Advances in radioimmunotherapy 2

Rational combinations of immunotherapy with radiotherapy in ovarian cancer

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Except for its use in palliative care, radiotherapy has been largely abandoned in the management of ovarian cancer because of the recognised efficacy and lower toxicity of systemic chemotherapy compared with radiotherapy. New data have emerged that show synergy of radiotherapy with immunotherapy to control or eradicate cancer. Different doses of hypofractionated radiotherapy have been shown to induce immunogenic cell death and in-situ vaccination in several tumour models. However, doses less than 2 Gy can also reprogramme the tumour microenvironment. This Series paper discusses the past and present use of radiotherapy for ovarian cancer, and the mechanisms by which radiotherapy can mobilise anticancer immunity. We provide emerging preclinical and clinical data for combining immunotherapy with radiotherapy for ovarian cancer treatment and offer a clinical development roadmap to guide the next generation of clinical trials for this combination strategy for this disease.

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Introduction

Ovarian cancer is the fourth leading cause of cancer-related death in women, with 140 000 deaths per year.[1] Nearly 75% of patients are diagnosed at a late stage with widespread intra-abdominal disease. Cytoreductive surgery and primary chemotherapy remain cornerstone treatments for this disease.[2,3] Despite advances in combinatorial chemotherapy regimens,[3] targeted therapy,[4,5] and the advent of intraperitoneal chemotherapy,[6] current therapeutic options for ovarian cancer are not appropriate or are insufficient to confer long-term survival benefit. Although clinical remission for ovarian cancer is commonly attainable, the majority (over 70%) of patients will relapse, with 5-year survival rates of approximately 30%[7] and the proportion of patients who remain cancer-free at 10 years is less than 15%.[8] Immunotherapy has emerged as a therapeutic option with huge curative potential, and immune checkpoint inhibitors have gained an important place in the treatment of several disease cancer types.[9] However, ovarian cancer remains poorly responsive to immunotherapy.[10] Historically, the clinical efficacy of ionising radiation has been attributed to its ability to induce DNA damage, which can result in direct tumour cell death. However, the existence of radiation-induced antitumour immunity and its potential to synergise with immunotherapy, has been increasingly recognised as a potential therapy.[11] Under certain circumstances, radiotherapy can induce an immune-mediated abscopal effect, whereby radiation to a metastatic deposit induces tumour regression outside the irradiated field.[12] The biological mechanisms underlying the abscopal effect are yet unknown, but several case reports have shown that immunotherapy when combined with radiotherapy can leverage this effect.[11,13,14] Irradiated tumour cells can undergo so-called immunogenic cell death, a particular form of cell death that exposes tumour-associated antigens.[14–16] Which can be recognised and engulfed by antigen-presenting cells (APCs),[17,18] and then presented to CD8 T cells (figure 1).[14,19,20] Radiation can also induce a profound reprogramming of the tumour micro-environment through the upregulation of cytokines and chemokines,[21] and normalisation of the tumour vasculature.[22] Together, these events promote antitumour T-cell responses (figure 2) and thus provide evidence of the abscopal effects of radiotherapy. Clinical reports support this idea, with objective responses reported in patients with metastatic cancer who are undergoing radiotherapy and immunotherapy treatment (table 1).[13,28] The results of the PACIFIC phase 3 randomised trial are intriguing as they also suggest that checkpoint immunotherapy following conventional chemoradiation might be beneficial.[29] In this study, 713 patients who received at least two cycles of platinum-based chemotherapy with radiotherapy and did not develop disease progression were randomly assigned (2:1) to receive the PD-L1 inhibitor durvalumab (10 mg/kg every 2 weeks up to 12 months) or placebo. With a median follow up of 14·5 months, the median time to death or distant metastasis was 23·2 months (95% CI, 23·2 to not reached) with durvalumab versus 14·6 months (10·6–18·6) with placebo (hazard ratio 0·5; 0·4–0·7; two-sided p<0·001).[29] Although in this study PD-L1 blockade was given sequentially to chemoradiotherapy, a potential late-onset synergy effect between these two treatments cannot be excluded.

In-vitro radiosensitivity data of ovarian cancer cells and patient tumour-derived spheroids support the view that ovarian cancer tumours are responsive to radiation, with a mean inactivation dose between 1·31 Gy and 2·80 Gy.[30] Historically, whole-abdominal radiotherapy has been used in ovarian cancer to sterilise anatomical areas that are at a high risk of recurrence.[31] However, the high-dose irradiation schema applied (typically 22·5–33 Gy to the whole abdomen plus a complementary dose of up to 45–50 Gy to the pelvis) was limited by several severe acute and late toxic effects.[31] Radiation-mediated toxic effects

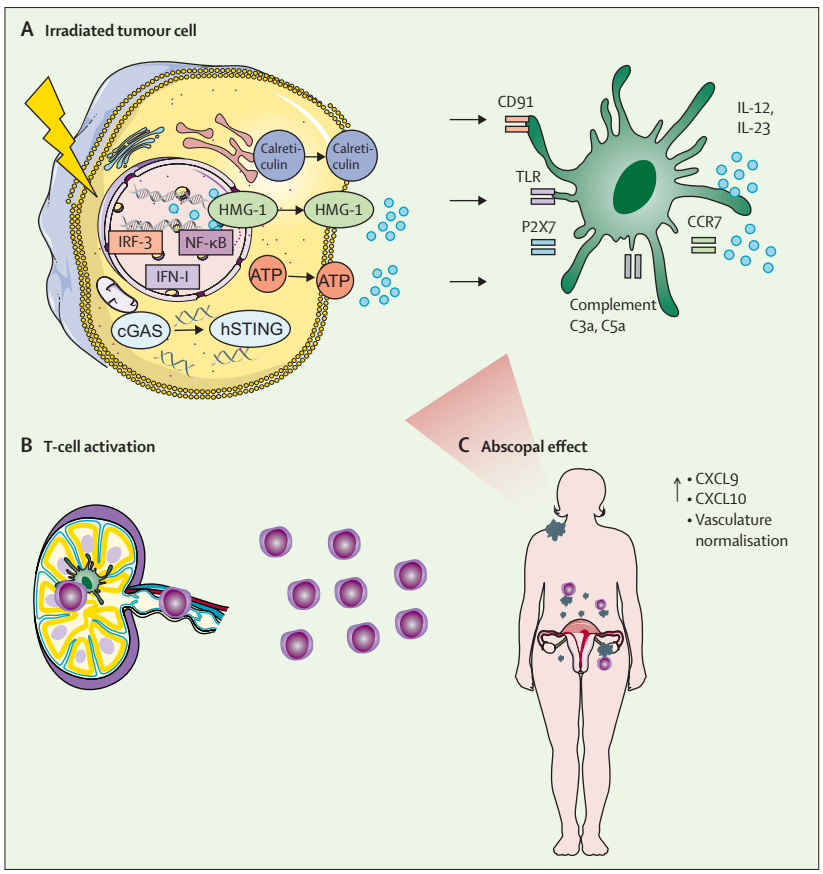

Figure 1: **Immune activation following radiotherapy**
(A) Apoptotic tumour cells release various immunological mediators in the form of ATP, HMG-1, calreticulin, and complement. DNA accumulation in the cancer cell's cytosol activates an IFN-I pathway via the cGAS/hSTING pathway. (B) Together these processes result in a potent inflammatory response promoting dendritic cell migration to the lymph node. (C) Activation of the adaptive immune response and elimination of tumours. IFN-I=type I interferon. NF-κB=nuclear factor κB. TLR=toll-like receptor.

were exacerbated if full-dose cisplatin and paclitaxel chemotherapies had been added to the treatment, either together or in sequence.[32] Thus, the use of whole-abdominal radiotherapy at these doses has declined because of high toxicity, particularly when compared with new available chemotherapy regimens. New radiotherapy modalities, like intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT), along with studies showing synergy between whole-abdominal low-dose irradiation combined with chemotherapy[33,34] or PARP (poly[ADP-ribose] polymerase) inhibitors,[35] have renewed the interest in using low-dose whole-abdominal radiotherapy after primary debulking in ovarian cancer. Similarly, in the oligometastatic setting, stereotactic body radiotherapy (SBRT), a novel treatment modality that implements real-time imaging and high-dose radiation beams, has shown a high rate of local control (90–100%) in properly selected patient populations with metastatic ovarian cancer, with mild or no toxic effects.[36] This wealth of evidence related to radiotherapy, and its immunogenic potential prompted us to re-examine the role of radiotherapy in ovarian cancer. In this Series paper, we review the clinical experiences and clinical trial results of two different forms of radiotherapy for ovarian cancer treatment—namely whole-abdominal radiotherapy and SBRT. We will then focus our discussions on the biological mechanisms that these two different forms of radiotherapy might elicit to mobilise the immune system. We then offer a roadmap that could guide future clinical development and research opportunities of novel combination therapies in ovarian cancer.

History of whole-abdominal radiotherapy for ovarian cancer

Radiotherapy has extensively been used with a curative intent as an adjuvant therapeutic option for early-stage and minimal residual advanced-stage ovarian carcinoma of all tumour subtypes. However, in the 1980s whole-abdominal radiotherapy was mainly abandoned in favour of systemic platinum-based chemotherapy on the basis that cisplatin was a highly active systemic agent, with possibly higher efficacy than whole-abdominal radiotherapy; whole-abdominal radiotherapy could not be conveniently combined with full-dose platinum chemotherapy because of severe myeloid toxic effects; and large radiation fields were intolerable for the gastrointestinal and genitourinary tracts[31] and inefficacious in eradicating bulky residual peritoneal carcinomatosis.[37] Nevertheless, multiple trials have suggested that whole-abdominal radiotherapy is still an active option for ovarian cancer management. In 1979, Dembo and colleagues[38] randomly assigned 190 patients with stage I–III ovarian cancer with minimal residual disease to postoperative whole-abdominal radiotherapy (22·5 Gy in ten fractions given to the pelvis, followed immediately by 22·5 Gy in ten fractions to the whole abdomen and pelvis using cobalt with moving-strip technique) versus pelvis irradiation (45 Gy in 20 fractions) and concomitant chlorambucil (6 mg per day for 2 years).[38] The study showed a 10-year survival advantage of 64% versus 40% in favour of whole-abdominal radiotherapy.[39] However, no survival benefit was observed for patients with gross (>2 cm) residual disease.[39] In a Canadian trial done between 1981 and 1990, 125 stage I–III patients with ovarian cancer were randomly assigned to receive two doses of whole-abdominal radiotherapy, 22·5 Gy in 22 fractions or 27·5 Gy in 27 fractions, both followed by a pelvic boost of 22·5 Gy in ten fractions.[40] No difference was found in terms of survival, tumour control, or toxic effects between high-dose and low-dose abdominopelvic radiotherapy, and the authors concluded that doses higher than 22·5 Gy are unlikely to improve outcomes.[40]

Four other randomised trials have compared whole-abdominal radiotherapy with chemotherapy, although only two studies involved cisplatin-based chemotherapy. Most of these trials showed no significant differences between whole-abdominal radiotherapy and chemotherapy groups. The most important trials summarising the role of whole-

abdominal radiotherapy in the postoperative setting are presented in table 2. In the study published by Dembo and colleagues,[39] patients with stage III ovarian cancer with high-grade histology and residual disease after debulking surgery had worse prognoses and derived less benefit from whole-abdominal radiotherapy. In this subgroup of patients, the group from Princess Margaret Hospital, tested a combined chemotherapy strategy (50 mg/m² cisplatin, 50 mg/m² doxorubicin, and 500 mg/m² cyclophosphamide every 3 weeks) followed by consolidation whole-abdominal radiotherapy.[37] This group was compared with a historical group of high-risk patients who received only whole-abdominal radiotherapy. The median survival for the combined group was 5·7 years compared with 2·4 years in the control group.[37] Although the difference was not statistically significant due to the small number of patients involved, this finding served to highlight that whole-abdominal radiotherapy was tolerable following systemic chemotherapy. More recently, a population-based study from British Columbia, Canada, suggested that whole-abdominal radiotherapy confers a survival benefit when added to chemotherapy, particularly in clear-cell carcinoma.[45] Using retrospective data, this report showed that patients with stage IC–II ovarian clear-cell carcinoma treated with carboplatin and paclitaxel followed by whole-abdominal radiotherapy had statistically superior disease-free survival compared with patients who had no radiotherapy after initial chemotherapy. The absolute increase in disease-free survival following radiotherapy was 20% at 5 years. The results of these trials should be interpreted with caution due to the small number of patients included in the analysis, methodological issues (eg, unbalanced selection criteria, comparison with historical series), and the suboptimal surgery, chemotherapy, or radiotherapy technology applied in some of these studies. Nevertheless, these reports provide important experience regarding the use of whole-abdominal radio-therapy in ovarian cancer.

