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Dynamic crosstalk within the tumor microenvironment of uterine cervical carcinoma: baseline network, iatrogenic alterations, and translational implications

Rosalba De Nola [a,b,c,*], Vera Loizzi [b], Ettore Cicinelli [b], Gennaro Cormio [b,c]

[a] Department of Tissues and Organs Transplantation and Cellular Therapies, D.E.O.T., University of Bari "Aldo Moro", Piazza G. Cesare, 11-Policlinico, 70124, Bari, Italy
[b] Department of Biomedical and Human Oncological Science, Division of Obstetrics and Gynecology, University of Bari "Aldo Moro", Piazza G. Cesare, 11-Policlinico, 70124, Bari, Italy
[c] Gynecologic Oncology Unit, IRCCS, Istituto Tumori Giovanni Paolo II, 70142, Bari, Italy

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ABSTRACT

Uterine cervical cancer is the fourth most frequent gynecological tumor worldwide. The tumor microenvironment of cervical cancer is the result of persistent high-risk human papillomavirus infection together with stromal activation of estrogen receptor alpha and the pro-angiogenic and pro-inflammatory activity of immune cells, mainly T-helper 17 cells and tumor-associated macrophages. Therapeutic management (e.g., immunotherapy, especially in advanced cases) may be influenced by the translational implications of tumoral stroma crosstalk and an abundance of tumor-infiltrating lymphocytes within the tumor microenvironment. The prognosis of cervical cancer is inversely correlated with microvessel density, making anti-angiogenic strategies with agents such as bevacizumab crucial for improving both progression-free survival and overall survival in patients with advanced and recurrent tumors.

1. Introduction

Uterine cervical cancer (UCC) is the fourth most frequent gynecological tumor worldwide, with 569 847 new diagnoses in 2018 (International Agency for Research on Cancer) (Cohen et al., 2019) and a projected total of 44.4 million cases by the year 2069 in the absence of further intervention (Simms et al., 2019; Brisson and Drolet, 2019). The implementation of screening campaigns together with broad-spectrum human papillomavirus (HPV) vaccination could potentially prevent about 7 million cases in the next half century (Simms et al., 2019).

The newest guideline issued by the *Féderation Internationale de Gynécologie et d'Obstétrique* (FIGO) outlines a series of imaging techniques, surgery, and pathology examination as tools for staging cervical cancer, representing as a step forward from the previous exclusively clinical approach (Bhatla et al., 2019). However, a clinical approach is still considered acceptable in low-middle income countries given the demanding costs of additional exams (Bhatla et al., 2019).

The therapeutic management of UCC should be decided by a multidisciplinary board that considers desired fertility, disease stage, pathological features, and patient risk factors as thoroughly illustrated in the most recent guidelines jointly developed by the European Society of Medical Oncology (ESMO) (Marth et al., 2017), the European Society of Gynaecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP) (Cibula et al., 2018).

With regard to biological context, UCC is characterized by persistent high-risk HPV infection and resultant cancerous lesions, a specialized tumor microenvironment (TME) together with the estrogen *milieu*, and the immunological host response (Galliverti et al., 2020; Kumar et al., 2016; Shew et al., 2002; Hong et al., 2017; Spurgeon et al., 2017). In a previous review, we explored the biological aspects and translational relevance of the crowded crosstalk between cancer cells and the surrounding stroma in gynecological malignancies (De Nola et al., 2019). The present review provides a framework for the main biological pathways governing tumor-associated cells in the UCC microenvironment. Specifically, we discuss the biological mechanisms underlying the

* Corresponding author at: Department of Tissues and Organs Transplantation and Cellular Therapies, D.E.O.T., University of Bari "Aldo Moro", Piazza G. Cesare, 11-Policlinico, 70124, Bari, Italy.
E-mail address: rosalba.denola@uniba.it (R. De Nola).

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iatrogenic effects of chemotherapy, radiotherapy (RT), concurrent chemo-radiotherapy (CCRT), and new biological agents on the TME in UCC, especially in squamous cell UCC and adenocarcinoma (ACC).

2. An updated synopsis of crosstalk between epithelial cells and their stromal *milieu* in the uterine cervix under the effect of estrogens and HPV

The stromal microenvironment of UCC harbors peculiar features among all solid tumors due to persistent high-risk HPV (hr-HPV) infection, especially with strains 16 and 18 (Liang et al., 2018). The tumoral *milieu* is mostly populated by estrogen receptor alpha (ERα)-positive myofibroblasts and fibroblasts, leukocytes, tumor-associated macrophages (TAMs), and lymphocytes positive for the following clusters of differentiation (CD): $CD4^+$, $CD8^+$, $CD103^+$, and $CD17^+$ (Kumar et al., 2016; Liang et al., 2018; Chung et al., 2008; Ding et al., 2014; Chen et al., 2017; Zhang et al., 2006). CD 103^- natural killer (NK) cells are also present, with a mild representation of dendritic cells (DCs), the primary antigen-presenting cells (Walch-Ruckheim et al., 2015, 2019). Activation of stromal ERα induces the release of inflammatory chemokines, extracellular matrix (ECM) proteinases, and anti-apoptotic/pro-angiogenic molecules from myofibroblasts (De Nola et al., 2019) (Fig. 1 and Table 1).

