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Improved survival associated with local tumor response following multi-site radiotherapy and pembrolizumab: secondary analysis of a phase I trial

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Abstract

Background: Multi-site stereotactic body radiotherapy followed by pembrolizumab (SBRT+P) has demonstrated safety in advanced solid tumors (AST). However, no studies have examined the relationships between irradiated tumor response, SBRT-induced tumor gene expression, and overall survival (OS).

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Conflict of Interest:

JJL declares Data and Safety Monitoring Board: TTC Oncology, Scientific Advisory Board: 7 Hills, Actym, Alphamab Oncology, Array, BeneVir, Mavu, Tempest, Consultancy: Aduro, Astellas, AstraZeneca, Bayer, Bristol-Myers Squibb, Castle, CheckMate, Compugen, EMD Serono, IDEAYA, Immunocore, Janssen, Jounce, Leap, Merck, Mersana, NewLink, Novartis, RefleXion, Spring Bank, Syndax, Tempest, Vividion, WntRx, Research Support: (clinical trials unless noted) AbbVie, Array (Scientific Research Agreement; SRA), Boston Biomedical, Bristol-Myers Squibb, Celldex, CheckMate (SRA), Compugen, Corvus, EMD Serono, Evelo (SRA), Delcath, Five Prime, FLX Bio, Genentech, Immunocore, Incyte, Leap, MedImmune, MacroGenics, Novartis, Pharmacyclics, Palleon (SRA), Merck, Tesaro, Xencor, Travel: Array, AstraZeneca, Bayer, BeneVir, Bristol-Myers Squibb, Castle, CheckMate, EMD Serono, IDEAYA, Immunocore, Janssen, Jounce, Merck, Mersana, NewLink, Novartis, RefleXion, Patents: (both provisional) Serial #15/612,657 (Cancer Immunotherapy), PCT/US18/36052 (Microbiome Biomarkers for Anti-PD-1/PD-L1 Responsiveness: Diagnostic, Prognostic and Therapeutic Uses Thereof).

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Methods: Patients with AST received SBRT (30–50 Gy in 3–5 fractions) to 2–4 metastases followed by pembrolizumab (200mg IV every 3 weeks). SBRT was prescribed to a maximum tumor volume of 65cc. Small metastases received the complete prescribed coverage (complete-Rx), while larger metastases received partial-coverage (partial-Rx). Treated metastasis control (TMC) was defined as a lack of progression for an irradiated metastasis. Landmark analysis was used to assess the relationship between TMC and OS. Thirty-five biopsies were obtained from 24 patients: 19 pre-SBRT and 16 post-SBRT (11-matched) prior to pembrolizumab and were analyzed via RNA microarray.

Results: 68 patients (139 metastases) were enrolled with a median follow-up of 10.4 months. One-year TMC was 89.5% with no difference between complete-Rx or partial-Rx. On MVA, TMC was independently associated with a reduced risk for death (hazard ratio, 0.36; 95% CI, 0.17–0.75; $P=0.006$). SBRT increased expression of innate and adaptive immune genes and concomitantly decreased expression of cell cycle and DNA repair genes in the irradiated tumors. Elevated post-SBRT expression of *DNASE1* correlated with increased expression of cytolytic T cell genes and irradiated tumor response.

Conclusions: In the context of SBRT+P, TMC independently correlates with OS. SBRT impacts intratumoral immune gene expression associated with TMC. Randomized trials are needed to validate these findings.

Keywords

PD-1; PD-L1; Immune checkpoint inhibitor; Immunotherapy; Pembrolizumab; SBRT; Stereotactic Ablative Radiotherapy; Partial tumor irradiation; Phase 1 Trial

Introduction:

Tumors with sustained responses to immunotherapy demonstrate a T cell-inflamed tumor microenvironment characterized by tumor infiltrating lymphocytes (TILs) and type I/II interferon (IFN) gene expression profiles^{1,2}. By contrast, tumors that fail to respond to immunotherapy frequently exhibit a non-T cell-inflamed tumor phenotype. High-dose stereotactic body radiation (SBRT) has been reported to promote immunogenic cell death through activation of innate and adaptive immunity orchestrated by IFN signaling^{3,4}. These observations have led to several investigations exploring the use of radiation to enhance response to immunotherapy.

We previously reported the initial results of a clinical trial investigating the safety of treating metastases in multiple body sites with SBRT followed by pembrolizumab⁵. Dose-limiting toxicities (DLTs) were seen in <10% of enrolled advanced solid tumor patients. We observed a RECIST objective response rate (ORR) of 13% in a population of predominately programmed death ligand-1 (PD-L1)-low tumor types, which was similar to the ORR of PD-L1-positive cancer patients treated with pembrolizumab alone on KEYNOTE-28⁶. Our finding indicates that the primary effect of combination SBRT+P was localized to irradiated metastases and not to systemic, non-irradiated metastases. Additionally, we observed >90% control rates of irradiated metastases at four months even though many metastases received the prescription dose to only a portion of the tumor volume to minimize potential toxicity,

which has historically resulted in poor local control. Therefore, a secondary analysis of this trial was performed to investigate the biological effects of SBRT as they relate to pembrolizumab response, we analyzed gene expression profiles of irradiated tumors and their impact on treated metastasis control (TMC). Here, we report outcomes based on local tumor response and tumor gene expression patterns.

