Human Endogenous Retrovirus as Therapeutic Targets in Neurologic Disease

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Abstract: Human endogenous retroviruses (HERVs) are ancient retroviral DNA sequences established into germline. They contain regulatory elements and encoded proteins few of which may provide benefits to hosts when co-opted as cellular genes. Their tight regulation is mainly achieved by epigenetic mechanisms which can be altered by environmental factors, e.g. viral infections, leading to HERV activation. Aberrant expression of HERVs associates with neurological disease, such as multiple sclerosis (MS) or amyotrophic lateral sclerosis (ALS), inflammatory processes and neurodegeneration. This review summarizes recent advances on the epigenetic mechanisms controlling HERV expression and the pathogenic effects triggered by HERV derepression. The article ends describing new promising therapies targeting HERV elements, one of which, temelimab, has completed phase II trials with encouraging results in treating MS. The information gathered here may turn helpful in the design of new strategies to unveil epigenetic failures behind HERV-triggered disease, opening new possibilities for druggable targets and/or for extending the use of temelimab to treat other associated diseases.

Keywords: amyotrophic lateral sclerosis; epigenetics; HERV-K; HERV-W; monoclonal antibody; multiple sclerosis; neurodegeneration; temelimab.

1. Introduction

At least 45% of the human genome is comprised of transposable elements (TEs). TEs are classified into DNA transposons and retrotransposons. The first can change their position by excising from one region to integrate into another, while retrotransposons "make a copy" of themselves which is then integrated into another position of the genome, while retaining the original copy. Retrotransposons can be categorized according to the presence/absence of long terminal repeats (LTRs) as LTR retrotransposons and non-LTR retrotransposons, respectively. Long Interspersed Nuclear Elements (LINE) and Short Interspersed Nuclear Elements (SINE) make the non-LTR group and represent around 33% of the human genome, whereas human endogenous retroviruses (HERVs), flanked by LTRs, represent almost 8% [1-3].

HERVs are remnants of ancient exogenous viral infections which established into the Vertebrates germline millions of years ago [4-7]. They classify into different families named after the amino acid one-letter code corresponding to the aminoacyl-tRNA used by the reverse transcriptase to prime their retrotranscription [2]. HERVs typically encompass three main proviral genes, *pol*, *gag* and *env*, flanked by two LTR sequences. The *gag* gene encodes structural proteins; the *pol* gene a reverse transcriptase, a protease and an integrase; and the *env* gene, an envelope protein. Since their introduction into the germline, some HERVs have accumulated mutations in their proviral genes making them replication-defective because of frameshifts, the appearance of early stop codons or

deletions/insertions of sequences. Homologous recombination between both LTR sequences also led to the complete loss of proviral genes in some cases, leaving the HERV represented by its LTR only [3-5,7,8]. This mechanism has left copies with variable genomic content in the human population, such as the newly discovered HERV-W copy on chromosome 18 that may be homozygote solo LTR after complete proviral elimination, heterozygote with one allele consisting in the remaining proviral genes and one solo LTR, and homozygote with both alleles retaining the proviral genes [9]. Despite all these events, some families remain transcriptionally active, mostly expressed in a tissue-specific manner [10-12], but also contribute to human genome heterogeneity [9,13].

Throughout evolution, HERVs have occasionally provided their hosts with beneficial effects. They contributed to genomic variability, provided additional gene regulatory elements, such as alternative promoters or alternative splice sites [14-17] or may now rule important physiological processes, contributing to transcriptional regulation with non-coding RNAs [18,19] or through the expression of "domesticated" viral proteins. For example, an element of the HERV-W family, ERVWE1, encodes a protein, Syncytin-1, which is essential for embryo implantation [20,21]. Also, some HERVs are proposed to protect the fetus by suppressing potential maternal immunological reactions [22,23]. Nonetheless, if uncontrolled and/or abnormally activated, HERVs can lead to detrimental effects in their host organisms by altering gene expression profiles, expressing pathogenic nucleic acids/proteins or even inducing deleterious mutations [2,24,25].

2. Epigenetic control of HERVs

Expression of HERVs can be controlled at different levels but one of the most important involves epigenetic regulatory mechanisms [26,27] (Figure 1). Although most HERVs are constitutively repressed in the human genome, some of them remain transcriptionally active but their activation is limited to specific time frames and tissues [20]. To achieve this context-specific regulation, some HERVs harbor binding sequences for KRAB-containing zinc finger proteins (KRAB-ZFPs). Interestingly, the number of KRAB-ZFPs correlates with LTR transposon load across species [28]. In fact, these transcriptional repressors can benefit to the host via a role in domesticating TEs, including HERVs [29].

KRAB-ZFPs are comprised of an array of C2H2 zinc fingers, which confers them specificity to bind DNA sequences within HERV elements, and a Krüppel-associated box (KRAB) that recruits the corepressor KRAB-associated protein 1 (KAP1), also called TRIM28 [30-35]. KAP1 serves as a scaffold for the assembly of heterochromatin epigenetic modifiers like the histone methyl transferase SETDB1 (also known as ESET) [36], the heterochromatin protein 1 (HP1) [37] or DNA methyl transferases (DNMTS) [38,39] (Figure 1, number 1).

Three molecules of KAP1 bind to the KRAB domain of the KRAB-ZFPs through their N-terminal RING-B box-coiled coil (RBCC) region, while the C-terminal PDH domain of KAP1 functions as an intramolecular E3 ligase that sumoylates several lysines in its bromodomain. Sumoylation is a post-translational modification that consists on the covalent attachment of a Small Ubiquitin-like Modifier (SUMO) to a lysine residue. KAP1 bromodomain sumoylation enhances its co-represor activity and enables its interaction with SETDB1 [30,31,33,36,40]. Furthermore, it enhances SETDB1 activity [40] (Figure 1, number 1). When SETDB1 interacts with KAP1, it methylates histone H3 at lysine 9 (H3K9me) through its C-terminal ubiquitinated SET domain [36,41]. This mechanism leads to repression of a subset of HERVs where H3K9me heterochromatin has not yet been established, as it has been shown that SETDB1 *knock-out* activates expression of a wider group of HERVs than *knock-out* of KAP1 [27].

Further, SETDB1 can directly bind, in a KAP1-independent way, to H3K9me1/K14ac or H3K9me2/K14ac histone tails through its N-terminal triple Tudor domain (TTD) to deacetylate K14

via SETDB1-associated histone deacetylases (HDACs) and further methylate K9 to achieve a trimethylated (H3K9me3) state [42] (Figure 1, number 2). SETDB1 takes part in silencing retroelements in differentiated cells, including B cells [43] and brain tissue [44] as well as innate immune genes [35].

Introduction of H3K9me3 by SETDB1 also allows the binding of HP1 to the genome, where it interacts with KAP1 as well [31,36] (Figure 1, number 1). HP1 proteins prevent transcription factor binding and help maintain the heterochromatic structure [45], indeed, HP1 isoform α may be required to repress expression of both immune-related genes and HERVs [46]. Besides SETDB1 and HP1, KAP1 also recruits other repressor proteins such as the NuRD HDAC complex, which removes transcription-promoting histone acetylation [47] (Figure 1, number 1). Moreover, since the axis KRAB/KAP1/SETDB1 localizes to restricted regions within HERVs, and H3K9me3 marks must spread all over HERV sequences to ensure their silencing, KAP1 may form a complex with HP1 and histone methyltransferases all over the HERV region to mediate the spreading of heterochromatin [48,49].
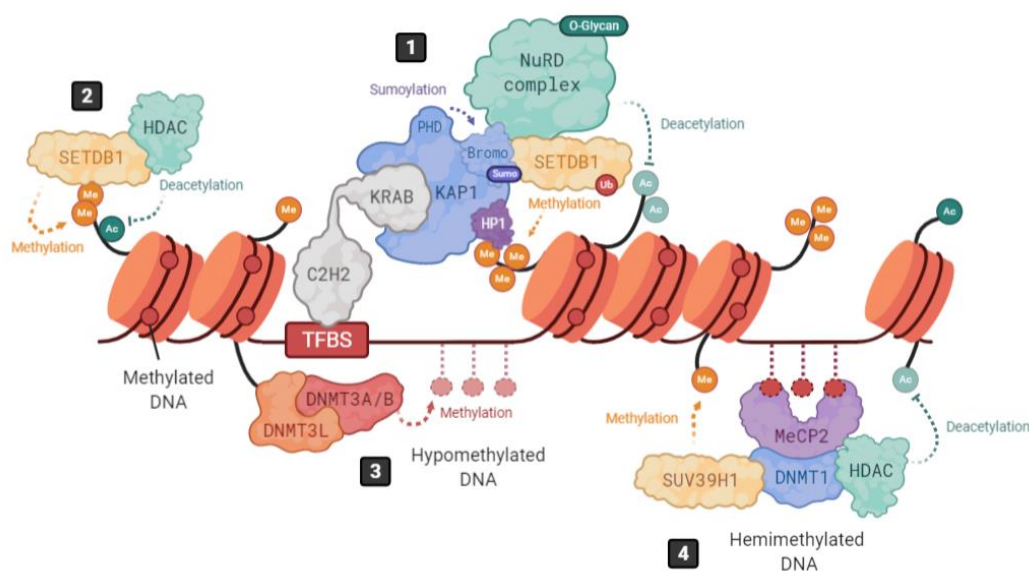
Figure 1. Epigenetic silencing mechanisms of HERVs. The expression of HERVs is mainly controlled by DNA methylation and histone tail modifications. Histone modifications are driven by KRAB-containing zinc finger proteins (KRAB-ZFPs) which bind to transcription factor binding site (TFBS) within HERV elements, recruiting the corepressor KRAB-associated protein 1 (KAP1), also called TRIM28 (1) [31,33]. KAP1, in turn, serves as a scaffold molecule for the assembly of heterochromatin modifiers like the histone methyltransferase SETDB1 (also known as ESET) [36], the heterochromatin protein 1 (HP1) [37] or the NuRD histone deacetylase complex [47]. Moreover, SETDB1 can also bind in a KAP1-independent way to H3K9me/K14ac marks to drive histone methylation (2) [42]. DNA methylation is driven by DNA methyltransferases (DNMTs). DNMT3L binds to histone 3 tails and recruits DNMT3A and DNMT3B to establish *de novo* methylation in hypomethylated DNA (3) whereas hemimethylated DNA is bound by methyl binding proteins like MeCP2, which recruits repressor complexes such as DNMT1, histone deacetylases (HDACs) and methyltransferases like SUV39H1 to stabilize chromatin structure repressing gene expression (4) [26,50-52]. Created with Biorender.com.

Although some studies have highlighted the importance of histone post-translational modifications (PTMs) in regulating HERV expression in terminally differentiated cells [32,39,43], DNA methylation seems to also play a relevant role in achieving their regulation [26,52,53]. Furthermore, depending on the cell type and the evolutionary age of HERVs, the impact of DNA methylation-dependent mechanisms may vary. For example, histone PTMs mediated by SETDB1, and not DNA methylation, are essential for HERV repression in neural progenitor cells [44,54] and B

cells [43]. Also, younger LTRs are found to be suppressed by DNA methylation while older ones are mainly silenced by histone PTMs [55]. By contrast, both mechanisms seem to play important roles in embryonic stem cells [56].

DNA methylation is a modification carried out by DNA methyltransferases (DNMTs) that consists in the transfer of a methyl group onto carbon 5 (C5) of cytosines to form 5-methylcytosine, thus recruiting methyl-CpG binding proteins (MBP) and/or preventing the binding of transcription factors [50]. It has been shown that brain contains some of the highest DNA methylation levels in the body and MBPs are important for normal neuronal function, being specifically highly expressed in the brain as well [57]. DNA methylation can be promoted at specific regions of the genome through transcription factor or RNA interference (RNAi)-mediated mechanisms [58], or simply spread across susceptible genomic regions. In hypomethylated DNA, DNMT3L binds to histone 3 tails and recruits DNMT3A and DNMT3B to establish *de novo* methylation [50,59,60] (Figure 1, number 3).

Also, DNMT3A/B can bind hypomethylated DNA regions, unprotected by transcription factors. When DNA is hemimethylated, MBPs like MeCP2, bind to DNA and recruit various repressor complexes such as DNMT1, or H3K9 methyltransferases like SUV39H1, to achieve DNA and histone methylation [50,57,61-64], and histone deacetylases (HDAC) to remove activating histone modifications [65] (Figure 1, number 4). Thus, DNA methylation and histone modifications work closely to induce a heterochromatic repressive state. In fact, one study demonstrated that DNMT3L facilitates the binding of TRIM28 to various repressive epigenetic modifiers [66].

In addition to DNA methylation and histone modifications, transcription factor PTMs play an important role in HERV silencing as well. As mentioned earlier, KAP1 sumoylation enhances its recruitment to retroelements and also the recruitment of SETDB1 to mediate H3K9 trimethylation [40] (Figure 1, number 1). In addition, the inhibitory activity of KAP1-associated repressive chromatin factors, such as the HDAC1 of the NuRD complex [67,68], is enhanced by methylation-dependent O-linked glycosylation, a PTM introduced by the O-linked β-N-acetylglucosamine transferase (OGT) [69] (Figure 1, number 1). HERVs can also be post-transcriptionally regulated by RNA binding proteins, such as the RNA-Binding Motif Protein 4 (RBM4) [70]. RBM4 regulates HERV-K (HML-2) and HERV-H transcript levels by binding at CGG consensus elements present on 5' and 3' LTRs. However, the mechanism by which RBM4 mediates HERV downregulation remains unknown. Thus, a combination of multiple heterochromatin modifiers, their PTMs and the post-transcriptional regulation of HERV transcripts may prevent ectopic and/or pathogenic expression of their encoded nucleic acid sequences and proteins that could be sensed by the immune system triggering inflammatory responses [24,25].

3. Transactivation of HERVs

Despite their tightly controlled regulation, pathogenic HERV proteins have been detected in chronic inflammatory or degenerative diseases of the nervous system, such as multiple sclerosis (MS) [71,72] amyotrophic lateral sclerosis (ALS) [73] or schizophrenia [74-76]. The main families being related to the mentioned neurological diseases are the HERV-W and HERV-K [77].

