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REVIEW

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High-risk HPV oncoproteins E6 and E7 and their interplay with the innate immune response: Uncovering mechanisms of immune evasion and therapeutic prospects

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Abstract

Human papillomaviruses (HPVs) are double-stranded DNA (dsDNA) tumor viruses causally associated with 5% of human cancers, comprising both anogenital and upper aerodigestive tract carcinomas. Despite the availability of prophylactic vaccines, HPVs continue to pose a significant global health challenge, primarily due to inadequate vaccine access and coverage. These viruses can establish persistent infections by evading both the intrinsic defenses of infected tissues and the extrinsic defenses provided by professional innate immune cells. Crucial for their evasion strategies is their unique intraepithelial life cycle, which effectively shields them from host detection. Thus, strategies aimed at reactivating the innate immune response within infected or transformed epithelial cells, particularly through the production of type I interferons (IFNs) and lymphocyte-recruiting chemokines, are considered viable solutions to counteract the adverse effects of persistent infections by these oncogenic viruses. This review focuses on the complex interplay between the high-risk HPV oncoproteins E6 and E7 and the innate immune response in epithelial cells and HPV-associated cancers. In particular, it details the molecular mechanisms by which E6 and E7 modulate the innate immune response, highlighting significant progress in our comprehension of these processes. It also examines forward-looking strategies that exploit the innate immune system to ameliorate existing anticancer therapies, thereby providing crucial insights into future therapeutic developments.

KEYWORDS
HPV-associated cancers, human papillomavirus (HPV), immunotherapy, innate immunity, PRR agonists

1 | INTRODUCTION

Human papillomavirus (HPV)-associated cancers in the genital and head & neck (HN) regions account for ~5% of all cancers worldwide and are expected to remain a major health concern for the foreseeable future, thereby requiring novel effective therapeutic solutions. The current treatment modalities for these tumors, which include radiotherapy, chemotherapy, and surgery, often result in severe consequences on the targeted anatomical sites, highlighting the urgent need for alternative antiviral therapies that offer fewer side effects and improve patient outcomes.[1–12]

A major challenge in fighting HPV-associated cancers is the ability of HPVs to circumvent host immune defenses at various stages, establishing persistent infections and lifelong diseases. Central to this immune evasion is the action of two HPV viral oncoproteins, E6 and E7, which specifically target and weaken the initial defense mechanisms mounted by keratinocytes, the primary cell types infected by HPVs. This manipulation creates a tumor-promoting environment that increases the resistance of cancer cells to conventional radio-chemotherapy treatments, posing a significant challenge to existing cancer treatment protocols.[13–21]

This difficulty in overcoming immune evasion by HPVs underscores the limitations of existing cancer immunotherapies, even those that remove checkpoint restraints on adaptive immunity. Therefore, there is a growing interest in reactivating immune pathways able to induce immunogenic cell death in HPV-associated tumors through the production of lymphocyte-recruiting chemokines and type I interferons (IFNs). These IFNs are secreted when innate immune receptors—particularly, pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and cytosolic DNA sensors (CDSs) like cGAS—detect microbial RNA and DNA. These receptors are expressed across various innate and adaptive immune cells, as well as tumor cells, and their activation appears to be a promising therapeutic target for cancer immunotherapy.[22–28]

In this context, PRR agonists are gaining attention for their ability to stimulate cytokine and chemokine secretion from both tumors and nearby immune cells. They can also regulate immune cell polarization and reprogram the immunosuppressive tumor microenvironment, thus promoting a robust immune response to cancer.[29]

This review explores the complex strategies employed by high-risk (hr)HPV E6 and E7 oncoproteins to bypass innate immune defenses and the encouraging role of PRR agonists in overcoming this evasion to strengthen the immune response against HPV-induced cancers. By examining the complex interplay between the mechanisms of immune evasion of HPV and the therapeutic potential of PRR activation, we aim to highlight existing and emerging strategies to improve treatment outcomes for HPV-associated malignancies.

This review will not cover the mechanisms of viral DNA detection during the initial phase of HPV infection, as this topic has already been extensively reviewed in existing literature.[13–16]

2 | HPV INFECTION AND CANCER DEVELOPMENT

Human papillomaviruses (HPVs) are small, non-enveloped double-stranded DNA viruses responsible for the development of squamous cell carcinoma (SCC) of the anogenital and upper aerodigestive tract, with an incidence of ~5% among all cancers worldwide. These widespread, sexually transmitted viruses are grouped into different genera, species, and types.[1–4]

Over the past two decades, substantial evidence has emerged demonstrating the etiological role of specific HPVs, particularly in a subset of head and neck cancers (HNCs), namely oropharyngeal squamous cell carcinomas (OPSCCs). While nearly all cervical cancers are caused by HPVs, about 20% of OPSCC cases are believed to arise from HPV infection—mainly HPV16.[5,7]

HPV^+ HNC is considered a separate oncological entity from its HPV^- counterpart, which is largely associated with the consumption of tobacco-based products, whose prevalence is expected to rise especially in Western countries.[30,31]

As for treatment, nearly all OPSCC patients are diagnosed with locally advanced disease. They typically undergo a combination of chemotherapy and radiotherapy. While response and survival rates are very high, the adverse effects caused by these treatment modalities can be particularly harmful and lead to permanent damage.[9]

On the preventive front, HPV-driven cancers could be theoretically averted by means of vaccination against oncogenic HPV types. Presently, three prophylactic vaccines offer effective protection against the most common oncogenic hrHPV types, including HPV16 and HPV18. These hr types are together responsible for nearly 70% of cervical cancer cases, with HPV16 being the predominant genotype in OPSCC. However, despite this encouraging outlook, there are still important issues to be dealt with, such as vaccine hesitancy and shortage of health resources in low-income countries—only a small minority (7.5%) of females worldwide, aged 10–20 years, are estimated to have received at least one shot of an HPV vaccine. In addition, given that HPV-driven carcinogenesis is the result of persistent infection with oncogenic HPVs, often lasting several decades, it is highly likely that HPV-associated tumors will remain a major health concern for the foreseeable future, thus requiring novel effective therapeutic solutions.[6,10,11,30,31]

The HPV genome is a circular double-stranded DNA episome of approximately 8000 bp containing one regulatory region and two early (E) and late (L) ORFs. Among early proteins, E6 and E7, the only two viral genes consistently found in cervical tumors, are required for the development and maintenance of HPV-associated cancer. High-risk HPV genotypes, particularly HPV16 and HPV18, have developed mechanisms to persist for years or decades, driving cell proliferation in the basal layers of the stratified squamous epithelium. This environment is not conducive to viral production as high-grade squamous intraepithelial lesions (HSILs) represent a state of abortive infection. During this phase, the usual process of viral gene expression, necessary for creating virus particles, is interrupted. This

leads to an abnormal increase and deregulation in the expression of E6 and E7 oncogenes.[14,18–20,32,33]

The transforming activity of these two oncoproteins is primarily mediated through their interactions with cellular proteins, fostering a replication-competent environment that can eventually lead to cancer. Among the plethora of cellular proteins targeted by HPV oncoproteins, it is worth mentioning that HPV E6 specifically targets the p53 tumor suppressor protein for degradation, thereby preventing p53 from promoting cell cycle arrest and apoptosis in response to cellular stress signals. On the other hand, hrHPV E7 promotes the degradation of the retinoblastoma tumor suppressor (pRb) protein, thereby eliciting E2F-mediated transcriptional activation of S-phase genes. The peculiarity of E6/E7 activity is consistent with the observation that, while most human tumors harbor p53 or pRb mutations, HPV^{+} cancer cells maintain unaltered p53 and pRb genes. Indeed, p53 or pRb mutations do not confer any growth advantage or transformation potential to cancer cells where these pathways are already disrupted by E6 and E7.[3,21,34–39]

3 | THE COMPLEX INTERPLAY BETWEEN E6/E7 ONCOPROTEINS AND THE INNATE IMMUNE RESPONSE

The innate immune response is the first line of defense against microbial pathogens. To recognize and counteract these intruders, cells employ specialized receptor proteins known as PRRs. These receptors identify conserved pathogen structures, called pathogen-associated molecular patterns (PAMPs), as well as host damage-associated molecular patterns (DAMPs). PAMPs include a range of viral components, such as double-stranded RNA, single-stranded RNA, CpG unmethylated DNA, and 5′ triphosphorylated RNA (5′ppp-RNA). All these molecules are recognized by PRRs, which are strategically positioned either on the cell surface or within specific intracellular compartments of the cytosol, allowing for the effective detection of these signals.[22–24,26–28,40–44]

