

REVIEW

Human endogenous retroviruses and their implication for immunotherapeutics of cancer

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Human endogenous retroviruses (HERVs) have recently caught increased attention as a potential internal trigger to sensitize tumor cells to immunotherapies. HERVs are remnants of retroviral germline infections that resulted in chromosomal integration into all the cells of the progeny. Today, HERVs constitute ~8% of the human genome, but most elements are highly degenerated, under strict epigenetic regulation, and rarely expressed in healthy tissues. However, cancer cells are specifically prone to reactivate the expression of HERV elements due to epigenetic dysregulation that accumulate during malignant transformation and when using epigenetic therapies. HERV expression can induce an interferon response due to induction of the viral defense pathway, so-called 'viral mimicry'. By mimicking viral infections, HERVs could function as an 'intrinsic adjuvant', possibly sensitizing cancer cells to immunological recognition. Furthermore, translated HERV elements may in themselves form a valuable pool of tumor-associated antigens. Epitopes derived from HERVs have been recognized by cytotoxic CD8⁺ T cells, leading to cancer cell recognition. The combination of 'viral mimicry' and T-cell recognition could provide a powerful combination with existing immune stimulatory therapies, such as checkpoint inhibition. This combination is currently being evaluated in clinical trials in a large number of cancers.

Key words: cancer immunotherapy, HERV, combination therapy, immune checkpoint inhibitors, DNA methyltransferase inhibitors, tumor-associated antigens

Introduction

Human endogenous retroviruses (HERVs) constitute ~8% of the human genome, distributed in ~700 000 elements [1]. They are remnants of retroviral germline infections that resulted in chromosomal integration into all the cells of the progeny, but their viral replication is defective in the present-day human genome [2]. Most HERVs are highly degenerated through evolutionary pressure due to accumulation of mutations or homologous recombination between proviral long terminal repeats (LTRs) [3]. Although most HERVs are under strict epigenetic repression and the protein coding capacity is limited, some HERVs are transcribed due to the strong promoter activity of the flanking LTRs [1, 3]. The fixation of HERVs in the human genome is not the end of the immunological conflict between the host and retrovirus. HERVs' retroviral origin, together with the fact that they constitute a large amount of the human genome, makes them

interesting from an immunological perspective [3]. Replication intermediates from HERVs can induce an interferon (IFN) response in a mechanism called 'viral mimicry' [4, 5]. By mimicking viral infections, HERVs could function as an 'intrinsic adjuvant', possibly sensitizing cancer cells for immune recognition [3]. Tumor-associated reactivation of HERVs has been reported in cancer tissues [6], and HERVs may play a prominent role in cancer immunogenicity, either by activating the viral defense pathway (viral mimicry) [4, 5] or by forming a novel pool of tumor-associated antigens that can serve as T-cell targets on tumor cells (Table 1). The interactions between HERVs and the adaptive immune system could be important for exploiting HERVs as targets in anticancer immunotherapy. However, some HERV elements, the HERV-encoded Envelope (Env) proteins, have immunosuppressive properties that are important in fetal-maternal tolerance [3] and the mechanism could potentially be

Table 1. Cytotoxic T-cell responses against HERV-derived epitopes reported in the literature					
HERV	Epitope	Location	HLA	Cancer type	References
ERV-4	FLHKTSVREV	6q15	A0201	Renal clear cell carcinoma	[19]
	SLNITSCYV				
ERVH-2	LLLQIMRSFV	6q15 Xp22.32	A11 A0201	Renal clear cell carcinoma	[11]
	ATFLGSLTWK				
	CLYPFSAFL			Colorectal cancer	[20]
	PLLSVSLPLL ^a SLNFNSFHFL				
ERVK			A0201	Breast cancer	[10]
ERVK3 ^b	FLQFKTWWI	16	A0201	Seminoma	[18]
	MLAVISCAV		A2	Melanoma	[17]

All the reported cytotoxic T-cell responses are found in malignant tissues.
^aMinor response reported.
^bHERV-K MEL is an antigenic protein that is encoded by a short open reading frame from a processed ERVK3.

used to shield tumor cells from the host’s immunological attacks [7]. In this review, we discuss how HERVs interact with the adaptive immune system with a particular focus on T-cell responses. In particular, CD8⁺ T-cell responses are of particular importance in the defense against viral infection, which HERV expression could mimic, and they are key mediators of cancer cell destruction in cancer immunotherapy. For HERV annotations, the revised nomenclature by Mayer et al. [1] was used in this review whenever the primary literature provided sufficient information to support the use of this nomenclature.