The HERV-W family is present in the human genome in multiple copies, most of them being defective and replication- incompetent [78,79], even though LINE-1 elements could, in principle, ease their transposition [79,80]. Although some complete proviral genes have been detected in these elements [72,81], they do not seem to encode complete proteins, mainly due to the presence of early stop codons [82,83]. The only known HERV-W family member encoding a complete *env* gene that transcribes and translates into a complete Env protein is ERVWE-1, a replication-incompetent HERV-W element located on chromosome 7q21.2 [78,84] which encodes Syncytin-1, a protein found only intracellularly or on the plasma membrane, therefore cell-associated. Syncytin-1 plays an important

role during early pregnancy by facilitating trophoblastic cells fusion to allow embryo implantation [20,85]. It consists of a 538 aa polypeptide (GenBank accession no. AF072506) comprising a cell surface domain (SU) and a transmembrane domain (TM). The SU domain is known to interact with the sodium-dependent neutral amino acid transporters ASCT-1 and ASCT-2 expressed by trophoblasts [86], while the TM domain promotes cell-cell fusion to mediate syncytium formation [87].

By contrast, MS-associated retrovirus (MSRV), now referred to as HERV-W/MSRV, have been found extracellularly, in the cerebrospinal fluid (CSF) of an MS patient [71,82,83,88], as genomic RNA in proviral particles [71,88]. The origin of HERV-W/MSRV remains uncertain since any known HERV-W family member could, in principle, yield viral particles and no human genome locus encoding HERV-W/MSRV has been yet found in the human genome [78]. Thus, it has been suggested that it originates from complementation of different HERV-W elements, by reversing the stop codon in the Xq22.3 locus [83,89], from a non-ubiquitous and/or unfixed copy [9,90] or even be an exogenous member of the HERV-W family [88,91].

The HERV-W/MSRV envelope protein (GenBank accession no. AF331500), named pHERV-W ENV from pathogenic HERV-W envelope protein in MS [92], consists of a 542 aa polypeptide with six potential N-glycosylation sites and is divided by a cleavage site into a SU domain of 290 aas and a TM domain of 252 aas [88]. Interestingly, and likewise all HERV-W sequences, pHERV-W ENV length only differs from Syncytin-1´s by the presence of 4 additional amino acids. Despite a reported identity of 81% [21], several important differences between these two proteins are detected. Firstly, while Syncytin-1 TM domain has fusogenic properties, the pHERV-W ENV fusogenic region is non-functional. Secondly, Syncytin-1 is a membrane-associated protein while pHERV-W ENV can be found membrane-associated, within viral particles or in a hexameric soluble form, although monomer and trimers have also been described [93,94]. In the hexameric soluble form, the hydrophobic TM domain of each monomer is located toward the inside, at the core of the complex, whereas the hydrophilic SU domain is exposed to the surrounding biological fluids [93,94]. Thirdly, HERV-W/MSRV is the main family member associated with chronic inflammatory diseases, though ectopic pathogenic Syncytin-1 expression has been reported in schizophrenia [95].

With regard to the HERV-K family, the youngest in human genome [6], it is comprised of ten groups (HML-1 to HML-10), although an HML-11 group has been more recently identified [96]. The HML-2 group comprises the most recent set of integrations for this element in humans, with more than 90 proviruses that maintain ORFs (open reading frames) encoding functional proteins [96]. HERV-K is the only known family able to produce retroviral particles, with the exception of the MSRV element from the HERV-W family. However, the particles produced by HERV-K seem not to be infectious [97-100]. Although it is not clear which genomic sequences are responsible for viral particle production, it is known that HERV-K (HML-2) is abnormally expressed in neurons of ALS patients [101].

The activation of HERV-W and HERV-K sequences may probably derive from dysregulation of the epigenetic mechanisms controlling their expression, since inhibition of DNMTs and HDACs leads to HERV reactivation [102]. As epigenetic mechanisms can be influenced by environmental stimuli, it seems possible that some environmental factors may distort HERV silencing mechanisms reactivating their expression, thereby inducing or contributing to disease. In fact, some viral infections have been shown to lead to reactivation of HERVs and interestingly, are believed to be co-factors of HERV associated diseases. For example, *Herpesviridae* infections are associated with a higher risk for MS [72,103-105] or, for schizophrenia along with Influenza, especially when infection or exposure occurs during prenatal stages [106-109].

Influenza virus has been shown to transactivate HERV-W elements by impairing the KRAB-ZFP/KAP1/SETDB1 axis, which plays an important role in controlling HERV expression as described above (Figure 1, number 1). *In vitro*, influenza virus triggers loss of sumoylations on KAP1 and lowers

the levels of H3K9 trimethylation and SETDB1, without affecting promoter DNA methylation status. This leads to derepression of HERV-W elements like Syncytin-1 [110-112].

Besides, herpes viruses like Herpes Simplex Virus type 1 (HSV-1), Human Herpes Virus type 6 (HHV6) and Epstein-Barr Virus (EBV) have been shown to be efficient at activating HERV-W elements even on methylated promoters [93,113]. In fact, *in vitro* infection by HSV-1 was shown to transactivate HERV-W by stimulating LTR-directed transcription [114], inducing its expression in neuronal and brain endothelial cells [115]. Another HERV-W activator is HHV6 [84,116]. For Epstein Bar virus (EBV), *in vitro* experiments showed that the binding of EBV major envelope protein gp350 activates the transcription of HERV-W/MSRV and Syncytin-1 in peripheral blood mononuclear cells (PBMCs) and astrocytes, without requiring EBV entry [72]. Indeed, *in vivo* infection triggered a higher MSRV activation in PBMCs when patients had or had passed through symptomatic infectious mononucleosis, the clinical manifestation of EBV [117].

EBV and HHV6 not only transactivate HERV-W elements but also HERV-K´s [118-122]. Further, first-episode schizophrenia is associated with a reduction of HERV-K methylation in PBMCs [123]. However, not only viral infections have the capacity for HERV activation, but cytokines can also induce their expression. HERV-K LTRs appear to be responsive to pro-inflammatory stimuli in ALS [124]. This opens the intriguing possibility that activation of HERVs in inflammatory diseases serves to create a feedback loop amplifying abnormal immune responses when pathogenic HERVs have already been epigenetically de-repressed and activated. This may be readily observed when HERVs are not constitutively repressed as it occurs with certain HERV-K copies that are accessible to cytokine-triggered signaling pathways [125], or HERV-W elements in epigenetically primed EBV-transformed B-cells [91].

As for MS, no single environmental stimuli or single gene mutation have been described as the disease-causing agents for schizophrenia or ALS, but a combination of environmental factors and genetic susceptibility backgrounds have been proposed as pathogenic factors [126,127]. It is tempting to propose that HERV activation may represent the common denominator to those risk factors, with particular combinations leading to the reactivation of specific HERV elements. In this hypothetic scenario, depending on the HERV(s) element(s) derepressed and the cell type(s) involved, the resulting symptoms, and thereby disease, triggered would be one or another.

4. HERV-associated pathogenesis

4.1. HERV-W related diseases

Both HERV-W envelope proteins, pHERV-W ENV and Syncytin-1, have been shown to possess immunopathogenic features *in vitro* and *in vivo* [24,71,128-130]. One of the most studied pathologies linked to abnormal HERV expression is MS, an autoimmune neurodegenerative disease that affects myelin sheaths, leading to neuronal loss [71,131]. The most commonly used experimental model for MS is the mouse autoimmune model EAE (Experimental Autoimmune Encephalomyelitis) [132], from which derives most of the knowledge about neuroinflammation and neurodegeneration, along with many other studies. EAE is induced by immunization with myelin or central nervous system (CNS)-derived peptides and disease is mediated by activated CNS-specific CD4+ T cells in the periphery which enter the CNS by crossing the endothelial blood-brain barrier (BBB). By contrast, neuroinflammation in MS now reveals to be initiated in the CNS with the activation of microglia [133,134]. Under physiological conditions, microglia protect the brain by sensing pathogens, phagocytosing cell debris and promoting remyelination. However, under MS pathological conditions, microglia become activated and secrete proinflammatory molecules that directly damage myelin sheaths and oligodendrocytes, causing inflammation and neurodegeneration [135,136] (Figure 2, number 1). This proinflammatory environment attracts peripheral monocyte-derived

macrophages and dendritic cells (DC), as well as peripheral autoreactive T lymphocytes, which enter the CNS through the BBB localized in CNS micro vessels [133,137-139] (Figure 2, number 2). Infiltrating T lymphocytes become activated by microglia, which also act as antigen presenting cells (APCs), phagocytosing myelin debris and expressing major histocompatibility complex (MHC) I and II with costimulatory molecules (Figure 2, number 3). Infiltrated monocyte-derived DC also act as APCs, presenting myelin antigen to T cells along with microglia [140] (Figure 2, number 3). Activated T lymphocytes differentiate into T helper cell type 1 (Th1) (producing IFN- γ) and Th17 cells (producing IL-17, IL-22, IL-21) [133,135,136,141,142] (Figure 2, number 3). The crosstalk between neuroimmune and peripheral immune systems exacerbates neuroinflammation, further activating microglia and astrocytes through cytokine production, as well as facilitating the infiltration of other immune cells such as activated B cells, monocytes and macrophages. The release of proinflammatory cytokines, nitric oxide, reactive oxygen and nitrogen species [143], together with some other processes like phagocytosis of myelin by macrophages and antibody-dependent cytotoxicity, contribute to inflammatory processes within the CNS, oligodendrocyte loss and demyelination of the white and gray matter [144].

Although neurodegeneration has traditionally been thought to be a consequence of inflammation in the CNS, this concept has been challenged by the poor efficacy of immunomodulatory treatments on preventing demyelination and axonal degeneration [145]. Now, we know that HERV-W/MSRV, through the expression of pHERV-W ENV, may be participating in the development of MS by triggering a potent immune response as well as impairing the myelin repairing process [24,25,92, 146-149], which could explain why immunomodulatory treatments failed at preventing neurodegeneration. Furthermore, pHERV-W ENV contribution to MS is supported by the observation that administered pHERV-W ENV with myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide triggers EAE in mice [149].

pHERV-W ENV RNA and protein have been detected in PBMCs of MS patients [84,91,150,151], showing their highest RNA levels in natural killer (NK) cells, followed by B cells and monocytes. The last increasing their expression with their differentiation to macrophages. By contrast, no ENV encoding RNA or protein is detected in T cells [72,130]. pHERV-W ENV has also been detected on astrocytes [72,84], infiltrated macrophages and activated microglia in the brains [150,152,153] of MS patients, and its soluble form in their serum, plasma and CSF [71,151,154-157]. Indeed, the presence of HERV-W RNA in CSF is considered as a negative prognostic marker in MS and its load increases with MS duration and parallels clinical stages, being a sign of worst prognosis in the following years when it is detected early [155,156,158,159].

The SU domain of the pHERV-W ENV, either associated to viral particles or in its soluble form, has been shown to trigger proinflammatory responses *in vitro*, in human PBMCs and dendritic cells (DC) [25]; and *in vivo*, in humanized severe combined immunodeficiency (SCID) mice, which die from brain hemorrhage with overexpression of TNF-α after pHERVW-ENV injection [24,129]. The inflammatory reaction induced by pHERV-W ENV is mediated by the activation of Toll-like receptor (TLR) 4 and its coreceptor CD14 [25,149]. TLRs are expressed by different cell types such as B cells [160], macrophages, monocytes, DCs [161], oligodendrocytes [162], astrocytes [163] or microglia [164], among others, where they mediate part of the innate immune response against invading pathogens [165-166].
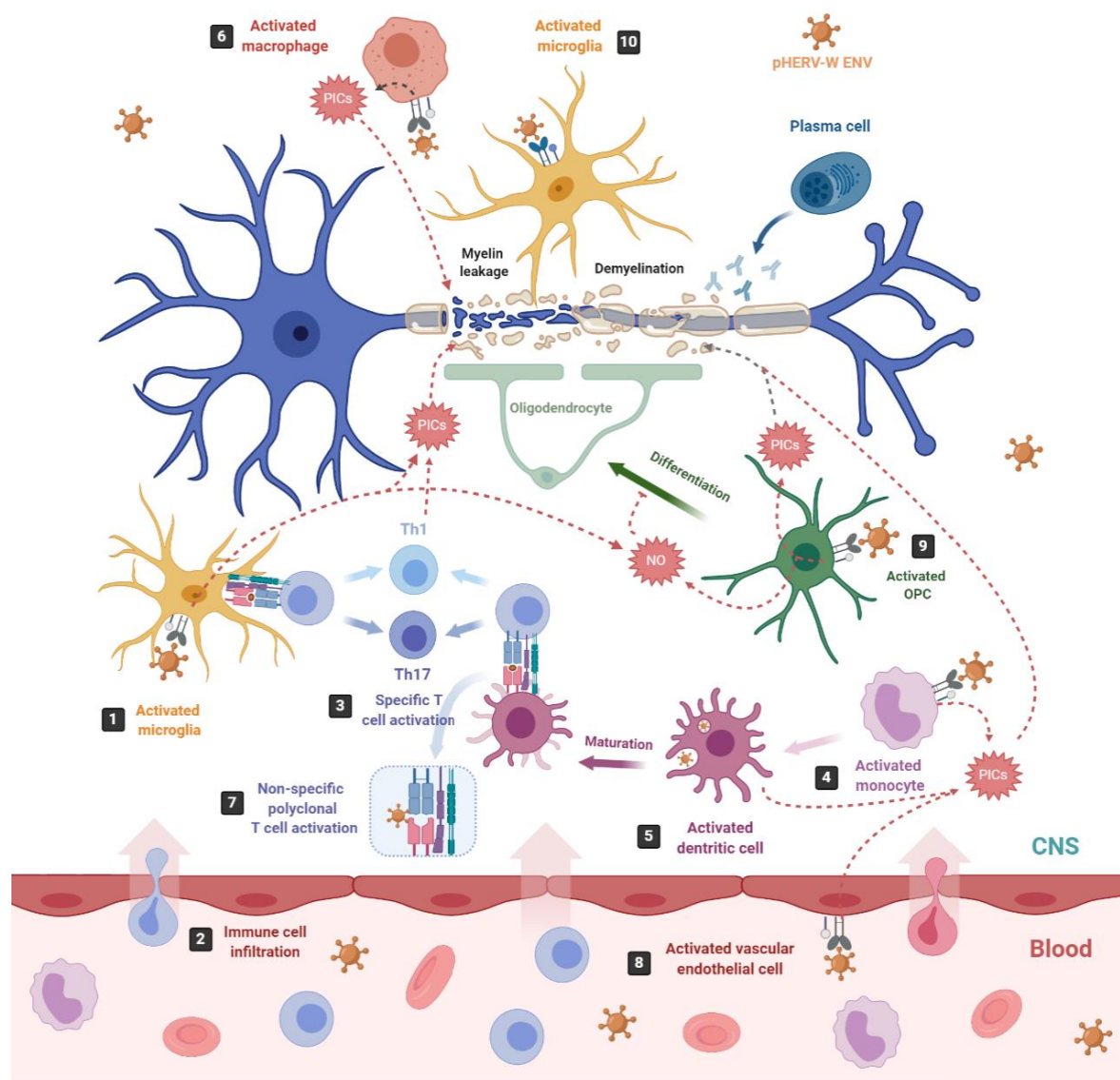
Figure 2. pHERV-W-ENV drives inflammation and neurodegeneration in MS.