The families of PRRs comprise toll-like receptors (TLR) and C-type lectin receptors (CLR), which are found at the surface of cells or in endocytic compartments, as well as nucleotide-binding oligomerization domain (NOD)-like receptors (NLR), RIG-I–like receptors (RLR), and cytosol CDS, residing in the cytoplasm to sense intracellular pathogens. Among the DNA sensors there are AIM2-like receptors and the enzyme cyclic guanosine monophosphate–adenosine monophosphate (cyclic GMP–AMP) synthase (cGAS). Once bound to dsDNA, cGAS initiate signaling by producing cGAMP. This small-molecule second messenger, in turn, binds to and activates the endoplasmic reticulum (ER)-localized adapter STING, a scaffold protein localized on the ER membrane. The activation of these pathways triggers an intracellular cascade that leads to proinflammatory cytokine production through NF-κB and/or type I IFN secretion mediated by the interferon regulatory transcription factor 3 (IRF3) or IRF7. The release of type I IFN induces paracrine and autocrine pathways leading to the activation of signaling pathways that culminates in the induction and release of IFN-stimulated genes (ISGs).[45–55]

Keratinocytes, which constitute the stratified squamous epithelium of the skin and mucosal sites, such as the ano-genital or upper respiratory tract, are the natural targets of HPVs. Despite the presence in these cells of multiple PRRs capable of detecting viral pathogens and initiating the innate immune response, HPVs have evolved complex strategies to make these cells unresponsive, fostering viral persistence and tumorigenesis. By manipulating their cellular environment, HPVs effectively suppress immune surveillance, establishing a milieu conducive to tumor growth.[56–62] Advances in understanding these evasion strategies have led to the development of immunomodulatory treatments, which have shown encouraging initial results in individuals with HPV-associated cancers.

Studies dating back to the early 2000s have consistently demonstrated that hrHPVs can inhibit the transcriptional activation of numerous ISGs, predominantly through the actions of the E6 and E7 viral proteins. Intriguingly, a significant number of these suppressed ISGs are integral components of the host's antiviral response machinery, suggesting that hrHPVs can maintain a persistent state of infection by dodging the immune surveillance of the host, thus creating a cancer-promoting environment.

Extensive research has revealed the complex molecular mechanisms by which E6 and E7 interfere with the immune response. These viral proteins can bind to and effectively neutralize key transcription factors involved in the innate immune response, such as IRF1, IRF3, and STAT1. By doing so, they affect the transcriptional activation of IFN genes and the downstream IFN receptor signaling pathways, ultimately impairing the activation of ISGs. These insights, coupled with a deeper understanding of how the induction of IFN by PRRs is regulated, have underscored the role of E6 and E7 in inducing the cellular alterations that promote an immune-evasive, uncontrolled proliferative state.[63–72]

The following sections summarize current knowledge on the impact of E6/E7 on the three major innate immune signaling pathways: cGAS/STING/TANK binding kinase-1 (TBK1), RIG-I/MAVS/TBK1, and TLRs.

3.1 | Manipulation of the cGAS-STING pathway by the E6/E7 oncoproteins

The cGAS-STING pathway is a conserved antiviral mechanism that is activated upon cytosolic DNA detection, characteristic of DNA virus infection. Following DNA binding, cGAS catalyzes the transformation of adenosine 5′-triphosphate (ATP) and guanosine 5′-triphosphate (GTP) into cyclic GMP–AMP (2′3′-cGAMP), which functions as a secondary messenger that binds to and activates STING. Once activated, STING recruits and activates the kinase TBK1, which in turn phosphorylates IRF3, increasing the expression of type I IFNs and proinflammatory cytokines (Figure 1, left-hand panel). The activation of this signaling cascade leads to the upregulation of cell adhesion proteins, costimulatory factors, and MHC class I and II