The analysis of the side-effects of whole-abdominal radiotherapy in 598 patients with ovarian cancer who were treated between 1971 and 1985 showed that the most frequent acute complications were nausea and vomiting (in 364 patients [61%]), diarrhoea (in 407 patients [68%]), and cystitis (in 38 patients [6·4%]).[31] 25 patients (4·2%) had serious late bowel complications. Treatment interruptions were frequent and were observed in 136 patients (23%), the most common cause being myelosuppression.[31] Many of the toxic effects of whole-abdominal radiotherapy are due to the large volume of tissue receiving a high dose of radiotherapy with little sparing of healthy organs. Over the past few years, newer radiotherapy techniques have been implemented that enable the sparing of healthy tissues. Arians and colleagues[46] reported a phase 2 study of 20 patients treated with IMRT to the whole-abdominal cavity with effective sparing of healthy organs. Only one patient experienced acute grade 4 haematological toxic effects. No gastrointestinal acute toxic effects above grade 2 were observed. Importantly, quality of life (QOL; mean global health status) decreased by 18·1 points (95% CI 7·1–29·0), but had recovered to baseline 6 weeks after whole-abdominal radiotherapy. A similar observation was found for all function scale scores. Our group and others have been able to spare large volumes of active bone marrow from high-radiation doses in gynaecological tumours with the aim of reducing acute and late haematological toxic effects.[47,48] Rochet and colleagues[49] showed substantial sparing of kidney and liver tissue using a form of VMAT called TomoTherapy (Accuray, Sunnyvale, CA, USA). We summarise several dosimetric studies on the differences among three-dimensional conformal radiation therapy, IMRT, and VMAT on whole-abdominal radiotherapy delivery for ovarian cancer treatment (appendix pp 1–2).

New strategies using radiotherapy at lower doses as a biological response modifier could improve tolerance and increase the efficacy of chemotherapy, targeted therapy, and immunotherapy. As an example, the Gynecology Oncology Group administered low-dose whole-abdominal radiotherapy (0·6 Gy in two fractions daily on days 1 and 4 of each week for 6 weeks) as a chemosensitiser for dose-escalated weekly docetaxel in

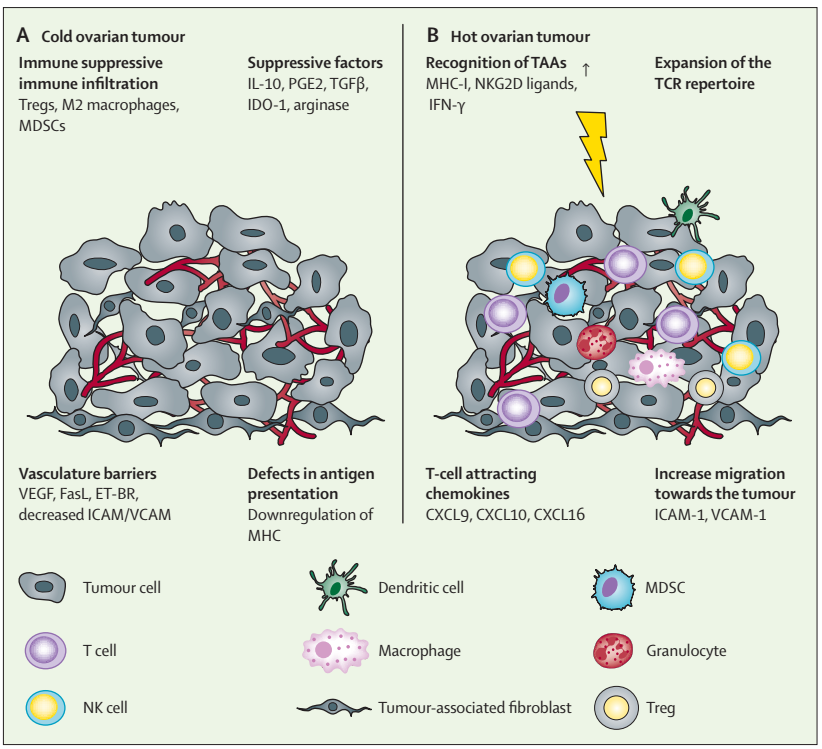

Figure 2: **Tumour inflammatory profiles**
Tumours can be divided into so-called hot (T-cell inflamed) or cold (T-cell non-inflamed) tumours according to the presence of immune cells. (A) In cold tumours, barriers to T-cell infiltration and activity exist. (B) In these cold tumours, radiotherapy can reprogramme the tumour microenvironment to make them suitable for checkpoint blockade cancer immunotherapies. TAA=tumour-associated antigen. TCR=T-cell receptor. Treg=regulatory T cell. MDSC=myeloid-derived suppressor cell. MHC-I=MHC class I. NK cell=natural killer cell. PGE2=prostaglandin E2.

See Online for appendix

	Patients (n)	Histology	Primary tumour site	Previous treatments	Type of immune therapy	Radiotherapy dose	Abscopal effects	Time between abscopal effects and radiotherapy (months)	Duration of response	Patient outcomes
Ohba et al, 1998[23]	1	Hepatocellular carcinoma	Liver	Surgery and chemoembolisation	..	36 Gy	Hepatic metastases	10	29 months	Alive with minimal disease
Nam et al, 2005[24]	1	Hepatocellular carcinoma	Liver	NR	..	30 Gy	In skull, liver, ribs, and sternum	10	60 months	Alive without disease
Siva et al, 2013[25]	1	Adenocarcinoma	Lung	Chemotherapy and radiotherapy	..	60 Gy in 30 fractions and one fraction of 26 Gy	In adrenal gland and bone	12	15 months	In progression
Maity et al, 2018[26]	12	Non-small-cell lung cancer and melanoma	Lung, adrenal gland, liver, lymph node, and skin	Surgery and chemotherapy	Patients were progressing on anti-PDL-1 or anti-CTLA-4 therapy	24 Gy in three fractions and 17 Gy in one fraction	In lymph nodes and lung	6	9·2 months and 28 months	Two patients in partial response (overall response rate, 17%)
Grimaldi et al, 2014[27]	21	Melanoma	Skin	Surgery	Patients were progressing after having received anti-CTLA-4 therapy	30 Gy in ten fractions or one fraction of 20–24 Gy for brain; 20 Gy in five fractions, 30 Gy in ten fractions, or 50 Gy in 25 fractions for extracranial sites	62% brain metastases; 38% extracranial metastases	NR	Median overall survival for patients with abscopal responses, 22·4 months vs 8·3 months without	Partial response, 43%; stable disease, 10%
Postow et al, 2012[13]	1	Melanoma	Skin	Surgery	Patient was progressing on anti-CTLA-4 therapy	28·5 Gy in three fractions	Hilar lymphadenopathy and splenic lesions	4	NR	Complete response 10 months after radiotherapy

Abscopal effects of radiation administered alone and in combination with immunotherapy in patients that were progressing on immune checkpoint inhibitors. NR=not reported.

Table 1: **Abscopal effects in published clinical cases**

women with recurrent ovarian, fallopian tube, or peritoneal cancers. Ten (77%) of 13 patients included had radiologically measurable disease. Of the ten radiologically evaluable patients, six (60%) had stable disease and four (40%) had progressive disease. Three (30%) of these patients were disease free for at least 6 months. Of the three patients (23% of the 13 cases) with radiologically non-measurable disease, one patient (33%) had a disease-free interval of 21·2 months. Dose-limiting toxic effects were primarily haematological but also included grade 3 diarrhoea in one patient.[33] Reducing radiotherapy doses to the whole abdomen while potentiating synergy with systemic therapies was also shown in other studies. A phase 1 trial that enrolled 12 patients with optimally debulked stage III/IV endometrial cancer tested low-dose whole-abdominal radiotherapy in combination with weekly cisplatin. The results suggested feasibility of using low-dose whole-abdominal radiotherapy (0·50 and 0·75 Gy per fraction) as a novel chemosensitising modality for weekly cisplatin (40 mg/m², maximum 70 mg intravenously).[34] Three patients in each cohort had grade 3 acute haematological events. One patient had grade 4 neutropenia. The estimated median time to recurrence was 18·2 months (95% CI 7·2 to not reached). The estimated median survival time was 27·5 months (10·4 months to not reached).[34] Similar results were seen in a cohort of patients with gastrointestinal malignancies and peritoneal carcinomatoses that were treated with low-dose whole-abdominal radiotherapy and gemcitabine.[50] In the past 2 years, low-dose whole-abdominal radiotherapy (0·6 Gy in two daily fractions on day 1 and day 5 of each week for 3 weeks) was combined with the PARP inhibitor veliparib used in a dose-escalated manner twice daily in patients with epithelial ovarian, fallopian tube, or peritoneal cancers.[35] Of the 32 patients, one patient with platinum-sensitive *BRCA*-mutated ovarian cancer achieved a partial response. The overall survival in the platinum-sensitive population was 11 months compared with 6 months in the platinum-resistant patients. The treatment was well tolerated with no major side-effects.[35] These data indicate that low-dose irradiation to the whole-abdominal cavity is well tolerated.

Hypofractionated radiotherapy in the oligometastatic setting of ovarian cancer

For many years, radiotherapy has been predominantly used in the palliative setting with the aim to offer symptom control in ovarian cancer. In the past 10 years, several studies have reported the use of localised high-dose irradiation (>5 Gy per fraction) to treat patients with oligometastatic ovarian cancer. Oligometastatic disease is an intermediate clinical state of cancer dissemination where few metastases are detectable by current imaging methods.[51] SBRT is an innovative new approach for ablative or salvage radiotherapy that allows for precise high-dose radiotherapy to the tumour, with minimal

	Patients (n)	Stage	Treatment groups	Overall survival (%)			p value
				WART	Chemotherapy	No further treatment	
Dembo et al, 1979[38]	147	I–III	WART (45·0 Gy in 20 fractions for pelvis and 22·5 Gy in ten fractions for abdomen) *vs* 6 mg per day chlorambucil	85%	53%	NA	<0·05
Sell et al, 1990[41]	118	I–II	WART (45·0 Gy in 20 fractions for pelvis and 22·5 Gy in ten fractions for abdomen) *vs* 200 mg/m² cyclophosphamide per day for 4 weeks	55%	63%	NA	NS
Chiara et al, 1994[42]	70	I–III	WART (43·2 Gy in 24 fractions for pelvis and 30·2 Gy in 24 fractions for abdomen) vs 50 mg/m² cisplatin and 600 mg/m² cyclophosphamide; 6 cycles every 28 days from day 1	53%	71%	NA	NA
Sorbe et al, 2003[43]	98	III–R0	Group A, WART*; group B, chemotherapy†; group C, no further treatment	69%	57%	65%	<0·001
Sorbe et al, 2003[43]	172	III–R1	Group A, WART*; group B, no further treatment	32%	NA	41%	0·112
Pickel et al, 1999[44]	32	IC–IV (R0 surgery followed by chemotherapy)‡	WART (30·0 Gy for abomen, 51·6 Gy for pelvis, and 42·0 Gy for para-aortic irradiation) *vs* no further treatment	59%	NA	33%	0·029

WART=whole-abdominal radiotherapy. NA=not applicable. NS=not significant. R0=no evidence of microscopic disease after surgery. R1=microscopic disease after surgery. *WART consisted of 50·4 Gy in 32 fractions for pelvis and 20 Gy in 20 fractions for the abdomen. †Chemotherapy consisted of 50 mg/m² cisplatin plus 50 mg/m² doxorubicine or 60 mg/m² epirrubicine. ‡Chemotherapy consisted of 400 mg/m² carboplatin and 70 mg/m² epirubicin on day 1 and 100 mg/m² prednimustine orally on days 3–7 at 1-month intervals.

