The Impact of Estrogen in the Tumor Microenvironment

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Abstract

Tumor immune escape is now a hallmark of cancer development, and therapies targeting these pathways have emerged as standard of care. Specifically, immune checkpoint signal blockade offers durable responses and increased overall survival. However, the majority of cancer patients still do not respond to checkpoint blockade immune therapy leading to an unmet need in tumor immunology research. Sex-based differences have been noted in the use of cancer immunotherapy suggesting that sex hormones such as estrogen may play an important role in tumor immune regulation. Estrogen signaling already has a known role in autoimmunity, and the estrogen receptor can be expressed across multiple immune cell populations and effect their regulation. While it has been well established that tumor cells such as ovarian carcinoma, breast carcinoma, and even lung carcinoma can be regulated by estrogen, research into the role of estrogen in the regulation of tumor-associated immune cells is still emerging. In this chapter, we discuss the role of estrogen in the tumor immune microenvironment and the possible immunotherapeutic implications of targeting estrogen in cancer patients.

Keywords

Tumor microenvironment (TME) · 17β-estradiol (E2) · Estrogen receptor α (ERα) or estrogen receptor β (ERβ) · Cancer-associated fibroblasts (CAFs) · Tumor-associated macrophages (TAMs) · Myeloid-derived suppressor cells (MDSCs) · Immune T cells · CD4+ T lymphocytes · CD8+ T lymphocytes · Regulatory T cells (Tregs) · Programmed death-1 (PD1) · Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) · Immune B cells · Natural killer (NK) cells · Dendritic cells (DCs) · Fibroblast growth factor (FGF) · Epidermal growth factor (EGF) · Vascular endothelial growth factor (VEGF)

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2.1 Introduction

The tumor microenvironment (TME) is made up of multiple cell types beyond only tumor cells including immune cells, stromal cells including pericytes, and extracellular molecules all regulating tumor growth. These cells have been well established as mechanisms of resistance and have been targets for cancer therapy [1–4]. While these therapeutic strategies have been promising, de novo, and acquired resistance leading to inevitable tumor progression remains an ongoing problem [5–7]. Therefore, alternative regulatory pathways have become necessary to evaluate for possible avenues for future therapeutic research. Female gender has been suggested in retrospective meta-analyses to be associated with decreased response to checkpoint blockade therapy [8–10]. Given the known role of estrogen and other sex hormones effecting immune responses, these findings warrant the evaluation of estrogen signaling in the TME [11]. Estrogen is a steroid hormone that has many physiological functions associated with reproduction, metabolism, and even immune regulation [12]. The main biological endogenous estrogen, 17β-estradiol (E2), is synthesized from androgens by aromatase (CYP19A1) and binds estrogen receptor α (ERα) or estrogen receptor β (ERβ) to exert its effects through both genomic and non-genomic mechanisms [12–17]. Estrogen has been long established as a driver of malignancy in hormone-sensitive carcinomas such as ovarian, breast, endometrial, lung, colon, and even prostate [18]. The oncogenic function of ER is due to the ability of tumor cells to enable transcriptional upregulation of proliferation and cell-survival genes via growth factors such as insulin growth factor (IGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) [19–23]. Therapy targeting these aspects of E2 signaling in cancer has been in use clinically for decades. These therapeutics include selective estrogen receptor modulators or degraders (SERMs or SERDs) and aromatase inhibitors (AIs), and are typically utilized in hormone-positive breast cancer, but their utility is being evaluated in other relevant solid tumors [24]. However, most of the studies have focused primarily on the tumoral signaling of E2, while the remainder of the TME has gone unexplored.

E2 signaling and ER expression are not limited to tumor cells but also found on immune cells where they have distinct functions of immune regulation [25–28]. The link between E2 and autoimmunity has been established since findings of sex disparities in patients with systemic lupus erythematosus (SLE), and multiple, current reviews outline E2 regulation of immune cell function and expansion [29–32]. While the link between E2 and immune regulation has been well characterized and tumor immunology is growing as a field, there is a missing connection between E2 pathways and tumor immunology. This chapter will discuss the current findings in the literature exploring the impact of E2 and tumor immunology, as well as the future therapeutic implications of targeting the E2 pathway in the cancer immunotherapy era.

