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Invited Review

Immunotherapy and radiation combinatorial trials in gynecologic cancer: A potential synergy?

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HIGHLIGHTS

- In an abscopal response, tumor regression occurs at a distant metastatic site following local radiation treatment.
- Radiation therapy can prime the immune system by creating a T-cell mediated response that acts locally and distally.
- Immune checkpoint blockade may synergize with radiotherapy to enhance local tumor control and systemic response.
- Prospective trials will evaluate the combination of radiation and immunotherapy in the definitive and metastatic setting.
- Th optimal timing, radiation dose, and technique in combination with immunotherapy have yet to be determined.

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ABSTRACT

Immunotherapy (IO) is an important new pillar in the treatment of solid tumors, and the integration of IO agents with chemotherapy, targeted therapy, surgery and radiation has yet to be defined. As preclinical and clinical studies have described synergistic activity with the combination of radiation and immunotherapy, many clinical trials are underway to explore both the safety and efficacy of this approach both in the metastatic and definitive setting. Through immune priming, radiation may enhance local tumor control at the irradiated site, as well as induce a systemic response to control distant metastasis, known as the abscopal effect. On a mechanistic level, radiation therapy releases tumor neoantigens and activates an adaptive immune response that is mediated by cytotoxic T-cells, which then hone to sites of irradiated tumor as well as non-irradiated tumor metastases to induce immunogenic tumor cell death. Although the abscopal effect is rare in clinical practice, strategies that combine immune checkpoint blockade with radiation are being studied to overcome immune tolerance or suppression and increase systemic response rates to IO agents. Gynecologic cancers are attractive targets for immune checkpoint blockade, and IO agents may be used in combination with definitive chemoradiotherapy to enhance radiosensitivity and thus local control for unresected disease as well as control distant micrometastatic spread. For patients with metastatic disease, immune checkpoint blockade in combination with stereotactic radiotherapy is being evaluated as a strategy for immune activation and tumor cytoreduction. In this review, we highlight the current use of IO agents in gynecologic cancer, describe the immunogenic potential of radiation through clinical observation and preclinical study, and discuss strategies for combining IO and radiation in reported and ongoing clinical trials.

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1. Introduction

The successful clinical introduction of immune checkpoint blockade is rapidly changing treatment paradigms in oncology. In the metastatic setting, durable clinical responses have been reported in a variety of tumor types, such as melanoma, non-small cell lung cancer, renal cell carcinoma, and bladder cancer. For patients with metastatic disease that has progressed through conventional treatment and was once considered chemotherapy-refractory, immune checkpoint blockade has multiple approved indications for an expanding list of both solid and hematologic tumors. This class of immunotherapy (IO) agents includes antibodies directed against CTLA-4, PD-1 and PD-L1, which act against inhibitor pathways on activated T-cells and amplify the immune response. As single agents, the response rates for immune checkpoint inhibitors that have gained clinical approval are on the order of 20 to 40%. However, for women with gynecologic malignancies, clinical activity of immune checkpoint blockade has been modest with single agents, although a noteworthy exception is the use of PD-1 inhibitors for the treatment of microsatellite instability-high (MSI-High) or mismatch-repair-deficient (dMMR) metastatic endometrial cancer, which was granted accelerated FDA approval based on biomarker status and agnostic of treatment site [1]. Conditional approval for the use of a PD-1 inhibitor was also recently granted for patients with recurrent or metastatic cervical cancer selected for positive tumor staining for the ligand PD-L1. The overall response rate (ORR) on independent review was 14% for patients with PD-L1 positive tumors, most of which were durable responses lasting >6 months, and there was no clinical activity for those with PD-L1 negative tumors [2]. In order to enhance response rates to IO agents and broaden the indications for treatment, new approaches with combinatorial therapy are needed, and the addition of radiation has synergistic potential given its inherent immunogenic properties. The biological mechanisms of immune activation by radiation are important to understand as the basis for the combination of immune checkpoint blockade and radiation, as it is now well understood that radiation-induced cell damage is regulated by immune mechanisms through action on the cancer cell as well as the tumor microenvironment. This review will discuss the use of IO agents in gynecologic cancers as well as the role and rationale of combined IO and radiation strategies in clinical trials, including those that have been reported and studies underway.

2. Immune checkpoint blockade in gynecologic cancers

2.1. Ovarian cancer

The immunogenicity of ovarian cancer has been shown to be an important prognostic determinant for patient survival, and therefore a promising tumor type for the study of IO agents. In advanced disease, T-cell infiltration of tumors, and specifically effector CD8+ T cells, is independently associated with improved overall survival [3–6]. Furthermore, upregulation of immune checkpoints such as PD-L1 on tumor cells may represent an important mechanism to evade immune surveillance through inhibitory pathways that block activated T-cells. PD-L1 expression has been shown to be inversely correlated with CD8 + T cell infiltration, and in a cohort of 70 ovarian cancer patients, 5-year survival rates were significantly worse for those patients with high PD-L1 expression compared to those with low expression (53% vs. 80%, respectively, $p = 0.016$) [3].

Single agent immune checkpoint blockade has shown low to modest efficacy in unselected patients with recurrent ovarian cancer with response rates ranging from 6 to 15% [7–9]. Single agent pembrolizumab (anti-PD-1) was studied in a large 2-cohort phase II study (KEYNOTE-100) that enrolled 378 patients with recurrent advanced ovarian cancer following platinum-based chemotherapy, and stratified by number of prior chemotherapy lines and platinum-free interval [10]. PD-L1 expression was quantified by a combined positive score (CPS) assay, which evaluates PD-L1 staining on both tumor and immune cells. Among the 97 patients enrolled in the training set, the ORR to pembrolizumab was 9% in unselected patients, 14% for those with a CPS score of 1 or higher, and 25% with a score of 10 or higher. For cohorts A and B, the ORR was 17.1% for those with a CPS score of 1 or higher. Many of these IO agents are now being evaluated in the front-line setting in combination with platinum-based chemotherapy, as well as in patients with platinum-resistant and refractory disease with second-line regimens including anti-angiogenic agents.