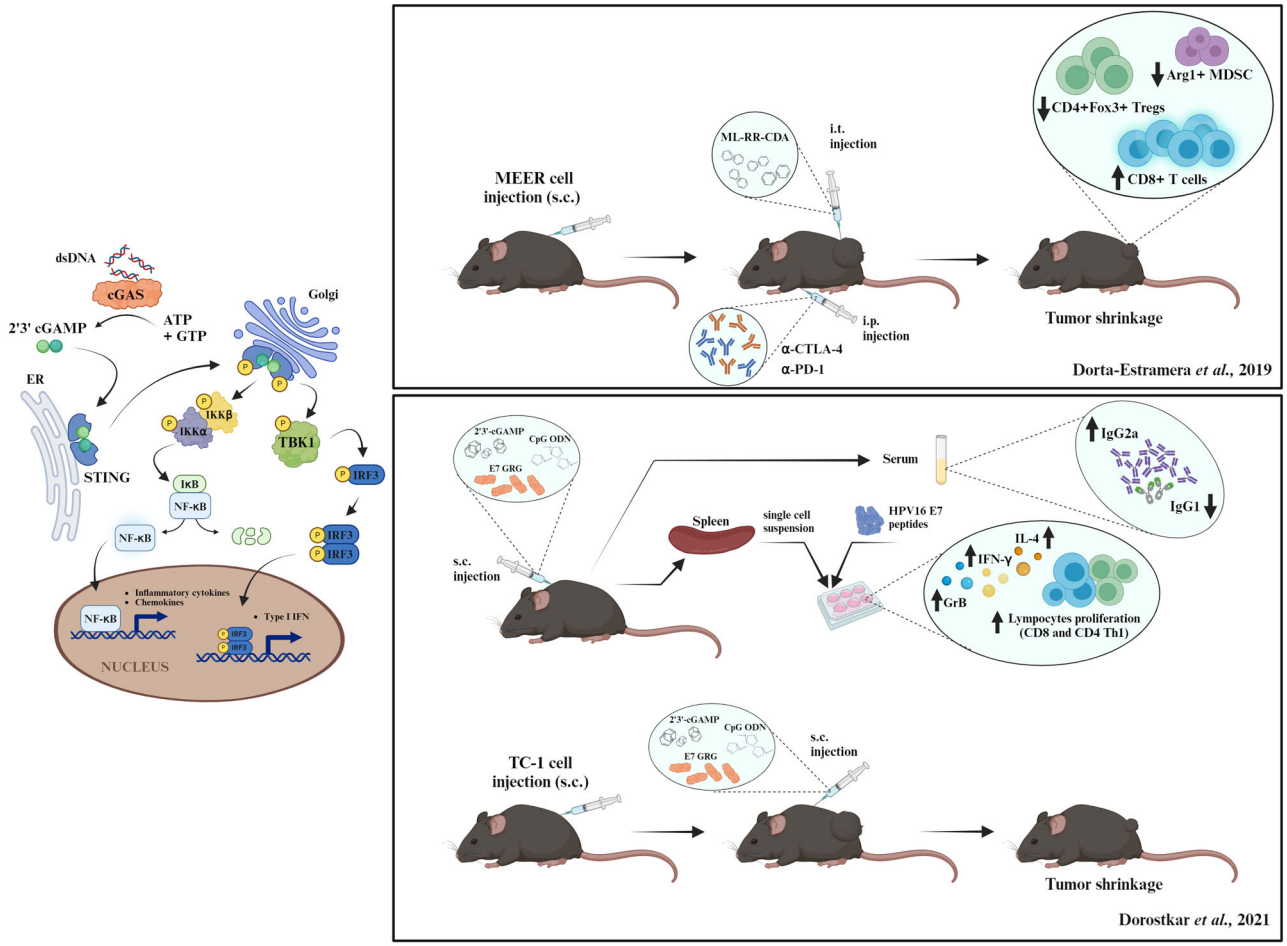

FIGURE 1 Schematic outline of the STING agonist action in HPV16-based syngeneic mouse cancer models. The cGAS/STING signaling pathway is shown in the left-hand part of the figure. Briefly, upon binding to DNA, cGAS undergoes structural changes activating it to produce the second messenger cyclic GMP–AMP (2′3′-cGAMP) using ATP and GTP. This 2′3′-cGAMP molecule acts as a ligand for the adapter molecule STING. Binding to 2′3′-cGAMP causes STING to relocate from the endoplasmic reticulum (ER) compartment to the ER–Golgi intermediate compartment (ERGIC) and the Golgi apparatus, triggering the activation of downstream signaling pathways. Specifically, TBK1, once autophosphorylated and recruited by STING, phosphorylates STING, enabling the transcription factor IRF3 to bind to the phosphorylated STING residue. The ensuing TBK1-dependent phosphorylation of IRF3 leads to the dimerization of phosphorylated IRF3, followed by its nuclear translocation, where phosphorylated IRF3 activates transcription. Right-end part. In a model of HPV^{+} HNC consisting of orthotopic—into the base of the tongue—or heterotopic—subcutaneously in the flank—injection of the MEER cells, a C57BL/6-derived tonsillar epithelial cells (MTECs) stably transduced with the HPV16 E6 and E7 genes, the intratumoral injection of the STING agonist (ML-RR-CDA), combined with systemic α-CTLA-4 and α-PD-1 administration, decreases the number of infiltrating CD4^{+} Foxp3^{+} Treg and Arg1^{+} myeloid derived suppressor cells (MDSCs), while increasing the frequency of infiltrating CTLs in both the flank and tongue tumors (right-hand upper panel). These changes in tumor microenvironment are accompanied by tumor shrinkage. The lower panel shows that immunizing mice with a modified E7 protein (E7GRG) combined with PRR agonists 2′3′-cGAMP and CpG-C increases the IgG2a/IgG1 ratio in serum. Spleen lymphocytes from these treated mice, when exposed in vitro to the HPV16 E7 peptide, secrete higher levels of IL-4, IFN-γ, and granzyme B compared to spleen lymphocytes from singly treated mice. In the HPV16-driven syngeneic mouse cancer model, based on subcutaneous injection of TC-1 cells, significant tumor reduction is observed following intraperitoneal administration of this combined treatment (right-handed lower panel). This figure was created with Biorender.com. Abbreviations: cGAS, cyclic GMP–AMP synthase; dsDNA, double-stranded DNA; ATP, adenosine triphosphate; GTP, guanosine triphosphate; 2′3′-cGAMP, cyclic GMP–AMP; ER, endoplasmic reticulum; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; IKK, IκB kinase; IκB, inhibitor of kappa BM; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IRF3, interferon regulatory factor 3; P, phosphorylation; IFN, interferon; CTLA-4, cytotoxic T-lymphocyte antigen 4; programmed cell death protein 1; MDSC, myeloid-derived suppressor cells; i.t., intratumoral; i.p., intraperitoneal; s.c., subcutaneous; IL, interleukin, GrB, granzyme B; Ig, immunoglobulin; Tregs, regulatory T cells. Symbols: , CD4^{+}FOX3^{+} Tregs; , Arg1^{+} MDSC; , CD8^{+} T cells; , IFNγ; , IL-4; , GrB; , IgG2A; , IgG1.

molecules, fostering an immunostimulatory milieu. This environment facilitates the recruitment and activation of both innate and adaptive immune effector cells, resulting in robust cytotoxic T-cell infiltration aimed to counteract viral infection.[13,22,23,26,43,44,73–83]

In addition to its crucial role in fighting infections caused by various pathogens, recent research has shed light on additional functions of the cGAS–STING pathway, particularly in response to cellular stress under sterile conditions, such as those present in cancer cells undergoing chromosomal abnormalities, genomic DNA damage, and hyperproliferation. Furthermore, the crucial influence of the cGAS–STING axis on the dynamics of antitumor immune responses is underscored by the common occurrence of micronuclei or cytoplasmic DNA fragments in cancerous cells, all signals capable of activating this DNA-sensing pathway.

Given its importance in generating inflammatory and immune-stimulatory responses with antitumor capabilities, including the activation of cytokines and NK cell ligands, viruses, such as HPVs, have developed strategies to inhibit this pathway, thereby circumventing host immune surveillance. This evasion tactic is particularly evident in the suppression of STING-dependent IFN responses by HPV18 and HPV16 E7 protein, albeit through distinct mechanisms. Specifically, HPV18 E7, but not HPV18 E6, binds to STING via its LCXCE motif to inhibit its function, particularly inhibiting type I IFN production in response to exogenous DNA stimulation.[84] In addition, it has also been recently shown that HPV18 E7, but not HPV6 and HPV11 E7, selectively antagonizes the cGAS-STING pathway by inhibiting NF-κB activation and the expression of STING-induced NF-κB-related genes. More specifically, HPV18 E7 interferes with NF-κB signaling by blocking STING-mediated nuclear translocation of p65 while not affecting IRF3 activation.[85]

In addition, alternative mechanisms have been also reported in HNSCC-derived cell lines that interfere with STING signaling involving NLRX1, a distinctive member of the nucleotide-binding domain and leucine-rich repeat (NLR) family showing an ability to negatively regulate IFN antiviral immunity upon viral infection. Mechanistically, NLRX1 mediates the K48-linked polyubiquitination of MAVS, leading to MAVS protein degradation through a proteasome-dependent pathway. In HPV^+ cell lines, the NLRX1-centered autophagy-promoting molecular complex regulates dsDNA virus–induced immune activation by accelerating the turnover of autophagosome cargos, including STING. Through this strategy, HPV16 E7 functions as an effective "degrader" of this adapter molecule.[86] Fittingly, findings from the same study show that NLRX1 depletion improves type I IFN-dependent T-cell infiltration and tumor control in an HPV16 E6/E7-expressing HNSCC mouse model (MOC2-E6/E7). Subsequent research has also shown that, in a panel of $HPV16^+$ HNSCC cell lines, dampening of the cGAS-STING pathway is mediated by the LCXCE domain in HPV16 E7.[87] Accordingly, in these cell lines, the induction of IFNβ in response to salmon sperm or 2′3′-cGAMP, both potent agonists of this pathway, was significantly reduced when compared to HPV^- HNSCC-derived cell lines.

Impairment of the cGAS-STING axis, and, to a lower extent, the RIG-I pathway has also been observed by our group in normal immortalized keratinocytes (NIKS) harboring episomal HPV18 genomes and in HeLa cells, an $HPV18^+$ human carcinoma-derived cell line. In these cell lines, we found that HPV18 persistence in keratinocytes hampers the production of both type I and III IFNs in response to exogenous DNA ligands.[88] Moreover, we demonstrated that this downregulation occurs at the transcriptional level and is mediated by the H3K9-specific methyltransferase SUV39H1, which induces the accumulation of repressive heterochromatin markers, mainly H3K9me2, at the promoter region of RIG-I, cGAS, and STING genes in an E7-dependent manner.[88,89] Accordingly, pharmacological inhibition or gene silencing of SUV39H1 promotes the transcriptional activation of RIG-I and cGAS genes, subsequently enhancing the release of type I and III IFNs upon poly(dA:dT) transfection.[89] Epigenetic regulation of the STING gene has also been reported in breast cancer cell lines by Wu and co-workers. They found that STING mRNA expression is epigenetically downregulated by the histone H3K4 lysine demethylases KDM5B and KDM5C, whereas it is activated by H3K4 methyltransferases.[90] Indeed, KDM5 blockade boosted STING expression and, consequently, a robust IFN response in a cytosolic DNA-dependent manner. More recently, the expression levels of cGAS and those of its downstream effectors STING and IRF3 were found to be significantly increased in keratinocytes expressing HPV31 E6 or both E6 and E7 in response to cGAMP or poly(dA:dT) compared to similarly treated normal human keratinocytes or those expressing only E7, indicating that E6 alone is sufficient to increase cGAS levels.[91]

Collectively, these findings underscore the critical role of HPV oncoproteins in disrupting the cGAS-STING pathway in cancer cells, a prime determinant of HPV-induced carcinogenesis. Indeed, the suppression of the cGAS-STING pathway in HPV^+ cells creates an unreactive cellular milieu that allows cells with high genomic instability to keep proliferating despite the accumulation of genetic mutations, thus increasing the risk of cancer progression.