Methods

Study Design and Participants

Patients with advanced solid tumors were enrolled between January 2016 and March 2017 ([clinicaltrials.gov: NCT02608385](https://clinicaltrials.gov/ct2/show/study/NCT02608385))⁵. Study enrollment began after the institution internal review board approved the study, written informed consent was obtained, and pursued per Declaration of Helsinki. Data lock occurred on 5/01/18. Patients received SBRT to at least two measurable metastases with each receiving 30–50 Gy over 3–5 fractions^{5,7}. Metastases were prioritized based on the largest size and/or those causing the most morbidity. The SBRT prescription dose was limited to a maximum of 65cc such that tumors >65cc received the prescription dose to part of the metastasis (partial-Rx). Otherwise, tumors received SBRT to the entire metastasis (complete-Rx). Pembrolizumab (200mg IV every three weeks) began within seven days following SBRT (SBRT+P). Tumor assessments were performed every 2–3 months using Response Evaluation for Solid Tumors 1.1 (RECIST) principles. Osseous and spinal metastases were considered controlled as part of the TMC measurement as per MD Anderson response criteria⁸. Under FDA guidance, we attributed dose-limiting toxicities (DLTs) to the combination of SBRT+P therapy by anatomical organ system, and not to each specific therapy.

Tumor Tissue Analysis

Thirty-five tumor biopsies were collected prior to and/or within 7 days following SBRT, but prior to pembrolizumab, from 24 patients: 19 pre-SBRT and 16 post-SBRT (11 matched samples from the same tumor) (Table S1). RNA was extracted and analyzed using Affymetrix Human Clariom™ D microarrays. Computational gene expression deconvolution methods were used for genome-wide expression analyses following SBRT and correlated with irradiated tumor response. We utilized Ingenuity Pathway Analysis (IPA) to predict the upstream regulators of pathways involved in the gene expression changes. See Supplemental Data for details regarding baseline PD-L1 expression, microarray data pre-processing, and detection of treatment-responsive gene expression changes. A TaqMan RT-PCR assay (ThermoFisher) was used to validate *DNASE1* gene expression utilizing an exon-spanning probe (Hs00173736_m1) overlapping the Clariom D transcript cluster TC1600011345.hg. Expression values for *DNASE1* were normalized to the geometric mean of β -actin and 18S rRNA.

Statistical Analysis

Baseline characteristics were compared between patients who received complete-Rx (to all treated lesions) and partial-Rx (to at least one of the lesions) using *t*-tests and Pearson's χ^2 test for continuous and discrete variables, respectively. TMC was defined as a lack of progression for an irradiated metastasis^{8–10}, and duration of control was estimated using

the Kaplan-Meier method. Death without irradiated tumor progression led to censoring at time of death¹⁰. TMC and overall treatment response (i.e. CR, PR, SD, or PD) were evaluated using shared frailty Cox regression models and generalized estimating equations (GEE) to account for correlation of individual metastasis responses within patients. At the first scan, patients who had a 30% or greater decrease (50% for osseous/spine) in the maximum transverse diameter of each irradiated metastasis were classified as responders, and those who had a 20% or greater growth (25% for osseous/spine) in the maximum transverse diameter of one or more irradiated metastases were non-responders. Patients who did not fit either category were deemed to have stable disease (mixed responders). We used the Kaplan-Meier method to estimate progression-free survival (PFS) utilizing the time from SBRT to either progression or death from any cause and overall survival (OS) using the time from SBRT to death. Landmark analysis at 2 months was used to avoid a guarantee time bias when examining the association of early irradiated metastasis response with OS¹¹. We stratified outcomes using clinical, prognostic, and pathologic variables and compared differences using the log-rank test and Cox regression modeling. Baseline PD-L1 staining was available from 51 analyzable patients, and a cut-off of 1% was used to define PD-L1 positivity. P-values <0.05 were considered statistically significant. Multivariable Cox regression analysis was performed for covariates with univariate p-values <0.10. Data were analyzed using Stata software version 15.0 and JMP version 14 (SAS Institute Inc.).