As many ovarian cancers may induce an immunosuppressive tumor microenvironment, dual immune checkpoint inhibition may improve response rates and clinical outcomes by inhibiting 2 distinct pathways of immune modulation. In a recently reported analysis of the NRG clinical trial NRG-GY003, the addition of ipilimumab (CTLA-4 inhibitor) to nivolumab (PD-1 inhibitor) improved tumor response and progression-free survival (PFS) compared to nivolumab alone among 100 patients with recurrent epithelial ovarian cancer [11]. Within 6 months of randomization, the response rate for the combination was 31% compared to 12% for nivolumab alone (odds ratio 3.3), and the hazard ratio for PFS was 0.5, favoring combined checkpoint blockade.

2.2. Cervical cancer

Cervical cancer is a promising target for IO agents given its association with HPV infection and persistence and the presence of viral oncoproteins. In addition, PD-L1 or PD-L2 expression has been identified in a large proportion of tumors (67%), supporting the rationale for PD-1/PD-L1 blockade in cervical cancer patients [12]. Pembrolizumab was recently granted conditional FDA approval for PD-L1 positive advanced cervical cancer following progression on first line systemic therapy [13]. Among the 77 recurrent cervical patients with PD-L1 positive disease, the ORR was 14.3% and 91% had an ongoing response after

>6 months of follow-up. The median duration of response had not yet been reached (range, 4.1 to 18.6 months). Nivolumab is also being tested in combination with ipilimumab in HPV-positive cervical, vaginal and vulvar cancer.

2.3. Endometrial cancer

PD-1 inhibition has demonstrated clinical activity in women with MSI-high or dMMR recurrent endometrial cancer, and this indication has received FDA approval based on biomarker status independent of disease site. Due to an increased load of somatic mutations, tumors with mismatch-repair deficiency may be particularly susceptible to immune checkpoint blockade. In a phase II trial of 41 patients, pembrolizumab led to an improved immune-related PFS rate of 78% for dMMR colorectal cancers compared to 11% for those with mismatch-proficient disease [1]. Patients with dMMR non-colorectal cancer had similar responses, which included 2 patients with endometrial cancer. Median PFS and OS were not met for those with dMMR disease. Based on the molecular subgroups put forth by The Cancer Genome Atlas [14], the Polymerase E (*POLE*)-ultramutated and MSI-hypermutated subgroups fit within the classification of MSI-high or dMMR tumors. These subgroups have been shown to have high neoantigen loads and increased number of tumor infiltrating lymphocytes (TILs) with overexpression of PD-1 and PD-L1 in TILs and intraepithelial immune cells [15]. Trials of IO agents that incorporate predictive biomarkers, such as endometrial cancer molecular subtypes and PD-L1 immunostaining, are underway. As in ovarian cancer, IO agents will also be combined with first-line chemotherapy regimens both in unselected and selected populations, including those with MSI-high, dMMR and/or POLE-mutated cancers.

3. Immune activation by radiation therapy

A more substantial understanding of the immunogenic potential of radiation has developed over the last several decades through clinical observation, preclinical experimentation, and more recently, prospective study, as discussed herein. Radiation therapy is a complex therapeutic agent, which acts by the principle mechanism of double-strand DNA damage that ultimately leads to cell death. The most well-recognized pathway of radiation-induced cellular lethality is mitotic catastrophe, although alternative pathways of cell death exist, including apoptosis, necrosis, autophagy and senescence, all of which lead to a complex interplay between the host immune system and the tumor microenvironment [16]. In 1979, a seminal series of preclinical experiments by Stone et al. established the importance of host immunocompetence as a determinant of radiation response [17]. In a syngeneic mouse model of fibrosarcoma, the radiation dose required to control tumors was significantly lower in immunocompetent mice compared to those that were immunodepleted through whole body radiation or thymectomy. These findings suggested that local tumor control by radiation was dependent on a functional immune system. When the immune system of the mouse model was stimulated by bacterial injection, the radiation dose required to control tumors was further decreased. Furthermore, the immunodepleted mice were more likely to develop distant metastasis, suggesting that the immune system is a determinant of both local tumor control as well as distant metastatic spread. It is now well understood that radiation therapy can prime the immune system by generating a cytotoxic adaptive immune response that is now recognized as immunogenic cell death. Immunostimulation by radiation leads to a cascade of molecular events both at the local level as well as through systemic immune mediators, and creates a pro-inflammatory environment through increased tumor antigen uptake and presentation by dendritic cells, release of proinflammatory cytokines and chemokines, cytotoxic T-cell activation, and upregulation of immune receptors on tumor cells [18].

4. Systemic effects of immune activation by radiation

4.1. Clinical reports of the abscopal effect

The abscopal effect is a rare but intriguing clinical phenomenon whereby tumor regression occurs at a distant metastatic site or sites following local radiation treatment. Derived from the Latin “ab scopus”, or away from the target, the abscopal effect was first reported in the clinical literature in 1953, [19] and has since been described in case reports of diverse tumor types, such melanoma, renal cell carcinoma, adenocarcinoma of the lung and esophagus, and hepatocellular carcinoma [20]. The abscopal effect is now understood to be an immune-mediated response to radiation, although its incidence is exceedingly rare, as a systematic review recently identified an estimated 46 cases in the medical literature from 1969 through 2014 [21]. In this clinical review, the abscopal effect was observed across diverse tumor types and patients, including both men and women, with ages ranging from 28 to 83 years. The radiation treatment parameters varied significantly with cumulative delivered dose as low as 0.5 Gy and as high as 61 Gy, to visceral (lung, liver, kidney), skin, bone, and nodal sites. Most patients were treated with external beam radiation, although abscopal responses have also been reported with high-dose-rate (HDR) brachytherapy. Most patients were treated with conventional fractionation of 1.8 to 2 Gy per day, although some received stereotactic body radiotherapy (SBRT) to doses of 26–40 Gy in 1–5 fractions. The median timing of the abscopal response was 2 months (range, 0–24 months), and the site of the observed response included liver, skin, bone, lung and nodal sites. Several of these tumor types, such as melanoma and renal cell carcinoma, have well known immunogenic potential, whereas spontaneous regression of the primary site or metastatic disease has been observed in extremely rare instances [22,23]. Among the gynecologic cancers, an abscopal response has been reported in one patient with local advanced cervical cancer, a 69 year old woman with FIGO IIIB squamous cell carcinoma of the cervix who received pelvic radiation and HDR brachytherapy [24]. Due to cost limitations, concurrent chemotherapy and planned sequential para-aortic radiation for bulky nodal disease were not delivered. Remarkably, radiographic regression of the untreated bulky para-aortic nodes was observed, and the patient's serum SCC antigen normalized, although the duration of response and clinical follow-up time were not reported.