3.2 | Disruption of the RIG-I/MAVS pathway by HPV oncoproteins and therapeutic implications

The retinoic acid-inducible gene I-like receptors (RLRs) act as primary sensors for cytosolic detection of viral RNA. These receptors are DExD/H box-containing RNA helicases and are expressed ubiquitously in the cytoplasm, with RIG-I being the founding member of this receptor family. RLRs typically contain a central helicase domain and a C-terminal domain fundamental for RNA recognition. RIG-I additionally displays a tandem caspase recruitment domain (CARD) mediating its interaction with the downstream signaling adapter protein mitochondrial antiviral-signaling (MAVS). This interaction between RIG-I and MAVS recruits other downstream signaling molecules, such as TNF receptor-associated factor (TRAF)3/6 and inhibitor of NF-κB kinase (IKK) family members, which in turn activate IRF3/7- and NF-κB-dependent transcription of ISGs and proinflammatory factors. Specifically, RIG-I can recognize short double strand RNAs with a 5′-triphosphate or -diphosphate groups (5′-pppRNA or -ppRNA) (Figure 2, left-hand panel). Although initially identified as a critical sensor only for RNA viruses, mounting evidence suggests that

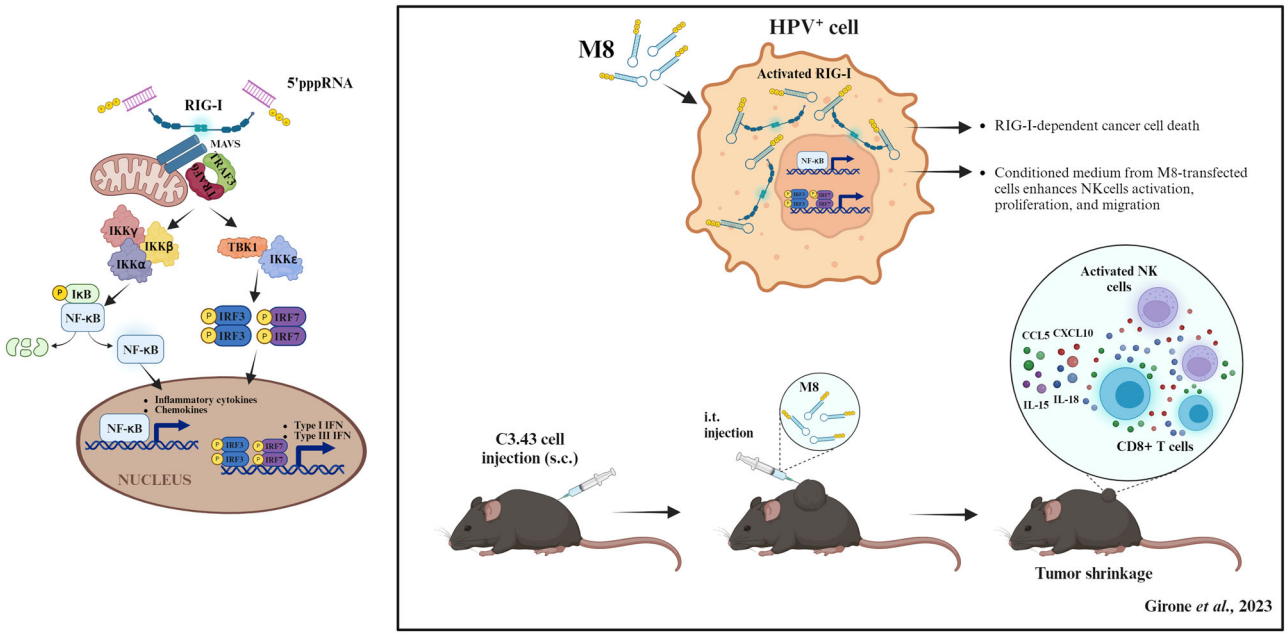

FIGURE 2 Schematic outline of the RIG-I agonist action in HPV$^+$ cells and in a HPV16-based syngeneic mouse cancer model. The left-hand part of the figure displays the RIG-I signaling pathway. Briefly, upon sensing and binding to 5′ triphosphate double-stranded RNA (5′-ppp-RNA), RIG-I interacts with the adapter protein MAVS located on the outer membrane of the mitochondria. MAVS activation initiates a signaling cascade involving TRAF3 and TRAF6, subsequently activating TBK1, IKKε, and the IKKα/β/γ complex. This leads the activation of IRF3/7 and NF-κB, respectively, which then translocate into the nucleus to induce expression of interferons (IFNs) and proinflammatory cytokines (left-hand panel). In HPV$^+$ cellular models, such as HeLa and CaSki cell lines, transfection with the RIG-I agonist M8 induces apoptosis, which is significantly reduced in cells lacking RIG-I. The conditioned medium from M8-transfected CaSki cells boosts NK cell proliferation, activation, and migration in a RIG-I-dependent, tumor cell-intrinsic manner (upper part of the Figure). Intratumoral injection of M8 in a syngeneic HPV16-driven mouse cancer model, based on subcutaneous injection of C3.43 cancer cells harboring an integrated HPV16 genome, increases CD8$^+$ and NK cell recruitment in the tumor microenvironment and upregulates IL-15, IL-18, CCL-5, and CXCL-10 mRNA expression levels (right-hand panel). These changes in tumor microenvironment are accompanied by tumor shrinkage. This figure was created with Biorender.com. Abbreviations: RIG-I, retinoic acid inducible gene I; 5′-ppp-RNA, 5′ triphosphate double-stranded RNA; MAVS, adapter mitochondrial antiviral signaling protein; TRAF, TNF receptor associated factor; IKK, IκB kinase; TBK1, TANK-binding kinase 1; IRF, interferon regulatory factor; IκB, inhibitor of kappa B; NF-κB, nuclear factor-κB; P, phosphorylation; IFN, interferon; i.t., intratumoral; NK, natural killer; IL, interleukin; CCL5, Chemokine (C-C motif) ligand 5; C-X-C Motif Chemokine Ligand 10. Symbols: , activated NK cells; , CD8$^+$ T cells; , CCL5; , CXCL10; , IL15; , IL-18.

RIG-I can also play a role in indirectly detecting certain DNA viruses by recognizing RNA species transcribed by RNA polymerase III.[15,50,92–103]

While there is currently no direct evidence of the involvement of RIG-I in HPV sensing, the inactivation of this signaling pathway through distinct mechanisms in HPV$^+$ cells has been reported. Among others, Chiang et al. demonstrated that HPV16 E6 forms a complex with TRIM25 and its upstream regulator, the ubiquitin-specific protease 15 (USP15). In their experiments involving immortalized human embryonic kidney HEK293T cells and cervical-carcinoma-derived HPV$^-$C33A cells expressing FLAG-tagged E6 of HPV16, the authors observed that E6 binds to both exogenous TRIM25 and USP15, resulting in the formation of a ternary E6-TRIM25-USP15 complex. This enhanced E6-driven TRIM25 polyubiquitination results in reduced TRIM25 protein stability. Importantly, this ability of E6 to form a ternary complex was also observed using other hr and low-risk (lr) HPVs from the alpha genus. In contrast, E7 from the same genotypes fails to bind to TRIM25, indicating the specificity of this inhibitory cascade. As a result, the poor stability of TRIM25 in cells expressing HPV16 E6 impairs the interaction between RIG-I and MAVS during Sendai virus infection, with HPV16 E6, but not E7, significantly inhibiting RIG-I-mediated ISG induction.[104,105] Along these lines, Akgul's group has recently demonstrated that, in primary human keratinocytes (PHKs), ectopic expression of HPV16 E6 transcriptionally downregulates the expression of several PRRs, including RIG-I. Importantly, they also found that the oncoproteins from the oncogenic cutaneous beta genotype HPV8 similarly target RIG-I.[106–108] Moreover, we have shown that the RIG-I/MAVS/TBK1 pathway is still functional in HPV$^-$transformed cells and exhibits a strong responsive upon transfection with the 5′ppp-RNA agonist M8, leading to a massive production of type I and III interferons (IFNs). This suggests that specifically targeting this pathway could be a promising strategy to potentiate the efficacy of radio- and chemotherapy in eliminating cancer cells.[88,89,109,110]

3.3 | TLR signaling evasion in HPV oncogenesis

Toll-like receptors (TLRs), a family of transmembrane receptors belonging to pattern recognition receptors (PRRs), are localized on the plasma or endosomal membranes. Their function is to recognize

extracellular and endosomal PAMPs and DAMPs, thereby activating immune and host defense mechanisms. In humans, 10 TLR family members have been identified, each responding to a distinct set of ligands. For instance, TLR4 is known for its binding to bacterial lipopolysaccharide (LPS), whereas TLRs 1, 2, and 6 form heterodimers (i.e., TLR1/2, TLR2/6) that recognize several PAMPs, such as lipopeptides and other components of Gram-positive bacterial cells. TLR5 detects bacterial flagellin to trigger an immune response against microorganisms. On the other hand, TLR3 is specific to viral-double stranded RNA (dsRNA), while TLR7, 8 and 9, localized on the cell membrane of endosomes, are sensitive to nucleic acids like ssRNA and unmethylated CpG-containing DNA from viruses and bacteria. TLR3 acts through the TRIF-dependent pathway, activating IRF3, which then induces the production of type I IFNs. Conversely, TLR7, 8 and 9 engage the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway, which leads to the activation of NF-κB or IRF7 and, consequently, to the induction of proinflammatory cytokines or type I IFNs, respectively (Figure 3).[45,111–116]