In MS, inflammation starts in the CNS with the activation of microglia, which releases proinflammatory cytokines (PICs) that directly damage myelin sheaths (1). Cell debris and PICs attract peripheral immune cells to the CNS (2), among them T cells, which are then presented by APCs like microglia or infiltrating monocyte-derived dendritic cells (3) [133]. In this sense, pHERV-W ENV stimulates innate immune system by engaging TLR4/CD14 receptors in microglia (1) monocytes (4), dendritic cells (5) and macrophages (6) inducing the release of PICs. In addition, it stimulates specific T cell activation (3) and abnormal polyclonal T cell activation (7) activation [24, 25], acting like a superantigen. Furthermore, pHERV-W ENV activates vascular endothelial cells (8), making the migration of circulating cells possible at the level of ongoing brain lesions initiated by perivascular macrophages and/or microglia [146]. On another hand, pHERV-WENV impairs the remyelination process by inducing the release of PICs and nitrosative stress in oligodendrocyte precursor cells (OPCs) (9) and microglia (1), inhibiting the differentiation of OPCs to oligodendrocytes and damaging the myelin sheath, ultimately leading to neuronal loss [153]. As well, pHERV-W ENV drives microglial cells to physically interact with myelinated axons and induce leakage of intra-axonal and myelin proteins (10) [92]. Created with Biorender.com.

On the one hand, pHERV-W ENV activates monocytes, DCs and macrophages (Figure 2, number 4-6). Activated monocytes produce proinflammatory cytokines, including TNFα, IL-1β, IL-6 and IL-12p40 (Figure 2, number 4). Interestingly, levels of IL-6 and IL-12p40 induced in cultured

PBMCs directly correlate with MS disease severity [25]. Activated DCs not only release the proinflammatory cytokines IL-6, TNFα, IL-12p40, and IL-12p70, but also upregulate antigen presentation, activating antigen-specific T cells. Release of IL-12 by DCs activates naïve T cells and promotes their differentiation into IFN γ-secreting Th1 cells [25] (Figure 2, number 3). In turn, released INF-γ and TNF-α upregulate pHERV-W ENV secretion by PBMCs [91]. Thus, pHERV-W ENV not only activates the innate immune system but also the adaptive immune system. However, it also acts like a superantigen [24], stimulating abnormal polyclonal T cell activation through its binding to Vβ chains and expansion of T-cell receptor β-chain (TCR Vβ) cells, leading to non-specific oligoclonal Vβ+T cell activation [24,167] (Figure 2, number 7).

In addition, pHERV-W ENV activates vascular endothelial cells within the BBB, leading to the release of proinflammatory cytokines IL-6 and IL-8 and to the overexpression of ICAM-1, thus, enhancing the migration of circulating cells towards the brain (Figure 2, number 8) [146]. In the brain, a pHERV-W ENV-SU domain triggers CNS innate immunity through TLR4 binding, activating microglia and perivascular macrophages, which release proinflammatory cytokines leading to neuroinflammation and neurodegeneration [147] (Figure 2, number 1). Also, pHERV-W ENV produced by surrounding cells like microglia or macrophages [84], engages TLR4 receptors present on brain resident oligodendrocyte precursor cells (OPCs), activating them. OPCs role is to migrate to the lesion sites, where oligodendrocytes are depleted, and differentiate into mature oligodendrocytes to remyelinate axons after an injury [168]. After migration, OPCs transiently express TLR4 receptors, which become downregulated during the OPCs differentiation process to oligodendrocytes [153]. TLR4 activation does not affect OPC survival, however, induces the release of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, as well as overexpression of inducible nitric oxide synthase (iNOS), leading to an increase in nitric oxide (NO) levels that triggers nitrosative stress, which, in turn, affects myelin protein expression and reduces OPCs differentiation capacity [153] (Figure 2, number 9). NO and TNF-α are well-established mediators of axonal injury and demyelination [169,170]. Interestingly, TLR4 activation in OPCs seems to be sensitive to the cyclin dependent kinase inhibitor p57kip2, which participates in controlling TLR4´s surface expression [148].

On the other hand, pHERV-W ENV induces a degenerative phenotype in microglial cells characterized by the expression of the same set of proinflammatory cytokines detected on pHERV-W ENV -activated OPCs (TNF-α, IL-1β and IL-6), and by the overexpression of iNOS [92] (Figure 2, number 1). While reducing anti-inflammatory and neuroprotective proteins and downregulating expression of genes involved in myelin debris phagocytosis, it affects neurorepair and inhibits OPC differentiation. Furthermore, pHERV-W ENV protein stimulates microglia proliferation and drives physical interaction between microglial cells and myelinated axons to induce leakage of intra-axonal and myelin proteins [92] (Figure 2, number 10). Of note, the multifaceted effects of pHERV-W ENV are quite always due to its ability to strongly bind TLR4 receptors on expressing cells and, thereby, to activate underlying signaling pathways leading to various effects with a pro-inflammatory common point in different cells [171]

Furthermore, pHERV-W-ENV inflammatory and demyelinating consequences can also be observed in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), a rare immune disease of the peripheral nervous system (PNS) [150]. In these patients, pHERV-W ENV is found in PBMCs and peripheral nerve lesions, especially in Human Schwann Cells (HSCs) in which it exerts pathogenic effects by TLR4 engagement, inducing the release of proinflammatory IL-6 and the macrophage and T cell chemoattractant CXCL10 from HSCs [172], triggering activation of the innate immune system.

Another HERV-W related disease is schizophrenia [173,174], a psychiatric disorder that highly impacts patient´s quality of life. In reference to the mechanisms underlying this disease, several are the hypotheses raised, including the disturbance of the dopaminergic neurotransmission or its relation to neurodevelopment problems. However, the idea that autoimmune processes within the

nervous system could be playing an important role in its pathophysiology has been gaining more and more acceptance [175]. Some studies have shown elevated expression of TLRs [176] and abnormal expression of proinflammatory cytokines like IL-6, IL-8 and the C-reactive protein (CRP) in the serum of these patients [177,178]. CRP is released in the blood under inflammatory conditions and its overexpression supports that immune mechanisms play a role in schizophrenia symptoms beyond cognitive decline [179]. Interestingly, the levels of HERV-W mRNA were found to associate with a proinflammatory phenotype in these patients [180].

HERV-W expression is particularly associated with recent-onset schizophrenia [74,173,181,182]. Both HERV-W/MSRV and Syncytin-1 were reported to be increased in schizophrenia [95,183], and HERV-W/MSRV was shown to be unequally represented in the genome of an affected and its non-affected monozygotic twin [184]. HERV-W GAG and ENV RNA or protein have been found on circulating blood of these patients [174,181-183,185], correlating GAG protein levels with disease severity [185] and Syncytin-1 or (MSRV) pHERV-W ENV with CRP levels [182], providing a link between HERV-W envelopes and the status of systemic inflammation of the patients [95,174,182]. Activation of CRP in microglia and astrocytes, and the consequent inflammation, can be triggered by Syncytin-1 through direct interaction with TLR3 receptor [95] and IL-6 release. Furthermore, HERV-W presence has also been detected in CSF [74] and in cortical tissue [186], indicating a pathogenic role in brain. In fact, HERV-W/MSRV induces activation of the Brain-derived neurotrophic factor (BDNF) and Dopamine receptor D3 (DRD3) genes [183], both implicated in providing a higher risk for suffering schizophrenia [187]. Thus, HERV-W Syncytin-1 and MSRV may determine and/or promote the development of schizophrenia. In this regard it should be mentioned that by combining single-molecule tracking, calcium imaging, and behavioral approaches, Johansson et al. [76] showed that the mechanism used by HERV-W-ENV to alter glutamate synapse maturation and generate behavioral deficits depends on N-methyl-d-aspartate receptor (NMDAR) organization and cytokine-dependent changes, further supporting an etiological role for this HERV product in the development of psychosis.

HERV-K related diseases

The main HERV-K related disease is ALS. It is a progressive motor neurodegenerative disease leading to cortical and spinal neurons degeneration, causing muscle atrophy, weakness, and ultimately death [188]. About 10% of ALS cases are familial (fALS) and out of them, 20% are associated with a mutation in the superoxide dismutase (SOD1) gene. However, the remaining ALS cases, which comprise the majority, are considered sporadic (sALS) because no genetic component has been identified [189]. Instead, sALS are related to the presence of pathological DNA binding protein-43 (TDP-43) neuronal cytoplasmic aggregates, which are not present in SOD1- mutant fALS, suggesting different disease mechanisms [190,191].

TDP-43 was initially identified as a protein able to bind TAR DNA sequence motifs in the human immunodeficiency virus (HIV) [192]. Later, it was found to be located in the nucleus of neurons and glial cells such as oligodendrocytes [191,193], however, its physiological role in the brain remains unknown. Besides HIV, another retroviral element which contains TDP-43 binding sites is the endogenous HERV-K (HML-2). TDP-43 levels in cortical and spinal neurons of ALS patients correlate and colocalize with HERV-K expression [73,101]. Although TDP-43 has been found to bind HERV-K LTRs, only the ALS-associated pathological TDP-43 aggregates seem to have the potential to induce HERV-K transcription in cortical neurons [73,194]. The transcripts of HERV-K *GAG, POL* and *ENV* genes have been detected in neuronal cells of postmortem ALS brains [73] and, particularly, the expression of HERV-K Env protein triggered neurite retraction in *in vitro* neuronal cultures. Furthermore, its overexpression *in vivo* decreased the number of cortical and spinal neurons of transgenic mice, which also presented with shorter dendrites, less spines and a lower complexity, leading to motor dysfunction and eventually death [73]. The mechanisms by which HERV-K Env protein exerts those neurotoxic effects are unknown. However, it has been suggested that it could be

impairing the normal function of the nucleolar protein nucleophosmin, leading to nucleolar dysfunction, a feature observed in ALS patients with mutations on the ORF 72 gene of chromosome 9 (*C9ORF72*), the most frequent genetic defect in ALS [73,195].

5. HERV targeted treatments

With the discovery of the implication of HERVs in chronic diseases such as MS, schizophrenia or ALS, a new avenue for the development of targeted therapies directed against HERV proteins opened. In this direction, the Swiss company GeNeuro developed the first HERV-directed treatment, named GNbAC1 or temelimab, that consists of a humanized immunoglobulin (Ig) G4/ kappa (κ) isotype monoclonal antibody (mAb), with an approximate molecular weight of 147 kDa, directed against the pHERV-W ENV. Like other antibodies, temelimab is comprised of two heavy chains (HC), from class IgG subtype 4, bound to each other by disulfide bonds with a light chain (LC) type κ bound to each HC [196]. However, site-directed mutagenesis was performed to stabilize the interchain disulfide bridges. At the same time, each HC and LC are constituted of a constant domain, that determines the biological properties of the antibody, and a variable domain which determines the binding specificity. While the LC includes a variable domain (VL) and a constant domain (CL), the HC includes a variable domain (VH) and three constant domains (CH1, CH2 and CH3). Moreover, at N-terminal of the VL and VH, lies the complementarity determining regions (CDRs), which define the structural complementarity between the antibody and the specific antigen they bind. The LC and HC each have three CDRs, shaping the antigen binding site. Of note, temelimab is a humanized mAb because the variable and constant regions come from a human antibody but only the CDRs derived from the parental murine antibody remain, which specifically binds the pHERV-W ENV.

Temelimab targets a linear non-glycosylated epitope of the SU domain of the HERV-W/MSRV Env [197-199], blocking its interaction with the TLR4 receptor and, hence, the proinflammatory cascade and the inhibition of the myelin repair process. Its testing *in vitro* proved temelimab could prevent pHERV-W ENV mediated PBMC activation and the consequent TNF-α release without blocking TLR4 neither inhibiting its activation by other ligands like lipopolysaccharide (LPS) [25]. In addition, this antibody also prevents pHERV-W ENV mediated release of proinflammatory cytokines, especially of TNF-α, by OPCs and the induction of nitrosative stress, neutralizing pHERV-W ENV associated inhibition of OPC differentiation [92,148]. Furthermore, when tested *in vivo* in the EAE mouse model [149], all temelimab-treated mice healed and survived, in fact, even remyelination of CNS lesions was observed [198,199]. These results further confirmed the pathogenic role of HERV-W/MSRV and pointed out a double therapeutic effect of temelimab which confers neuroprotective properties. On one hand, temelimab prevents the release of pro-inflammatory cytokines induced by pHERV-W ENV in the blood and in the CNS and the consequent damage to myelin sheaths. On another hand, it allows OPC differentiation into oligodendrocytes to remyelinate demyelinated axons preventing neuronal death. These properties make temelimab a potential targeted treatment for those neurological diseases in which HERV-W/MSRV causes inflammation and neurodegeneration such as MS.

After the promising preclinical results, temelimab has been assessed in clinical trials as a new MS treatment under development. In a randomized, double-bind, placebo-controlled, dose-escalation, phase I, temelimab safety was assessed in a total of 88 healthy male volunteers, with the administration of different doses ranging from 0.0025 to 6 mg/kg (ClinicalTrials.gov Identifier: NCT01699555) [197], 6 to 36 mg/Kg (ClinicalTrials.gov Identifier: NCT02452996) [200] and 36 to 110 mg/Kg (ClinicalTrials.gov Identifier: NCT03574428) in the three groups tested. All intravenous administered doses were well tolerated, showing non-specific minor adverse events. Its mean half-life was estimated to be between 19 and 28 days, depending on the dose, in line with the average of 3 weeks for IgG4 antibodies, so its application should follow a monthly regular basis [197, 200]. In a phase IIa trial, the safety and pharmacokinetics of temelimab were assessed again but this time in 10

MS patients with monthly repeated doses of 2 mg/Kg or 6 mg/Kg for 6 months (ClinicalTrials.gov Identifier: NCT01639300) [198,201]. Of those MS patients, one had relapsing-remitting MS (RRMS), three had primary progressive MS (PPMS), and six had secondary progressive MS (SPMS). Doses of 2 and 6 mg/Kg were selected to ensure a sustained pHERV-W ENV neutralization during 4 weeks. As in previous studies, temelimab was well tolerated, its pharmacokinetics were dose-linear and its mean half-life was between 27 and 37 days.