Renewed interest in the abscopal effect has emerged with the successful clinical introduction of immune checkpoint blockade. In a seminal case report published in 2012, [25] a female patient with metastatic melanoma was treated with palliative radiotherapy to a paraspinal mass while on a maintenance course of ipilimumab over a 19-month period. Although the patient had experienced progression while on immunotherapy, within 4 months of radiation treatment imaging revealed regression at sites both within and outside of the treated radiation field. The authors also reported changes in peripheral blood immune cell populations and antibody titers to various antigens after radiation treatment that correlated with clinical response and postulated that radiotherapy may have an immunomodulatory role in expanding an activated T-cell population. This case report was the first evidence of an abscopal response by radiation in a patient who was receiving a CTLA-4 inhibitor and has since led to the development of many new trials exploring the combination of immunotherapy and radiation to increase systemic response rates in the metastatic setting.

In the gynecologic cancer literature, another report describes a patient with metastatic endometrial cancer who experienced multiple abscopal responses following several courses of palliative radiotherapy while on nivolumab [26]. The patient was initially diagnosed with FIGO stage IIIC2 grade 1 endometrial cancer following surgical staging, and experienced disease progression on carboplatin and paclitaxel chemotherapy. Following additional treatment with doxorubicin, topotecan, then oral melphalan with disease progression, she received nivolumab in the setting of a tumor with presumed microsatellite

instability (absent expression of the MMR proteins MLH1 and PMS2). Following a palliative course of radiotherapy to the left thigh of 25 Gy in 5 fractions, there was clinical resolution of fungating masses involving the thigh, as well as a decrease in the size of hepatic metastasis, retroperitoneal lymphadenopathy and a sigmoid nodule. Subsequent imaging 2 months later showed progression, including a large symptomatic L3 vertebral metastasis for which palliative radiotherapy was again delivered with radiographic response both within and outside the radiation field, a pattern that was repeated over 5 courses of radiation. Although initial pseudoprogression may also explain the clinical response, the authors note the patient had further progression on nivolumab at 15 months, and further response following the fourth and fifth course of palliative radiation that led to clinical improvement and ongoing survival at last follow-up at 28 months. The mechanism of the abscopal effect in this patient is unknown, although may be related to MMR deficiency in the primary tumor with a high mutational burden and tumor-specific neoantigens, which are known prognostic determinants for immunotherapy response that could synergize with radiotherapy. A second case report describes a patient with metastatic neuroendocrine cervical cancer and progression following first-line cisplatin and etoposide [27]. In the setting of clinical deterioration related to her disease burden, SBRT was delivered to an abdominal mass with a dose of 20 Gy in 4 fractions with concurrent nivolumab and sandostatin. The patient had a remarkable response to treatment with disease regression both within and outside the radiation field and a near-complete radiographic response at 10 months. Genomic profiling revealed high tumor mutational burden as well as an MMR gene defect.

4.2. Mediators of the abscopal response

An established body of preclinical work has shown that the abscopal effect is mediated by cytotoxic T-cells, and that radiation can boost the abscopal response by increasing tumor immunogenicity. On a mechanistic level, radiation therapy results in release of tumor neoantigens through cellular damage and necrosis, which are then taken up by antigen presenting cells (APCs) such as dendritic cells. With a substantially increased number and diversity of tumor antigens released by radiation, immune system priming occurs when APCs travel to regional lymph nodes to present tumor-specific antigens to cytotoxic (CD8+) T-cells. Activated T-cells then hone to the site of the irradiated tumor as well as non-irradiated tumor metastases to induce immunogenic tumor cell death, which enhances local tumor control as well as abscopal responses at distant non-irradiated sites (Fig. 1). Local radiation also releases cellular danger-associated molecular patterns, known as DAMPs, as well as cytokines, which enhance immune cell trafficking though induction of an inflammatory response [28].

Despite the immunogenic potential of radiation, the rarity of the abscopal response in clinical practice is likely related to mechanisms of immune tolerance or suppression within the tumor microenvironment. Tumors can evade the host immune system through release of immunosuppressive cytokines such as TGF-β, or upregulate receptors that result in immune tolerance such as PD-L1 [29]. The recruitment of regulatory T-cells also has an immunosuppressive effect, through the release of immunosuppressive cytokines or upregulation of CTLA-4. In addition to tumor-related factors, determinants of the abscopal responses are likely dependent on clinical factors, including the degree of myelosuppression, specifically lymphopenia, as well as prior treatment with radiation and/or chemotherapy [30].

