# HUMAN ENDOGENOUS RETROVIRUS AND CLINICAL TREATMENTS OF NEUROLOGICAL DISEASE

Bibliography

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## HERVs: THEIR ASSOCIATION WITH NEUROLOGICAL DISEASE

Human endogenous retroviruses (HERVs) are DNA sequences from ancient viral germ line infections which comprise 8% of the human genome. They consist of two long terminal repeats (LTR) encompassing three proviral gene open reading frames: *gag*, *pol* and *env*, although some have lost some, or even all, the proviral genes. HERVs can control gene expression of nearby genes, being necessary for some physiological processes. However, their expression needs to be tightly regulated to avoid potential harmful effects, such as neurological disease **(Table 1)**. HERVs transcription is restricted by epigenetic mechanisms such as DNA methylation and histone modifications **(Figure 1)**, modulated by environmental factors like nutritional deficits or viral infections, among other. Under neuroinflammatory pathological conditions, such as in Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS), two diseases whose primary cause remains unknown, aberrant HERV expression is clearly evidenced, and its presence linked as causative.

**Table 1. HERVs associated with neurological disease**

| DISEASE | RETROTRANSPOSON | ELEVATED CYTOKINES |
|---|---|---|
| **Multiple Sclerosis (MS)** | HERV-W, HERV-H | IFN-γ, IL-6, TNF-α |
| **Fibromyalgia (FM)** | HERV-K, HERV-H, HERV-W | INF-β, INF-γ |
| **Attention deficit hyperactivity disorder (ADHD)** | HERV-H | IL-6, TNF-β |
| **Aicardi-Goutieres syndrome (AGS)** | LINE-1 | TNF-α, IL-15, IFN-α |
| **Rett syndrome (RTT)** | LINE-1 | IL-6, IL-8 |
| **Autism spectrum disorder (ASD)** | HERV-H, HERV-W, LINE-1 | IFN-γ, IL-1β, IL-6 |
| **Bipolar disorder (BD)** | HERV-W, HERV-K | IL-6, TNF-α, IL-10 |
| **Sporadic amyotrophic lateral sclerosis (ALS)** | HERV-K | TNF-α, IL-6, IL-8, IL-1β |
| **Chronic fatigue syndrome/ myalgic encephalomyelitis (CFS/MS)** | HERV-K | IFN-γ, IL-4, IL-5, IL-7, IL-13 |
| **Schizophrenia** | HERV-W, HERV-K | IL-6, TNF-α, IL-1β, IL-2, IFN-γ, IL-8 |

