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ORIGINAL RESEARCH ARTICLE

Repurposing Licensed Drugs with Activity Against Epstein–Barr Virus for Treatment of Multiple Sclerosis: A Systematic Approach

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Abstract

Background Epstein–Barr virus (EBV) is implicated as a necessary factor in the development of multiple sclerosis (MS) and may also be a driver of disease activity. Although it is not clear whether ongoing viral replication is the driver for MS pathology, MS researchers have considered the prospect of using drugs with potential efficacy against EBV in the treatment of MS. We have undertaken scientific and lived experience expert panel reviews to shortlist existing licensed therapies that could be used in later-stage clinical trials in MS.

Methods A list of therapies with anti-EBV effects was developed from existing reviews. A detailed review of pre-clinical and clinical data was undertaken to assess these candidates for potential usefulness and possible harm in MS. A 'drug-CV' and a plain language version focusing on tolerability aspects was created for each candidate. We used validated criteria to score each candidate with an international scientific panel and people living with MS.

Results A preliminary list of 11 drug candidates was generated. Following review by the scientific and lived experience expert panels, six yielded the same highest score. A further review by the expert panel shortlisted four drugs (famciclovir, tenofovir alafenamide, maribavir and spironolactone) deemed to have the best balance of efficacy, safety and tolerability for use in MS.

Conclusions Scientific and lived experience expert panel review of anti-EBV therapies selected four candidates with evidence for efficacy against EBV and acceptable safety and tolerability for potential use in phase III clinical trials for MS.

Key Points

Infection with Epstein–Barr virus (EBV) is necessary for the development of multiple sclerosis (MS).

Four antiviral drug candidates were identified for potential repurposing in clinical trials in MS.

These were selected by people with MS and scientific expert panels on the basis of safety, tolerability and evidence for activity against EBV.

Extended author information available on the last page of the article

1 Introduction

Multiple sclerosis (MS) is a complex immune-mediated, neurodegenerative condition with protean manifestations. It arises as the result of genetic and environmental factors including Epstein–Barr virus (EBV) infection, low vitamin D and/or sun exposure, smoking and obesity [1–3]. Risk of MS is associated with a history of infectious mononucleosis (IM), [4] in particular at a later age [5]. There is a linear relationship between the age at which IM develops and the age of onset of MS with a latency of 10 years [6]. Serological evidence of infection with EBV (the commonest cause of IM) is essentially universal in adults with MS, compared with 90% of the general adult population [7]. MS is associated with higher titres of antibodies to Epstein–Barr nuclear antigen 1 (EBNA1) [8]. A recent study of US military personnel confirmed the strong

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association between EBV and MS and provided arguments that the association is causal [9]. Thus, the evidence now suggests that EBV is likely 'essential, but insufficient' to cause MS.

EBV is a gamma herpesvirus that, following acute infection, enters a latent phase within B cells and persists for life. EBV can also infect T cells, epithelial cells and natural killer cells, albeit at lower efficiency. EBV-infected B cells can be found in the brains of people with MS [10] and there is enrichment of these cells in cortical lesions of people with progressive MS [11], although not all studies have found this to be the case [12, 13].There is also evidence for molecular mimicry between EBNA1 and several antigens present in the central nervous system (CNS) [14–16]. EBV gains access to human tissue via B cell-specific binding proteins (gp350, gH/gL, gp42 and gB). Complement receptor 2 on B cells is used by gp350 to gain entry and EBV can also use HLA class II molecules as entry receptors [17]. Following the resolution of acute infection and lytic phase replication, the virus enters a latent state in B cells where additional antigens (including EBNA1) are produced, but only when dividing [18]. This latent phase assists EBV in evading the immune system throughout the life of the infected individual. Importantly, reactivation of lytic phase replication from latent virus may also occur periodically, associated with expression of BZLF-1 and BRLF-1 open reading frames (ORF). Accordingly, expression of the former in MS lesions along with CD8 T cells targeting the antigen is noteworthy [19].

Understanding the link between EBV and MS has led to the investigation of multiple strategies to target EBV through antiviral medications [20], vaccines [21] and T cell therapy [22]. Given the considerable costs and timeframe involved in new drug discovery, repurposing existing drugs for new indications is an attractive strategy to accelerate treatment development. A recent systematic review highlighted the low quality of evidence for antiviral treatment of acute IM emphasising the need for further research, and for carefully designed clinical trials of antivirals for EBV-related diseases going forward [23].

In this paper we describe a systematic approach to identify and select repurposed drugs with anti-EBV effects for the treatment of MS. This initiative was undertaken in response to a specific call for funding from the Medical Research Future Fund in Australia for a phase III clinical trial of putative anti-EBV therapy in MS. The aim was to identify the most appropriate drugs for potential use in late phase clinical trials in MS with respect to both efficacy and tolerability.

2 Methods

Between 3 June 2022 and 12 December 2022, a scientific committee comprising national and international experts in neurology, neuroscience, virology and immunology was convened to undertake a systematic process of treatment selection (Fig. 1). This process aimed to select drugs with efficacy against EBV that could be evaluated in a phase III clinical trial in people with MS. The specific objective was to 'accelerate the development or repurposing of therapeutic candidates (including antivirals) to treat EBV to prevent or treat conditions, such as MS'.

