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Low-dose irradiation for reversing immunotherapy resistance: how to translate?

Maria Ochoa-de-Olza,[1,2] Jean Bourhis,[3] George Coukos,[1,2] Fernanda G Herrera [1,2,3]

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[1]Ludwig Institute for Cancer Research Lausanne branch, Lausanne, Switzerland
[2]Immuno-oncology Service, Department of Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
[3]Radiation Oncology Service, Department of Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Correspondence to
Dr Fernanda G Herrera; fernanda.herrera@chuv.ch

ABSTRACT

The use of low-dose irradiation (LDI) for mobilizing innate and adaptive immunity is gaining interest among the scientific community. Recent evidence suggests that LDI can reprogramme the tumor microenvironment, induce inflammation and turn cold tumors susceptible to immunecheckpoint blockade therapy. Translating immuno-radiation preclinical findings in the clinic is more challenging than expected. We propose therapeutic strategies for combining LDI with immunotherapy, and emphasize the importance of pursuing clinical research to determine optimal radiation dosage, fractionation, volumes, and sequencing to stimulate immune-mediated tumor responses.

Low-dose irradiation (LDI) has gained interest in the scientific community over the last few months in light of recent preclinical and clinical publications, which suggest that LDI can effectively mobilize innate and adaptive immunity.[1–3] Indeed, these new data point to LDI's ability to reprogramme the tumor microenvironment (TME), inducing inflammation and making cold tumors susceptible to immunecheckpoint blockade (ICB).

Despite these promising preliminary findings, the contribution of LDI was not confirmed in a recently published phase 2 randomized clinical trial.[4] Indeed, Schoenfeld *et al* presented the results of a randomized phase 2 clinical trial, in which two different radiotherapy (RT) regimens known to enhance immune responses in preclinical models[5 6] were tested in non-small-cell lung carcinoma (NSCLC) patients with innate or acquired resistance to previous PD-1 or PD-L1 inhibitors in combination with dual ICB including durvalumab, an anti-PD-L1 antibody, and tremelimumab, an anti-CTLA-4 antibody. We congratulate the authors for incorporating these preclinical concepts into a well-designed, randomized phase 2 trial. Unfortunately, neither LDI nor high-dose irradiation (HDI) enhanced the overall response rate, which was the study's primary endpoint, and progression-free survival along with overall survival remained unchanged.

These outcomes are clearly disappointing and require some reflection, lest enthusiasm on immuno-radiation combinations is tempered. Indeed, these are now added to a previous study in colorectal cancer where repeat fractionated LDI or oligofractionated RT were combined with ICB,[7] and recent results with stereotactic oligofractionated radiation and ICB in head and neck cancer,[8] which were also disappointing. Certainly, translating immuno-radiation preclinical findings in the clinic is more complicated than expected. We offer some points of reflection about the NSCLC study that we would like to share with the authors and the readers.

One of the study's arm treated patients with a hyperfractionated LDI schema of 0.5 Gy delivered twice a day during two consecutive days (total 2 Gy/cycle), repeated for each of the first four cycles of therapy for a total dose of 8 Gy. The other treatment arm delivered hypofractionated RT consisting of three 8 Gy fractions for a total dose of 24 Gy every other day during the first cycle of therapy only. One week after durvalumab–tremelimumab administration, RT was delivered in both arms. The former scheme of LDI is inspired by previous work by Klug *et al*,[5] who were the first to demonstrate that radiation doses ranging from 0.5 to 2 Gy (administered as single fractions, oligo-RT) promoted M1 macrophage polarization, normalization of tumor vasculature and increased infiltration of adoptively transferred T cells in a mouse model of neuroendocrine pancreatic cancer. The latter scheme is based on preclinical data, where the delivery of three fractions of 8 Gy combined with anti-CTLA-4 effectively induced type I interferon (IFN-I) secretion by cancer cells, resulting in tumor recruitment of professional dendritic cells (DCs) and subsequent T cell-mediated rejection

of irradiated and synchronous non-irradiated murine breast tumors.[6] A recent phase II randomized study in early stage surgically operable NSCLC patients using stereotactic body radiation therapy (SBRT) in combination with durvalumab or durvalumab alone showed a statistically significant major pathological response rate of 53.3% (95% CI 34.3% to 71.7%) vs 6.7% (95% CI 0.8% to 22.1%), respectively. Importantly, in patients with major pathological response in the dual therapy group, significantly higher MHC-I gene expression was found in tumor specimens after therapy.[9] Furthermore, when the same approach was used to treat metastatic NSCLC patients, $CD8^+$ T cells recognized tumor neoantigens upregulated by RT.[10]

With the rather encouraging prior results of this irradiation scheme, what could explain the combination's lack of efficacy in this trial?

