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Combining novel agents with radiotherapy for gynecologic malignancies: beyond the era of cisplatin

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ABSTRACT

Therapeutic strategies combining radiation therapy with novel agents have become an area of intense research focus in oncology and are actively being investigated for a wide range of solid tumors. The mechanism of action of these systemic agents can be stratified into three general categories: (1) enhancement or alteration of the immune system; (2) disruption of DNA damage response mechanisms; and (3) impediment of cellular signaling pathways involving growth, angiogenesis, and hypoxia. Pre-clinical data suggest that radiation therapy has immunogenic qualities and may optimize response to immuno-oncology therapies by priming the immune system, whereas other novel systemic agents can enhance radiosensitivity through augmentation of genomic instability and alteration of central signaling pathways related to growth and survival. Gynecologic cancers in particular have the potential for synergistic response to combination approaches incorporating radiation therapy and novel systemic therapies. Several clinical trials have been proposed to elucidate the efficacy and safety of such approaches. Here we discuss the mechanisms of novel therapies and the rationale for these combination strategies, reviewing the relevant pre-clinical and clinical data. We explore their optimal use with respect to indications, interactions, and potential synergy in combination with radiation therapy and review ongoing trials and active areas of investigation.

INTRODUCTION

Radiation therapy is essential for the definitive treatment of locally advanced or unresectable gynecologic malignancies, such as cervical, vaginal, vulvar, and uterine cancer. Radiation therapy with concurrent cisplatin is the standard of care for locally advanced cervical cancer after multiple clinical trials testing radiation therapy versus radiation therapy and concurrent platinum chemotherapy demonstrated an overall survival benefit.[1] The use of concurrent cisplatin has been extrapolated to other gynecologic malignancies when radiotherapy is delivered to improve local control and survival. While concurrent chemoradiotherapy has led to improved clinical outcomes, it is not without associated normal tissue toxicity. Toxicity concerns, in particular duodenal toxicities,[2] are worsened when extended-field irradiation is necessary due to common iliac and para-aortic nodal metastases, as is common in many gynecologic cancers. As a result, there is significant interest in investigating novel therapies.

The past two decades have witnessed an expansion in systemic therapy options for the concurrent and sequential management of other solid malignancies; by targeting specific cellular pathways involved in malignant transformation and progression, many of these novel therapeutic agents also have the potential to improve outcomes in gynecologic malignancies. Due to their mechanisms of action, their toxicity profile may be independent of radiotherapy, and as a result there is significant interest in testing these agents in clinical trials. Mechanistically, these agents can be considered in three general categories: (1) enhancement or alteration of the immune system; (2) disruption of DNA damage response mechanisms; and (3) impediment of cellular pathways involved in processes such as signaling, growth, and angiogenesis. Herein, we explore the mechanisms underlying several of these novel strategies that are currently being tested in clinical trials in gynecologic cancers, as well as their optimal use with respect to indications, interactions, and potential synergy in combination with radiation therapy.

IMMUNOTHERAPY

Checkpoint Blockade

The development of anti-tumor immunity is a complex process involving: (1) the exposure of dendritic cells and other antigen-presenting cells to tumor antigens (including products of oncogenic mutations or overexpressed non-mutated proteins); (2) stimulation and maturation of antigen-presenting cells; (3) presentation of antigens to cytotoxic T-cells, followed by their activation; (4) migration of activated T-cells to tumor sites; and finally (5) cytotoxic targeted killing of malignant cells.[3] Therapies developed to focus on this process are aimed at either enhancing the immune system's natural efficacy or impeding a tumor's intrinsic ability to evade these steps; however, the bulk of modern immunotherapy relies more on the latter—specifically, the molecular blockade of targeted immune checkpoints such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death

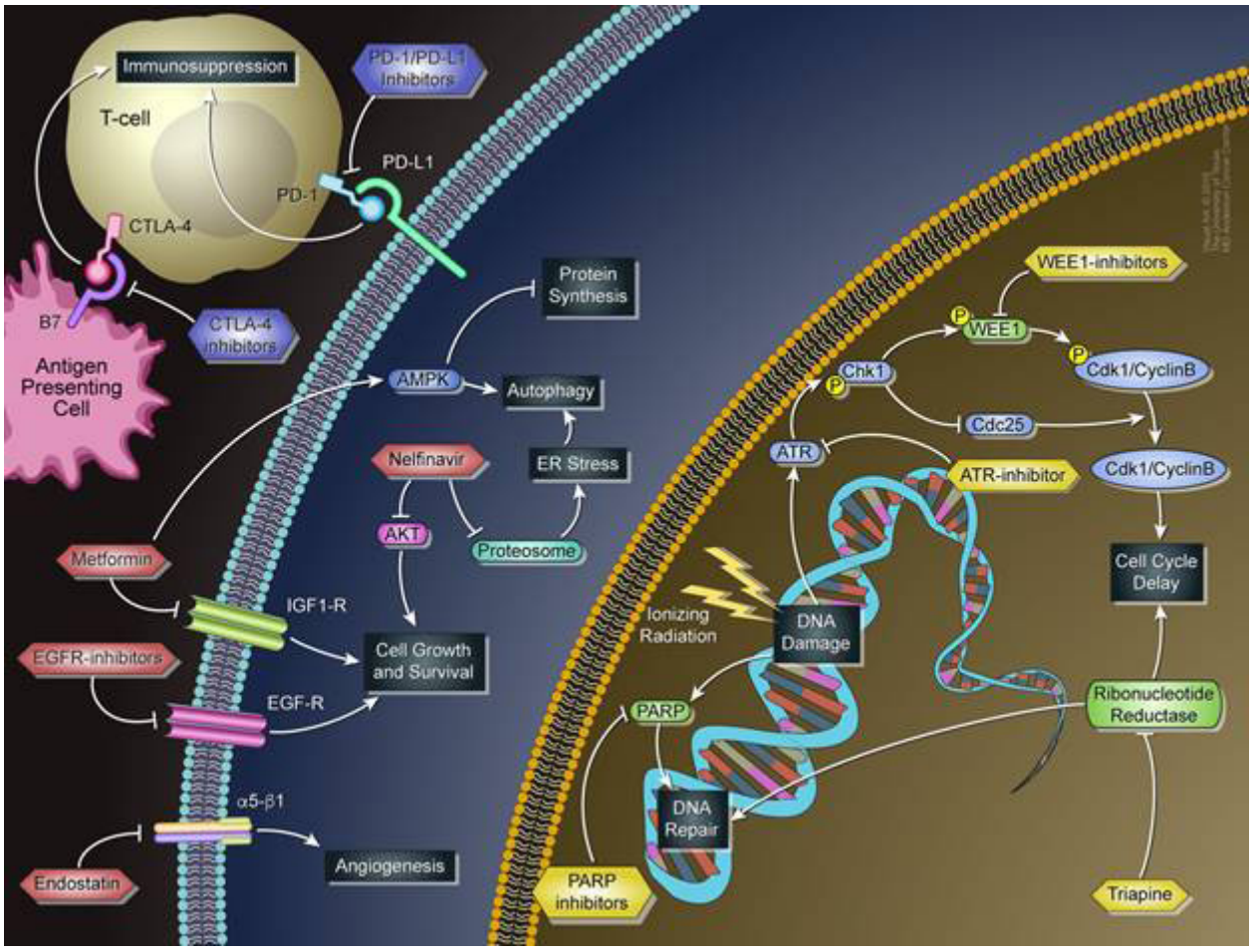

Figure 1 Current combinatorial radiation therapy strategies in gynecologic oncology. AMPK, 5' adenosine-monophosphate-activated protein kinase; ATR, ataxia telangiectasia and Rad3-related; Chk1, checkpoint kinase 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; IGF1, insulin-like growth factor 1; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

protein 1 (PD-1), and programmed death-ligand 1 (PD-L1).[4] CTLA-4 is a receptor molecule found on T-cells, most predominantly on regulatory T-cells, which when bound to its corresponding ligand functions to send inhibitory signals to the T-cells, particularly at the early induction phase after antigen stimulation.[3 4] Similarly, PD-1 is a receptor found on T-cells that when bound to its ligand (PD-L1) impedes T-cell activation; however, unlike CTLA-4, most of its effects impact cytotoxic CD8 T-cells in the later effector phase of immune activity.[3 5] Although these molecular blockades have critical roles in normal immune tolerance and inhibition of autoimmune pathology,[6] malignant cells exploit these mechanisms through increased expression of immune blockade to functionally downregulate T-cell activation and differentiation (Figure 1).[5]