Table 2: **Studies comparing WART with chemotherapy**

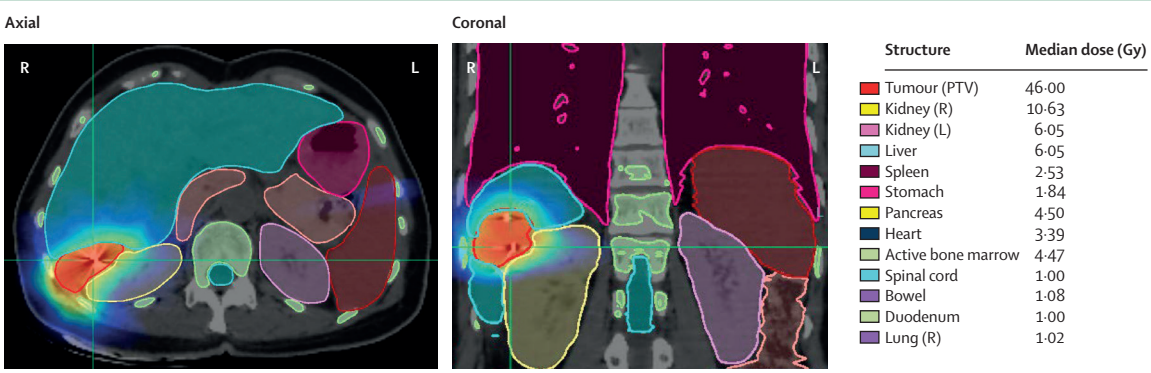

Figure 3: **SBRT on an ovarian cancer tumour**
Example of SBRT dose wash for a patient with oligometastatic ovarian cancer. The tumour has a dose wash of 46 Gy (prescribed dose 45·5 Gy in seven fractions of 6·5 Gy at 80% isodose line). Fiducial markers were placed in the tumour for robotic assistance. Median dose to the organs at risk and the tumour are described. L=left. PTV=planning target volume. R=right. SBRT=stereotactic body radiotherapy.

dose to organs in close proximity, and is ideal for patients with little metastatic disease (figure 3). The use of SBRT at oligometastatic sites was shown to increase progression-free survival by 5 months[52] and 7 months,[53] in two randomised trials that included patients with metastatic non-small-cell lung cancer.[52,53] Furthermore, in stage I inoperable non-small-cell lung cancer, SBRT increased local disease control when compared with standard fractionated radiotherapy.[54] In 2015, a phase 2 randomised trial that included patients with breast, prostate, colorectal, or lung cancer showed that the use of SBRT in patients with controlled primary tumours and one to five oligometastases achieved a 13 month improvement in overall survival.[55]

Multiple studies of SBRT in the management of metastatic gynaecological malignancies have been published. Lazzari and colleagues[36] evaluated 82 oligometastatic, platinum-resistant patients with ovarian cancer who had a median of three prior systemic treatment regimens. A median of two lesions per patient were irradiated with 24 Gy in three fractions. For the whole cohort of patients, 156 treated lesions were radiologically evaluated. Complete radiological response was observed in 91 (58%) lesions, partial response in 26

(17%) lesions, stable disease in 24 (15%), and progressive disease in 11 (7%) irradiated lesions. This study confirmed the radiosensitivity of ovarian cancer even in the context of platinum resistance. Importantly, the study showed the potential role of SBRT to delay systemic therapy with a median systemic treatment-free interval after SBRT of 7·4 months. The pattern of recurrence after SBRT was predominantly outside the irradiated area. Kunos and colleagues[56] treated 25 patients with oligometastatic, platinum-resistant ovarian cancer (less than four metastatic sites) with 24 Gy in three fractions and showed 100% local control and a disease-free survival of 7·8 months. Stereotactic body radiosurgery was well tolerated in this study despite a heavily pretreated patient population. Similarly, Iftode and colleagues[57] treated 26 patients with SBRT. A median of two metastatic locations per patient were irradiated (44 metastatic lesions treated in total). 28 lesions (63·6%) were located in the lymph nodes, 14 lesions (31·8%) in the liver and two lesions (4·5%) were located in the lung. At a median follow-up of 28·5 months, the local control rate was 92% and median disease-free survival was 19 months. This study provided evidence that endometrioid and clear-cell histology are also radiosensitive. Importantly, no severe adverse events were reported.[57] A group at MD Anderson Cancer Center reported a series of 102 carefully selected patients with ovarian cancer whom, despite having been pretreated with a median of three chemotherapy lines, underwent local irradiation of oligometastases with curative doses (≥45 Gy).[58] 72 patients (71%) achieved disease control in the irradiated area and 35 patients (35%) had no evidence of disease outside the irradiated area at a median of 38 months after irradiation.[58] 5-year overall survival after radiotherapy was 40% and disease-free survival was 24%. Patients benefited from longer chemotherapy-free periods after receiving radiotherapy (median chemotherapy-free period 6 months after radiotherapy *vs* 2 months before radiotherapy). No major acute side-effects were reported. Ten patients had bowel obstruction and five patients had ureteral stenosis, although most of these complications were attributed to disease progression rather than radiotherapy.[58]

The results of these studies deserve caution as the sample sizes are small, patients were not randomly assigned, and an important selection bias might exist for patients with ovarian cancer treated with this modality. It is also worth noting that despite excellent local control in the irradiated area, progression outside of the targeted lesions remained high, and in one study was reported to occur in 58 (72%) of 81 patients.[36] The challenge is determining which patients will benefit most from SBRT. Patients with clear-cell histology were more likely to be continuously without systemic disease after irradiation (six [75%] of eight patients) than other patients (19 [26%] of 74 patients; p=0.003).[58] These high rates of distant progression prompted a phase 1 study with 12 women (seven with primary ovarian cancer) testing the safety of sequential carboplatin and gemcitabine followed by SBRT.[59] This study showed that carboplatin area under curve 2 or 4 and 600 mg/m² gemcitabine can be delivered the day before SBRT using CyberKnife (Accuray, Sunnyvale, CA, USA) with acceptable toxic effects by use of a regimen of three fractions of 8 Gy.[59] These results are reassuring and might lead to the use of radiosensitisers, targeted agents, and immunotherapy in combination with SBRT.

Immunotherapy in ovarian cancer

Although immunotherapy with immune checkpoint inhibitors has been accepted as a new and important cancer treatment, not all patients, nor all tumour types, draw clinical benefit. A phase 1 study of 20 patients with platinum-resistant ovarian cancer treated with nivolumab (1–3 mg/kg), a checkpoint inhibitor targeting PD-1, showed a modest overall response rate of 15% and a disease control rate of 45%.[60] Similarly, an analysis of patients with ovarian cancer who had been treated with one or fewer lines of therapy and given avelumab, an anti-PD-L1 drug, administered at 10 mg/kg every 2 weeks, showed an overall response of 21% compared with only 9% in individuals who were heavily pretreated and had more advanced disease.[61] Other trials using PD-1 or PD-L1 inhibitors showed similar results.[62] Regarding biomarker predictors of tumour response, Disis and colleagues[61] analysed PD-L1 expression in archival tumour tissues. No correlation was found between PD-L1 expression and benefit from avelumab treatment. The proportion of patients with PD-L1-positive tumours (PD-L1 expression cutoff of 1%) who achieved an overall response was 12% (nine of 76 patients). In the nivolumab trial,[60] tumour specimens from 16 (80%) of 20 patients showed high expression of PD-L1 (15 patients had pathology scores of +2 and one patient had a pathology score of +3). An objective response occurred in two of 16 patients (12·5%) with tumours that showed high expression of PD-L1, whereas no response occurred in two of the four patients with tumours with low expression of PD-L1. Similarly, in other trials, responses occurred irrespective of tumour PD-L1 status.[62]

Responses to immune checkpoint inhibitors are contingent upon the presence of tumour-specific T cells that can be reinvigorated in the tumour microenvironment (tumour-infiltrating lymphocytes [TILs]).[63,64] Thus, the key objective of current cancer immunotherapy is to convert so-called cold or immune-desert tumours to so-called hot tumours to unleash tumour immune responses. In preclinical models of ovarian cancer, Duraiswamy and colleagues[65,66] confirmed that absence of T-cell infiltration at baseline predicts resistance to PD-L1 blockade-based combinations, an association confirmed in patients with melanoma who received PD-1 blockade.[64] In published ovarian cancer trials, baseline TIL status of patients is unknown, and thus no correlation

with disease control rate or overall response rate has been reported. However, overall disease control rate reported in patients with ovarian cancer receiving PD-1 or PD-L1 blockade monotherapy (38–52%)[61,67] is lower or similar to previously reported proportions of patients with ovarian cancer whose tumours exhibit intraepithelial TILs (roughly 55%).[68] Therefore, approximately half of ovarian cancer tumours that do not have pre-existing TILs would not be expected a priori to respond to PD-1 blockade or combinations that primarily focus on boosting T effector cell functions. The immune-inflamed phenotype might also feature TILs that have attenuated effector cell functions because of coexpression of additional coinhibitory T-cell receptors (eg, LAG-3, TIM-3, BTLA [B- and T-lymphocyte attenuator], or CTLA-4), which have already been described in human ovarian tumours,[66,69] and combination of antibodies blocking more than one of these receptors could reduce adaptive resistance to PD-1 blockade.

In advanced ovarian tumours, several immune suppressive factors might attenuate T effector cell functions, dampening the response to PD-1 blockade. In this context, T cells might be subject to suppression by regulatory T cells,[70] myeloid-derived suppressor cells (MDSCs),[71] tumour-associated macrophages,[72] and a plethora of soluble factors including IL (interleukin)-10,[73] prostaglandins,[74] and TGFβ.[75] In addition, negative metabolic regulators, such as IDO-1 (indoleamine 2,3-dioxygenase 1)[76] and arginase-1[65,77] can suppress T cells through depletion of L-tryptophan and L-arginine in the tumour microenvironment. In other instances, T cells are excluded from the tumour islets because of vascular barriers like the upregulation of FasL (tumor necrosis factor ligand superfamily member 6) on endothelial cells, which selectively kills T cells leaving regulatory T cells (Tregs) unaffected, or the decrease or deregulation of endothelial adhesion molecules ICAM-1 (intracellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1), which are key for T-cell extravasation.[78–80] Reprogramming the immune microenvironment of ovarian cancer is thus crucial to unleash tumour responses to immune checkpoint inhibitors.

