Review Article on Synergy in Action: Novel Approaches to Combining Radiation Therapy and Immunotherapy

Combination radiation and immunotherapy in gynecologic malignancies—a comprehensive review

Colton Ladbury[1], Elizabeth Germino[1], Jennifer Novak[1], Jason Liu[1], Zachary Horne[2], Brandon Dyer[3], Scott Glaser[1]

[1]Department of Radiation Oncology, City of Hope National Cancer Center, Duarte, CA, USA; [2]Department of Radiation Oncology, Allegheny General Hospital, Pittsburgh, PA, USA; [3]Department of Radiation Oncology, University of Washington, Seattle, WA, USA

Contributions: (I) Conception and design: S Glaser, C Ladbury; (II) Administrative support: S Glaser; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: C Ladbury; (V) Data analysis and interpretation: C Ladbury; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Scott Glaser. Department of Radiation Oncology, City of Hope National Cancer Center, 1500 East Duarte Road, Duarte, CA 91010, USA. Email: sglaser@coh.org.

Abstract: Definitive and adjuvant radiation and chemoradiation have been mainstays in the management of multiple gynecologic malignancies for decades. However, despite these treatments, the prognosis of patients with locally advanced, recurrent, refractory, and metastatic disease continues to be poor. Over the last decade, immune checkpoint inhibitors have emerged as a promising therapeutic modality, but response rates to monotherapy are low. Mounting basic science and translational research suggests that immunotherapy and radiation may act synergistically with the potential to improve clinical outcomes across multiple disease sites relative to monotherapy with either radiation or immunotherapy alone. Results from early clinical trials in other disease sites, and burgeoning trials within the gynecologic malignancies space hold promise for combined modality treatment. With increasing clinical data supporting combined modality therapy, there is interest in reevaluating treatment paradigms in gynecologic malignancies to improve the current standards of care. In this review, current proposed mechanisms, rationale, and evidence for treatment of gynecologic malignancies with combined radiation and immunotherapy, specifically immune checkpoint inhibitors, will be discussed. Additionally, although currently early and limited, existing clinical data will be summarized as it applies to cervical, endometrial, ovarian, and vulvar cancers. The status of current clinical trials investigating the sequencing, dosing, and fractionation of combined radiation and immunotherapy in these disease sites will also be reviewed.

Keywords: Immunotherapy; radiation; gynecologic cancer; checkpoint inhibitor; intensity modulated radiation therapy (IMRT); SBRT; stereotactic ablative radiotherapy (SABR); cervical cancer; endometrial cancer; ovarian cancer; vulvar cancer; immunoradiotherapy

Submitted Oct 10, 2020. Accepted for publication Dec 24, 2020.
doi: 10.21037/tcr-20-3019
View this article at: http://dx.doi.org/10.21037/tcr-20-3019

Introduction

Radiation therapy plays a central role in the treatment of most gynecologic malignancies, in both the definitive and palliative setting. Historically, radiation therapy has frequently been given with concurrent systemic therapy to augment the local effects of radiotherapy and improve systemic disease control. As with other disease sites, patients with locally advanced, recurrent, refractory, or metastatic disease continue to have poor clinical outcomes without significant advancement. More recently, cell cycle immune checkpoint inhibition has emerged as a promising systemic treatment in several disease sites, including gynecologic malignancies, for patients with advanced, recurrent, or

Table 1 Classification of immunotherapies currently investigated in clinical trials by target

PD-1	PD-L1	CTLA-4
Nivolumab	Atezolizumab	Ipilimumab
Pembrolizumab	Avelumab	Tremelimumab
TSR-042	Durvalumab	

metastatic disease.

Broadly, the goal of immunotherapy is to harness the ability of the immune system to recognize and eliminate tumor cells (1). Therefore, by modulating the immune system using antibodies against protein receptors such as CTLA-4, PD-1, and PD-L1, the anti-tumor immune response can be amplified, leading to improved systemic response to malignant cells (2). However, tumor responses to immunotherapy monotherapy are low, in the 20–30% range (3-6). This is thought to be due to immune-evasive properties of tumors through selective pressure, tumor evolution, and tumor genetic heterogeneity, resulting in reduced tumor immunogenicity or induction of immune tolerance (anergy) (1).

Radiation can increase antigen quantity, variety, and presentation to increase the immune system's ability to respond to a tumor through both innate and adaptive immune mechanisms and induction of anti-tumor T-cell responses. Therefore, the therapeutic intent behind combined radioimmunotherapy is to augment the immune response and circumvent immune evasion. The thought is that radiation can increase antigen generation/presentation, T-cell priming, dendritic cell activation, and upregulation of pro-inflammatory cytokines. Meanwhile, immunotherapy can reverse T-cell exhaustion, broaden T-cell receptor recognition, and enhance T-cell activation. Hence, immunotherapy and radiation may have a synergistic effect.

This hypothesis has only just begun to be explored in gynecologic malignancies but is supported by the first reported clinical trials. For example, GOG 9929, a Phase I clinical trial published in 2019 investigating sequential immunotherapy following chemoradiation (CRT) in women with locally-advanced cervical cancer showed an increase in T-cells expressing PD-1 following CRT, and offers a rationale for further investigation into neoadjuvant, concurrent, and adjuvant treatment with immunotherapies such as PD-1/PD-L1 and CTLA-4 inhibitors (7).

The optimal sequencing, dosing, and fractionation of radiation and immunotherapy remains unknown and is the topic of several clinical trials throughout disease sites, including gynecologic malignancies. Herein, we review the current utilization of radiation combined with immune checkpoint inhibitors for treatment of gynecologic malignancies. We will then describe current and potential future investigations into their combined efficacy.