CTLA-4 Inhibitors

While the US Food and Drug Administration (FDA)-approved indications for CTLA-4 inhibitors are currently limited to advanced, metastatic, or refractory melanoma, renal cell carcinoma, and colorectal carcinoma, this family of immunotherapeutic agents currently is being tested in clinical trials for multiple gynecologic cancers.[4 5] Ipilimumab, the first CTLA-4 inhibitor approved for clinical use in 2011, serves as one of the earliest examples of CTLA-4 inhibitors in conjunction with radiation therapy. A 2012 case report of a patient with metastatic melanoma on ipilimumab, who received palliative radiotherapy for slowly progressive disease, noted tumor regression at 4 months after palliative radiotherapy both within and outside the radiation treatment field.[7] This report contributes to a small body of evidence for the hypothesized "abscopal response", defined as tumor regression outside a localized radiation therapy field thought to be initiated by a radiation-triggered immune response; such case reports have been corroborated by evidence of changes in immune-cell population distribution and antibody titers after radiation therapy representing the expansion of activated T cells.[4 7]

The hypothesized efficacy of CTLA-4 inhibitors for gynecologic malignancies, such as cervical cancer, stems from the notion that human papillomavirus (HPV)-positive malignancies (eg, head and neck and cervical cancers) have well-characterized immune-escape mechanisms.[8 9] A recent phase I/II trial of ipilimumab monotherapy in 42 women with recurrent or metastatic cervical cancer demonstrated a median progression-free survival time of 2.5 months and a median overall survival time of 8.5 months, with four patients experiencing grade 3 diarrhea and no dose limiting toxicities observed in the run-in cohort.[10] Among the 34 patients assessed using Response Evaluation Criteria In Solid Tumors (RECIST), one patient had a partial response and 10 patients had

stable disease, thus not meeting the pre-specified response rate of 20% with biopsy samples showing no relationship between patient response and pre-treatment levels of PD-L1. GOG-9929, a phase I study of four cycles of ipilimumab after chemoradiotherapy for pelvic or para-aortic lymph node positive cervical cancer, found a 12 month overall survival and progression-free survival rate of 90% and 81%, respectively.[11] There were two self-limiting grade 3 toxicities (lipase increase and dermatitis).[11] Collectively, these studies indicate reasonable tolerability; however, given the limited response rates observed in the metastatic/recurrent setting, ipilimumab as monotherapy has a limited role. On the other hand, CTLA-4 inhibitors combined with PD-L1 or PD-1 inhibitors have shown promise, as discussed further in the section 'Combined Checkpoint Inhibitors' below.

PD-1/PD-L1 Inhibitors

A separate class of immunotherapy agents includes drugs that inhibit the PD-1/PD-L1 cell surface signaling pathway. Nivolumab was the first PD-1 inhibitor to be approved for clinical use in unresectable or metastatic melanoma and metastatic squamous non-small cell lung cancer. In CheckMate 358, a phase I/II study of nivolumab in HPV-associated gynecologic cancers including cervical, vulvar, and vaginal cancers, nivolumab was found to have an overall response rate of 21% (26% for those with cervical cancer) and a median progression-free survival time of 5.5 months, with only four (17%) of 24 patients experiencing a grade 3–4 toxicity .[12] A Japanese phase II trial found similarly positive results with nivolumab monotherapy for patients with platinum-resistant ovarian cancer, with a disease control rate of 45%, a median progression-free survival interval of 3.5 months, and a median overall survival time of 20 months; however, eight (40%) of 20 patients experienced grade 3–4 treatment related toxicity, and the most common toxicity was lymphocytopenia.[13]

Analogous to ipilimumab, several case reports indicate potential synergy between nivolumab and radiation therapy,[14 15] with abscopal responses noted in patients with metastatic endometrial cancer[14] and progressive neuroendocrine cervical cancer.[15] On the basis of these promising findings, several studies are ongoing to evaluate nivolumab's potential synergy with concurrent radiation therapy in locally advanced cervical cancer (International Federation of Gynecology and Obstetrics (FIGO) stage IB–IVA) (NCT03527264, NCT03298893; Table 1). Another PD-1 inhibitor, pembrolizumab, has garnered similar interest via case reports of patients with metastatic renal cell carcinoma[16] or brain metastases.[17] These case reports were more formally evaluated in a phase I trial of multisite stereotactic ablative radiation therapy (SABR) with concurrent pembrolizumab in patients with metastatic solid tumors, demonstrating an overall objective response rate of 13%; a median progression-free survival time of 3.1 months; and a median overall survival time of 9.6 months, with six (7.6%) of 79 patients experiencing a grade 3 treatment-related toxicity including pneumonitis, colitis, and hepatic toxicity.[18] SABR, also referred to as stereotactic body radiotherapy, precisely delivers a high dose of radiotherapy in limited fractions (1–5 fractions) to the tumor while limiting the dose to surrounding normal tissues. A separate phase I study assessing pembrolizumab with a hypofractionated radiation therapy regimen for patients with solid metastatic cancer found that two (8.3%) of 24 patients had an increased immune response after anti-PD-1 therapy, leading to tumor regression in non-irradiated sites, a positive abscopal effect, and no dose-limiting toxicities observed.[19]

Pembrolizumab has also garnered interest for use in gynecologic malignancies. The KEYNOTE trials have shown pembrolizumab's efficacy for tumors at a variety of sites, including gynecologic cancers in the KEYNOTE-028,[20] KEYNOTE-158,[21] and KEYNOTE-100 trials.[22] KEYNOTE-028, a phase Ib trial of pembrolizumab for patients with advanced cervical cancer, showed a median progression-free survival time of 2 months and an overall survival time of 9 months; five (28%) of 18 patients experienced grade 3 toxicity, the most common being rash.[20] These results led to expansion of this trial into the phase II trial KEYNOTE-158, which confirmed these initial promising endpoints in 98 patients with advanced, progressive cervical cancer (median progression-free survival of 2.1 months and overall survival of 9.4 months).[21] The phase II trial KEYNOTE-100 for advanced, recurrent ovarian cancer showed an overall progression-free survival time advantage of 2.1 months and a median overall survival time of 17.6 months in patients with disease refractory to 4–6 previous lines of treatment, simultaneously providing a platinum-free or treatment-free interval of ≥3 months for these heavily pre-treated patients; of note, 19.7% of patients experienced a grade 3 or higher toxicity with fatigue being the most common.[22] Additional trials continue to evaluate combinations of pembrolizumab with radiation therapy for women with gynecologic malignancies including cervical and uterine cancers (NCT02635360, NCT03192059, NCT03932409; Table 1), to determine whether adding radiation therapy to checkpoint inhibition can improve response rates. Other drugs in this class are also being explored in the same context, including the PD-1 inhibitor TSR-042 (NCT03955978) and the PD-L1 inhibitors durvalumab (NCT03830866), atezolizumab (NCT03738228, NCT03614949), and avelumab (NCT03312114). Further details are summarized in Table 1.