High tumour mutational burden and consequently neoantigen load have also been associated with tumour response to PD-1 and PD-L1 blockade in melanoma, and non-small-cell lung cancer.[81,82] Furthermore, patients with mismatch repair-deficient colorectal cancer, which exhibits a hypermutated phenotype, had higher response rates to PD-1 blockade than patients with mismatch repair-proficient tumours.[83,84] Conversely, in patients with ovarian cancer, the number of non-synonymous mutations and the incidence of mismatch repair deficiency is relatively low.[84,85] This low incidence of mutations correlated with unresponsiveness to anti-PD-1 or anti-PD-L1 therapy for ovarian cancer in two studies.[86,87]

Immune modulatory effects of radiotherapy

In-situ vaccination induced by radiotherapy

Radiation can induce multiple forms of DNA damage, which has been observed with doses as low as 1 Gy.[88] Radiation-generated double-strand break DNA fragments are sensed by cGAS (cyclic GMP-AMP synthase), a pattern-recognition receptor that triggers type I interferon (IFN-I) production via hSTING (downstream adaptor stimulator of interferon genes, also known as MITA).[17] The induction of IFN from cGAS/hSTING signalling is required to achieve optimal dendritic cell recruitment and cross-priming of T effector cells, the essential steps to convert the tumour into an in-situ vaccine. Similarly, irradiated stressed cells might release danger signals that can ignite an inflammatory reaction, and undergo a so-called immunogenic death which will effectively expose tumour-associated antigens.[89] Hallmarks of immunogenic cell death upon irradiation include the translocation of calreticulin from the endoplasmic reticulum to the cell surface, which acts as a so-called eat-me signal inducing maturation of dendritic cells, with subsequent release of cytokines such as IL-6 and TNF (tumor necrosis factor)-alpha.[90] In addition, radiation-damaged tumour cells activate APCs through the release of damage-associated molecular pattern molecules (DAMPs), which include HMG-1 (high-mobility group box 1), a chromatin nuclear protein that is released mainly after necrotic cell death and serves as a TLR (toll-like receptor) 4 ligand on APCs,[19] and the release of ATP, which acts as a so-called find-me signal for monocytes and dendritic cells,[91] leading to the secretion of proinflammatory cytokines such as IL-1 beta and IL-18.[92] Similarly, complement anaphylatoxins, released following complement activation by radiotherapy-induced IgM binding to necrotic tumour cells, might directly contribute to dendritic cell recruitment and maturation, and ultimately to T-cell immunity (figure 1).[93]

Immune reprogramming by radiotherapy

The in-situ vaccination process contributes to the effective recognition of tumour-associated antigens by dendritic cells, which will then migrate to lymph nodes or tertiary lymph node structures, where they present such antigens to T cells and exert potent immunomodulatory effects. Furthermore, the Batf3 (basic leucine zipper transcriptional factor ATF-like 3)-dependent dendritic cell subset has been shown to be essential for the cross-priming of CD8 T cells, which are key effectors in antitumour immunity.[94] Indeed, *Batf3*$^{-/-}$ mice exhibit an impaired ability to crossprime cytotoxic T lymphocytes against tumour antigens.[94] This process requires peptide MHC recognition by cognate T-cell receptors. Radiotherapy upregulates MHC class I molecules on tumour cells, enabling enhanced presentation of tumour-associated antigens.[15,95] In addition, local high-dose radiotherapy can trigger production of type I IFN, which initiates a cascade of events able to activate innate and adaptive immunity against the tumour.[96] NKG2D

(NKG2-D type II integral membrane protein) receptor ligands, induced upon irradiation, act as activating receptors for the adaptive immune system.[97,98] Radiotherapy can also upregulate the expression of death receptors of the TNF family on tumour cells, including FasL, TNF-apha receptors,[99] TRAIL-R1 (death receptor 4), and TRAIL-R2 (death receptor 5)[100] on tumour cells. The ligands for these receptors (FasL and TRAIL) are expressed on activated cytotoxic T lymphocytes or secreted by them (TNF-alpha). Thus, radiotherapy can sensitise tumour cells to cytotoxic T lymphocyte-mediated apoptosis.

Many of these acute responses to radiotherapy exposure can either directly or indirectly attract or activate cytotoxic T lymphocytes, thus explaining its potential for turning a non-inflamed tumour into one that is responsive to immunotherapy.[89] For example, CXCL16, which has been shown to be induced by radiotherapy (via IFN-γ and TNF-alpha), promotes the recruitment of CD8 T effector cells and T helper 1 CD4 cells.[101] IFN-γ also induces CXCL9 and CXCL10, which recruit T cells into the tumour microenvironment.[102] In addition to providing chemoattractants to recruit T cells, radiotherapy can also help their homing into the tumour bed via the upregulation of adhesion molecules, such as ICAM-1 on the tumour vasculature endothelium, which facilitates leucocyte endothelial transmigration (figure 2).[103]

A roadmap for the development of radiotherapy and immunotherapy combinations in ovarian cancer

This clinical experience offers important knowledge for rethinking radiotherapy in the era of immunotherapy. Indeed, both SBRT and whole-abdominal radiotherapy could be repurposed as partners for immunomodulatory therapies to achieve improved tumour control in specific clinical populations; SBRT has emerged as an important intervention for in-situ vaccination, while low-dose whole-abdominal radiotherapy could be envisioned as a means to achieve subdiaphragmatic so-called in-field tumour reprogramming in the context of immunotherapy schemes. However, the exact dose, volumes, and fractionation schemes of radiotherapy require optimisation to maximise the benefits in combination with immunotherapy.[89] We describe two clinical scenarios where radiotherapy can be tested in clinical trials in combination with immunotherapy in ovarian cancer.

SBRT to induce in-situ vaccination in combination with immunotherapy

Hypofractionated SBRT releases immunogenic tumour-associated antigens over the course of several days[14,15] together with endogenous DAMP ligands that can stimulate TLRs on APCs.[19] Vanpouille-Box and colleagues[104] showed that double-stranded DNA fragments accumulated in the cytoplasm with hypofractionated doses below 10 Gy. Above that radiation therapy dose threshold, induction of TREX1 (three-prime repair exonuclease 1), an enzyme that degrades cytoplasmic DNA, mediates rapid degradation of cytosolic DNA, precluding the activation of the cGAS/hSTING pathway and abrogating the abscopal effect of radiation and synergy with CTLA-4 blockade.[104]

This finding provides the basis for developing rational radioimmunotherapy combinations. Although SBRT itself can promote tumour antigen release and APC stimulation, tumour resident APCs might be strongly polarised towards tolerogenic functions, such that SBRT alone might be insufficient to produce effective in-situ vaccination. Further activation of APCs through CD40 or TLR agonists could be used to maximise immunogenic antigen presentation.[105,106] Immune checkpoint inhibition of the PD-1 and PD-L1 axis, and CLTA-4 could provide further synergy to the combination by removing inhibition on T cells and enhancing their priming capacity.[107,108] A combination of SBRT, CD40, or TLR agonists, plus immune checkpoint inhibitors, might be sufficient to trigger effective in-situ vaccination in a subset of patients with ovarian cancer. This combination could serve as one component of combinatorial immunotherapy to mobilise immunity. Clearly, additional immune suppressive mechanisms that are active will need to be neutralised, including T-cell intrinsic immune checkpoints but also extrinsic suppressive mechanisms from the tumour microenvironment. The therapeutic toolbox is progressively increasing, with a large number of agents under clinical testing.

Immediate opportunities to target immune suppressive pathways exist. For example, Tregs, which have an important role in suppressing T-cell immunity in ovarian cancer,[70] can be attenuated through metronomic cyclophosphamide,[109] while VEGF blockade can normalise the tumour vasculature,[79] and in combination with aspirin, can significantly enhance T-cell homing in ovarian tumours.[80]

The combination of metronomic cyclophosphamide (50 mg delivered orally) with bevacizumab (15 mg/kg delivered intravenously) together with the anti-PD-1 antibody pembrolizumab (200 mg delivered intravenously) produced important clinical responses (15 partial response and 13 stable disease) in 40 patients with advanced recurrent ovarian cancer.[110] The 6-month progression-free survival rates for the platinum-sensitive population were 70% and 59% for non-sensitive patients (p=0·024).Other agents under current clinical testing could prove very useful, including antibodies targeting TGFβ, which might be used to overcome stromal barriers,[111] and IL-2, which at the peak of tumour antigen release by radiotherapy could help boost the expansion and function of mobilised T cells.[112] We summarise the preclinical evidence of SBRT and different combinatorial immunotherapies to activate tumour responses in different tumour models (table 3) and published clinical trials of SBRT and immunotherapy (table 4).

The combination of PD-1 and CTLA-4 inhibition with nivolumab and ipilimumab was reported to produce statistically higher response rates (31·4%) than nivolumab alone (12·2%) in a phase 2 study with 100 patients with persistent or recurrent ovarian cancer.[128] Importantly, the administration of immune checkpoint inhibitors targeting PD-1 or PD-L1 as well as CTLA-4 in concomitance with SBRT was proven safe.[129] Overall, SBRT studies in ovarian cancer have shown that hypofractionated radiation is well tolerated without clinically significant side-effects. However, the potential for complications should not be minimised, particularly for patients with ovarian cancer harbouring metastatic disease close to the bowel, kidney, bladder, or ureters. In the PLUMMB trial, combining pembrolizumab (100 mg every 3 weeks) and radiotherapy (36 Gy in six fractions, weekly) in patients with localised bladder cancer, grade 3 dose-limiting urinary toxicity was reported in two out of the first five patients enrolled, and the trial was stopped prematurely without reporting efficacy outcomes.[130] The authors advised caution when

	In-vivo tumour model	Radiation dose	Immunotherapy details	Results
Chakravarty et al, 1999[113]	Mice bearing spontaneous tumour model of subcutaneous Lewis lung carcinoma	One fraction of 60 Gy	After radiotherapy, 500 µg/kg bodyweight intraperitoneal Flt3L per day for 10 days	Improved survival and reduced lung metastases compared with control or Flt3L alone
Teitz-Tennenbaum et al, 2003[114]	Mice bearing subcutaneous MCA 205 sarcoma or D5 melanoma	42·5 Gy (five fractions of 8·5 Gy)	Before and after radiotherapy, four intravenous deliveries of 1×10^6 intratumoral dendritic cells	Increased overall response rate compared with control or dendritc cell administration alone
Demaria et al, 2004[11]	Mice bearing subcutaneous 67NR mammary carcinoma	One fraction of 2–6 Gy	After radiation therapy, ten deliveries of 0·5 mg/kg bodyweight intraperitoneal Flt3L	Synergism through T-cell-mediated abscopal effect
Demaria et al, 2005[115]	Mice bearing subcutaneous 4T1 mammary carcinoma	12 fractions of 2 Gy	After radiation therapy, three intraperitoneal doses of 200 µg anti-CTLA-4	Increased tumour responses compared with control or CTLA-4 alone
Lee et al, 2009[116]	Mice bearing subcutaneous 4T1 mammary carcinoma and B16-CCR7 melanoma	Two fractions of 12 Gy	During and after radiation therapy, delivery of intratumoral adenovirus expressing TNFSF14	Increased abscopal effect was T-cell mediated
Dewan et al, 2009[117]	Mice bearing subcutaneous TSA mammary adenocarcinoma and MCA38 colon carcinoma	Five fractions of 6 Gy, three fractions of 8 Gy, or one fraction of 20 Gy	During or after radiation therapy, three intraperitoneal doses of 200 µg anti-CTLA-4	Fractionated but no single fraction radiotherapy with CTLA-4 monoclonal antibody induced abscopal effect
Deng et al, 2014[118]	Mice bearing subcutaneous TUBO mammary carcinoma and MCA38 colon carcinoma	One fraction of 12 Gy	Before, during, and after radiotherapy, four intraperitoneal doses of 200 µg anti-PD-L1	Increased T-cell infiltration boosts abscopal effect
Twyman-Saint et al, 2015[119]	Mice bearing subcutaneous TSA mammary adenocarcinoma and subcutaneous B16-F10 melanoma	Three fractions of 8 Gy or one fraction of 20 Gy	Before, during, and after radiotherapy, 200 µg intraperitoneal anti-CTLA4, anti-PD1, and anti-PD-L1	CTLA-4 decreased Tregs, PD-L1 reinvigorates exhausted T cells, and radiotherapy increases the TCR repertoire
Rodriguez-Ruiz et al, 2006[120]	Mice bearing subcutaneous MC38 colon adenocarcinoma	Three fractions of 8 Gy	After each radiation therapy fraction, 300 µg intraperitoneal anti-PD1, CD137 agonist, or both	Potentiation of abscopal effect
Reits et al, 2006[15]	Subcutaneous MC38 adenocarcinoma	One fraction of 10 Gy	Three intravenous adoptive transfers of 1×10^6 gp70-specific CTLs	Radiotherapy significantly enhanced the efficacy of adoptive T-cell transfer in vivo
Honeychurch et al, 2003[121]	B-cell lymphoma line injected intravenously into BALB/c mice	One fraction of 5 Gy total body irradiation	1 mg intravenous CD40 agonist	Combination resulted in increased survival with long-term T-cell-mediated protection in more than 80% of animals
Verbrugge et al, 2012[122]	4T1·2 mouse subcutaneous syngeneic breast tumours	One fraction of 12 Gy	100 µg intraperitoneal CD137 agonist, agonistic CD40, or anti-PD-1 on days 0, 4, 8, and 12 relative to radiation therapy	Enhanced antitumor effect mediated by CD8 and natural killer cells
Dovedi et al, 2013[123]	A20 cell line model of subcutaneous B-cell lymphoma and EL4 cell line model of T-cell lymphoma line	One fraction of 10 Gy	3 mg/kg intravenous TLR7 agonist R848 starting with radiation therapy and repeated weekly for up to 5 weeks	Increased CD8-mediated tumour control
Vanpouille-Box et al, 2015[124]	4T1 mouse model of subcutaneous mammary carcinoma	Five fractions of 6 Gy	200 µg intraperitoneal 1D11 (a pan-isoform anti-TGFβ; every other day from day 12–28 before and after radiotherapy), anti-PD1 (on days 18, 22, 26, and 30 after radiotherapy), or both	Increased tumour responses and survival
Rech et al, 2018[105]	KPC.4662 mouse model of spontaneous pancreatic ductal carcinoma and mice bearing subcutaneous B16-F10 tumours	One fraction of 20 Gy	200 mg intraperitoneal anti-PD-1 and anti-CTLA-4 administered before, concomitant, and after irradiation and CD40 agonist on day 3 after radiotherapy	Increased survival and abscopal effect
Mason et al, 2005[125]	C3Hf/KamLaw mice developing fibrosarcoma induced by methylcholanthrene	Two fractions of 10 Gy	100 µg peritumoral CpG oligodeoxynucleotide 1826 (TLR9 agonist) per mouse	Increased tumour control and survival