Radiation and immunotherapy sequencing, dose, and fractionation

The optimal sequence, dose, and fractionation schedule for combining radiation and immunotherapies remains unclear and depends on the proposed mechanism of synergy of the two modalities. Pre-clinical data suggests that a close or concurrent sequencing may be beneficial in the case of PD-L1 inhibitors. This setting has been supported by murine models, where immune checkpoint blockade correlates with peak T-cell infiltration of tumors after radiation, leading to superior outcomes with concurrent administration (8-10). Conversely, other data suggest benefits to the neoadjuvant use of CTLA-4 inhibitors, theoretically due to CTLA-4 inactivating intratumoral regulatory T-cells (11). Specific agents being studied in ongoing clinical trials of combined radiation and immunotherapy for gynecologic malignancies are summarized in *Table 1*.

In addition to optimizing the sequencing of therapies, radiation dose and fractionation may influence therapeutic synergy. Early murine models suggest hypofractionated radiation may be favorable through a resultant increase in antigen presentation, mature CD8 T-cell tumor infiltration, and enhanced effector T-cell reactivity (12,13). This has been attributed to the superior cytoreductive capability of hypofractionated radiation, resulting in reduced tumor burden, which in turn increases reinvigoration of exhausted T-cells and, consequently, radiosensitivity (8,9,14). Other contributors may be increased upregulation of checkpoint molecules and inflammatory cytokines, although it is not yet clear how fractionation schemes influence these factors (15). In the ongoing trials summarized below, sequencing, dose, and fractionation are all reported. Unfortunately, few of them are specifically designed to identify an optimal regimen, and indeed there may not be a one-size-fits-all approach. Future work investigating these parameters in both the pre-clinical and clinical setting will be needed to guide the combined use of radiation treatment with immunotherapy. The ongoing trials investigating combined radiation and chemotherapy, and the existing data and rationale behind them, are reviewed below broken down by

disease site, and are summarized in *Table 2*.

Cervical cancer

Cervical cancer affects an estimated 13,800 women per year in the United States, accounting for 4,290 deaths annually, and is also the most common gynecologic malignancy worldwide (16). The current standard of care for definitive management of locally advanced cervical cancer is concomitant platinum-based CRT (17-21). This treatment paradigm has remained relatively unchanged for two decades; however, overall survival (OS) and progression-free survival (PFS) for patients with the locally advanced, node positive, and metastatic disease remains decidedly poor. More recently, chemotherapy intensification was evaluated with the addition of adjuvant chemotherapy in hopes of improving clinical outcomes for patients with high-risk disease. A Phase III trial showed improved PFS (74% *vs.* 65%) with "outback" cisplatin/gemcitabine, but had significant increased Grade 3/4 toxicity (22). Chemotherapy intensification continues to be the topic of investigation of OUTBACK (GOG-0274/RTOG 1174) and INTERLACE trials, which are evaluating adjuvant and neoadjuvant platinum-taxane regimens, respectively. In a similar vein, there is also significant interest in investigating immunotherapy combined with radiation to treat cervical cancers to determine if PFS and OS can be improved without excessive toxicity.

A rationale for treatment of cervical cancers with immunotherapy arises from most cervical malignancies developing out of immune tolerance/anergy in response to persistent infection with human papillomavirus (HPV). This virally-driven malignancy results in dysplasia progressing to neoplasia, then carcinoma, cellular immortalization, and immune evasion. The resulting immune downregulation, via mechanisms such as increased PD-1 and PD-L1 expression, is higher in virus-associated cancers such as HPV-associated cancers (23-25). This provides a strong preclinical rationale in HPV-mediated cervical cancers to target modulation of PD-1/PD-L1 in hopes of decreasing immune escape mechanisms.

In June 2018, based on data from KEYNOTE-158, pembrolizumab was approved for patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy. In this trial, 98 patients received 200 mg of pembrolizumab every three weeks, and results demonstrated an objective response rate in 12.2% of patients (three complete and nine partial responses) (26). Of note, there were no responses in patients whose tumors did not express PD-L1. Bastilimab ± zalifrelimab is currently under FDA fast track designation as second-line therapy for patients with recurrent, unresectable, and metastatic cervical cancer with progressive disease (NCT03894215). However, as with treatment of other disease site with immunotherapy alone, these existing data suggest low objective response rates (ORR), necessitating either improved patient selection or investigation of other combined therapies.

GOG 9929 is a recent Phase 1 trial examining pelvic CRT followed by sequential ipilimumab in the management of node-positive cervical cancer (7). Patients with FIGO Stage IB2 and IIA disease with positive para-aortic lymph nodes and patients with Stage IIB, IIIB, or IVA disease with positive para-aortic or pelvic lymph nodes were eligible. They were treated with standard pelvic CRT followed by four sequential escalating doses of ipilimumab. Twenty-one patients received at least two cycles of ipilimumab (18 received four cycles and three received two cycles). Only two patients had grade 3 toxicity, with one episode each of self-limited lipase increase and dermatitis. Overall Survival at 12 months was 90% and PFS was 81%. Interestingly, following CRT there was a significant increase in CD4 and CD8 positive T cell expression of PD-1. This suggests that CRT has the potential to expand immune surveillance populations and anti-cancer immune responses (27). Furthermore, the increase in PD-1 expression was maintained following CRT with ipilimumab, suggesting that the CTLA-4 blockade has the potential to generate a durable adaptive anti-tumor response.