2.2 E2 Signaling Pathways on Tumor Cells

While ER expression and E2 pathways are canonically associated with tumor cells from hormone-sensitive tumors such as ovarian, breast, and endometrial, there are almost 30 tumor types that are also associated with the E2 pathway [33, 34]. These findings are also associated with changes in outcome for the disease further conveying the importance of understanding this pathway across multiple relevant tumor types. For example, nuclear ERα expression in breast cancer, ovarian cancer, or endometrial cancer is correlated with improved overall survival (OS) compared to cancer patients that are ERα-negative [25, 35–38], while some of the breast cancer patients that were ERα-positive also had increased disease burden. Conversely, cytoplasmic ERα expression in non-small-cell lung cancer (NSCLC) cells is correlated with worse OS [39–41]. Aromatase and ERβ expression in tumor cells are more controversial with studies varying on whether they convey a survival benefit [42–47]. These mixed opinions in the literature are

possibly due to the lack of standardized and clinically validated staining ERβ antibody, as well as the multitude of ERβ splice variants and post-translational modifications [26, 48, 49]. While these findings are consistent with understanding E2 on tumor cells, there is still the need to evaluate the remainder of the TME.

2.3 The E2 Pathway in Tumor-Associated Stromal Cells and Immune Cells

Within the TME, ERs and aromatase are in notable concentrations in stromal and immune cells in addition to neoplastic cells (Table 2.1). A myriad of studies in the past decade have detailed key interactions between neoplastic cells and their recruited stromal cells that are responsible for tumorigenic potentiation (reviewed in [4, 47]). Cellular architecture complicit in this potentiation is heterogeneous between and within tumor cells, but generally includes cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), immune T and B cells, natural killer (NK) cells, and endothelial cells [4]. Transitively, the association of hormonal protein expression in TME stromal and immune cells serves to underlie a potential immunomodulatory role of ER signaling in cancer biology, demonstrated by cell types listed in Table 2.1.

2.3.1 Tumor-Infiltrating Lymphocytes (TIL)

There exists a notable interplay between cancer type and lymphocyte composition of the TME. It is often opposing immune infiltrates within a given primary tumor that promote neoplastic evolution and antitumor immunity [65]. For example, $CD4^+$ T-cell polarization has been identified as a mediator of tumor immune surveillance. Specifically, T helper 1 (Th1) T cell responses are associated with tumor suppression while T helper 2 (Th2) exhibit tumor activation via IFNγ and IL-12 upregulation and IL-4 expression, respec-

Table 2.1 Estrogen receptor (ER) and aromatase expression in stromal and immune cells in the tumor microenvironment

TME cell type	Cancer type	Human expression	Murine expression	Method of evaluation	Reference
Stromal	Breast	Aromatase	ERα	PCR, IHC	[50, 51]
	Melanoma		ERα	IHC	[51]
	Lung		ERα	IHC	[51]
	Endometrial	Aromatase		IHC	[52]
CAF	Breast	ERα		PCR	[53]
	Prostate	ERα, ERβ		IHC	[54, 55]
	Endometrial	ERα, ERβ		PCR	[56]
	Ovarian	ERα		IHC	[57]
TAM	Ovarian	ERα, ERβ		IF,IHC	[58]
	Breast	Aromatase		IHC, PCR	[59]
	Lung	Aromatase	Aromatase	IHC	[17, 60]
MDSC	Ovarian	ERα	ERα	PCR, Western	[57]
NK cells	Breast	ERα, ERβ		IHC	[61]
Effector CD4+/CD8+ T cells	Breast Nonmalignant	ERα, ERβ		IHC	[27, 62]
Tregs	Cervical	ERα		IHC	[63]

Table adapted from [64]

Studies were identified by PubMed searches using keywords: ERα, ERβ, aromatase, stromal, CAF, TAM, MDSC, expression, cancer. *CAF* cancer-associated fibroblast, *TAM* tumor-associated macrophage, *MDSC* myeloid-derived suppressor cell, *IHC* immunohistochemistry, *PCR* polymerase chain reaction, *IF* immunofluorescence, *Western*: Western blotting analysis

tively [66, 67]. Interestingly, several murine and human studies have reported an induction of Th2 response and IL-4 production in settings of elevated E2 [29, 32]. Further support of ER's role in tumorigenesis was illuminated by a recent in silico study showing an increase in Th1 T cells, B cells, and cytotoxic T lymphocytes (CTLs) in ER-negative breast tumors relative to ER-positive breast tumors [68]. This study additionally saw an inverse correlation between ER activity and immune infiltration of these cell types in breast cancer tissues. The inverse correlation observed affirmed previous reports that increased TIL, specifically $CD8^+$ T cells, in ER-negative tumors correlated with improved OS [68, 69]. Additionally, post hoc analysis in ER-positive breast cancer patients treated with letrozole showed increased infiltration of B and Th1 cells both at the initiation and at the end of treatments [68].