Surprisingly, it was observed that administration of temelimab also decreased the genomic expression of HERV-W/MSRV *env* and *pol* mRNAs on PBMCs upon three and six months of treatment. This was unexpected as temelimab is a mAb designed to bind pHERV-W ENV, not its mRNA nor other HERV-W genomic sequences or features [201]. However, this effect on *env* mRNA has also been reported in other MS treatments such as interferon β (IFN-β) [91,157] and the monoclonal antibody natalizumab [202]. Thus, importantly, temelimab, unlike other MS treatments, has been shown not to impair the immune system [203].

Later phase IIb (CHANGE-MS) (ClinicalTrials.gov Identifier: NCT02782858) [204] and phase IIc (ANGEL-MS) (ClinicalTrials.gov Identifier: NCT03239860) studies tested temelimab efficacy on RRMS in the long term [205]. The results obtained were encouraging as neuroprotective and regenerative effects of temelimab were observed in RRMS patients indicating that a new RRMS treatment with neuroprotective and antiretroviral effects which does not impair the immune system may be available in a closer future. Furthermore, these results open the door to future clinical studies testing temelimab in progressive MS characterized by established neurodegeneration, and to test the efficacy of temelimab on other HERV-W/MSRV related diseases such as CIDP, where HERV-W/MSRV triggers an innate immunity response by engaging TLR4 receptor in HSCs, releasing IL-6 and CXCL10 [172], as earlier mentioned. In fact, in vitro experiments have demonstrated a neutralizing effect of temelimab on the HERV-W/MSRV mediated induction of HSCs, inhibiting the release of IL-6 and CXCL10 [172]. A phase II clinical trial is now ongoing in progressive MS with escalating doses of 18, 36 and 54 mg/Kg versus placebo (ClinicalTrials.gov Identifier: NCT04480307).

Furthermore, HERV-W/MSRV not only has an impact on neurological diseases but also in other autoimmune diseases, such as type 1 diabetes (T1D), where pHERV-W ENV has been detected in the serum, PBCMs, and pancreatic acinar cells of patients [206]. Interestingly, as previously mentioned for MS and schizophrenia, influenza and EBV also associate with T1D, as well as the enterotropic Coxsackie B virus infection which transactivates HERV-W [207,208]. *In vitro* experiments suggest that HERV-W/MSRV triggers an inflammatory response and also directly affects insulin production by pancreatic β-cells [206]. HERV-W/MSRV has been previously shown to induce potent inflammatory effects by engaging TLR4 [25,149], and pancreatic β-cells also express this receptor type [209]. Further, transgenic mice expressing HERV-W/MSRV displayed T1D clinical symptoms, such as hyperglycemia and hypoinsulinemia, and a strong increase in the release of IL-6 and TNF-α in blood [206,210]. These results evidence a role for HERV-W in T1D pathogenesis.

When temelimab was assayed by *in vivo* and *in vitro* models of T1D, both, the release of proinflammatory cytokines, and the toxic effect of HERV-W/MSRV on pancreatic β-cells were inhibited [211]. These results allowed to carry out a double-blind, placebo-controlled Phase IIa clinical trial RAINBOW-T1D (clinicaltrials.gov no NCT03179423) to assess the safety of temelimab on T1D patients and its effect on the autoimmune process. Temelimab was administered at a dose of 6 mg/kg in 60 patients for a year on a monthly regular basis [211,212]. As with MS patients, temelimab was well tolerated in T1D patients. Furthermore, it reduced the frequency of hypoglycemia events and the levels of anti-insulin autoantibodies after six months of treatment.

In summary, temelimab represents the first HERV directed treatment that also does not impair the immune system [203] unlike other MS treatments, especially for the RRMS, which is the most common form of MS. As RRMS is mainly driven by inflammation, most treatments act on immune

system cells trying to reduce immune responses and inflammation. For example, natalizumab prevents BBB crossing of lymphocytes, or rituximab depletes mature B cell pools, even fingolimod reduces the number of circulating lymphocytes by preventing them to leave secondary lymphoid organs [213]. Thus, most potential treatments have several safety concerns, that indeed have even led to their withdrawal, as it is the case of daclizumab. In this sense, temelimab shows an improved performance, with minimal adverse events on the so far completed clinical trials. Furthermore, unlike other MS treatments, temelimab shows neuroprotective effects in MS patients due to the neutralization of the pHERV-W ENV-induced blockade of OPC differentiation into oligodendrocytes. Beyond MS, temelimab seems to be effective in other HERV-W/MSRV related diseases such as CIDP or T1D. Therefore, it might be of interest to assess its efficacy in the treatment of additional diseases such as fibromyalgia or chronic fatigue syndrome, where the upregulated expression of HERV-W has recently been reported [214,215].

Lastly GeNeuro company is presently developing an anti-HERV-K Env mAb for the treatment of ALS (patent number US20200308258) [216]. Its application *in vitro* shows neuroprotective effects like described for temelimab. It restores the activity of motor neurons, increases their viability and neurite length as compared to untreated controls [216]. Thus, temelimab seems to represent   the beginning of a promising new generation of antibody-based drugs directed specifically against HERV retroviral proteins with pathogenic properties.

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References

1. Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; et al. Initial sequencing and analysis of the human genome. *Nature*. **2001**, *409*, 860–921. DOI: 10.1038/35057062

2. Griffiths, D.J. Endogenous retroviruses in the human genome sequence. *Genome Biol.* **2001**, *2*, 1017.1–1017.5. DOI: 10.1186/gb-2001-2-6-reviews1017

3. Saleh, A.; Macia, A.; Muotri, A.R. Transposable elements, inflammation, and neurological disease. *Front Neurol.* **2019**, *10*, 894. DOI: 10.3389/fneur.2019.00894

4. Löwer, R.; Löwer, J.; Kurth, R. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. *Proc. Natl. Acad. Sci. USA*. **1996**, *93*, 5177–5184. DOI: 10.1073/pnas.93.11.5177

5. Sverdlov, E.D. Retroviruses and primate evolution. *Bioessays*. **2000**, *22*, 161–171. DOI: 10.1002/(SICI)1521-1878(200002)22:2<161::AID-BIES7>3.0.CO;2-X

6. Bannert, N.; Kurth, R. The evolutionary dynamics of human endogenous retroviral families. *Annu. Rev. Genomics Hum. Genet.* **2006**, *7*, 149–173. DOI: 10.1146/annurev.genom.7.080505.115700

7. Johnson, W.E. Origins and evolutionary consequences of ancient endogenous retroviruses. *Nat. Rev. Microbiol.* **2019**, *17*, 355–370. DOI: 10.1038/s41579-019-0189-2

8. Belshaw, R.; Watson, J.; Katzourakis, A.; Howe, A.; Woolven-Allen, J.; Burt, A.; Tristem, M. Rate of recombinational deletion among human endogenous retroviruses. *J. Virol.* **2007**, *81*, 9437–9442. DOI: 10.1128/JVI.02216-06

9. Thomas, J.; Perron, H.; Feschotte, C. Variation in proviral content among human genomes mediated by LTR recombination. *Mob. DNA*. **2018**, *9*, 36. DOI: 10.1186/s13100-018-0142-3

10. Seifarth, W.; Frank, O.; Zeilfelder, U.; Spiess, B.; Greenwood, A.D.; Hehlmann, R.; Leib-Mösch, C. Comprehensive analysis of human endogenous retrovirus transcriptional activity in human tissues with a retrovirus-specific microarray. *J. Virol.* **2005**, *79*, 341–352. DOI: 10.1128/JVI.79.1.341-352.2005

11. Stauffer, Y.; Theiler, G.; Sperisen, P.; Lebedev, Y.; Jongeneel, C.V. Digital expression profiles of human endogenous retroviral families in normal and cancerous tissues. *Cancer Immun.* **2004**, *4*, 2.

12. Oja, M.; Peltonen, J.; Blomberg, J.; Kaski, S. Methods for estimating human endogenous retrovirus activities from EST databases. *BMC Bioinformatics.* **2007**, *8*, S2-S11. DOI: 10.1186/1471-2105-8-S2-S11

13. Shin, W.; Lee, J.; Son, S.Y.; Ahn, K.; Kim, H.S.; Han, K. Human-specific HERV-K insertion causes genomic variations in the human genome. *PLoS One* **2013**, *8*, e60605. DOI: 10.1371/journal.pone.0060605

14. Medstrand, P.; Landry, J.R.; Mager, D.L. Long terminal repeats are used as alternative promoters for the endothelin B receptor and apolipoprotein C-I genes in humans. *J. Biol. Chem.* **2001**, *276*, 1896–1903. DOI: 10.1074/jbc.M006557200

15. Jern, P.; Coffin, J.M. Effects of retroviruses on host genome function. *Annu. Rev. Genet.* **2008**, *42*, 709–732. DOI: 10.1146/annurev.genet.42.110807.091501

16. Suntsova, M.; Garazha, A.; Ivanova, A.; Kaminsky, D.; Zhavoronkov, A.; Buzdin, A. Molecular functions of human endogenous retroviruses in health and disease. *Cell Mol. Life Sci.* **2015**, *72*, 3653–3675.

17. Ito, J.; Sugimoto, R.; Nakaoka, H.; Yamada, S.; Kimura, T.; Hayano, T.; Inoue, I. Systematic identification and characterization of regulatory elements derived from human endogenous retroviruses. *PLoS Genet.* **2017**, *13*, e1006883. DOI: 10.1371/journal.pgen.1006883

18. Hu, T.; Pi, W.; Zhu, X.; Yu, M.; Ha, H.; Shi, H.; et al. Long non-coding RNAs transcribed by ERV-9 LTR retrotransposon act in cis to modulate long-range LTR enhancer function. *Nucleic Acids Res.* **2017**, *45*, 4479-4492. DOI: 10.1093/nar/gkx055

19. Wilson, K.D.; Ameen, M.; Guo, H.; Abilez, O.J.; Tian, L.; Mumbach, M.R.; et al. Endogenous Retrovirus-Derived lncRNA BANCR Promotes Cardiomyocyte Migration in Humans and Non-human Primates. *Dev. Cell* **2020** *54*, 694-709. DOI: 10.1016/j.devcel.2020.07.006

20. Mi, S.; Lee, X.; Li, X.; Veldman, G.M.; Finnerty, H.; Racie, L.; LaVallie, E.; Tang, X.Y.; Edouard, P.; Howes, S.; et al. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature.* **2000**, *403*, 785–789.

21. Mallet, F.; Bouton, O.; Prudhomme, S., Cheynet, V.; Oriol, G.; Bonnaud, B.; et al. The endogenous retroviral locus ERVWE1 is a bona fide gene involved in hominoid placental physiology. Proc Natl Acad Sci U S A. **2004**, *101*, 1731–1736. DOI: 10.1073/pnas.0305763101

22. Larsson, E.; Andersson, G. Beneficial role of human endogenous retroviruses: facts and hypotheses. *Scand. J. Immunol.* **1998**, *48*, 329–338. DOI: 10.1046/j.1365-3083.1998.00428.x

23. Mangeney, M.; Renard, M.; Schlecht-Louf, G.; Bouallaga, I.; Heidmann, O.; Letzelter, C.; Richaud, A.; Ducos, B.; Heidmann, T. Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc. Natl. Acad. Sci. USA.* **2007**, *104*, 20534-20539. DOI: 10.1073/pnas.0707873105.

24. Perron, H.; Jouvin-Marche, E.; Michel, M.; Ounanian-Paraz, A.; Camelo, S.; Dumon, A.; Jolivet-Reynaud, C.; Marcel, F.; Souillet, Y.; Gebuhrer, L.; et al. Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal Vbeta16 T-lymphocyte activation. *Virology.* **2001**, *287*, 321–332. DOI: 10.1006/viro.2001.1045

25. Rolland, A.; Jouvin-Marche, E.; Viret, C.; Faure, M.; Perron, H.; Marche, P.N. The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. *J Immunol.* **2006**, *176*, 7636–7644. DOI: 10.4049/jimmunol.176.12.7636

26. Hurst, T.P.; Magiorkinis, G. Epigenetic control of human endogenous retrovirus expression: Focus on regulation of long-terminal repeats (LTRs). *Viruses.* **2017**, *9*, 130. DOI: 10.3390/v9060130

27. Groh, S.; Schotta, G. Silencing of endogenous retroviruses by heterochromatin. *Cell Mol. Life Sci.* **2017**, *74*, 2055–2065. DOI: 10.1007/s00018-017-2454-8

28. Thomas, J.H.; Schneider, S. Coevolution of retroelements and tandem zinc finger genes. *Genome Res.* **2011**, *21*, 1800–1812. DOI: 10.1101/gr.121749.111

29. Ecco, G.; Imbeault, M.; Trono, D. A tale of domestication: the endovirome, its polydactyl controllers and the species-specificity of human biology. *Development.* **2020**, *144*, 2719-2729. DOI:10.1242/dev.132605

30. Peng, H.; Begg, G.E.; Schultz, D.C.; Friedman, J.R.; Jensen, D.E.; Speicher, D.W.; Rauscher, F.J.3rd. Reconstitution of the KRAB-KAP-1 repressor complex: a model system for defining the molecular anatomy

of RING-B box-coiled-coil domain-mediated protein-protein interactions. *J. Mol. Biol*. **2000**, *295*, 1139–1162. DOI: 10.1006/jmbi.1999.3402

31. Iyengar, S.; Farnham, P.J. KAP1 protein: an enigmatic master regulator of the genome. *J. Biol. Chem*. **2011**, *286*, 26267–26276. DOI: 10.1074/jbc.R111.252569

32. Ecco, G.; Imbeault, M.; Trono, D. KRAB zinc finger proteins. *Development*. **2017**, *144*, 2719–2729. DOI: 10.1242/dev.132605

33. Stoll, G.A.; Oda, S-I.; Chong, Z-S.; Yu, M.; McLaughlin, S.H.; Modis, Y. Structure of KAP1 tripartite motif identifies molecular interfaces required for retroelement silencing. *Proc. Natl. Acad. Sci. USA*. **2019**, *116*, 15042–15051. DOI: 10.1073/pnas.1901318116

34. Grassi, D.A.; Jonsson, M.E.; Brattas, P.L.; Jakobsson, J. TRIM28 and the control of transposable elements in the brain. *Brain Res*. **2019**, *1705*, 43–47. DOI: 10.1016/j.brainres.2018.02.043