Results

Baseline Characteristics and Toxicity

Seventy-nine patients were enrolled, and 11 patients were excluded from the analysis due to: not receiving SBRT (n=3); refusing pembrolizumab (n=3); or lost to follow-up (n=5). The overall median follow-up time (n=68) at data lock was 10.4 months (range 2.3–24.1), and was 33.4 months (range 15.3–42.5) for the 17 patients alive past the data lock. Sixty-eight patients with 26 different, non-radiosensitive and/or generally non-PD-1 responsive, solid tumor types who received SBRT to 139 metastases were analyzed (Table S2). Patients had previously received a median of 5 lines of systemic therapy, and 3 had received checkpoint therapy. Table 1 summarizes baseline patient-level (n=68) characteristics stratified based on the receipt of partial-Rx to at least one metastasis. A representative example of partial-Rx versus complete-Rx treatment is shown in Figure S1. In 50 patients (73%), 102 metastases received the radiation prescription dose to the entire tumor volume (complete-Rx), whereas in 18 patients (26%) at least one metastasis (21 of 37 metastases) received the prescription dose to less than the entire tumor volume (partial-Rx). There were no differences in tumor histology (p=0.19), baseline PD-L1 status (p=0.92), or prior lines of therapy (p=0.54) between patients stratified by who received all complete-Rx versus at least one partial-Rx. There were only 6 DLTs: 6 (all metastases complete-Rx), 0 (at least one metastasis partial-Rx), for an overall rate of 8.8%.

Treated Metastasis Control

The 1-year Kaplan-Meier TMC rate was 89.5% (Figure 1A) consistent with other reports following complete volumetric tumor coverage with high-dose SBRT^{12–14}. However, 21 of the 139 treated metastases received partial-Rx SBRT+P. The partial-Rx treated metastases

(Table S4) were larger at baseline (mean volume: 157.6 cc vs. 12.8 cc, $p<0.001$) and more likely to be located in the abdomen or pelvis ($p<0.001$) (Table S3). We observed no significant difference in TMC between complete-Rx vs. partial-Rx SBRT+P (Figure 2) despite differences in the metastasis target volume receiving at least 95% of prescribed radiation dose (median volume for partial-Rx vs. complete-Rx: 67.2% vs. 100%), however the low number of events limits statistical power to draw firm conclusions. Moreover, there were no significant differences in TMC when stratified by baseline PD-L1 status ($p=0.81$, Figure 1B), or tumor volume irradiated ($p=0.86$, Figure 1C). By contrast to typical SBRT plans¹⁵, we observed similar TMC rates independent of the metastasis size or volume receiving the complete prescription dose when delivered prior to pembrolizumab.

Association of SBRT+P Response and Survival

We analyzed treatment responses of individual metastases and at the patient-level (Figure S2). Seven metastases (5%) had a complete response and 42 (30.2%) metastases had a partial response for an overall treated metastasis response rate of 35.2%. Eighty-one metastases (58.3%) were stable with 9 (6.5%) demonstrating immediate progression following SBRT+P. No differences in SBRT+P response were observed between patients who received complete-Rx versus partial-Rx SBRT+P ($p=0.15$). Partial-Rx SBRT+P did not affect local treatment response ($p=0.35$, Figure S3A), nor did tumor volume ($p=0.44$, Figure S3B), baseline PD-L1 status ($p=0.12$, Figure S3C) or histology ($p=0.41$, Figure S3D). Table S5 summarizes all factors analyzed in this regression model.

The ORR of the non-irradiated metastases was 13.2% as previously reported. The median PFS was 3.1 months (95% CI 2.9–3.5) (Figure S4A) and the median OS was 9.9 months (95% CI 7.0–13.5) (Figure S4B). No covariates were predictive of OS on univariate Cox regression (Table S6). Also, no significant differences in OS were observed between patients stratified by partial-Rx status (Figure S5A) or baseline PD-L1 expression (Figure S5B). These findings demonstrated that TMC to SBRT+P occurred independent of baseline or treatment characteristics.

We analyzed OS based on irradiated tumor response (landmark analysis). This is supported by a direct relationship between the change in size of unirradiated lesions as a function of response of irradiated lesions (Figure S6). Only one patient died prior to their first scan, and of the 67 patients who survived beyond the 2-month landmark time, the median OS was 3.5 months for non-responders (95% CI, 2.3–9.6 months), 9.0 months (95% CI, 6.5–12.9 months) for mixed responders, and 17.8 months (95% CI, 7.7–37.7 months) for responders ($p=0.0007$, Figure 3). Moreover, on post-landmark Cox regression, a larger irradiated tumor response, coded as an ordinal variable (0=no response, 1=mixed response, 2=response), decreased the risk for death (hazard ratio, 0.44; 95% CI, 0.23–0.85; $P = 0.015$). These results demonstrate that patients whose tumors exhibited a local response to the combination of SBRT+P were more likely to experience extended survival.