Importantly, in both irradiation arms of Schoenfeld and *et al*'s clinical trial, one to two metastases per patient were treated with RT. The rationale to irradiate a small number of metastases during ICB treatment is based on the assumption that isolated RT triggers a local in situ vaccination effect, exposing immunogenic tumor antigens and can therefore synergize with systemic immune activation. This is supported by exciting preclinical findings in mice where delivery of RT to one tumor deposit triggers distant immune-mediated tumor regression. This effect is conspicuously displayed in animal models known to be highly immunogenic (ie, the CT26 colon tumor model), with limited abscopal effects found in tumors known to be less immunogenic, such as the B16 melanoma model.[11 12] Furthermore, isolated case reports demonstrating remarkable abscopal effects in patients undergoing single-site RT in combination with ICB further heightened interest in this therapeutic strategy. However, the incidence of abscopal effects may have been overestimated. Indeed, the demonstration of abscopal effects when RT is administered alone has long remained elusive in clinical practice, but hopes for finally attaining it were reinvigorated with the possibility of combining ICB. However, randomized clinical trials have now convincingly demonstrated that abscopal effects are inherently incidental even with ICB combinations.[8] Therefore, the expectation that in situ vaccination through localized delivery of RT to one tumor lesion can overcome resistance to ICB in distant lesions may be overoptimistic. Indeed, if the desired effect of RT is antigen release, multisite partial tumor volume irradiation may be sufficient with fewer side effects. Luke *et al* implemented this strategy in a phase I/II clinical trial and reported that partial volume SBRT maintained local disease control while increasing the expression of innate and adaptive immune genes in responder patients.[13]

An innovative study from MD Anderson Cancer Center implemented hypofractionated RT (20–70 Gy) to 1–2 metastatic lesions to trigger in situ vaccination, delivered with or without LDI (1–10 Gy delivered in fractions of 0.5–2 Gy), to stimulate immunological reprogramming in the remaining tumor deposits.[14] The majority of patients in the trial had progressed on anti-PD1 or anti-PD-L1 therapy, which they continued during radiation. Despite the fact that the combination group had a higher overall response rate than the hypofractionated RT group (26% vs 13%, respectively), this did not translate into improved progression free survival nor overall survival. This could imply that the combined therapy induces local TME reprogramming but is insufficient to stimulate a systemic anti-tumor immune response. Combinatorial immunotherapy strategies, such as a CD40 or TLR agonists, may be required to maximize the HDI in situ vaccination effect, while ICB is required for increasing T cell cytotoxicity, and blocking radiation-induced TGF-β may decrease epithelial cell proliferation and immune evasion.[15]

Similarly, a phase I clinical study (RACIN, NCT03728179) from our group which tested 0.5 or 1 Gy to all visible tumor lesions administered every 2 weeks demonstrated tumor size reduction in 37.5% of the irradiated lesions in an immunotherapy-naïve population of patients with metastatic ovarian, prostate, gallbladder or colon carcinoma.[16] Importantly, this LDI schema was able to induce de novo T cell infiltration into immune "cold" tumors and triggered immune gene signatures associated with IFN-I response, immune-cell activation, antigen presentation, T cell receptor activation, and effector memory phenotype in responder patients. Underscoring the key contribution of locally delivered LDI, responder patients showed regression of all irradiated metastases, whereas subsequent progression was only observed in lesions outside the irradiated field. These findings suggest that the observed synergy between LDI and immunotherapy was not due to a systemic abscopal effect, but rather to the local effects mediated by the direct modulation of the TME.[16] Presumably, delivering LDI to a low number of metastases by Schoenfeld *et al* failed to reprogram the TME in non-irradiated lesions sufficiently to facilitate an effective anti-tumor immune response. Furthermore, only 12% of irradiated patients in the study received RT on liver metastasis, while the most common site of irradiation was the lung (62%). Recent work indicates that patients with liver metastases are less responsive to ICB,[17] therefore, liver metastases are in greatest need for TME reprogramming. Within the liver, activated antigen-specific Fas^+CD8^+ T cells undergo apoptosis following interactions with $FasL^+CD11b^+F4/80^+$ monocyte-derived macrophages. Indeed, delivering 8 Gy in a single fraction to liver metastasis in mice has shown to overcome the immune suppressive effect of hepatic macrophages, increasing hepatic T cell survival and decreasing apoptosis of T cells.[17] Nevertheless, several RT regimens used so far for targeting liver metastases of patients in combination with ICB (24 Gy in 3 fractions, 8 Gy in 0.5 Gy per fraction,[7] 50 Gy in 4 fractions, 60 Gy in 10 fractions[18]) have thus far failed to demonstrate increased response rates. Consequently, improved RT regimens and combinations directed to liver metastasis of patients merit further investigation.