2.3.1.1 Cytotoxic T Cells and Natural Killer Cells

Granule-mediated exocytosis of serine proteases, such as granzyme B, is a major pathway CTLs and NKCs initiate caspase-dependent apoptosis to eliminate pathogenic and tumor cells [70, 71]. Jiang et al. cultured ERα-expressing human liver carcinoma cells with E2 resulting in upregulated expression of the granzyme B inhibitor, proteinase inhibitor-9 (PI-9). This upregulation protected the tumor cells against granule-mediated exocytosis by these cells per DNA fragmentation assays [72]. A similar study illustrated E2-induced PI-9 expression was also observed in ERα-positive MCF7 breast cancer cells with the same protection, while PI-9 knockdown blocked E2's protective effect [73]. Cumulatively, these studies suggest a component of E2 immunosuppression is via inhibition of NK- and CTL-mediated tumor cell elimination.

2.3.1.2 Regulatory T Cells

T cell activation and effector differentiation are integral to the adaptive immune response. $FoxP3^-$ expressing Tregs subdue neoplastic activity, as well as responder T cell expansion, through secretion of immunosuppressive cytokines [74]. Administration of physiologic doses of E2 to immunocompetent, ovariectomized mice has been observed to expand $CD4^+CD25^+$ Treg concentration, as well as Foxp3 expression in various tissue types [75]. Furthermore, fluorescence-activated cell sorting (FACs) assays revealed acquisition of CD25 in E2-incubated ERα-expressing $CD4^+CD25^-$ cells [75]. These transformed $CD4^+CD25^+$ T cells then exhibited an immunosuppressive Treg phenotype in vitro that significantly downregulated T cell concentration [75–78]. Additional studies have reported E2-stimulated Foxp3 expression in murine Tregs, expression of which is vital to Treg functionality. High $FoxP3^+$ Tregs in the TME is a negative prognostic indicator in a variety of cancers. For example, early-stage NSCLC with nuclear ERα expression has a relatively higher risk of both recurrence and $FoxP3^+$ lymphocyte infiltrate [79]. Furthermore, a recent meta-analysis reported $FoxP3^+$ Treg infiltration correlated negatively with OS in ER-positive breast cancer patients and positively in ER-negative patients [80]. Conversely, studies of ERα-positive breast tumors treated with letrozole in vivo demonstrated a resulting reduction of $FoxP3^+$ Tregs [81].

E2 appears to suppress Treg expression in both physiologic and ERα/ERβ knockout mice, with the former group having increased expression of programmed-death 1 (PD-1) and the latter having decreased PD-1 expression [82]. E2 treatment of ERα-positive endometrial and breast cancer cells also stimulates in vitro expression of the PD-1 ligand (PD-L1) via activation of PI3K signaling [83]. $PD\text{-}L1^+$ tumor cells exhaust $PD\text{-}1^+$ cytotoxic T lymphocytes (CTLs) through this protein interaction, resulting in tumor immune evasion [84]. Given E2's upregulation of both PD-1 and PD-L1, the hormone appears to have an important influence on the pathway and its role in the TME.

2.3.2 Stromal Cells

Tumor evolution is heavily dependent on malignant tissue as well as recruited stromal cells that

interact between and within the TME. Via an in vivo murine model, ERα expression in stromal cells was observed within the context of tumor-cell-independent ER signaling in the TME. E2 interactions with stromal ERα has also been seen to accelerate neoplastic growth and blood vessel density in ovariectomized, syngeneic mice transplanted with ER-negative melanoma, breast, or lung cancer cells [51]. The same study found this E2-stimulated tumor growth demonstrated a relative increase in immunocompromised mice, reflecting closer association with E2 modulation of innate immunity [51]. Aromatase expression appears to also modulate the TME in certain neoplasms. Perineoplastic endometrial stromal cells' expression of aromatase also correlates with more advanced disease and, transitively, worse OS [52, 85]. Similarly, perineoplastic breast adipocytes' expression of aromatase appears to be complicit in tumorigenesis in obese patients via inflammation and modification of the TME [50, 86, 87]. Additionally, type 2 pericytes have also been associated with tumorigenesis and vascular formation for tumors [88]. Pericytes recruited for vascular formation have been associated with ERα expression and E2-dependent signaling during function [89, 90].

