# Targeting Epstein–Barr virus in multiple sclerosis: when and how?

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**Purpose of review**
Epidemiological evidence implicates Epstein–Barr virus (EBV) as the cause of multiple sclerosis (MS). However, its biological role in the pathogenesis of MS is uncertain. The article provides an overview of the role of EBV in the pathogenesis of MS and makes a case for targeting EBV as a treatment strategy for MS.

**Recent findings**
EBV potentially triggers autoimmunity via molecular mimicry or immune dysregulation. Another hypothesis, supported by immunological and virological data, indicates that active EBV infection via latent-lytic infection cycling within the central nervous system or periphery drives MS disease activity. This supports testing small molecule anti-EBV agents targeting both latent and lytic infection, central nervous system-penetrant B-cell therapies and EBV-targeted immunotherapies in MS. Immunotherapies may include EBV-specific cytotoxic or chimeric antigen receptors T-cells, therapeutic EBV vaccines and immune reconstitution therapies to boost endogenous EBV-targeted cytotoxic T-cell responses.

**Summary**
EBV is the probable cause of MS and is likely to be driving MS disease activity via latent-lytic infection cycling. There is evidence that all licensed MS disease-modifying therapies target EBV, and there is a compelling case for testing other anti-EBV strategies as potential treatments for MS.

**Keywords**
CD19-targeted CAR T-cells, Epstein–Barr virus antiviral drugs, Epstein–Barr virus vaccine, Epstein–Barr virus-targeted immunotherapies, Epstein–Barr virus, latent-lytic cycling

## INTRODUCTION

Epstein–Barr virus (EBV) plays a causal role in multiple sclerosis (MS). EBV is necessary but insufficient for someone to develop MS [1▪,2]. EBV seronegative people are largely protected from developing MS [3]; if they develop MS in the future, they seroconvert before the clinical onset of MS [4▪▪]. In this large cohort study of young adults in the US military, the risk of MS increased 32-fold after infection with EBV. In comparison, the risk was unchanged after infection with other viruses, including cytomegalovirus (CMV), a closely related herpes virus with similar patterns of transmission [4▪▪]. Only one out of 801 MS cases with samples available to assess EBV infection was EBV-negative. Thirty-five people who were subsequently diagnosed with MS and 107 controls were EBV-negative at baseline, and all but one of these 35 cases seroconverted during the follow-up, and all did so before the onset of MS [4▪▪]. In the MS cases, blood neurofilament light chain levels, a marker of neurodegeneration [5], increased in study subjects, but only after EBV infection, indicating likely presymptomatic MS disease onset [4▪▪]. These findings confirm the temporal link and further strengthen the direct involvement of EBV in the causal chain between EBV infection and MS. These observations underpin the need to explore EBV vaccination to try and prevent primary EBV infection, or at least infectious mononucleosis, and the subsequent development of MS [1▪,2]. Despite this evidence that EBV is the likely cause of MS, it is unknown how EBV causes MS biologically. Current hypotheses include molecular mimicry, immune dysregulation, active EBV infection, and the activation of human endogenous retroviruses (HERVs). Whether EBV triggers MS and then does not play a further role in disease pathogenesis (hit-and-run

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## KEY POINTS

- Epstein–Barr virus (EBV) is necessary but not sufficient to develop multiple sclerosis.
- An EBV vaccine to prevent asymptomatic or symptomatic EBV infection (infectious mononucleosis) will be explored as an multiple sclerosis (MS) prevention strategy.
- How EBV causes MS is at present unknown.
- Immunological and virological evidence suggests that latent-lytic cycling of EBV may be driving MS disease activity, supporting a case for testing EBV antivirals as a treatment for MS.

hypothesis) or is causing the disease process via active infection (the driver hypothesis) is unknown [6]. In addition, the pathogenic site of EBV latent-lytic cycling is unknown (Fig. 1). Despite the uncertainty about how EBV causes MS, several observations support the need for targeting EBV as a potential treatment for MS both in the periphery or systemic compartment and within the central nervous system (CNS) and meninges (Fig. 1).

## MOLECULAR MIMICRY AND IMMUNE DYSREGULATION

Molecular mimicry as the causal mechanism is favoured by immunologists to explain how EBV causes MS [7–10] (Fig. 2). Antigens from EBV result in cross-reactive immune responses to self-antigens that trigger MS. Multiple autoantigens have been shown to have cross-reactive immune responses between putative autoantigens and EBV proteins [7–10]. The latest autoantigens to be identified include proteolipid protein (PLP) [11] and glialCAM [12]. However, many of the proposed autoantigens are expressed outside of the central nervous system (CNS), are intracellular, and several have sequences homologous with other viruses that are not associated with MS, which questions their specific role as an MS trigger.