Many current clinical trials are focused on combinatorial therapy to overcome immune suppression and enhance the response rates to immunotherapy. These immunologic properties of radiation may compliment the immune stimulatory effects of both CTLA-4 and PD-1 pathway inhibition, as preclinical studies have demonstrated synergies with both classes of agents and radiation [31] in terms of improved local and distant control, including abscopal regression of established tumors outside of the radiation treatment field [32]. Preclinical results of radiation combined with PD-1 inhibitors have shown clinical promise, although the highest response rates have been observed with the combination of radiation and dual checkpoint blockade. In preclinical study by Twyman-Saint Victor et al., focal radiation and CTLA-4 and

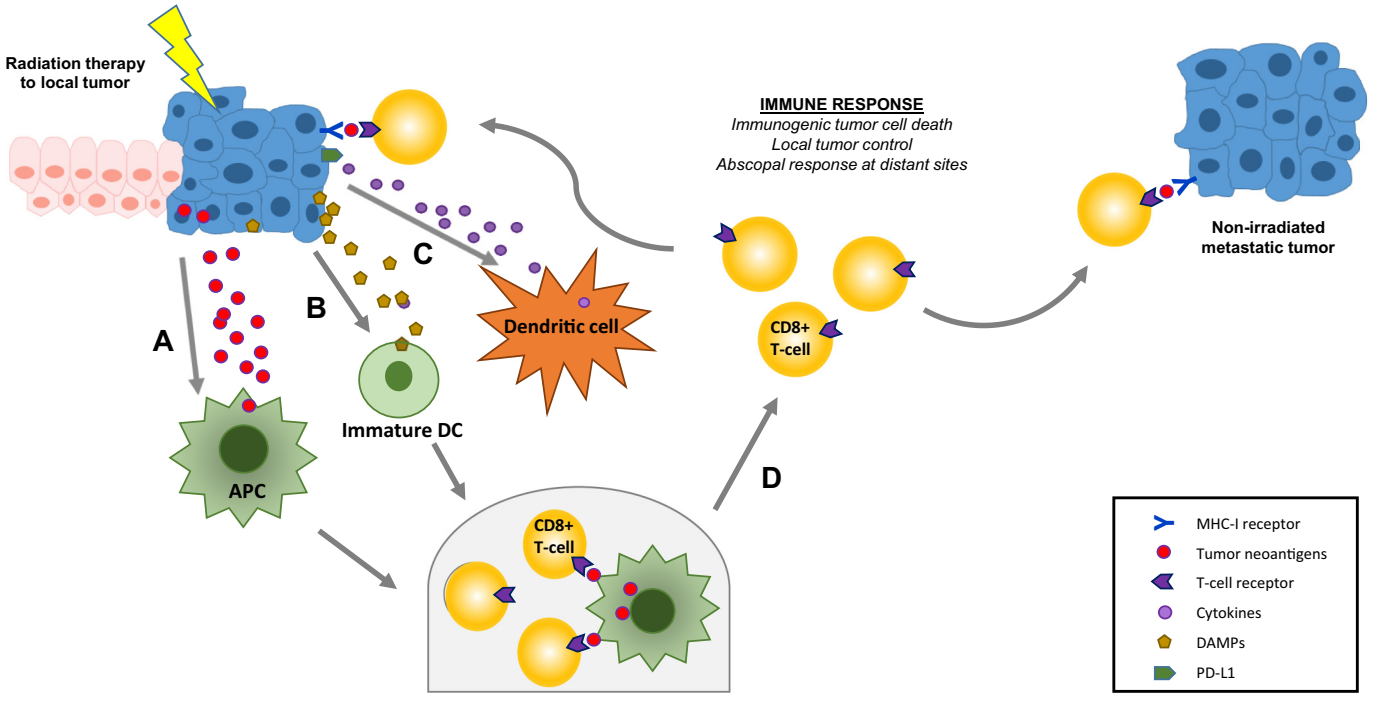

Fig. 1. Mechanism of the abscopal effect and generation of an adaptive immune response. A) Radiation therapy to a local tumor releases tumor neoantigens, which are taken up by antigen presenting cells (APCs) such as macrophages and dendritic cells. B and C) Radiotherapy also results in the release of damage-associated molecular patterns (DAMPs) and pro-inflammatory cytokines that leads to maturation and activation of dendritic cells. Upregulation of PD-L1 and MHC class I on tumor cells also occurs. The APCs then migrate to lymph nodes to present the tumor neoantigens to cytotoxic (CD8+) T-cells. D) Activated T-cells induce immunogenic tumor cell death by acting on primary irradiated tumor as well as distant metastatic sites. Abbreviations: MHC, major histocompatibility complex.

Table 1
Comparison of radiation treatment parameters for involved field radiotherapy versus stereotactic body or ablative radiotherapy.

	Involved field RT	SBRT/SABR
Technique	3D conformal, IMRT or VMAT	IMRT or VMAT
Fractionation	Conventional (1.8–2 Gy per fraction)	Hypofractionation (5–10 Gy per fraction)
Cumulative dose and # fractions	45–65 Gy in 25–35 fractions	24–60 Gy in 3–5 fractions; 18–24 Gy in 1 fraction
Length of RT	5–7 weeks	1 day to 2 weeks
Timing of RT	5 days per week	2–3 fractions per week
Dose gradient	Less steep	Very steep
Treatment volume	Large fields with primary site and elective nodal radiation	Involved site only; no elective nodal radiation
Treatment site	Pelvic RT, extended field RT	Liver, lung and bone metastasis

Abbreviations: IMRT: intensity modulated radiotherapy; RT: radiotherapy; SABR: stereotactic ablative radiotherapy; SBRT: stereotactic body radiotherapy; VMAT: volumetric modulated arc therapy.

PD-1 inhibition led to 60–80% response rates across tumor models, [33] significantly greater than any single component or the combination of dual checkpoint blockade without radiation.

5. Clinical trials of combined immunotherapy and radiation

Prospective clinical studies of IO agents and radiation are limited, although phase III trials have been reported in the metastatic and adjuvant setting for prostate and lung cancer. The clinical trials that have evaluated the combination of immunotherapy and radiation have not led to a consensus on the optimal timing, dose or setting. Most trials initiated to date have selected hypofractionated regimens with delivered doses >6 Gy per fraction and delivery of radiation within 1 week of initiation of immunotherapy. A relatively small number of trials have evaluated radiation dose through dose escalation or randomized comparison, or the sequencing of radiation and immunotherapy [34].