Similar to their impact on other primary innate immune signaling pathways, HPVs also manipulate elements of the TLR signaling cascade to evade the immune response. Specifically, TLR9 was found to be downregulated at both the mRNA and protein levels in HPV16 E6/E7-transduced keratinocytes, whereas in HPV18 E6/E7-transduced cells this downregulation was less pronounced, indicating a lower efficiency of HPV18 in inhibiting TLR9 transcription. In addition, HPV16 E7 has been implicated in the formation of an inhibitory transcriptional complex on the TLR9 promoter in in vitro models, thus negatively impacting its transcription.[117] Such down-regulation of TLR9 was also observed in human cervical cancer biopsies by immunohistochemistry.[118] Other studies have assessed TLR mRNA expression levels in human cervical specimens. For example, Halec and colleagues examined the mRNA expression levels of TLR2, TLR3, TLR7, TLR8, and TLR9 genes in cervical cytobrush samples, showing that higher expression levels of TLR3 or TLR7 mRNAs at an HPV16$^+$ visit significantly predicted viral clearance by the following visit. In addition, increased mRNA levels of TLR2, TLR7, and TLR8 genes were associated with regression of cervical intraepithelial neoplasia (CIN)2.[119–121]

Altogether, these findings highlight the critical role of TLRs in modulating the HPV$^-$driven oncogenic process. In response, HPVs have evolved multiple strategies to circumvent host immunity, including the impairment of TLRs, thereby facilitating viral persistence.

3.4 | The role of HPV in shaping the tumor microenvironment

The immune system plays a crucial role in determining the course of cancer and its progression. The response of the immune system to cancer is complex, with the potential to both inhibit and facilitate the growth and spread of this disease. This dual capacity is due to the intricate interplay between innate and adaptive immunity. The innate immune system, in particular, contributes to cancer immunity by supporting an immunostimulatory state that enables T cell immuno-surveillance. However, in the tumor environment, innate immune cells often display immune-suppressive properties, thereby creating a tolerogenic niche that interferes with the cytotoxic potential of tumor antigen-specific T cells.

Against this backdrop, targeting effector T cells has become a fundamental aspect in the immunotherapy of various cancers, more recently including those associated with HPVs. Given the ability of HPV to induce a state of immune suppression and evasion, understanding how to activate, sustain, and prevent the exhaustion of T cells in the context of HPV-associated cancers is a crucial aspect of immunotherapy research. This focus on effector T cells is particularly pertinent in light of recent findings regarding the immune landscape within HPV-positive tumors. For instance, several reports have highlighted an elevated presence of tumor-infiltrating lymphocytes (TILs) in HPV$^+$ tumors. A study on TILs in 12 human cervical tumors revealed that nine of them displayed CD4$^+$ T cells, while 8 harbored CD8$^+$ T cells specifically targeting HPV antigens when exposed to overlapping peptides from E6 and E7 ex vivo, with most patients showing polyclonal responses.[122] Intriguingly, these cells, despite being reactive ex vivo, are ineffective against HPV-infected cells in vivo, underscoring the complex relationship between HPV and the immune system.

Further complicating this scenario are large-scale genetic studies that have identified both inherited (germline) and acquired (somatic) genetic alterations in genes associated with immune function in HPV-associated cancers. Data from The Cancer Genome Atlas (TCGA) on cervical cancer indicated that approximately 8% of patients carry previously unidentified somatic mutations in HLA-A and 6% in HLA-B. Furthermore, 8% of cervical cancers harbor a gain-of-function mutation in CD274, the gene coding for the programmed cell death ligand-1 (PD-L1).[123] In head and neck cancers, TCGA data revealed immune pathway disruptions due to somatic mutations, affecting 7% of all HPV$^-$ tumors and 11% of HPV$^+$ tumors.[124] Although these mutations are present in a small fraction of patients, they can provide us with useful information on the mechanisms through which HPV can circumvent immune detection and control.

Expanding upon these genetic insights, additional research has also demonstrated how HPV can interfere with the immune system at the cellular level. In particular, HPV has been shown to disrupt specific HLA molecules, rendering NK cells unable to eliminate virus-infected cells. Studies indicate that persistent HPV infection in cervical samples markedly reduces the expression of HLA-A, HLA-B, and HLA-C molecules, while increasing the levels of HLA-E, which in turn binds to the CD94-NKG2A inhibitory receptor on NK cells, affecting their activity.[125,126] Even though this review is focused on the action of the E6 and E7 oncoprotein, it is worth mentioning that also the E5 protein, another early protein with documented carcinogenetic properties, can impair surface HLA class I expression levels.[125] Despite being downregulated, NK cell cytotoxicity is not completely suppressed in advanced HPV$^+$ head and neck squamous cell carcinoma (HNSCC) cases. Indeed, high infiltration rates of