More than 99 % of UCCs express hr-HPV oncogenic proteins, namely E6 and E7, which disrupt the tumor suppressor functions of protein 53 (p53) and retinoblastoma protein (Rb-p) (Eun-Kyoung Yim, 2005). Specifically, E6 binds p53 and marks it for degradation via ubiquitination, whereas E7 mainly targets Rb-p, resulting in apoptosis escape and the loss of cell-cycle blockade (Eun-Kyoung Yim, 2005). A recent preclinical study demonstrated tumor suppression in a murine model of

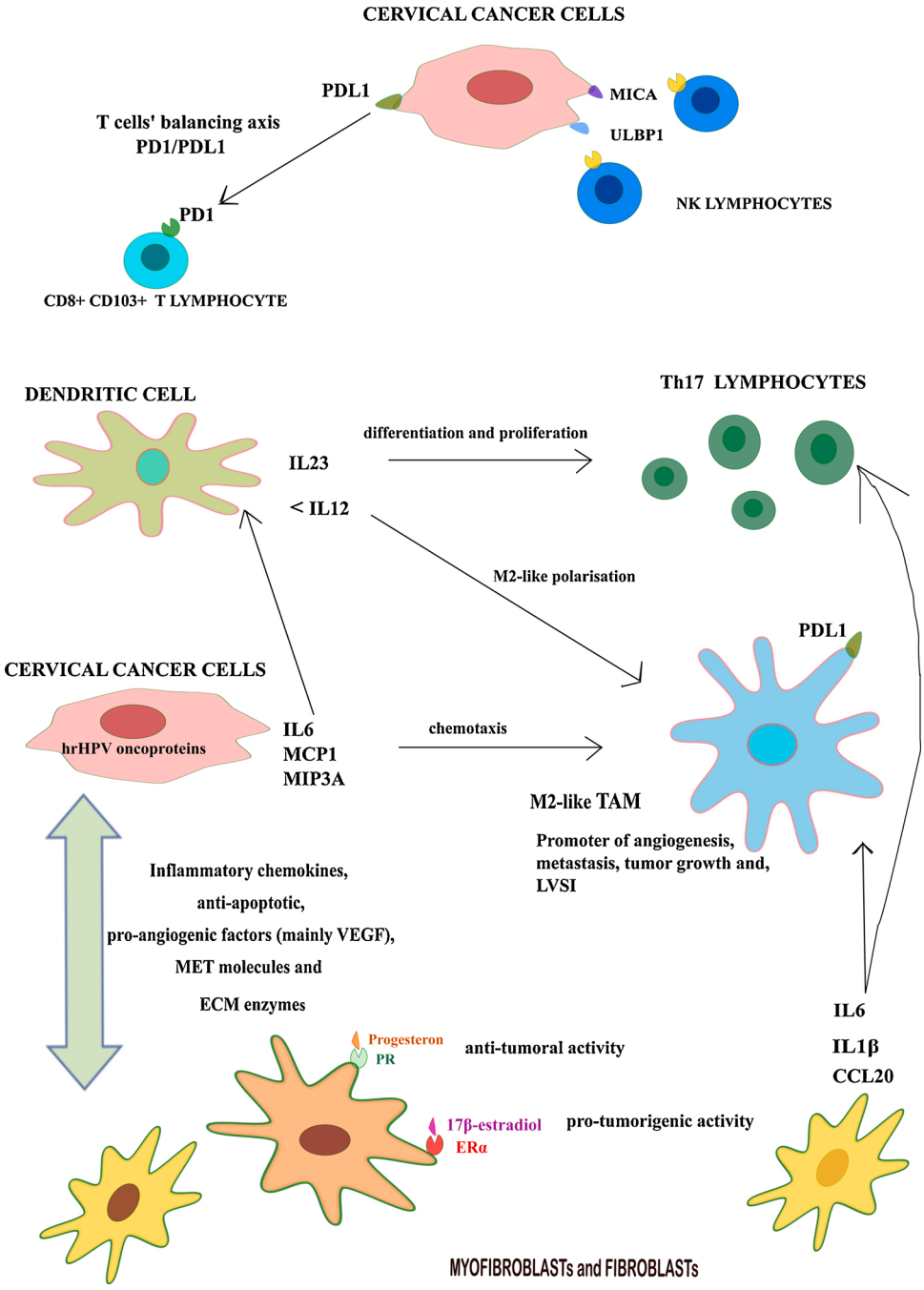

Fig. 1. Crosstalk between tumoral and stromal cells within the cervical cancer microenvironment. Cervical cancer stromal cells mainly consist of $ERα^+$ fibroblasts and myofibroblasts, tumor-associated macrophages (TAMs), and tumor-infiltrating lymphocytes (TILs). 17-β-Estradiol (E2) binds the stromal ERα on the surface of fibroblasts and myofibroblasts, triggering the secretion of inflammatory chemokines, anti-apoptotic factors, pro-angiogenic factors, and extracellular matrix enzymes. Epithelial cells with persistent high-risk HPV + infection (pre-cancerous cells) chemoattract monocytes (MCP1 and MIP3A), Th17 lymphocytes (CCL20, IL6), and natural killer (NK) cells. The oncoproteins (E6 and E7) of high-risk HPV strains are thought to trigger a cascade of events via host cells of the cervix epithelium. In detail, these cells participate in the mesenchymal-to-epithelial (MET) transition by inducing proliferative, pro-angiogenic, and pro-inflammatory effects in concert with the stromal ERα /E2 pathway, but are contrasted by the stromal progesterone cascade via the progesterone receptor (PR). Th17 lymphocytes exert a chronic pro-inflammatory and pro-tumorigenic action within tumor microenvironment (TME), whereas NK lymphocytes have a direct anti-tumoral function. Tumor cells express MICA/B and ULBP1 that bind NKG2D expressed on NK cells. M2-like TAMs play a crucial role in the TME since they promote angiogenesis, lympho-vascular space invasion (LVSI), lymph node metastasis, and tumor overgrowth. Dendritic cells (DCs) that present the antigens from cancer cells characterized by HPV protein expression are polarized towards a pro-tumorigenic pattern due to the presence of IL6 released by cancer cells and fibroblasts/myofibroblasts. In this phenotype, DCs reduce their secretion of IL12 and increase the release of IL23, inducing the Th17 polarization of T-helper cells within the stroma. In the end, a key potential pathway for escaping the cytotoxic activity of $CD8^+$ T lymphocytes after the effects of chronic hr-HPV infection involves M2-like TAMs: the PD/PDL1 axis.