A preliminary list of putative candidate treatments was generated through a review of published literature on agents that have, at a minimum, in vitro activity against EBV. It was deemed expeditious to utilise two recent reviews to generate this preliminary list [20, 24]. Further characteristics prioritised at this stage included in vitro

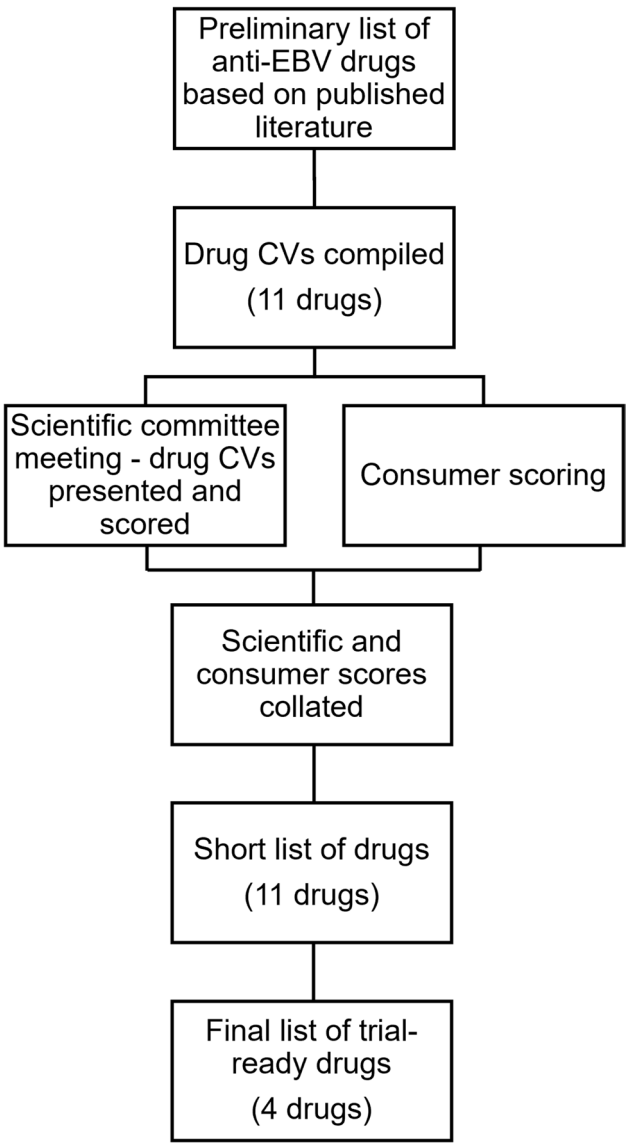

Fig. 1 Summary of the process of selecting repurposed anti-EBV drugs for treatment of MS. See text for details of 'drug CV'. CV, curriculum vitae; EBV, Epstein–Barr virus; MS, multiple sclerosis

efficacy against EBV, ability to cross the blood–brain barrier (BBB) and existing evidence in people with MS. The BBB is a multi-layered filtering system which normally protects the brain from the passage of large molecules and immune cells [25]. For efficacy against the pial lymphoid tissue, high CNS and BBB penetrance would be desirable. Drugs that were not yet licensed for other indications and would not be suitable for a phase III clinical trial were excluded. Disease-modifying therapies already licensed for MS were also excluded as they would not yield new therapies for MS.

A detailed 'drug CV' (a 'curriculum vitae' summarising the approved indications, molecular mechanistic target, evidence for anti-EBV effects, dosage, formulation, pharmacological properties, BBB penetrance, side effects, interactions, contraindications and cost) for each compound on the preliminary list was compiled by two scientific committee members (V.L. and F.M.). This comprised information on existing indications, regulatory status, molecular or mechanistic target, evidence from preclinical studies, evidence in MS, dosage, formulation and administration, pharmacokinetics and pharmacodynamics, CNS penetration, adverse effects and relevant drug interactions, adjustments in renal or hepatic failure, contraindications, monitoring requirements, safety in pregnancy and breastfeeding, side effect profile and cost.

The drug CVs were distributed to the scientific committee and each candidate agent presented at a workshop. Each drug was scored on the basis of its efficacy and safety, with an overall evaluation based on readiness to be taken to phase III clinical trials in MS. People with lived experience of MS also participated in the drug selection process. The group comprised six national advocates of MS Australia, the national peak body supporting MS research and advocacy. Advocates were selected as experienced participants in MS research studies with high health literacy.

A detailed survey was developed for the Lived Experience Panel. This contained information on the rationale for treating EBV in MS and plain language explanations of the currently approved therapeutic indication in Australia, potential side effects (very common, > 10%; common, 1–10%; uncommon, < 1%; and rare but serious, < 0.1%) and route of administration for each of the drugs. The survey was administered online using Survey Monkey® (Momentive®; Waterford, NY, US) from 3 to 10 January 2023.

The scores from the scientific committee and lived experience panel were collated to generate a final shortlist of drugs. These were reconsidered by the scientific committee, taking into consideration pharmacokinetics, feasibility, cost and potential trial designs leading to a final shortlist.