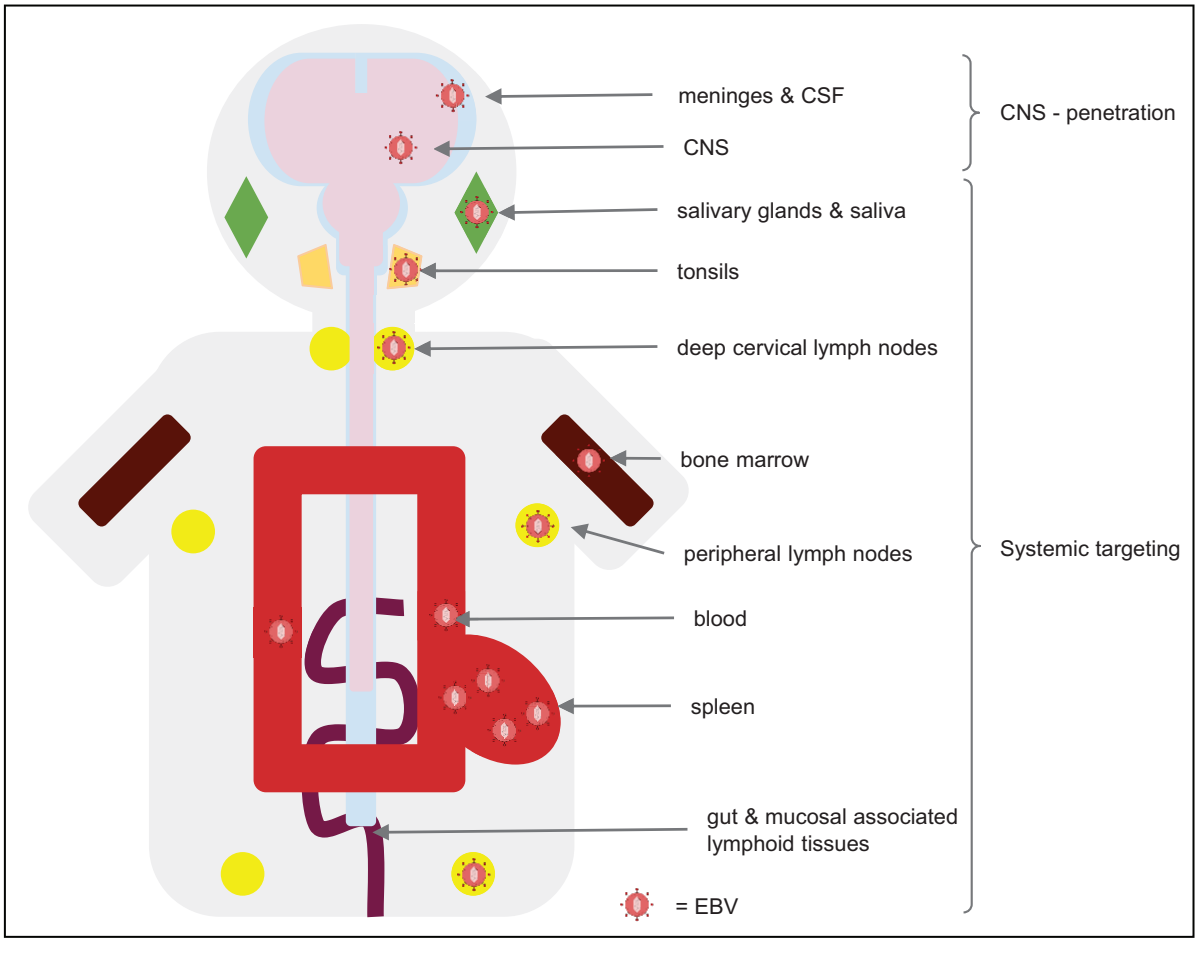


**FIGURE 1.** A cartoon illustrating potential sites where EBV latent-lytic infections cycling may be driving MS disease activity. CNS penetrant strategies for central nervous system (CNS) involvement will be required. EBV, Epstein–Barr virus; MS, multiple sclerosis.

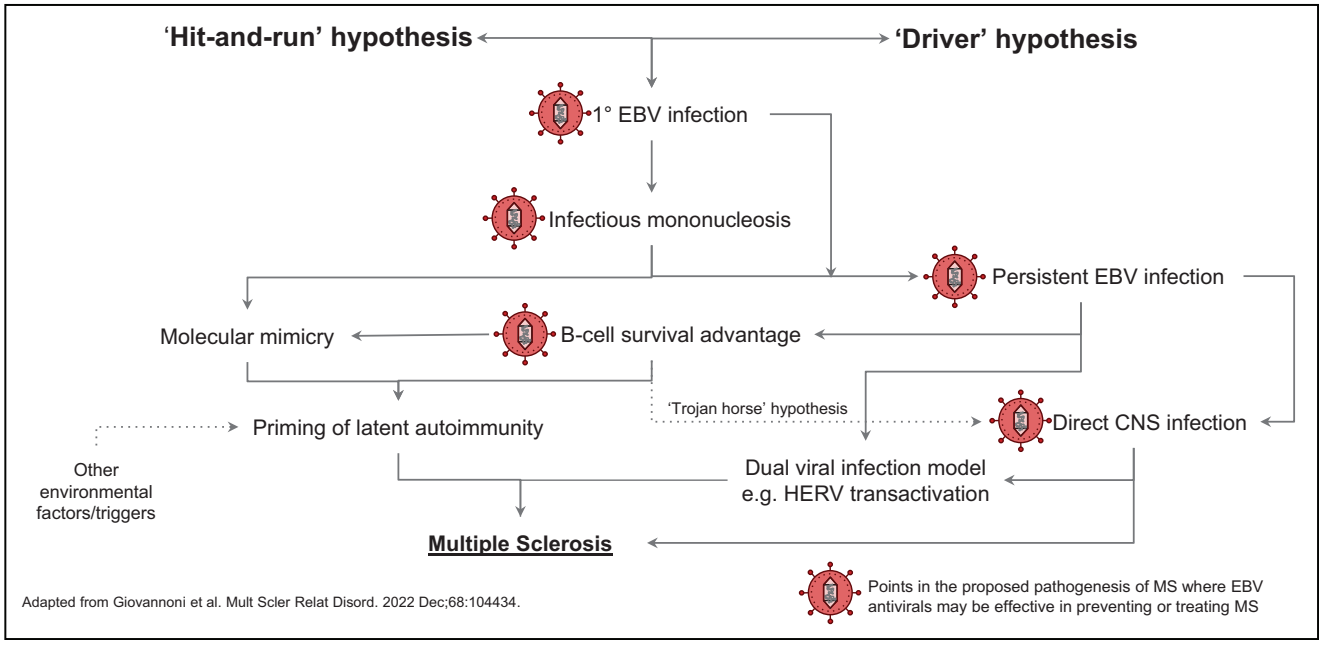


**FIGURE 2.** A simple schematic of the potential role EBV plays in the pathogenesis of MS and at which points EBV latent-lytic cycling may be targeted with EBV antivirals to prevent or treat MS. Please note that if EBV is triggering MS disease activity in the CNS, then CNS-penetrant antivirals or EBV immunotherapies will be required. This figure has been adapted from a previous figure in an MSARD editorial commentary [6]. CAR, chimeric antigen receptors; CNS, central nervous system; EBV, Epstein–Barr virus; MS, multiple sclerosis.