Gene Expression Analysis

We investigated radiation-induced gene expression changes associated with local response in serial biopsies acquired from irradiated metastases. First, we performed a genome-wide

analysis to characterize gene expression changes following SBRT from 11 patients with both pre- and post-SBRT biopsies (matched samples). SBRT increased the expression of pathways enriched for innate and adaptive immunity, such as B-cell development, antigen presentation and dendritic cell maturation, while concomitantly reducing the expression of pathways involved in G2/M cell cycle progression and DNA damage repair processes (Figure 4A). Immune genes in the activated pathways were predicted to be regulated by type I/II interferon signaling, whereas down-regulation of cell cycle and DNA repair processes were predicted to be in response to RAD21 and CDKN2A signaling pathways (Figure 4A)¹⁶. Furthermore, the response to SBRT was associated with suppression of histone subunits and genes mediating mitosis and cell division, such as *CDC20*, *CENPM*, *MCM5*, and *PRC1* (Figure S7). By contrast, SBRT led to the induction of *CXCR3*, which has shown to regulate leukocyte trafficking and to promote Th1 and CD8+ effector cell migration into inflamed tissues^{17,18}. These results are consistent with preclinical models demonstrating that high-dose SBRT impacts pathways associated with cell cycle, DNA repair, and antitumor immunity.

We then examined 16 patients with post-SBRT (pre-pembrolizumab) biopsies to determine if the same changes in gene expression were associated with irradiated tumor response. Among the 30 top-ranked genes in terms of correlation with irradiated tumor response, we found that elevated expression of immune genes, including *DNASE1* ($p=0.00029$) and *CCR10* ($p=0.00069$), were associated with increased irradiated tumor response, while elevated expression of *TGFBR3L* ($p=0.0014$) and *SIGLEC15* ($p<0.0001$) were strongly associated with decreased tumor response (Figure 4B)^{19–21}.

DNASE1 has been shown to promote DNA degradation in the response to ionizing radiation, which may influence activation of cGAS-STING signaling in tumor cells^{3,22–24}. Increased *DNASE1* levels following SBRT were associated with concomitant increases in the expression of cytolytic T cell genes (granzyme A and perforin)²⁵ (Figure 4C) and a 2.1-fold improved local tumor response (Figure 4D). RT-PCR analysis showed that increased *DNASE1* expression following SBRT was associated with a reduction in size of irradiated metastases in the response to SBRT+P. Furthermore, the patient with the highest *DNASE1* expression exhibited the greatest reduction in tumor burden (Figure S8). By contrast, increased *TREX1* (*DNase III*) expression levels following SBRT were associated with decreased cytolytic gene expression (Figure 4C) in concert with a 2.4-fold reduced local tumor response (Figure 4D)²⁶. These findings indicated that SBRT induces gene expression changes in immune pathways, which are associated with local tumor response in the presence of adjuvant pembrolizumab.

Discussion

We initially reported the safety of multi-site SBRT+P in patients with advanced solid malignancies⁵. Here, we report that ORR, PFS, and OS did not differ between patients who received complete-Rx versus partial-Rx SBRT to multiple sites in the context of pembrolizumab. Also, clinical responses at the irradiated site could be induced without irradiation of an entire metastasis. Moreover, gene expression changes correlating with SBRT+P treatment response were enriched for innate and adaptive immune pathways.

Finally, we found that the local response of irradiated metastases to SBRT+P was associated with overall survival.

Since TMC following radiotherapy in the absence of immunotherapy is a function of the overall dose, the dose per fraction, and the overall time during which the radiation is given^{12–15,27–29}, we examined the relationship between irradiated tumor volume and radiation dose. Previous studies have established the minimum dose required for local control following SBRT alone is 36 Gy in 3 fractions^{10,30}. While in our partial-Rx cohort, the median value for tumor minimum dose was 9 Gy in 3 fractions, which is expected to have poor rates of local control^{31–33}. Moreover, partial-Rx treatment increased normal tissue sparing compared to the complete-Rx group (See Supplemental Methods for Biological Effective Dose (BED) calculations). Thus, partial-Rx treatment in combination with pembrolizumab is feasible and should be examined further.

The correlation of survival with the local response of the irradiated tumors, and not with baseline patient characteristics suggesting that multi-site SBRT can limit progression of existing metastases while also augmenting antitumor immune responses to potentially improve outcomes in metastatic patients treated with pembrolizumab. Cytoreduction through radiation may be important, particularly given the clinical observation indicating that patients with lower disease burden treated on prospective immune checkpoint therapy trials have improved outcomes³⁴.

We also found that clinical responses could be induced, irrespective of tumor PD-L1 status, without irradiation of an entire metastasis and also resulted in less toxicity. Despite the partial treatment of these metastases, their inherently larger size, and the reduced overall dose to the metastasis, we did not detect any difference in TMC when compared to tumors that received the prescribed radiation dose to the entirety of the lesion. Specifically, 15/16 (94%) metastases in our partial-Rx group responded despite receiving lower radiation doses. Based on prior studies using single modality SBRT prescribed to the entire metastasis volume in which 25.5 Gy was prescribed in 3 fractions, we expected few metastases receiving partial-Rx to have radiographic response³⁵. We propose the response seen in our study is likely multifactorial and related to both cytoreduction and local immune enhancement as previously demonstrated in preclinical models when stereotactic radiation was combined with immune checkpoint therapy³⁶. However, we await further prospective evidence to further understand the contribution of these two potential mechanisms. Of note, the high TMC rate seen was not associated with increased toxicities given that no DLTs occurred in the partial-Rx group. Collectively, these data suggest that a multi-site SBRT approach, including partial-Rx, followed by immunotherapy may have broad applicability by enhancing response and limiting adjacent organ toxicity in advanced cancer patients.