Adaptive immune response to HERVs

The majority of HERVs have degenerated proviral genomes, which prevent transcription of their retroviral genes. Still, some HERVs have retained their open reading frames (ORFs) within the human genome with the potential to allow transcription and translation, and hence contribute their viral proteins to the human proteome [3]. While the level of HERV expression in healthy human tissues is limited [6], it may be induced during malignant transformation or due to epigenetic therapy. Since these proteins have retroviral origins, they may form antigens that can be recognized by T cells and B cells. Animal and human studies have demonstrated both humoral and cell-mediated immune responses against ERV antigens [8–10], which could indicate that immunological tolerance to HERVs is incomplete; however, these antigens’ thymic expression is still poorly understood, and consequently the level of tolerance toward such antigens remains to be determined.

Presentation of HERV-derived antigens by the major histocompatibility complex (MHC) class I is of particular interest to determine the potential immune recognition of HERVs. CD8⁺ T cells specific for HERV-derived epitopes have been isolated and expanded from the blood of patients with regressing renal cell carcinoma (RCC) following nonmyeloablative allogeneic hematopoietic stem cell transplantation. Such HERV-specific T-cell mediated the killing of patient-derived tumor cells *in vitro* [11].

Rolland et al. [12] demonstrated that the ERVW Env could induce dendritic cell maturation through an innate immune mechanism, which resulted in a T_H1-like immune response. T_H1 immune responses are important in the defense against viral infection, which is mimicked by HERV expression. In the literature, several specific CD8⁺ T-cell responses against HERV-derived antigens in cancer have been reported (Table 1), indicating the potential importance of HERVs as a target for cell-mediated cytotoxicity in cancer immunotherapy.

Intuitively, humoral immune responses would not be of great importance since HERV expression is intracellular; however, the retroviral Env proteins are transported to the cell surface and could serve as direct targets for humoral immune responses [10]. Antibody responses may also be driven by inflammatory cell death and uptake, and both humoral and cell-mediated immunity against the ERVK Env protein have been detected in breast cancer patients [10]. Antibody-mediated immune responses were also reported in ovarian cancer patients and reverse transcriptase activity was observed in the plasma of ovarian cancer patients [13]. To mount an antibody-driven immune response, either direct secretion of HERV antigens from tumor cells or uptake of dying tumor cells with HERV expression should take place [10]. In many cases, antibody immune responses are accompanied by a CD8⁺ T-cell response. The lytic potential of such T cells may explain the potential shedding of HERV antigens from dying tumor cells and the presence of reverse transcriptase activity in plasma, as observed in ovarian cancer patients [13]. In particular, antibody responses against the retroviral group-specific antigen (Gag) and Env proteins have been reported in the literature, which could indicate that these viral proteins are highly immunogenic.

Immune tolerance to HERVs

HERV expression exhibits tissue specificity [6], which is determined mainly by epigenetic regulation and the availability of tissue-specific transcription factors [14]. Expression of HERVs in the thymus is not well described, though the intrathymic

expression of HERVs is believed to be incomplete mainly due to epigenetic silencing [3]. It has been speculated that HERVs share strong similarities with exogenous retroviruses and therefore incomplete immunological tolerance could be necessary to defend against exogenous retroviral infections [3]; however, the basal expression of HERVs in healthy tissues [6, 15] could indicate the need for tolerance toward HERVs to avoid autoimmune responses. Hence, peripheral tolerance and ignorance mechanisms may play prominent roles in the control of HERV-specific T-cell recognition in healthy individuals [3]. HERV activation of innate receptors, which mimics viral infections, can potentially induce an inflammatory state, leading to the production and secretion of T-cell-activating chemokines and cytokines [4, 5, 16] and thereby bypass the potential immunological ignorant state. CD8⁺ T-cell responses against HERV-derived antigens have been reported in the literature (Table 1), strongly indicating an incomplete clonal deletion of HERV-antigen-specific T cells in the thymus. Understanding the differences in HERV expression in the thymus and peripheral tissues would be of great importance for the use of HERVs as immunotherapeutic targets.