3 Results

3.1 Summary of Evidence for a Preliminary List of Drugs

The pre-existing literature yielded 11 potential drugs [20, 24]. The mechanisms of action, evidence for anti-EBV effect, BBB penetration and evidence in treating MS, and key side effects are summarised in Table 1. There were drugs in development specifically targeting the latent phase of EBV (e.g. EBNA1 inhibitors), but none of these were at a stage of development suitable for evaluation in phase III clinical trials. Existing disease modifying therapies for MS with potential anti-EBV effects (e.g. teriflunomide) were similarly excluded on the basis that these drugs already have proven efficacy in MS and have an approved indication for MS.

3.1.1 Mechanisms of Action and Anti-EBV Effects

All the selected treatments had evidence of in vitro effectiveness against EBV. Most of the anti-viral drugs were indicated primarily for other herpesvirus infections. These predominantly act by targeting the viral DNA polymerase, thereby preventing DNA replication. However, the in vitro anti-EBV effects of these drugs were typically lower than the activity against alpha herpesviruses, such as herpes simplex or varicella. Maribavir, which is licensed for cytomegalovirus (CMV) infection, had higher anti-EBV activity than aciclovir, cidofovir, ganciclovir and foscarnet [26].

By contrast, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) are licensed, either alone or in combination, for the treatment of chronic hepatitis B and human immunodeficiency virus (HIV). Spironolactone is indicated for treating cardiac failure, hyperaldosteronism, hypertension and nephrotic syndrome. TDF, TAF and spironolactone inhibit late lytic EBV replication (Table 1 and discussed below).

3.1.2 BBB Penetration

Data on BBB penetration was variable, with limited penetration reported for cidofovir and tenofovir (5% of plasma concentrations), a mean of 31% for aciclovir and 20% for valaciclovir (though valaciclovir has two to three times the bioavailability of aciclovir) [31, 36, 49], and comparable levels for ganciclovir/valganciclovir [49, 50]. Penciclovir, for which famciclovir is the prodrug, was detectable in the brain in rodent models, reaching 41.5% of the concentration in muscle after 5 h [41]. Penetration coefficient was variable for foscarnet, ranging from 5 to 72% after a single infusion,

Table 1 Summary of key features of 11 preliminarily listed anti-EBV drugs

Drug	Mechanism of action	Evidence EBV	Evidence MS	BBB penetration	Significant side effects
Aciclovir	Competes with viral DNA polymerase to stop viral DNA synthesis and lytic replication	In vitro: inhibited EBV DNA synthesis in lymphoblastoid cell line [27] In vivo: reduced EBV shedding in people with IM treated whilst on treatment; no effect on clinical course or EBV shedding after cessation of treatment [28] Case series of 45 people with severe manifestations of IM treated with aciclovir demonstrated cure/improvement in majority (17 of 21 with CNS involvement) [29]	Non-significant reduction in annualised relapse rate in people with RRMS treated with PO 800 mg TDS for 2 years versus placebo; statistically significant when grouped into low, medium or high relapse rates [30]	Mean CSF/serum concentration ratio 31% [31]	Common: gastrointestinal, fatigue, headache, dizziness, skin rash, joint/muscle aches, increased liver enzymes Rare but serious: anaphylaxis, kidney injury, neurotoxicity, thrombotic thrombocytopaenic purpura [32]
Valaciclovir	Prodrug of aciclovir; competes with viral DNA polymerase to stop viral DNA synthesis and lytic replication	In vivo: reduced frequency of EBV-infected B cells in healthy volunteers receiving 500 mg daily for 1 year versus no treatment [33]	No difference in new MRI lesions or clinical endpoints over 24 weeks in people with RRMS on 100 mg TDS versus placebo. In people with > 1 active lesion at baseline, valaciclovir reduced development of further new lesions [34] No effect on progression, time to relapse, relapse rate, MRI measures in people with RRMS treated with 1000 mg TDS for 2 years versus placebo [35]	Mean CSF/serum aciclovir ratio ~20% after oral valaciclovir 1000 mg TDS for 6 days [36]	
Famciclovir	Prodrug of penciclovir which is phosphorylated to active metabolite penciclovir triphosphate which blocks viral DNA synthesis	In vitro: penciclovir inhibits the productive replication cycle of EBV in cell culture [37] In vivo: case reports of successful treatment of severe infectious mononucleosis [29, 38] and oral hairy leucoplakia [39] using famciclovir	Phase II add-on trial of famciclovir 500 mg BD to existing disease-modifying therapy (natalizumab) for 12 weeks in relapsing-remitting MS did not significantly reduce salivary shedding of EBV in this small pilot study [40] (NCT05283551; see text for further detail)	In rodent model, CSF/gastrocnemius muscle (which corresponds to plasma concentration) ratio of penciclovir at 0–5 h after IV administration was 0.096, and after oral administration as famciclovir was 0.143 [41]	Common: headache, fatigue, nausea, vomiting, diarrhoea, abnormal liver function, rash, pruritis Uncommon: confusion and somnolence (predominantly in elderly) Rare but serious: severe skin reaction [42]

Table 1 (continued)