TNFS14=tumor necrosis factor ligand superfamily member 14 (also known as LIGHT). CTL=cytotoxic T lymphocyte. Treg=regulatory T cell. TCR=T-cell receptor. SBRT=stereotactic body radiotherapy.

Table 3: **Preclinical studies of SBRT and immunotherapy**

	Disease	Patients (n)	Radiation therapy	Immune checkpoint inhibitor	Schedule	Abscopal
Twyman-Saint Victor et al, 2015[119]	Melanoma	22	Two to three fractions of 6 Gy or two to three fractions of 8 Gy	Four doses of 3 mg/kg ipilimumab every 3 weeks	Ipilimumab 3–5 days after radiation therapy	18%; no complete responses
Hiniker et al, 2012[28]	Melanoma	22	Four fractions of 50 Gy, three fractions of 24 Gy, or ten fractions of 40 Gy	Four doses of 3 mg/kg ipilimumab every 3 weeks	Radiation therapy within 5 days of ipilimumab	Complete response, 14%; partial response, 14%; stable disease, 23%
Tang et al, 2017[126]	Non-small-cell lung cancer, colorectal cancer, renal cell carcinoma, and other cancers	35	50 Gy in four fractions; ten fractions of 6 Gy at one site	Four doses of 3 mg/kg ipilimumab every 3 weeks	Radiation therapy 1 day after ipilimumab or 1 week after second ipilimumab	Partial response, 10%; stable disease, 13%; no complete response
Luke et al, 2018[127]	Ovarian cancer, endometrial cancer, colorectal cancer, and other cancers	73	Three to five fractions of 10 Gy at two to four sites	200 mg pembrolizumab every 3 weeks until progression, death, or toxic effects	Pembrolizumab 7 days after SBRT	One complete response; eight partial responses; 21 with stable disease
Maity et al, 2018[26] (cohort 1)	Non-small-cell lung cancer or melanoma (progression on anti-PD-1)	12	Three fractions of 8 Gy to the first six patients and one fraction of 17 Gy to the following patients at one site	Six doses of 200 mg pembrolizumab every 3 weeks	SBRT 6–10 days after pembrolizumab	Two partial responses; nine progressive disease; one not evaluable
Maity et al, 2018[26] (cohort 2)	Pancreatic cancer, breast cancer, head and neck cancer, colon cancer, or kidney cancer (no previous anti-PD-1)	12	Three fractions of 8 Gy to the first six patients and one fraction of 17 Gy to the following patients at one site	Six doses of 200 mg pembrolizumab every 3 weeks	SBRT 6–10 days after pembrolizumab	One complete response; one stable disease; nine progressive disease

SBRT=stereotactic body radiotherapy.

Table 4: **Clinical trials of SBRT and immune checkpoint inhibitors in different disease types**

combining radiotherapy and immune checkpoint inhibition, particularly when radiotherapy is given at a high dose per fraction for pelvic tumours.[130] Grade 3 anaemia was observed in four (18%) of 18 patients in a trial that combined SBRT with CTLA-4 in patients with metastatic melanoma.[119] Radiotherapy can also contribute to severe late sequelae, including bowel obstruction and ureteral strictures. Therefore, clinicians should consider four major principles when delivering SBRT: proper target and healthy organ localisation (eg, by use of MRI, PET/CT, or both, when appropriate); management of breathing-related motion, which is essential to assure tight planning margins; use of imaging-guided radiotherapy, which enables automatic correction of patient position through translation and rotation of the treatment coach; and use of a real-time method for motion management during treatment (eg, tracking devices with fiducial markers implanted near the target volume) to avoid as much as possible any internal organ motion. Similar measurements should be considered when irradiating the supra-clavicular or mediastinal areas, which are frequent locations of oligometastatic disease outside the abdomen. SBRT can cause important late adverse effects if delivered near the main airways, the oesophagus, main blood vessels, or nerves. To avoid these severe toxic effects, investigators should apply dose constraints to healthy organs.

Low-dose fractionated whole-abdominal radiotherapy to reprogramme the tumour microenvironment

Low-dose ionising irradiation might increase immunogenicity of cold tumours by triggering inflammatory mechanisms that enable T-cell attack.[131] Low-dose irradiation is able to induce DNA damage,[88] and numerous studies have reported that it can activate dendritic cells, increase antigen uptake, and enhance T-cell stimulation.[132–136] Similarly, low-dose irradiation (0·94 Gy) might induce substantial apoptosis in Tregs compared with T effector cells.[137] In a mouse model of pancreatic cancer, a single fraction of localised low-dose irradiation (ie, 0·5–2·0 Gy) could reprogramme the tumour microenvironment, inducing reprogramming of tumour-associated macrophages towards an iNOS-positive phenotype, which in turn produced the appropriate chemokines to recruit T effector cells.[103] In addition, M1 macrophages drove normalisation of the tumour vasculature, increasing CD31 and VCAM-1 expression, and allowing T-cell infiltration in tumours. Together, these effects enabled T-cell-mediated tumour rejection.[103] Moreover, the results were corroborated in patients with pancreatic adenocarcinomas treated in the neoadjuvant setting, where a single dose of 2 Gy was sufficient to increase T cells in the tumour microenvironment.[103] Notably, large volumes of low-dose radiation have been shown to enhance antitumour control. For instance, rats with hepatocarcinoma irradiated with 0·2 Gy to the whole body had significantly fewer lung metastases than controls, with a significant increase in tumour-infiltrating CD8 cells and IFN-γ and TNF-alpha expression in the lung tumour micro-environment.[138] Spary and collegues[139] showed that 0·6–2·4 Gy radiation enhanced T-cell function by increasing T-cell proliferation, T-cell receptor signalling, and the polyfunctionality of CD8 cells.

These observations are provocative and support the re-examination of whole-abdominal radiotherapy approaches. Given that in the majority of patients, ovarian cancer remains localised to the subdiaphragmatic region, whole-abdominal radiotherapy could be repurposed as an interesting approach to so-called in-field immunomodulation in the context of combinatorial immunotherapy. In this setting, low-dose (metronomic) whole-abdominal radiotherapy could neutralise some of the important immunosuppressive circuitries operating in ovarian cancer, such as Tregs,[137] immunosuppressive tumour-associated macrophages,[103] and tolerogenic dendritic cells,[140] and offer a convenient therapeutic platform for immunotherapy by fostering reprogramming of macrophages and APCs, and promoting T-cell infiltration into tumours. In this case, irradiation to the whole-abdominal cavity of patients with ovarian cancer could be accompanied by pharmacological interventions to further increase APC activation (eg, using CD40 agonists or TLR agonists) and by immune checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-CTLA-4, or anti-LAG-3. These schemes are supported by preclinical studies and early clinical experiments. For example, an intratumoral TLR9 agonist, combined with 2 Gy radiation in two fractions, produced T-cell infiltration and important clinical responses in patients with advanced lymphoma.[106] Furthermore, low-dose whole-body irradiation (1–2 Gy) favoured maturation of peritoneal APCs that showed high expression of CD80 and CD86 and production of IL-12. These events were accompanied by a significant upregulation of CD28 on T cells that was observed with both 1 Gy and 2 Gy. CTLA-4 on T cells was increased with 2 Gy and significantly reduced with 0·075 Gy.[141] This preclinical study supports the hypothesis that the combination of large volumes of low-dose irradiation with a CTLA-4 inhibitor could eventually be synergistic in patients. Future preclinical studies of large volumes of low-dose irradiation could reveal whether radiotherapy induces higher expression of other coinhibitory receptors like PD-1, TIM-3, and LAG-3 to justify the combination of immune checkpoint inhibitors targeting these receptors with radiotherapy in patients. Low-dose whole-abdominal radiotherapy should be tested clinically in combination with immunotherapy in the advanced setting, but also in the adjuvant setting with curative intent. The tolerability of low-dose whole-abdominal radiotherapy with PARP inhibitors[35] could also position whole-abdominal radiotherapy as a potential important partner of combinations with PARP inhibitors and immune checkpoint inhibition.

The best radiotherapy delivery for optimal immunotherapy synergy might require both SBRT and low-dose whole-abdominal radiotherapy. In this case, SBRT is used to cause the release of tumour antigen to ultimately elicit competent cytotoxic T lymphocytes while, in parallel, low-dose whole-abdominal radiotherapy of the remaining tumour deposits is used to reprogramme the tumour microenvironment, enabling robust T-cell infiltration in ovarian tumours.

Late chronic radiation toxicity has always been a major concern of whole-abdominal radiotherapy, therefore it is of major importance that radiation oncologists deliver this technique using IMRT or VMAT that has been shown to provide better target coverage with substantial sparing of the liver, bone marrow, and kidneys.[49] Whole abdominal radiotherapy can also be myelosuppressive and this characteristic might have important consequences for patients receiving immune checkpoint inhibitor therapy. IMRT could also help spare the bone marrow in addition to reducing gastrointestinal and urinary toxicity.[47] In 2019, Pike and colleagues[142] reported a clinically significant lymphopenia in patients receiving three-dimensional conformal radiotherapy to the spine, lung, mediastinum or chest wall. The lymphopenia persisted and became severe after initiation of immune checkpoint inhibitors and was associated with increased mortality on multivariate analysis. Despite the lower doses proposed, caution should be exercised with the delivery of low-dose radiation in large treatment volumes. Therefore, similar principles to SBRT apply to whole-abdominal radiotherapy delivery, and include the precise localisation of the target and organs at risk and the management of respiratory motion. To avoid irradiation of large abdominal volumes, an alternative could be the delivery of low-dose irradiation uniquely to the macroscopically visible tumour deposits. A phase 1a/1b clinical trial (NCT03728179) is currently testing the combination of low-dose image-guided radiotherapy delivered to all metastatic tumour deposits using TomoTherapy in patients with cold tumours in combination with low-dose metronomic cyclophosphamide, anti-CTLA-4, anti-PD1 monoclonal antibody, and aspirin.