T-cell responses against HERVs in tumors

Schiavetti et al. was the first to report a CD8⁺ T-cell response against an HERV-derived epitope in a melanoma patient. They identified in ERVK3 (HERV-K MEL), a translated region encoding the peptide sequence (MLAVISCAV) presented in HLA-A2 and verified CD8⁺ T-cell recognition of autologous tumor cells targeting this peptide-MHC complex. ERVK3-specific CD8⁺ T cells were found in two melanoma patients where the autologous tumor cell lines expressed ERVK3, but no ERVK3-specific CD8⁺ T cells were detected in the blood of three healthy individuals analyzed in parallel [17]. This study was followed by Rakoff-Nahoum et al. who, *in silico*, predicted 15 peptides from ERVK Gag for prevalent HLA alleles and divided these peptides into four pools for an initial screening of T-cell responses in patients with seminoma. T-cell responses to the ERVK peptide pools were found in the peripheral blood mononuclear cells (PBMCs) of seminoma patients at a much higher frequency than in healthy controls [18]. Some cancer types seem to have high and preferential expression of certain HERVs; in RCC, ERVE-4 has been shown to be prominently and preferentially expressed in comparison to other tumor types and to normal tissues, where no expression is observed [6, 19]. The long-conserved ORFs in ERVE-4 *env* could indicate a retained capacity to produce partial Env proteins in RCC tumors [19]. In several studies, HERV-specific T-cell recognition was detected in RCC. From the ERVE-4 locus, four epitopes have been identified that give rise to specific cytotoxic T-cell responses [11, 19]. Of these, Cherkasova et al. show that three HLA-A0201 *in silico*-predicted peptides from the putative ERVE-4 Env protein could expand CD8⁺ T cells *in vitro* [19]. Takahashi et al. reported a regression of metastatic RCC in patients following nonmyeloablative allogeneic hematopoietic stem cell transplantation with the detection of tumor-reactive, donor-derived CD8⁺ T cells in the blood of the patients. With the use of cDNA cloning, they identified an epitope from ERVE-4 as responsible for the tumor-reactivity of the donor-derived CD8⁺ T cells [11]. T-cell recognition of HERVs has likewise been

described in a few other cancer types. Mullins and Linnebacher demonstrated the presence of T cells that reacted to the ORF of ERVH-2 in patients with gastrointestinal cancers. ERVH-2 has previously been shown to be strongly expressed in a subset of gastrointestinal cancers while having low expression in other tumor types and healthy tissue. They further used 10 ERVH-2-derived peptides for the *in vitro* stimulation of T cells from healthy donors and demonstrated the induction of ERVH-2-specific T cells capable of lysing a HLA-A0201 colorectal cancer cell line expressing ERVH-2 [20]. Also in breast cancer patients, T-cell responses against ERVK were demonstrated following stimulation of PBMCs with autologous dendritic cells pulsed with ERVK Env antigens. T-cell proliferation was observed together with cytokine secretion, that is IFN- γ , interleukin-2 (IL-2), IL-6, IL-8, and chemokine CXCL10, only in breast cancer patients, indicating the presence of ERVK Env T-cell recognition in response to disease. Moreover, T cells with lytic capacity toward ERVK Env antigen were found in breast cancer patients but not in healthy females [10].

Together, these studies summarized in Table 1 suggest that HERVs can be immunogenic and have the ability to activate adaptive T-cell responses capable of tumor cell recognition, particularly the *env* gene, which has been suggested to be highly immunogenic. This antigen could be a promising candidate for future immunotherapeutic strategies such as cancer vaccines; however, among the group of HERVs, there may be substantial differences in their biological effects, potential roles in immune sensitization, and ability to form an antigen reservoir. Early data point toward distinct features for the expression profiles of different HERVs [6], but these characteristics remain to be fully elucidated.

Reactivation of HERVs in cancer

Many studies have associated HERV expression with various cancer types, such as breast cancer [21, 22], melanoma [23–25], and kidney cancer [11, 19]. The elevated expression of distinct HERVs observed in cancer cell lines could indicate a potential oncogenic role of HERVs, but the causative involvement of HERVs in cancer remains controversial (review the potential oncogenic role of HERVs in Kassiotis [26]).

A study by Rooney et al. [6] mapped the 66 HERVs from Mayer et al. to RNA-sequencing (RNAseq) data from 18 different tumor types from The Cancer Genome Atlas (TCGA) and corresponding healthy tissue controls from the Genotype-Tissue Expression project and TCGA. By mapping the RNAseq data to the HERV transcripts, they demonstrated that numerous HERVs showed enhanced transcription in tumor tissues compared with healthy tissues. Out of the 66 HERV loci, they highlighted three HERV loci (ERVH-5, ERVH48-1, and ERVE-4) with minimal to undetectable transcription in normal tissues and highly elevated expression in certain tumor tissues. Hence, the authors characterized these as tumor-specific endogenous retroviruses [6]. ERVH-5 is especially prominently expressed in bladder, colorectal, head and neck, lung squamous, ovarian, stomach, and uterine cancers and ERVH48-1 is especially prominently expressed in bladder cancer and prostate cancer, whereas ERVE-4 can be found specifically in RCC. Interestingly, cytotoxic T-cell activity against