EBV infection may lead to immune dysregulation, which sets the stage for the later development of autoimmunity (Fig. 2). For example, latent EBV infection provides infected B-cells with a survival advantage, which allows autoreactive B-cells to escape immune tolerance. In support of this argument is the association of EBV with several autoimmune diseases and MS [13,14].

## ONGOING EPSTEIN–BARR VIRUS INFECTION

Molecular mimicry and immune dysregulation imply that EBV acts via a hit-and-run event; i.e. once autoimmunity is primed, targeting EBV will not impact the course of MS (Fig. 2). This may not necessarily be correct. Latent-lytic infection cycling of EBV will intermittently expose the immune system to EBV antigens, which may drive molecular mimicry via antigenic spread or maintain the immune system in a dysregulated state. EBV may drive MS activity by cycling through its latent and lytic infection phases [15] directly with the CNS, by intermittently stimulating autoreactive T and B cells, or potentially by upregulating MS-associated HERVs [16], which in turn acting downstream of EBV cause tissue damage.

## IMMUNOLOGY AND VIROLOGY OF EPSTEIN–BARR VIRUS IN MULTIPLE SCLEROSIS

Subjects with MS have higher titres and broader antibody repertoires to both latent [17] and lytic [18] EBV proteins. In addition to elevated antibody titres against the EBNA complex [17], people with MS have more EBNA1 reactive $CD4^+$ T-cells [19] and a more expansive repertoire of T-cells reacting against epitopes distributed across the EBNA-1 protein [19]. In comparison, T-cells from healthy controls only react to the immunodominant portion of the protein [20]. This suggests patients with MS have a problem controlling EBV in its latent state.

A recent *in silico* study showed that pwMS have more T-cell receptors that recognise EBNA-1 and a broader array of epitopes in the EBNA1 protein than control subjects [19,21]. Identical twins concordant for having MS had a more expansive T-cell receptor repertoire than twins discordant for the disease [21]. Data from twins avoid confounding by genetic factors and imply that these results are likely to be due to having MS. In subjects without MS, blood and cerebrospinal fluid (CSF) derived EBV-specific T cells consist mainly of effector-memory cells [21]. In comparison, the cerebrospinal fluid also contained EBV-specific central-memory T cells in patients with MS [21]. This would imply these cells had recently

been exposed to EBV antigens, which in the case of cells in the CSF is likely to have taken place within the CNS of patients with MS.

The observation that patients with MS are more likely to generate spontaneous EBV-associated lymphoblastoid cell lines (LCLs) from peripheral blood B cells [22–24] and that both children and adults shed EBV in their saliva more frequently than control subjects [25,26], indicates that patients with MS control EBV poorly. These immunological and viral data support testing peripheral and CNS-penetrant anti-EBV strategies in MS. This includes EBV-specific antiviral agents, immunotherapies and therapies directed at CNS resident memory [27].

## THE CASE FOR TARGETING B-CELLS

The memory B-cell is the reservoir for the latent EBV virus. Therefore, targeting memory B-cells will affect EBV biology. The observation that most licensed MS disease-modifying therapies (DMTs) either reduce or stop memory B-cells from trafficking into the CNS [28–31] supports this premise. Interestingly, atacicept [32] binds to and neutralises BAFF and APRIL B-cell survival factors, and infliximab and lenercept, anti-TNF-alpha therapies [33], increase MS disease activity. Both these treatment classes have been shown to expand peripheral memory B-cell numbers [28], support the memory B-cell as the cell driving MS disease activity and help explain some of the treatment effects of DMTs in MS.

## EPSTEIN–BARR VIRUS ANTIVIRALS

Whether we need to target lytic EBV infection, latent EBV infection, or both and in which compartment, the CNS, peripheral compartment, or both, is unknown. I predict the need to target latent and lytic EBV infection with agents working in the periphery and CNS. Please see Table 1 for potential EBV antiviral agents for treating MS.

## ELIMINATING EPSTEIN–BARR VIRUS

One therapeutic strategy is eliminating EBV from the body by depleting latently infected B-cells or at least reducing the EBV viral load. This can be done using cell-depleting therapies such as alemtuzumab or cladribine, B-cell-directed depleting monoclonal antibodies such as anti-CD20 and anti-CD19 or small molecules. The latter potentially include EBNA1 inhibitors [34], DNA hypomethylating agents that inhibit DNA methyltransferases (DNMTs) or histone deacetylases (HDAC) inhibitors in combination with an EBV antiviral. An example of an HDAC inhibitor in combination with an EBV antiviral is nanatinostat or chidamide used with a nucleoside antiviral such as valganciclovir or tenofovir, respectively [35,36]. DNMTs, such as decitabine, induce the synthesis of EBV antigens, including LMP1, EBNA2, and EBNA3C, which could theoretically sensitise cells to lysis by EBV- specific cytotoxic T-lymphocytes (CTLs) [37]. Therefore, DNMTs could be used in combination with cell-based EBV-targeted immunotherapies. This strategy is similar to what has been proposed for HIV, using a treatment to induce the expression of latent virus, which then allows the immune system to eliminate infected cells [38]. EBNA1, HDAC, and DNMT inhibitors have not been tried in MS.