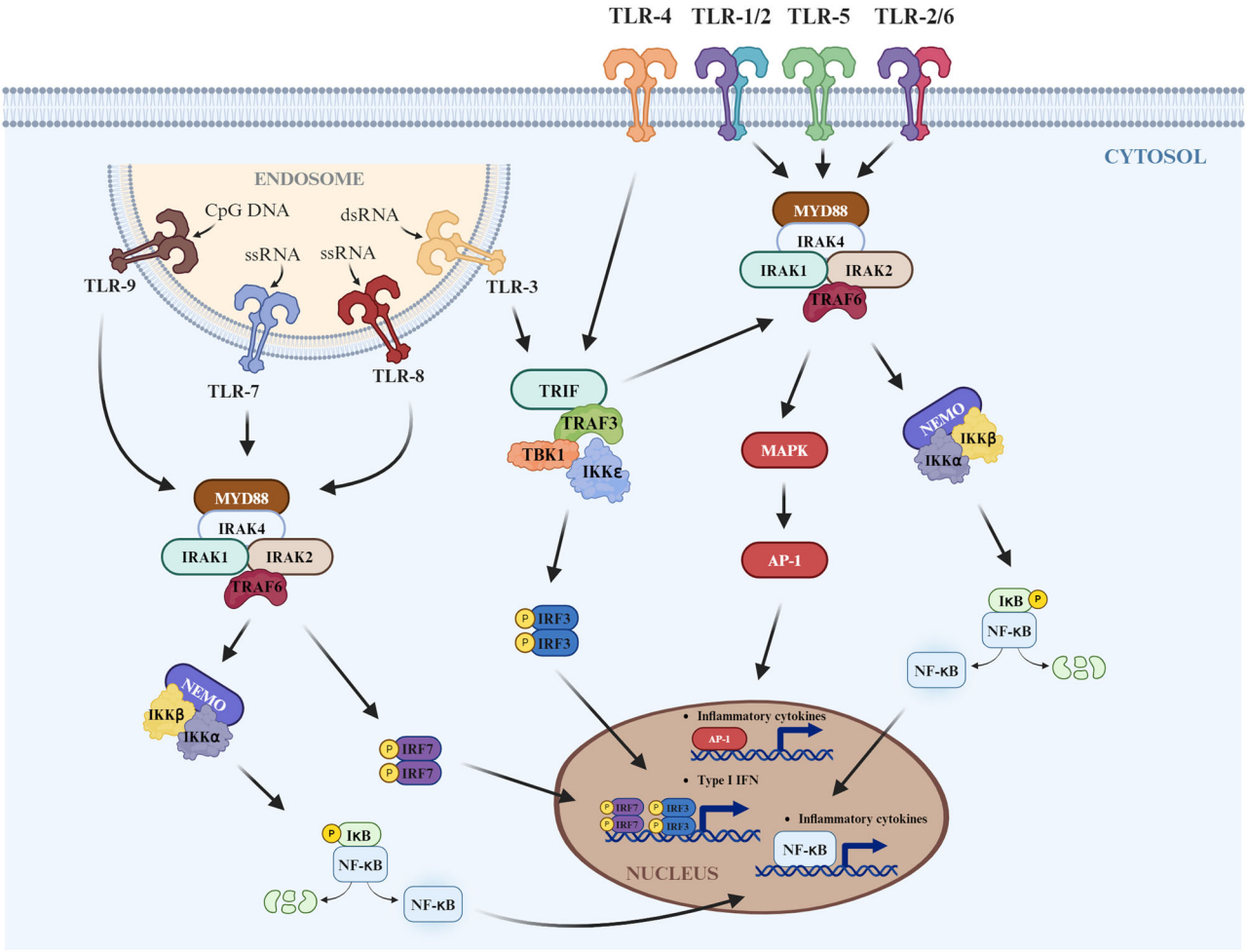

FIGURE 3 TLR signaling pathways. TLR5, TLR4, and the heterodimers of TLR2–TLR1 or TLR2–TLR6 predominantly recognize the membrane components of pathogens at the cell surface, whereas TLR3, TLR7–TLR8, and TLR9 are localized to endosomes, where they detect nucleic acids from both host and foreign microorganisms. Upon binding to their respective nucleic acid targets, TLRs dimerize and their cytoplasmic TIR domains oligomerize, initiating the recruitment of signaling adapters. TLR3 and TLR4 interacts sequentially with TRIF and TRAF3, activating the TBK1/IKKε/IRF3 pathway to promote transcriptional activation of type I interferons. On the other hand, TLRs 1/2, 5, 2/6, 7, 8, and 9 form a complex with MyD88, facilitating the assembly of the Myddosome complex, comprising MyD88, IRAK4, IRAK1, and IRAK2. Once activated, this complex stimulates IRAKs and the ubiquitin E3 ligase TRAF6, initiating NF-κB- and IRF7-mediated transcriptional activation of type I interferons and proinflammatory cytokines. Abbreviations: TLR, Toll-like receptor; dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; dsDNA, double-stranded DNA; TRIF, TIR-domain-containing adapter-inducing interferon-β; TRAF, TNF receptor-associated factor; IKK, IκB kinase; TBK1, TANK-binding kinase 1; MyD88, Myeloid differentiation primary response 88; IRAK, interleukin-1 receptor-associated kinase; IRF, interferon regulatory factor; NF-κB, nuclear factor-κB; IκB, inhibitor of kappa B; Ub, ubiquitination; P, phosphorylation; IFN, interferon; HPV, human papillomavirus.

CD56dim, indicative of cytotoxic NK cell phenotype, correlated with better survival outcomes. Moreover, HPV$^+$ oropharyngeal carcinoma cases showed significantly greater infiltration of CD56$^+$ cells compared to that found in HPV$^-$ HNSCCs, and its extent correlated with improved clinical outcomes.[127,128]

Moving from the cellular impact of HPV on immune modulation, clinical observations have further underscored the significance of CD4$^+$ T cell phenotypes over their absolute count in fighting HPV infection. Analysis of cervical cancers revealed a wide spectrum in the total number of tumor infiltrating CD4$^+$ T cells, with no clear association with patient survival. However, the proportion of a particular subset of CD4$^+$ T cells, namely CD4$^+$CD161$^+$ T cells, positively correlated with patient survival.[129,130] Surprisingly, cervical cancers generally have fewer CD4$^+$CD161$^+$ effector T cells compared to oropharyngeal cancers.[129,130] Similar studies on HPV-related oropharyngeal cancers found no correlation between the absolute count of CD4$^+$TILs and clinical outcomes.[131] Of note, patients with cervical cancer often exhibit an imbalance in their CD4$^+$ T-helper (Th) cell response, favoring a Th2 response (associated with humoral immunity) over a Th1 one (associated with cell-mediated immunity), which is accompanied by decreased levels of peripheral interferon-gamma (IFNγ).[132] Furthermore, these patients display altered cytokine profiles in their peripheral blood, indicative of a Th2-biased response. However, the extent of this shift has not been

thoroughly investigated in HPV$^+$ HNSCC.[133] Consistent with these findings, a Th1 response involving CD161$^+$ and CD103$^+$ T cells correlates with better outcomes in HPV$^+$ oropharyngeal SCC patients. Lastly, a protumorigenic IL17-associated Th17 response, induced by stromal fibroblasts secreting CCL20, has been recently identified in cervical cancer, correlating with progression from high-grade cervical neoplasia to invasive cancer. The persistence of this Th17 response in invasive cancer stages and its potential as a therapeutic target are still being investigated.[134]

Building on these immunological insights, FOXP3$^+$ regulatory T cells (Tregs), a distinct subset of CD4$^+$ T cells, have been identified as crucial modulators within the immune landscape of HPV-associated cancers. These cells play a significant role in suppressing the antitumor immune response by limiting the activation and expansion of effector T cells. Tregs are widely recognized for their essential function in mitigating the host's immune response in conditions like autoimmune diseases and viral infections. Interestingly, an elevated presence of Tregs has been observed in CIN and cervical cancers, with their abundance correlating with disease severity. This correlation implies a potential role for Tregs in interfering with anti-HPV immunity. Supporting this theory, research indicates that tumor-infiltrating Tregs in cervical cancer patients are often specific for HPV antigens. Moreover, these patients possess CD4$^+$T lymphocytes with a regulatory phenotype that exhibit reduced proliferative capacity. These cells are present not only in the primary tumor site but also in lymph node metastases and peripheral blood, suggesting a systemic induction of immune tolerance that may facilitate the spread of metastases.[135]

The interaction between the programmed death-1 receptor (PD-1) and its ligand PD-L1 highlights another critical aspect of immune regulation in the context of HPV-associated cancers. This immune checkpoint acts as a conserved inhibitory mechanism that maintains immune balance and prevents autoimmunity. Numerous cancers, including those associated with HPV, have evolved to hijack this pathway, upregulating PD-1/PD-L1 expression to induce immune tolerance. The clinical success of checkpoint inhibitors targeting PD-1 and PD-L1 underscores the importance of this pathway in cancer immunotherapy. Research has demonstrated that HPV-positive cancers are characterized by elevated levels of PD-L1 on both tumor and immune cells, suggesting a strategic adaptation to suppress the host immune response. Nonetheless, distinguishing this increase from the general enhancement of immune infiltration in these tumors remains quite challenging.[136–141]

4 | PATTERN RECOGNITION RECEPTOR AGONISTS AGAINST HPV-INDUCED CANCER

Over the past two decades, there has been growing interest in the development of PRR agonists, moving from antiviral to cancer therapeutic applications. The ability of the immune system to target solid tumors through specialized immune cells that detect unique tumor-specific antigens is quite compelling. Yet, this powerful defense often encounters significant hurdles, primarily stemming from the immunosuppressive microenvironment surrounding the tumor. To overcome this challenge, an innovative strategy has emerged based on direct injection of immune modulators into the core of the tumor. This approach not only initiates a local immune response against the tumor but also promotes the infiltration of immune cells bearing potent anticancer capabilities into remote tumor sites.[142,143]

Since PRRs mediates the immune response to infections and activate the immune system as needed, targeting these sensors may represent a promising therapeutic approach for managing chronic inflammatory diseases, fighting infections, and enhancing the efficacy of vaccines as adjuvants. In the context of cancer therapy, the activation of PRRs within the tumor environment by these immune modulators serves as a signal to the immune system, alerting it to the presence of a tumor, and eliciting a robust immune response against cancer cells. This immunostimulatory cascade triggers not just a localized response but also a systemic mobilization of anti-cancer immune cells across various sites. Therefore, employing immune modulators that PRRs can detect constitutes a strategic approach to tap into the natural ability of the immune system to recognize danger and respond accordingly. In other words, by mimicking the signals associated with infection or tissue damage, these compounds can enhance the immune response against solid cancers, offering a new paradigm in cancer treatment, including HPV-associated cancer.[144–148]

Below, we summarize the recent findings on the novel interventions designed to enhance our body's ability to recognize and eliminate HPV-infected/transformed cells through PRR agonists.