Combined Checkpoint Inhibitors

One of the limitations of PD-1/PD-L1 blockade has been the difficulty of mounting a protective immune response against poorly immunogenic or "cold" tumors. The primary proposed mechanism of resistance to immune checkpoint inhibitors used as monotherapy involves the compensatory upregulation of alternative immune checkpoint pathway molecules. Thus, combination strategies have been proposed and evaluated as a solution to circumvent this resistance mechanism.[23] For example, although CTLA-4 and PD-1/PD-L1 inhibitors are both immune checkpoint agents, each act through non-redundant mechanisms and thus may complement one another (Figure 1).

Twyman-Saint Victor et al were the first to show proof-of-concept for dual checkpoint blockade combined with radiation therapy.[24] They reported finding that although tumor regression was observed in patients with metastatic melanoma and in mouse models treated with anti-CTLA-4 and radiation therapy, a high degree of resistance remained that was thought to be attributable to upregulation of PD-L1 and T-cell exhaustion. These investigators found a synergistic relationship between dual checkpoint inhibition and radiation therapy, whereby anti-CTLA-4 inhibited T-regulatory cells, radiation therapy enhanced T-cell receptor diversity, and PD-L1 blocked T-cell exhaustion and encouraged further T-cell expansion.[24] Other studies have shown similar findings, including

Table 1 Clinical trials of radiotherapy and immunotherapy

Identifier	Phase	N	Title	Disease	Interventions	Radiation details	Primary outcome	Secondary outcomes
Not yet recruiting								
NCT03932409	I	20	Frontline Immunotherapy Combined With Radiation and Chemotherapy in High Risk Endometrial Cancer (FIERCE)	High and intermediate-risk endometrial cancer	**Pembrolizumab** given 1 week before CRT (paclitaxel every 3 weeks for 3 cycles)	Vaginal cuff brachytherapy	Proportion of patients completing 3 cycles of pembrolizumab	PFS; OS; AE frequency
Recruiting								
NCT03738228	I	40	Atezolizumab Before and/or With Chemoradiotherapy in Immune System Activation in Patients With Node-Positive Stage IB2, II, IIIB, or IVA Cervical Cancer	Node-positive stage IB2, II, IIIB, or IVA cervical cancer	**Atezolizumab** q3w starting –21 days before standard cisplatin-based CRT; atezolizumab q3w starting at day 0 before standard cisplastin-based CRT	EBRT once daily for 25 fractions with image guided brachytherapy beginning in weeks 4, 5, or at the end of EBRT	T-cell receptor β clonal expansion in peripheral blood	Incidence of DLTs; frequency and severity of adverse events; T-cell receptor clonality, diversity and frequency; PD-L1 expression in tissue
NCT03955978	I	12	TSR-042 in Addition to Standard of Care Definitive Radiation for Inoperable Endometrial Cancer	Medically inoperable FIGO clinical stage I or II endometrial carcinoma	**TSR-042** given over 4 doses: first dose given 21 days before first brachytherapy fraction, second at time of first fraction, third dose at time of fourth fraction, final dose 1 week after sixth fraction	Brachytherapy 36 Gy in 6 fractions, given weekly	AE at 6 weeks	PFS
NCT03452332	I	18	Stereotactic Body Radiation Therapy, Tremelimumab and Durvalumab in Treating Participants With Recurrent or Metastatic Cervical, Vaginal, or Vulvar Cancers	Recurrent or metastatic cervical, vaginal, or vulvar cancers	**Tremelimumab + durvalumab** q4w×4 cycles, followed by durvalumab alone for up to 8 cycles in absence of progression or severe toxicity; SABR on days 8, 10, 12 of cycle 1	SABR with 3 fractions separated by 48 hours	AE	Response to treatment; PFS; OS; TTNT
NCT03277482	I	32	Durvalumab, Tremelimumab+Radiotherapy in Gynecologic Cancer	Metastatic or unresectable endometrial, ovarian (ovarian epithelial, fallopian tube, primary peritoneal), cervical, vaginal, or vulvar cancer	**Tremelimumab** and **durvulumab** taken together every 4 weeks for 4 cycles, durvalumab continued further for 13 cycles or until disease progression, concurrent radiotherapy to start with first day of immmunotherapy; durvalumab q4w for 13 cycles or until progression with concurrent radiotherapy	Hypofractionated short course (either 1 or 5 days)	MTD	ORR; LRR, LCR, ARR, RD; PFS, OS

Continued

Table 1 Continued

Identifier	Phase	N	Title	Disease	Interventions	Radiation details	Primary outcome	Secondary outcomes
NCT03298893	I/II	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 (FIGO stage IB2–IVA)	**Nivolumab** every 2 weeks with concurrent CRT followed by 5 months of nivolumab alone	IMRT (including VMAT) at 45 Gy in 25 fractions with an additionally 54 Gy in 25 fractions to positive nodes; optional lateral pelvic dose to cover target volume	DLT (within 11 weeks after treatment start)	ORR; PFS; DFS; AE incidence; retrospective exome, RNA, and targeted sequence analysis; ctDNA heterogeneity; tumor micro-environment description; tumor PD-L1 immunohistochemistry
NCT03527264	II	24	BrUOG 355: Nivolumab to Tailored Radiation Therapy With Concomitant Cisplatin in the Treatment of Patients With Cervical Cancer	Locally advanced cervical cancer (FIGO stage IB–IVA)	**Nivolumab** q2w for 3 cycles starting at day 0 with concurrent CRT; nivolumab maintenance every 4 weeks after following CRT for 2 years; concurrent nivolumab with CRT (q2w for 3 cycles) and maintenance (every 4 weeks for 2 years)	45 Gy in 25 fractions, whole pelvis or extended field	AE within 6 months of treatment; AE within 30 days of treatment; PFS	
NCT03192059	II	43	Study of Pembrolizumab, Radiation and Immune Modulatory Cocktail in Cervical/ Uterine Cancer (PRIMMO)	Advanced or refractory cervical cancer, endometrial carcinoma, or uterine sarcoma	An immunomodulatory cocktail consisting of vitamin D (2000 IU), aspirin (325 mg), cyclophosphamide (50 mg), and 180 or 30 mg lansoprazole alternating weekly) plus curcumin with **pembrolizumab** every 3 weeks and RT	EBRT 24 Gy in 3 fractions, a fraction every 28 hours	ORR	Incidence of AE; best OR; PFS; median PFS; OS; median OS; HR-QoL
NCT02635360	II	88	A Randomized Phase II Study of Chemoradiation and Pembrolizumab for Locally Advanced Cancer	Advanced cervical cancer	Standard cisplatin-based CRT with concurrent **pembrolizumab**; standard cisplatin based CRT followed by pembrolizumab every 3 weeks	4–6 fractions of brachytherapy	Change in immunologic markers; DLTs	Metabolic response rate on imaging; incidence of distant metastases; PFS; OS
NCT03614949	II	26	SBRT and Atezolizumab in the Management of Recurrent, Persistent, or Metastatic Cervical Cancer	Recurrent or metastatic cervical cancer	**Atezolizumab** q3w 1 week following SBRT therapy	SBRT with 24 Gy in 3 fractions	ORR	PFS; OS
NCT03830866	III	714	Study of Durvalumab With Chemoradiotherapy for Women With Locally Advanced Cervical Cancer	Locally advanced cervical cancer	**Durvalumab** every 4 weeks and standard-of-care CRT followed by durvalumab monotherapy; placebo and CRT	EBRT and brachytherapy (standard-of-care)	PFS	OS; CR; PR; ORR; DoR in patients with CR; HR-QoL; PFS 3 years; AE, safety and tolerability (ECGs, vital signs, chemistry and hematology panels)