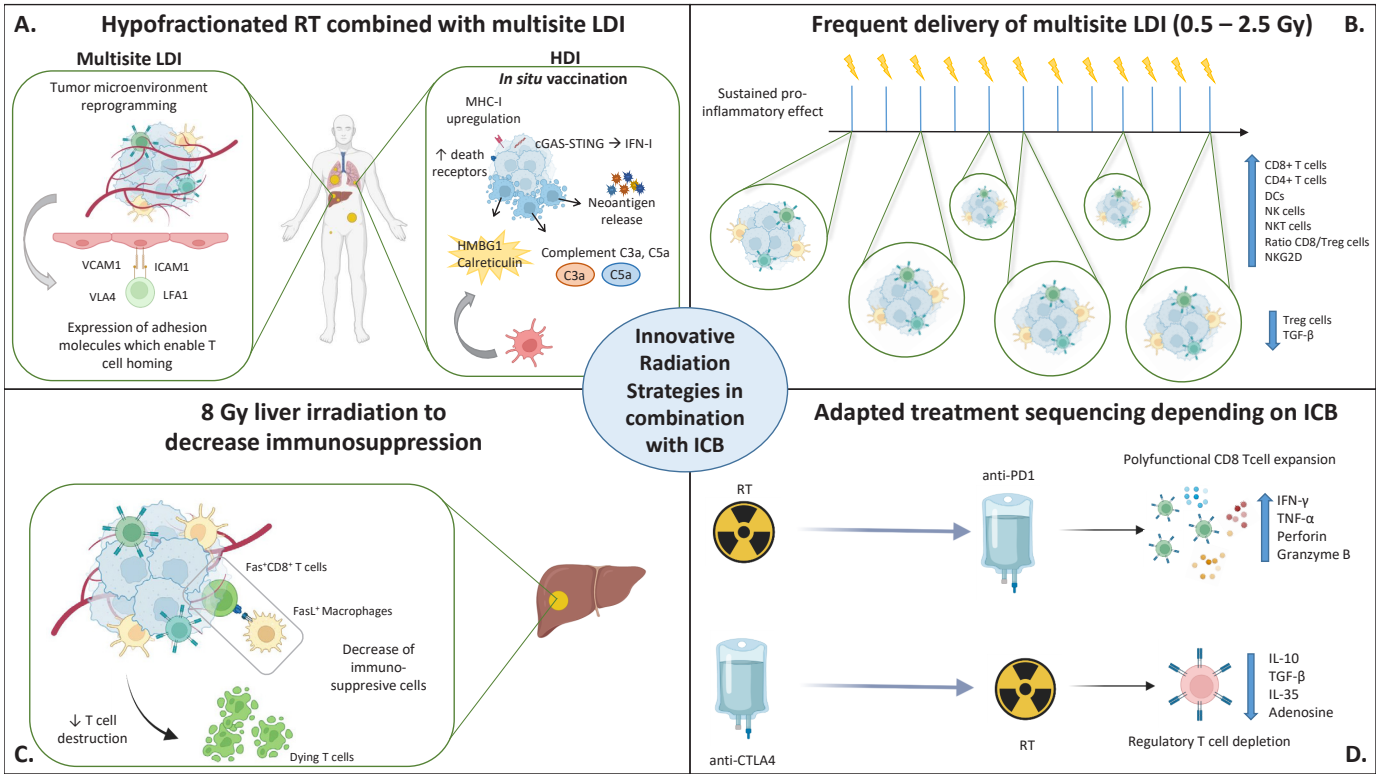

Figure 1 (A) HDI can induce an in situ vaccination effect through neoantigen release, upregulation of MHC-I, and cGAS-STING-mediated IFN-I production. Similarly, high-dose treated metastases generate danger signals, attracting DCs and leading to improved immune-mediated tumor control. The addition of multisite LDI can locally modulate the TME, increase immune cell infiltration through upregulation of vascular adhesion molecules, further boosting immune responses. (B) Weekly delivery of LDI is capable of inducing a sustained proinflammatory effect with increased DC, CD4 and CD8 T cell infiltration, as well as decreasing immunosuppressive T regulatory cells. (C) Delivering 8 Gy in a single fraction to liver metastasis in mice has shown to overcome the immune suppressive effect of hepatic macrophages and decreasing destruction of T cells by FasL$^+$ macrophages. (D) Therapy sequencing and schedule influences the TME and antitumor responses. RT delivered before anti-PD1 can induce polyfunctional CD8 T cell expansion, while delivering anti-CTLA4 before RT leads to a decrease in T regulatory cells, stressing the need of additional research for optimizing sequencing schemas. DCs, dendritic cells; HDI, high-dose irradiation; IFN, interferon; ICB, immunecheckpoint blockade; LDI, low-dose irradiation; RT, radiotherapy; TME, tumor microenvironment.

How to effectively target microscopic disseminated disease emerges as a key question in the field. Innovative approaches like targeted radionuclide therapy (TRT) can deliver RT to all metastatic deposits irrespective of whether they are visible by imaging, a clear shortcoming of external beam radiation therapy (EBRT). Patel *et al*[2] implemented ^{90}Y-NM600 in order to render immunologically cold syngeneic B78 melanoma tumors sensitive to ICBs. After low-dose TRT (2.5 Gy), a significant increase in tumor-infiltrating myeloid (CD11b$^+$) and natural killer (NK) cells, as well as an increase in the ratio of effector CD8$^+$ to suppressor CD4$^+$CD25$^+$FOXP3$^+$ T regulatory (Treg) cells were observed compared with controls, along with improved responses to ICB. Furthermore, coadministration of oligo-RT (12 Gy) to one lesion and ^{90}Y-NM600 together with CTLA-4 blockade further enhanced the therapeutic efficacy and induced more abscopal effects when compared with either treatment alone. Hence, TRT could be a means of irradiating all metastatic deposits, including micro-metastatic disease, consequently overcoming aforementioned EBRT limitations.