2.3.3 Cancer-Associated Fibroblasts

CAFs are one of the most integral stromal cell types in the TME for tumor survival and metastasis via paracrine-induced signaling pathways via chemokines and soluble growth factors [91, 92]. ERα expression in breast CAFs have been observed in vivo through nuclear receptor arrays comparing gene expression between CAFs and normal human breast adipose fibroblasts [53]. Interestingly, similar levels of ERα expression are seen in both malignant and physiologic fibroblasts, but with downstream upregulation of the direct transcriptional activator liver receptor homolog-1 (*LRH-1*) in the former [53]. The regulator serves to increase expression of the aromatase-encoding gene *CYP19A1* [93–95]. Co-expression of aromatase and LRH-1 in the breast TME suggests CAF-induced paracrine formation of E2 and subsequent ER-mediated oncogenesis [96]. Coculturing of endometrial CAFs with endometrial neoplastic cells have been seen to contribute to tumor progression, possibly attributed to CAFs' expression of ERα and ERβ [56]. This tumor progression mechanism is supported through in vitro upregulation of phosphatidylinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling networks, which are both well-known ER-mediated pathways in breast and lung cancer [56, 97–99].

Contrastingly, ER expression in prostate CAFs has contradicting evidence, with reports of ERα/ERβ expression portending advanced disease [54] and others suggesting ERα expression is a protective factor again neoplastic invasion macrophage infiltration [100, 101]. These latter in vitro studies conveyed that CAF ERα expression reduced murine and human prostate cancer cell invasion, as well as lymph node metastasis of orthotopically implanted human prostate cancer cells in mice [101]. These ERα-positive CAFs appeared to halt invasion and metastasis of human prostate cancer cells through downstream downregulation of the C-C motif chemokine ligand 5 (CCL5) and IL-6 chemokines, whose roles are involved in growth factor signaling, inflammation, and tumor recruitment [102, 103].

2.3.4 Tumor-Associated Macrophages

In a physiologic setting, macrophages regulate tissue-specific innate immune responses to fight foreign invaders through polarization by varied cytokines. However, TAMs have been complicit in tumor proliferation and migration, as well as inflammation in the TME [104, 105]. Physiologically, polarized M1 macrophages secrete the proinflammatory cytokines IFNγ, interleukin 12 (IL-12), and tumor necrosis factor (TNF)-α for tumor rejection and antigen presentation [106]. Alternatively, M2 macrophages produce interleukins 4, 5, 6, and 10 [106], which are known promoters of tumor cell growth and immune evasion [107]. TAMs within the TME

are often M2, with denser concentration demonstrating worse OS, thus offering a therapeutic opportunity for a variety of malignancies [108].

TAMs are an independent poor prognostic predictor for ovarian adenocarcinoma [109]. Relatedly, co-localized expression of both ERα/ERβ is reported in human high-grade serous ovarian cystadenocarcinoma (HGSOC) TAMs. Interestingly, HGSOC in premenopausal women demonstrates elevated TAM infiltration relative to that of postmenopausal women. The highest concentration of TAMs in this TME can be found in ERα-positive tumors [58]. The mechanism of this was elucidated by an IHC analysis revealing aromatase expression in the TME of breast TAMs, which was observed to increase E2 production and breast cell proliferation [59]. TAM proliferation, however, is relatively more prevalent in ER-negative breast malignancies [110, 111]. It is important to note, however, that quantification of TAM polarization was not analyzed in these studies. Interestingly, aromatase and ERβ expression in NSCLC TAMs have also been observed, specifically in infiltrating macrophages of preneoplastic, tobacco carcinogen-induced murine lung lesions [17, 60].

Although direct observation of ER expression in TAMs has been limited, E2 induction of M2 polarization and subsequent tumor spread has been studied. A polyomavirus middle T (PyMT), ER-positive breast cancer murine model demonstrated direct E2 stimulation of tumoral M2 TAM infiltration and vascular endothelial growth factor (VEGF) [112, 113]. Alternatively, untreated controls exhibited M1 TAM infiltration instead [112]. In a HGSOC, ovariectomized murine model, E2 induced growth of both ER-negative xenografts and M2 TAM infiltration [58]. In tobacco carcinogen-exposed mice, administration of E2 increased pulmonary TAM infiltration while mice receiving the aromatase inhibitor anastrozole had a significant reduction in pulmonary TAMs [114]. Further, E2-induced VEGF expression was also observed in this model [114]. Of note, E2-mediated TAM infiltration has been observed in vitro to be fed forward via M2 TAM-induced epigenetic ERα upregulation via interleukin 17A (IL-17A) in endometrial malignancy [115]. This positive feedback mechanism between E2 and M2 TAMs provides a potential therapeutic target, a concept recently addressed via effects of the phytoestrogen SERM resveratrol in a lung cancer xenograft model [116]. Resveratrol treatment appeared to suppress tumor proliferation through decreased signal transducer and activator of transcription 3 (STAT3) signaling and M2 polarization [116].