Table 1
The main cellular subpopulation of the UCC: pathogenesis and translational possibilities.

Cell-type	Pathogenetic role	Translational possibilities
Persistently high-risk HPV + keratinocytes	Anti-inflammatory action in early stages; chemoattraction for monocytes (via MCP1 and MIP3A), Th_{17} (CCL20, IL6) and also NK cells in advanced stages; pro-angiogenic action via upregulation of BDK/BDKR2 axis and consequent upregulation of VEGF	Targeting EGFR, CCL2 (i.e. MCP1); CCL20, (i.e. MIP3A). Using IL6 (increasing chemosensitivity) or anti-VEGF and anti-BDKRB2 E6/E7-targeted vaccine
PR^+ $ER\alpha^+$ fibroblasts and myofibroblasts	MET (via miR-9−5p) Secretion of pro-inflammatory chemokines, anti-apoptotic molecules, pro-angiogenic factors, and ECM enzymes	Targeting stromal ERα, IL1A and IL1B, FGF9, HBEGF, CXCR2 and its ligands CXCLs (mainly CXCL5 and CXCL1), MMP9. MPA (via upregulation of Hand2 and Mic/cyclin E after PR binding) could treat CIN preventing UCC Itraconazole (ITC) as an "h-h" inhibitor (the molecular bridge between estrogens and hr-HPV persistent infection)
CD 103 + CD 8+ T lymphocytes	Cytotoxic activity against cancer cells	E6/E7-targeted vaccine plus radiotherapy increase their TILs fraction via TGFβ. Higher CRT and immunotherapy responsiveness
CD163$^+$ CD68$^+$ M2 TAMs	Promote angiogenesis, LVSI, lymph node metastasis, tumor progression, and growth Create a cancer-tolerant immune-regulatory TME	Re-education toward an antitumor, immunostimulatory function (paclitaxel via TLR4 signaling or mAbs against E6/E7); blocking monocytes migration to the TME; activate the phagocytic activity of TAMs; blockade of PD-L1 on TAMs (avelumab, nivolumab pembrolizumab) in combination with traditional therapy (ideally two weeks after radiotherapy or RCT) as a final adjuvant.
Th_{17} lymphocytes	Chronic pro-inflammatory/pro-tumoral effect	mABs against IL1β, IL17α, IL17, and IL23p19 or IL6/C/EBPb/CCL20 targeting even in association with radiotherapy; re-education under IL2 *stimuli*
NK lymphocytes	Anti-tumoral activity towards cells MICA + and ULBP1+ (NKG2DLs)	Clonal autologous expansion; vaccines against MICA and ULBP1

re-adapted from De Nola et al. (2019)

cervical carcinoma after experimental monoclonal antibody (mAb) treatment with anti-HPV16E6 and anti-HPV18E6 or anti-HPV16E7 (Jiang et al., 2019). A significant reduction in tumor mass was observed after the first dose and the result after serial treatments was non-inferior to cisplatin therapy (Jiang et al., 2019). Pathological examination revealed that tumor necrosis and complement deposition depended on the number of treatments; mAbs targeted $E6^+/E7^+$ cancer cells that consequently underwent degradation via opsonization, releasing additional antigens (Jiang et al., 2019). Another suggested action of mAb treatment was the repolarization of TAMs towards the M1-like anti-inflammatory, anti-proliferative phenotype (Jiang et al., 2019). mAbs against viral oncogenic proteins require further assessment in phase I studies alone or in combination with RT and/or chemotherapy (Jiang et al., 2019).

In UCC, hr-HPV infection acts synergistically with the ERα biochemical cascade in cancer-associated fibroblasts (CAFs) within the stroma (Spurgeon et al., 2017). When stromal receptors are blocked or degraded, estrogens lose their pro-tumoral activity as their action at neighboring epithelial receptors is inconsequential for tumor progression (Son et al., 2018). The human keratin 14 (K14) promoter (Chung et al., 2008) of the E6/E7 viral oncoproteins induces hyperplasia of the basal layer of the cervical epithelium (Rojo-Leon et al., 2019). When E6/E7 transgenic mice were treated with 17-β-estradiol (E2), initial hyperplasia converted into UCC, consistent with epidemiological and pathological observations (Hong et al., 2017; Rojo-Leon et al., 2019) as well as previous preclinical evidence (Spurgeon et al., 2017).

The hormonal and viral pathways are bridged in part by hedgehog (h-h) signaling, controlled by the paracrine feedback of E2-ERα at progesterone receptors (PRs) that inhibits carcinogenesis (Rojo-Leon et al., 2019) (Fig. 1). Although HPV-positive pap-smears are associated with higher levels of ER transcript (Shew et al., 2002), only the stromal form of ERα plays a key role in UCC biology (Spurgeon et al., 2017). Accordingly, dysplasia and UCC are not detected in K14E7/ERα knockout (-/-) mice under E2 treatment (Chung et al., 2008). Indeed, ERα inhibition (e.g., with fulvestrant, raloxifene, or methylpiperidinopyrazole) produces tumor regression or prevents pre-tumoral alterations (atypical metaplasia, dysplasia) in the transgenic murine E6/E7 model (Spurgeon et al., 2017; Chung and Lambert, 2009). The h-h pathway cooperates with carcinogenic processes by activating the hypoxia molecular cascade and facilitating the cell-cycle re-entry of supra-basal cells (Rojo-Leon et al., 2019). The use of itraconazole (ITC) as an h-h inhibitor decreases the proliferation of supra-basal cells and dysplastic progression in transgenic E6/E7 mice under E2 treatment (Rojo-Leon et al., 2019). Briefly, ITC thins the epithelium, intercepting the carcinogenic crosstalk between hr-HPV-infected cells of the supra-basal layer and the surrounding $ER\alpha^+$ stromal cells (Kumar et al., 2016), mainly CAFs (De Nola et al., 2019).