4.1 | STING agonists

Emerging evidence suggests that the cGAS-STING pathway plays a critical role in inducing both innate and adaptive immune response resulting in either the suppression or promotion of cancer progression.[149,150] Despite this, a number of studies have shown that this pathway is frequently suppressed across various cancer types, leaving a degree of uncertainty regarding the targeting of this pathway for cancer therapy. cGAS-STING agonists, such as STING-binding molecules and cGAMP derivatives, have been developed and used to demonstrate that the intratumoral administration of cGAMP and other cyclic dinucleotides can result in decreased tumor volume and growth in mouse models of colon, brain, skin, pancreatic, breast, and B cell malignancies.[151–156]

In a study involving HPV$^+$ HNSCC, the STING agonist ML-RR-S2 CDA was combined with several immune checkpoint blockades (ICBs) in a dual model: one orthotopic, involving injection into the base of the tongue, and the other heterotopic, consisting of subcutaneous injection in the flank. These models used MEER cells, which are tonsillar epithelial cells (MTECs) derived from C57BL/6 mice stably transduced with the HPV16 E6 and E7 genes. The results showed

that administering the STING agonist directly into the tumor significantly enhanced the effectiveness of systemic checkpoint blockade therapy, leading to both tumor shrinkage and improved survival rates.

Specifically, combining the STING agonist with either α-PD-1 or α-CTLA-4 antibodies—two types of ICBs—triggered prolonged tumor reduction and activated the antitumor immune response. This co-treatment notably increased the ratio of cytotoxic $CD8^+$T cells to Tregs and functional myeloid-derived suppressor cells (MDSCs), indicating a potent antitumor immune environment (Figure 1, right-hand upper panel).[157] In addition, another report showed that the immunization of C57BL/6 mice with E7GRG, a modified E7 protein carrying C24G, L67R, C91G amino acid substitutions, in combination with the PRR agonists 2′3′-cGAMP and CpG-C (ODN-2395), increased the IgG2a/IgG1 ratio in serum. Lymphocytes from these immunized mice, derived from the spleen and stimulated in vitro with an HPV16E7 peptide, showed increased proliferation and higher levels of IL-4, IFN-γ and granzyme B. The subcutaneous injection of this combined treatment (i.e., E7GRG + 2′3′-cGAMP+CpG-C) in a syngeneic HPV16-driven preclinical cancer model, involving subcutaneous injection of TC-1 cells—a tumorigenic cell line derived from primary lung epithelial cells of C57BL/6 mice harboring E6 and E7 genes from HPV16—into the mouse flank, led to significant tumor growth inhibition (Figure 1, right-hand lower panel).[158]

While these findings suggest a potential role for STING agonists in potentiating the effects of immune-directed therapies, the efficacy of STING agonists as standalone treatments remains unconvincing and large clinical trials are still missing. More importantly, the discovery that the cGAS-STING pathway is suppressed in HPV^+ cells at various stages implies that the use of these agonists may not to be the most effective strategy.[84–89]

4.2 | RIG-I agonists

In recent years, the potential of RIG-I activation as a therapeutic approach has gained significant interest. Many reports, comprising both in vitro and in vivo research, have demonstrated the efficacy of RIG-I selective ligands in exerting anti-tumorigenic activity in different cancer models. Despite the rising concern over HPV-induced cancers—particularly in the head and neck region—as a global health issue, limited efforts have been made to assess these alternative therapeutic strategies against this cancer type. What is probably the most intriguing finding from research into RIG-I agonists across a spectrum of cancer models is their ability to induce the recruitment and activation of professional innate immune cells. In particular, several studies have documented the activation of DCs, NK cells, and $CD8^+$T lymphocytes upon RIG-I stimulation, leading to considerable anti-cancer effects.[110,159–161]

The aforementioned findings led to the hypothesis that RIG-I, being largely active in HPV^+ cells, could respond effectively to specific agonists in this cancer type. Indeed, our research and studies by other groups have shown that while hrHPVs significantly suppress the cGAS/STING pathway, the RIG-I protein remains functional and responsive to specific agonists.[17,84,86–89] In this regard, we have recently reported that engaging RIG-I through a specific 5′ppp-RNA agonist, namely M8, markedly reduced tumor burden in both in vitro and in vivo models.[162] In experiments with CaSki and HeLa cells, containing the HPV16 and HPV18 genomes, respectively, M8 transfection promoted intrinsic apoptotic cell death, which was significantly reduced in cells with RIG-I knockdown. Furthermore, RIG-I stimulation by M8 significantly potentiated the anti-cancer activity of the chemotherapeutic agent cisplatin, not only in HeLa and CaSki cells but also in a syngeneic mouse model of HPV16-induced cancer, created by dorsal subcutaneous injection of C3.43 cells, carrying an integrated HPV16 genome, into C57BL/6J mice. Remarkably, additional in vivo studies using the same mice confirmed that intertumoral M8 injection boosted the efficacy of cisplatin, leading to tumor reduction and an increase in $CD45^+$ leukocyte infiltration. Specifically, the presence of activated NK cells within the tumor was significantly augmented following M8 injection, as evidenced by increased expression of the NK activation markers CD11b and CD69. Consistently, a range of cytokines and chemokines known to be involved in NK activation and recruitment were found to be transcriptionally upregulated after M8 treatment. The ability of M8 to induce an inflamed tumor microenvironment was further demonstrated using conditioned media from M8-treated CaSki cells, which enhanced NK cell proliferation, cytotoxicity, and migration (Figure 2, right-hand panel).[162] However, to the best of our knowledge, agonists of this type have not yet been tested in clinical trials for HPV-associated cancers.

4.3 | TLR agonists

As TLRs can regulate essential processes for T-cell immunity, such as antigen uptake, processing, and presentation by antigen presenting cells (APCs), along with transcriptional activation of genes required for T-cell activation, their therapeutic stimulation offers a viable option to reactivate antitumor immunity. Although monotherapies targeting TLRs have shown little success in clinical settings, combining TLR agonists with ICBs has shown more encouraging outcomes, enhancing their overall therapeutic efficacy (Table 1). For example, the TLR7 (1V270) and TLR9 (SD-101) agonists, when used in combination with PD-1 blockade in the HPV16 E6/E7-expressing MTEC syngeneic mouse model of HNSCC (MEER), not only contributed to tumor reduction but also increased the ratio of M1 to M2 tumor-associated macrophages (TAMs) and promoted the infiltration of tumor-specific IFNγ-producing $CD8^+$ T cells, while anti–PD-1 treatment increased T cell receptor (TCR) clonality of $CD8^+$ T cells.[165]

Several TLR agonists have already been tested in clinical trials targeting HPV-associated diseases. In particular, the TLR7 agonist imiquimod, the first topically active TLR agonist approved for the treatment of warts caused by lrHPVs, actinic keratosis, and superficial basal cell carcinoma (BCC), has also proven effective in treating hrHPV-induced vulvar intraepithelial neoplasia.[169,170] Its mechanism of action consists in inducing the maturation of

TABLE 1 The TLR agonists used in HPV-based mouse cancer models.

Agonists	Targeted TLR	Combined treatments	HPV16-driven syngeneic mouse cancer model	Effects on immune professional cells	Anticancer activity	References
Poly I:C (i.t.)	TLR3	HPV E7 peptide-based therapeutic vaccine and a pan HLA-DR epitope (i.t.)	TC-1 cells in C57BL/6 mice (s.c.)	• Enhanced frequency of E7-specific infiltrating $CD8^+$ T cells upon combined treatment	Better survival upon combined treatment	Wu et al., 2010[163]
Fusion protein EDA-HPVE7 (i.v.)	TLR4	• Poly I:C (i.v.) • cyclophosphamide (i.p.) and the TLR9 ligand CpG-B (i.v.) encapsulated in cationic lipid	TC-1 cells in C57BL/6 mice (s.c.)	• Enhanced antitumor $CD8^+$ T cell response upon EDA-HPVE7 and poly I:C co-treatment	Eradication of large TC-1 tumors following each co-treatment	Mansilla et al., 2012[164]
1V270 (i.t.) SD-101 (i.t.)	TLR7 TLR9	anti-PD-1 antibody (i.p.)	MEER cells in C57BL/6 mice (s.c.)	• Increased the ratio of M1 to M2 TAMs upon 1V270 injection • 1V270 injection in combination with anti-PD-1 antibody increases activated CD8 + T cells in tumors and spleen	Suppression of primary tumor growth and prevention of metastases upon either 1V270 or SD-101 injection in combination with an anti-PD-1 antibody	Sato-Kaneko et al., 2017[165]
CpG ODN	TLR9	CpG-enriched HPV16 E7 encoding DNA vaccine (i.m.)	C3.43 cells in C57BL/6 mice (s.c)	• Enhancement of the cytotoxic T cell response	Tumor growth inhibition	Olschlager et al., 2011[166]
		HPV16 E7 (s.c.)	TC-1 cells in C57BL/6 mice (s.c)	• Increased E7-specific antibody and T-helper cell proliferative responses • Increased IFN-γ production from spleen-derived CD8 + T cells	Suppression of tumor growth	Kim et al., 2002[167]
			TC-1 cells in C57BL/6 mice (s.c.) pre-immunized with recombinant E7 and ODN treated DC		Complete protection from tumor formation upon TC-1 s.c. challenging	Kim et al., 2004[168]