Current investigations

Relative to other gynecologic malignancies, cervical cancer is the topic of a large quantity of ongoing clinical trials, and will be helpful in better elucidating questions regarding efficacy, sequencing, dose, and fractionation. These are summarized in *Table 2*.

In the locally advanced definitive setting, a Phase II University of Virginia study evaluates the addition of pembrolizumab to standard of care chemoradiation (NCT02635360). Pembrolizumab is to be administered in three-week cycles concurrently with definitive chemoradiation and for three months in the adjuvant setting. Similarly, a larger phase III trial (MK-3475-A18/KEYNOTE-A18/ENGOT-cx11) is investigating definitive CRT with or without pembrolizumab per the same protocol as above except for permitting up to 20 cycles,

Table 2 Summary of current national and international trials examining combined radiation and immunotherapy for gynecologic malignancies

NCT Identifier	Phase	Target accrual	Study title	Disease site	Setting	Treatment arms	Radiation therapy	Immunotherapy sequencing	Primary endpoints	Secondary endpoints
NCT01711515*	I	34	GOG 9929: A Phase I Trial of Sequential Ipilimumab After Chemoradiation for the Primary Treatment of Patients With Locally Advanced Cervical Cancer Stages IB2/IIA With Positive Para-Aortic Lymph Nodes Only and Stage IIB/IIIB/IVA With Positive Lymph Nodes	Cervical	Advanced	ChemoRT with cisplatin and brachytherapy, then ipilimumab q3w	3D EFRT with SIB to gross nodes, weekly cisplatin, and brachytherapy	Adjuvant	Toxicity	ORR, OS, PFS, location of recurrence, chronic toxicity
NCT02635360	II	88	A Randomized Phase II Study of Chemoradiation and Pembrolizumab for Locally Advanced Cancer	Cervical	Advanced	Arm 1: ChemoRT then pembrolizumab; Arm 2: concurrent chemoRT with pembrolizumab	EBRT with weekly cisplatin and brachytherapy	Arm 1: Adjuvant; Arm 2: Concurrent	Change in immunologic markers, toxicity	Metabolic response rate, Incidence of distant metastases, OS, PFS
NCT03192059	II	43	PRIMMO: A Phase II Investigation of Pembrolizumab (Keytruda) in Combination With Radiation and an Immune Modulatory Cocktail in Patients With Cervical and Uterine Cancer	Cervical, Uterine	Advanced or refractory	Pembrolizumab, Lansoprazole, Curcumin, Vitamin D, Aspirin, Cyclophosphamide, every 3 weeks with RT	8 Gy ×3 q48h	Concurrent and adjuvant	ORR	Best OR, OS, PFS, Toxicity, QoL
NCT03277482	I	32	A Phase 1 Study of Durvalumab, Tremelimumab and Radiotherapy in Recurrent Gynecologic Cancer	Cervical, Ovarian, Uterine, Vulva	Recurrent or metastatic	Safety Lead-in: Durvalumab q4w for max of 13 doses + RT. Dose Evaluation: durvalumab q4w for max of 13 doses, tremelimumab q4w for max of 4 doses + RT	5 Gy ×5 or 8 Gy ×1	Concurrent and adjuvant	MTD	ORR, LRR, LCR, Abscopal response rate, response duration, OS, PFS
NCT03298893*	I	21	NiCOL: A Phase-I Study of Nivolumab in Association With Radiotherapy and Cisplatin in Locally Advanced Cervical Cancers Followed by Adjuvant Nivolumab for up to 6 Months	Cervical	Advanced	ChemoRT with nivolumab, then nivolumab	IMRT with SIB to gross nodes, weekly cisplatin, and brachytherapy	Adjuvant	Toxicity	Incidence of AEs and serious AEs, ORR, PFS, DFS, tumor microenvironment description, tumor PD-L, ctDNA heterogeneity, validation of molecular alterations
NCT03312114~	II	5	Phase II Trial of Concurrent Anti-PD-L1 and SAbR for Patients With Persistent or Recurrent Epithelial Ovarian, Primary Peritoneal or Fallopian Tube Cancer (With Safety lead-in)	Ovarian	Persistent, recurrent, or metastatic	Avelumab with SABR	SABR	Concurrent	ORR	OS, CRR, TTP
NCT03452332	I	18	Phase I Multi-Center Study of Hypofractionated Radiotherapy in Combination With Durvalumab and Tremelimumab in Patients With Recurrent/Metastatic Advanced Cervical, Vaginal, or Vulvar Cancer	Cervical, Vulvar	Recurrent or metastatic	Tremelimumab and durvalumab q4w, SABR on days 8, 10, 12 of cycle 1	8 Gy ×3	Concurrent and adjuvant	Toxicity	ORR, PFS, OS, TTNT
NCT03527264	II	24	BrUOG 355: A Pilot Feasibility Study Incorporating Nivolumab to Tailored Radiation Therapy With Concomitant Cisplatin in the Treatment of Patients With Cervical Cancer	Cervical	Advanced	Cohort 1A: WP chemoRT with concurrent nivolumab; Cohort 1B: EF chemoRT with concurrent nivolumab; Cohort 2: chemoRT then nivolumab; Cohort 3: chemoRT with nivolumab, then nivolumab	WPRT or EFRT 45 Gy in 25 fractions with weekly cisplatin and brachytherapy	Cohort 1A and 1B: Concurrent; Cohort 2 and 3: Adjuvant	toxicity, PFS	None
NCT03612791	II	190	ATEZOLACC: Randomized Phase II Trial Assessing the Inhibitor of Programmed Cell Death Ligand 1 (PD-L1) Immune Checkpoint Atezolizumab in Locally Advanced Cervical Cancer	Cervical	Advanced	Arm 1: chemoRT Arm 2: chemoRT with atezolizumab, then atezolizumab	WPRT or EFRT using IMRT 45 Gy in 25 fractions (SIB to gross nodes) with weekly cisplatin and brachytherapy	Concurrent and adjuvant	PFS	None
NCT03614949	II	26	Phase II Study of Stereotactic Body Radiation Therapy and Atezolizumab in the Management of Recurrent, Persistent, or Metastatic Cervical Cancer	Cervical	Recurrent, refractory, or metastatic	SBRT, then atezolizumab q3w	8 Gy ×3 to ≥2 sites	Adjuvant	ORR	OS, PFS
NCT03738228*	I	40	NRG-GY017: Anti PD-L1 (Atezolizumab) as an Immune Primer and Concurrently With Extended Field Chemoradiotherapy for Node Positive Locally Advanced Cervical Cancer	Cervical	Advanced	Arm 1: atezolizumab day -21 then chemoRT with atezolizumab; Arm 2: chemoRT with atezolizumab	EFRT using IMRT 45 Gy in 25 fractions (SIB to gross nodes) with weekly cisplatin and brachytherapy	Arm 1: Neoadjuvant; Arm 2: Concurrent	T-cell receptor beta expansion	toxicity, T-cell receptor clonality, diversity, and frequency, PD-L1 expression
NCT03830866	III	714	CALLA: A Phase III, Randomized, Multi-Center, Double-Blind, Global Study to Determine the Efficacy and Safety of Durvalumab in Combination With and Following Chemoradiotherapy Compared to Chemoradiotherapy Alone for Treatment in Women With Locally Advanced Cervical Cancer	Cervical	Advanced	Arm 1: chemoRT with durvalumab, then durvalumab; Arm 2: chemoRT with placebo, then placebo	EBRT 45 Gy in 25 fractions (boost to gross nodes) with weekly cisplatin or carboplatin and brachytherapy	Arm 1: Concurrent and adjuvant; Arm 2: None	PFS	OS, CRR, ORR, DoR, Health-related QoL, PFS, PFS and OS in PD-L1 positive patients