Consistent with preclinical evidence and the recent ORIOLE phase 2 randomized trial, we observed that SBRT induced both innate and adaptive immune pathways, while concomitantly decreasing genes involved in cell cycle and DNA damage repair pathways^{3,4,37}. We found overexpression of the DNase I (*DNASE1*) endonuclease following SBRT was associated with favorable irradiated tumor response to SBRT+P, whereas overexpression of the DNase III (*TREX1*) exonuclease was associated with unfavorable

local tumor response. These findings suggest that differential DNA degradation in the response to SBRT might contribute to distinct treatment responses to SBRT+P. Moreover, overexpression of *SIGLEC15*, recently described as an important immune suppressor in the context of checkpoint blockade³⁸, following SBRT emerged as potential negative regulator of SBRT+P response in our analysis. Taken together, our finding that there is no difference in tumor control whether patients receive partial-Rx or complete-Rx, our work suggests that the ablative function of radiation may not be the only aspect of radiation, which should be considered. In fact, our work is novel in suggesting that the other aspects of the biological response to radiation (which are not accounted for in the α/β ratio) may be just as important.

While there is generally a lack of consensus regarding the optimal radiation dose (thought to be in the range of 8–12 Gy per fraction)^{10,26,30} and the degree of tumor coverage required to potentiate systemic immunity³⁹. In our study, we used higher radiation doses per treatment, and although we found an inverse relationship between treatment response and the post-SBRT expression of *TREX1*, we also observed that the majority of irradiated tumors responded to treatment and exhibited high levels of post-SBRT immune gene expression. We recognize that even in the context of higher fraction irradiation some portion of the tumor receives a lower dose, especially in partial-Rx tumors. However, when we investigated the percentage of the tumor that received 8–12 Gy we did not find an association with irradiated tumor or overall patient response. These findings suggest that, in humans, the optimal radiation dose and volume to synergize with immunotherapy remains undefined.

While these analyses were generated from a prospective clinical trial, there are several limitations of our investigation. For example, only 18 patients and 21 (of 37) metastases were treated with partial-Rx SBRT+P. Additional prospectively treated patients are needed to confirm the favorable local tumor response with partial-Rx SBRT+P. The single arm nature and heterogeneous patient population within the phase I trial also have limitations and randomized evaluation within a specific disease setting is required. Regarding gene expression analysis, RNA isolation was prioritized from tumor biopsies limiting the availability of additional tissue for exploratory analyses.