Other pathways upregulated in the stromal microenvironment that rely on the combined effect of E2 and hr-HPV include: inflammation, angiogenesis, the mesenchymal-to-epithelial transition (MET), apoptotic escape, invasion, and cellular growth (Shew et al., 2002; Spurgeon et al., 2017; De Nola et al., 2019; Chung et al., 2008; Son et al., 2018; Rojo-Leon et al., 2019) (see Fig. 1 and Table 1). The main molecules known to orchestrate these processes are listed as follows: chemokine C-C motif ligand 20 (CCL20) also known as macrophage inflammatory protein-3α (MIP3A), CCL2 also known as monocyte chemoattractant protein 1 (MCP1), chemokine C-X-C motif receptor (CXCR2) and its ligands (CXCLs), interleukins (IL1A, IL1B, IL6), heparin binding EGF-like growth factor (HBEGF), vascular endothelial growth factor (VEGF), fibroblast growth factor 9 (FGF9), and matrix metalloproteinase 9 (MMP-9) (Spurgeon et al., 2017; Son et al., 2018).

The action of estrogens is contrasted by progesterone, as demonstrated in epidemiological (Hong et al., 2017) and preclinical studies (Baik et al., 2019). Even if the progesterone receptor (PR) characterizes only 20–40 % of the UCC stroma, its expression is correlated with a better prognosis and lower likelihood of progression from cervical intraepithelial neoplasia (CIN) 3 to invasive cancer (Hong et al., 2017). A synthetical progestin, medroxyprogesterone acetate (MPA), has been recently studied in a murine model of induced preinvasive cervical lesions (mimicking various stages of CIN) and in transgenic E6/E7 mice after E2 treatment (UCC model) (Baik et al., 2019; Yoo et al., 2013) with successful results. Strikingly, MPA induces hypoplasia, apoptosis, and the regression of dysplastic cells (Hong et al., 2017). MPA also promotes the terminal differentiation of the cervical epithelial layer into Alcian blue-positive mucinous cells (Yoo et al., 2013). The effect of MPA on CIN and in preventing the progression of UCC from cervical mucinous

atrophy in preclinical studies is partly mediated by the upregulation of heart and neural crest derivatives expressed transcript 2 (Hand2) and/or hyperactivation of Myc via cyclin E (Baik et al., 2019; Yoo et al., 2013). In transgenic mice, hr-HPV-related lesions reappeared when MPA treatment was interrupted. Translated to a clinical context, women receiving MPA treatment might continue treatment until HPV infection is no longer detected on cervical pap-smear examination. PR knock-out mice show a higher frequency of atypical squamous cells; in this context, the protective effects of MPA might be mediated via other steroid receptors, especially glucocorticoid and androgen receptors (Baik et al., 2019). Since MPA is already used as a contraceptive agent and as hormonal therapy in PR^+ endometrial cancers (Baik et al., 2019), new clinical studies are both feasible and necessary to translate preclinical findings to clinical use in patients such as those with CIN lesions, for whom the standard of care is still too invasive to guarantee future fertility.

3. Chronic pro-tumorigenic inflammation in the stroma: crosstalk between cancer cells, fibroblasts, DCs, lymphocyte subpopulations, and monocytes/macrophages

Given the viral-driven biology of UCC, the immune system plays a critical role in the transition from initial immunotolerance of the precancerous lesion to chronic inflammation ("hot" tumors) and finally, in advanced stages, to a *scenario* of exhausted immune defense ("cold" tumors) characterized by the expression of CD137, cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed death cell protein 1 (PD1), and its ligand (PDL1) (De Nola et al., 2019; Walch-Ruckheim et al., 2015; Komdeur et al., 2017). Cancer cells instruct fibroblasts and myofibroblasts to chemoattract DCs in a paracrine fashion and together these cells induce leukocyte homing within the TME (Walch-Ruckheim et al., 2015). Once circulating T cells and monocytes have reached the TME, they undergo pro-tumorigenic polarization (Th17 and M2-like TAM polarization, respectively) as the result of specific cytokine stimulation (IL6, IL17, Il23, tumor growth factor beta [TGF-β]) (Walch-Ruckheim et al., 2015, 2019) (see Fig. 1 and Table 1).

IL6 released by cancer cells and CAFs plays a key role in Th17 chemoattraction and proliferation within the TME (Walch-Ruckheim et al., 2015). Specifically, IL6 can deregulate antitumoral Th1 polarization guided by DCs by inhibiting IL12 (see Fig. 1) (Walch-Ruckheim et al., 2019). Due to the paracrine action of IL1β derived from CAFs via CAAT/enhancer-binding protein b (C/EBPb), memory $CD83^+$ DCs increase the production of IL23 and, in turn, the differentiation and proliferation of Th17 cells (Walch-Ruckheim et al., 2019). At the same time, CAFs secrete additional CCL20 to attract Th17 cells (Walch-Ruckheim et al., 2015). Of note, the number of $CD83^+$IL23 subunit $p19^+$ (IL23p19) myeloid DCs and Th17 cells is positively correlated with higher FIGO stage, lymph node metastasis, and tumor recurrence, opening a wide range of translational possibilities for prognostic evaluation and personalized biological therapy (e.g., mAbs against IL1β, IL17α, IL17, and IL23p19 or IL6/C/EBPb/CCL20 targeting) (Walch-Ruckheim et al., 2015), even in association with RT (Walch-Ruckheim et al., 2019). IL6 may also reduce chemosensitivity through the IL6-signal transducer and activator of transcription 3 (STAT3) pathway (Walch-Ruckheim et al., 2019). Therefore, immunological therapy with anti-IL6 merits further assessment in UCC (Walch-Ruckheim et al., 2019).