Drug	Mechanism of action	Evidence EBV	Evidence MS	BBB penetration	Significant side effects
Ganciclovir	Ganciclovir is a synthetic 2-deoxyguanosine (nucleoside) analogue that is converted by EBV viral kinases (expressed only in lytic phase) into its cytotoxic active form, ganciclovir-triphosphate [43, 44] Inhibits viral DNA synthesis by competitive inhibition of viral DNA polymerases and direct incorporation into viral DNA causing DNA chain termination	In vitro: ganciclovir has potent anti-EBV activity in multiple assays [26] In vivo: ganciclovir and valganciclovir had significant prophylactic effect on primary EBV infection in paediatric renal transplant [45] and liver transplant [46] patients, and lowered EBV viral load In adult renal transplant, valganciclovir delayed primary EBV infection and reduced EBV-associated neoplasia [47]	Ganciclovir is a potent inhibitor of microglial proliferation and neuroinflammation in EAE [48]	CSF/serum in a nonhuman primate model was 0.155–0.071 [49], suggesting a CSF penetration similar to that of aciclovir [50]	Common: pancytopaenia, leukopenia, headache, mucous membrane disorder, pyrexia, rigors, sepsis, infection, anorexia, facial oedema, confusion, peripheral neuropathy, hypertension, pleural effusion, renal impairment, renal failure Potentially fatal side effects: bone marrow failure, pancreatitis, sepsis, multiple organ failure [51]
Valganciclovir	Valganciclovir is an L-valyl ester salt (prodrug) of ganciclovir which, after oral administration, is rapidly converted to ganciclovir	Valganciclovir reduced oral EBV shedding and viral load in adult men [52] Ganciclovir conferred a 38% risk reduction of early PTLD in the first year post renal transplant in a multicentre case-control study [53] In uveitis with presumed EBV activation, majority (9/14) responded to valganciclovir treatment [54]	No specific evidence but assumed to be similar to ganciclovir	No specific data but presumed to be similar to ganciclovir	As for ganciclovir

Table 1 (continued)

Drug	Mechanism of action	Evidence EBV	Evidence MS	BBB penetration	Significant side effects
Cidofovir	Cytosine (nucleotide) analogue which, when incorporated into viral DNA, acts as a chain terminator of DNA replication and thus an inhibitor of viral lytic replication [55] Cidofovir is converted to its active metabolite by host enzymes therefore metabolism is not dependent on, or facilitated by, viral infection [56]	In vitro: potent activity against EBV in a similar EC_{50} range to acyclovir and ganciclovir [26, 57] Decreased EBV oncoproteins and enhanced radiosensitivity in EBV-related malignancies [58] In vivo: successful treatment of locally recurrent EBV-associated nasopharyngeal carcinoma using cidofovir in two patients [59] Case reports of cidofovir used successfully in combination therapy to treat EBV-associated leukemic lymphoma [60] and EBV post-transplant lymphoproliferative disease with CNS involvement [61, 62]	γHV-68, an animal gammaherpes virus closely related to EBV, was shown to enhance symptoms in EAE. Suppression of lytic γHV-68 replication with cidofovir had no effect on phenotype and clinical disease compared with untreated γHV-68 mice [63] Case reports of cidofovir for other viral infections in MS, including JC virus (and associated progressive multifocal leukoencephalopathy) [64] and adenovirus post autologous haemopoietic stem cell transplant [65], often in combination with other antivirals or steroids	CSF penetration of cidofovir is negligible under normal circumstances (i.e. without inflammation of the BBB) [66]	Nephrotoxic and potential carcinogen in humans Very common: headache, neutropaenia, nausea, vomiting, hair thinning/loss, rash, proteinuria, weakness/fatigue, fever Common: renal impairment, iritis/uveitis, low intraocular pressure, potentially vision-threatening, shortness of breath, diarrhoea, chills Uncommon: Fanconi syndrome [67]
Foscarnet	Pyrophosphate analogue, directly inhibits viral DNA polymerase and reverse transcriptase [55]. Does not require activation by thymidine kinase or other kinases	In vitro: lowest anti-EBV potency of nine antiviral compounds tested including maribavir, cidofovir, ganciclovir [26] In vivo: in EBV lymphoproliferative disease, foscarnet is preferred over aciclovir and ganciclovir, as the latter require the EBV viral thymidine kinase, which is not regularly expressed in EBV lymphoproliferative tissue. In these cases, foscarnet resulted in continuous complete remissions [68]. Foscarnet has been used with success in EBV acute retinal necrosis [69] and severe infectious mononucleosis cases [70]	Case reports of foscarnet use in people with MS: two with bilateral acute retinal necrosis caused by VZV: one on alemtuzumab [71], and one on natalizumab [72]; and one with tumefactive MS and HHV-6 infection [73]. Administered with other antivirals with variable clinical outcomes in these studies	CSF/plasma concentration ratio was 66 (± 11)% [74] Foscarnet penetrates the BBB in the presence and absence of meningeal inflammation [50]	Requires intravenous administration and in-hospital monitoring owing to serious toxic side effects [75] Common: renal impairment, electrolyte disturbance, seizures, genital irritation, anaemia, leukopaenia, neutropaenia thrombocytopaenia, abnormal liver function, sepsis, ECG abnormalities, gastrointestinal pain, headache, paraesthesia, dizziness, nausea, vomiting Rare but serious: pancytopaenia, acidosis, angioedema renal tubular disorder, glomerulonephritis, nephrotic syndrome [76]