Table 2. Expression of HERV-encoded proteins detected in human cancers

Cancer type	HERV protein	Sample type	References
Breast cancer	ERVK Env	Tissue	[10]
	ERVK RT	Tissue	[31]
	ERVW Env	Cell line, tissue	[32]
Endometrial carcinoma	ERVW Env	Tissue	[33]
Colorectal cancer	ERVH-2 Env	Cell line	[20]
Germ cell tumor	ERVK Env	Cell line	[34, 35]
	ERVK Gag	Cell line, tissue	[35–37]
Lymphoma	ERVK Env	Plasma	[38]
	ERVK Gag	Plasma	[38]
Melanoma	ERVK Env	Cell line, tissue	[23, 24, 39]
	ERVK Gag	Cell line, tissue	[23, 24]
	ERVK Rec	Cell line, tissue	[23]
Ovarian cancer	ERVK Env	Cell line, tissue	[13, 40]
Prostate cancer	ERVK Gag	Tissue	[41]
Renal cell carcinoma	ERVE-4 Env	Cell line	[19]

Env, envelope; Gag, group-specific antigen; RT, reverse transcriptase.

ERVE-4 was reported previously in RCC [11, 19]. From the Rooney et al. [6] data, it can be deduced that distinct HERV loci exhibited tumor-associated rather than tumor-specific transcription, because basal HERV expression is observed in healthy tissue, depending on the HERV loci and tissue type. The Rooney et al.'s study is, to date, the most comprehensive analysis of HERV expression in different cancer tissues compared with healthy tissues. ERVE-4 expression in RCC is a well-described example of tumor-associated HERV expression. Inactivation of the tumor suppressor gene *von Hippel-Lindau* results in the overexpression of hypoxia-inducible transcription factor-2 α (HIF-2 α). HIF-2 α binds to HIF response elements, which are located in the 5'LTR of ERVE-4. This LTR was found to be hypomethylated in RCC tumors that expressed ERVE-4, compared with other tumors and normal tissues, indicating a tissue-specific epigenetic regulation in RCC [27, 28]. Cancer-specific transcription factors can be important for tumor-associated expression, for example ERVK has been demonstrated to be activated by the melanoma-specific transcription factor (MITF-M) [14, 29]. ERVK LTRs are additionally susceptible to stimulation by hormones, and hence ERVK expression has been suggested to be relevant in cancers of hormone-regulated tissues [30].

The tumor-associated expression of HERVs observed in cancer tissues has naturally given rise to one question: do HERVs have a causal role in the development of cancer or is their expression a byproduct of malignant cellular transformation, such as global DNA hypomethylation? Global DNA hypomethylation is a hallmark for malignant cellular transformation and could potentially be a major contributor to the oncogenic activation of HERVs [26]. As a result of the malignant transformation, the levels of HERV transcripts and proteins in human cancers are highly dysregulated compared with healthy tissues, where HERV expression is either low or absent [6]. HERV-encoded protein expression in human cancer specimens is summarized in Table 2 [10, 13, 19, 20, 23, 24, 31–41].

Exploiting HERV reactivation in cancer immunotherapy

HERVs exhibit tumor-associated expression in cancer cells [6] and could potentially be a novel pool of tumor-associated antigens to be exploited as targets for cancer immunotherapy (Table 2) [10]. Another attractive trait of HERVs is their retroviral origin, which can promote innate immune responses and result in an adjuvant-like behavior to potentially boost antitumor immunity [42]. The immunogenic potential of HERVs is further supported by reports of humoral and cell-mediated immunity in the literature [10, 19] (Table 1). However, immunotherapy targeting HERVs is still in its infancy, but preliminary steps are being taken to elucidate the potential of HERV antigens in immunotherapy.

HERVs used as targets in monoclonal antibody therapy

ERVK Env expression has been detected at much higher levels in malignant breast cancer cell lines than in healthy breast tissues [10]. Wang-Johanning et al. [43] observed that breast cancer cell lines and human primary breast cancers have abundant levels of ERVK Env on their cell surfaces, which makes them ideal targets for antibody therapy (Figure 1). Monoclonal ERVK Env antibodies inhibited the growth and proliferation of breast cancer cells lines *in vitro*. Induction of apoptosis was also observed in breast cancer cells treated with monoclonal ERVK Env antibodies [43]. Mice bearing xenograft tumors showed significantly reduced growth upon treatment with monoclonal ERVK Env antibodies, compared with control experiments [43]. Taken together, such data indicate the potential of monoclonal ERVK Env antibodies as a novel immunotherapeutic agent for the treatment of breast cancer. Intriguingly, ERVK-positive breast tumors develop lymph node metastasis more frequently than ERVK-negative breast tumors. Although the exact mechanism for this effect is poorly understood, it has been suggested that ERVK expression may have prognostic relevance in metastatic breast cancer [43]. Interestingly, initial clinical studies with a humanized monoclonal antibody targeting HERV Env protein as a treatment of multiple sclerosis demonstrated a favorable safety profile with no induction of immunogenicity [44]. These results are promising for future clinical use of HERV-targeting monoclonal antibodies, although the potential therapeutic efficacy needs to be confirmed in more comprehensive clinical studies.