## ANTI-CD20, ANTI-CD19, BISPECIFIC AND CENTRAL NERVOUS SUSTEM-PENETRANT MONOCLONAL ANTIBODY THERAPIES

Rituximab is widely used for EBV-associated lymphoproliferative disease and rapidly reduces peripheral EBV viral loads [39]. However, rituximab does not prevent EBV from being shed in the saliva [40]. This implies that an EBV viral pool will persist in the case of anti-CD20 and likely anti-CD19 targeted therapies. Hence, there is a need for combination or sequential EBV-specific antiviral therapies to prevent reinfection of the repopulating B-cell pool. This supports using an anti-CD20 or anti-CD19 monoclonal antibody as induction therapy followed by a maintenance antiviral therapy to prevent the reinfection of the reconstituted B-cell population. Sequential agents with anti-EBV effects could include dihydroorotate dehydrogenase (DHODH) inhibitors such as teriflunomide, leflunomide and vidofludimus [41], HAART (highly active antiretrovirals), such as tenofovir [42▪], famciclovir [43,44] or other antiviral agents [44]. Another advantage of using an induction-maintenance or sequential treatment strategy is that chronic immunosuppression associated with long-term B-cell depletion, particularly the development of hypogammaglobulinemia, can be prevented.

Many antiviral drugs do not penetrate the CNS, so there is a significant unmet need for further development of EBV antivirals for testing in MS. More potent B-cell-depleting monoclonal antibodies are being developed, including bispecific antibodies targeting CD20 and CD3 antibodies, for example, mosunetuzumab and glofitamab, which are proving to be more effective than anti-CD20 therapies in refractory B-cell lymphomas [45,46]. If CNS EBV infection drives MS disease activity, sufficient doses of anti-CD20 therapies are unlikely to penetrate the CNS to clear this compartment of EBV. Developing CNS penetrating anti-CD20 or anti-CD19 antibodies using the transferrin receptor

**Table 1.** Potential EBV targeted therapies

| Anti-EBV strategy | Class of therapy | Agents | Comment |
|---|---|---|---|
| B-cell therapies targeting latent infection | | | |
| | Selective B-cell-depleting monoclonal antibodies | • Anti-CD20 (rituximab, ocrelizumab, ofatumumab, ublituximab, ...)<br>• anti-CD19 (inebilizumab)<br>• bispecific monoclonal anti-CD20/CD3 antibodies (mosunetuzumab, glofitamab)<br>• brain shuttle CD20 inhibitor (RG6035) | Selective depletion of peripheral blood B-cells, variable depletion of deep tissue B-cells and unlikely to clear CNS resident pathogenic B-cells<br>Nonselective and depletes all B-cells regardless of EBV status |
| | Immune reconstitution therapies | mitoxantrone, alemtuzumab, cladribine, AHSCT, ..... | Nonselective peripheral lymphocyte depletion, including the B-cell population. Non-CNS penetrant except for cladribine<br>Hypothesised that EBV-targeted cytotoxic T-lymphocyte responses may be rejuvenated postimmune reconstitution |
| | CD19 targeted CAR-T cells | axicabtagene ciloleucel, tisagenlecleucel, lisocabtagene maraleucel, brexucabtagene autoleucel, ... | Selective depletion of CD19 expressing B-cells and plasmablasts. Based on results in refractory lymphoma, this strategy will have good deep tissue and CNS penetration. It is likely to be more effective than B-cell-targeted monoclonal antibodies<br>Clinical trials have been announced in multiple sclerosis |
| | EBNA1 inhibitors | VK2019, peptide inhibitors (JLP2 and L2P4), ... | Will target both latent and lytic-infected B-cells. A good theoretical target, but have yet to be tested in autoimmune diseases and multiple sclerosis. CNS penetration may be necessary |
| | HDAC (histone deacetylases) inhibitors | nantinostat, chidamide, .... | HDAC inhibitors drive latent EBV to become lytic and will need to be in combination with antivirals targeting lytic infection. This class of therapies have yet to be tried in autoimmune diseases |
| | DNMT (DNA methyltransferases) inhibitors | decitabine | DNMT inhibitors induce the synthesis of LMP1, EBNA2, and EBNA3C. They could theoretically sensitise cells to lysis by EBV-specific cytotoxic T-lymphocytes (CTLs). May need to be used in combination with EBV-targeted immunotherapies. This class of therapies have yet to be tried in autoimmune diseases. |
| | Bruton tyrosine kinase (BTK) inhibitors | evobrutinib, tolebrutinib, fenebrutinb, remibrutinib, orelabrutinib, GB7208, ... | Ibrutinib, a first-generation BTK inhibitor, has been shown to reduce EBV viral loads *in vivo* and *in vitro*. This is likely a class effect as EBV's LMP2a signals via BTK to bypass B-cell receptor signalling, providing a pro-survival signal to EBV-infected B-cells |
| Antivirals targeting lytic infection | | | |
| | DNA polymerase inhibitors | acyclovir/valacyclovir, penciclovir/famciclovir, ganciclovir/valganciclovir, omaciclovir/valomaciclovir, cidofovir/brincidofovir, cyclopropavir, foscarnet ... | Trial results from first-generation viral DNA polymerase inhibitors were negative, with moderate activity against EBV. However, a good case exists for testing newer, more effective DNA polymerase inhibitors in MS and other autoimmune diseases |
| | Antiretrovirals | zidovudine, tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), ... | Anecdotal evidence shows that this class of therapy may be effective as a disease-modifying therapy in MS. This, with *in vitro* data showing that these agents have anti-EBV activity, has catalysed some exploratory studies in MS |
| | Other antivirals | artesunate, maribavir, L-dioxolane thymidine derivatives (KAY-2–41 and KAH-39–149), teriflunomide/leflunomide and other dihydroorotate dehydrogenase inhibitors, ... | There are a large number of other potential small molecule drugs that are potentially active against EBV that have the potential to be tried in multiple sclerosis |
| | Monoclonal antibodies | anti-GP350, anti-GP350/CD89, ... | EBV antigen-specific neutralising monoclonal antibodies targeting EBV lytic infection are unlikely to prevent EBV viral reactivation but will likely prevent reinfection of naive EBV-negative B cells. Therefore, this strategy may need to be combined with other anti-EBV targeted therapies, such as an induction-maintenance strategy |