Table 1 (continued)

Drug	Mechanism of action	Evidence EBV	Evidence MS	BBB penetration	Significant side effects
Maribavir	Oral benzimidazole riboside with significant activity against both human CMV and EBV but not other herpesviruses [77, 78]. Effects are mainly through inhibition of the human CMV and EBV protein kinases [79, 80] Marked activity against EBV through a unique dual effect, inhibition of viral DNA replication and of virus transcription [81, 82]	In vitro: maribavir inhibits expression of multiple EBV RNAs [83]. Maribavir largely affects EBV transcript levels through inhibition of BGLF4, although maribavir does not directly affect the EBV protein kinase [84]. Considering that EBV BGLF4 has at least 20 viral targets, maribavir may also affect downstream targets indirectly [20] Activity of maribavir against EBV is high among the antivirals and more potent than activity reported against CMV (0.31 and 19.4 μM) [26]	No studies in MS or EAE found	Maribavir poorly penetrates the blood–retinal barrier and is not expected to cross the BBB in humans on the basis of results from nonclinical distribution studies [85] Maribavir does not cross the BBB in rats. In monkeys, maribavir levels in the brain, CSF and vitreous humour range from 4 to 20%, 1 to 2% and < 1% of plasma concentrations, respectively [86]	Toxicity of maribavir is low and includes manageable symptoms, such as taste disturbances, headache and nausea Very common: taste disturbance, nausea, diarrhoea, vomiting, fatigue, anaemia Common: headache upper abdominal pain, decreased appetite, weight loss, fever, acute kidney injury, neutropaenia, oedema Common but serious: diarrhoea (2%), kidney disorders (1%) [85]
Tenofovir alafenamide	Prodrugs of tenofovir, an acyclic nucleoside/nucleotide analogue that inhibits EBV lytic DNA replication by preventing incorporation of dATP into DNA by EBV DNA polymerase	In vitro: tenofovir alafenamide (TAF) reduced number of viral copies produced after lytic induction in EBV cell line by > 99.9%. TAF was around threefold more potent than TDF in inhibiting EBV DNA replication [87]	Eight published case studies of people with HIV and MS, treated with pro-drugs of tenofovir were reviewed [88] ($n = 6$ TDF; $n = 2$ sequential TAF and TDF, including [89]). In ~ 40.5 person-years of cumulative exposure to TAF/TDF for at least 3 months after a confirmed MS diagnosis, only 10% (2/21) of MRIs showed new lesions, with no new relapses (ARR < 0.025). Prior to TAF/TDF initiation, 68% (13/19) of MRIs showed new lesions, with 22 relapses during approximately 37 person-years (ARR 0.6) [88]. A clinical trial of TAF is currently actively recruiting (EUCT number: 2023-503814-62-00)	Median CSF/plasma concentration ratio 5% [90, 91]	Common: headache, gastrointestinal, fatigue, joint aches, skin rash Rare but serious: acute renal failure, Fanconi syndrome, osteoporosis [92]

Table 1 (continued)

Drug	Mechanism of action	Evidence EBV	Evidence MS	BBB penetration	Significant side effects
Tenofovir disoproxil fumarate	Prodrugs of tenofovir, an acyclic nucleoside/nucleotide analogue that inhibits EBV lytic DNA replication by preventing incorporation of dATP into DNA by EBV DNA polymerase	In vitro: tenofovir disoproxil fumarate (TDF) reduced number of viral copies produced after lytic induction in EBV cell line by > 99.9%. [83]	Case report of an HIV-negative person with MS experiencing improvements in fatigue, MRI stabilisation and no disease progression or relapses over 4 years on TDF treatment in the absence of MS DMTs [93]. A clinical trial of TDF/emtricitabine is currently actively recruiting (NCT05957913)	May have lower BBB penetration than TDF [94, 95]	As for TAF but greater risk of acute renal failure, Fanconi syndrome and osteoporosis
Spironolactone	Prodrug of 7α-thiomethyl-spironolactone and canrenone. Anti-mineralocorticoid, moderately antiandrogenic and weakly inhibits steroidogenesis	In vitro: inhibits function of essential EBV protein SM via degradation of xeroderma pigmentosum group B-complementing protein, a transcription factor involved in transcription of several late lytic antigens. Results in inhibition of viral capsid antigen synthesis and capsid formation [96, 97]	In vitro: reduced TNF-α levels of lipopolysaccharide-activated microglia by > 50% at non-toxic concentrations [98] In vivo: blockade of the mineralocorticoid receptor with spironolactone attenuated EAE development in aldosterone-treated mice [99] No studies in people with MS	Limited studies; active metabolite, canrenone, crosses BBB in animal model [100]	Common: hyperkalaemia, gynaecomastia, gastrointestinal, skin rash, fatigue, hair loss Rare but serious: hepatotoxicity, stomach ulceration [101]