35. Tie, C.H.; Fernandes, L.; Conde, L.; Robbez-Masson, L.; Sumner, R.P.; Peacock, T.; Rodriguez-Plata, M.T.; Mickute, G.; Gifford, R.; Towers, G.J.; et al. KAP1 regulates endogenous retroviruses in adult human cells and contributes to innate immune control. *EMBO Rep*. **2018**, *19*, e45000. DOI: 10.15252/embr.201745000

36. Schultz, D.C.; Ayyanathan, K.; Negorev, D.; Maul, G.G.; Rauscher, F.J. 3rd. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev*. **2002**, *16*, 919–932. DOI: 10.1101/gad.973302

37. Ryan, R.F.; Schultz, D.C.; Ayyanathan, K.; Singh, P.B.; Friedman, J.R.; Fredericks, W.J.; Rauscher, F.J.3rd. KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: A potential role for Krüppel-associated box–zinc finger proteins in heterochromatin-mediated gene silencing. *Mol. Cell Biol*. **1999**, *19*, 4366–4378. DOI: 10.1128/mcb.19.6.4366

38. Quenneville, S.; Turelli, P.; Bojkowska, K.; Raclot, C.; Offner, S.; Kapopoulou, A.; Trono, D. The KRAB-ZFP/KAP1 system contributes to the early embryonic establishment of site-specific DNA methylation patterns maintained during development. *Cell Rep*. **2012**, *2*, 766–773. DOI: 10.1016/j.celrep.2012.08.043

39. Turelli, P.; Castro-Diaz, N.; Marzetta, F.; Kapopoulou, A.; Raclot, C.; Duc, J.; Tieng, V.; Quenneville, S.; Trono, D. Interplay of TRIM28 and DNA methylation in controlling human endogenous retroelements. *Genome Res*. **2014**, *24*, 1260–1270. DOI: 10.1101/gr.172833.114

40. Ivanov, A.V.; Peng, H.; Yurchenko, V.; Yap, K.L.; Negorev, D.G.; Schultz, D.C.; Psulkowski, E.; Fredericks, W.J.; White, D.E.; Maul, G.G.; et al. PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. *Mol Cell*. **2007**, *28*, 823–837. DOI: 10.1016/j.molcel.2007.11.012

41. Ishimoto, K.; Kawamata, N.; Uchihara, Y.; Okubo, M.; Fujimoto, R.; Gotoh, E.; Kakinouchi, K.; Mizohata, E.; Hino, N.; Okada, Y.; et al. Ubiquitination of lysine 867 of the human SETDB1 protein upregulates its histone H3 lysine 9 (H3K9) methyltransferase activity. *PLoS One*. **2016**, *11*, e0165766. DOI: 10.1371/journal.pone.0165766

42. Jurkowska, R.Z.; Qin, S.; Kungulovski, G.; Tempel, W.; Liu, Y.; Bashtrykov, P.; Stiefelmaier, J.; Jurkowski, T.P.; Kudithipudi, S.; Weirich, S.; et al. H3K14ac is linked to methylation of H3K9 by the triple Tudor domain of SETDB1. *Nat. Commun*. **2017**, *8*. DOI: 10.1038/s41467-017-02259-9

43. Collins, P.L.; Kyle, K.E.; Egawa, T.; Shinkai, Y.; Oltz, E.M. The histone methyltransferase SETDB1 represses endogenous and exogenous retroviruses in B lymphocytes. *Proc. Natl. Acad. Sci. USA*. **2015**, *112*, 8367–8372. DOI: 10.1073/pnas.1422187112

44. Tan, S.L.; Nishi, M.; Ohtsuka, T.; Matsui, T.; Takemoto, K.; Kamio-Miura, A.; Aburatani H.; Shinkai, Y.; Kageyama, R. Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development. *Development*. **2012**, *139*, 3806–3816. DOI: 10.1242/dev.082198

45. Machida, S.; Takizawa, Y.; Ishimaru, M.; Sugita, Y.; Sekine, S.; Nakayama, J-I.; Wolf, M.; Kurumizaka, H. Structural basis of heterochromatin formation by human HP1. *Mol. Cell*. **2018**, *69*, 385-397. DOI: 10.1016/j.molcel.2017.12.011

46. Sharma, P.; Azebi, S.; England, P.; Christensen, T.; Møller-Larsen, A.; Petersen, T.; Batsché, E.; Muchardt, C. Citrullination of histone H3 interferes with HP1-mediated transcriptional repression. *PLoS Genet*. **2012**, *8*, :e1002934. DOI: 10.1371/journal.pgen.1002934

47. Schultz, D.C.; Friedman, J.R.; Rauscher, F.J. 3rd. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. *Genes Dev*. **2001**, *15*, 428–443. DOI: 10.1101/gad.869501

48. Stewart, M.D.; Li, J.; Wong, J. Relationship between histone H3 lysine 9 methylation, transcription repression, and heterochromatin protein 1 recruitment. *Mol. Cell Biol*. **2005**, *25*, 2525–2538. DOI: 10.1128/MCB.25.7.2525-2538.2005

49. Groner, A.C.; Meylan, S.; Ciuffi, A.; Zangger, N.; Ambrosini, G.; Dénervaud, N.; Bucher, P.; Trono, D. KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. *PLoS Genet*. **2010**, *6*, e1000869. DOI: 10.1371/journal.pgen.1000869

50. Moore, L.D.; Le, T.; Fan, G. DNA methylation and its basic function. *Neuropsychopharmacology*. **2013**, *38*, 23–38.

51. Geis, F.K.; Goff, S.P. Silencing and transcriptional regulation of endogenous retroviruses: An overview. *Viruses*. **2020**, *12*, 884. DOI: 10.3390/v12080884

52. Liang, G.; Chan, M.F.; Tomigahara, Y.; Tsai, Y.C.; Gonzales, F.A.; Li E.; Laird, P.W.; Jones, P.A. Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol. Cell Biol*. **2002**, *22*, 480–491. DOI: 10.1128/MCB.22.2.480-491.2002

53. Karimi, M.M.; Goyal, P.; Maksakova, I.A.; Bilenky, M.; Leung, D.; Tang, J.X.; Shinkai, Y.; Mager, D.L.; Jones, S.; Hirst, M.; et al. DNA methylation and SETDB1/H3K9me3 regulate predominantly distinct sets of genes, retroelements, and chimeric transcripts in mESCs. *Cell Stem Cell*. **2011**, *8*, 676–687. DOI: 10.1016/j.stem.2011.04.004

54. Fasching, L.; Kapopoulou, A.; Sachdeva, R.; Petri, R.; Jönsson, M.E.; Männe, C.; Turelli, P.; Jern, P.; Cammas, F.; Trono, D.; et al. TRIM28 represses transcription of endogenous retroviruses in neural progenitor cells. *Cell Rep*. **2015**, *10*, 20–28. DOI: 10.1016/j.celrep.2014.12.004

55. Ohtani, H.; Liu, M.; Zhou, W.; Liang, G.; Jones, P.A. Switching roles for DNA and histone methylation depend on evolutionary ages of human endogenous retroviruses. *Genome Res*. **2018**, *28*, 1147–1157. DOI: 10.1101/gr.234229.118

56. Sharif, J.; Endo, T.A.; Nakayama, M.; Karimi, M.M.; Shimada, M.; Katsuyama, K.; Goyal, P.; Brind'Amour, J.; Sun, M-A.; Sun, Z.; et al. Activation of endogenous retroviruses in dnmt1(−/−) ESCs involves disruption of SETDB1-mediated repression by NP95 binding to hemimethylated DNA. *Cell Stem Cell*. **2016**, *19*, 81–94 DOI: 10.1016/j.stem.2016.03.013

57. Amir, R.E.; Van den Veyver, I.B.; Wan, M.; Tran, C.Q.; Francke, U.; Zoghbi, H.Y. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet*. **1999**, *23*, 185–188. DOI: 10.1038/13810

58. Morris, K.V.; Chan, S.W-L.; Jacobsen, S.E.; Looney, D.J. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science*. **2004**, *305*, 1289–1292. DOI: 10.1126/science.1101372

59. Ooi, S.K.T.; Qiu, C.; Bernstein, E.; Li, K.; Jia, D.; Yang, Z.; Erdjument-Bromage, H.; Tempst, P.; Lin, S-P.; Allis, C.D.; et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*. **2007**, *448*, 714–717. DOI: 10.1038/nature05987

60. Zhang, Y.; Jurkowska, R.; Soeroes, S.; Rajavelu, A.; Dhayalan, A.; Bock, I.; Rathert, P.; Brandt, O.; Reinhardt, R.; Fischle, W.; et al. Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Res*. **2010**, *38*, 4246–4253. DOI: 10.1093/nar/gkq147

61. Rea, S.; Eisenhaber, F.; O'Carroll, D.; Strahl, B.D.; Sun, Z.W.; Schmid, M.; Opravil, S.; Mechtler, K.; Ponting, C.P.; Allis, D.; et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*. **2000**, *406*, 593–599. DOI: 10.1038/35020506

62. Fuks, F.; Hurd, P.J.; Deplus, R.; Kouzarides, T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res*. **2003**, *31*, 2305–2312. DOI: 10.1093/nar/gkg332

63. Fuks, F.; Hurd, P.J.; Wolf, D.; Nan, X.; Bird, A.P.; Kouzarides, T. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem*. **2003b**, *278*, 4035–4040. DOI: 10.1074/jbc.M210256200

64. Kimura, H.; Shiota, K. Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. *J. Biol. Chem*. **2003**, *278*, 4806–4812. DOI: 10.1074/jbc.M209923200

65. Fuks, F.; Burgers, W.A.; Brehm, A.; Hughes-Davies, L.; Kouzarides, T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet*. **2000**, *24*, 88–91. DOI: 10.1038/71750

66. Kao, T-H.; Liao, H-F.; Wolf, D.; Tai, K-Y.; Chuang, C-Y.; Lee, H-S.; Kuo, H-C.; Hata, K.; Zhang, X.; Cheng, X.; et al. Ectopic DNMT3L triggers assembly of a repressive complex for retroviral silencing in somatic cells. *J. Virol*. **2014**, *88*, 10680–10695. DOI: 10.1128/JVI.01176-14

67. Zhu, G.; Tao, T.; Zhang, D.; Liu, X.; Qiu, H.; Han, L.; Xu, Z.; Xiao, Y.; Cheng, C.; Shen, A. O-GlcNAcylation of histone deacetylases 1 in hepatocellular carcinoma promotes cancer progression. *Glycobiology.* **2016**, *26*, 820–833. DOI: 10.1093/glycob/cww025

68. Gao, J.; Yang, Y.; Qiu, R.; Zhang, K.; Teng, X.; Liu, R.; Wang, Y. Proteomic analysis of the OGT interactome: novel links to epithelial-mesenchymal transition and metastasis of cervical cancer. *Carcinogenesis.* **2018**, *39*, 1222–1234. DOI: 10.1093/carcin/bgy097

69. Boulard, M.; Rucli, S.; Edwards, J.R.; Bestor, T.H. Methylation-directed glycosylation of chromatin factors represses retrotransposon promoters. *Proc. Natl. Acad. Sci. USA.* **2020**, *117*, 14292–14298. DOI: 10.1073/pnas.1912074117

70. Foroushani, A.K.; Chim, B.; Wong, M.; Rastegar, A.; Barbian, K.; Martens, C.; Hafner, M.; Muljo, S.A. Post-transcriptional regulation of human endogenous retroviruses by RNA-Binding Motif Protein 4, RBM4. *Proc. Natl. Acad. Sci. USA.* **2020**, *117*, 26520-26430 DOI: 10.1073/pnas.2005237117

71. Perron, H.; Garson, J.A.; Bedin, F.; Beseme, F.; Paranhos-Baccala, G.; Komurian-Pradel, F.; Mallet, F.; Tuke, P.W.; Voisset, C.; Blond, J.L.; et al. Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. The Collaborative Research Group on Multiple Sclerosis. *Proc. Natl. Acad. Sci. USA.* **1997**, *94*, 7583–7588. DOI: 10.1073/pnas.94.14.7583

72. Mameli, G.; Poddighe, L.; Mei, A.; Uleri, E.; Sotgiu, S.; Serra, C.; Manetti, R.; Dolei. A. Expression and activation by Epstein Barr virus of human endogenous retroviruses-W in blood cells and astrocytes: inference for multiple sclerosis. *PLoS One.* **2012**, *7*, e44991. DOI: 10.1371/journal.pone.0044991

73. Li, W.; Lee, M-H.; Henderson, L.; Tyagi, R.; Bachani, M.; Steiner, J.; Campanac, E.; Hoffman, D.A.; Geldern, G.V.; Johnson, K.; et al. Human endogenous retrovirus-K contributes to motor neuron disease. *Sci Transl. Med.* **2015**, *7*, 307ra153. DOI: 10.1126/scitranslmed.aac8201

74. Karlsson, H.; Bachmann, S.; Schröder, J.; McArthur, J.; Torrey, E.F.; Yolken, R.H. Retroviral RNA identified in the cerebrospinal fluids and brains of individuals with schizophrenia. *Proc. Natl. Acad. Sci. USA.* **2001**, *98*, 4634–4639. DOI: 10.1073/pnas.061021998

75. Slokar, G.; Hasler, G. Human endogenous retroviruses as pathogenic factors in the development of schizophrenia. *Front. Psychiatry.* **2015**, *6*, 183. DOI: 10.3389/fpsyt.2015.00183

76. Johansson, E.M.; Bouchet, D.; Tamouza, R.; Ellul, P.; Morr, A.S.; Avignone, E.; et al. Human endogenous retroviral protein triggers deficit in glutamate synapse maturation and behaviors associated with psychosis. *Sci. Adv.* **2020** *6*, eabc0708. DOI: 10.1126/sciadv.abc0708

77. Kury, P.; Nath, A.; Creange, A.; Dolei, A.; Marche, P.; Gold, J.; et al. Human Endogenous Retroviruses in Neurological Diseases. *Trends Mol. Med.* **2018**, *24*, 379-394. DOI: 10.1016/j.molmed.2018.02.007

78. Blond, J.L.; Besème, F.; Duret, L.; Bouton, O.; Bedin, F.; Perron, H.; Mandrand, B.; Mallet, F.. Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. *J. Virol.* **1999**, *73*, 1175–1185. DOI: 10.1128/JVI.73.2.1175-1185.1999

79. Pavlícek, A.; Paces, J.; Elleder, D.; Hejnar, J. Processed pseudogenes of human endogenous retroviruses generated by LINEs: their integration, stability, and distribution. *Genome Res.* **2002**, *12*, 391–399. DOI: 10.1101/gr.216902

80. Costas, J. Characterization of the intragenomic spread of the human endogenous retrovirus family HERV-W. *Mol. Biol. Evol.* **2002**, *19*, 526–533. DOI: 10.1093/oxfordjournals.molbev.a004108

81. Voisset, C.; Bouton, O.; Bedin, F.; Duret, L.; Mandrand, B.; Mallet, F.; Paranhos-Baccala, G. Chromosomal distribution and coding capacity of the human endogenous retrovirus HERV-W family. *AIDS Res. Hum. Retroviruses.* **2000**, *16*, 731–740. DOI: 10.1089/088922200308738

82. de Parseval, N.; Lazar, V.; Casella, J-F.; Benit, L.; Heidmann, T. Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. *J. Virol.* **2003**, *77*, 10414–10422. DOI: 10.1128/jvi.77.19.10414-10422.2003

83. Roebke, C.; Wahl, S.; Laufer, G.; Stadelmann, C.; Sauter, M.; Mueller-Lantzsch, N.; Mayer, J.; Ruprecht, K. An N-terminally truncated envelope protein encoded by a human endogenous retrovirus W locus on chromosome Xq22.3. *Retrovirology.* **2010**, *7*, 69. DOI: 10.1186/1742-4690-7-69

84. Mameli, G.; Astone, V.; Arru, G.; Marconi, S.; Lovato, L.; Serra, C.; Sotgiu, S.; Bonetti, B.; Dolei, A. Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not Human herpesvirus 6. *J. Gen. Virol.* **2007**, *88*, 264–274. DOI: 10.1099/vir.0.81890-0

85. Kim, H-S.; Kim, D-S.; Huh, J-W.; Ahn, K.; Yi, J-M.; Lee, J-R.; Hirai, H. Molecular characterization of the HERV-W env gene in humans and primates: expression, FISH, phylogeny, and evolution. *Mol. Cells*. **2008**, *26*, 53–60.