**Table 1** (*Continued*)

| Anti-EBV strategy | Class of therapy | Agents | Comment |
|---|---|---|---|
| EBV immunotherapies targeting latent and lytic infection | | | |
| | Autologous and allogeneic EBV-targeted T-cell therapies | tabelecleucel, ATA188, … | These cellular therapies are based on preliminary open-label studies of autologous EBV-specific cytotoxic T-cells. Please note a phase 2 trial of ATA188 in progressive MS was negative |
| | Therapeutic EBV vaccine | mRNA component vaccines | Based on the theory of a dysfunctional or senescent EBV-specific cytotoxic T-cell response results in poor control of EBV in patients with MS. Vaccines covering both latent and lytic EBV antigens will boost anti-EBV immunity and potentially controlling the virus<br>There is a theoretical risk that add EBV therapeutic vaccination may trigger MS disease activity via molecular mimicry |
| | EBV-antigen-targeted CAR-T cells | GP350 and EBNA-1 | Using EBV antigen-specific CAR T-cells will likely require EBV antigens to be expressed on the surface of infected cells. Because latent EBV proteins are intracellular antigens and EBV's role in driving MS is likely to be intermittent lytic re-activation, this strategy is unlikely to be effective unless autologous EBV-targeted CAR T-cells persist *in vivo*. The current evidence suggests that CAR T-cells do not persist long-term |

CAR, chimeric antigen receptors; CNS, central nervous system; EBV, Epstein–Barr virus; MS, multiple sclerosis.

shuttle to get higher doses of antibody across the blood-brain barrier is one potential solution to this problem [47–49].

## ANTIVIRALS TARGETING LYTIC INFECTION

Acyclovir and the prodrug valacyclovir were unsuccessful in small controlled MS trials [50,51]. There have been no trials of famciclovir, ganciclovir, omaciclovir, valganciclovir, cidofovir, brincidofovir, cyclopropavir, artesunate, tenofovir, maribavir or foscarnet targeting EBV in MS. Maribavir, which is licensed for CMV infection, has better activity against EBV than acyclovir, cidofovir, ganciclovir and foscarnet, but it has also not yet been tested against EBV [52]. The observation that HIV-positive people with co-incident MS who are started on highly active antiretroviral therapy (HAART) do well [53–57] and that having HIV and being on HAART protects people from developing MS [58▪,59,60] has raised the possibility of whether or not antiretroviral therapies may be effective treatments for MS. This is particularly relevant for zidovudine [56], tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) [42▪], which are effective against EBV lytic infection *in vitro*. As a result, two clinical trials will be exploring these agents as potential anti-EBV agents in patients with MS: tenofovir disoproxil fumarate (TDF) (ClinicalTrials.gov ID: NCT05957913) in the US and tenofovir alafenamide (TAF) in Norway (personal communication Øivind Torkildsen, Norway).

## EBNA1 INHIBITORS

Small molecule inhibitors of the DNA binding activity of EBV nuclear antigen 1 (EBNA1) have been developed. The efficacy of VK2019, an EBNA1 inhibitor, has been studied in EBV-dependent xenograft models [34,61] and is currently being evaluated in patients with EBV-associated nasopharyngeal carcinoma (ClinicalTrials.gov Identifier: NCT04925544). Another approach uses peptide inhibitors to disrupt the EBNA1 [62]. Modified versions of this peptide inhibitor have been shown to induce the EBV lytic cycle, thereby rendering infected cells more susceptible to T cell recognition [63]. EBNA-1 inhibitors need to be tried in MS.

## EPSTEIN–BARR VIRUS IMMUNOTHERAPIES

### Epstein–Barr virus reactive T-cell therapies

Patients with MS are reported to have exhausted EBV-targeted T-cell responses, particularly the $CD8^+$ CTL response, which leads to poor EBV control [64,65]. EBV-targeted immunotherapies have, therefore, been proposed as a treatment for MS. The adoptive transfer of in ex vivo expanded autologous EBV-specific $CD8^+$ CTLs directed against viral latent proteins has been tried. In an open-label trial of patients with progressive MS who received escalating doses of ex vivo expanded autologous EBV-specific CTLs targeting EBNA1, LMP1 and LMP2A, 7/10 showed

improvement [66]. This approach was further explored using adoptively transferred partially MHC-matched allogenic EBV-specific T-cells (Atara Bio, Tevogen) [67] (clinicaltrials.gov NCT03283826). Unfortunately, a phase 2 trial using allogeneic EBV targeting CTLs in nonactive progressive MS was negative [68]. Whether this trial failed because of the poor survival of these allogeneic cells or poor trial design is a moot point [69].

### Therapeutic Epstein–Barr virus vaccination

Another immunotherapy approach is vaccination to boost the immune response to EBV using mRNA vaccines (Moderna). Immune reconstitution therapies like autologous haematopoietic stem cell transplantation (AHSCT) or alemtuzumab may work similarly. The depletion-reconstitution cycle of an IRT may work by reactivating EBV that stimulates exhausted and/or generates new EBV-specific CTL responses (Pender *et al.*, 2017 [64]), which then suppress EBV immunologically [70]. Almost all MS patients undergoing AHSCT develop detectable EBV viraemia in the peripheral blood, supporting this potential mechanism of action of AHSCT [71].

### Anti- Epstein–Barr virus-targeted monoclonal antibodies

Therapeutic antibody therapies came of age during the COVID-19 pandemic, with several antibodies targeting the SARS-CoV-2 envelope protein being licensed to prevent serious COVID-19 [72]. A similar approach is being explored to target EBV. For example, the humanised antigp350 neutralising monoclonal antibody 72a1, targeting a critical EBV envelope protein required for cell fusion, prevents *in vitro* EBV infection of B cells [73]. Similarly, a bispecific antibody targeting the EBV's envelope protein gp350, paired with a monoclonal antibody against CD89 or FcαRI for redirecting macrophages and neutrophils, enables phagocytosis of EBV and killing of gp350 + lymphoma cells in the presence of mononuclear cells [74]. The bispecific antibody dramatically reduces the viral load in blood, solid organs and tissues of treated mice [74]. These monoclonal antibodies constitute a starting point for developing anti-EBV therapeutic antibodies for EBV-associated diseases, including MS.