Multi-site SBRT, including partial-Rx SBRT to large lesions, in combination with immunotherapy may provide a benefit to the irradiated metastases, irrespective of PD-L1 status. This treatment paradigm is feasible and clinically available to patients in the standard-of-care setting. Furthermore, the association of treatment response to radiation (TMC) with OS suggests a potential clinical and radiographic biomarker that is easily evaluable. Finally, gene expression profiling changes should similarly be assessed in other studies using a range of radiation doses to identify the optimal dose and schedule for immune potentiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Trujillo JA, Sweis RF, Bao R, Luke JJ. T Cell-Inflamed versus Non-T Cell-Inflamed Tumors: A Conceptual Framework for Cancer Immunotherapy Drug Development and Combination Therapy Selection. *Cancer Immunol Res.* 2018;6(9):990–1000. doi:10.1158/2326-6066.CIR-18-0277 [PubMed: 30181337]
2. Ayers M, Lunceford J, Nebozhyn M, et al. IFN- γ -related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest.* 2017;127(8):2930–2940. doi:10.1172/JCI91190 [PubMed: 28650338]
3. Deng L, Liang H, Xu M, et al. STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors. *Immunity.* 2014;41(5):843–852. doi:10.1016/j.immuni.2014.10.019 [PubMed: 25517616]
4. Liang H, Deng L, Hou Y, et al. Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. *Nat Commun.* 2017;8(1):1736. doi:10.1038/s41467-017-01566-5 [PubMed: 29170400]
5. Luke JJ, Lemons JM, Karrison TG, et al. Safety and Clinical Activity of Pembrolizumab and Multisite Stereotactic Body Radiotherapy in Patients With Advanced Solid Tumors. *J Clin Oncol Off J Am Soc Clin Oncol.* 2018;36(16):1611–1618. doi:10.1200/JCO.2017.76.2229
6. Ott PA, Bang Y-J, Berton-Rigaud D, et al. Safety and Antitumor Activity of Pembrolizumab in Advanced Programmed Death Ligand 1-Positive Endometrial Cancer: Results From the KEYNOTE-028 Study. *J Clin Oncol Off J Am Soc Clin Oncol.* 2017;35(22):2535–2541. doi:10.1200/JCO.2017.72.5952
7. Salama JK, Hasselle MD, Chmura SJ, et al. Stereotactic body radiotherapy for multisite extracranial oligometastases: final report of a dose escalation trial in patients with 1 to 5 sites of metastatic disease. *Cancer.* 2012;118(11):2962–2970. doi:10.1002/cncr.26611 [PubMed: 22020702]
8. Costelloe CM, Chuang HH, Madewell JE, Ueno NT. Cancer Response Criteria and Bone Metastases: RECIST 1.1, MDA and PERCIST. *J Cancer.* 2010;1:80–92. [PubMed: 20842228]
9. Milano MT, Katz AW, Zhang H, Okunieff P. Oligometastases treated with stereotactic body radiotherapy: long-term follow-up of prospective study. *Int J Radiat Oncol Biol Phys.* 2012;83(3):878–886. doi:10.1016/j.ijrobp.2011.08.036 [PubMed: 22172903]
10. Wong AC, Watson SP, Pitroda SP, et al. Clinical and molecular markers of long-term survival after oligometastasis-directed stereotactic body radiotherapy (SBRT). *Cancer.* 2016;122(14):2242–2250. doi:10.1002/cncr.30058 [PubMed: 27206146]
11. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. *J Clin Oncol Off J Am Soc Clin Oncol.* 1983;1(11):710–719. doi:10.1200/JCO.1983.1.11.710
12. Mahadevan A, Blanck O, Lanciano R, et al. Stereotactic Body Radiotherapy (SBRT) for liver metastasis - clinical outcomes from the international multi-institutional RSSearch® Patient Registry. *Radiat Oncol Lond Engl.* 2018;13(1):26. doi:10.1186/s13014-018-0969-2
13. McCammon R, Schefter TE, Gaspar LE, Zaemisch R, Gravidahl D, Kavanagh B. Observation of a dose-control relationship for lung and liver tumors after stereotactic body radiation therapy. *Int J Radiat Oncol Biol Phys.* 2009;73(1):112–118. doi:10.1016/j.ijrobp.2008.03.062 [PubMed: 18786780]
14. Ohri N, Tomé WA, Méndez Romero A, et al. Local Control After Stereotactic Body Radiation Therapy for Liver Tumors. *Int J Radiat Oncol Biol Phys.* Published online January 6, 2018. doi:10.1016/j.ijrobp.2017.12.288
15. Yamada Y, Katsoulakis E, Laufer I, et al. The impact of histology and delivered dose on local control of spinal metastases treated with stereotactic radiosurgery. *Neurosurg Focus.* 2017;42(1):E6. doi:10.3171/2016.9.FOCUS16369

16. Burnette BC, Liang H, Lee Y, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Res.* 2011;71(7):2488–2496. doi:10.1158/0008-5472.CAN-10-2820 [PubMed: 21300764]
17. Harlin H, Meng Y, Peterson AC, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res.* 2009;69(7):3077–3085. doi:10.1158/0008-5472.CAN-08-2281 [PubMed: 19293190]
18. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer Cell.* 2017;31(5):711–723.e4. doi:10.1016/j.ccell.2017.04.003 [PubMed: 28486109]
19. Formenti SC, Lee P, Adams S, et al. Focal Irradiation and Systemic TGF β Blockade in Metastatic Breast Cancer. *Clin Cancer Res Off J Am Assoc Cancer Res.* 2018;24(11):2493–2504. doi:10.1158/1078-0432.CCR-17-3322
20. Barcellos-Hoff MH. Radiation-induced transforming growth factor beta and subsequent extracellular matrix reorganization in murine mammary gland. *Cancer Res.* 1993;53(17):3880–3886. [PubMed: 8358713]
21. Barcellos-Hoff MH, Cucinotta FA. New tricks for an old fox: impact of TGF β on the DNA damage response and genomic stability. *Sci Signal.* 2014;7(341):re5. doi:10.1126/scisignal.2005474
22. Woo S-R, Fuertes MB, Corrales L, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity.* 2014;41(5):830–842. doi:10.1016/j.immuni.2014.10.017 [PubMed: 25517615]
23. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. *Proc Natl Acad Sci U S A.* 2012;109(47):19386–19391. doi:10.1073/pnas.1215006109 [PubMed: 23132945]
24. Weichselbaum RR, Liang H, Deng L, Fu Y-X. Radiotherapy and immunotherapy: a beneficial liaison? *Nat Rev Clin Oncol.* 2017;14(6):365–379. doi:10.1038/nrclinonc.2016.211 [PubMed: 28094262]
25. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity. *Cell.* 2015;160(1):48–61. doi:10.1016/j.cell.2014.12.033 [PubMed: 25594174]
26. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun.* 2017;8:15618. doi:10.1038/ncomms15618 [PubMed: 28598415]
27. Duijm M, Nuytens J, Schilleman W, et al. The Contribution of Underdosage to the Local Control for Central Tumors in the Lung Treated With Stereotactic Radiation Therapy. *Int J Radiat Oncol Biol Phys.* 2016;96(2):E465. doi:10.1016/j.ijrobp.2016.06.1797
28. Onishi H, Shirato H, Nagata Y, et al. Hypofractionated stereotactic radiotherapy (HypoFXSRT) for stage I non-small cell lung cancer: updated results of 257 patients in a Japanese multi-institutional study. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer.* 2007;2(7 Suppl 3):S94–100. doi:10.1097/JTO.0b013e318074de34
29. Ball D, Mai GT, Vinod S, et al. Stereotactic ablative radiotherapy versus standard radiotherapy in stage I non-small-cell lung cancer (TROG 09.02 CHISEL): a phase 3, open-label, randomised controlled trial. *Lancet Oncol.* 2019;20(4):494–503. doi:10.1016/S1470-2045(18)30896-9 [PubMed: 30770291]
30. Rusthoven KE, Kavanagh BD, Burri SH, et al. Multi-institutional phase I/II trial of stereotactic body radiation therapy for lung metastases. *J Clin Oncol Off J Am Soc Clin Oncol.* 2009;27(10):1579–1584. doi:10.1200/JCO.2008.19.6386
31. Liu F, Tai A, Lee P, et al. Tumor control probability modeling for stereotactic body radiation therapy of early-stage lung cancer using multiple bio-physical models. *Radiother Oncol J Eur Soc Ther Radiol Oncol.* 2017;122(2):286–294. doi:10.1016/j.radonc.2016.11.006
32. Laufer I, Iorgulescu JB, Chapman T, et al. Local disease control for spinal metastases following “separation surgery” and adjuvant hypofractionated or high-dose single-fraction stereotactic radiosurgery: outcome analysis in 186 patients. *J Neurosurg Spine.* 2013;18(3):207–214. doi:10.3171/2012.11.SPINE12111 [PubMed: 23339593]