5.1. Metastatic setting: harnessing the abscopal response

In patients with metastatic disease, several clinical studies have evaluated the combination of checkpoint blockade and multi-site SBRT to target metastatic sites with ablative doses of radiation. As clinical response rates to immunotherapy remain low and have been shown to be correlated with disease burden, [35] the use of SBRT may be a promising strategy for tumor cytoreduction as well as to enhance immune response through tumor antigen release, increased T-cell infiltration and activation of innate immunity [36,37]. A phase III trial of 799 patients with metastatic castrate and chemotherapy-resistant prostate cancer randomized patients to ipilimumab or placebo following a single 8 Gy dose of radiation to up to 5 bony metastatic sites [38]. Although there was no difference in overall survival, post hoc subset analysis revealed a survival benefit for patients with good prognostic features, although a follow-up study in this population did not confirm these findings [39]. A prospective phase I study has also evaluated the safety of pembrolizumab with multisite SBRT in 79 patients with metastatic solid tumors that had progressed on standard treatment. Radiation was delivered to 2–4 sites with doses of 30 to 50 Gy delivered in 3 to 5 fractions with pre-defined dose de-escalation in the setting of toxicity [40]. Most patients received treatment to 2 sites, which were most commonly lung or mediastinum (45%), abdomen or pelvis (19%), liver (16%) or bone (11%). The study population included 9 patients with ovarian/fallopian tube cancer, 6 with endometrial cancer, and 1 patient each with cervical and vaginal cancer. Six dose-limiting toxicities (DLTs) were observed, including grade 3 pneumonitis, grade 3 colitis and grade 3 hepatic toxicity. The overall DLT rate was <10% and similar to that reported for patients who previously received radiotherapy who were treated with pembrolizumab [41]. The observed toxicities were most commonly within the irradiated field, and therefore difficult to determine if the adverse event (AE) was related to radiation, immunotherapy or the combination. Overall, the treatment was considered well tolerated with acceptable toxicity and there were no radiation dose reductions. The overall objective response rate was 13.2%, and out-of-field response rate for non-irradiated target metastases by RECIST was 14%. The abscopal response rate, defined as a 30% reduction in any single non-irradiated RECIST target lesion, was 27%. From these data, it remains unclear if SBRT can potentiate the efficacy of PD-1/PD-L1/CTLA-

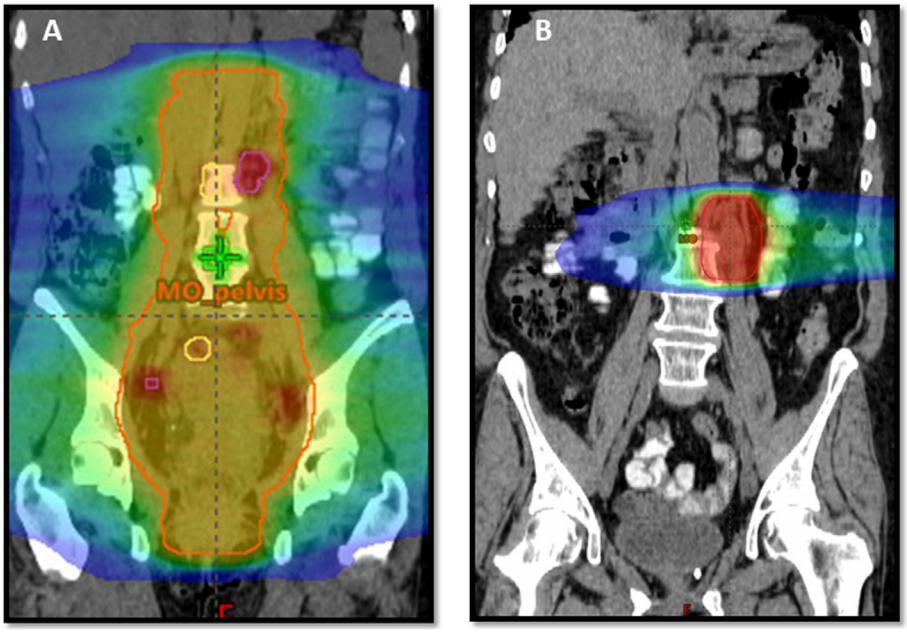

Fig. 2. Comparison of radiation dose distribution for (A) extended field radiotherapy with lymph node boost delivered by conventional fractionation and (B) SBRT to involved para-aortic lymph node. The planning target volume is represented by red contour. Extended field radiotherapy includes elective nodal radiation of pelvic and para-aortic lymph nodes with additional boost dose to involved nodes (purple contour).

4 inhibition as the ORRs were relatively low and comparable to other unselected, heavily pretreated populations with advanced solid tumors.

For patients with metastatic gynecologic cancer, radiation could be added to immunotherapy when given for approved indications, such as MSI-high or dMMR endometrial cancer and PD-L1 positive cervical cancer, or in populations with low clinical response rates in hopes of enhancing systemic response through the abscopal effect. To evaluate the potential synergy of radiation and immunotherapy observed in preclinical studies, radiation may be delivered to one or more metastatic sites concurrently with checkpoint blockade, including for tumors which are known to be poorly immunogenic, such as ovarian cancer. As shown in Table 2, several phase II trials will evaluate the overall response rate for the combination of a PD-1 or PD-L1 inhibitor and stereotactic radiation therapy for patients with recurrent, persistent or metastatic ovarian, endometrial and cervical cancer. There are also 2 phase I studies that will evaluate the safety and tolerability of dual checkpoint blockade (anti-PD-L1 and anti-CTLA-4) and hypofractionated radiotherapy for recurrent gynecologic cancer, one of which will report the abscopal response rate for non-irradiated RECIST target lesions.