Importantly, irradiation was given 1 week following ICB infusion in Schoenfeld *et al*'s clinical trial. Indeed, the timing and sequencing of RT and ICB is complex, and the abscopal effect of hypofractionated RT has been shown to be schedule-dependent in preclinical models. Dovedi *et al* demonstrated that concurrent but not sequential administration of anti-PD-L1 with RT is essential for enhanced tumor control and survival.[19] Furthermore, when anti-PD1 was administered prior to RT, the abscopal effect was abolished.[20] Similarly, radiation significantly increased Tregs with elevated CTLA4 expression,[21] and pretreatment with an anti-CTLA4 antibody provided efficient tumor control in CT26 tumor bearing mice.[22]

The mechanisms underlying such schedule sensitivity of the positive interaction between ICB and RT may be multiple. For instance, ionizing radiation increases response to tumor neoantigens,[10] which are crucial determinants of ICB response. Yet, radiation-induced MHC class I expression begins 18 hours after RT but declines 72 hours later.[23] As a result, T cell reinvigoration by anti-PD-1 should be carefully timed to occur at the same time as

the peak of tumor antigen presentation induced by RT.[10] Furthermore, loss of the antitumor effect of the combination may be due to increased vulnerability to radiation in tumor-specific $CD8^+$ cells activated by PD-1 blockade.[20] These interactions may become time-sensitive especially when RT is given at low doses, where its immune effects may be transient. We reported that following a single fraction of 1 Gy RT in the ID8 ovarian cancer mouse model, RT-induced T cell inflammation in tumors vanished within a week and repeated weekly administrations of 1 Gy were required to maintain immune infiltration in tumors.[16] These time-sensitive effects included NKG2D-dependent interactions, with influx into tumors of T cells that expressed the NKG2D-costimulatory receptor along with a subpopulation of DCs expressing RAE1, a stress signaling ligand to the NKG2D receptor. The costimulatory signal delivered by NKG2D to T cells was critical, as inhibiting NKG2D prevented radiation-induced antitumor immunological responses. Although the half-life of ICB antibodies is relatively long, therapy sequencing and schedule appear to be particularly important. This stresses the need of additional research optimizing sequencing schemas. In the trial of Schoenfeld *et al.*, RT doses were spaced widely apart, this schedule being likely insufficient to maintain the pro-inflammatory effects of RT.