Table 2 (*continued*)

Table 2 (*continued*)

NCT Identifier	Phase	Target accrual	Study title	Disease site	Setting	Treatment arms	Radiation therapy	Immunotherapy sequencing	Primary endpoints	Secondary endpoints
NCT03833479	II	132	ATOMICC: A Randomized, Open Label, Phase II Trial of Anti-PD1, TSR-042, as Maintenance Therapy for Patients With High-risk Locally Advanced Cervical Cancer After Chemo-radiation	Cervical	Advanced	Arm 1: no further treatment; Arm 2: TSR-042 q6w for up to 24 months	Curative intent chemoRT with >4 doses weekly cisplatin prior to enrollment	Arm 1: None; Arm 2: Adjuvant	PFS	AEs, OS, Health-related QoL
NCT03932409	I	20	FIERCE: A Phase Ib Trial of Vaginal Cuff Brachytherapy + Pembrolizumab (MK3475) Followed by 3 Cycles of Dose Dense Paclitaxel/q 21 Day Carboplatin + Pembrolizumab (MK3475) in High Intermediate Risk Endometrial Cancer	Endometrial	High-intermediate risk	Pembrolizumab at -7 days then vaginal cuff brachytherapy then pembrolizumab/cisplatin q3w x3	Vaginal cuff brachytherapy	Neoadjuvant and adjuvant	Completion of 3 cycles of pembrolizumab	PFS, OS, AEs
NCT03955978	I	12	A Phase I Study of PD-1 Inhibition With TSR-042 in Addition to Standard of Care Definitive Radiation for Inoperable Endometrial Cancer	Endometrial	Early-stage inoperable	TSR042 on day -21 then q3w. 1 fraction brachytherapy 6 Gy every week	6 Gy ×6 Brachytherapy	Neoadjuvant, concurrent, and adjuvant	toxicity	PFS
NCT04221945	III	980	KEYNOTE-A18/ENGOT-cx11: A Randomized, Phase 3, Double-Blind Study of Chemoradiotherapy With or Without Pembrolizumab for the Treatment of High-risk, Locally Advanced Cervical Cancer	Cervical	Advanced	Arm 1: chemoRT with pembrolizumab, then pembrolizumab for 20 total cycles; Arm 2: chemoRT with placebo, then placebo for 20 total cycles	EBRT 45-50 Gy then 25–30 Gy brachytherapy. Total radiation treatment <56 days	Arm 1: Concurrent and adjuvant; Arm 2: None	PFS, OS	2 yr PFS, 3 yr OS, CRR, ORR, PFS, OS, QoL, AEs
NCT04430699	II	24	A Phase 2 Study of Combined Chemo-immunotherapy With Cisplatin-pembrolizumab and Radiation for Unresectable Vulvar Squamous Cell Carcinoma	Vulvar	Unresectable, incompletely resected, recurrent, or metastatic	Cisplatin q1w + pembrolizumab q3w + daily radiation, pembrolizumab to be continued after radiation until 12 cycles given	SOC up to 8 weeks	Concurrent and adjuvant	ORR	RFS

*Trial Accrued, ~Closed due to poor accrual; AE, adverse event; CRR, clinical response rate; DoR, duration of response; LCR, local control rate; LRR, local response rate; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; QoL, quality of life; TTP, time to progression; TTNT, time to next treatment.