5.2. Adjuvant setting: controlling micro-metastatic disease

Systemic control with immunotherapy may be most effective in the adjuvant setting when immune activation is harnessed to control distant micro-metastatic disease. In the phase III PACIFIC trial, 713 patients with stage III, locally advanced and unresectable non-small cell lung cancer were randomized to receive the PD-L1 inhibitor durvalumab or placebo following definitive chemoradiotherapy with a platinum-based regimen. The administration of durvalumab commenced within 42 days after chemoradiotherapy and was delivered every 2 weeks until disease progression for up to 12 months. The co-primary endpoint of PFS was met with the addition of durvalumab, which was a median of 16.8 months compared to 5.6 months with placebo, and led to FDA approval for the use of adjuvant durvalumab after standard treatment. The PFS benefit was observed across all prespecified subgroups, and in contrast to the metastatic setting, was independent of biomarker status, as patients with both PD-L1 positive and negative tumors experienced clinical benefit. The incidence of new brain metastasis was also lower with durvalumab (5.5% vs 11%) as were new metastatic lesions (20% vs 32%), [42] suggestive of a promising role in systemic control. In an updated follow-up, durvalumab also significantly prolonged overall survival, which at 24 months was 66% with the study drug and 56% with placebo [43].

The addition of ipilimumab following definitive chemoradiation for advanced cervical cancer has been evaluated in a phase I NRG study [44]. Given the high rate of systemic progression among patients with node positive disease, concurrent and/or adjuvant immunotherapy is a promising approach for future study. Following extended field radiotherapy and weekly cisplatin, sequential ipilimumab was given at 2 dose levels with a dose expansion cohort. The authors reported that outback ipilimumab was well tolerated, as 90% of patients received 4 cycles. Three patients (16%) had acute grade 3 toxicity, including elevated

Table 2
Ongoing clinical trials of combined immunotherapy and radiation for metastatic or recurrent gynecologic cancer.

Identifier	Phase	N	Title	Disease	Treatment	Radiation details	Primary outcome	Secondary outcomes
NCT03277482	I	32	Durvalumab, Tremelimumab, and Radiotherapy in Recurrent Gynecologic Cancer	Metastatic or recurrent gynecologic cancer	Durvalumab every 4 weeks for 1 year; tremelimumab every 4 weeks for 4 cycles; EBRT at day 1	25 Gy in 5 fractions to an abdominal or pelvic site (level 0), or 8 Gy in single fraction (level − 1)	Incidence of DLTs	• Abscopal RR • LC/LRR • OS • ORR • PFS • Response duration
NCT03452332	I	18	Stereotactic Ablative Radiotherapy (SABR) in Combination with Durvalumab and Tremelimumab in Patients with Cervical, Vaginal, or Vulvar Cancer	Cervical, vaginal or vulvar cancer	Durvalumab every 4 weeks up to 8 cycles; tremelimumab every 4 weeks for 4 cycles; EBRT at day 1	SABR (24 Gy in 3 fractions) given cycle 1, day 7	Incidence of DLTs Incidence of AEs	• Clinical RR • Objective response (CR or PR) • OS • PFS • Time to next treatment
NCT03192059	II	43	Study of Pembrolizumab, Radiation and Immune Modulatory Cocktail in Cervical/Uterine Cancer (PRIMMO)	Advanced and/or refractory cervical cancer, endometrial cancer, or uterine sarcoma	Vitamin D, aspirin, cyclophosphamide, lansoprazole, followed by pembrolizumab every 21 days and RT; daily curcumin supplement	Stereotactic RT (24 Gy in 3 fractions)	Objective RR at 26 weeks by irRC	• Best overall response • Incidence of DLTs • Objective RR by RECIST • OS • PFS • QOL assessment
NCT03312114	II	29	Anti—PD-L1 and SAbR for Ovarian Cancer	Recurrent or persistent epithelial ovarian, primary peritoneal or fallopian tube cancer	Avelumab and SABR	SABR	ORR per RECIST	• CR rate • OS • Median response duration • Time to progression
NCT03614949	II	26	SBRT and Atezolizumab in the Management of Recurrent, Persistent, or Metastatic Cervical Cancer	Recurrent, persistent or metastatic cervical cancer	SBRT followed by atezolizumab 1 week later, every 3 weeks	SBRT (24 Gy in 3 fractions) to 2 or more sites	ORR by immune-modified RECIST	• OS • PFS

Abbreviations: AE: adverse event; CR: complete response; DFS: disease-free survival; DLT: dose-limiting toxicity; EBRT: external beam radiotherapy; LC: local control rate; LRR: local response rate; ORR: overall response rate; OS: overall survival; PFS: progression-free survival; PR: partial response; QOL: quality of life; RR: response rate; RT: radiotherapy; SABR: stereotactic ablative radiotherapy; SBRT: stereotactic body radiotherapy.

lipase, rash, and neutropenia. Additional clinical studies are underway to evaluate the potential additive or synergistic effect of radiation with checkpoint blockade on both systemic response and local control for patients with locally advanced, unresectable disease, as discussed below.

5.3. Unresectable disease: enhancing local control

Immune checkpoint blockade is also an attractive option to enhance radiosensitivity and thus local control in patients with unresected disease. Radiation has been shown to increase the density of TILs in the irradiated site, and pre-clinical models have demonstrated improved tumor control in combination with both CTLA-4 and PD-1/PD-L1 inhibitors. In the PACIFIC trial, the use of adjuvant durvalumab following chemoradiation led to higher objective response rates compared to placebo (30% vs. 18%, $p < 0.001$), and among patients with a response, 74% of those in the durvalumab arm had an ongoing response at 18 months compared to 52% with placebo [43]. In the locally advanced lung cancer population, such gains in both local and distant tumor control may likely have an impact on tumor-related morbidity, and would be clinically meaningful among gynecologic cancer patients as well.