If multisite radiation is to be implemented, some technical constraints must be considered. For instance, treating discrete tumor volumes, each with a different isocenter, raises radiation scatter doses between volumes (eg, 3–5 Gy). The immediate consequences include moderate to severe acute toxicities, including hematological toxicity. Patients in a phase I clinical study who had palliative radiation to the spine, lung, mediastinum, or chest wall had clinically severe lymphopenia, which worsened after ICB was initiated and contributed to the patients' poor clinical outcomes,[24] an observation confirmed now by Shoenfeld *et al* with either low or HDI. As a result, radiation should be administered in accordance with SBRT principles such as precise target localization and motion-based management. In two ongoing clinical trials (NCT04643574 and NCT03728179), we administer LDI to all metastatic deposits using Tomotherapy Hi-Art allowing for 360° focused irradiation without the need of an isocenter and protecting as much of active bone marrow as possible.[25] The majority of patients in our trial (RACIN, NCT03728179) had peritoneal carcinomatosis, pleural effusion, or significant liver and lung metastases; these patients could not have received high-dose SBRT, but LDI was feasible and increased T cell infiltration in some of them without major toxicities. Moreover, further irradiation strategies to limit prohibitive scattered doses includes GRID therapy, which permits the delivery of multisite RT to bulky tumors while reducing detrimental doses to surrounding tissues.[26]

A second hurdle to multisite RT adoption is treatment time. To treat all lesions, Tomotherapy takes 30–45 min; if the same treatment were performed with different isocenters for each location, the treatment would take much longer and would most likely be unfeasible. Furthermore, patients may be unable to maintain a steady position for long periods of time, rendering breath-hold modeling unattainable with higher-than-expected doses to organs that move with respiration. Another downside of multisite RT is undeniably associated with workflow limits. Because therapy requires repeated simulation, contouring, dosimetry planning, and imaging evaluation, establishing and implementing treatment plans for several sites is time consuming. In future clinical trials, artificial intelligence-based automation could constitute a paradigm shift in the execution of multisite RT favoring a smooth workflow.

In light of these considerations, what lessons may be drawn for future development? First, both arms in the Schoenfeld study attempted to overcome resistance to PD-1/PD-L1 by exploiting the systemic abscopal effect of local RT, an expectation that may be overhyped. Hypofractionated RT could be used with the above intent, but should be best combined with orthogonal systemic approaches to reprogram the TME. In this setting, in addition to timing, the dose of hypofractionated RT matters.[6] If RT is used to reprogram the TME, all lesions should be included in the planning,[1 16] in which case LDI may be a rational option. Because of the short-lived effects,[16] frequent radiations could be applied, although the optimal schedule, dose and fractionation remain to be determined (figure 1).

We are persuaded that Schoenfeld *et al*'s study makes an important contribution to evaluating the role of RT in patients with innate or acquired ICB resistance. However, determining the optimal radiation dosage, fractionation, volumes, and sequencing to stimulate immune-mediated tumor responses remains a challenge.

Twitter George Coukos @CoukosGeorge and Fernanda G Herrera @fg_herrera

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ORCID iD

Fernanda G Herrera http://orcid.org/0000-0001-7984-0472

REFERENCES

1 Barsoumian HB, Ramapriyan R, Younes AI, *et al*. Low-dose radiation treatment enhances systemic antitumor immune responses by overcoming the inhibitory stroma. *J Immunother Cancer* 2020;8:e000537.
2 Patel RB, Hernandez R, Carlson P, *et al*. Low-dose targeted radionuclide therapy renders immunologically cold tumors