86. Lavillette, D.; Marin, M.; Ruggieri, A.; Mallet, F.; Cosset, F-L.; Kabat, D. The envelope glycoprotein of human endogenous retrovirus type W uses a divergent family of amino acid transporters/cell surface receptors. *J. Virol.* **2002**, *76*, :6442–6452. DOI: 10.1128/jvi.76.13.6442-6452.2002

87. Gong, R.; Peng, X.; Kang, S.; Feng, H.; Huang, J.; Zhang, W.; Lin, D.; Tien, P.; Xiao, G. Structural characterization of the fusion core in syncytin, envelope protein of human endogenous retrovirus family W. *Biochem. Biophys. Res. Commun*. **2005**, *331*, 1193–1200. DOI: 10.1016/j.bbrc.2005.04.032

88. Komurian-Pradel F, Paranhos-Baccala G, Bedin F, Ounanian-Paraz A, Sodoyer M, Ott C, et al. Molecular cloning and characterization of MSRV-related sequences associated with retrovirus-like particles. *Virology*. **1999**, *260*, 1–9. DOI: 10.1006/viro.1999.9792

89. Ruprecht, K.; Mayer, J. On the origin of a pathogenic HERV-W envelope protein present in multiple sclerosis lesions. *Proc. Natl. Acad. Sci. USA*. **2019**, *116*, 19791–19792. DOI: 10.1073/pnas.1911703116

90. Marchi, E.; Kanapin, A.; Magiorkinis, G.; Belshaw, R. Unfixed endogenous retroviral insertions in the human population. *J. Virol*. **2014**, *88*, 9529-9537. DOI: 10.1128/JVI.00919-14

91. Serra, C.; Mameli, G.; Arru, G.; Sotgiu, S.; Rosati, G.; Dolei, A. In vitro modulation of the multiple sclerosis (MS)-associated retrovirus by cytokines: Implications for MS pathogenesis. *J. Neurovirol*. **2003**, *9*, 637–643. DOI: 10.1080/13550280390246462

92. Kremer, D.; Gruchot, J.; Weyers, V.; Oldemeier, L.; Göttle, P.; Healy, L.; et al. pHERV-W envelope protein fuels microglial cell-dependent damage of myelinated axons in multiple sclerosis. *PNAS,* **2019**, *116*, 15216-1525. DOI: 10.1073/pnas.1901283116

93. Charvet, B.; Reynaud, J.M.; Gourru-Lesimple, G.; Perron, H.; Marche, P.N.; Horvat, B. Induction of Proinflammatory Multiple Sclerosis-Associated Retrovirus Envelope Protein by Human Herpesvirus-6A and CD46 Receptor Engagement. *Front. Immunol*. **2018**, *9*, 2803. DOI: 10.3389/fimmu.2018.02803

94. Charvet, B.; Pierquin, J.; Brunel, J.; Gorter, R.; Quétard, C.; Horvat, B.; et al. Human Endogenous Retrovirus Type W Envelope from Multiple Sclerosis Demyelinating Lesions Shows Unique Solubility and Antigenic Characteristics. *Virol. Sinica.* **2021**, in press. DOI: 10.1007/s12250-021-00372-0

95. Wang, X.; Liu, Z.; Wang, P.; Li, S.; Zeng, J.; Tu, X.; Yan, Q.; Xiao, Z.; Pan, M.; Zhu, F. Syncytin-1, an endogenous retroviral protein, triggers the activation of CRP via TLR3 signal cascade in glial cells. *Brain Behav. Immun*. **2018**, *67*, 324–334. DOI: 10.1016/j.bbi.2017.09.009

96. Subramanian, R.P.; Wildschutte, J.H.; Russo, C.; Coffin, J.M. Identification, characterization, and comparative genomic distribution of the HERV-K (HML-2) group of human endogenous retroviruses. *Retrovirology*. **2011**, *8*, 90.

97. Boller K, König H, Sauter M, Mueller-Lantzsch N, Löwer R, Löwer J, et al. Evidence that HERV-K is the endogenous retrovirus sequence that codes for the human teratocarcinoma-derived retrovirus HTDV. Virology. **1993**, *96*, 349–53. DOI: 10.1186/1742-4690-8-90

98. Bieda, K.; Hoffmann, A.; Boller, K. Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines. *J. Gen. Virol*. **2001**, *82*, 591–596. DOI: 10.1099/0022-1317-82-3-591

99. Dewannieux, M.; Blaise, S.; Heidmann, T. Identification of a functional envelope protein from the HERV-K family of human endogenous retroviruses. *J. Virol*. **2005**, *79*, 15573–15577. DOI: 10.1128/JVI.79.24.15573-15577.2005

100. Boller, K.; Schönfeld, K.; Lischer, S.; Fischer, N.; Hoffmann, A.; Kurth, R.; Tönjes, R.R. Human endogenous retrovirus HERV-K113 is capable of producing intact viral particles. *J. Gen. Virol*. **2008**, *89*, 567–572. DOI: 10.1099/vir.0.83534-0

101. Douville, R.; Liu, J.; Rothstein, J.; Nath, A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. *Ann. Neurol.* **2011**, *69*, 141–151. DOI: 10.1002/ana.22149

102. Daskalakis, M.; Brocks, D.; Sheng, Y-H.; Islam, M.S.; Ressnerova, A.; Assenov, Y.; Milde, T.; Oehme, I.; Witt, O.; Goyal, A.; et al. Reactivation of endogenous retroviral elements via treatment with DNMT- and HDAC-inhibitors. *Cell Cycle*. **2018**, *17*, 811–822. DOI: 10.1080/15384101.2018.1442623

103. Ascherio, A.; Munger, K.L. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann. Neurol*. **2007**, *61*, 288–299. DOI: 10.1002/ana.21117

104. Perron, H.; Lang, A. The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. *Clin. Rev. Allergy Immunol*. **2010**, *39*, 51–61. DOI: 10.1007/s12016-009-8170-x

105. Cusick, M.F.; Libbey, J.E.; Fujinami, R.S. Multiple sclerosis: autoimmunity and viruses. *Curr. Opin. Rheumatol*. **2013**, *25*, 496–501. DOI: 10.1097/BOR.0b013e328362004d

106. Limosin, F.; Rouillon, F.; Payan, C.; Cohen, J-M.; Strub, N. Prenatal exposure to influenza as a risk factor for adult schizophrenia: Influenza and schizophrenia. *Acta Psychiatr. Scand*. **2003**, *107*, 331–335. DOI: 10.1034/j.1600-0447.2003.00052.x

107. Shi, L.; Fatemi, S.H.; Sidwell, R.W.; Patterson, P.H. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J. Neurosci.* **2003**, *23*, 297–302. DOI: 10.1523/JNEUROSCI.23-01-00297.2003

108. Ebert, T.; Kotler, M. Prenatal exposure to influenza and the risk of subsequent development of schizophrenia. Isr Med Assoc J. **2005**, *7*, 35–38. DOI: 10.1034/j.1600-0447.2003.00052.x

109. Brown, A.S.; Derkits, E.J. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am. J. Psychiatry*. **2010**, *167*, 261–80. DOI: 10.1176/appi.ajp.2009.09030361

110. Nellåker, C.; Yao, Y.; Jones-Brando, L.; Mallet, F.; Yolken, RH.; Karlsson, H. Transactivation of elements in the human endogenous retrovirus W family by viral infection. *Retrovirology*. **2006**, *3*, 44. DOI: 10.1186/1742-4690-3-44

111. Li, F.; Nellåker, C.; Sabunciyan, S.; Yolken, R.H.; Jones-Brando, L.; Johansson, A-S.; et al. Transcriptional derepression of the ERVWE1 locus following influenza A virus infection. *J. Virol*. **2014**, *88*, 4328–4337. DOI: 10.1128/JVI.03628-13

112. Schmidt, N.; Domingues, P.; Golebiowski, F.; Patzina, C.; Tatham, M.H.; Hay, R.T.; Hale, B.G. An influenza virus-triggered SUMO switch orchestrates co-opted endogenous retroviruses to stimulate host antiviral immunity. *PNAS*. **2019**, *16*, 17399-17408. DOI: 10.1073/pnas.1907031116

113. Bhende, P.M.; Seaman, W.T.; Delecluse, H-J.; Kenney, S.C. The EBV lytic switch protein, Z, preferentially binds to and activates the methylated viral genome. *Nat. Genet*. 2004;36(10):1099–104. DOI: 10.1038/ng1424

114. Lee, W.J.; Kwun, H.J.; Kim, H.S.; Jang, K.L. Activation of the human endogenous retrovirus W long terminal repeat by herpes simplex virus type 1 immediate early protein 1. *Mol. Cells*. **2003**, *15*, 75–80.

115. Ruprecht, K.; Obojes, K.; Wengel, V.; Gronen, F.; Kim, K.S.; Perron, H.; Schneider-Schaulies, J.; Rieckmann, P. Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: implications for multiple sclerosis. *J. Neurovirol.* **2006**, *12*, 65–71. DOI: 10.1080/13550280600614973

116. Moore, F.G.A.; Wolfson, C. Human herpes virus 6 and multiple sclerosis: Human herpes virus 6 and multiple sclerosis. *Acta Neurol. Scand.* **2002**, *106*, 63–83. DOI: 10.1034/j.1600-0404.2002.01251.x

117. Mameli, G.; Madeddu, G.; Mei, A.; Uleri, E.; Poddighe, L.; Delogu, L.G.; Maida, I.; Babudieri, S.; Serra, C.; Manetti, R.; et al. Activation of MSRV-type endogenous retroviruses during infectious mononucleosis and Epstein-Barr virus latency: the missing link with multiple sclerosis? *PLoS One*. **2013**, *8*, e78474. DOI: 10.1371/journal.pone.0078474

118. Sutkowski, N.; Conrad, B.; Thorley-Lawson, D.A.; Huber, B.T. Epstein-Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen. *Immunity*. **2001**, *15*, 579–589. DOI: 10.1016/s1074-7613(01)00210-2

119. Sutkowski, N.; Chen, G.; Calderon, G.; Huber, B.T. Epstein-Barr virus latent membrane protein LMP-2A is sufficient for transactivation of the human endogenous retrovirus HERV-K18 superantigen. *J. Virol*. **2004**, *78*, 7852–7860. DOI: 10.1128/JVI.78.14.7852-7860.2004

120. Hsiao, F.C.; Lin, M.; Tai, A.; Chen, G.; Huber, B.T. Cutting edge: Epstein-Barr virus transactivates the HERV-K18 superantigen by docking to the human complement receptor 2 (CD21) on primary B cells. *J. Immunol*. **2006**,*177*, 2056–2060. DOI: 10.4049/jimmunol.177.4.2056

121. Tai, A.K.; Luka, J.; Ablashi, D.; Huber, B.T. HHV-6A infection induces expression of HERV-K18-encoded superantigen. *J. Clin. Virol.* **2009**, *46*, 47–48. DOI: 10.1016/j.jcv.2009.05.019

122. Turcanova, V.L.; Bundgaard, B.; Höllsberg, P. Human herpesvirus-6B induces expression of the human endogenous retrovirus K18-encoded superantigen. *J. Clin. Virol*. **2009**, *46*, 15–19. DOI: 10.1016/j.jcv.2009.05.015

123. Mak, M.; Samochowiec, J.; Frydecka, D.; Pełka-Wysiecka, J.; Szmida, E.; Karpiński, P.; Sasiadek, M.M.; Piotrowski, P.; Samochowiec, A.; Misiak, B. First-episode schizophrenia is associated with a reduction of HERV-K methylation in peripheral blood. *Psychiatry Res*. **2019**;*271*, 459–463. DOI: 10.1016/j.psychres.2018.12.012

124. Manghera, M.; Ferguson-Parry, J.; Lin, R.; Douville, R.N. NF-κB and IRF1 induce endogenous retrovirus K expression via interferon-stimulated response elements in its 5′ long terminal repeat. *J. Virol.* **2016**;*90*, 9338–9349. DOI: 10.1128/JVI.01503-16

125. Saito, T.; Miyagawa, K.; Chen, S.Y.; Tamosiuniene, R.; Wang, L.; Sharp, O.; et al. Upregulation of HERV-K is Linked to Immunity and Inflammation in Pulmonary Arterial Hypertension. *Circulation* **2017**, *136*, 1920-1935 DOI: 10.1161/CIRCULATIONAHA.117.027589

126. Brown, A.S. The environment and susceptibility to schizophrenia. *Prog. Neurobiol.* **2011**, *93*, 23-58. DOI: 10.1016/j.pneurobio.2010.09.003

127. Waubant, E.; Lucas, R.; Mowry, E.; Graves, J.; Olsson, T.; Alfredsson, L.; Langer-Gould, A. Environmental and genetic risk factors for MS: an integrated review. *Ann. Clin. Transl. Neurol*. **2019**, *6*, 1905–1922. DOI: 10.1002/acn3.50862.