Abbreviations: TLR, toll like receptor; Poly I:C, Polyinosinic: polycytidylic acid; i.t., intratumoral, s.c., subcutaneous; HLA, human leukocyte antigens; EDA, Extra domain A; TAM, tumor associated- macrophages i.v., intravenous; i.p., intraperitoneal; PD1, programmed death-1 receptor; IFN, interferon; i.m., intramuscular; IFN, interferon; ODN, oligodeoxynucleotide.

monocyte-derived dendritic cells (mdDCs) maturation with enhanced antigen-presenting activity and IL-12 production. Furthermore, in patients with genital warts, imiquimod has been shown to activate innate immune cells, leading to the production of IFNs and other cytokines improving antigen presentation and promoting an antigen-specific Th1 cell-mediated immune response.[171–174]

In a separate study, poly I:C, serving as a TLR3 agonist, was combined with an HPV E7 peptide-based therapeutic vaccine and, in some cases, with an HLA-DR epitope peptide in an HPV16$^+$ TC-1-based mouse tumor model. This approach resulted in the generation of E7-specific CD8$^+$ T cells and enhanced antitumor effects, demonstrating a more significant impact when both treatments were used together compared to each treatment applied individually.[163]

TLR9 agonists, such as CpG oligodeoxynucleotide (ODN), have been shown to boost type I IFN release by pDCs, promoting the expression of costimulatory molecules such as CD80 and CD86. This action subsequently induces the secretion of cytokines and chemokines, activating NK cells, Th1, and cytotoxic T lymphocytes.[175] Similarly to TLR3 agonists, TLR9 agonists have also been tested in experimental models of therapeutic vaccines against HPV-driven cancer. Noteworthy, the administration of CpG-enriched HPV16 E7 encoding DNA vaccine (HPV16 E7SH), acting as a TLR9 agonist surrogate, elicited stronger IFN-γ and granzyme B responses, leading to enhanced tumor regression in HPV16-C3.43 syngeneic mice.[166] In addition, treating HPV16-TC-1 syngeneic mice with a fusion protein containing the extra domain A (EDA) from fibronectin, a natural ligand for TLR4, in combination with the HPV E7 protein (EDA-HPVE7) enhanced CD8$^+$ T cell antitumor response. Likewise, intravenous administration of EDA-HPVE7 alongside poly I:C, or with low doses of cyclophosphamide and the TLR9 ligand CpG-B encapsulated in cationic lipids, proved capable of eradicating large established TC-1 tumors.[164] Finally, coinjection of recombinant E7 with CpG ODN activated CD4$^+$ and, predominantly, CD8$^+$ T-cells, significantly reducing tumor formation in a TC-1 syngeneic mouse model.[167,168]

Altogether, these data underscore the potential of TLR agonists to induce CD8$^+$ T cells and sustain a long-term immune response, making them viable anticancer therapeutic options for HPV-associated diseases. Furthermore, increasing evidence suggests that TLR agonists effectively enhance cancer immune surveillance as immunological enhancers. This is supported by several ongoing clinical trials assessing the efficacy of combining TLR agonists with chemotherapy, radiotherapy, or various other immunotherapies, also in the context of HPV-associated cancers.[176–178] Lastly, their efficacy suggests they could serve as adjuvants for the production of therapeutic HPV vaccines aimed at promoting cellular responses while disrupting the anti-inflammatory microenvironment generated by HPV$^+$ cells.[179]

5 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The establishment of persistent infections by HPVs in human stratified squamous epithelia, both in the genital and upper aerodigestive regions, is the *condictio sine qua non* for cancer progression. The viral life cycle during these persistent infections is characterized by the transcriptional deregulation of the viral oncoproteins E6 and E7, whose aberrant expression progressively augments as lesions progresses from CIN-1 to CIN-3.[2–4,37,38] Notably, HPV-induced cancers are highly dependent on these viral oncoproteins, underscoring their critical role in promoting and sustaining the cancerous phenotype. This dependency on viral persistence and cancer progression relies on the ability of E6/E7 to evade innate immune surveillance.[13,14,18–21,32–35] The suppression of the innate immune response is essential for the creation of a cellular environment that supports viral persistence while allowing cells overexpressing E6 and E7 to proliferate despite accumulating chromosomal instability and DNA damage. This include preventing the cytoplasmic sensors, known as PRRs, from detecting DNA in the cytoplasm.[22–26,29] Circumventing this detection in infected keratinocytes ensures the creation and maintenance of a cellular environment that is unresponsive and conducive to transformation driven by deregulated E6/E7 expression.

In this review, we have explored current insights into the molecular mechanisms by which E6/E7 manipulate the innate immune response, emphasizing how these effects may be partly counteracted using emerging immunotherapies designed to reinvigorate the innate immune response as a means to mitigate these effects.[13–17,69,84–91,118–121,179] In this regard, we have summarized a large body of literature that attest the potential of PRR agonists to reactivate effectively innate immunity pathways in HPV$^+$ cancer cells by targeting the cGAS/STING, RIG-I, or TLR signaling pathways. These agonists not only promote cancer cell death in vitro and in vivo but also alter the tumor microenvironment in HPV16-based mouse cancer models in immunocompetent hosts, thereby enhancing systemic antitumor immunity.[157,158,162–170,179] Overall, the findings reviewed here are very promising; however, especially for STING and RIG-I agonists, appropriate human trials are necessary to establish their efficacy.

Despite the implementation of HPV vaccination programs for both genders in several countries in recent years, HPV-associated cancers will continue to pose a significant threat for the next two to three decades until the full benefits of comprehensive, gender-neutral vaccination become evident. This scenario is further complicated by various barriers to vaccination, such as parental concerns over vaccine safety, socioeconomic factors, and an overall lack of awareness. Thus, based on current vaccination rates in the USA, the incidence of HPV$^+$ OPSCC is expected to increase in the near future. Since this cancer is among those with the fastest-growing incidence rates in high-income countries, we can anticipate significant morbidity, mortality, and broader societal costs underscoring the need for novel therapeutic interventions against HPV-associated cancers, especially those affecting the head and neck region, which present unique phenotypic and clinical challenges.[1,4–6,8–12,30,31] Moreover, there has been a noticeable increase in anal cancer cases over the past few decades, especially among men. Factors such as specific sexual behaviors, the number of sexual partners, the frequency of receptive anal intercourse, and HIV

infection contribute to increased risk exposure to hrHPV genotypes. The global incidence of anal cancer is expected to continue rising in the near future, particularly among high-risk groups, such as HIV-positive men who have sex with men, with an annual incidence rate exceeding 131 cases per 100,000, and women with a history of HPV-related cancers.[180]

In this vein, gaining a comprehensive understanding of the mechanisms behind HPV-associated immune evasion could pave the way for the development of novel immunotherapeutic tools that can effectively restore antiviral and antitumoral immune responses. However, current research faces some limitations, including the need for further exploration of the action of PRR agonists in preclinical models of HPV-associated cancer, as well as in clinical trials.

It is our hope that the insights and perspectives shared here will inspire interdisciplinary research efforts aimed to elucidate the functional role of viral oncoproteins at the intersection of immune evasion and abnormal proliferation in HPV-associated cancers, with the ultimate goal of discovering novel targets for therapeutic development.

AUTHOR CONTRIBUTIONS

Conceptualization, Irene Lo Cigno and Marisa Gariglio; writing—original draft preparation, Irene Lo Cigno, Federica Calati, Carlo Girone, Marta Catozzo, and Marisa Gariglio; writing—review and editing, Irene Lo Cigno and Marisa Gariglio; supervision, Marisa Gariglio; funding acquisition, Irene Lo Cigno and Marisa Gariglio. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

All references to the original literature sources have been provided in the references section. No additional data are provided as the authors did not create new data or original experimental data for this review.

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