33. Alghamdi M, Tseng C-L, Myrehaug S, et al. Postoperative stereotactic body radiotherapy for spinal metastases. *Chin Clin Oncol Vol 6 Suppl 2 Sept 2017 Chin Clin Oncol Adv Stereotact Radio Surg-Guest Ed Kevin M Chua David B H Tan Melvin K Chua Simon Lo*. Published online 2017. Accessed January 1, 2017. <http://cco.amegroups.com/article/view/16251>
34. Huang AC, Postow MA, Orlowski RJ, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature*. 2017;545(7652):60–65. doi:10.1038/nature22079 [PubMed: 28397821]
35. Bondiau P-Y, Courdi A, Bahadoran P, et al. Phase 1 clinical trial of stereotactic body radiation therapy concomitant with neoadjuvant chemotherapy for breast cancer. *Int J Radiat Oncol Biol Phys*. 2013;85(5):1193–1199. doi:10.1016/j.ijrobp.2012.10.034 [PubMed: 23332384]
36. Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys*. 2013;86(2):343–349. doi:10.1016/j.ijrobp.2012.12.025 [PubMed: 23462419]
37. Phillips R, Shi WY, Deek M, et al. Outcomes of Observation vs Stereotactic Ablative Radiation for Oligometastatic Prostate Cancer: The ORIOLE Phase 2 Randomized Clinical Trial. *JAMA Oncol*. Published online March 26, 2020. doi:10.1001/jamaoncol.2020.0147
38. Wang J, Sun J, Liu LN, et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med*. 2019;25(4):656–666. doi:10.1038/s41591-019-0374-x [PubMed: 30833750]
39. Markovsky E, Budhu S, Samstein RM, et al. An Antitumor Immune Response Is Evoked by Partial-Volume Single-Dose Radiation in 2 Murine Models. *Int J Radiat Oncol Biol Phys*. 2019;103(3):697–708. doi:10.1016/j.ijrobp.2018.10.009 [PubMed: 30342090]

Translational Relevance:**Question:**

Does irradiated tumor response correlate with survival in patients with advanced solid tumors treated with SBRT and pembrolizumab (SBRT+P), and does radiation alter the tumor microenvironment?

Results:

In this phase I trial, irradiated tumor response was associated with overall survival. Large tumors treated with a partial radiation prescription dose achieved similar local control to smaller, completely irradiated tumors. The primary effect of combination SBRT+P was localized to irradiated metastases and not to systemic, non-irradiated metastases. SBRT induced innate and adaptive immune responses in tumors, and post-SBRT gene expression correlated with tumor control.

Implication for Future Cancer Medicine:

Survival after multi-site SBRT and pembrolizumab was associated with irradiated tumor response, and SBRT induced immune gene expression within the tumor microenvironment. These results support a randomized trial examining this approach.