Active, not recruiting

Continued

Table 1 Continued

Identifier	Phase	N	Title	Disease	Interventions	Radiation details	Primary outcome	Secondary outcomes
NCT03312114	II	29	Anti-PD-L1 and SABR for Ovarian Cancer	Metastatic fallopian tube cancer, primary peritoneal carcinoma, recurrent epithelial cancer of ovary	**Avelumab**	Stereotactic treatment (eg, SABR/SBRT)	ORR	OS; CR; TTP; median response duration

AE, adverse event; ARR, abscopal response rate; CR, complete response; CRT, chemo-radiation therapy; DLT, dose-limiting toxicity; DoR, duration of response; EBRT, external-beam radiation therapy; ECG, electrocardiogram; FIGO, International Federation of Obstetrics and Gynecology; HR-QoL, health-related quality of life; IMRT, intensity-modulated radiation therapy; LCR, local control rate; LRR, local-regional recurrence; ORR, overall response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; q2w, once every 2 weeks; q3w, once every 3 weeks; q4w, once every 4 weeks; RD, response duration; SABR, stereotactic ablative radiation therapy; SBRT, stereotactic body radiation therapy ; TTNT, time to next treatment; TTP, time to progression; VMAT, volumetric modulated arc therapy.

synergistic anti-tumor effects and enhanced changes in the microenvironment.[25] This improved efficacy, however, may come at the cost of increased toxicity, as exemplified by the dose-dependent immune-related gastrointestinal inflammation found in macaque models treated with combinations of checkpoint inhibitors.[25]

Combined checkpoint blockade with ipilimumab and nivolumab has also shown superior efficacy over nivolumab alone for recurrent epithelial ovarian cancer, with the NRG-GY003 trial showing a 31.4% response rate at 6 months (compared with 12.2% with nivolumab monotherapy; odds ratio 3.28).[26] In CheckMate-358, combined ipilimumab and nivolumab resulted in durable clinical activity in patients with recurrent or metastatic cervical cancer independent of PD-L1 expression.[27] A total of 91 patients with squamous cell carcinoma of the cervix with two or fewer lines of prior systemic therapy for recurrent or metastatic disease were randomized to either nivolumab at 3 mg/kg every 2 weeks plus ipilimumab at 1 mg/kg every 6 weeks (nivo3+ipi1) or nivolumab at 1 mg/kg plus ipilimumab at 3 mg/kg, given every 3 weeks for four doses followed by nivolumab at 240 mg every 2 weeks (nivo1+ipi3). For patients in the nivo3+ipi1 arm after a median follow-up of 10.7 months, the median progression-free survival was 13.8 months for treatment-naive patients versus 3.6 months in the patients treated with systemic therapy. For the patients in the nivo1+ipi3 arm after a median follow-up of 13.9 months, the progression-free survival was 8.5 months in treatment-naive patients versus 5.8 months in those with previous systemic therapy. In the nivo3+ipi1 arm, the objective response rate was 31.6% vs 21.3% in patients without prior systemic therapy versus with prior systemic therapy. In the nivo1+ipi3 arm, the corresponding objective response rate for patients without and with prior systemic therapy was 45.8% and 36.4%, respectively. Durvalumab (a PD-L1 inhibitor) and tremelimumab (a CTLA-4 inhibitor) have also been tested in combination, with excellent anti-tumor effects and manageable toxicity profiles in phase I trials of lung cancer[28] and other solid tumors, including ovarian cancer.[29] Trials assessing this tremelimumab/durvalumab combination with radiation therapy are nascent at present, and include a pilot study of SABR with combined immunotherapy for patients with metastatic pancreatic cancer, with no patients experiencing dose-limiting toxicity.[30] Further trials assessing this combination in metastatic gynecologic cancers combined with hypofractionated radiotherapy are ongoing (NCT03452332, NCT03277482; Table 1).

Proposed Mechanisms Underlying the Synergy between Radiation Therapy and Immunotherapy

Immune checkpoint inhibitors have demonstrated anti-tumor effects at a variety of anatomic sites, and the addition of radiation therapy has the potential to enhance these effects further. Various strategies for patient selection and sequencing of concurrent therapy are being explored in attempts to optimize the inherent immunogenicity of radiation therapy.[31] Ionizing radiation induces cell death through a variety of mechanisms: primarily apoptosis, but also through necrosis, autophagy, mitotic catastrophe, and senescence. Each of these mechanisms is initially precipitated by the genomic instability caused by radiation therapy, via direct and indirect DNA damage, the latter of which entails generation of free radical products.[32]

In addition to having direct cytotoxic effects on cancer cells, radiation enhances the release of tumor antigens and "danger" molecules from the so-called damage-associated molecular patterns.[33] Radiation further leads to enhanced tumor antigen uptake and cross-presentation by dendritic cells and the increased secretion of lymphocyte-stimulating cytokines and chemokines.[34 35] Indeed, kinetic studies of intra-tumoral immune cell activation during radiation therapy for cervical cancer indicate dynamic changes at the tumor site, specifically temporal variations in populations of CD3+, CD8+, CD4+, activated CD11c+CD11b- dendritic cells, and proliferating CD8+ T-cells expressing Ki67 and CD69.[36] Collectively, this process results in a complex activation of the immune system and modulation of the tumor micro-environment through several pathways: cytokine cascades (tumor necrosis factor α (TNF-α), interleukin 1α (IL-1α), IL-1β, IL-6, and transforming growth factor β (TGF-β)); increased signal transduction (eg, NF-κB and STAT3); and hypoxia and receptor tyrosine kinase mechanisms, as discussed below.[32]

These immune sequelae provide a strong rationale for evaluating combinations of radiation therapy with systemic immune checkpoint blockade. In mouse models, radiation therapy combined with CTLA-4 or PD-L1 blockade leads to inhibition of systemic metastases.[37 38] Through amplification of such immune processes, radiation therapy has been proposed to enhance antigen presentation and thus response to checkpoint inhibition.[31]

Optimal Sequencing of Radiation Therapy and Checkpoint Inhibition

The optimal timing, dose, and fractionation schedules for radiation therapy to be used in combination with immunotherapy remain unclear. Questions regarding sequencing and timing allude to two proposed mechanisms for optimizing synergistic effect: (1) if the role of radiation therapy is to simply re-invigorate *exhausted* intratumoral CD8 T-cells, then temporal overlap and specific timing are not critical; (2) alternatively, if the predominant role of radiation therapy is to potentiate naïve T-cell activation, then close or concurrent sequencing would be vital.[31]

In general, pre-clinical data support the latter strategy of close or concurrent sequencing. For example, anti-PD-L1 inhibitors optimally enhance T-cell-mediated clearance of infections if administered early during T-cell differentiation.[39] Other studies have demonstrated that T-cell tumor infiltration peaks between 5–8 days after radiation therapy, and the highest ratio of effector-to-regulatory T cells is also noted at 5 days after radiation therapy, supporting the importance of close sequencing and timing of radiation therapy.[40 41] In murine models, concurrent administration of PD-L1 with fractionated radiation therapy has demonstrated superiority over sequential administration.[42]

Unlike anti-PD-1 inhibitors, which may benefit from concurrent or close adjuvant administration with respect to radiation therapy, CTLA-4 inhibition has been shown to have enhanced effects with neoadjuvant administration (before radiation therapy). For example, pre-clinical data demonstrate superior efficacy from CTLA-4 given 7 days before radiation therapy (20 Gy single fraction) versus 1 or 5 days after radiation therapy. This discrepancy has been attributed to the ability of CTLA-4 to deplete regulatory T-cells within the tumor.[43] Each of these proposed mechanisms requires further evaluation in clinical trials.