### Chimeric antigen receptors T cells

The report of successfully using CD19-targeted chimeric antigen receptors (CAR) T cells in patients with treatment-resistant SLE (systemic lupus erythematosus) [75] has generated interest in using this strategy to treat MS. Autologous CAR T cells are T-cells harvested from an individual's peripheral blood, genetically engineered outside the body to express an artificial T cell receptor that targets a self- or onco-antigen, in this case, CD19 that is expressed on B-cells. Once expanded, these CD19-targeted T-cells are reinfused into the patient after the patient has had a cycle of immunodepleting therapy [76]. CAR T-cells then find CD19-expressing cells and kill them. CD19-targeting CAR T cells will have an advantage over anti-CD19 or anti-CD20 monoclonal antibody therapy because cells penetrate the deep tissues and the central nervous system and kill B-cells in the brain, spinal cord and meninges. The latter is supported by the recent success of using CD19-targeted T-cells to treat EBV-associated B-cell lymphomas in the CNS [77]. It has recently been announced that two CD19-targeted CAR T-cell trials in MS will start.

## CONCLUSION

In conclusion, from an epidemiological perspective, EBV's role in the pathogenesis of MS is likely to be causal. EBV vaccines for primary prevention are being planned to try and prevent MS. In parallel, immunological, clinical and pathological studies suggest that EBV may drive MS disease activity through latent lytic infection cycling. Therefore, there is a strong case for testing anti-EBV strategies as a treatment for MS. This includes CNS penetrant small molecule antiviral agents, B-cell targeting therapies and EBV-targeted immunotherapy. Importantly, all licensed MS disease-modifying therapies may work via mechanisms targeting EBV.

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## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest


1. Aloisi F, Giovannoni G, Salvetti M. Epstein–Barr virus as a cause of multiple
■ sclerosis: opportunities for prevention and therapy. Lancet Neurol 2023; 22:338–349.

A state-of-the-art review on the role of EBV plays in the causal pathway that leads to the development of multiple sclerosis.

2. Giovannoni G, Hawkes CH, Lechner-Scott J, *et al.* Is EBV the cause of multiple sclerosis? Mult Scler Relat Disord 2022; 58:103636.
3. Pakpoor J, Disanto G, Gerber JE, *et al.* The risk of developing multiple sclerosis in individuals seronegative for Epstein–Barr virus: a meta-analysis. Mult Scler 2013; 19:162–166.
4. Bjornevik K, Cortese M, Healy BC, *et al.* Longitudinal analysis reveals high
■■ prevalence of Epstein–Barr virus associated with multiple sclerosis. Science 2022; 375:296–301.

A landmark study shows people have to be infected with EBV before developing MS. The finding is specific to EBV and not other viruses. This temporal sequence is critical for proving EBV is the cause of MS.