HERVs used in chimeric antigen receptor T-cell therapy

Adoptive cellular immunotherapy with chimeric antigen receptor (CAR)-expressing T cells targeting ERVK Env has been investigated for melanoma [45] and breast cancer [46] (Figure 1). Krishnamurthy et al. reported a decrease in tumor burden in a murine metastatic melanoma model when mice were treated with ERVK Env-specific CAR T cells. *In vitro*, these CAR T cells exhibited the specific lysis of tumor cells expressing ERVK Env on their surfaces [45]. Zhou et al. [46] demonstrated that ERVK Env-specific CAR T cells could reduce tumor growth and tumor weight in xenograft breast cancer murine models. *In vitro* experiments showed that ERVK Env-specific CAR T cells exhibited a

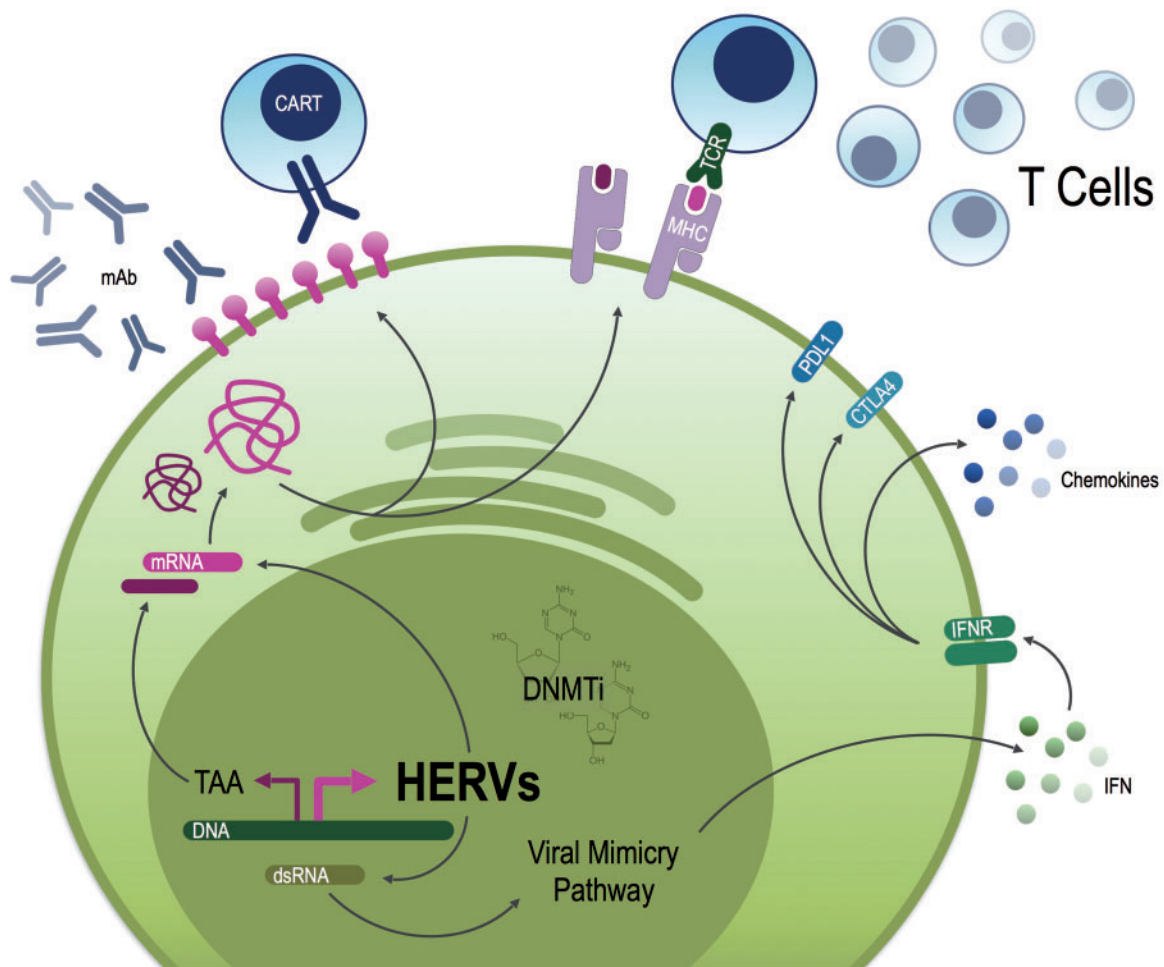


Figure 1. DNA methyltransferase inhibitors (DNMTis) alleviate the epigenetic repression of human endogenous retroviruses (HERVs) by removing DNA methylation, causing HERV double-stranded RNA (dsRNA) to activate innate immune pathway mimicking viral infections resulting in secretion of interferons (IFN). Stimulation of interferon receptors (IFNR) activate JAK/STAT signaling and up-regulate expression of chemokines, programmed death-ligand 1 (PD-L1), cytotoxic T-cell-associated protein 4 (CTLA-4), and components of the antigen presentation pathway. Separately, distinct HERVs exhibit tumor-associated expression similar to tumor-associated antigens (TAA) and may serve as targets for T-cell recognition, when HERV-derived peptides are presented on the cell surface in MHC class I. Additionally, HERV envelop (Env) expression on the cell surface make it susceptible to targeting by monoclonal antibodies (mAb) or chimeric antigen receptor T cells (CART).

cytotoxic effect on breast cancer cells but not healthy breast tissue. The antitumor effect observed for ERVK Env-specific CAR T cells was significantly reduced in breast cancer cells treated with either control T cells or short hairpin RNA (shRNA) to induce the knockdown of ERVK Env. Furthermore, multiple cytokines, including IFN- γ , tumor necrosis factor- α , and IL-2, were detected in the culture media of breast cancer cell lines treated with ERVK Env-specific CAR T cells, suggesting T-cell-mediated lytic activity [46]. In this same study, a significant reduction or complete inhibition of metastasis development was observed in the liver, brain, lungs, and lymph nodes of murine models treated with ERVK Env-specific CAR T cells, compared with control T cells. Interestingly, they observed that ERVK Env interacted with the p53 and RAS pathway, which could indicate an oncogenic effect of ERVK Env protein [46]. Together, these two studies show the promise of CAR T cells targeting ERVK Env in treating melanoma and breast cancer, but further studies are needed to translate these results into clinical use.

HERV-derived antigens as vaccination targets