Radiation Dose and Fractionation

Various radiation therapy dose and fractionation schedules have been proposed for optimal combination with immunotherapy, with studies in murine models favoring hypofractionated regimens. For example, Lugade et al found increased T-cell interferon γ (IFN-γ) secretion, antigen-presenting cell infiltration, and CD8 T-cell infiltration among mice engrafted with B12 melanoma cell lines treated with 15 Gy in a single-fraction relative to 5 Gy in three separate fractions[44]; mirroring these results, Schaue et al found that 15 Gy in two to five fractions enhanced effector T-cell reactivity without increasing regulatory T-cell activity.[45] Several hypothesized mechanisms support hypofractionation over conventional fractionation in combination with immunotherapy. The simplest of these mechanisms attributes efficacy to the optimal cytoreductive ability of stereotactic ablative regimens; because a significant tumor burden can diminish the re-invigoration of circulating exhausted CD8 T-cells,[46] SABR can thus enhance immunomodulatory effects by maximally reducing the overall tumor burden ratio. Limiting the total number of fractions (as opposed to conventional daily fractionation) is also thought to limit the elimination of activated and infiltrated lymphocytes, given their inherent radiosensitivity.[40 41] Other factors supporting the use of SABR include the upregulation of checkpoint molecules, activation of the cGAS-STING pathway, and upregulation of inflammatory cytokines with stereotactic ablative regimens.[31] These findings have led to several studies exploring the use of hypofractionated radiation therapy for gynecologic malignancies (NCT03614949, NCT03955978, NCT03452332, NCT03277482; Table 1).

DNA DAMAGE RESPONSE INHIBITORS

As malignant cells carry large burdens of inherent genomic instability, both radiation therapy and traditional chemotherapy function to augment DNA damage, resulting in tumor death. As a result, the capacity of malignant cells to repair such damage can contribute to treatment resistance. Thus, inhibition of DNA repair toxicity via DNA damage response (DDR) inhibitors in combination with radiotherapy are actively being investigated, given the potential for additional synergy with increased mutational burden.[47 48]

The DDR network consists of molecules that both regulate DNA repair such as poly(ADP-ribose) polymerases (PARPs) as well as cell cycle regulators including ataxia telangiectasia and RAD3 related (ATR) kinase, WEE1, and ribonucleotide reductase. Deregulation of this system results in aberrant firing of replication origins resulting in insufficient nucleotide pools, which can lead to further replication fork stalling and ultimately increased DNA damage, genomic instability, and triggering of cell death.[49] Additionally, it has been found that cancerous cells often have deficient G_1 checkpoint mechanisms and thus rely more heavily on the G_2 checkpoint.[50] Given that this class of drugs preferentially targets G_2 checkpoint regulators, they may serve to both enhance genomic instability, in synergy with radiation therapy, as well as abrogate adverse effects on non-cancerous cells and thus limiting toxicity

PARP Inhibitors

An increasingly popular oncologic drug target in recent years is PARP, a chromatin-associated enzyme important for repair of single-stranded DNA breaks.[51] This mechanism has led to PARP

inhibitors being considered for their potential radiosensitization effects, which have been observed in xenograft mouse models of BRCA-1-deficient high-grade serous ovarian carcinoma,[52] breast cancer,[53] and esophageal squamous cell carcinoma, which share similar histopathologic characteristics with cervical cancers.[54]

Early clinical trials based on these pre-clinical data have had mixed results, with some promise accompanied by toxicity concerns. For example, a phase II trial of veliparib (ABT-888) with carboplatin and paclitaxel for advanced/metastatic non-small cell lung cancer demonstrated a favorable trend in both progression-free and overall survival over traditional carboplatin–paclitaxel chemotherapy alone.[55] However, veliparib combined with temozolomide, an alkylating chemotherapy agent, and radiation therapy was found to have intolerable hematologic toxicity in adults with glioblastoma multiforme.[56] A separate trial of olaparib (a PARP inhibitor), cetuximab (an epidermal growth factor receptor inhibitor), and radiation therapy in patients with locally advanced head and neck cancer and heavy smoking history observed a 2 year overall survival of 72%, while previous studies of similar cohorts had an expected overall survival of approximately 60%.[57] The most common grade 3 and 4 toxicities observed were mucositis (69%) and dermatitis (38%).[57] Among patients with gynecologic cancer, a phase I trial of veliparib and low-dose fractionated whole-abdominal radiation therapy for ovarian and fallopian cancer with peritoneal carcinomatosis demonstrated acceptable tolerability; however, out of 32 patients, only one was observed to have a partial response.[58] Other studies of PARP inhibitors in combination with radiation therapy are ongoing, including a phase I trial of niraparib for metastatic, invasive cervical cancer, with niraparib delivered concurrent with pelvic radiotherapy after induction carboplatin and paclitaxel chemotherapy for 3–6 cycles (NCT03644342; Table 2), and a phase I study of talazoparib and fractionated radiotherapy for limited abdominopelvic recurrences from gynecologic malignancies (NCT03968406; Table 2). At least seven PARP inhibitors are currently in clinical trial use or development.[59]

ATR Inhibitors

Another important component in the DNA damage response system includes ataxia telangiectasia and Rad3 related (ATR) kinase. ATR is activated in the presence of single-stranded DNA damage and works to regulate G_2/M cell cycle progression, stabilization of replication forks, and control of replication origin, playing a critical role in maintaining genomic stability following DNA damage.[60] ATR acts through downstream targets to promote cell survival by arresting the cell cycle and promoting DNA repair.

Pre-clinical results have shown ATR inhibitors to have remarkable cytotoxic activity in cancer cells, especially when used concurrently with other DNA-damaging agents such as cisplatin and ionizing radiation.[61] Multiple studies have demonstrated that ATR inhibitors can sensitize cells to standard cancer therapies including radiation and chemotherapy in vitro. Of note, sensitization was almost absent in normal cells, suggesting a high therapeutic ratio.[61] The combination of cisplatin, radiotherapy, and ATR inhibitors are currently being assessed in phase I trials in a variety of sites including solid tumors (NCT02223923, NCT03641547; Table 2) and head and neck cancers (NCT02567422, NCT03641547) among others.

Sun et al have recently demonstrated that radiotherapy or chemotherapy (cisplatin) can also significantly induce cell surface PD-L1 expression in multiple cancer types[62]; however, this effect is blocked by depletion or pharmacologic inhibition of ATR, suggesting a possible synergistic role in combining immune checkpoint blockade with ATR inhibition. Additionally, Vendetti et al have shown in a genetically engineered mouse model of colorectal cancer that AZD6738, an ATR inhibitor, when combined with conformal radiotherapy, can potentiate CD8+ T-cell activity in the tumor micro-environment.[63] Therefore, the use of combined checkpoint inhibition with radiation and ATR inhibition may potentiate an anti-tumor response.