Barriers to the implementation of radioimmunotherapy combinations

The ability of radiotherapy to activate antitumour immunity explains the synergy of radiotherapy with immune checkpoints well documented in mouse tumour models, and in patients who were previously refractory to checkpoint inhibitor therapy and subsequently responded after receiving radiotherapy. However, abscopal responses remain relatively rare in the clinic, the majority of them having been observed in immunoreactive tumours such as melanoma, kidney, and lung cancers (table 1). This observation suggests that although radiotherapy might release tumour antigens, the activation of APCs might be suboptimal in many combinations, while barriers to T-cell homing, engraftment, and function remain in distant tumour deposits. Thus, identifying and overcoming such barriers, which could either pre-exist or be induced by radiotherapy in the tumour microenvironment is necessary. For instance, tumour cells undergoing irradiation release ATP,[91] which is rapidly catabolised into adenosine in the tumour microenvironment by ectoenzymes CD39 and CD73 expressed on tumour

Search strategy and selection criteria

References for this Series paper were identified through searches of PubMed using the search terms "ovarian cancer", "immunotherapy", "dendritic cells", "T cells", "microenvironment", "radiation therapy", "whole abdominal radiation therapy", stereotactic body radiation therapy", "intensity modulated radiation therapy", "PARP inhibitors", and "abscopal effect". No date limits were applied. Articles were also identified through searches of the authors' own files. Only papers published in the English language were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Series paper.

cells,[143] stromal cells,[144] and immune cells.[145] Local accumulation of extracellular adenosine suppresses dendritic cells and T effector cells while promoting proliferation of Tregs[146] and a suppressive phenotype in tumour-associated macrophages and MDSCs.[147] Pharmacological blockade of the adenosine pathway with a CD73 antibody synergises with anti-PD-1 or anti-CTLA-4 and promotes antitumour immune responses in mouse models of locally advanced fibrosarcoma and metastatic breast cancer.[148] Future research in preclinical studies and translational research in patients should address the role of adenosine in inducing immune suppression from radiotherapy. Homoeostatic repair mechanisms induced by radiotherapy are responsible for the accumulation of Tregs in the tumour microenvironment[149] and the increased expression or bioavailability of TGFβ,[111] which locally curtails the positive immunomodulatory effects of radiation since TGFβ and IL-10 (also produced by Tregs) can dampen activation of dendritic cells and proper priming of T cells.[150] Cyclophosphamide is effective in reducing Tregs in vivo;[109] however, studies have now shown that these cells repopulate in the periphery very rapidly, highlighting the transient and possibly limiting nature of this approach.[151] Therefore, clinical trials assessing the effect of a cyclophosphamide dosing schedule on Treg depletion should be carefully planned. Agents in clinical development blocking TGFβ or IL-10 could also be useful in this setting.

Other immunosuppressive loops in the tumour microenvironment might represent obstacles for the abscopal effect. Upregulation of PD-L1 following radiotherapy has been reported in several preclinical studies[122,124,152] and is mediated via increased production of IFN-γ by T cells that infiltrate the tumour microenvironment following radiotherapy, which in turn induces PD-L1 expression on tumour cells. If radiotherapy alone or in combination with immune checkpoint inhibitors elicits T-cell responses that are insufficient to eliminate the tumour, then the upregulation of other immune checkpoints will limit tumour rejection by adaptive resistance.[152]

Genomic variations might create challenges to the expected synergy between radiotherapy and immunotherapy. For example, cell stress mechanisms activated by radiation require functional p53 (cellular tumor antigen p53), which is involved in the upregulation of NKG2D ligands. Thus, in the absence of p53, radiotherapy might not result in enhanced tumour immune recognition.[153] Similarly, a TLR4 polymorphism (Asp299Gly) that affects binding to HMG-1 predicted early relapse after radiotherapy in patients with breast cancer.[131] Finally, tumour heterogeneity and divergent clonal evolution might be a major factor contributing to the emergence of escape variants resisting the favourable immune response that can be elicited by the combination of radiotherapy and immunotherapy. Genetic variants affecting neoantigen expression, processing and presentation,[154] oncogenic pathways,[155] and IFN signalling[156,157] have been described as mechanisms of immune escape.

In addition to biological barriers, one must also consider the feasibility of the combinatorial approach. The implementation of phase 1 clinical trials that combine radiotherapy with three or more immuno-modulatory drugs will require clear prespecified radiotherapy-associated and drug-attributed side-effects to properly define dose-limiting toxic effects. Dose-escalation studies of radiotherapy or the administered drugs might help refine the recommended phase 1b or 2 dose. Such trials should test clinical and radiological endpoints of efficacy and be sufficiently enriched by translational interrogation of patients and tumour biopsies obtained before treatment and longitudinally during treatment to provide rigorous scientific evidence of the effects of the treatment in the tumour microenvironment. Lastly, considering the risk of toxic effects, such trials will require evaluation of the health-related quality of life of patients.[158]

Conclusion

The combination of radiotherapy with immunotherapy is a paradigm shift for radiation oncology as the aim of radiotherapy is progressing from direct tumour sterilisation to tumour microenvironment reprogramming and immune modulation. Although radiotherapy can be applied to promote many aspects of the tumour immunity cycle, such as activating tumour antigen presentation, vasculature normalisation, and T-cell and dendritic cell recruitment and activation, a variety of suppressive mechanisms, including influx of Tregs and MDSCs, and stroma repair are also set into motion, which will have to be countered by suitable combinations of radiation and immunotherapy treatments. Combining immunomodulatory agents with low-dose whole-abdominal radiotherapy, or with high-precision SBRT, can further promote the activity of favourable immune cells and block or reprogramme inhibitory ones (ie, MDSCs, M2 macrophages, and Tregs). These radiotherapy modalities represent new opportunities in ovarian cancer treatment, promising to enhance the efficacy of immunotherapy in

this disease. Much remains to be determined, including biomarkers to select the best combinations of immunomodulatory agents, and the optimal radiation doses, volumes, and fractionations to be used. Clinical studies testing combinations must be designed to carefully test radiotherapy doses and schedules, taking into consideration the key biological and safety concerns. In addition, a careful evaluation of immunomodulatory drugs should balance feasibility and biological opportunity to ask important biological efficacy and clinical safety questions. Rapid clinical development of these combinations will require the use of reliable preclinical tumour models along with neoadjuvant window-of-opportunity clinical studies that can test radiotherapy doses and drug combinations. Detailed tissue analyses should help elucidate the mechanisms of action of the combinations, and hopefully lead to the development of biomarkers for patient selection. The kinetics of the cellular and molecular events triggered in the tumour microenvironment by different radiotherapy modalities and sequences of treatments should provide valuable clues as to the optimal time window for radiotherapy to potentiate immunomodulatory inter-ventions that might be tumour-type specific.

Contributors

FGH and GC devised the concept of this Series paper. All authors contributed to writing and revising the manuscript. FGH and GC designed and created the figures.

Declaration of Interests

GC has received grants or research support or is coinvestigator in clinical trials by Bristol-Myers Squibb, Celgene, Boehringer Ingelheim, Roche, Iovance, and Kite Pharma; has received honoraria for consultations or presentations by Roche, Genentech, Bristol-Myers Squibb, AstraZeneca, Sanofi, NextCure, and Geneos Therapeutics; has patents in the domain of antibodies and vaccines targeting the tumour vasculature as well as technologies related to T-cell expansion and engineering for T-cell therapy; and receives royalties from the University of Pennsylvania. FH reports grants from Prostate Cancer Foundation, Bristol-Myers-Squibb, Accuray, BioProtect, and Roche (ImFlame Network) during the conduct of the study and outside the submitted work; and non-financial support from the European Organisation for Research and Treatment of Cancer during the conduct of the study and outside the submitted work. MI and LEK declare no competing interests.

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References

1 Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136:** E359–86.

2 Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol* 2002; **20:** 1248–59.

3 Kyrgiou M, Salanti G, Pavlidis N, Paraskevaidis E, Ioannidis JP. Survival benefits with diverse chemotherapy regimens for ovarian cancer: meta-analysis of multiple treatments. *J Natl Cancer Inst* 2006; **98:** 1655–63.

4 Perren TJ, Swart AM, Pfisterer J, et al. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011; **365:** 2484–96.

5 Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med* 2018; **379:** 2495–505.

6 Armstrong DK, Bundy B, Wenzel L, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* 2006; **354:** 34–43.

7 Matz M, Coleman MP, Carreira H, et al. Worldwide comparison of ovarian cancer survival: histological group and stage at diagnosis (CONCORD-2). *Gynecol Oncol* 2017; **144:** 396–404.

8 Hoppenot C, Eckert MA, Tienda SM, Lengyel E. Who are the long-term survivors of high grade serous ovarian cancer? *Gynecol Oncol* 2018; **148:** 204–12.

9 Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol* 2014; **11:** 24–37.

10 Coukos G, Tanyi J, Kandalaft LE. Opportunities in immunotherapy of ovarian cancer. *Ann Oncol* 2016; **27**(suppl 1)**:** i11–15.

11 Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biop Phys* 2004; **58:** 862–70.

12 Mole RH. Whole body irradiation; radiobiology or medicine? *Br J Radiol* 1953; **26:** 234–41.

13 Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med* 2012; **366:** 925–31.

14 Formenti SC, Rudqvist NP, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med* 2018; **24:** 1845–51.

15 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.

16 Panaretakis T, Kepp O, Brockmeier U, et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J* 2009; **28:** 578–90.

17 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:** 843–52.

18 Gupta A, Probst HC, Vuong V, et al. Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation. *J Immunol* 2012; **189:** 558–66.

19 Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007; **13:** 1050–59.

20 Panaretakis T, Joza N, Modjtahedi N, et al. The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. *Cell Death Differ* 2008; **15:** 1499–509.

21 Matsumura S, Wang B, Kawashima N, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. *J Immunol* 2008; **181:** 3099–107.

22 Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J Immunol* 2005; **174:** 7516–23.

23 Ohba K, Omagari K, Nakamura T, et al. Abscopal regression of hepatocellular carcinoma after radiotherapy for bone metastasis. *Gut* 1998; **43:** 575–77.

24 Nam SW, Han JY, Kim JI, et al. Spontaneous regression of a large hepatocellular carcinoma with skull metastasis. *J Gastroenterol Hepatol* 2005; **20:** 488–92.

25 Siva S, Callahan J, MacManus MP, Martin O, Hicks RJ, Ball DL. Abscopal [corrected] effects after conventional and stereotactic lung irradiation of non-small-cell lung cancer. *J Thorac Oncol* 2013; **8:** e71–72.

26 Maity A, Mick R, Huang AC, et al. A phase I trial of pembrolizumab with hypofractionated radiotherapy in patients with metastatic solid tumours. *Br J Cancer* 2018; **119:** 1200–07.

27 Grimaldi AM, Simeone E, Giannarelli D, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. *Oncoimmunology* 2014; **3:** e28780.

28 Hiniker SM, Reddy SA, Maecker HT, et al. A prospective clinical trial combining radiation therapy with systemic immunotherapy in metastatic melanoma. *Int J Radiat Oncol Biol Phys* 2016; **96:** 578–88.

