mouse; NOD.Cg-Prkdcscid Iilitgtm1Sug/JicTac, Taconic Animal Laboratory, Germantown, NY), age 4 to 6 weeks.

Wild animals
The study did not involve wild animals.

Field-collected samples
No field-collected samples were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics
Woman (n=23) and men (n=16) age 48 to 97 with metastatic non-small cell lung cancer and at least 2 distinct measurable metastatic sites, an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less, and at least one prior therapy.

Recruitment
Patients with metastatic NSCLC who presented at NYU for medical care and fulfilled the eligibility criteria (see online methods) as per approved protocol were offered participation in the study.
Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: See methods

Instrument: Samples were acquired on a MACSquant Analyser 10 (Miltenyi Biotec)

Software: Data was analyzed using the FlowJo software version 10.1 (Treestar).

Cell population abundance: Not applicable.

Gating strategy: Gating strategy is illustrated in Supplementary Figure 6.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.