2.3.5 Myeloid-Derived Suppressor Cells

MDSCs are another myeloid cell present in the TME known to interfere immune surveillance and facilitate tumor growth [117]. ERα expression in human ovarian adenocarcinoma MDSCs has been identified by IHC and confirmed through PCR and immunoblotting [57]. In an E2-insensitive syngeneic ovarian cancer model, ovariectomized mice exhibited improved survival compared to non-ovariectomized mice following tumor challenge. Contrastingly, E2 supplementation in these mice accelerated tumor progression and reversed the protective effect found in estrogen-depleted mice [57]. Of note, this study found that T-cell-deficient mice lost survival benefit of estrogen depletion, suggesting adaptive immunocompetence to be mechanistically integral [57]. Estrogen's effect on the two legs of immunity was also observed in E2-treated mice, which were found to have notably decreased concentrations of helper and cytotoxic T cells, and significantly increased concentrations of granulocytic MDSCs in spleen and tumor beds [57]. ER-dependence of MDSC expansion was further studied with in vitro administration of the ERα antagonist methylpiperidino pyrazole (MPP) to inhibit MDSC proliferation [57]. Ovarian tumor-bearing mice treated with E2 had measurable JAK2 and SRC upregulation with downstream STAT3 signaling, a regulator of myeloid differentiation and development [118]. In syngeneic lung and breast cancer murine models, E2-stimulated tumor growth was mitigated by MDSC depletion after treatment with anti-Gr1 antibodies [57, 119, 120]. Patients with cervical cancer that were pregnant with high E2 had increased expansion

of MDSCs and shorter PFS. These findings were further evaluated in mouse models [120].

2.3.6 Inflammatory Cytokines and Eicosanoids

Chronic inflammation has been accepted as a common factor in tumorigenesis and spread. TME facilitates neoplastic progression primarily through cytokine-induced oncogenic pathway activation, leading to cell proliferation, immune evasion, and infiltration [121]. IL-6 from TAFs has been observed to assist ERα-positive breast cancer proliferation and immune evasion [122] via STAT3 activation in vitro and in vivo [123]. TNFα in ERα-positive breast cancer cells has been observed to regulate gene expression for metastasis [124]. This cytokine has also been shown to upregulate aromatase expression in cultured human adipose stromal cells [125]. Neoplastic implication of these inflammatory markers is evidenced by data showing TNFα and IL-6 correlate closely with aromatase expression in human breast cancer tissue and not in adjacent noncancerous tissue [126]. Aromatase has similar transcriptional correlation with cyclooxygenase-2 (COX-2) [126]. COX-2 mediates the inflammatory response by producing eicosanoids such as prostaglandin E2 (PGE2) [127], which upregulates aromatase expression through cyclic adenosine monophosphate (cAMP) in breast malignancy [128]. Despite conflicting reports, a case–control study demonstrated regular administration of the nonsteroidal anti-inflammatory drug (NSAID) aspirin reduced the risk of developing ERα-positive breast cancers (hazard ratio (HR) = 0.74; 95% CI, 0.60–0.93), but not ERα-negative cancers (HR = 0.97; 95% CI, 0.67–1.40) [129].

ERα, TNFα, and NF-κB protein expression correlate closely in breast cancer tissues [130]. NF-κB signaling, a proinflammatory cytokine associated with IL-6 and TNFα, is often constitutively activated in many tumor types [131]. High levels of the cytokine are also implicated in SERM resistance in ERα-expressing human breast cancer cells [132, 133]. E2 also enhanced pulmonary inflammation through increased NF-κB, VEGF, and IL-17A in a murine model evaluating tobacco carcinogen-induced lung cancer [114]. E2 inhibition with combined AI/NSAID treatment served to noticeably decrease pulmonary malignancy in these mice. Notable pathways affected included IL-17A expression, IL-6 concentration, as well as STAT3 and MAPK [114]. Cumulatively, there appears to be a potential target for the E2 pathway as it interacts with tumorigenesis via inflammation.