128. Lafon, M.; Jouvin-Marche, E.; Marche, P.N.; Perron, H. Human viral superantigens: to be or not to be transactivated? *Trends Immunol*. **2002**, *23*, 238–239. DOI: 10.1016/s1471-4906(02)02207-x

129. Firouzi, R.; Rolland, A.; Michel, M.; Jouvin-Marche, E.; Hauw, J.J.; Malcus-Vocanson, C.; Lazarini, F.; Gebuhrer, L.; Seigneurin, J.M.; Touraine, J.L.; et al. Multiple sclerosis-associated retrovirus particles cause T lymphocyte-dependent death with brain hemorrhage in humanized SCID mice model. *J. Neurovirol.* **2003**, *9*, 79–93. DOI: 10.1080/13550280390173328

130. Brudek, T.; Christensen, T.; Aagaard, L.; Petersen, T.; Hansen, H.J.; Møller-Larsen, A. B cells and monocytes from patients with active multiple sclerosis exhibit increased surface expression of both HERV-H Env and HERV-W Env, accompanied by increased seroreactivity. *Retrovirology*. **2009**, *6*, 104. DOI: 10.1186/1742-4690-6-104

131. Garcia-Montojo, M.; Dominguez-Mozo, M.; Arias-Leal, A.; Garcia-Martinez, Á., De las Heras, V.; Casanova, I.; Faucard, R.; Gehin, N.; Madeira, A.; Arroyo, R.; et al. The DNA copy number of human endogenous retrovirus-W (MSRV-type) is increased in multiple sclerosis patients and is influenced by gender and disease severity. *PLoS One*. **2013**, *8*, e53623. DOI: 10.1371/journal.pone.0053623

132. Constantinescu, C.S.; Farooqi, N.; O'Brien, K.; Gran, B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br. J. Pharmacol.* **2011**, *164*, 1079-1106. DOI: 10.1111/j.1476-5381.2011.01302.x

133. Luo, C.; Jian, C.; Liao, Y.; Huang, Q.; Wu, Y.; Liu, X.; et al. The role of microglia in multiple sclerosis. *Neuropsychiatr. Dis. Treat.* **2017**, *13*, 1661-1667. DOI: 10.2147/NDT.S140634

134. Goldmann, T.; Prinz, M. Role of microglia in CNS autoimmunity. *Clin. Dev. Immunol.* **2013**, *2013*, 209093. DOI: 10.1155/2013/208093

135. Guerrero, B.L.; Sicotte, N.L. Microglia in Multiple Sclerosis: Friend or Foe? *Front. Immunol.* **2020**, *11*,374. DOI: 10.3389/fimmu.2020.00374

136. Monoghan, K.L.; Zheng, W., Hu, G.; Wan, E.C.K. Monocytes and Monocyte-Derived Antigen-Presenting Cells Have Distinct Gene Signatures in Experimental Model of Multiple Sclerosis. *Front. Immunol.* **2019**, *10*, 2779. DOI: 10.3389/fimmu.2019.02779

137. Engelhardt, B.; Ransohoff, R.M. apture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends. Immunol.* **2012**, *33*, 579-589. DOI: 10.1016/j.it.2012.07.004

138. Ransohoff, R.M.; Engelhardt, B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.* **2012**, *12*, 623-635. DOI: 10.1038/nri3265.

139. Ransohoff, R.M. How neuroinflammation contributes to neurodegeneration. *Science*. **2016**;*353*, 777–783. DOI: 10.1126/science.aag2590

140. Ludewig, P.; Gallizioli, M.; Urra, X.; Behr, S.; Brait, V.H.; Gelderblom, M.; et al. Dendritic cells in brain diseases. *Biochim. Biophys. Acta.* **2016**, *1862*, 352-367. DOI: 10.1016/j.bbadis.2015.11.003

141. McFarland, H.F.; Martin, R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat. Immunol*. **2007**, *8*, 913–919. DOI: 10.1038/ni1507

142. Martino, G.; Adorini, L.; Rieckmann, P.; Hillert, J.; Kallmann, B.; Comi, G.; et al. Inflammation in multiple sclerosis: the good, the bad, and the complex. *Lancet Neurol.* **2002**, *1*, 499-509. DOI: 10.1016/s1474-4422(02)00223-5

143. Hemmer, B.; Nessler, S.; Zhou, D.; Kieseier, B.; Hartung, H-P. Immunopathogenesis and immunotherapy of multiple sclerosis. *Nat. Clin. Pract. Neurol*. **2006**, *2*, 201–11. DOI: 10.1038/ncpneuro0154

144. Popescu, B.F.G.; Lucchinetti, C.F. Pathology of demyelinating diseases. *Annu. Rev. Pathol.* **2012**, *7*, 185–217. DOI: 10.1146/annurev-pathol-011811-132443

145. Sospedra, M., Martin, R. Immunology of multiple sclerosis. *Semin. Neurol.* **2016**, *36*, 115–127. DOI: 10.1055/s-0036-1579739

146. Duperray, A.; Barbe, D.; Raguenez, G.; Weksler, B.B.; Romero, I.A.; Couraud, P-O.; Perron, H.; Marche, P.N. Inflammatory response of endothelial cells to a human endogenous retrovirus associated with multiple sclerosis is mediated by TLR4. I*nt. Immunol.* **2015**, *27*, 545–53. DOI: 10.1093/intimm/dxv025

147. Lehnardt, S.; Massillon, L.; Follett, P.; Jensen, F.E.; Ratan, R.; Rosenberg, P.A.; Volpe, J.K.; Vartanian, T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc. Natl. Acad. Sci. USA.* **2003**, *100*, 8514–8519. DOI: 10.1073/pnas.1432609100

148. Göttle, P.; Förster, M.; Gruchot, J.; Kremer, D.; Hartung, H.P.; Perron, H.; Küry, P. Rescuing the negative impact of human endogenous retrovirus envelope protein on oligodendroglial differentiation and myelination. *Glia.* **2019**, *67*, 160–70. DOI: 10.1002/glia.23535

149. Perron, H.; Dougier-Reynaud, H-L.; Lomparski, C.; Popa, I.; Firouzi, R.; Bertrand, J-B.; Marusic, S.; Portoukalian, J.; Jouvin-Marche, E.; Villiers, C.L.; et al. Human endogenous retrovirus protein activates innate immunity and promotes experimental allergic encephalomyelitis in mice. *PLoS One.* **2013**, *8*, e80128. DOI: 10.1371/journal.pone.0080128

150. Perron, H.; Germi, R.; Bernard, C.; García-Montojo, M.; Deluen, C.; Farinelli, L.; et al. Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. *Mult. Scler.* **2012**, *18*, 1721-1736. DOI: 10.1177/1352458512441381

151. Nowak, J.; Januszkiewicz, D.; Pernak, M., Liweń, I.; Zawada, M.; Rembowska, J.; Nowicka, K.; Lewandowski, K.; Hertmanoswka, H.; Wender, M. Multiple sclerosis-associated virus-related pol sequences found both in multiple sclerosis and healthy donors are more frequently expressed in multiple sclerosis patients. *J. Neurovirol.* **2003**, *9*, 112–7. DOI: 10.1080/13550280390173355

152. Perron, H.; Lazarini, F.; Ruprecht, K.; Péchoux-Longin, C.; Seilhean, D.; Sazdovitch, V.; Créange, A.; Battail-Poirot, N.; Sibaï, G.; Santoro, L.; et al. Human endogenous retrovirus (HERV)-W ENV and GAG proteins: physiological expression in human brain and pathophysiological modulation in multiple sclerosis lesions. *J. Neurovirol.* **2005**, *11*, 23–33. DOI: 10.1080/13550280590901741

153. Kremer, D.; Schichel, T.; Förster, M.; Tzekova, N.; Bernard, C.; van der Valk, P.; Horssen, J.V.; Hartung, H-P.; Perron, H.; Küry, P. Human endogenous retrovirus type W envelope protein inhibits oligodendroglial precursor cell differentiation: Retroviruses and Myelin Repair. *Ann. Neurol.* **2013**, *74*, 721–732. DOI: 10.1002/ana.23970

154. Garson, J.A.; Tuke, P.W.; Giraud, P.; Paranhos-Baccala, G.; Perron, H. Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. *Lancet.* **1998**, *351*, 33. DOI: 10.1016/s0140-6736(98)24001-3

155. Dolei, A.; Serra, C.; Mameli, G.; Pugliatti, M.; Sechi, G.; Cirotto, M.C.; Rosati, G.; Sotgiu, S. Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients. *Neurology.* **2002**, *58*, 471–473. DOI: 10.1212/wnl.58.3.471

156. Sotgiu, S.; Arru, G.; Mameli, G.; Serra, C.; Pugliatti, M.; Rosati, G.; Dolei, A. Multiple sclerosis-associated retrovirus in early multiple sclerosis: a six-year follow-up of a Sardinian cohort. *Mult Scler.* **2006**, *12*, 698–703. DOI: 10.1177/1352458506070773.

157. Mameli, G.; Serra, C.; Astone, V.; Castellazzi, M.; Poddighe, L.; Fainardi, E.; Neri, W.; Granieri, W.; Dolei, A. Inhibition of multiple-sclerosis-associated retrovirus as biomarker of interferon therapy. *J. Neurovirol.* **2008**;*14*, 73–77. DOI: 10.1080/13550280701801107

158. Sotgiu, S.; Pugliatti, M.; Sanna, A.; Sotgiu, A.; Castiglia, P.; Solinas, G.; Dolei, A.; Serra, C.; Bonetti, B.; Rosati, G. Multiple sclerosis complexity in selected populations: the challenge of Sardinia, insular Italy. *Eur. J. Neurol.* **2002**, *9*, 329–341. DOI: 10.1046/j.1468-1331.2002.00412.x

159. Sotgiu, S.; Mameli, G.; Serra, C.; Zarbo, I.R.; Arru, G.; Dolei, A. Multiple sclerosis-associated retrovirus and progressive disability of multiple sclerosis. *Mult. Scler.* **2010**, *16*, 1248–1251. DOI: 10.1177/1352458510376956

160. Gerondakis, S.; Grumont, R.J.; Banerjee, A. Regulating B-cell activation and survival in response to TLR signals. *Immunol. Cell Biol.* **2007**, *85*, 471–475. DOI: 10.1038/sj.icb.7100097

161. Kaisho, T.; Akira, S. Toll-like receptor function and signaling. *J. Allergy Clin. Immunol.* **2006**, *117*, 979–987. DOI: 10.1016/j.jaci.2006.02.023

162. Bsibsi, M.; Ravid, R.; Gveric, D.; van Noort, J.M. Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.* **2002**, *61*, 1013–1021. DOI: 10.1093/jnen/61.11.1013

163. Bowman, C.C.; Rasley, A.; Tranguch, S.L.; Marriott, I. Cultured astrocytes express toll-like receptors for bacterial products. *Glia*. **2003**, *43*, 281–291. DOI: 10.1002/glia.10256

164. Olson, J.K.; Miller, S.D. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J. Immunol*. **2004**, *173*, 3916–3924. DOI: 10.4049/jimmunol.173.6.3916

165. Janeway, C.A. Jr.; Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol*. **2002**, *20*, 197–216. DOI: 10.1146/annurev.immunol.20.083001.084359

166. Akira, S.; Hemmi, H. Recognition of pathogen-associated molecular patterns by TLR family. *Immunol. Lett*. **2003**, *85*, 85–95. DOI: 10.1016/s0165-2478(02)00228-6

167. Planas, R.; Metz, I.; Martin, R, Sospedra, M. Detailed Characterization of T Cell Receptor Repertoires in Multiple Sclerosis Brain Lesions. *Front Immunol*. **2018**, *9*, 509. DOI: 10.3389/fimmu.2018.00509.

168. Franklin, R.J.M.; Ffrench-Constant, C. Regenerating CNS myelin - from mechanisms to experimental medicines. *Nat. Rev. Neurosci*. **2017**, *18*, 753–769. DOI: 10.1038/nrn.2017.136

169. Touil, T.; Deloire-Grassin, M.S.; Vital, C.; Petry, K.G.; Brochet, B. In vivo damage of CNS myelin and axons induced by peroxynitrite. *Neuroreport*. **2001**, *12*, 3637–3644. DOI: 10.1097/00001756-200111160-00052

170. Karamita, M.; Barnum, C.; Möbius, W.; Tansey, M.G.; Szymkowski, D.E.; Lassmann, H.; Probert, L. Therapeutic inhibition of soluble brain TNF promotes remyelination by increasing myelin phagocytosis by microglia. *JCI Insight*. **2017**, *2*. DOI: 10.1172/jci.insight.87455

171. Madeira, A.; Burgelin, I.; Perron, H.; Curtin, F.; Lang, A.B.; Faucard, R. MSRV envelope protein is a potent, endogenous and pathogenic agonist of human toll-like receptor 4: Relevance of GNbAC1 in multiple sclerosis treatment. *J. Neuroimmunol*. **2016**, *291*, 29-38. DOI: 10.1016/j.jneuroim.2015.12.006

172. Faucard, R.; Madeira, A.; Gehin, N.; Authier, F-J.; Panaite, P-A.; Lesage, C.; Burgelin, I.; Bertel, M.; Bernard, C.; Curtin, F.; et al. Human endogenous retrovirus and neuroinflammation in chronic inflammatory demyelinating polyradiculoneuropathy. *EBioMedicine*. **2016**, *6*, 190–198. DOI: 10.1016/j.ebiom.2016.03.001

173. Christensen, T. HERVs in neuropathogenesis. *J. Neuroimmune Pharmacol*. **2010**, *5*, 326–35. DOI: 10.1007/s11481-010-9214-y

174. Leboyer, M.; Tamouza, R.; Charron, D.; Faucard, R.; Perron, H. Human endogenous retrovirus type W (HERV-W) in schizophrenia: a new avenue of research at the gene-environment interface. *World J. Biol. Psychiatry*. **2011**, *14*, 80–90. DOI: 10.3109/15622975.2010.601760

175. Howes, O.D.; McCutcheon, R. Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization. *Transl. Psychiatry*. **2017**, *7*, e1024–e1024. DOI: 10.1038/tp.2016.278

176. Müller, N.; Wagner, J.K.; Krause, D.; Weidinger, E.; Wildenauer, A.; Obermeier, M.; Dehning, S.; Gruber, R.; Shwarz, M.J. Impaired monocyte activation in schizophrenia. *Psychiatry Res*. **2012**, *198*, 341–346. DOI: 10.1016/j.psychres.2011.12.049

177. Potvin, S.; Stip, E.; Sepehry, A.A.; Gendron, A.; Bah, R.; Kouassi, E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biol. Psychiatry*. **2008**, *63*, 801–808. DOI: 10.1016/j.biopsych.2007.09.024

178. Misiak, B.; Stańczykiewicz, B.; Kotowicz, K.; Rybakowski, J.K.; Samochowiec, J.; Frydecka, D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. *Schizophr. Res*. **2018**, *192*, 16–29. DOI: 10.1016/j.schres.2017.04.015

179. Dickerson, F.; Stallings, C.; Origoni, A.; Boronow, J.; Yolken, R. C-reactive protein is associated with the severity of cognitive impairment but not of psychiatric symptoms in individuals with schizophrenia. *Schizophr. Res*. **2007**, *93*, 261–265. DOI: 10.1016/j.schres.2007.03.022

180. Melbourne, J.K.; Chase, K.A.; Feiner, B.; Rosen, C.; Sharma, R.P. Long non-coding and endogenous retroviral RNA levels are associated with proinflammatory cytokine mRNA expression in peripheral blood cells: Implications for schizophrenia. *Psychiatry Res*. **2018**, *262*, 465–468. DOI: 10.1016/j.psychres.2017.09.025

181. Karlsson, H.; Schröder, J.; Bachmann, S.; Bottmer, C.; Yolken, R.H. HERV-W-related RNA detected in plasma from individuals with recent-onset schizophrenia or schizoaffective disorder. *Mol. Psychiatry*. **2004**, *9*, 12–13. DOI: 10.1038/sj.mp.4001439.