ARR, annualised relapse rate; BBB, blood brain barrier; BD, twice daily; BGLF-4, EBV-encoded protein kinase; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; dATP, deoxyadenosine triphosphate; DMT, disease modifying therapy; DNA, deoxyribonucleic acid; EAE, experimental autoimmune encephalomyelitis; EBV, Epstein–Barr virus; EC50, half maximal effective concentration; ECG, electrocardiogram; γHV-68, gamma herpes virus 68; HHV-6, human herpes virus 6; HIV, human immunodeficiency virus; IM, infectious mononucleosis; IV, intravenous; JC, John Cunningham (virus); MRI, magnetic resonance imaging; MS, multiple sclerosis; PO, per os (by mouth) ; PTLD, post-transplant lymphoproliferative disorder; RNA, ribonucleic acid; RRMS, relapsing-remitting multiple sclerosis; SM, EBV protein; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TDS, three times per day; TNF-α, tumour necrosis factor alpha; VZV, varicella zoster virus

and depended on the integrity of the blood–brain barrier [41]. Evidence was mixed or inconclusive for spironolactone and maribavir.

3.1.3 Evidence in Treating MS

The evidence for efficacy in people with MS was limited. Aciclovir and valaciclovir had been tested in a small number of randomised controlled trials in MS (Table 1). There have been no studies of famciclovir, ganciclovir, valganciclovir, cidofovir or foscarnet targeting EBV in MS. A small number of case reports describe the use of cidofovir and foscarnet in people with MS to treat other viral infections (varicella zoster virus, human herpes virus 6, John Cunningham virus).

3.2 Scoring of Drugs on the Preliminary List

Results of scoring by 15 members of the scientific committee, based on efficacy against EBV, evidence in MS, and administration/toxicity are shown in Fig. 2 (raw data in Online Resource 1), with most drugs scoring 1 out of 2 on both criteria. Notably, maribavir scored most highly in terms of anti-EBV effect, but has not been tested in people with MS. The Lived Experience Panel survey response and tabulated data are given in Online Resource 2 and 3.

3.3 Lived Experience Review

In total, six drugs achieved the maximum score of 2, whilst ganciclovir, cidofovir and foscarnet were scored 0 on the basis of their adverse side effect profiles, significant toxicities and intravenous administration (Fig. 2). Tenofovir disoproxil fumarate, which has a greater likelihood of bone and renal side effects, was also rated lower than tenofovir alafenamide due to safety concerns. There was good concordance between the scientific and lived experience reviewers regarding acceptability of route of administration.

3.4 Final Selection

Combining the scientific and lived experience scores produced six drugs which all scored 6 out of 8: aciclovir, valaciclovir, famciclovir, maribavir, spironolactone and tenofovir alafenamide. The list was reviewed again by the scientific committee before a final selection was made to prioritise famciclovir, maribavir, spironolactone and tenofovir alafenamide for consideration in clinical trials of MS.

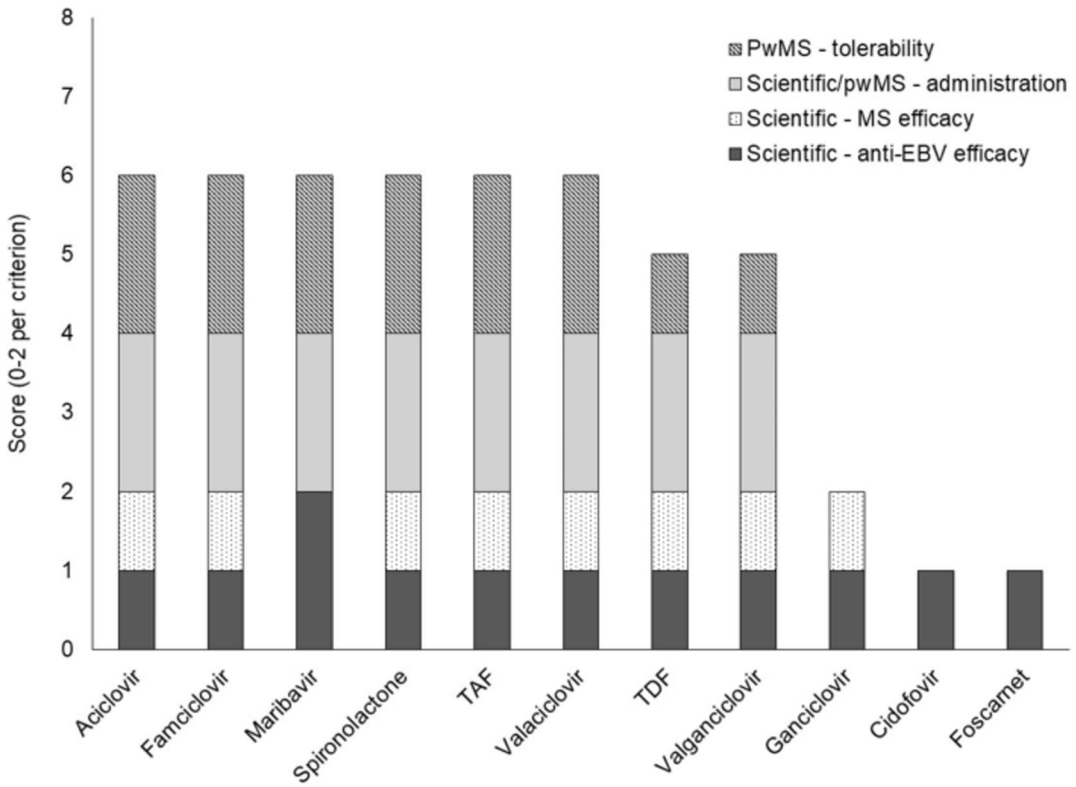

Fig. 2 Scoring of anti-EBV drugs by the scientific committee based on evidence of efficacy against EBV in MS, and by people with MS on route/convenience of administration for participating in trials of these agents. EBV, Epstein–Barr virus; MS, multiple sclerosis; pwMS, people with multiple sclerosis; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate

3.4.1 Famciclovir

Compared with aciclovir and valaciclovir, famciclovir was chosen because of its greater bioavailability, higher intracellular concentration and greater persistence in infected cells [102]. Famciclovir is a prodrug that is phosphorylated to the active metabolite penciclovir triphosphate. Penciclovir is phosphorylated by herpesvirus thymidine kinase, which is only found in herpesvirus-infected cells. Penciclovir inhibits EBV replication in cell culture [37] and brain concentrations in rats were 41.5% of the concentration in muscle 5 h after administration [41]. The related drug aciclovir has been used for the treatment of severe acute EBV infection [29], and famciclovir has been reported in one case to successfully treat overwhelming IM [38].

A randomised, double-blind, placebo-controlled trial of valaciclovir (the prodrug of aciclovir) in MS has shown a reduction in the number of new active lesions on MRI in those with active disease, although formation of active lesions in patients with RRMS who had two or more relapses during previous 2 years was not reduced [34]. Trends towards a reduction in annualised relapse rate and EDSS progression were also seen with valaciclovir treatment [35]. An analysis of salivary EBV DNA in people with MS found a significant reduction in proportion of people with detectable levels after 24 weeks of valaciclovir treatment [103]. A randomised double-blind, placebo-controlled trial of aciclovir showed a trend towards reduction in annualised relapse rate, which when dichotomised to low or high relapse rate became statistically significant [30]. A small phase II add-on trial of 500 mg of famciclovir administered twice daily to existing disease-modifying therapy for 12 weeks in relapsing-remitting MS to assess effects on viral shedding, viral load and anti-EBV serology has been completed (NCT05283551). While no significant effect of famciclovir on salivary EBV shedding was seen compared with untreated epochs (10/21 at baseline; 7/21 on-treatment), shedding in this natalizumab-treated cohort was lower than those reported in previous studies and the study requires replication [40]. Salivary EBV shedding is primarily an indicator of lytic EBV activity and may not be the most appropriate measure of latent EBV load. However, it does provide an indication of anti-EBV effect and alternatives are problematic. EBNA1 titres are of uncertain relevance and direct detection of EBV DNA in blood is technically very challenging in the latent infection stage.

3.4.2 Maribavir

Maribavir does not target the viral DNA polymerase, but rather its inhibitory effects are mainly produced through inhibition of the HCMV and EBV protein kinases [84]. It has more specific antiviral properties and fewer adverse side effects compared with other currently approved anti-human CMV drugs [26]. It has marked activity against EBV through a unique dual effect, comprising inhibition of viral DNA replication and multiple viral transcripts.

3.4.3 Spironolactone

Spironolactone inhibits EBV replication in the late lytic phase by blocking SM protein [96]. This occurs as a result of inhibition of xeroderma pigmentosum group B-complementing protein, a component of human transcription factor II H which EBV recruits in the transcription of several late lytic proteins [97]. In a case report, spironolactone was effective in controlling EBV in a case of non-HIV acquired immunodeficiency syndrome [104]. In a pilot study of nine people with MS, a combination of spironolactone and aldosterone was effective in improving symptoms [105].

3.4.4 Tenofovir Alafenamide

Tenofovir is an acyclic nucleotide analogue that inhibits EBV lytic DNA replication in vitro and is more potent than aciclovir. Tenofovir alafenamide, the prodrug of tenofovir, inhibits transcription of late lytic EBV viral genes [87]. Compared with tenofovir disoproxil fumarate, it has fewer bone and renal side effects, and reaches higher concentrations in peripheral blood mononuclear cells. A recent review of eight published cases of people with MS and human immunodeficiency virus (HIV) found a marked reduction in relapse rates and new lesions during periods in which they were treated with tenofovir prodrugs, including tenofovir alafenamide [88]. A phase II clinical trial of tenofovir alafenamide versus placebo in combination with ocrelizumab or rituximab in RRMS had been proposed but was later withdrawn owing to lack of funding (NCT04880577). A phase II clinical trial of tenofovir alafenamide in MS is currently actively recruiting.

4 Discussion

Through a systematic approach incorporating international experts from a wide range of fields and people with MS, we have determined that the most appropriate anti-EBV agents to trial in MS are famciclovir, maribavir, spironolactone and tenofovir alafenamide. Maribavir appears to be the most potent against EBV and maribavir and spironolactone may have the greatest potential effect on the latent phase of EBV. All were deemed to be acceptable in terms of their tolerability and side effect profile. Spironolactone is inexpensive, famciclovir and tenofovir alafenamide are intermediate in cost. Maribavir is the most recently licenced and its cost may prove prohibitive for longer duration studies without commercial sponsorship.