2.3.7 The Impact of Supraphysiologic Estrogen

Esterified estrogen, specifically estrone, is significantly increased in the setting of obesity. Aromatase in adipocytes serves to increase estrone secretion in the setting of hypertrophy. The effect this supraphysiologic estrogen has on tumorigenesis has been controversial [134, 135]. Recent findings suggest that while immune dysfunction and tumor progression are associated with obesity, improved response to immunotherapy may also be associated with obesity, supporting the immune-mediated link between obesity and cancer [136]. Chronic inflammation from obesity is integral to carcinogenesis and tumor evolution, as observed in postmenopausal, ER- and progesterone receptor (PR)-expressing breast malignancy [137]. It is important to note that studies suggesting protumor effects of estrogen in estrogen-depleted mammals have been performed primarily in the setting of hormone replacement therapy (HRT).

Tumorigenesis, progression, and infiltration in the setting of HRT in estrogen-depleted mammals remain controversial. There is a paucity of studies demonstrating proinflammatory changes with hormone replacement therapy in murine models. In contrast, there are many studies conveying a protective effect of exogenous estrogen. Specifically, ERβ-expression has been observed to prevent progression of human colorectal carcinoma (CRC) [134, 138]. Mechanistic protection against carcinogenesis with exogenous estrogen in postmenopausal patients appears to primarily

be through a decrease in the natural postmenopausal increase in Th1/Th2 ratio [139, 140]. Specifically, Th2 cytokines are quantifiably stable until late postmenopausal stage, while production of Th1 cytokines is progressively increased in women after menopause. HRT prevents this increased Th1/Th2 ratio, thereby improving the aberration of Th1/Th2 balance that is implicated in an inadequate immune response and neoplastic conditions [140]. Substantiation of this antitumoral concept was provided through an in vivo, placebo-controlled study regarding postmenopausal human breast cancer cell demonstrating estrogen's notable decrease in IL-6 production [141].

2.4 Clinical Implications of Targeting the Estrogen Pathway in the Tumor Microenvironment

Immunotherapy is a developing and effective treatment avenue in the world of cancer; yet the TME and its immunosuppressive mechanisms is a deterrent for large-scale success. As it stands, the immune checkpoint modulators of cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and PD-1/PD-L1 are the most studied immunotherapies [142]. These revolutionary options have had dramatic impacts on OS relative to standard-of-care chemotherapies [143–146]. Even so, response rates are limited to 20–35% of cases, closely dependent on tumor type, stage, and PD-L1 expression [147]. Moreover, 25–33% of melanoma patients often demonstrate delayed relapse during treatments attributed to tumor cell adaptation [5, 6].

There appears to be a balance of tumoral mutations and immunoediting that facilitate immune evasion, and subsequently, failure of checkpoint therapy. On the one hand, damaged DNA repair mechanisms, increased non-synonymous somatic mutational load, and neoantigen presentation cripple immune evasion and improve OS [2, 3, 148]. On the other hand, damage to antigen-presenting mechanisms, as well as recurrence of nonantigenic mutations, appears to facilitate immune evasion [149, 150]. Studies identifying these mechanisms provide insight into measurable biomarkers to assess tumor responsiveness to current and, inevitably necessary, novel immunotherapies. A potential investigative therapy is endocrinological agents that modulate E2 and its protumoral pathway to abrogate tumor immune evasion. Specifically, anti-estrogen therapy may reduce TME immunosuppression while increasing E2-sensitive tumor responsiveness.

Recently, a high-throughput screening assay in human lung cancer cells demonstrated fulvestrant, an anti-estrogen agent, as the most efficacious compound in increasing tumor sensitivity to immune-mediated lysis [151]. Fulvestrant additionally has few interactions and overlapping toxicities with anti-PD-1/PD-L1 agents. Thus, anti-E2 therapies to target the immunosuppressive TME could increase efficacy and duration of response of current immune checkpoint inhibitors (ICI) [119, 152] (Fig. 2.1).

Based on the well-established evidence of sex-driven dimorphism in immune function and response, patient sex has been postulated to have an influence on the efficacy of ICIs [9]. This sexual dimorphism plays an important role in the disparity of cancer immunoediting in females and males and could not only explain differences in progression and mortality observed between male and female cancer patients but also sex differences in response rates, toxicity patterns, and outcomes to treatment with ICIs. In support of this concept, the PD-1/PD-L1 pathway is modulated by multiple X-linked microRNAs (miRNAs), which crosstalk with the estrogen-ERα axis, suggesting an important role of the estrogen pathway and response to ICIs [153, 154]. Further since estrogen modulation of the PD-1/PD-L1 pathway has been demonstrated in animal models [82, 155], it is reasonable to expect that immunotherapy efficacy may vary according to patient sex.