29 Antonia SJ, Villegas A, Daniel D, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018; **379:** 2342–50.

30 Griffon G, Marchal C, Merlin JL, Marchal S, Parache RM, Bey P. Radiosensitivity of multicellular tumour spheroids obtained from human ovarian cancers. *Eur J Cancer* 1995; **31A:** 85–91.

31 Fyles AW, Dembo AJ, Bush RS, et al. Analysis of complications in patients treated with abdomino-pelvic radiation therapy for ovarian carcinoma. *Int J Radiat Oncol Biol Phys* 1992; **22:** 847–51.

32 Wong R, Milosevic M, Sturgeon J, et al. Treatment of early epithelial ovarian cancer with chemotherapy and abdominopelvic radiotherapy: results of a prospective treatment protocol. *Int J Radiat Oncol Biol Phys* 1999; **45:** 657–65.

33 Kunos CA, Sill MW, Buekers TE, et al. Low-dose abdominal radiation as a docetaxel chemosensitizer for recurrent epithelial ovarian cancer: a phase I study of the Gynecologic Oncology Group. *Gynecol Oncol* 2011; **120:** 224–28.

34 Wrenn DC, Saigal K, Lucci JA, 3rd, et al. A phase I study using low-dose fractionated whole abdominal radiotherapy as a chemopotentiator to full-dose cisplatin for optimally debulked stage III/IV carcinoma of the endometrium. *Gynecol Oncol* 2011; **122:** 59–62.

35 Reiss KA, Herman JM, Armstrong D, et al. A final report of a phase I study of veliparib (ABT-888) in combination with low-dose fractionated whole abdominal radiation therapy (LDFWAR) in patients with advanced solid malignancies and peritoneal carcinomatosis with a dose escalation in ovarian and fallopian tube cancers. *Gynecol Oncol* 2017; **144:** 486–90.

36 Lazzari R, Ronchi S, Gandini S, et al. Stereotactic body radiation therapy for oligometastatic ovarian cancer: a step toward a drug holiday. *Int J Radiat Oncol Biol Phys* 2018; **101:** 650–60.

37 Ledermann JA, Dembo AJ, Sturgeon JF, et al. Outcome of patients with unfavorable optimally cytoreduced ovarian cancer treated with chemotherapy and whole abdominal radiation. *Gynecol Oncol* 1991; **41:** 30–35.

38 Dembo AJ, Bush RS, Beale FA, et al. Ovarian carcinoma: improved survival following abdominopelvic irradiation in patients with a completed pelvic operation. *Am J Obstet Gynecol* 1979; **134:** 793–800.

39 Dembo AJ. Abdominopelvic radiotherapy in ovarian cancer. A 10-year experience. *Cancer* 1985; **55**(suppl)**:** 2285–90.

40 Fyles AW, Thomas GM, Pintilie M, Ackerman I, Levin W. A randomized study of two doses of abdominopelvic radiation therapy for patients with optimally debulked stage I, II, and III ovarian cancer. *Int J Radiat Oncol Biol Phys* 1998; **41:** 543–49.

41 Sell A, Bertelsen K, Andersen JE, Stroyer I, Panduro J. Randomized study of whole-abdomen irradiation versus pelvic irradiation plus cyclophosphamide in treatment of early ovarian cancer. *Gynecol Oncol* 1990; **37:** 367–73.

42 Chiara S, Conte P, Franzone P, et al. High-risk early-stage ovarian cancer. Randomized clinical trial comparing cisplatin plus cyclophosphamide versus whole abdominal radiotherapy. *Am J Clin Oncol* 1994; **17:** 72–76.

43 Sorbe B, Swedish-Norgewian Ovarian Cancer Study Group. Consolidation treatment of advanced (FIGO stage III) ovarian carcinoma in complete surgical remission after induction chemotherapy: a randomized, controlled, clinical trial comparing whole abdominal radiotherapy, chemotherapy, and no further treatment. *Int J Gynecol Cancer* 2003; **13:** 278–86.

44 Pickel H, Lahousen M, Petru E, et al. Consolidation radiotherapy after carboplatin-based chemotherapy in radically operated advanced ovarian cancer. *Gynecol Oncol* 1999; **72:** 215–19.

45 Hoskins PJ, Le N, Gilks B, et al. Low-stage ovarian clear cell carcinoma: population-based outcomes in British Columbia, Canada, with evidence for a survival benefit as a result of irradiation. *J Clin Oncol* 2012; **30:** 1656–62.

46 Arians N, Kieser M, Benner L, et al. Adjuvant intensity modulated whole-abdominal radiation therapy for high-risk patients with ovarian cancer (International Federation of Gynecology and Obstetrics Stage III): first results of a prospective phase 2 study. *Int J Radiat Oncol Biol Phys* 2017; **99:** 912–20.

47 Elicin O, Callaway S, Prior JO, Bourhis J, Ozsahin M, Herrera FG. [(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.

48 Mell LK, Kochanski JD, Roeske JC, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients treated with concurrent cisplatin and intensity-modulated pelvic radiotherapy. *Int J Radiat Oncol Biol Phys* 2006; **66:** 1356–65.

49 Rochet N, Sterzing F, Jensen AD, et al. Intensity-modulated whole abdominal radiotherapy after surgery and carboplatin/taxane chemotherapy for advanced ovarian cancer: phase I study. *Int J Radiat Oncol Biol Phys* 2010; **76:** 1382–89.

50 Regine WF, Hanna N, Garofalo MC, et al. Low-dose radiotherapy as a chemopotentiator of gemcitabine in tumors of the pancreas or small bowel: a phase I study exploring a new treatment paradigm. *Int J Radiat Oncol Biol Phys* 2007; **68:** 172–77.

51 Hellman S, Weichselbaum RR. Oligometastases. *J Clin Oncol* 1995; **13:** 8–10.

52 Iyengar P, Wardak Z, Gerber DE, et al. Consolidative radiotherapy for limited metastatic non-small-cell lung cancer: a phase 2 randomized clinical trial. *JAMA Oncol* 2018; **4:** e173501.

53 Gomez DR, Blumenschein GR Jr, Lee JJ, et al. Local consolidative therapy versus maintenance therapy or observation for patients with oligometastatic non-small-cell lung cancer without progression after first-line systemic therapy: a multicentre, randomised, controlled, phase 2 study. *Lancet Oncol* 2016; **17:** 1672–82.

54 Ball D, Mai GT, Vinod S, et al. Stereotactic ablative radiotherapy versus standard radiotherapy in stage 1 non-small-cell lung cancer (TROG 09.02 CHISEL): a phase 3, open-label, randomised controlled trial. *Lancet Oncol* 2019; **20:** 494–503.

55 Palma DA, Olson R, Harrow S, et al. Stereotactic ablative radiotherapy versus standard of care palliative treatment in patients with oligometastatic cancers (SABR-COMET): a randomised, phase 2, open-label trial. *Lancet* 2019; **393:** 2051–58.

56 Kunos CA, Brindle J, Waggoner S, et al. Phase II clinical trial of robotic stereotactic body radiosurgery for metastatic gynecologic malignancies. *Front Oncol* 2012; **2:** 181.

57 Iftode C, D'Agostino GR, Tozzi A, et al. Stereotactic body radiation therapy in oligometastatic ovarian cancer: a promising therapeutic approach. *Int J Gynecol Cancer* 2018; **28:** 1507–13.

58 Brown AP, Jhingran A, Klopp AH, Schmeler KM, Ramirez PT, Eifel PJ. Involved-field radiation therapy for locoregionally recurrent ovarian cancer. *Gynecol Oncol* 2013; **130:** 300–05.

59 Kunos CA, Sherertz TM, Mislmani M, et al. Phase I trial of carboplatin and gemcitabine chemotherapy and stereotactic ablative radiosurgery for the palliative treatment of persistent or recurrent gynecologic cancer. *Front Oncol* 2015; **5:** 126.

60 Hamanishi J, Mandai M, Ikeda T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. *J Clin Oncol* 2015; **33:** 4015–22.

61 Disis ML, Taylor MH, Kelly K, et al. Efficacy and safety of avelumab for patients with recurrent or refractory ovarian cancer: phase 1b results from the JAVELIN solid tumor trial. *JAMA Oncol* 2019; published online Jan 24. DOI:10.1001/jamaoncol.2018.6258.

62 Pietzner K, Nasser S, Alavi S, et al. Checkpoint-inhibition in ovarian cancer: rising star or just a dream? *J Gynecol Oncol* 2018; **29:** e93.

63 Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515:** 563–67.

64 Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515:** 568–71.

65 Duraiswamy J, Freeman GJ, Coukos G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. *Cancer Res* 2013; **73:** 6900–12.

66 Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Res* 2013; **73:** 3591–603.

67 Varga A, Piha-Paul S, Ott PA, et al. Pembrolizumab in patients with programmed death ligand 1-positive advanced ovarian cancer: analysis of KEYNOTE-028. *Gynecol Oncol* 2019; **152:** 243–50.

68 Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003; **348:** 203–13.

69 Huang RY, Eppolito C, Lele S, Shrikant P, Matsuzaki J, Odunsi K. LAG3 and PD1 co-inhibitory molecules collaborate to limit CD8+ T cell signaling and dampen antitumor immunity in a murine ovarian cancer model. *Oncotarget* 2015; **6:** 27359–77.

70 Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10:** 942–49.

71 Taki M, Abiko K, Baba T, et al. Snail promotes ovarian cancer progression by recruiting myeloid-derived suppressor cells via CXCR2 ligand upregulation. *Nat Comm* 2018; **9:** 1685.

72 Hagemann T, Wilson J, Burke F, et al. Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. *J Immunol* 2006; **176:** 5023–32.

73 Wu L, Deng Z, Peng Y, et al. Ascites-derived IL-6 and IL-10 synergistically expand CD14(+)HLA-DR(-/low) myeloid-derived suppressor cells in ovarian cancer patients. *Oncotarget* 2017; **8:** 76843–56.

74 Brencicova E, Jagger AL, Evans HG, et al. Interleukin-10 and prostaglandin E2 have complementary but distinct suppressive effects on Toll-like receptor-mediated dendritic cell activation in ovarian carcinoma. *PLoS One* 2017; **12:** e0175712.

75 Hu Q, Hisamatsu T, Haemmerle M, et al. Role of platelet-derived tgfbeta1 in the progression of ovarian cancer. *Clin Cancer Res* 2017; **23:** 5611–21.

76 Inaba T, Ino K, Kajiyama H, et al. Role of the immunosuppressive enzyme indoleamine 2,3-dioxygenase in the progression of ovarian carcinoma. *Gynecol Oncol* 2009; **115:** 185–92.

77 Bak SP, Alonso A, Turk MJ, Berwin B. Murine ovarian cancer vascular leukocytes require arginase-1 activity for T cell suppression. *Mol Immunol* 2008; **46:** 258–68.

78 Buckanovich RJ, Facciabene A, Kim S, et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat Med* 2008; **14:** 28–36.

79 Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat Rev Immunol* 2011; **11:** 702–11.

80 Motz GT, Santoro SP, Wang LP, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014; **20:** 607–15.

81 Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 2017; **171:** 934–49e16.

82 Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348:** 124–28.

83 Le DT, Uram JN, Wang H, et al. PD-1 Blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015; **372:** 2509–20.

84 Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357:** 409–13.

85 Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature* 2013; **500:** 415–21.

86 Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017; **377:** 2500–01.

87 Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; **362:** eear3593.

88 Joiner MC, Marples B, Lambin P, Short SC, Turesson I. Low-dose hypersensitivity: current status and possible mechanisms. *Int J Radiat Oncol Biol Phys* 2001; **49:** 379–89.

89 Herrera FG, Bourhis J, Coukos G. Radiotherapy combination opportunities leveraging immunity for the next oncology practice. *CA Cancer J Clin* 2017; **67:** 65–85.

90 Obeid M, Panaretakis T, Joza N, et al. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. *Cell Death Differ* 2007; **14:** 1848–50.