to evaluate the safety and efficacy of combine CRT and pembrolizumab compared to placebo (NCT04221945). A similar study is also underway as part of the CALLA study, a Phase III study using durvalumab plus CRT for locally-advanced cervical cancer (NCT03830866). In this study, the experimental arm consists of durvalumab given in four-week cycles, up to 24 cycles, given concurrently and then adjuvantly with definitive chemoradiation. Similar studies using nivolumab (NCT03298893, NCT03527264), atezolizumab (NCT03612791, NCT03738228), and TSR-042 (NCT03833479) in combination with radiation are also currently underway. Of note, the timing of anti-PD-L1 therapy is being investigated on NRG GY017 with a randomization to concurrent vs neoadjuvant and concurrent CRT + atezolizumab.

In the metastatic setting, Moffit Cancer center is investigating combining SBRT followed by atezolizumab (NCT03614949). In this trial, SBRT is administered to at least 2 sites to a total dose of 24 Gy in 3 fractions and is then followed by atezolizumab given every three weeks. There are other trials not limited to cervical cancer that will be discussed below.

Endometrial cancer

Endometrial cancer is the most common gynecologic malignancy in the United States, affecting an estimated 65,620 women per year and accounting for 12,590 deaths annually (16). Endometrial cancer is typically diagnosed early, with approximately 75% of new diagnoses being Stage I/II, which can often be managed with surgery alone. However, there is a subset of these patients classified as high-intermediate risk (defined by PORTEC-2 as having at least two of the following: outer half myometrium invasion, Grade 3, or Age >60), who are traditionally treated with adjuvant vaginal cuff brachytherapy, and have a non-insignificant rate of 10-year pelvic recurrence and distant metastasis rate of 6% and 10% respectively. Furthermore, patients that are locally-advanced disease have suboptimal outcomes, despite treatment with chemotherapy, with 5-yr OS, FFS, and DMFS rates in PORTEC-3 being 81%, 77%, and 79% respectively (28). The addition of immunotherapy to these existing paradigms might optimally improve on these results.

In endometrial cancers, approximately 40% of tumors genetically express DNA polymerase epsilon (POLE) or microsatellite instability high (MSI-H) phenotypes. These tumor phenotypes are immunogenic and potentially amenable to immunotherapeutic treatment approaches (although patients whose tumors have POLE mutations already have excellent outcomes even with radiation alone) (29-32). In order to examine microsatellite instability, MLH1 methylation and mismatch repair (MMR) protein expression was examined in specimens from GOG 210. In this study, cases with epigenetic and probable mutations in MMR where associated with higher grade and lymphovascular space invasion (33). MMR deficient (dMMR) tumors were associated with a significantly worse PFS, with a HR of 1.37. Further exploratory analysis showed that tumors with probable MMR mutations had a four-fold advantage to adjuvant therapy compared to non-mutant MMR tumors, measured by response rate. These results were supported by another single-institution study, which found dMMR to be associated with advanced stage, tumor size, higher grade, presence of lymphovascular space invasion, and older age (34). Stage III/IV patients with dMMR had significantly decreased recurrence-free survival (47.4% *vs.* 3.4%) despite receiving similar adjuvant therapies. This relationship was further investigated in high-intermediate risk patients, with MMR defects being associated with worse recurrence rates (28% *vs.* 11%), with distant recurrences being particularly notable (14.1% *vs.* 3%) (35). This difference held even when excluding isolated vaginal recurrences (18.8% *vs.* 4.5%). 5-year RFS was 66% in dMMR tumors compared to 89% in non-mutant MMR tumors.

Immunotherapy for dMMR tumors was first investigated in patients with colon cancer. This led to accelerated FDA approval of pembrolizumab in May 2017 for patients with dMMR or microsatellite instability-high solid tumors who progressed on prior therapy, and provided an opportunity to treat patients with progressive endometrial cancer with that biomarker under such a paradigm (36,37). Beyond the potential targeting of microsatellite instability, the strong influence excess estrogen signaling has on endometrial cancer development, with associated activation of alternative signaling pathways such as MAPK, IGF-1, and cAMP, also provides a possible therapeutic target (38-40).

In 2019, combined pembrolizumab and lenvatinib was approved for patients with metastatic endometrial cancer with disease progression after receiving no more than two systemic therapies. This was based on data from KEYNOTE-146, where patients were treated with oral lenvatinib daily plus pembrolizumab every three weeks. 108 patients were enrolled, with an objective response rate of 38% with 11% complete and 28% partial response

rates (41). Again, while not trivial, these response rates are objectively low, and combining immunotherapy with radiation is aimed at improving these disease responses, and is the topic of multiple ongoing trials investigating treatment of endometrial cancer.

Current investigations

Ongoing trials combining radiation and immune checkpoint inhibitors are summarized in *Table 2*. The benefit of immunotherapy in the management of patients with high-intermediate risk endometrial cancers will be examined in the Phase I FIERCE trial from the University of Oklahoma (NCT03932409). This trial will investigate the use of neoadjuvant pembrolizumab given seven days prior to the initiation of definitive vaginal brachytherapy, followed by combined chemotherapy and pembrolizumab.

In the setting of medically inoperable Stage I/II endometrial cancer, anti PD-L1 in the form of TSR-042 is being investigated at Washington University (NCT03955978). In this trial, TSR-042, a monoclonal antibody against PD-L1, will be administered both neoadjuvantly every three weeks and concurrently with six fractions of definitive intracavitary brachytherapy for a total of four cycles.