Prophylactic vaccination against HERV-derived antigens could potentially elicit long-lived T-cell responses, in an otherwise tolerant host, that could eliminate early malignancies [10]. Therapeutic vaccine strategies have been suggested for breast cancer, ovarian cancer, kidney cancer, and other tumors where HERV-derived epitopes have been demonstrated to be processed and presented, forming targets for T-cell recognition [10, 13, 19]. To test the vaccination efficacy against cancer, Kraus et al. [47] generated a recombinant vaccinia virus that expressed the ERVK Env protein and a syngeneic mouse tumor model that introduced ERVK Env expression in murine renal carcinoma cells (RLZ-HKenv cells). Intravenous injection of RLZ-HKenv cells into BALB/c mice resulted in the formation of pulmonary metastases, which could be prevented by a single administration of the vaccine [47]. A prophylactic strategy was also tested, which showed complete protection against tumor development when using the ERVK Env vaccination, compared with wild-type vaccinia virus

[47]. Vaccinated mice exhibited cell-mediated cytotoxic activity against RLZ-HKenv cells, suggesting that T-cell-mediated tumor cell killing was induced by the vaccine [47]. Kraus et al. [2] also demonstrated, with a similar study, that ERVK Gag could potentially be a valuable target for cancer immunotherapy. A potential concern of HERV-based vaccine treatment could be hazardous ERVK expression in, for example hematopoietic stem cells [2]. Sacha et al. [9] investigated the safety of the vaccine strategy in rhesus macaques by immunization with simian endogenous retrovirus (SERV) Gag and Env proteins demonstrating expansion of polyfunctional T cells to both SERV proteins and induction of antibody responses to SERV Env protein; no adverse effects, including a lack of autoimmunity, were reported. ERV antigen-based vaccines show potential as a safe immunotherapeutic option against cancer in preclinical animal models [2, 9, 47]; however, clinical human studies are needed to test both the efficacy and safety of the vaccine and facilitate further therapeutic development.

DNA hypomethylating agents and immunotherapy combinatory treatments

Alleviating the epigenetic silencing of distinct HERV loci in various cancer types may result in the reactivation of HERVs in tumor tissues, and this may happen following the intrinsic hypomethylating profile seen in many tumors [6]. A similar phenomenon could be induced using DNA hypomethylating agents, such as DNA methyltransferase inhibitors (DNMTi), for example azacitidine, decitabine, or guadecitabine. Recently, Brocks et al. [48] showed that DNMTi and histone deacetylase inhibitor (HDACi) treatment resulted in the induction of the *de novo* transcription of LTRs in cancer cell lines rather than altering the expression of canonical genes. Ohtani et al. demonstrated an epigenetic switch from DNA methylation to histone silencing depending on the evolutionary age of LTRs. LTRs from evolutionarily young HERVs such as the ERVK family are predominately silenced by DNA methylation, whereas LTRs from intermediate age HERVs are silenced by histone modifications [49].