In an effort to identify patient characteristics linked to ICI effectiveness, several meta-analyses have been conducted to evaluate sex-differential effects in efficacy of ICIs. Conforti et al. evaluated the effect of patient's sex on the efficacy of

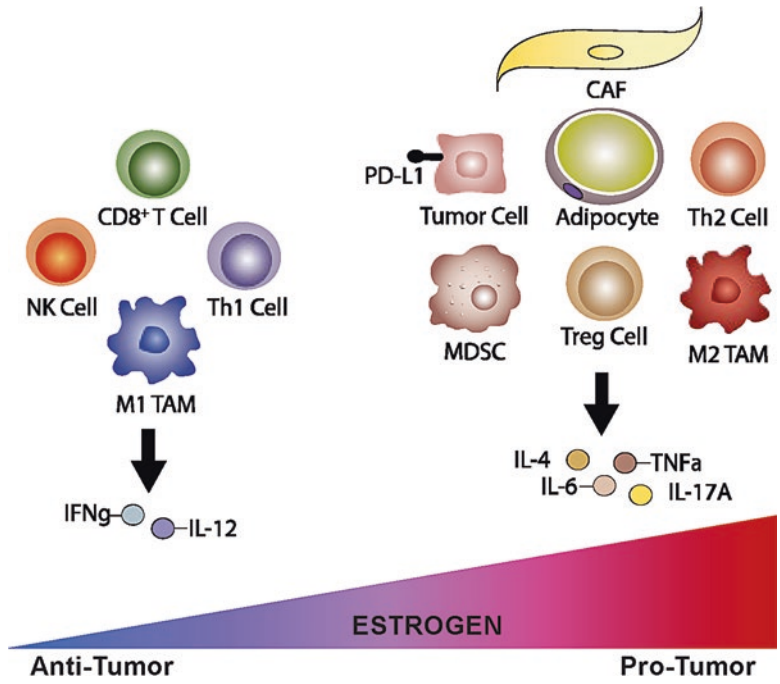

Fig. 2.1 Increasing estrogen promotes a pro-tumor TME via increased Th2 responses, increased production of tumor-promoting cytokines (IL-4, IL-6, TNFα, and IL-17A), M2 TAM infiltration, decreased Th1 cytokines (IL-12 and IFNγ), and M1 TAM infiltration. E2 has also been associated with increased Treg and MDSC proliferation, increased PD-L1 expression on tumor cells, and decreased $CD8^+$ T cell and NK cell proliferation. CAFs and adipocytes may also serve as pro-tumor as they can supply E2 and IL-6. (Adapted from [64])

ICIs measured in terms of OS on different tumor types [156]. This study included 11,351 patients (67% men and 33% women) enrolled in 20 Phase II and III randomized controlled trials that evaluated CTLA4 inhibitors, as well as PD-1/PD-L1 inhibitors in patients with different tumor types, mostly melanoma and NSCLC. Results showed that male patients who received ICIs alone had a reduced risk of death compared to men in the control arms (HR = 0.72, 95% CI 0.65–0.79). Similar findings were observed in female patients, but the difference in risk reduction was smaller between the treatment and the control arm (HR = 0.86, 95% CI 0.79–0.93). Although there was a significant difference in the efficacy of ICIs between male and female patients, the heterogeneity test for this sex-related interaction was not quite significant.

A subsequent meta-analysis evaluated the differences in outcomes based on sex in lung cancer patients who received targeted therapy or immunotherapy [10]. This study included a total of 12 Phase III clinical trials evaluating EGFR, ALK, and PD-1 inhibitors versus chemotherapy. Of the 12 trials included in this meta-analysis, five compared PD-1 inhibitors versus chemotherapy, two of which compared pembrolizumab versus chemotherapy (KEYNOTE 010 and KEYNOTE 024), and three compared nivolumab versus chemotherapy (CheckMate 017, CheckMate 026, CheckMate 057) [144, 157–159]. The studies that compared ICIs versus chemotherapy included 1028 female and 1435 male lung cancer patients. While there was significant heterogeneity between STUDIES, OS was favorable in male patients treated with ICIs compared to chemotherapy (HR = 0.76; 95% CI 0.068–0.86; $p < 0.00001$). There was no significant difference in survival in female lung cancer patients receiving chemotherapy compared to ICIs (HR = 1.03; 95%CI 0.89 to 1.03; $p = 0.69$). In a separate study focused on metastatic NSCLC, El-Ostra et al. evaluated results from eight randomized clinical trials for predictors of benefit to single agent ICIs over chemotherapy [8]. NSCLC patients treated with ICIs had significant progression-free survival (PFS) superiority in ever-smokers, male patients, and patients with PD-L1-positive tumors. In contrast, female NSCLC patients had comparable PFS between ICIs and chemotherapy.