Our treatment selection process for anti-EBV agents was based on the systematic approach established by the UK MS Society expert consortium for progressive MS clinical trials to test repurposed treatments in an adaptive platform trial [106]. Given the smaller number of candidate drugs, we only undertook one round of review and voting to shortlist the treatments to be taken forward. Engaging people with lived experience of MS was a critical element and strength of our approach. Scores from people with MS on safety and administration made up 50% of the total score for each drug. This aspect of the selection process is critical to ensure potential participants are willing to take the selected treatments and minimise dropouts. The limitations of this study are that prior reviews of potential drugs with activity against EBV were used to formulate our initial list of candidate drugs, rather than conducting a fresh literature review. As a result, some potential drugs of interest may have been missed. Our rating scale for putative drug efficacy was rather blunt and may have failed to separate drugs effectively. However, the feeling of those reviewing the evidence was that in the quality of the evidence was mixed and more fine-grained determinations would not have been appropriate. In addition, it was felt important to balance efficacy with consumer acceptability in an equitable manner.

Whilst EBV may be essential to develop MS, its role in ongoing disease activity is unknown. The concept of using anti-EBV drugs to treat MS is predicated on the assumption that EBV replication or persistence of latently infected autoreactive B cells contributes to persistent neuroinflammation in people with MS. There is currently no evidence to support this hypothesis. The drugs being considered all primarily act on the lytic phase of EBV and therefore may have no effect on latent-EBV infected B cells in blood or the brain. Any trial of anti-EBV therapy in MS could fail owing to either the drug being ineffective against EBV or suppression of EBV replication having no effect on CNS inflammation. Ultimately, EBV may simply act as a trigger and have no discernible effect on further disease activity [107].

The optimal clinical trial design to test anti-EBV therapies in MS is yet to be determined. A preventive approach targeting the earliest stages of MS (clinically isolated syndrome) when T cell responses to EBV remain relatively high [108] may be appropriate. Evidence for sequestration of autoreactive, EBV-infected, B cells within lymphoid tissue in the CNS as a primary driver of continuing low-grade inflammation and disability would support use in progressive forms of MS [11]. There is growing evidence for the role of EBV in causing fatigue both in MS and in other conditions [109], making such symptoms a suitable substrate for monitoring treatment response. Given significant differences between anti-EBV therapies in their in vivo pharmacokinetics, drug metabolism and mechanism of action, clinical studies should seek to evaluate multiple candidates as the requirements for an antiviral drugging EBV in MS remain unknown. A further consideration is the choice of sensitive and reliable markers of anti-EBV effect. EBNA-1 antibody titres and salivary EBV DNA have been determined to be suitable measures of EBV activity [110]. These measures have been used in previous studies of people with MS, such as teriflunomide which reduced salivary EBV shedding over 3 months [111], and was associated with less cortical atrophy in people with the greatest reductions in EBNA-1 IgG titres over 12 months [112]. If specific medications are successful in future clinical studies, these may allow exploratory development of novel biomarkers for EBV in MS.

5 Conclusions

We have outlined a multidisciplinary, evidence-based process, informed by the lived experience of MS throughout, to identify promising drugs with anti-EBV effects that are ready to be evaluated in a clinical trial with the potential to be repurposed for treatment of MS. This information can be used to inform treatment selection for future clinical trials of anti-EBV therapy in EBV related diseases, such as MS.

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Declarations

Conflicts of Interest V.L., F.McK., D.T., C.S., R.K., J.L.-S., A.L., B.T., J.M., L.S., G.G., S.S. and T.H. are recipients of or members of partner organisations on research grants for clinical trials of antiviral treatments for EBV in MS from the Australian Government Medical Research Future Fund (MRFF, 2023). D.T. has received research funding from the MRFF, Australian Research Council, the Australian National University and the Defence Materials Technology Centre. C.S. reports research and consultancy funding from Atara Biotherapeutics and has acted as a consultant for ATA188 program developing EBV immunotherapy for multiple sclerosis. He is a recipient of grants from MRFF and holds international patents or patent applications that cover CMV epitope sequences and their use in adoptive immunotherapy. R.K. is on the scientific advisory board of and is a consultant of Atara Biotherapeutics; is the editor-in-chief of Clinical and Translational Immunology; has patents or patents pending for T cell epitopes and immunotherapy for virus-associated diseases including malignancies and autoimmune diseases; received research support from Atara Biotherapeutics and National Health and Medical Research Council (NHMRC); and holds stock options in and received licensing payments from Atara Biotherapeutics. J.L.-S. has accepted travel com-

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Author contributions S.B. conceived the idea; S.B., S.H. and G.G. proposed drug candidates for consideration from existing reviews; V.L. and F.McK. prepared scientific and plain language drug summaries; V.L., wrote the first draft of the manuscript; S.B., V.L. and F.McK. analysed the data; A.P., N.C., L.S., E.B. and B.F. reviewed plain language summaries and completed detailed tolerability surveys. The expert scientific committee included S.B., V.L., D.T., R.K., C.S., W.R., A.L., B.T., L.S., G.G., J.L.-S., S.H. and T.H. All authors revised and approved the final manuscript.

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