$CD163^+$ M2-like TAMs are a crucial component of the immunological response within the TME, orchestrating a wide range of pro-tumorigenic actions as follows: angiogenesis mainly via VEGF release, lympho-vascular space invasion (LVSI), migration, and distant invasion (De Nola et al., 2019). A lower M1/M2 TAM ratio together with a higher frequency of PD-$L1^+$ TAMs are a negative prognostic factor for ACC (De Nola et al., 2019). Responses to immunotherapy suggest three main cancer phenotypes: (1) an "inflamed" immune profile with high infiltration by $CD4^+$ or $CD8^+$ lymphocytes and $PDL1^+$ immune or tumoral cells; (2) an immune-excluded profile where immune cells remain in the stroma without reaching the epithelial mass of tumoral cells; and (3) an immune-desert phenotype, with poor infiltration by leukocytes and lower rates of CD8 positivity within the TME (Chen and Mellman, 2017). The first type, rich in $CD8^+$ cells, is the most sensitive to immunotherapy (specifically anti-PD1/PDL1 therapy) and is associated with the best prognosis and overall survival (OS) (Chen and Mellman, 2017). However, there is a negative correlation between response to immunotherapy and the number of $CD68^+$ TAMs and $PD1^+$ or $PDL1^+$ TILs (Martins et al., 2019). Therefore, the proportion of $CD8^+$ cells in the TME is a potential prognostic factor for immunotherapy response ($r = 0.67$, $p = 0.033$); these cells may increase their activation state ($PDL2^+$) in response to tumoral genomic instability, as seen in immunotherapy-responders (Martins et al., 2019). The function of PDL2 is somewhat unclear, as it may act as a co-inhibitor or co-activator of $CD8^+$ lymphocytes depending on the TME (Zhang et al., 2006). Interestingly, chemo-radiotherapy response is also positively correlated with the first phenotype and is at the same time associated with increased CD8 positivity and PD1/PDL1/2 expression (Zhang et al., 2006). Yet, the ratio of $CD8^+$/$PD\text{-}1^+$ is lower in responders than in non-responders, whereas $PD\text{-}1^+$ cells and TAMs are higher among non-responders (Martins et al., 2019).

The adaptive immune system is highly activated in cervical cancers, especially in the advanced stages with LVSI and/or lymph node metastasis. Adaptive immune activation is associated with the increased presence of $CD8^+$ and $PD1/PDL1^+$ cells (Zhang et al., 2006; Meng et al., 2018; Heeren et al., 2016). In one study, patients who underwent neo-adjuvant chemotherapy (NACT) had increased proportions of these cellular subpopulations compared to those who did not (NACT vs. control; PD-L1: 87.50 % vs. 43.75 %, $p < 0.01$; PD-1: 87.5 % vs 53.12 %, $p = 0.001$; CD8: 87.50 % vs. 50.00 %, $p < 0.01$) (Meng et al., 2018). One may consider possible bias in this patient population, since the indication for NACT rather than surgery/RT/CRT implies a more advanced stage (Marth et al., 2017). The immune escape pathway sustained by $PDL1^+$ cells is more relevant in the case of CIN 1/2 (95 %), where cytotoxic cells are partly inhibited to create HPV tolerance until the lesion becomes cancerous (50 %) with re-population of $CD8^+$ cells and subsequent chronic inflammation (Meng et al., 2018). The distribution of cytotoxic cells and their balance with the PD1/PDL1 axis is mainly organized as follows: $PD1^+$ and $CD8^+$ cells are typically targeted to stromal nests, whereas PDL1 is mainly expressed on the surface of cancer cells (Meng et al., 2018). Since HPV18 is characterized by a higher expression of PDL1 than HPV16 (83 % vs. 42 %), HPV type also plays a role in the immune response (Meng et al., 2018), possibly leading to different biological behaviors and varying degrees of immune-tolerance and inflammation. Therefore, biological therapy with mAbs such as nivolumab (BMS-936,558) and pembrolizumab (MK-3475) may be useful for patients affected by cervical cancer with risk factors (e.g., LVSI), a history of NACT, advanced FIGO stage, and HPV18 positivity (Meng et al., 2018). Moreover, a checkpoint blockade strategy should consider the utility of CTLA-4 inhibitors such as ipilimumab (Komdeur et al., 2017). Immunotherapy may be as useful for pre-invasive lesions, especially in CIN 1/2, as in advanced stages given the immune response and immune escape in cervical cancer biology orchestrated by chronic hr-HPV infection (Jiang et al., 2019).

An abundance of $CD103^-$ NK cells exists at the edge of the tumoral stroma as the result of chemoattractants secreted by cancer cells, especially in advanced stages of disease (Komdeur et al., 2017). In cases with a more favorable prognosis, NK cells induce cancer cell death via major histocompatibility complex class I-related chains (MIC) A and B (MICA/B) and UL-16 binding proteins (ULPB1), which bind the NK group 2, member D (NKG2D) receptor on NK cells (De Nola et al., 2019) (see Table 1). The presence of few $CD103^+$/$CD8^-$/$NkP46^-$/$FoxP3^-$ TILs indicates the presence of a $CD3^+CD4^+$ lineage without any relationship to NK cells or T regulatory cells (Komdeur et al., 2017).