5. Giovannoni G. Peripheral blood neurofilament light chain levels: the neurologist's C-reactive protein? Brain 2018; 141:2235–2237.
6. Giovannoni G, Hawkes CH, Lechner-Scott J, *et al.* What are T-cells telling us about how EBV causes MS? Mult Scler Relat Disord 2022; 68:104434.
7. Lanz TV, Robinson WH, Ho PP, Steinman L. Roadmap for understanding mechanisms on how Epstein–Barr virus triggers multiple sclerosis and for translating these discoveries in clinical trials. Clin Transl Immunol 2023; 12: e1438.
8. Läderach F, Münz C. Altered immune response to the Epstein–Barr virus as a prerequisite for multiple sclerosis. Cells 2022; 11:2757.
9. Sospedra M, Martin R. Molecular mimicry in multiple sclerosis. Autoimmunity 2006; 39:127–147.
10. Martin R, Sospedra M, Eiermann T, Olsson T. Multiple sclerosis: doubling down on MHC. Trends Genet 2021; 37:784–797.
11. Owens GP, Fellin TJ, Matschulat A, *et al.* Pathogenic myelin specific antibodies in multiple sclerosis target conformational proteolipid protein 1 anchored membrane domains. J Clin Invest 2023; 133:e162731.
12. Lanz TV, Brewer RC, Ho PP, *et al.* Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. Nature 2022; 603:321–327.
13. Houen G, Trier NH. Epstein–Barr virus and systemic autoimmune diseases. Front Immunol 2020; 11:587380.
14. Jog NR, James JA. Epstein–Barr virus and autoimmune responses in systemic lupus erythematosus. Front Immunol 2020; 11:623944.
15. Giovannoni G, Vanderdonckt P, Hartung H-P, *et al.* EBV and multiple sclerosis: setting the research agenda. Mult Scler Relat Disord 2022; 67:104158.
16. Küry P, Nath A, Créange A, *et al.* Human endogenous retroviruses in neurological diseases. Trends Mol Med 2018; 24:379–394.
17. Levin LI, Munger KL, Rubertone MV, *et al.* Temporal relationship between elevation of Epstein–Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 2005; 293:2496–2500.
18. Persson Berg L, Eriksson M, Longhi S, *et al.* Serum IgG levels to Epstein–Barr and measles viruses in patients with multiple sclerosis during natalizumab and interferon beta treatment. BMJ Neurol Open 2022; 4:e000271.
19. Lünemann JD, Edwards N, Muraro PA, *et al.* Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. Brain 2006; 129:1493–1506.
20. Ruprecht K, Wunderlich B, Gieß R, *et al.* Multiple sclerosis: the elevated antibody response to Epstei−-Barr virus primarily targets, but is not confined to, the glycine-alanine repeat of Epstein–Barr nuclear antigen-1. J Neuroimmunol 2014; 272:56–61.
21. Schneider-Hohendorf T, Gerdes LA, Pignolet B, *et al.* Broader Epstein–Barr virus-specific T cell receptor repertoire in patients with multiple sclerosis. J Exp Med 2022; 219:e20220650.
22. Fraser KB, Haire M, Millar JH, McCrea S. Increased tendency to spontaneous in-vitro lymphocyte transformation in clinically active multiple sclerosis. Lancet 1979; 2:175–176.
23. Munch M, Møller-Larsen A, Christensen T, *et al.* B-lymphoblastoid cell lines from multiple sclerosis patients and a healthy control producing a putative new human retrovirus and Epstein–Barr virus. Mult Scler 1995; 1:78–81.
24. Tørring C, Andreasen C, Gehr N, *et al.* Higher incidence of Epstein–Barr virus-induced lymphocyte transformation in multiple sclerosis. Acta Neurol Scand 2014; 130:90–96.
25. Holden DW, Gold J, Hawkes CH, *et al.* Epstein Barr virus shedding in multiple sclerosis: similar frequencies of EBV in saliva across separate patient cohorts. Mult Scler Relat Disord 2018; 25:197–199.
26. Yea C, Tellier R, Chong P, *et al.,* Canadian Pediatric Demyelinating Disease Network. Epstein–Barr virus in oral shedding of children with multiple sclerosis. Neurology 2013; 81:1392–1399.
27. Baker D, Jacobs BM, Gnanapavan S, *et al.* Plasma cell and B cell-targeted treatments for use in advanced multiple sclerosis. Mult Scler Relat Disord 2019; 35:19–25.
28. Baker D, Marta M, Pryce G, *et al.* Memory B cells are major targets for effective immunotherapy in relapsing multiple sclerosis. EBioMedicine 2017; 16:41–50.
29. Baker D, Pryce G, Amor S, *et al.* Learning from other autoimmunities to understand targeting of B cells to control multiple sclerosis. Brain 2018; 141:2834–2847.
30. Baker D, Pryce G, James LK, *et al.* Failed B cell survival factor trials support the importance of memory B cells in multiple sclerosis. Eur J Neurol 2020; 27:221–228.
31. Ceronie B, Jacobs BM, Baker D, *et al.* Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells. J Neurol 2018; 265:1199–1209.
32. Kappos L, Hartung H-P, Freedman MS, *et al.* Atacicept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Neurol 2014; 13:353–363.
33. Paty DW, Li DKB; the UBC MS/MRI Study Group, and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple-sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 2011; 77:1431–11431.
34. Messick TE, Smith GR, Soldan SS, *et al.* Structure-based design of small-molecule inhibitors of EBNA1 DNA binding blocks Epstein–Barr virus latent infection and tumor growth. Sci Transl Med 2019; 11:eaau5612.
35. Haverkos B, Alpdogan O, Baiocchi R, *et al.* Targeted therapy with nanatinostat and valganciclovir in recurrent EBV-positive lymphoid malignancies: a phase 1b/2 study. Blood Adv 2023; 7:6339–6350.
36. Xu L, Zhang M, Tu D, *et al.* Chidamide induces Epstein–Barr Virus (EBV) lytic infection and acts synergistically with tenofovir to eliminate EBV-positive Burkitt lymphoma. J Pharmacol Exp Ther 2023; 387:288–298.
37. Dalton T, Doubrovina E, Pankov D, *et al.* Epigenetic reprogramming sensitizes immunologically silent EBV+ lymphomas to virus-directed immunotherapy. Blood 2020; 135:1870–1881.
38. Qi J, Ding C, Jiang X, Gao Y. Advances in developing CAR T-cell therapy for HIV cure. Front Immunol 2020; 11:361.
39. Rouce RH, Louis CU, Heslop HE. Epstein–Barr virus lymphoproliferative disease after hematopoietic stem cell transplant. Curr Opin Hematol 2014; 21:476–481.
40. Hoover SE, Kawada J, Wilson W, Cohen JI. Oropharyngeal shedding of Epstein–Barr virus in the absence of circulating B cells. J Infect Dis 2008; 198:318–323.
41. Zheng Y, Li S, Song K, *et al.* A broad antiviral strategy: inhibitors of human DHODH pave the way for host-targeting antivirals against emerging and re-emerging viruses. Viruses 2022; 14:928.
42. Drosu NC, Edelman ER, Housman DE. Tenofovir prodrugs potently inhibit
■ Epstein–Barr virus lytic DNA replication by targeting the viral DNA polymerase. Proc Natl Acad Sci USA 2020; 117:12368–12374.

Critical study showing some antiretroviral drugs are effective against EBV.