Figure 1: Treated Metastasis Control was independent of PD-L1 status, tumor size, and tumor histology

Kaplan-Meier estimates of (A) Overall Treated Metastasis Control (TMC) (n=139). (B) TMC by PD-L1 Status (n=105). (C) TMC by treated metastasis quartile volume prior to stereotactic body radiation therapy. In B and C, P-values were determined using shared-frailty Cox and then fit to a flexible parametric model to generate figures. Values along the x-axis are the number of metastases at risk among 68 evaluable patients. NSCLC = non-small cell lung cancer, cholangio = cholangiocarcinoma.

Figure 2: Treatment outcomes stratified by prescription radiotherapy dose volume

Treated Metastasis Control (TMC) by prescription stereotactic body radiation therapy dose covering the entire metastasis (cRx) compared to partial metastasis coverage (pRx) (n=139). P-values were determined using shared-frailty Cox Regression and then fit to a flexible parametric model. Values along the x-axis are the number of metastases at risk among 68 evaluable patients.

Figure 3: Kaplan-Meier overall survival stratified by SBRT response

Overall survival stratified by irradiated tumor response (responders, mixed responders, and non-responders) from those that survived to the 2-month landmark time (n=67). HR 0.44; 95% CI 0.23–0.85; p=0.015. SBRT, stereotactic body radiation therapy; HR = hazard ratio; mo = month.

Figure 4: Gene expression analysis of irradiated tumors following SBRT and association with irradiated tumor response

(A) Enriched canonical pathways and predicted upstream activators following SBRT. P-values were determined using Fisher's exact tests.

(B) Association of irradiated tumor response with post-SBRT gene expression. Pearson correlation coefficients plotted against statistical significance of correlation for all protein-coding genes.

(C) Change in intratumoral cytolytic gene expression (*PRF1* + *GZMA* expression) in relation to change in *DNASE1* (upper panel) or *TREX1* (lower panel) expression after SBRT. >0 indicates increased expression, while <0 indicates decreased expression. Tumors were dichotomized as low or high expressors based on the median expression value of *DNASE1* and *TREX1*. P-values were determined using Mann-Whitney U tests.

(D) Change in irradiated tumor size ($[(\text{post-treatment largest diameter} / \text{pre-treatment largest diameter}) - 1] \times 100\%$) after SBRT + pembrolizumab as a function of post-SBRT *DNASE1* and *TREX1* expressions in matched tumor specimens. Tumors were dichotomized as low or high expressors based on the median expression value of *DNASE1* and *TREX1*. P-values were determined using Mann-Whitney U tests. PD, progressive disease; SD, stable disease; CR/PR, complete response/partial response based on RECIST criteria.

Table 1:**Patient-Level Baseline and Treatment Characteristics**

Patient Characteristic	Complete-Rx 50 Pts 102 Mets	Partial-Rx 18 Pts 37 Mets
Partial-tumor SBRT (Metastasis-level)	0% (0/102 Mets)	56.8% (21/37 Mets)
Follow up (mo) mean (SD)	10.8 (6.5)	8.9 (6.0)
Age (yrs), mean (SD)	60.5 (13.25)	61.0 (15.9)
Gender		
F	28 (55%)	13 (72%)
M	22 (44%)	5 (28%)
ECOG Performance Status		
0	25 (50%)	7 (39%)
1	25 (50%)	11 (61%)
Smoking Status		
Current	2 (4%)	1 (6%)
Former	23 (46%)	7 (39%)
Never	25 (50%)	10 (56%)
Baseline Albumin, median (range)	4.0 (2.4–4.5)	4.0 (3.2–4.4)
Primary Cancer Histology		
Other	18 (36%)	9 (50%)
Ovarian/fallopian tube	7 (14%)	2 (11%)
Non-small-cell Lung	5 (10%)	1 (6%)
Breast	5 (10%)	1 (6%)
Cholangiocarcinoma	2 (4%)	4 (22%)
Endometrial	6 (12%)	0 (0%)
Colorectal	3 (6%)	1 (6%)
Head and Neck	4 (8%)	0 (0%)
PD-L1 Status		
<1%	19 (39%)	7 (39%)
>1%	19 (39%)	6 (33%)
Missing	12 (24%)	5 (28%)
# of Prior Treatments, median (range)	5 (0–13)	3 (0–10)
Prior Immunotherapy?		
No	47 (94%)	18 (100%)
Yes	3 (6%)	0 (0%)
Total RECIST lesions followed (mean, SD)	3.4 (1.4)	3.2 (1.1)
Sites Treated with SBRT		
2 Metastases	48 pts (96%)	17 pts (94%)
3 Metastases	2 pts (4%)	1 pts (6%)
Cycles of Pembrolizumab, median (range)	4(1–27)	5 (1–34)

Data are n (%) unless otherwise stated.

SD = standard deviation

SBRT = stereotactic body radiation therapy.

Mets= treated metastases, pts = patients.

Partial-Rx = prescription SBRT dose to part of the metastasis volume.

Complete-Rx = prescription SBRT dose to the entire metastasis volume.

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