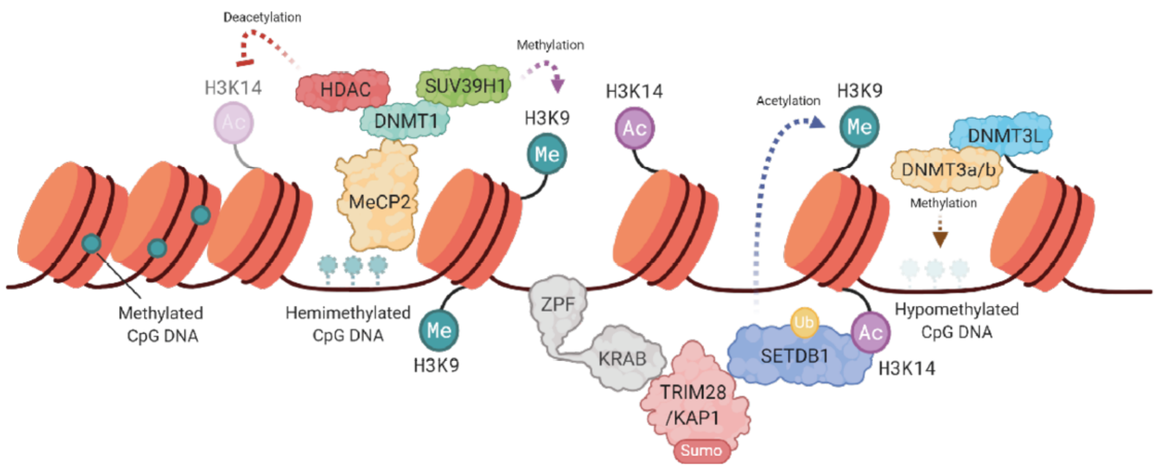


**Figure 1. Retroelements silencing mechanisms.**

In adult tissues, retroelements are repressed by epigenetic modifications. DNA methylation impairs transcription factors binding, inhibiting gene expression. It is controlled by DNA methyltransferases (DNMTs). DNMT3L binds to histone 3 (H3) tails and recruits DNMT3a and DNMT3b to establish *de novo* methylation in hypomethylated DNA. Also, DNMT3a/b bind to DNA hypomethylated regions, not protected by transcription factors. When DNA is hemimethylated, methyl-CpG binding proteins (MBD), like MeCP2, bind to methylated DNA and recruit various repressor complexes. MeCP2 recruits DNMT1 to perform DNA methylation and can also bind to histone deacetylases (HDAC) and H3K9 methyltransferases like SUV39H1 to stabilize chromatin structure repressing gene expression. Moreover, MBD proteins expression is higher in brain than in other tissues. Another family of proteins, KRAB zinc finger proteins (KRAB-ZFPs) are essential in retroelement silencing. They bind to specific positions and recruit the corepresor TRIM28/ KAP1, which also recruits the SETDB1 methyltransferase. SETDB1 binds to K9 methylated or K14 acetylated tails of H3 to repress retroelements in brain cells, B lymphocytes and T lymphocytes. (Created with Biorender.com).

## HERV-K AND AMYOTROPHIC LATERAL SCLEROSIS (ALS)

In ALS motor neurons from the brain and/or spinal cord progressively degenerate causing muscle weakness that lead to paralysis and death of the patient. ALS can be sporadic (sALS) (90% of the cases) or genetic (10%) due to specific mutations. It has been shown that the HERV-K *env* gene is highly expressed in cortical and spinal neurons of sALS, and not in other neurodegenerative diseases such as Alzheimer's or Parkinson's disease, indicating its expression is specific to sALS. Furthermore, *in vitro* expression of HERV-K *env* in human neurons causes neurotoxicity and *in vivo* expression in transgenic mice leads to progressive motor dysfunction. Interestingly, increased incidence (3.5 out of 1.000) in HIV-infected persons, HIV-associated ALS (HALS), is found compared to sALS (4-6 out of 100.000). Clinical manifestation of HALS is identical to sALS despite it occurs at an earlier age and its clinical progression is faster. In fact, it has been shown that HIV-Tat protein induces the activation of HERV-K. When HALS patients are treated with antiretroviral therapy, HERV-K expression decreases, and symptoms related to ALS are relieved. However, it remains unclear whether HERV-K expression is directly inhibited by the antiretroviral therapy or if it is a consequence of HIV inactivation. Interestingly, HERV-K seems to be the only HERV capable of retrotranscription in the human genome. *In vitro* studies testing FDA approved anti-HIV inhibitors of reverse transcriptase (tenofovir, abacavir, lamivudine) or integrase (dolutegravir) also show a reduction of HERV-K expression, while protease inhibitors (Darunavir or Lopinavir) are not as effective. Currently the NCT02437110 clinical trial (**Table 2**) aimed at learning how anti-HIV drugs affect HIV-negative sALS patients is ongoing.