responsive to immune checkpoint blockade. *Sci Transl Med* 2021;13. doi:10.1126/scitranslmed.abb3631. [Epub ahead of print: 14 07 2021].
3 Herrera FG, Romero P, Coukos G. Lighting up the tumor fire with low-dose irradiation. *Trends Immunol* 2022;43:173–9.
4 Schoenfeld JD, Giobbie-Hurder A, Ranasinghe S, *et al*. Durvalumab plus tremelimumab alone or in combination with low-dose or hypofractionated radiotherapy in metastatic non-small-cell lung cancer refractory to previous PD(L)-1 therapy: an open-label, multicentre, randomised, phase 2 trial. *Lancet Oncol* 2022;23:279–91.
5 Klug F, Prakash H, Huber PE, *et al*. Low-dose irradiation programs macrophage differentiation to an iNOS$^+$/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell* 2013;24:589–602.
6 Vanpouille-Box C, Alard A, Aryankalayil MJ, *et al*. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* 2017;8:15618.
7 Monjazeb AM, Giobbie-Hurder A, Lako A, *et al*. A randomized trial of combined PD-L1 and CTLA-4 inhibition with targeted low-dose or Hypofractionated radiation for patients with metastatic colorectal cancer. *Clin Cancer Res* 2021;27:2470–80.
8 McBride S, Sherman E, Tsai CJ, *et al*. Randomized phase II trial of nivolumab with stereotactic body radiotherapy versus nivolumab alone in metastatic head and neck squamous cell carcinoma. *J Clin Oncol* 2021;39:30–7.
9 Altorki NK, McGraw TE, Borczuk AC, *et al*. Neoadjuvant durvalumab with or without stereotactic body radiotherapy in patients with early-stage non-small-cell lung cancer: a single-centre, randomised phase 2 trial. *Lancet Oncol* 2021;22:824–35.
10 Formenti SC, Rudqvist N-P, Golden E, *et al*. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med* 2018;24:1845–51.
11 Zhong W, Myers JS, Wang F, *et al*. Comparison of the molecular and cellular phenotypes of common mouse syngeneic models with human tumors. *BMC Genomics* 2020;21:2.
12 Ngwa W, Irabor OC, Schoenfeld JD, *et al*. Using immunotherapy to boost the abscopal effect. *Nat Rev Cancer* 2018;18:313–22.
13 Luke JJ, Onderdonk BE, Bhave SR, *et al*. Improved survival associated with local tumor response following multisite radiotherapy and pembrolizumab: secondary analysis of a phase I trial. *Clin Cancer Res* 2020;26:6437–44.
14 Patel RR, He K, Barsoumian HB, *et al*. High-dose irradiation in combination with non-ablative low-dose radiation to treat metastatic disease after progression on immunotherapy: results of a phase II trial. *Radiother Oncol* 2021;162:60–7.
15 Herrera FG, Bourhis J, Coukos G. Radiotherapy combination opportunities leveraging immunity for the next oncology practice. *CA Cancer J Clin* 2017;67:65–85.
16 Herrera FG, Ronet C, Ochoa de Olza M, *et al*. Low-dose radiotherapy reverses tumor immune desertification and resistance to immunotherapy. *Cancer Discov* 2022;12:108–33.
17 Yu J, Green MD, Li S, *et al*. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. *Nat Med* 2021;27:152–64.
18 Welsh JW, Tang C, de Groot P, *et al*. Phase II trial of ipilimumab with stereotactic radiation therapy for metastatic disease: outcomes, toxicities, and low-dose radiation-related Abscopal responses. *Cancer Immunol Res* 2019;7:1903–9.
19 Dovedi SJ, Adlard AL, Lipowska-Bhalla G, *et al*. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res* 2014;74:5458–68.
20 Wei J, Montalvo-Ortiz W, Yu L, *et al*. Sequence of αPD-1 relative to local tumor irradiation determines the induction of abscopal antitumor immune responses. *Sci Immunol* 2021;6.
21 Muroyama Y, Nirschl TR, Kochel CM, *et al*. Stereotactic radiotherapy increases functionally suppressive regulatory T cells in the tumor microenvironment. *Cancer Immunol Res* 2017;5:992–1004.
22 Young KH, Baird JR, Savage T, *et al*. Optimizing timing of immunotherapy improves control of tumors by Hypofractionated radiation therapy. *PLoS One* 2016;11:e0157164.
23 Reits EA, Hodge JW, Herberts CA, *et al*. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 2006;203:1259–71.
24 Pike LRG, Bang A, Mahal BA, *et al*. The impact of radiation therapy on lymphocyte count and survival in metastatic cancer patients receiving PD-1 immune checkpoint inhibitors. *Int J Radiat Oncol Biol Phys* 2019;103:142–51.
25 Elicin O, Callaway S, Prior JO, *et al*. [(18)F]FDG-PET standard uptake value as a metabolic predictor of bone marrow response to radiation: impact on acute and late hematological toxicity in cervical cancer patients treated with chemoradiation therapy. *Int J Radiat Oncol Biol Phys* 2014;90:1099–107.
26 Yan W, Khan MK, Wu X, *et al*. Spatially fractionated radiation therapy: history, present and the future. *Clin Transl Radiat Oncol* 2020;20:30–8.