6. Definitive chemoradiation and immunotherapy for gynecologic cancer

Building upon the phase I concept of adding outback ipilimumab following definitive chemoradiotherapy for node positive cervical cancer, several phase I and II studies will evaluate the safety as well as efficacy of definitive chemoradiation, including external beam radiotherapy, weekly cisplatin and brachytherapy, with concurrent or adjuvant PD-1 or PD-L1 inhibition (Table 3). Within the NRG cooperative group, the timing of atezolizumab (anti-PD-L1) as an immune primer will be evaluated in a randomized phase I study, delivered either 3 weeks prior and/or concurrently with chemoradiotherapy in patients with high risk, advanced stage cervical cancer. The primary endpoint will evaluate atezolizumab sequencing on immune activation through the measurement of T-cell receptor clonal expansion in peripheral blood. An alternate sequencing strategy will be evaluated in a phase II study of pembrolizumab (anti-PD-1) delivered either concurrently or sequentially with chemoradiation in a similar population of locally advanced cervical cancer. These 2 trials, as well as a phase I study of chemoradiation and concurrent nivolumab, will evaluate the incidence of DLTs, disease-related outcomes and correlative endpoints such as changes in

Table 3
Ongoing clinical trials of definitive radiation with concurrent and adjuvant immunotherapy for locally advanced cervical cancer.

Identifier	Phase	N	Title	Disease	Treatment	Radiation details	Primary outcome	Secondary outcomes
NCT03298893	I	21	Nivolumab in Association with Radiotherapy and Cisplatin in Locally Advanced Cervical Cancers Followed by Adjuvant Nivolumab for up to 6 Months (NiCOL)	Locally advanced cervical cancer (stages IB2-IVA)	Nivolumab every 2 weeks (240 mg or 1 mg/kg) with CRT and 5 months thereafter	IMRT with or without nodal boost and weekly cisplatin followed by brachytherapy	Incidence of DLTs	• ctDNA heterogeneity • DFS • Incidence of AEs • Incidence of SAEs • ORR • PFS • Tumor molecular analysis • Tumor PD-L1 expression
NCT03738228	I	40	Anti PD-L1 (Atezolizumab) as an Immune Primer and Concurrently with Extended Field Chemoradiotherapy for Node Positive Locally Advanced Cervical Cancer	Stage IB/IIA cervical cancer with PALN or stage IIB/III/IVA cervical cancer with pelvic and/or PALN	Randomized to day −21 or day 0 atezolizumab with CRT every 3 weeks for 3 cycles	Extended field RT with weekly cisplatin, image-guided brachytherapy	Measurements in T-cell receptor clonal expansion in peripheral blood	• Correlation of PD-L1 expression and post--treatment PET-CT • 2-year DFS • Frequency and severity of AEs • Incidence of DLTs • Serial measurements of T-cell clonality and diversity
NCT02635360	II	88	Pembrolizumab and Chemoradiation Treatment for Advanced Cervical Cancer	Locally advanced cervical cancer	Randomized to concurrent or sequential pembrolizumab with CRT every 21 days for 3 months	EBRT with weekly cisplatin followed by brachytherapy	Changes in immunologic markers at 6 and 12 weeks Incidence of DLTs	• Incidence of distant metastasis • Metabolic RR by PET/CT at 12 weeks • OS • PFS
NCT03612791	II	190	Trial Assessing the Inhibitor of Programmed Cell Death Ligand 1 (PD-L1) Immune Checkpoint Atezolizumab (ATEZOLACC)	Stage IB2-IIA cervical cancer with positive pelvic nodes, stage IIB-IVA, or stage IVB with para-aortic nodes	Randomized to CRT alone or CRT with concurrent and adjuvant atezolizumab every 21 days for up to 20 cycles	Pelvic +/− para-aortic EBRT with nodal boost and weekly cisplatin followed by brachytherapy	PFS	–

Abbreviations: AE: adverse event; CRT: chemoradiation; ctDNA: circulating tumor DNA; DFS: disease-free survival; DLT: dose-limiting toxicity; EBRT: external beam radiotherapy; ORR: overall response rate; OS: overall survival; PALN: para-aortic lymph nodes; PFS: progression-free survival; RR: response rate; RT: radiotherapy; SAE: serious adverse event.

serum immunologic markers, tumor molecular analysis and PD-L1 expression. The largest study (ATEZOLACC, Gustave Roussy) is a randomized phase II trial of definitive chemoradiation with or without concurrent and adjuvant atezolizumab (anti-PD-L1) with a planned accrual of 190 patients with high-risk or advanced cervical cancer with a primary study endpoint of PFS. Such combinatorial and sequencing approaches could also be evaluated in patients who receive definitive radiation in the salvage setting, such as vaginal and/or nodal recurrence related to endometrial cancer, given the relatively high rates of local and systemic failure, [45] as well as promising clinical activity for dMMR tumors.

7. Optimizing radiation parameters with immunotherapy

Radiation technique and delivery has been evolving rapidly over the last decade, with the introduction of highly conformal radiation approaches, such as intensity modulated radiotherapy (IMRT), volumetric-modulated arc therapy (VMAT) and particle beam therapy such as proton beam, with the goal of enhancing the therapeutic index of radiation. Image-guided brachytherapy with CT, MRI and/or US guidance has also led to improved local control rates for patients with advanced gynecologic cancer [46,47]. These techniques allow for the delivery of higher radiation doses while maintaining or lowering the associated acute and long-term toxicity risk. The increased conformality of the delivered dose with associated steep dose drop off to adjacent uninvolved tissues has also allowed a shift from conventional fractionation of 1.8–2 Gy daily to hypofractionated approaches that may range from 5 to 10 Gy daily, and up to 24 Gy in a single dose (Table 1, Fig. 2). Improved immobilization, advanced imaging modalities such as MRI and PET-CT for radiation planning, as well as the ability to track tumor and organ motion, has allowed for further dose escalation and the adoption of radiation delivery that is considered ablative in nature, including stereotactic radiosurgery (SRS) for brain metastases and SBRT or stereotactic ablative radiotherapy (SABR) for oligometastatic disease, including sites in the lung, liver, abdomen and bone. Hypofractionated radiation, such as SBRT, SABR and HDR brachytherapy, are considered especially promising modalities for combination with immunotherapy, as higher dose per fraction is more likely to induce tumor cell death via necrosis and senescence, which are considered more pro-inflammatory to induce an immunogenic response. Most preclinical studies have shown that hypofractionated regimens of 24 Gy delivered in 3 fractions and 30 Gy in 5 fractions are superior to a single ablative dose of 20 Gy [48].