182. Perron, H.; Mekaoui, L.; Bernard, C.; Veas, F.; Stefas, I.; Leboyer, M. Endogenous retrovirus type W GAG and envelope protein antigenemia in serum of szhizophrenia patients. *Biol. Psychiatry*. **2008**, *64*, 1019-1023. DOI: 10.1016/j.biopsych.2008.06.028

183. Huang, W.; Li, S.; Hu, Y.; Yu, H.; Luo, F.; Zhang, Q.; Zhu, F. Implication of the env gene of the human endogenous retrovirus W family in the expression of BDNF and DRD3 and development of recent-onset schizophrenia. *Schizophr. Bull*. **2011**, *37*, 988–1000. DOI: 10.1093/schbul/sbp166

184. Deb-Rinker, P.; Klempan, T.A.; O'Reilly, R.L.; Torrey, E.F.; Singh, S.M. Molecular characterization of a MSRV-like sequence identified by RDA from monozygotic twin pairs discordant for schizophrenia. *Genomics*. **1999**, *61*, 133–144. DOI: 10.1006/geno.1999.5946

185. Yao, Y.; Schröder, J.; Nellåker, C.; Bottmer, C.; Bachmann, S.; Yolken, R.H.; Karlsson, H. Elevated levels of human endogenous retrovirus-W transcripts in blood cells from patients with first episode schizophrenia. *Genes Brain Behav*. **2008**, *7*, 103–112. DOI: 10.1111/j.1601-183X.2007.00334.x

186. Yolken, R.H.; Karlsson, H.; Yee, F.; Johnston-Wilson, N.L.; Torrey, E.F. Endogenous retroviruses and schizophrenia. *Brain Res. Rev*. **2000,** *31*, 193–199. DOI: 10.1016/s0165-0173(99)00037-5

187. Gourion, D.; Goldberger, C.; Leroy, S.; Bourdel, M-C.; Olié, J-P.; Krebs, M-O. Age at onset of schizophrenia: interaction between brain-derived neurotrophic factor and dopamine D3 receptor gene variants. *Neuroreport*. **2005**, *16*, 1407–1410. DOI: 10.1097/01.wnr.0000175245.58708.6b

188. Zarei, S.; Carr, K.; Reiley, L.; Diaz, K.; Guerra, O.; Altamirano, P.F.; Pagani, W.; Lodin, D.; Orozco, G., Chinea, A. A comprehensive review of amyotrophic lateral sclerosis. *Surg. Neurol. Int*. **2015**, *6*, 171. DOI: 10.4103/2152-7806.169561

189. Gros-Louis, F.; Gaspar, C.; Rouleau, G.A. Genetics of familial and sporadic amyotrophic lateral sclerosis. *Biochim. Biophys. Acta*. **2006**, *1762*, 956–972. DOI: 10.1016/j.bbadis.2006.01.004

190. Mackenzie, I.R.A.; Bigio, E.H.; Ince, P.G.; Geser, F.; Neumann, M.; Cairns, N.J.; Kwong, L.K.; Forman, M.S.; Ravits, J.; Stewart, H.; et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann. Neurol*. **2007**, *61*, 427–434. DOI: 10.1002/ana.21147

191. Neumann, M.; Sampathu, D.M.; Kwong, L.K.; Truax, A.C.; Micsenyi, M.C.; Chou, T.T.; Bruce, J.; Schuck, T.; Grossman, M.; Clark, C.M.; et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. **2006**, *314*, 130–133. DOI: 10.1126/science.1134108

192. Ou, S.H.; Wu, F.; Harrich, D.; García-Martínez, L.F.; Gaynor, R.B. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *J. Virol*. **1995**, *69*, 3584–3596. DOI: 10.1128/JVI.69.6.3584-3596.1995

193. Neumann, M.; Kwong, L.K.; Truax, A.C.; Vanmassenhove, B.; Kretzschmar, H.A.; Van Deerlin, V.M.; Clarck, c.M.; Grossman, M.; Miller, B.L.; Trojanowski, J.Q.; et al. TDP-43-positive white matter pathology in frontotemporal lobar degeneration with ubiquitin-positive inclusions. *J. Neuropathol. Exp. Neurol*. **2007**, *66*, 177–183. DOI: 10.1097/01.jnen.0000248554.45456.58

194. Manghera, M.; Ferguson-Parry, J.; Douville, R.N. TDP-43 regulates endogenous retrovirus-K viral protein accumulation. *Neurobiol. Dis*. **2016**, *94*, 226–236. DOI: 10.1016/j.nbd.2016.06.017

195. Haeusler, A.R.; Donnelly, C.J.; Periz, G.; Simko, E.A.J.; Shaw, P.G.; Kim, M-S.; Maragakis, N-J.; Troncoso, J.C.; Pandey, A.; Sattler, R.; et al. C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature*. **2014**, *507*, 195–200.

196. Janeway, C.A.; Travers, P.; Walport, M.; Shlomchik, M.J. The immune system in health and disease. In *Immunobiology*, 6th ed. London, England: Churchill Livingstone; 2004.

197. Curtin, F.; Lang, A.B.; Perron, H.; Laumonier, M.; Vidal, V.; Porchet, H.C.; Hartung, H-P. GNbAC1, a humanized monoclonal antibody against the envelope protein of multiple sclerosis-associated endogenous retrovirus: a first-in-humans randomized clinical study. *Clin. Ther*. **2012**, *34*, 2268–2278. DOI: 10.1016/j.clinthera.2012.11.006

198. Curtin, F.; Perron, H.; Kromminga, A.; Porchet, H.; Lang, A.B. Preclinical and early clinical development of GNbAC1, a humanized IgG4 monoclonal antibody targeting endogenous retroviral MSRV-Env protein. *MAbs*. **2015**, *7*, 265–275. DOI: 10.4161/19420862.2014.985021

199. Curtin, F.; Perron, H.; Faucard, R.; Porchet, H.; Lang, A.B. Treatment against human endogenous retrovirus: a possible personalized medicine approach for multiple sclerosis. *Mol. Diagn. Ther*. **2015**, *19*, 255–265. DOI: 10.1007/s40291-015-0166-z

200. Curtin, F.; Vidal, V.; Bernard, C.; Kromminga, A.; Lang, A.B.; Porchet, H. Serum pharmacokinetics and cerebrospinal fluid concentration analysis of the new IgG4 monoclonal antibody GNbAC1 to treat multiple sclerosis: A Phase 1 study. *MAbs*. **2016**, *8*, 854–860. DOI: 10.1080/19420862.2016.1168956

201. Derfuss, T.; Curtin, F.; Guebelin, C.; Bridel, C.; Rasenack, M.; Matthey, A.; Pasquier, R.D.; Schluep, M.; Desmeules, J.; Lang, A.B.; et al. A phase IIa randomized clinical study testing GNbAC1, a humanized monoclonal antibody against the envelope protein of multiple sclerosis associated endogenous retrovirus in multiple sclerosis patients - a twelve month follow-up. *J. Neuroimmunol*. **2015**, *285*, 68–70. DOI: 10.1177/1352458514554052

202. Arru, G.; Leoni, S.; Pugliatti, M.; Mei,A.; Serra, C.; Delogu, L.G.; et al. Natalizumab inhibits the expression of human endogenous retroviruses of the W family in multiple sclerosis patients: a longitudinal cohort study. *Mult. Scler.* **2014**, *20*, 174–182.

203. Zimmermann, M.; Sanderson, N.S.R.; Rasenack, M.; Lalive, P.H.; Lang, A.B.; Curtin, F.; Lindberg, R.L.P.; Kappos, L.; Derfuss, T. Immunologic monitoring during a phase 2a trial of the GNbAC1 antibody in patients with MS. *Neurol. Neuroimmunol. Neuroinflamm*. **2015**, *2*, e144. DOI: 10.1212/NXI.0000000000000144

204. Curtin, F.; Porchet, H.; Glanzman, R.; Schneble, H.M.; Vidal, V.; Audoli-Inthavong, M.L.; et al. A placebo randomized controlled study to test the efficacy and safety of GNbAC1, a monoclonal antibody for the treatment of multiple sclerosis - Rationale and design. *Mult. Scler. Relat. Disord*. **2016**, *9*, 95–100. DOI: 10.1016/j.msard.2016.07.002

205. Kremer, D.; Weyers, V.; Gruchot, J.; Göttle, P.; Hartung, H. P.; Perron, H.; Küry, P. (2020). Meeting report:"Human endogenous retroviruses: HERVs or transposable elements in autoimmune, chronic inflammatory and degenerative diseases or cancer", Lyon, France, november 5th and 6th 2019–an MS scientist's digest. *Mult. Scler. Relat. Disord.* **2020**, *42*, 102068. DOI: 10.1016/j.msard.2020.102068

206. Levet, S.; Medina, J.; Joanou, J.; Demolder, A.; Queruel, N.; Réant, K.; Normand, M.; Sefals, M.; Dimier, J.; Germi, R.; et al. An ancestral retroviral protein identified as a therapeutic target in type-1 diabetes. *JCI Insight*. **2017**, *2*. DOI: 10.1172/jci.insight.94387

207. Ruiz, P.L.D.; Tapia, G.; Bakken, I.J.; Håberg, S.E.; Hungnes, O.; Gulseth, H.L.; Stene, L.C. Pandemic influenza and subsequent risk of type 1 diabetes: a nationwide cohort study. *Diabetologia*. **2018**, *61*, 1996–2004. DOI: 10.1007/s00125-018-4662-7

208. Tauriainen, S.; Oikarinen, S.; Oikarinen, M.; Hyöty, H. Enteroviruses in the pathogenesis of type 1 diabetes. *Semin. Immunopathol*. **2011**, *33*, 45–55. DOI: 10.1007/s00281-010-0207-y

209. Garay-Malpartida, H.M.; Mourão, R.F.; Mantovani, M.; Santos, I.A.; Sogayar, M.C.; Goldberg, A.C. Toll-like receptor 4 (TLR4) expression in human and murine pancreatic beta-cells affects cell viability and insulin homeostasis. *BMC Immunol*. **2011**, *12*, 18. DOI: 10.1186/1471-2172-12-18

210. Qiao, Y-C.; Chen, Y-L.; Pan, Y-H.; Tian, F.; Xu, Y.; Zhang, X-X.; Zhao, H-L. The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. *PLoS One*. **2017**, *12*, e0176157. DOI: 10.1371/journal.pone.0176157

211. Curtin, F.; Bernard, C.; Levet, S.; Perron, H.; Porchet, H.; Médina, J.; Malpass, S.; Lloyd, D.; Simpson, R.; investigators R.T. A new therapeutic approach for type 1 diabetes: Rationale for GNbAC1, an anti-HERV-W-Env monoclonal antibody. *Diabetes Obes. Metab*. **2018**, *20*, 2075–2084. DOI: 10.1111/dom.13357

212. Curtin, F.; Champion, B.; Davoren, P.; Duke, S.; Ekinci, E.I.; Gilfillan, C.; Morbey, C.; Nathow, T.; O'Moore-Sullivan, T.; O'Neal, D.; et al. A safety and pharmacodynamics study of temelimab, an antipathogenic human endogenous retrovirus type W envelope monoclonal antibody, in patients with type 1 diabetes. *Diabetes Obes. Metab*. **2020**, *22*, 1111–1121. DOI: 10.1111/dom.14010

213. Tintore, M.; Vidal-Jordana, A.; Sastre-Garriga, J. Treatment of multiple sclerosis - success from bench to bedside. *Nat. Rev. Neurol*. **2019**, *15*, 53–58. DOI: 10.1038/s41582-018-0082-z

214. Ovejero, T.; Sadones, O.; Sánchez-Fito, T.; Almenar-Pérez, E.; Espejo, J.A.; Martín-Martínez, E.; Nathanson, L.; Oltra, E. Activation of transposable elements in immune cells of fibromyalgia patients. Int J Mol Sci. **2020**, *21*, 1366. DOI: 10.3390/ijms21041366

215. Rodrigues, L.S.; da Silva Nali, L.H.; Leal, C.O.D.; Sabino, E.C.; Lacerda, E.M.; Kingdon, C.C.; Nacul, L.; Romano, C.M. HERV-K and HERV-W transcriptional activity in myalgic encephalomyelitis/chronic fatigue syndrome. *Auto Immun. Highlights*. **2019**, *10*, 12. DOI: 10.1101/693465

216. Perron, H.; Medina, J.; Nath, A.; Steiner, J.P.; Wenxue, L.I.; Myoung-Hwa, L.E.E. US20200308258 - ANTI-HERV-K ENVELOPE ANTIBODY AND USES THEREOF [Internet]. Patent. 16903947, 2020. Available from: https://patentscope.wipo.int/search/en/detail.jsf?docId=US306969352&tab=NATIONALBIBLIO&_cid=P22-KJQZUJ-45299-1