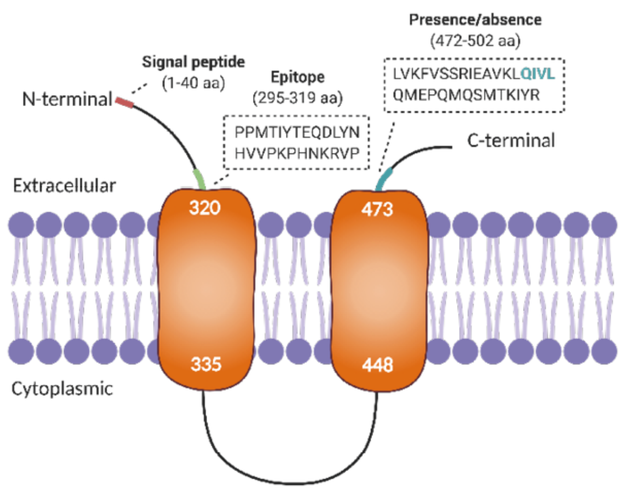


**Figure 2. GNbAC1 targets both MSRV and Syncytin-1**

HERVW-Env protein (GenBank: AF331500) structure has been predicted by Phyre2 software. The GNbAC1 epitope amino-terminal location is shown in light green. Epitope amino acid sequence and residue positions are indicated. The 4 amino acid sequence that differentiates the MS -related HERVW-Env protein (MRSV) from the HERVW/Syncytin-1 are shown in dark green. The positions of these 4 amino acids in MRSV primary sequence are shown and their carboxy-terminal disposition in the structure is labeled in dark green. This shows that the GNbAC1 antibody does not differentiate between either or the two HERVW-Env proteins cited. (Created with Biorender.com)

## HERV-W AND MULTIPLE SCLEROSIS (MS)

On the other hand, MS is a chronic inflammatory and neurodemyelinating disease whose association with HERV-W has been demonstrated. MS development is characterized by an acute inflammatory process at early stages mediated by penetrating T and B cells in the brain while impairment of myelin integrity and axonal damage are taking place. With improved knowledge of MS, new therapies with fewer secondary effects and better efficacy have arisen. From IFNβ injectables or fingolimod oral treatment to monoclonal antibodies like natalizumab or ocrelizumab, all therapies target immune cells. However, and, despite their contribution to MS symptoms amelioration, none showed neuroprotective effects. A recently developed monoclonal antibody against HERVW- Env protein, GNbAC1 (also called temelimab), is currently being tested in clinical trials **(Table 2)** constituting the first biological directed therapy against an HERV-encoded product. As GNbAC1 seems to have remyelinating benefits but does not seem to be immunomodulator, it would be interesting to test a combinatorial therapy between monoclonal antibodies targeting B cells and GNbAC1 antibody. A current clinical trial which studies the effects of GNbAC1 administration following rituximab therapy is ongoing (NCT04480307, **Table 2**).

## CONCLUDING REMARKS

In addition to the observed biomarker value of HERVs in neurological disease, this focused review for ALS and MS updated treatments points at the potential for HERV-directional therapies for additional diseases associating with HERV activation. Further research on the epigenetic mechanisms involved in the expression landscape of HERVs genome-wide may bring light to their particular implication in each of the neurological diseases examined which present with clearly distinctive symptomatology.

**Table 2. Clinical trials on HERV-K and HERV-W expression**

| DISEASE | CLINICAL TRIAL | DRUGS | TYPE | HERV | MECHANISM OF ACTION | RESULTS | RESPONSIBLE PARTY |
|---|---|---|---|---|---|---|---|
| **ALS** | NCT02868580 | Triumeq (dolutegravir, abacavir, lamivudine) | Small molecule | HERV-K | Dolutegavir: HIV-1 integrase inhibitor<br>Abacavir: carbocyclic nucleoside with potent selective anti-HIV activity.<br>Lamivudine: reverse transcriptase inhibitor | Safe and well tolerated. Favorable response on HERV-K expression levels. | Neuroscience Trials Australia |
| | NCT02437110 | Darunavir, ritonavir, dolutegravir, tenofovir alafenamide (TAF) | Small molecule | HERV-K | Darunavir: HIV protease inhibitor<br>Ritonavir: HIV protease inhibitor<br>Dolutegravir: HIV integrase inhibitor<br>TAF: reverse transcriptase inhibitor | Not available | National Institute of Neurological Disorders and Stroke (NINDS) |
| **MS** | NCT02782858 | Temelimab (GNbAC1) | Biological | HERV-W | Humanised IgG4 monoclonal antibody that targets the envelope protein (Env) of the human endogenous multiple sclerosis-associated retrovirus (HERV-W) | Safe and well tolerated. Favorable response on HERV-W expression levels | GeNeuro SA |
| | NCT03239860 | Temelimab (GNbAC1) | Biological | HERV-W | | Reduction in brain atrophy of 40% under 18 mg/kg GNbAC1 and remyelinating effects | GeNeuro SA |
| | NCT04480307 | Temelimab (GNbAC1) | Biological | HERV-W | | Not available | GeNeuro SA |