Treatment with DNMTi has been shown to increase HERV transcription, including HERV-derived double-stranded RNA (dsRNA), in tumor cell lines [4, 5]. HERV-derived dsRNA can trigger the viral defense pathway through the MDA5-MAVS dsRNA recognition pathway, resulting in an interferon response that resembles that of a viral infection [4, 5]. Interestingly, Ohtani et al. observed an increase in histone silencing of LTRs following DNMTi therapy, which suggest that the epigenetic silencing of HERVs can be rescued by histone silencing. This rationalizes a synergistic effect of inhibition of both DNA methylation and histone silencing modifications. Dual depletion by azacitidine and knockdown of specific histone modifying enzymes increased the expression of viral defense genes. This was probably due to activation of LTRs from young HERVs as knockdown of histone-modifying enzymes alone did not up-regulate viral defense genes, indicating that activation of LTRs from intermediate age HERVs is not sufficient to induce a viral mimicry response [49]. This viral mimicry can lead to increased antigen processing and presentation, secretion of immune-attracting chemokines, expression of interferon-stimulated genes, up-regulation of cancer testis antigens, and up-regulation of immune

checkpoints [programmed death-ligand 1 (PD-L1) and cytotoxic T-cell-associated protein 4 (CTLA-4)] [16, 48]. These characteristics may result in induced T-cell attraction, as observed upon viral infection, and the up-regulation of CTLA-4 and PD-L1 upon treatment with DNMTi can lead to a sensitization toward treatment with immune checkpoint inhibitors (Figure 1) [50]. In addition, PD-1 expression on the surface of T cells has been shown to be up-regulated following DNMTi therapy [51].

RNAseq data from melanoma patients treated with CTLA-4 antibodies showed that the long-term benefit of immune checkpoint therapy correlates with high basal expression levels of the azacitidine-induced viral defense gene signature in tumor samples [4]. This finding was confirmed in a murine melanoma model, which showed that low doses of azacitidine sensitize tumors to treatment with CTLA-4 antibodies [4]. Additionally, a synergy of decitabine and CTLA-4 antibodies was observed in murine ovarian cancer models, where a low dose of decitabine resulted in the up-regulation of chemokines that attract natural killer cells and CD8⁺ T cells. Combining epigenetic therapy with CTLA-4 antibody therapy promotes the differentiation of naive T cells into effector T cells and enhances cytotoxic T-cell responses together with increased survival in mice models [52]. Treatment of epithelial ovarian cancer and non-small-cell lung cancer cell lines with azacitidine resulted in increased surface expression of PD-L1 as an upstream consequence of activating the viral interferon response pathway [53, 54] and may additionally have a direct impact on PD-1 expression in T cells [51]. Although PD-L1 expression generally serves as a negative regulator for T-cell-mediated tumor recognition, this marker also reflects the inflammatory state and hence high expression of PD-L1 correlates with good responses to PD-1 antibody therapy [50]. The combination of epigenetic therapy and immunotherapy, such as immune checkpoint inhibitors, could potentially be of great clinical importance [50]. The ectopic expression of HERVs upon DNMTi treatment could potentially result in novel antigens being recognized by cytotoxic T cells and possibly contributing to an enhanced effect of the combinatory treatment [48]. This could be of great importance, specifically in cancer types with a low mutational burden. The combination of epigenetic therapy and therapeutic vaccine strategies or adoptive T-cell transfer that both target HERV-derived antigens could be of particular interest as future cancer therapy strategies.

Discussion

Concluding remarks

Immunotherapy against cancer relies on the tight association of target antigen expression in diseased tissue with none to minimal expression in healthy tissues [47], and consequently there is great interest and therapeutic potential in the identification of antigens with a distinct tumor-associated profile. Certain HERV groups or loci offer such tumor-associated properties [6], and their expression may be further induced through already marketed anti-cancer therapies, such as DNMTi. These characteristics, together with their retroviral origin and the incomplete tolerance, make them a promising target for future cancer immunotherapy.

Table 3. Clinical studies investigating the potential benefits of combining immune checkpoint inhibitors and DNA methyltransferase inhibitors (DNMTi) in cancer treatment based on ClinicalTrials.gov.