In the advanced and refractory setting, the PRIMMO study (NCT03192059) will examine the use of an immunomodulatory cocktail of vitamin D, aspirin, lansoprazole, cyclophosphamide, and curcumin in combination with pembrolizumab and radiation. The pembrolizumab is given in three-week cycles starting on the first day of radiation, which is given every other day in 8 Gy fractions to a total dose of 24 Gy for patients with uterine and cervical malignancies.

Ovarian cancer

Ovarian cancer is the deadliest gynecologic malignancy in the United States, affecting an estimated 21,750 women per year in the United States and accounting for 13,940 deaths annually (16). Ovarian cancer is often diagnosed at late stages, with poor prognosis despite systemic therapy, and provides an opportunity for the advancement of immunotherapy to improve clinical outcomes. The immunogenicity of ovarian cancer is an important prognostic determinant, with CD8+ T-cell infiltration of tumors being associated with improved OS (2). Furthermore, associated PD-L1 expression inversely correlates with CD8+ T-cell infiltration and, correspondingly, survival (42). This therefore provides rationale for use of immunotherapy to decrease PD-L1 induced immune inhibition.

Single agent immune checkpoint blockade in unselected patients with recurrent ovarian cancer has low to modest efficacy, with response rates ranging from 6% to 15% (2). Use of single-agent pembrolizumab was investigated as part of a Phase 2 study, KEYNOTE-100. PD-L1 expression was determined using a combined positive score (CPS) that examined staining on the tumor and immune cells. When stratified by CPS, ORR was 9% in unselected patients, 14% in patients with a CPS score of 1 or higher, and 25% in patients with a CPS score of 10 or higher (43). Dual immune checkpoint inhibitors have also been investigated. Based on NRG-GY003, adding ipilimumab to nivolumab improved response rate from 12% to 31%, with PFS HR of 0.5 (44). The addition of radiation aims to augment those response rates.

Current investigations

Given that radiotherapy has a limited role in managing early-stage ovarian cancers, existing trials combining radiation and immunotherapy are largely focused on the metastatic setting, and are detailed in *Table 2*. One such trial from UT Southwestern, which unfortunately closed early due to poor accrual, was investigating combined stereotactic ablative radiotherapy (SABR) given concurrently with avelumab (NCT03312114). Ovarian cancer has also been included in multiple, site agnostic trials evaluating combined radiation and immunotherapy for metastatic disease; however, enrollment of these patients on trial has been limited.

Vulvar cancer

Vulvar cancer is the least common gynecologic malignancy in the United States, affecting an estimated 6,120 women per year in the United States and accounting for 1,350 deaths annually (16). Patients with locally advanced, metastatic, and recurrent disease have poor prognosis, with 5 year OS of 53%, 19%, and 15% respectively (45,46). Treatment for such patients is highly individualized but typically involves some combination of surgery, radiation, and platinum-based chemotherapy. Given the poor prognosis associated with metastatic vulvar cancer, there is significant interest in the development of new clinical

treatment paradigms to prevent disease progression, recurrence, or the development of metastases. As with other gynecologic malignancies, many cases arise out of chronic infection/irritation, immune anergy/dysfunction, and/or HPV infection, and there is optimism that adding immunotherapies to current treatment regimens will improve on historic outcomes.

In vulvar cancer, membranous PD-L1 expression is present in about a quarter of vulvar squamous cell carcinoma, and is associated with HPV negativity (47). The increasing degree of PD-L1 was found to be correlated with poor outcomes. It has also been shown to be predictive of tumor stage (48). Additionally, tumors with more activated T-cells, largely present in HPV associated tumors and absent in p53-mutant tumors, are associated with superior survival (49). In combination, these findings provide rationale for a potential benefit for treatment with cell checkpoint inhibitors.

There are limited studies examining immunotherapy for the management of vulvar cancer. One study, the Dual Anti-CTLA-4 and Anti-PD-1 Blockade in Rare Tumors (DART) trial, does include vulvar cancer as an eligible cancer subtype, and is a Phase II trial investigating combined ipilimumab and nivolumab (NCT02834013). Additionally, there is evidence for off-label use of cemiplimab, which had an approximately 50% response rate among patients with advanced and metastatic cutaneous squamous cell carcinoma (50).

Current investigations

The incidence of vulvar malignances makes accrual for radioimmunotherapy trials difficult, but there are ongoing trials, as listed in *Table 2*. A newly opened Phase II trial based out of Massachusetts General Hospital (NCT04430699) seeks to evaluate combined radiation, cisplatin, and pembrolizumab for unresectable or metastatic vulvar squamous cell carcinoma. In this trial, standard-of-care radiation will be delivered with concurrent pembrolizumab every three weeks and weekly cisplatin. Patients will also receive adjuvant pembrolizumab every three weeks for up to 12 cycles. A separate, broader trial from MD Anderson is investigating tremelimumab and durvalumab combined with SBRT for recurrent or metastatic advanced cervical, vaginal, or vulvar cancer (NCT03452332). In this trial, tremelimumab and durvalumab are given every four weeks, with tremelimumab given up to four cycles and durvalumab up to eight cycles. SBRT is given on days 8, 10, and 12 of cycle 1, to a total dose of 24 Gy in 3 fractions.