The tumoral stroma in younger patients with squamous histology is characterized by the presence of $CD103^+$/$CD8^+$ T cells defining the so-

called "hot tumor" (Komdeur et al., 2017). These cells are mainly located along the mesenchymal edges near the neoplastic epithelium (pushing type) or within the carcinoma of more invasive desmoplastic tumors without a clear distinction between stroma and epithelium (Komdeur et al., 2017). Other TILs exhibit T-cell (CD3, CD2) and B-cell markers (CD19), exhaustion molecules (PD1), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif [ITIM] domain or TIGIT, antigen-presenting molecules (HLA-DR, DQ), and TGF-β receptor 1 (TGFβR1) (Komdeur et al., 2017). The pushing phenotype is also characterized by $CD8^{-}/CD103^{+}$ TILs; this cell population easily overflows into desmoplastic masses, notwithstanding their invasive behavior (Komdeur et al., 2017). This process likely relies on the wide net of reticulum between the stroma and epithelium, which upregulates CD103 via T cell receptor activation (Komdeur et al., 2017). Only a portion of $CD103^{+}$ TILs in pushing phenotype tumors belongs to the $CD8^{+}$ population (Komdeur et al., 2017). Notably, higher rates of $CD103^{+}$ encoded by the ITGAE gene are correlated with a better prognosis (p value < 0.0001, TCGA data set), especially after RT, and this benefit exceeds that associated with $CD8^{+}$ cells (Komdeur et al., 2017). Indeed, the proportion of $CD103^{+}$ TILs decreases as FIGO stage increases (Komdeur et al., 2017). In a multivariate analysis, Komdeur et al. demonstrated that stage (HR = 2.43, $p < 0.006$), CCRT (HR = 1.30, $p < 0.001$), and proportion of $CD103^{+}$ cells (HR = 0.67, p value < 0.027) are independent prognostic factors for cervical carcinoma (Komdeur et al., 2017). Therefore, immunological "hot" cervical cancers usually present with initial FIGO staging, whereas "cold" cancers have a more invasive growth pattern (locally advanced disease) that necessitates immediate surgery and subsequent adjuvant CCRT in cases with certain risk factors (e.g., infiltrated resection margins or positive lymph nodes) (Komdeur et al., 2017). Physical contact between hr-HPV^{+} cancer cells represents the first strike in the biological cascade of chemoattraction and expansion of $CD8^{+}/CD103^{+}$ TILs (Komdeur et al., 2017). Immunotherapy can therefore target HPV E6 and E7 using $CD103^{+}$ accumulation as a response biomarker (Komdeur et al., 2017). Komdeur et al. illustrated this mechanism in a preclinical murine study where E6/E7-targeted SFV vaccine treatment enhanced $CD103^{+}/CD8^{+}$ TILs via the TGF-β pathway, showing a synergistic increase after RT (Komdeur et al., 2017). Translation to the clinical setting might include a $CD103^{+}$ assessment at the first diagnostic cervical biopsy to personalize therapy and predict the biological behavior of the disease. Another possibility might be to combine anti-HPV therapy (similar to E7-directed immunotherapy in the K14HPV16/H2b mouse model) with immune checkpoint blockade (Galliverti et al., 2020; Komdeur et al., 2017). This therapeutic combination was recently demonstrated to be more effective than monotherapy with an anti-HPV agent (Galliverti et al., 2020).

The stromal expression of PD/PDL1 is inversely correlated with the number of $CD8^{+}$ TILs, but directly correlated with the number of $CD68^{+}$ TAMs (Berenguer Frances et al., 2020). Encouraging results from preclinical models highlight the potential utility of direct modifications to the stroma induced by the placement of brachytherapy devices (Berenguer Frances et al., 2020). Berenguer et al. demonstrated that HDR brachytherapy after CCRT regulated the MET process, attaining a maximum remodeling effect after 2 weeks (Berenguer Frances et al., 2020). At this time, there was a clear peak in PDL1 expression, suggesting a possible timing for the introduction of immune checkpoint blockade therapy as a final adjuvant therapy (Berenguer Frances et al., 2020).

4. The crucial role of angiogenesis in tumor progression and dissemination

The angiogenic switch is fundamental in cervical cancer when the size of the lesion is bigger than 1−2 mm in order to maintain a sufficient supply of oxygen and nutrients (Tomao et al., 2014a). This process is the target of antiangiogenic therapy, especially in advanced or recurrent cervical cancers (Tomao et al., 2014a, b).

The exact role of neo-angiogenesis is deducible from available evidence regarding the clinical, histopathological, and molecular aspects of UCC (Minion and Tewari, 2018). At first colposcopic evaluation, the pathological vessel arbor is visible as a typical feature even on abnormal pap-smear examination, visualized as mosaicism, punctuation, and atypical vessels (Minion and Tewari, 2018). Beyond the colposcopy, the pathologist can estimate the intra-tumoral micro-vessel density (IMD) of a surgical specimen as a valuable prognostic assessment, since the rate of 5-year survival decreases as IMD increases (Obermair et al., 1998). $CD105^{+}$ (i.e., endoglin^{+}) aberrant blood vessels are a negative prognostic factor, whereas well-structured CD31 $^{+}$ endothelial sheets are a positive prognostic factor (Obermair et al., 1998; Randall et al., 2009). Noteworthy, IMD is also positively correlated with VEGF expression and disease stage (Minion and Tewari, 2018). Therefore, the anti-VEGF mAb bevacizumab is an instrumental tool for the management of UCC (Minion and Tewari, 2018).