Wallis et al. also conducted a meta-analysis that included 23 randomized clinical trials (67.9% men and 32.1% women) that compared ICIs (both ICI alone and ICI plus chemotherapy trials) to standard-of-care treatment in advanced solid tumors (including NSCLC, SCLC, urothelial carcinoma, head and neck squamous carcinoma, melanoma, mesothelioma, clear cell renal carcinoma, and gastric or gastroesophageal carcinoma). In this study, no difference in OS between men and women who received immunotherapy was observed ($I^2 = 38\%$; $p = 0.6$) [160]. The conflicting results and limitations in these meta-analyses suggest that further investigation of the efficacy of ICIs and patients' sex is warranted in future studies. While the majority of the trials included in these studies were underpowered to detect clinically relevant sex differences in outcome, these results indicate that the hormonal milieu may have some effect on treatment response (Table 2.2).

The current best predictive markers of therapeutic response to ICIs are high PD-L1 expression and high tumor mutational burden (TMB). The difference between PD-L1 expression between men and women has been evaluated in some cancer patient cohort with a reported increased PD-L1 expression in male patients [161–163]. TMB has also been shown to be lower in women compared to men ($p = 0.0349$), across multiple studies [164, 165]. TMB is predictive of response to ICI in lung cancer and is lower in female lung cancer patients compared to male lung cancer patients [165]. Similarly, sex differences in immune-related adverse events (irAEs) have also been noted in ICI trials [166, 167]. The gut microbiome and obesity are emerging areas of interest that may predict response to ICIs [168]. Whether or not these factors interact with sex hormones in the context of anti-cancer immunity is yet to be determined.

2.5 Conclusions and Perspective

The E2 pathway is an identified promoter of tumorigenesis in several cancers, largely for its genomic, epigenomic, and transcriptional effects on tumor cells and the TME. The reciprocal interactions of the peritumoral and tumoral environment are becoming more evident, with E2 playing a major role in modulation of primarily protumoral pathways. With immunoediting being a culprit in E2-mediated protumoral activity, it appears to be an important deterrent for checkpoint blockade immunotherapy success. Thus, inhibition of the E2 pathway may augment current immunotherapy response rates.

Carcinogenesis from obesity and its related illnesses are thought to be primarily driven through proinflammatory cytokine secretion. Supraphysiologic estrogen from adipocyte aromatase expression may also play a role, but as of now, it is difficult to distinguish. However, estrogen replacement therapy in postmenopausal women appears to have a relatively protective effect via immune modulation. Stabilization of immunologic aberrancies, notably in the adaptive immune system, is protective against age-related malignancies such as colorectal carcinoma and breast cancers. Based on the above discussion, future studies are war-

Table 2.2 Selected trials evaluating the combination of Estrogen pathway targeting agents with ICIs

Malignancy	Selected study drugs	*n* =	Clinical trial number
ER+/Her2- Breast cancer	Exemestane and durvalumab/tremelimumab	240	NCT02997995
ER+/Her2- Breast cancer	Pembrolizumab, letrozole, and palbociclib	22	NCT02778685
ER+/Her2- Breast cancer	Atezolizumab and fulvestrant	126	NCT03280563
ER+/Her2- Breast cancer	Pembrolizumab and exemestane	25	NCT02990845
ER+/Her2- Breast cancer	Pembrolizumab and AI	37	NCT02971748
ER+/Her2- Breast cancer	Pembrolizumab and letrozole, exemestane anastrozole	56	NCT02648477
AR+/ER- Breast cancer	Pembrolizumab and enobosarm	29	NCT02971761

Selected ongoing trials evaluating ICI in combination with therapeutic agents targeting the E2 pathway. Disease type, selected study agents, predicted accrual size, and clinical trial number are provided

ranted to assess responsiveness to current ICIs across sex, menopausal status, and BMI in order to isolate E2 pathway contribution to immune evasion.

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