WEE1 Inhibitors

WEE1 inhibitors represent yet another DDR inhibitor class with good anti-tumor potential. WEE1 is a tyrosine kinase that regulates cell cycle progression by inhibiting CDK2 during the S-phase, which reduces the time for DNA repair to occur, and inhibiting CDK1 at the G_2/M checkpoint, resulting in premature mitotic entry and increased chance of mitotic catastrophe leading to cellular death.[64] WEE1 inhibition has also been shown to impair homologous recombination[65] and may thus serve as a radiosensitizer, particularly in combination with PARP inhibition.[66 67] Radiosensitization with dual inhibition of WEE1 and PARP has been demonstrated in several cancer types, including endometrial and ovarian cancer cell lines.[68] Clinically, a phase I trial of adavosertib (a WEE1 inhibitor), gemcitabine, and radiation therapy demonstrated tolerability and favorable progression-free and overall survival in patients with pancreatic carcinoma relative to historic controls.[69] A phase I trial of adavosertib combined with whole-pelvis external-beam radiation therapy and weekly cisplatin is underway for patients with newly diagnosed gynecologic cancers, including cervical cancer (CT1B-3B, N0/1, M01), upper 1/3 vaginal epithelial carcinoma (T1-3, N0/1, M01), and endometrioid adenocarcinoma (cT1-3, N0/1, M0 unsuitable for primary surgery; NCT03345784; Table 2).

Triapine

Triapine (NSC #663249) is an inhibitor of the M2 subunit of ribonucleotide reductase, the rate-limiting enzyme required for DNA-building deoxyribonucleotides.[70] Through this molecular inhibition, triapine can prolong DNA repair time and arrest cancer cells at the G_1/S cell cycle restriction checkpoint, thereby increasing their vulnerability to radiation therapy-induced death.[71] The activity and tolerability of triapine has been evaluated in colon[72] and pancreatic cancer,[73] and triapine has shown promise in cervical cancer, which is associated with overactive ribonucleotide reductase.[71] The National Cancer Institute undertook two single-arm prospective trials, phase I (7336) and phase II (8327), to evaluate the combination of triapine and cisplatin with radiation therapy for advanced cervical cancer; this combination resulted in auspicious results including a 3 year pelvic loco-regional relapse rate of 4%, disease-free survival of 80%, and overall survival of 82%, with only 25% of patients experiencing an acute attributable grade 3 or 4 toxicity (mostly transient cytopenias) and 8% experiencing late toxicity (mostly vaginitis).[70] From these promising findings, this triple-combination therapy is being evaluated in an ongoing randomized trial for women with American Joint Committee on Cancer (6th and 7th editions) stage IB2, II, or IIIB–IVA cervical cancer or stage II–IVA vaginal cancer (NCT02466971; Table 2).

Table 2 Clinical trials of radiotherapy and DNA damage response inhibitors

Identifier	Phase	N	Title	Disease	Interventions	Radiation details	Primary outcome	Secondary outcomes
Recruiting								
NCT03641547	I	65	A Phase 1 Dose Escalation Safety Study Combining the ATR Inhibitor M6620 With Chemoradiotherapy in Oesophageal Cancer & Other Solid Cancers Using Time to Event Continual Reassessment Method (CHARIOT)	Adenocarcinoma or squamous cell carcinoma of the esophagus; advanced, metastatic, or unresectable solid tumor	**M6620** (an ATR inhibitor) with radiotherapy; M6620 with chemotherapy; M6620 with CRT	Palliative radiotherapy	Best tolerated treatment schedule	Incidence of AE; proportion of patients completing 75%, 90%, and 100% of radiotherapy dose; objective tumor response
NCT02223923	I	100	A Phase I Study to Assess the Tolerability, Safety and Biological Effects of ATR Inhibitor (AZD6738) as a Single Agent and in Combination With Palliative Radiation Therapy in Patients With Solid Tumours (Patriot)	Solid tumors refractory to conventional treatment	**AZD6738** (an ATR inhibitor) alone and with radiotherapy	Palliative radiotherapy (20 or 30 Gy)	MTD	Incidence of AE; single and multiple dose pharmacokinetics; tumor response
NCT03345784	I	33	A Phase I Study of the Wee 1 Kinase (Wee 1) Inhibitor AZD1775 in Combination With Radiotherapy and Cisplatin in Cervical, Upper Vaginal and Uterine Cancers	Cervical, vaginal, or uterine cancer	Daily EBRT with **adavosertib** (daily or on days 1, 3, and 5) with weekly cisplatin for up to 5 weeks	Whole pelvic radiotherapy to a total dose of 45 Gy	Recommended phase 2 dose (dose level with <1/6 of patients with DLT)	ORR; pharmacodynamic effects (CKC2, Ki67, γH2AX, pH3, CC3 biomarkers); PFS; AE incidence
NCT03968406	I	24	Phase I Study of Talazoparib in Combination With Radiation Therapy for Locally Recurrent Gynecologic Cancers	Locally recurrent gynecologic cancers	**Talazoparib** on days −10 and −7 before radiotherapy and concurrent with radiotherapy unless unacceptable toxicity, continuing for up to 8 weeks in the absence of disease progression	Radiotherapy 5 days a week for up to 7 weeks	MTD	AE incidence; response rate; LCR; TTP; PFS; OS; level of PAR inhibition; γH2Ax and RAD51 foci formation; HR-QoL
NCT03644342	I/II	20	Phase I/II Study of Niraparib With Radiotherapy for Treatment of Metastatic Invasive Carcinoma of the Cervix	Distant metastatic cervical cancer (FIGO stage IV)	3–6 cycles of Induction-style carboplatin and paclitaxel followed by definitive pelvic radiotherapy with **niraparib**	Whole pelvic radiotherapy	MTD; local PFS	AE profile; HR-QoL; tumor response
NCT02466971	III	348	Radiation Therapy and Cisplatin With or Without Triapine in Treating Patients With Newly Diagnosed Stage IB2, II, or IIIB–IVA Cervical Cancer or Stage II–IVA Vaginal Cancer	Newly diagnosed AJCC v6 and v7 stage IB2, stage II, IIIB, or IVA cancer of the uterine cervix or stage II–IVA vaginal cancer	**Triapine** (IV over 2 hours on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 31, and 33) in addition to CRT (cisplatin-based)	EBRT in 25 fractions followed by LDR or HDR brachytherapy	PFS, assessed up to 5 years	OS (assessed up to 5 years); metabolic complete response; acute and chronic AEs, compliance

AE, adverse event; AJCC, American Joint Committee on Cancer; ATR, ataxia telangiectasia and Rad3 related; CRT, chemo-radiation therapy; DLT, dose-limiting toxicity; EBRT, external-beam radiation therapy; FIGO, International Federation of Obstetrics and Gynecology; HDR, high dose rate; HR-QoL, health-related quality of life; IV, intravenously; LDR, low dose rate; LRC, local-regional control; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

CELLULAR PATHWAY INHIBITORS AND OTHER AGENTS: SIGNALING, GROWTH, ANGIOGENESIS, AND HYPOXIA

The final class of agents discussed act by inhibiting cellular signaling pathways involved in growth, angiogenesis, and hypoxia. Interestingly, some of the drugs to be discussed are used to treat diseases other than cancer and are readily available in the healthcare market with well-established tolerability and toxicity profiles.