According to a recent study, a higher level of circulating tumor cells predicts better response to anti-angiogenic therapy with bevacizumab in terms of progression-free survival (PFS; HR 0.59; 95 % CI, 0.36–0.96) (Minion and Tewari, 2018). Moreover, bevacizumab was associated with benefits for OS and PFS in women with advanced or recurrent UCC (phase II GOG 227C, phase II GOG 240) independent of the associated chemotherapy regimen (Moore et al., 2010; Monk et al., 2009). In one study, bevacizumab was associated with a median OS benefit of 3.7 months (HR 0.71, $p = 0.0035$) (Tomao et al., 2014a; Monk et al., 2009).

The biological roots of neo-angiogenesis in UCC are located in the hr-HPV oncogenic proteins (E6 and E7), which inactivate the tumor suppressor function of p53 and retinoblastoma protein (Rb-p), leading to the upregulation of hypoxia-inducible factor 1-alpha (HIF1α) and, in turn, the suppression of thrombospondin (an anti-angiogenic factor) and the upregulation of VEGF, a key driver of tumoral neo-angiogenesis (Minion and Tewari, 2018).

Most pro-angiogenic factors are directly released from tumor cells (e.g., VEGF, basic fibroblast growth factor [bFGF], and angiopoietins) and engage the surrounding stroma through the autocrine and paracrine actions of platelet-derived growth factor (PDGF)-A, PDGF-C, or TGF-β to recruit bone marrow-derived angiogenic cells (Minion and Tewari, 2018). Meanwhile, vimentin^{+} fibroblasts, αSMA^{+} myofibroblasts, TAMs positive for the endothelial-specific receptor tyrosine kinase 2 (Tie2), Th17 TILs, and $PDGFR^{+}$ endothelial cells enrich the TME with other pro-angiogenic molecules. These molecules mainly include bFGF, hepatocyte growth factor (HGF), stromal-derived factor-1 (SDF-1), and PDGF (De Nola et al., 2019; Minion and Tewari, 2018). Together these factors induce the chemoattraction of pericytes and the subsequent first step towards vessel construction (De Nola et al., 2019; Minion and Tewari, 2018) (see Fig. 1 and Table 1). Next, bFGF feeds the cycle of neo-angiogenesis by inducing the release angiopoietin-2, proteases, and additional VEGF from tumoral and stromal cells (Minion and Tewari, 2018). The crucial interaction of Tie2 and its ligand, angiopoietin-2, can be disrupted by mAb treatment, as demonstrated by (Oliner et al. (2004)). Once the process of aberrant vasculogenesis is initiated, cancer cells can promote tumor growth, invasion, and distant metastasis, as in other solid tumors (Minion and Tewari, 2018).

Tumor neovascularization mainly relies on the VEGF signaling pathway and its modulator, the kallikrein-kinin system (Zhou et al., 2019). In 2019, Zhou et al. demonstrated the key role of the bradykinin B2 receptor (BDKRB2) in the kallikrein-kinin system *ex vivo*, *in vitro*, and *in vivo* (Zhou et al., 2019). The authors reported that BDKRB2, the cognate receptor of bradykinin (BDK), was upregulated in cervical tumors. Moreover, serum BDK was significantly higher in patients with UCC than in patients with CIN ($p < 0.05$) or healthy control subjects ($p < 0.01$), and significantly decreased after surgery ($p < 0.0001$) (Zhou et al., 2019). The stromal expression of BDKRB2 was higher in patients with CIN than in healthy controls, whereas serum levels of BDK were similar between these groups (Zhou et al., 2019). While serum BDK was a good marker for the presence of UCC, there were no differences in BDK

among patients with different prognostic features (i.e., lymph node status, histotype, stage, age) (Zhou et al., 2019). Moreover, the squamous cell carcinoma (SCC) antigen was higher in cervical SCC than in ACC ($p < 0.01$), and higher in UCC with positive lymph nodes than in UCC with negative lymph nodes ($p < 0.05$) (Zhou et al., 2019). There was also a trend for increased serum BDK in ACC (Zhou et al., 2019). Since the area under the receiver operating characteristic curve of the combined SCC and BDK test was 0.752, these two prognostic factors can be used for screening purposes in circumstances where the pap-smear and/or HPV test are considered too expensive (Zhou et al., 2019). The role of the BDK/BDKR2 axis was further evaluated *in vivo:* mice inoculated with a UCC cell line overexpressing BDKRB2 exhibited faster tumoral growth, a higher degree of vasculogenesis, and lower survival compared to those inoculated with the unmodified cell line (Zhou et al., 2019). *In vitro*, Zhou et al. demonstrated that the upregulation of BDKRB2 in UCC cells induced higher expression of VEGF and therefore increased angiogenesis, whereas the presence of a direct BDKRB2 antagonist (HOE140) inhibited tubulogenesis in human umbilical vein endothelial cells (HUVECs) (Zhou et al., 2019). Similarly, deletion of the BDKRB2 gene caused the downregulation of VEGF (Zhou et al., 2019) (see Table 1).

Another biological pathway that stimulates HUVEC tubulogenesis requires the activity of a specific microRNA (miR-9−5p) that promotes the proliferation and invasion of cervical cancer cells, probably via a neoangiogenic effect and via inhibition of the tumor suppressor gene SOCS5 (Wei et al., 2019). miR-9−5p is involved in the MET of the cervical stroma, likely by upregulating associated factors such as N-cadherin, vimentin, Snail, and p-mTOR and by downregulating E-cadherin (Wei et al., 2019). The process of MET is linked to wider remodeling of the TME driven by HPV oncoproteins, estrogens, tumoral cells, and CAFs (De Nola et al., 2019). In particular, CAFs can release HBEGF to bind the epidermal growth factor receptor (EGFR), both of which are potential targets for biological therapies (De Nola et al., 2019).