Drug combination		Conditions	Phase	NCT #
Azacitidine:	Atezolizumab	MDS	I	NCT02508870
	Avelumab	AML, B-cell lymphoma	I, II, III	NCT02953561, NCT03390296, NCT02951156
	Durvalumab	MDS, colorectal carcinoma, ovarian cancer, breast cancer, AML, T-cell lymphoma, head and neck cancer, NSCLC	I, II	NCT02281084, NCT02811497, NCT02775903, NCT03161223, NCT03019003, NCT02117219, NCT02250326
	Ipilimumab	Leukemia, MDS	II	NCT02397720, NCT02530463
	Nivolumab	MDS, AML	II, III	NCT03092674
	Pembrolizumab	Pancreas cancer, colorectal cancer, melanoma, MDS, NSCLC, head and neck cancer, urothelial carcinoma, AML, epithelial ovarian cancer	I, II	NCT03264404, NCT03182894, NCT02260440, NCT02816021, NCT03094637, NCT02959437, NCT02845297, NCT02546986, NCT02900560, NCT02512172
Decitabine:	Tremelimumab	Head and neck cancer, MDS	I, II	NCT03019003, NCT02117219
	Avelumab	AML	I	NCT03395873
	Ipilimumab	MDS, AML	I	NCT02890329
	Nivolumab	NSCLC, MDS, AML, leukemia	I, II, III	NCT02664181, NCT03358719, NCT03092674, NCT02599649, NCT02397720, NCT02530463, NCT01928576, NCT03259516
Guadecitabine:	Pembrolizumab	AML, T-cell lymphoma, NSCLC, breast cancer	I, II	NCT02996474, NCT03240211, NCT03233724, NCT02957968
	Atezolizumab	Leukemia, MDS, AML, fallopian tube carcinoma, ovarian carcinoma, primary peritoneal carcinoma, urothelial carcinoma	I, II	NCT02935361, NCT03206047, NCT02892318, NCT03179943
	Durvalumab	Kidney cancer, clear cell renal cell carcinoma, hepatocellular carcinoma, cholangiocarcinoma, pancreatic cancer, small cell lung cancer	I, II	NCT03308396, NCT03257761, NCT03085849
	Ipilimumab	Melanoma	I	NCT02608437
	Pembrolizumab	Lung cancer, fallopian tube carcinoma, ovarian carcinoma, primary peritoneal carcinoma, prostatic cancer, NSCLC	I	NCT03220477, NCT02901899, NCT02998567
	Tremelimumab	Small cell lung cancer	I	NCT03085849
Total: 46				

Following immune checkpoint inhibitors were included; pembrolizumab (anti-PD1), nivolumab (anti-PD1), durvalumab (anti-PDL1), atezolizumab (anti-PDL1), avelumab (anti-PDL1), tremelimumab (anti-CTLA4), and ipilimumab (anti-CTLA4). Following DNMTis were included; azacitidine, decitabine and guadecitabine. Search results from all combinations of the two groups in the field 'Intervention/treatment' (e.g. nivolumab and azacitidine) were included in the table if a combination treatment of immune checkpoint inhibitors and DNMTis was used to treat cancer. The table shown here is a comprised version of the search results, the full table is available in [supplementary Table S1](#), available at *Annals of Oncology* online.

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; NSCLC, non-small-cell lung carcinoma.

Different immunotherapeutic strategies have been applied to HERV-antigens *in vitro* and *in vivo*, for example monoclonal antibodies, prophylactic and therapeutic vaccination, CAR T cells, and epigenetic therapy combined with immune checkpoint inhibitors, which validate the great potential of HERV antigens. The combination of epigenetic drugs and immunotherapy is exciting for future cancer therapy, especially for cancer types with low mutational burden that respond poorly to immunotherapy or cancer types with poor responses to immune checkpoint inhibitor treatment [48]. More than 46 clinical studies are currently

addressing the safety and efficacy of such combination therapies (Table 3 and [supplementary Table S1](#), available at *Annals of Oncology* online). These studies were retrieved from Clinicaltrials.gov by searching for the combination of DNMTis and immune checkpoint inhibitors for treatment of cancer. In-depth analyses of tumor and immune cells from the patients enrolled in these trials will provide critical information regarding the mechanistic role of HERV expression and the following effects, in terms of both viral mimicry and the generation of tumor antigens. Additionally, future proteomic studies that

reveal the natural expression characteristics and levels of thymic HERV expression will be important in the process of developing safe and efficient strategies for targeting HERV expression in tumors. Furthermore, the knowledge of T-cell recognition of HERVs is currently very limited. Only a few T-cell epitopes have been characterized in patient samples from a variety of cancers, and little is known about the expression of the different HERV families in various cancer types. Hence the extent of HERV–T-cell recognition in cancer is not yet known, as well as the potential existence of immunological hotspots in the HERV-derived proteins that may be the most valuable for targeting. Thus, in-depth analyses of the immunological mechanisms and, in particular, the role of HERV-derived antigen presentation will hopefully shed light on the potential interaction between epigenetic drugs and immunotherapy in stimulating immune responses in cancer.

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Disclosure

The authors have declared no conflicts of interest.

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