Conventional, large field radiation therapy is commonly used for patients with bulky or locally advanced cervical and vulvar cancer, vaginal cancer, and advanced or recurrent endometrial cancer. For these indications, the radiation field comprehensively treats the pelvis to encompass the draining lymphatics as well as the primary site, often including the entire gynecologic tract. An unintended consequence of comprehensive pelvic radiation, or in some cases extended field radiotherapy to treat the para-aortic lymph nodes, is bone marrow suppression, which often leads to lymphopenia and neutropenia during treatment. Large radiation fields not only expose a greater volume of functional bone marrow to its cytotoxic effects, but also the circulating lymphocytes, which are exquisitely sensitive to radiation doses on the order of 0.5 Gy, and the lymph node basins where immune priming of tumor antigen would occur. Protracted radiation courses over 5–7 weeks may also be more likely to exhaust T-cell populations. Nevertheless, preclinical studies have also demonstrated immunologic effects and potential efficacy of low-dose fractionated radiation therapy [49]. Among clinical reports of melanoma patients treated with radiation and ipilimumab, a systemic review found that patients who received multiple fraction radiation regimens (≤3 Gy per fraction) had the most favorable response of index lesions outside the radiation field. As there is preclinical and limited clinical evidence to support both low dose and high dose approaches, [36] the optimal timing, radiation dose and technique in combination with immunotherapy have yet to be determined. Other parameters such as the specific target site and the sequencing and timing with IO agents also require study in the context of a clinical trial.

8. Potential toxicities of immunotherapy and radiation

The immune-related adverse events (ir-AEs) of checkpoint inhibitors are related to an inflammatory or immune-mediated mechanism that may affect any normal tissue or organ. The most common immune-mediated toxicities include colitis, pneumonitis, dermatitis, and hypo/hyperthyroidism, and less frequently nephritis, hepatitis, pancreatitis, and myositis [50]. Myocarditis and hypophysitis may also occur and are considered rare. The incidence of severe ir-AEs is higher with CTLA-4 inhibitors compared to PD-1 or PD-L1 inhibitors alone, and increases greatly with the combination [51]. With the addition of radiation, concern for increased toxicity is related to the site of radiation, such as pneumonitis risk for the treatment of lung cancer, and colitis for patients with pelvic malignancies. To date, the available data do not suggest an increased risk of immune-related toxicity with radiation [52]. In the randomized trial of ipilimumab and radiation for prostate cancer, the frequency and severity of gastrointestinal AEs were similar for those who received bone-directed radiotherapy to the pelvis and those who did not [38]. In the phase III lung cancer trial, grade 3 or 4 AEs of any cause occurred in 31% of the patients who received adjuvant durvalumab compared to 26% of those in the placebo group [43]. Lung toxicity did not appear to be increased with the addition of sequential durvalumab, with pneumonitis rates of 1.3% in both treatment arms, and pneumonia rates of 1.1% and 1.3% with durvalumab and placebo, respectively. A comprehensive toxicity assessment has also been performed in a retrospective study of 133 patients with metastatic lung, melanoma or renal cell cancer who received 286 courses of radiation and at least 1 cycle of a CTLA-4 or PD-1 inhibitor [53]. Most patients (50%) received radiation prior to immune checkpoint blockade at a median of 71 days, and the most common site was whole brain radiotherapy (58%) followed by thoracic (13%) and spine radiation (11%). As expected, patients who received both CTLA-4 and PD-1 inhibitors had significant higher rates of ir-AEs than those with monotherapy (71% vs. 29%, respectively, $p < 0.01$), and grade 3–4 toxicities were highest among patients receiving a CTLA-4 inhibitor compared to a PD-1 inhibitor (20% vs. 4%). Notably, patients who received radiation during or after checkpoint blockade had similar rates of ir-AEs compared to those who received radiation prior to checkpoint blockade. Of interest, there was no association between the specific ir-AE and the site that was irradiated. As many patients with recurrent or metastatic gynecologic cancer receive palliative radiotherapy for indications such as bleeding or pain, ongoing toxicity reporting from prospective and retrospective studies is warranted, including for patients with endometrial and cervical cancer who are receiving PD-1/PD-L1 inhibitors for approved indications.

9. Conclusions

Immunotherapy, and specifically immune checkpoint blockade, represents an important new therapeutic modality for many patients with advanced and treatment-refractory cancer. As response rates to IO agents have been modest in the gynecologic cancer population, novel combinatorial approaches with hypofractionated radiation or definitive chemoradiation are currently under investigation. Through immune system priming, radiation treatment has the potential to enhance systemic response rates to immunotherapy through the abscopal effect, as well as increase local tumor control at the irradiated site. Careful prospective study will be required to determine the optimal integration of immunotherapy, chemotherapy and radiotherapy, as there are many unanswered questions related to the radiation treatment site, volume and delivered dose, as well as sequencing with IO agents. In the

metastatic setting, patients with PD-L1 negative cervical cancer and MSI-low or MMR-proficient endometrial cancer are not candidates for anti-PD-1 therapy, and future trials should consider combinatorial strategies for these populations, as well as for patients who experience disease progression during or following exposure to IO agents. Many trials have and will continue to incorporate correlative endpoints to identify predictive and prognostic factors of response, as well as treatment-resistance, which promises to better select patients for immunotherapy combinations and guide future trial development.

Author contribution

L.L. wrote the manuscript text and generated the figures and tables. U.M. provided intellectual and editorial contributions.

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Conflict of interest statement

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