5. Conclusions

The TME of the UCC is unique relative to many other solid tumors given the action of persistent hr-HPV infection together with stromal activation of ERα and the influence of pro-inflammatory, pro-angiogenic immune cells, mainly Th17 cells and $CD163^+/CD68^+/Tie2^+/PDL\text{-}1^+/VEGF^+$ TAMs. From a translational perspective, immunotherapies targeting HPV E6 and E7 or the $PD1^+/PDL1^+$ axis might expand therapeutic reach, even in advanced cases. The present review provides a simple but comprehensive overview of recent studies characterizing cellular subpopulations of the TME in UCC, the combined effect of chronic hr-HPV infection and the estrogen *milieu* on the immune system, the effects of standard therapies (CCRT, RT, chemotherapy, brachytherapy) on the TME, the relevance of anti-angiogenic drugs (e.g., bevacizumab) in advanced-stage disease, and the rise of precision medicine with the experimental association of standard therapies with immune-checkpoint blockade (anti PDL1/PD1, anti-CTL4) and the E6/E7-targeted SFV vaccine. Our review also reveals a crucial knowledge gap about non-invasive therapy in the initial stages of UCC, which frequently affect younger women who have not yet started a family or might desire childbearing in the future.

The prognosis of cervical cancer is inversely correlated with IMD, consistent with the observation in phase II studies that bevacizumab increases both PFS and OS in advanced and recurrent tumors. Hormonal therapy (progestins such as MPA) combined with the E6/E7-targeted SFV vaccine, ITC, and/or immune-checkpoint blockade have potential utility in preinvasive neoplasia as fertility-sparing strategies to prevent progression. Over the next decade, therapeutic research efforts should focus on preventing cancer progression from the early stages by targeting chronic hr-HPV infection via mAbs against viral proteins together with hormonal and immune modulation of the TME towards an anti-tumoral switch.

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Author contributions

Rosalba De Nola: Conceptualization, methodology, data curation, writing - original draft preparation, writing - tables and figure drafting, writing - review and editing. **Vera Loizzi:** data curation, visualization. **Ettore Cicinelli:** writing - review and editing, supervision. **Gennaro Cormio:** Conceptualization, writing - review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Rosalba De Nola Degree in Medicine and Surgery at the University of Bari, Italy, in 2012 with full marks. From 2013 to 2018, residency program in Obstetrics and Gynaecology at the University of Bari with full marks. Fellowship at the European Institute of Oncology in Milan, Italy (2014–2015). The poster of a published article was one of the winners awarded at the Congress of "Associazione Ginecologi Universitari Italiani" (AGUI) in 2016. Since November 2018, Ph.D. course at the Department of Emergency and Organ Transplantation of the University of Bari, Italy. From July 2020 to December 2020, Researcher at the University of Bari, Obstetrics and Gynaecology Division. From January 2021, Medical Manager at the Di Venere Hospital in Bari, Obstetrics and Gynaecology Division. Sub-investigator for many clinical experimental projects under good clinical practice. Her previous review was rewarded as one of the most cited articles for the year 2019 of the IJMS. Three more articles are about to be published in international journals with high IF. Recently, she acquired the IELTS certification and a Short Master in Biostatistics. Reviewer for some Scientific Journals.

Vera Loizzi Degree in Medicine 1998 with full marks, University of Bari, Italy. Residency in Gynecology and Obstetrics 2003 with full marks, University of Bari, Italy. Fellowship in Gynecology Oncology at the University of California Irvine (USA) (2000–2003). Researcher at the University of Bari, Gynecology and Obstetrics Division (from 2006) and Adjunct Professor (from 2014) after national eligibility to associate professor and then, in 2017, to full professor. Co-Investigator in several phase II-III studies and clinical research activity conducted under GCP (Good Clinical Practice). Author of more than 200 scientific articles on journals with IF. "Jeremy J. Hoffman Postgraduate Prize Paper" at the International Congress of Endoscopic Gynecology (Las Vegas, 1999); Prize UNESCO "L'Orèal Italia per le Donne e la Scienza" (2004).

Ettore Cicinelli Graduated in Medicine at University of Bari, Italy, 1982. Residency in Gynecology and Obstetrics (1982) and Anesthesiology and Rianimation (1989) at the University of Bari. Full Professor and Director of 4th Dept. of Gynecology and Obstetrics from 2005 to 2011. Director of one of Dept.s Obstetrics and Gynecology (from March 2011 to October 2019), University of Bari. Since the 1 st Nov 2019, Director of 1 st and 2nd Dept. Gynecology and Obstetrics, University of Bari. Dean of the Post-Graduate Specialisation School in Gynecology and Obstetrics at University of Bari since 2002. Since 2014, Delegate of Dean of the University of Bari for Postgraduate Schools. Since 18 Feb. 2020, Head of the Action Line for Medicine Specialization Schools of the University of Bari. Author of more than 380 scientific papers published in international and national journals and of 2 monographs. Member of the directory board of SIGO (Italian Society of Obstetrics and Gynecology), AGUI, SIM (Italian Society for Menopause), and SEGI (Italian Society of Gynecological Endoscopy).

Gennaro Cormio Degree in Medicine 1991, University of Bari, Italy; Residency in Gynecology and Obstetrics 1995, University of Bari, Italy; Ph. D. "Oncological Science in Gynecology", University of Rome, Italy. 1998. Training at the Department of Obstetrics and Gynecology, University of Monza 1994−95 and 1997 Fellowship at the Department of Obstetrics and Gynecology University of Foggia, 1999−2000. Principal and Co-Investigator in several phase II-III studies and clinical research activity conduced under GCP (Good Clinical Practice). Last GCP course certification 4/2/2016. Senior Consultant of the Gynecologic Oncology Unit, National Cancer Institute, Bari. Authors of 160 scientific papers (PubMed). Member of the European Society of Gynecologic Oncology. Reviewer for many Scientific Journals.