43. Goldani LZ. Treatment of severe infectious mononucleosis with famciclovir. J Infect 2002; 44:92–93.
44. Rafailidis PI, Mavros MN, Kapaskelis A, Falagas ME. Antiviral treatment for severe EBV infections in apparently immunocompetent patients. J Clin Virol 2010; 49:151–157.
45. Dickinson MJ, Carlo-Stella C, Morschhauser F, *et al.* Glofitamab for relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2022; 387:2220–2231.
46. Lucijanic M, Sabljic A, Vlasac Glasnovic J, *et al.* Heavily pretreated patient with nodal marginal zone lymphoma successfully treated with mosunetuzumab. Ann Hematol 2023; 103:1015–1016.
47. Walsh FS, Wicher K, Szary J, *et al.* Abstract 3631: delivery of a CD20 transferrin receptor VNAR bispecific antibody to the brain for CNS lymphoma. Cancer Res 2017; 77:3631–13631.
48. Daniels-Wells TR, Widney DP, Leoh LS, *et al.* Efficacy of an antitransferrin receptor 1 antibody against aids-related non-Hodgkin lymphoma: a brief communication. J Immunother 2015; 38:307–310.
49. Genentech website. RG6035, RO7121932 Brain Shuttle CD20 inhibitor. https://www.gene.com/medical-professionals/pipeline/rg6035-ro7121932-brain-shuttle-cd20-inhibitor (Accessed 18-March-2024).
50. Lycke J, Svennerholm B, Hjelmquist E, *et al.* Acyclovir treatment of relapsing-remitting multiple sclerosis. A randomized, placebo-controlled, double-blind study. J Neurol 1996; 243:214–224.
51. Friedman JE, Zabriskie JB, Plank C, *et al.* A randomized clinical trial of valacyclovir in multiple sclerosis. Mult Scler 2005; 11:286–295.
52. Chemaly RF, Hill JA, Voigt S, Peggs KS. In vitro comparison of currently available and investigational antiviral agents against pathogenic human double-stranded DNA viruses: a systematic literature review. Antiviral Res 2019; 163:50–58.
53. Maruszak H, Brew BJ, Giovannoni G, Gold J. Could antiretroviral drugs be effective in multiple sclerosis? A case report. Eur J Neurol 2011; 18:e110–e111.
54. Labella F, Acebrón F, Blanco-Valero MDC, *et al.* HIV infection and multiple sclerosis: a case with unexpected 'no evidence of disease activity' status. J Int Med Res 2021; 49:300060521999577.

**55.** Torkildsen , Myhr K-M, Skogen V, *et al.* Tenofovir as a treatment option for multiple sclerosis. Mult Scler Relat Disord 2020; 46:102569.
**56.** Drosu NC, Edelman ER, Housman DE. Could antiretrovirals be treating EBV in MS? A case report. Mult Scler Relat Disord 2018; 22:19–21.
**57.** Chalkley J, Berger JR. Multiple sclerosis remission following antiretroviral therapy in an HIV-infected man. J Neurovirol 2014; 20:640–643.
**58.** McKay KA, Wijnands JMA, Manouchehrinia A, *et al.* Risk of multiple sclerosis in ■ people living with HIV: an international cohort study. Ann Neurol 2024; 95:487–494.
Important confirmatory study showing that having HIV protects people from developing MS.
**59.** Nexø BA, Pedersen L, Sørensen HT, Koch-Henriksen N. Treatment of HIV and risk of multiple sclerosis. Epidemiology 2013; 24:331–332.
**60.** Gold J, Goldacre R, Maruszak H, *et al.* HIV and lower risk of multiple sclerosis: beginning to unravel a mystery using a record-linked database study. J Neurol Neurosurg Psychiatry 2015; 86:9–12.
**61.** Soldan SS, Anderson EM, Frase DM, *et al.* EBNA1 inhibitors have potent and selective antitumor activity in xenograft models of Epstein–Barr virus-associated gastric cancer. Gastric Cancer 2021; 24:1076–1088.
**62.** Jiang L, Lan R, Huang T, *et al.* EBNA1-targeted probe for the imaging and growth inhibition of tumours associated with the Epstein–Barr virus. Nat Biomed Eng 2017; 1:1–10.
**63.** Jiang L, Lung HL, Huang T, *et al.* Reactivation of Epstein–Barr virus by a dual-responsive fluorescent EBNA1-targeting agent with Zn-chelating function. Proc Natl Acad Sci USA 2019; 116:26614–26624.
**64.** Pender MP, Csurhes PA, Burrows JM, Burrows SR. Defective T-cell control of Epstein-Barr virus infection in multiple sclerosis. Clin Transl Immunology 2017; 6:e126.
**65.** Pender MP, Csurhes PA, Pfluger CM, Burrows SR. Deficiency of $CD8^+$ effector memory T cells is an early and persistent feature of multiple sclerosis. Mult Scler 2014; 20:1825–1832.
**66.** Pender MP, Csurhes PA, Smith C, *et al.* Epstein–Barr virus-specific T cell therapy for progressive multiple sclerosis. JCI Insight 2018; 3:124714.
**67.** Bar-Or A, Pender MP, Khanna R, *et al.* Epstein–Barr virus in multiple sclerosis: theory and emerging immunotherapies. Trends Mol Med 2020; 26:296–310.
**68.** Giovannoni G, Hawkes CH, Lechner-Scott J, *et al.* Emboldened or not: The potential fall-out of a failed anti-EBV trial in multiple sclerosis. Mult Scler Relat Disord 2024; 81:105364.
**69.** Giovannoni G, Hawkes CH, Lechner-Scott J, *et al.* Emboldened or not: the potential fall-out of a failed anti-EBV trial in multiple sclerosis. Mult Scler Relat Disord 2023; 81:105364.
**70.** Muraro PA, Douek DC, Packer A, *et al.* Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med 2005; 201:805–816.
**71.** Nicholas RS, Rhone EE, Mariottini A, *et al.* Autologous hematopoietic stem cell transplantation in active multiple sclerosis: a real-world case series. Neurology 2021; 97:e890–e901.
**72.** Yang X. Passive antibody therapy in emerging infectious diseases. Front Med 2023; 17:1117–1134.
**73.** Tanner JE, Hu J, Alfieri C. Construction and characterization of a humanized anti-Epstein–Barr virus gp350 antibody with neutralizing activity in cell culture. Cancers 2018; 10:E112.
**74.** He H, Lei F, Huang L, *et al.* Immunotherapy of Epstein–Barr virus (EBV) infection and EBV-associated hematological diseases with gp350/CD89-targeted bispecific antibody. Biomed Pharmacother 2023; 163:114797.
**75.** Mackensen A, Müller F, Mougiakakos D, *et al.* Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med 2022; 28:2124–2132.
**76.** Ledford H. The race to supercharge cancer-fighting T cells. Nature 2023; 613:626–628.
**77.** Siddiqi T, Wang X, Blanchard MS, *et al.* CD19-directed CAR T-cell therapy for treatment of primary CNS lymphoma. Blood Adv 2021; 5:4059–4063.