Nelfinavir

Nelfinavir, an anti-retroviral protease inhibitor prescribed for the treatment of HIV infection, has known radiosensitizing properties. Pre-clinical data suggest that this radiosensitization could result from nelfinavir's inhibition of Akt phosphorylation and thus the downregulation of the PI3k/Akt signaling pathway, a central signal transduction pathway vital for cellular proliferation, migration, angiogenesis, overall growth, and survival.[74 75] Pore et al previously demonstrated synergy between nelfinavir and radiation therapy for head-and-neck and lung carcinoma cell lines, showing that therapeutic doses prescribed for HIV infection are sufficient for downregulating Akt phosphorylation in nude mice.[76] Other proposed mechanisms include reduction of hypoxiainducible factor 1α, which has downstream effects on vascular endothelial growth factor and functional inhibition of angiogenesis. Nelfinavir has also resulted in enhanced oxygenation (and thus increased radiosensitivity) in tumor xenograft models.[76]

Clinically, nelfinavir has been administered in conjunction with chemo-radiation for a wide range of solid malignancies, including pancreatic, rectal, non-small cell lung cancer, glioblastoma multiforme, and cervical cancer.[77–80] Its tolerability and activity have been demonstrated in phase I/II clinical trials.[77–80] For example, Brunner et al conducted the first phase I trial of nelfinavir in conjunction with chemo-radiation therapy (with cisplatin/gemcitabine) for locally advanced pancreatic cancer, finding a 20% increase in response rate over historical controls (50% vs 30%).[81] Other studies have also shown promising results; one phase I/II trial of nelfinavir with concurrent chemo-radiation therapy for locally advanced non-small cell lung cancer showed an objective response rate of 94% (31 of 33 patients, 95% confidence interval (95% CI) 86% to 100%), a median progression-free survival time of 11.7 months (95% CI 6.2 to 17.1 months), and a median overall survival time of 41.1 months (95% CI 19.0 to 63.1 months), with no dose-limiting toxicities noted.[82] Additionally, a phase I study of nelfinavir with cisplatin and pelvic radiotherapy for locally advanced cervical cancer has been completed.[83] Eleven patients with squamous cell carcinoma of the cervix were enrolled. There was one dose limiting toxicity of grade 3 diarrhea at a dose level 1 (875 mg oral twice daily) with no recurrences within the prior radiotherapy fields and two patients with out-of-field recurrences. The recommended phase II dose was identified to be 1250 mg twice daily which is the FDA approved dosing of nelfinavir as an anti-retroviral agent. One patient who had an isolated para-aortic recurrence was salvaged with definitive radiotherapy to the para-aortic node. A second patient developed both para-aortic and lung metastases soon after completion of chemo-radiation therapy and received salvage chemotherapy and died of disease. These results suggest that nelfinavir holds promise as a radiosensitizer and that further studies are warranted.

Nelfinavir is currently being tested in phase I/II trials of head-and-neck cancer, and a phase III trial (NCT03256916) of nelfinavir with chemo-radiation therapy is ongoing for locally advanced cervical cancer (Table 3). A phase I study of nelfinavir and weekly cisplatin with fractionated radiation therapy for women with locally advanced unresectable vulvar cancer will be activated soon at The University of Texas MD Anderson Cancer Center (NCT04169763; Table 3).

Metformin and Endostar

Metformin, a common, relatively non-toxic drug used to treat diabetes, is thought to have anti-neoplastic effects and thus potential value as an adjunct in definitive oncologic management. In retrospective studies, metformin has been associated with improved outcomes in metformin users compared with diabetic non-metformin users including improved overall survival in patients with laryngeal cancer (OR 3, 95% CI 1.04 to 8.4; p=0.04), improved distant metastases-free survival in those with oropharyngeal cancer (adjusted hazard ratio (HR) 0.54, 95% CI 0.32 to 0.93; p=0.03), and improved cancer-specific survival in HPV-associated head-and-neck malignancies (HR 2.33, 95% CI 1.49 to 6.16; p<0.01), as seen in a Surveillance Epidemiology and End Results–Medicare dataset study.[84] A phase I dose-escalation study of metformin with chemo-radiation therapy for locally advanced head-and-neck cancers resulted in encouraging 2 year overall and progression-free survival rates (90% and 84%, respectively).[85]

The precise mechanism of action of metformin remains in debate, but pre-clinical data suggest a radiosensitizing effect,[86] specifically through reduction of tumor hypoxia (as has been shown in animal studies). In cervical cancer, poor tumor oxygenation is associated with inferior survival and radioresistance, and a retrospective study of cervical cancer indicated a potential decrease in mortality from metformin use.[87] Metformin also indirectly inhibits central growth and proliferation cell-signaling pathways, including the PI3K/Akt pathway, which may have a role in its anti-tumor effects.[88] Given its promise, the potential benefit of adding metformin to chemo-radiation therapy is being evaluated in a phase II randomized trial of patients with locally advanced (FIGO stage IB2–IVA) cervical cancer (NCT02394652, Table 3).

Another hypoxia modifier, Endostar (recombinant human endostatin), is a novel artificially synthesized anti-angiogenic agent that has been approved for clinical use. Endostar has been found to enhance the anti-tumor effects of radiation therapy by altering the tumor's energy metabolism and micro-environment through hypoxia modification, as demonstrated in murine models.[89] The addition of Endostar to chemo-radiation therapy is being evaluated in both a phase II study of patients with postoperative high-risk early-stage cervical cancer (NCT03622827, Table 3) and a multi-institutional phase III study of women with cervical cancer (FIGO (2009) stage Ib, IIa2, IIb–IVa) (NCT03086681, Table 3).

Tyrosine-Kinase Inhibitors

Tyrosine-kinases are a large enzymatic class of molecules that function in a wide variety of vital cellular signaling pathways[90]; prime among these includes the epidermal growth factor receptor (EGFR).[91] EGFR expression has been observed in a majority of cervical cancer patient tumors and found to be correlated with tumor aggressiveness.[92] Additionally, EGFR inhibition has proven to be an effective adjuvant therapy for HPV-associated head and neck cancers.[93 94] Collectively, these factors established a strong

Table 3 Clinical trials of radiotherapy and cell-signaling inhibitors and hypoxia sensitizers

Identifier	Phase	N	Title	Disease	Interventions	Radiation details	Primary outcome	Secondary outcomes
Not yet recruiting								
NCT04169763	I	18	Nelfinavir, Cisplatin, and External Beam Radiation Therapy for the Treatment of Locally Advanced Vulvar Cancer That Cannot Be Removed by Surgery	Unresectable T2-4, N0-3 vulvar carcinoma	**Nelfinavir** twice daily for 8 weeks, cisplatin-based CRT weeks 2–8	EBRT (5 days a week, weeks 2–8)	Recommended phase II dose of nelfinavir; incidence of AE	PFS; OS
NCT03086681	III	120	A Multicenter, Randomized Controlled Clinical Trial Comparing Endostar With Concurrent Chemoradiotherapy vs Concurrent Chemoradiotherapy in the Treatment of Locally Advanced Cervical Carcinoma	Locally advanced cervical cancer (FIGO 2009 Ib, IIa2, IIb–IVa)	**Endostar** (daily for days 1–10, repeated q15 days for four cycles) with CRT (cisplatin based); CRT (cisplatin based)	IMRT 45–50 Gy	Short-time effect (image assessment of cancer status)	OS; PFS
Recruiting								
NCT02363829	I	6	A Phase I Study of Nelfinavir Added to Cisplatin Chemotherapy Concurrent With Pelvic Radiation for Locally Advanced Cervical Cancer (II–IVA)	Locally advanced cervical cancer (FIGO stage II–IVA	Twice daily **nelfinavir** with CRT (cisplatin based)	Whole-pelvis EBRT and intracavitary brachytherapy	AE	
NCT02394652	II	48	The Potential for Metformin to Improve Tumor Oxygenation in Locally Advanced Cervix Cancer: A Phase II Randomized Trial	Locally advanced cervical cancer (FIGO stage IB2–IVA)	**Metformin** administration 1 week before CRT (cisplatin based); standard CRT (cisplatin)	EBRT	Change in fractional hypoxic volume of the tumor via FAZA-PET scan	DFS; acute and late GI and GU toxicities; effect on endogenous hypoxia and other markers; biomarkers of metformin response
NCT03622827	II	120	Postoperative Concurrent Chemoradiotherapy Combined With Recombinant Human Endostatin for High-risk Early Stage Cervical Cancer: A Phase II Pilot Study (ChESS)	High-risk early stage cervical cancer	CRT with cisplatin and 5-flurouracil q3w for two cycles with **Endostar** (recombinant human endostatin) given 3 days before chemotherapy for both cycles	IMRT to 45–50 Gy in 6 weeks	3 year DFS; AE	Time to distant metastasis survival; LRR; OS; HR-QoL
NCT03256916	III	300	A Phase III Randomized Clinical Trial to Study the Radiosensitizing Effect of Nelfinavir in Locally Advanced Carcinoma of Uterine Cervix	FIGO stage IIIB carcinoma cervix	**Nelfinavir** 5–7 days before CRT (cisplatin based); standard CRT (cisplatin)	Pelvic EBRT 46 Gy in 23 fractions	3 year DFS	LRC; OS; AE incidence; change in Akt levels in the tumor; tumor hypoxia via PET/MRI; Cmax (variability of distribution); clearance of nelfinavir; half-life of nelfinavir