General gynecologic malignancies

Current investigations

Beyond current disease-site specific trials, there are also currently trials investigating the role of combined radiation and immunotherapy in gynecologic malignancies as a whole, found in *Table 2*. One such trial out of Dana Farber Cancer Institute is investigating the use of durvalumab with or without tremelimumab concurrent with 25 Gy EBRT in 5 fractions for recurrent, advanced, and metastatic gynecologic malignancies for which standard curative or palliative measures do not exist or are no longer effective (NCT03277482).

Toxicity

Although there is considerable interest in combination radiation and immunotherapy for gynecologic malignancies to improve clinical outcomes, combined therapy often comes at the cost of increased morbidity and toxicity. Immunotherapy alone has a diverse side effect profile including but not limited to uveitis, hypophysitis, dry mouth, hypothyroidism, pneumonitis, enterocolitis, hepatitis, pancreatitis, auto-immune diabetes, adrenal insufficiency, dermatitis, vitiligo, and arthralgias, although rates of grade 3–5 toxicity are less than 20% (51). There is therefore concern for multimodality combined radiotherapy and immunotherapy having synergistic toxicity profile, although currently available clinical data does not show increased risk of adverse events (27).

In the treatment of gynecologic malignancies, the close anatomic proximity of radiation targets to organs at risk leads to concern for synergistic gastrointestinal toxicity. In a Phase 1 trial using CRT extended-field pelvis without intensity modulation and ipilimumab for node positive cervical cancer in 21 women, there was Grade 1 and 2 diarrhea, but only one case of Grade 3 abdominal pain, nausea, and vomiting as well as an incidence of asymptomatic lipase elevation (7). This toxicity could be further mitigated using either a traditional pelvic field or intensity modulated radiation therapy (IMRT) and image guidance. As data from ongoing clinical trials become available, the toxicity profile of combined radiotherapy and immunotherapy will be better understood and inform future treatment dosing, sequencing, and overall management. In

the meantime, as treatment of gynecologic malignancies with multimodal immunotherapy combinations increase, patients should be closely monitored for toxicity.

Conclusions

Combined radiation and immunotherapy is an active area of investigation in modern oncology, and is the topic of multiple ongoing clinical trials for gynecologic malignancies. As outlined above, patients—particularly those with high-risk, locally advanced, recurrent, and metastatic gynecologic malignancies—have the most to potentially gain from combined modality therapy. Newer radioimmunotherapy approaches have the potential to realize meaningful clinical improvements in disease control and patient survival through augmented anti-tumor immune response via increased antigen presentation, phagocytosis, cell death, and immune-mediated tumor surveillance. Although current data is limited, such treatment shows promise as a safe and effective treatment paradigm. The optimal radiation dose, timing, sequencing, and immunotherapy drug(s) and treatment sequencing continue to evolve to best optimize outcomes and associated toxicities.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors (Dr. Arya Amini and Dr. Tyler Robin) for the series "Synergy in Action: Novel Approaches to Combining Radiation Therapy and Immunotherapy" published in *Translational Cancer Research*. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-3019). The series "Synergy in Action: Novel Approaches to Combining Radiation Therapy and Immunotherapy" was commissioned by the editorial office without any funding or sponsorship. Dr. Glaser reports that he is Co-chair of Elsevier clinical gynecological pathways. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Kalbasi A, June CH, Haas N, et al. Radiation and immunotherapy: A synergistic combination. J Clin Invest 2013;123:2756-63.
2. Lee L, Matulonis U. Immunotherapy and radiation combinatorial trials in gynecologic cancer: A potential synergy? Gynecol Oncol 2019;154:236-45.
3. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627-39.
4. Plimack ER, Bellmunt J, Gupta S, et al. Safety and activity of pembrolizumab in patients with locally advanced or metastatic urothelial cancer (KEYNOTE-012): a non-randomised, open-label, phase 1b study. Lancet Oncol 2017;18:212-20.
5. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803-13.
6. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. Lancet Oncol 2015;16:375-84.
7. Mayadev JS, Enserro D, Lin YG, et al. Sequential Ipilimumab after Chemoradiotherapy in Curative-Intent Treatment of Patients with Node-Positive Cervical Cancer. JAMA Oncol 2020;6:92-9.
8. Hettich M, Lahoti J, Prasad S, et al. Checkpoint antibodies but not T cell-recruiting diabodies effectively synergize with TIL-inducing γ-irradiation. Cancer Res 2016;76:4673-83.
9. Frey B, Rückert M, Weber J, et al. Hypofractionated irradiation has immune stimulatory potential and induces a timely restricted infiltration of immune cells in colon