91 Michaud M, Martins I, Sukkurwala AQ, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* 2011; **334:** 1573–77.

92 Perregaux DG, McNiff P, Laliberte R, Conklyn M, Gabel CA. ATP acts as an agonist to promote stimulus-induced secretion of IL-1 beta and IL-18 in human blood. *J Immunol* 2000; **165:** 4615–23.

93 Surace L, Lysenko V, Fontana AO, et al. Complement is a central mediator of radiotherapy-induced tumor-specific immunity and clinical response. *Immunity* 2015; **42:** 767–77.

94 Sanchez-Paulete AR, Cueto FJ, Martinez-Lopez M, et al. Cancer immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal antibodies requires BATF3- dependent dendritic cells. *Cancer Discov* 2016; **6:** 71–79.

95 Formenti SC, Rudqvist NP, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med* 2018; **24:** 1845–51.

96 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:** 2488–96.

97 Pilones KA, Aryankalayil J, Babb JS, Demaria S. Invariant natural killer T cells regulate anti-tumor immunity by controlling the population of dendritic cells in tumor and draining lymph nodes. *J Immunother Cancer* 2014; **2:** 37.

98 Ruocco MG, Pilones KA, Kawashima N, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. *J Clin Invest* 2012; **122:** 3718–30.

99 Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation. *Proc Natl Acad Sci USA* 1989; **86:** 10104–07.

100 Chinnaiyan AM, Prasad U, Shankar S, et al. Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. *Proc Natl Acad Sci USA* 2000; **97:** 1754–59.

101 Matsumura S, Demaria S. Up-regulation of the pro-inflammatory chemokine CXCL16 is a common response of tumor cells to ionizing radiation. *Radiation Res* 2010; **173:** 418–25.

102 Sung JH, Zhang H, Moseman EA, et al. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell* 2012; **150:** 1249–63.

103 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.

104 Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Comm* 2017; **8:** 15618.

105 Rech AJ, Dada H, Kotzin JJ, et al. Radiotherapy and CD40 activation separately augment immunity to checkpoint blockade in cancer. *Cancer Res* 2018; **78:** 4282–91.

106 Brody JD, Ai WZ, Czerwinski DK, et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. *J Clin Oncol* 2010; **28:** 4324–32.

107 Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017; **541:** 321–30.

108 Ngiow SF, Young A, Blake SJ, et al. Agonistic CD40 mAb-Driven IL12 reverses resistance to anti-PD1 in a T-cell-rich tumor. *Cancer Res* 2016; **76:** 6266–77.

109 Ghiringhelli F, Menard C, Puig PE, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* 2007; **56:** 641–48.

110 Zsiros EF, Frederick PJ, Akers SN, et al. A phase II trial of pembrolizumab in combination with bevacizumab and oral metronomic cyclophosphamide for recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer. Society of Gynecology Oncology 50th Annual Meeting 2019; Honolulu, HI, USA. March 16–19, 2019. LBA4.

111 Biswas S, Guix M, Rinehart C, et al. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest* 2007; **117:** 1305–13.

112 Seung SK, Curti BD, Crittenden M, et al. Phase 1 study of stereotactic body radiotherapy and interleukin-2—tumor and immunological responses. *Sci Transl Med* 2012; **4:** 137ra74.

113 Chakravarty PK, Alfieri A, Thomas EK, et al. Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer. *Cancer Res* 1999; **59:** 6028–32.

114 Teitz-Tennenbaum S, Li Q, Rynkiewicz S, et al. Radiotherapy potentiates the therapeutic efficacy of intratumoral dendritic cell administration. *Cancer Res* 2003; **63:** 8466–75.

115 Demaria S, Kawashima N, Yang AM, et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin Cancer Res* 2005; **11:** 728–34.

116 Lee Y, Auh SL, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. *Blood* 2009; **114:** 589–95.

117 Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 2009; **15:** 5379–88.

118 Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* 2014; **124:** 687–95.

119 Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015; **520:** 373–77.

120 Rodriguez-Ruiz ME, Rodriguez I, Garasa S, et al. Abscopal effects of radiotherapy are enhanced by combined immunostimulatory mAbs and are dependent on CD8 T cells and crosspriming. *Cancer Res* 2016; **76:** 5994–6005.

121 Honeychurch J, Glennie MJ, Johnson PW, Illidge TM. Anti-CD40 monoclonal antibody therapy in combination with irradiation results in a CD8 T-cell-dependent immunity to B-cell lymphoma. *Blood* 2003; **102:** 1449–57.

122 Verbrugge I, Hagekyriakou J, Sharp LL, et al. Radiotherapy increases the permissiveness of established mammary tumors to rejection by immunomodulatory antibodies. *Cancer Res* 2012; **72:** 3163–74.

123 Dovedi SJ, Melis MH, Wilkinson RW, et al. Systemic delivery of a TLR7 agonist in combination with radiation primes durable antitumor immune responses in mouse models of lymphoma. *Blood* 2013; **121:** 251–59.

124 Vanpouille-Box C, Diamond JM, Pilones KA, et al. TGFbeta is a master regulator of radiation therapy-induced anti-tumor immunity. *Cancer Res* 2015; **75:** 2232–42.

125 Mason KA, Ariga H, Neal R, et al. Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances tumor response to fractionated radiotherapy. *Clin Cancer Res* 2005; **11:** 361–69.

126 Tang C, Welsh JW, de Groot P, et al. Ipilimumab with stereotactic ablative radiation therapy: phase I results and immunologic correlates from peripheral T cells. *Clin Cancer Res* 2017; **23:** 1388–96.

127 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* 2018; **36:** 1611–18.

128 Burger R, Sill M, Zamarin D, et al. NRG-GY003 Oncology phase II randomized trial of nivolumab with or without ipilimumab in patients with persistent or recurrent ovarian cancer. 17th Biennial Meeting of the International Gynecological Cancer Society; Kyoto, Japan. Sept 14–16, 2018. OC11 2018.

129 Verma V, Cushman TR, Selek U, Tang C, Welsh JW. Safety of combined immunotherapy and thoracic radiation therapy: analysis of 3 single-institutional phase I/II trials. *Int J Radiat Oncol Biol Phys* 2018; **101:** 1141–48.

130 Tree AC, Jones K, Hafeez S, et al. Dose-limiting urinary toxicity with pembrolizumab combined with weekly hypofractionated radiation therapy in bladder cancer. *Int J Radiat Oncol Biol Phys* 2018; **101:** 1168–71.

131 Apetoh L, Ghiringhelli F, Tesniere A, et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunol Rev* 2007; **220:** 47–59.

132 Shigematsu A, Adachi Y, Koike-Kiriyama N, et al. Effects of low-dose irradiation on enhancement of immunity by dendritic cells. *J Radiat Res* 2007; **48:** 51–55.

133 Liao YP, Wang CC, Butterfield LH, et al. Ionizing radiation affects human MART-1 melanoma antigen processing and presentation by dendritic cells. *J Immunol* 2004; **173:** 2462–69.

134 Cao ZA, Daniel D, Hanahan D. Sub-lethal radiation enhances anti-tumor immunotherapy in a transgenic mouse model of pancreatic cancer. *BMC Cancer* 2002; **2:** 11.

135 El-Saghire H, Michaux A, Thierens H, Baatout S. Low doses of ionizing radiation induce immune-stimulatory responses in isolated human primary monocytes. *Int J Mol Med* 2013; **32:** 1407–14.

136 Ina Y, Sakai K. Activation of immunological network by chronic low-dose-rate irradiation in wild-type mouse strains: analysis of immune cell populations and surface molecules. *Int J Radiat Biol* 2005; **81:** 721–29.

137 Cao M, Cabrera R, Xu Y, Liu C, Nelson D. Different radiosensitivity of CD4(+)CD25(+) regulatory T cells and effector T cells to low dose gamma irradiation in vitro. *Int J Radiat Biol* 2011; **87:** 71–80.

138 Hashimoto S, Shirato H, Hosokawa M, et al. The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor-bearing rats. *Radiat Res* 1999; **151:** 717–24.

139 Spary LK, Al-Taei S, Salimu J, et al. Enhancement of T cell responses as a result of synergy between lower doses of radiation and T cell stimulation. *J Immunol* 2014; **192:** 3101–10.

140 Persa E, Szatmari T, Safrany G, Lumniczky K. In vivo irradiation of mice induces activation of dendritic cells. *Int J Mol Sci* 2018; **19:** e2391.

141 Liu SZ, Jin SZ, Liu XD, Sun YM. Role of CD28/B7 costimulation and IL-12/IL-10 interaction in the radiation-induced immune changes. *BMC Immunol* 2001; **2:** 8.

142 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.

143 Turcotte M, Spring K, Pommey S, et al. CD73 is associated with poor prognosis in high-grade serous ovarian cancer. *Cancer Res* 2015; **75:** 4494–503.

144 Xiong L, Wen Y, Miao X, Yang Z. NT5E and FcGBP as key regulators of TGF-1-induced epithelial-mesenchymal transition (EMT) are associated with tumor progression and survival of patients with gallbladder cancer. *Cell Tissue Res* 2014; **355:** 365–74.

145 Hyman MC, Petrovic-Djergovic D, Visovatti SH, et al. Self-regulation of inflammatory cell trafficking in mice by the leukocyte surface apyrase CD39. *J Clin Invest* 2009; **119:** 1136–49.

146 Whiteside TL, Mandapathil M, Schuler P. The role of the adenosinergic pathway in immunosuppression mediated by human regulatory T cells (Treg). *Curr Med Chem* 2011; **18:** 5217–23.

147 Ryzhov S, Novitskiy SV, Goldstein AE, et al. Adenosinergic regulation of the expansion and immunosuppressive activity of CD11b+Gr1+ cells. *J Immunol* 2011; **187:** 6120–29.

148 Allard B, Pommey S, Smyth MJ, Stagg J. Targeting CD73 enhances the antitumor activity of anti-PD-1 and anti-CTLA-4 mAbs. *Clin Cancer Res* 2013; **19:** 5626–35.

149 Qu Y, Jin S, Zhang A, et al. Gamma-ray resistance of regulatory CD4+CD25+Foxp3+ T cells in mice. *Radiat Res* 2010; **173:** 148–57.

150 Jarnicki AG, Lysaght J, Todryk S, Mills KH. Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. *J Immunol* 2006; **177:** 896–904.

151 Powell DJ Jr, de Vries CR, Allen T, Ahmadzadeh M, Rosenberg SA. Inability to mediate prolonged reduction of regulatory T Cells after transfer of autologous CD25-depleted PBMC and interleukin-2 after lymphodepleting chemotherapy. *J Immunother* 2007; **30:** 438–47.

152 Illidge T, Lipowska-Bhalla G, Cheadle E, et al. Radiation therapy induces an adaptive upregulation of PD-L1 on tumor cells which may limit the efficacy of the anti-tumor immune response but can be circumvented by anti-PD-L1. *Int J Radiat Oncol Biol Phys* 2014; **90:** S776 (abstr).

153 Iannello A, Thompson TW, Ardolino M, Lowe SW, Raulet DH. p53-dependent chemokine production by senescent tumor cells supports NKG2D-dependent tumor elimination by natural killer cells. *J Exp Med* 2013; **210:** 2057–69.

154 Rosenthal R, Cadieux EL, Salgado R, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature* 2019; **567:** 479–85.

155 Grasso CS, Giannakis M, Wells DK, et al. Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discov* 2018; **8:** 730–49.

156 Shin DS, Zaretsky JM, Escuin-Ordinas H, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov* 2017; **7:** 188–201.

157 Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 2016; **375:** 819–29.

158 Verma V, Sprave T, Haque W, et al. A systematic review of the cost and cost-effectiveness studies of immune checkpoint inhibitors. *J Immunother Cancer* 2018; **6:** 128.