AE, adverse events; CRT, chemo-radiation therapy; DFS, disease-free survival; EBRT, external-beam radiation therapy; FAZA-PET, 18F-fluoroazomycin arabinoside positron emission tomography; FIGO, International Federation of Obstetrics and Gynecology; GI, gastrointestinal; GU, genitourinary; HR-QoL, health-related quality of life; IMRT, intensity-modulated radiation therapy; LRC, local-regional control; LRR, local-regional recurrence; OS, overall survival; PFS, progression-free survival; qw3, once every 3 weeks.

rationale for the role of EGFR inhibition as a novel cervical cancer therapy; however, subsequent studies have shown mixed to poor results. Cetuximab, an inhibitory monoclonal EGFR antibody, was found to have no clinical activity when used as monotherapy for recurrent or persistent cervical cancer[95 96]; had no added benefit when combined with cisplatin over cisplatin alone;[97] and was found to have high toxicity rates when combined with radiation therapy and cisplatin, particularly for those needing extended field irradiation due to para-aortic nodal disease.[98] Other tyrosine-kinase inhibitors have been met with similar results. Both gefitinib[99] and erlotinib,[100] EGFR-specific tyrosine-kinase inhibitors, failed to produce any objective responses when used as monotherapy in those with advanced, metastatic, or recurrent cervical cancer. Multi-target tyrosine-kinase inhibitors such as pazopanib and lapatinib, which target multiple anti-angiogenic pathways, have shown some benefit in advanced and recurrent cervical cancer with reasonable toxicity; however, the overall response rate was <10% for both agents.[101] Nonetheless, these drugs continue to show efficacy in a variety of non-gynecologic sites, and while there are no ongoing or upcoming trials for this class in gynecologic malignancies, trials for cetuximab in particular are ongoing in head and neck cancers (NCT01969877, NCT00956007, NCT00265941). Further studies to elucidate their potential role in gynecologic cancer in combination with other agents may prove promising.

CONCLUSIONS

While chemo-radiation therapy with a platinum agent has been the standard of care for multi-modality therapy in gynecologic cancers for two decades, novel strategies with targeted agents and immunotherapy are being actively tested in clinical trials both in both the definitive and metastatic setting. Table 4 presents a summary of the agents in current clinical trials for women with locally advanced or metastatic gynecologic cancers, with a summary of their mechanisms and potential synergy with radiotherapy. The focus of these studies has largely been on novel immunotherapy agents or molecularly targeted agents combined with radiotherapy. Radiation,

Table 4 Current agents in clinical trials combined with radiotherapy

	Class	Mechanism	Potential synergy with radiation therapy	Status in gynecologic oncology
Immunotherapy	CTLA-4 inhibitors	Interrupts T-cell (particularly T-regulatory cells) inhibitory CTLA-4 signaling, resulting in increased T-cell activation at the early induction phase after antigen stimulation	Enhanced release, uptake, and cross-presentation of tumor antigens by dendritic cells and increased secretion of lymphocyte stimulating cytokines and chemokines	Phase I/II monotherapy trials performed; phase I trials of dual checkpoint inhibition and concurrent RT underway
	PD-1/PD-L1 inhibitors	Impedes inhibitory PD-1/PD-L1 interactions, resulting in increased T-cell activation, particularly cytotoxic CD8 T-cells in the late effector phase	Enhanced effector-to-regulatory T-cell ratio and T-cell infiltration after RT	Several phase I/II monotherapy trials completed and underway; phase I trials with dual checkpoint inhibitors and concurrent RT underway; monotherapy phase III trials underway; phase III trials with concurrent RT underway
DNA-damage response Inhibitor	PARP inhibitors	Inhibit PARP's essential role in single-stranded DNA repair; "trap" DNA resulting in replication fork collapse and DNA damage	Direct DNA-damage effects and increase in overall genomic instability	Multiple phase I–III monotherapy trials; phase I/II trials with concurrent RT underway
	WEE1 inhibitors	Regulation of cell cycle progression, decreasing time for DNA repair to occur		Phase I trial with concurrent RT underway
	ATR inhibitors	Inhibits ATR's regulation of cell cycle progression and DNA repair		Pre-clinical data; no pending clinical trials
	Triapine	Inhibits ribonucleotide reductase decreasing DNA repair time		Phase III trial with concurrent RT underway
Cell signaling modulators	Nelfinavir	Proteasome inhibition; inhibition of the PI3K/Akt signaling pathway central in cellular growth and survival; reduction of hypoxia-induced HIF-1α and subsequent reduction in angiogenesis	Direct cytotoxic effect; increased oxygenation and thus increased generation of free radical products	Phase I and III trials with concurrent RT underway/about to open
	Metformin	Indirect inhibition of the PI3K/Akt signaling pathway central in cellular growth and survival; reduction of tumor hypoxia		Phase II trial with concurrent RT
	Endostar	Alters energy metabolism primarily in the tumor micro-environment through hypoxia modification; inhibits angiogenesis		Phase III trial with concurrent RT

ATR, ataxia telangiectasia and Rad3-related; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RT, radiation therapy.

when combined with immunotherapy, has the potential to prime the immune system resulting in a synergistic response; however, multiple questions remain including the optimal immunotherapy combination with radiotherapy, the sequencing of therapy, as well as the dose of radiotherapy when given in the metastatic setting. In patients with locally advanced disease, careful clinical trials with appropriate stopping rules will need to be conducted, given the concerns with additional toxicities with combination immunotherapy or molecularly targeted agents with radiotherapy in the setting of high local control rates with standard chemo-radiation therapy. With a wealth of pre-clinical and early clinical data generated to date, the use of combination strategies involving radiation therapy and novel systemic agents is an exciting area of research in gynecologic oncology. Through the synergistic mechanisms described here, combination management approaches have the potential for optimizing outcomes in a wide range of patients with gynecologic cancers. We look forward to the results of ongoing clinical trials and additional evidence regarding their optimal use with respect to indications, sequencing, and timing, as well as further information on the mechanisms underlying the additional benefits of combinations of systemic agents with radiation therapy. Furthermore, with a deeper understanding of the interplay of mechanisms behind these combinatorial strategies, futures studies will need to focus on the identification of biomarkers to identify more effectively the optimal targeted or immunotherapy combination for patients, particularly those with metastatic disease. The discussion of this was beyond the scope of this review. Ultimately, we hope to mitigate toxicity and enhance efficacy to improve outcomes for those patients with gynecologic cancers.

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