cancer tumors. Front Immunol 2017;8:231.
10. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. Cancer Res 2014;74:5458-68.
11. Young KH, Baird JR, Savage T, et al. Optimizing timing of immunotherapy improves control of tumors by hypofractionated radiation therapy. PLoS One 2016;11:e0157164.
12. Lugade AA, Moran JP, Gerber SA, et al. 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.
13. Schaue D, Ratikan JA, Iwamoto KS, et al. Maximizing tumor immunity with fractionated radiation. Int J Radiat Oncol Biol Phys 2012;83:1306-10.
14. Huang AC, Postow MA, Orlowski RJ, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature 2017;545:60-5.
15. Buchwald ZS, Wynne J, Nasti TH, et al. Radiation, immune checkpoint blockade and the abscopal effect: A critical review on timing, dose and fractionation. Front Oncol 2018;8:612.
16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
17. Morris M, Eifel PJ, Lu J, et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med 1999;340:1137-43.
18. Eifel PJ, Winter K, Morris M, et al. Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: An update of Radiation Therapy Oncology Group Trial (RTOG) 90-01. J Clin Oncol 2004;22:872-80.
19. Rose PG, Bundy BN, Watkins EB, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med 1999;340:1144-53.
20. Pearcey R, Brundage M, Drouin P, et al. Phase III Trial Comparing Radical Radiotherapy With and Without Cisplatin Chemotherapy in Patients With Advanced Squamous Cell Cancer of the Cervix. J Clin Oncol 2002;20:966-72.
21. Keys HM, Bundy BN, Stehman FB, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med 1999;340:1154-61.
22. Dueñas-González A, Zarbá JJ, Patel F, et al. Phase III, open-label, randomized study comparing concurrent gemcitabine plus cisplatin and radiation followed by adjuvant gemcitabine and cisplatin versus concurrent cisplatin and radiation in patients with stage IIB to IVA carcinoma of the cervix. J Clin Oncol 2011;29:1678-85.
23. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res 2002;89:213-28.
24. Meng Y, Liang H, Hu J, et al. PD-L1 expression correlates with tumor infiltrating lymphocytes and response to neoadjuvant chemotherapy in cervical cancer. J Cancer 2018;9:2938-45.
25. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.
26. Chung HC, Ros W, Delord JP, et al. Efficacy and safety of pembrolizumab in previously treated advanced cervical cancer: Results from the phase II KEYNOTE-158 study. J Clin Oncol 2019;37:1470-8.
27. Dyer BA, Feng CH, Eskander R, et al. Current Status of Clinical Trials for Cervical and Uterine Cancer Using Immunotherapy Combined with Radiation. Int J Radiat Oncol Biol Phys 2021;109:396-412.
28. de Boer SM, Powell ME, Mileshkin L, et al. Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial. Lancet Oncol 2019;20:1273-85.
29. Dudley JC, Lin MT, Le DT, et al. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res 2016;22:813-20.
30. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67-73.
31. Naboush A, Roman CAJ, Shapira I. Immune checkpoint inhibitors in malignancies with mismatch repair deficiency: A review of the state of the current knowledge. J Investig Med 2017;65:754-8.
32. León-Castillo A, de Boer SM, Powell ME, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. J Clin Oncol. J Clin Oncol 2020;38:3388-97.
33. McMeekin DS, Tritchler DL, Cohn DE, et al. Clinicopathologic significance of mismatch repair defects in endometrial cancer: An NRG oncology/gynecologic oncology group study. J Clin Oncol 2016;34:3062-8.
34. Cosgrove CM, Cohn DE, Hampel H, et al. Epigenetic

silencing of MLH1 in endometrial cancers is associated with larger tumor volume, increased rate of lymph node positivity and reduced recurrence-free survival. Gynecol Oncol 2017;146:588-95.
35. Backes FJ, Haag J, Cosgrove CM, et al. Mismatch repair deficiency identifies patients with high-intermediate–risk (HIR) endometrioid endometrial cancer at the highest risk of recurrence: A prognostic biomarker. Cancer 2019;125:398-405.
36. 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.
37. 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.
38. Lösel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol 2003;4:46-56.
39. Kahlert S, Nuedling S, Van Eickels M, et al. Estrogen receptor a rapidly activates the IGF-1 receptor pathway. J Biol Chem 2000;275:18447-53.
40. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature 1977;265:69-72.
41. Makker V, Taylor MH, Aghajanian C, et al. Lenvatinib Plus Pembrolizumab in Patients With Advanced Endometrial Cancer. J Clin Oncol 2020;38:2981-92.
42. Hamanishi J, Mandai M, Iwasaki M, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A 2007;104:3360-5.
43. Matulonis UA, Shapira-Frommer R, Santin A, et al. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: Interim results from the phase 2 KEYNOTE-100 study. J Clin Oncol 2018;36:5511.
44. Zamarin D, Burger RA, Sill MW, et al. Randomized phase II trial of nivolumab versus nivolumab and ipilimumab for recurrent or persistent ovarian cancer: an NRG oncology study. J Clin Oncol 2020;38:1814-23.
45. Francis JA, Eiriksson L, Dean E, et al. No. 370-Management of Squamous Cell Cancer of the Vulva. J Obstet Gynaecol Can 2019;41:89-101.
46. Vulvar Cancer — Cancer Stat Facts. Available online: https://seer.cancer.gov/statfacts/html/vulva.html
47. Hecking T, Thiesler T, Schiller C, et al. Tumoral PD-L1 expression defines a subgroup of poor-prognosis vulvar carcinomas with non-viral etiology. Oncotarget 2017;8:92890-903.
48. Thangarajah F, Morgenstern B, Pahmeyer C, et al. Clinical impact of PD-L1 and PD-1 expression in squamous cell cancer of the vulva. J Cancer Res Clin Oncol 2019;145:1651-60.
49. Kortekaas KE, Santegoets SJ, Abdulrahman Z, et al. High numbers of activated helper T cells are associated with better clinical outcome in early stage vulvar cancer, irrespective of HPV or p53 status. J Immunother Cancer 2019;7:236.
50. Migden MR, Rischin D, Schmults CD, et al. PD-1 blockade with cemiplimab in advanced cutaneous squamous-cell carcinoma. N Engl J Med 2018;379:341-51.
51. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: A comprehensive review. Eur J Cancer 2016;54:139-48.

Cite this article as: Ladbury C, Germino E, Novak J, Liu J, Horne Z, Dyer B, Glaser S. Combination radiation and immunotherapy in gynecologic malignancies—a comprehensive review. Transl Cancer Res 2021;10(5):2609-2619. doi: 10.21037/tcr-20-3019

