1. JRMO Research Protocol for Interventional Studies

Full Title: **E**pstein **B**arr Virus Viral loads, **Me**mory B-Cells Repertoires, and Immune Response in People with and without a History of Infectious **M**ononucleosi**s**
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Glossary

EBV - Epstein-Barr Virus
IM - infectious mononucleosis
GAS - group A beta-haemolytic streptococcus
pwMS - people with MS
MS - multiple sclerosis
CTLs - cytotoxic T-lymphocytes
LCLs - lymphoblastoid cell lines
Ig's - immunoglobulins
DMTs - Disease-modifying therapies

3. Signature page

Chief Investigator Agreement

The study as detailed within this research protocol will be conducted in accordance with the principles of Good Clinical Practice, the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

Chief Investigator Name: **Ben Bloom**

Signature:

Date:

4. Summary and synopsis

Short title	EB-MEMs
Methodology	Prospective longitudinal cohort study with an exploratory open-label famciclovir intervention cohort. Participants with infectious mononucleosis, streptococcal pharyngitis, and healthy controls will undergo serial clinical, virological, immunological, and repertoire assessments over 24 months. An additional IM cohort will receive famciclovir for 7 days to evaluate its effects on EBV viral load and immune responses.
Research sites	• Blizard Institute, Queen Mary University of London • Royal London Hospital, Barts Health NHS Trust
Objectives / aims	• To compare deep immunophenotyping and EBV viral loads between subjects with infectious mononucleosis (IM), subjects with antibiotic-treated streptococcal pharyngitis, and healthy subjects who are either EBV-seronegative or EBV-seropositive following asymptomatic EBV exposure in the past. • To explore the effects of oral famciclovir (500 mg twice daily for 7 days) on EBV viral load, viral shedding and immunological biomarkers, including

	the B-cell repertoire and immune function, in participants with IM.
Number of participants	• Cohort 1 – approximately 60 subjects will be studied: 20 with EBV-associated IM, 20 with streptococcal pharyngitis and 20 controls without a history of IM. • Cohort 2 – approximately 20 subjects with EBV-associated IM will receive oral famciclovir (500mg BD x 7 days).

Inclusion criteria	• **Cohort 1 with IM:**
	  ○ Molecular and/or serological diagnosis of IM with symptom onset within the previous 14 days
	  ○ Aged 16 years or over
	  ○ Able to provide informed consent
	• **Cohort 1 with streptococcal pharyngitis:**
	  ○ Microbiologically confirmed (culture-positive) acute streptococcal pharyngitis
	  ○ Aged 16 years or over
	  ○ Able to provide informed consent
	• **Healthy controls:**
	  ○ No history of IM
	  ○ Age above 16 years
	  ○ Ability to provide Informed consent
	• **Cohort 2:**
	  ○ Molecular and/or serological diagnosis of IM with symptom onset within the previous 14 days
	  ○ Aged 16 years or over
	  ○ Able to provide informed consent
	  ○ Willing to take oral famciclovir 500 mg twice daily for 7 days

Exclusion criteria	• **Exclusion criteria for all subjects:**
	  ○ Unable to comply with study requirements
	  ○ Unable to provide informed consent
	  ○ Other pre-existing autoimmune diseases, for example, type I diabetes mellitus, autoimmune thyroid disease, inflammatory bowel disease, psoriasis, etc.
	• **Exclusion criteria for subjects with IM in cohort 2:**

	○ Unable to provide informed consent ○ Pre-existing autoimmune disease as specified in the protocol ○ Unwilling to take the 7-day course of famciclovir ○ Concomitant treatment with probenecid or raloxifene ○ Known contraindication, allergy or hypersensitivity to famciclovir, penciclovir or any component of the formulation ○ Pregnancy (self-declaration of pregnancy status and LMP more than 4 weeks or urine HCG test when pregnancy cannot be reasonably excluded)
Statistical methodology and analysis (if applicable)	● This is a longitudinal study with up to six repeated measurements per subject (D0, D7, D28, D183, D365, D730). The primary analysis approach is a linear mixed-effects model / mixed model for repeated measures (MMRM), which accounts for within-subject correlation and uses all available observations under a Missing At Random (MAR) assumption. For each continuous outcome (e.g., log-transformed EBV viral load), the model will include fixed effects for group, visit (categorical), and their interaction; a random intercept per subject; and, where feasible, an unstructured residual covariance matrix. Constrained longitudinal data analysis (cLDA) will model baseline and follow-up jointly. Within-group change from baseline and between-group differences in change will be estimated with 95% confidence intervals; the group × visit interaction provides the primary omnibus test. We will use generalised linear mixed models (negative binomial or binomial) for count and binary outcomes. Sensitivity analyses include multiple imputation under MNAR scenarios, generalised estimating equations, and per-subject area-under-the-curve summaries. For exploratory immunological endpoints, we will control the false discovery rate at 5% using the Benjamini-Hochberg procedure. We will perform analyses in SPSS

	(MIXED / GENLINMIXED), with R for repertoire diversity metrics and multivariate analyses.
Study duration	• Up to 36 months overall, with each participant followed for 24 months.

5. Introduction

5.1 Background

There is mounting evidence supporting a causal relationship between Epstein-Barr virus (EBV) infection in multiple sclerosis (MS) (Giovannoni et al. 2025). EBV infects at least 90% of the world's population, and approximately 90% of adults become antibody-positive before the age of 30 (Dunmire et al., 2015). A recent study of over 10 million U.S. military personnel found that nearly all individuals who developed MS had prior EBV infection, which suggests a universal association between EBV and MS (National Institutes of Health, 2022).

Although EBV infection appears to be necessary for the development of MS, it is not sufficient as most people infected with EBV do not develop MS. A history of IM is a more substantial risk factor for developing MS than asymptomatic EBV seroconversion. People with a history of IM have more than double the risk of getting MS compared to people without a history of IM. It has been argued that IM and MS are not causally related but rather manifestations of the same underlying propensity toward a dysregulated immune response. However, studies showing that EBV exposure is necessary before the onset of clinically apparent MS and the observation that EBV-seronegative people are protected from developing MS argue against this conjecture. This is why prophylactic EBV vaccination is being proposed as a strategy to prevent both IM and MS (Maple et al. 2022).

The substantial burden of the disease underscores the importance of understanding potentially modifiable risk factors for MS. More than 150,000 people are estimated to be living with MS in the United Kingdom, with approximately 7,100 new diagnoses each year (MS Society, 2024). MS imposes considerable economic costs that increase with disability progression, with annual per-person costs estimated at approximately £11,400, £22,700 and £36,500 for mild, moderate and advanced disability, respectively (Thompson et al., 2017).

Although most cases are self-limiting, some of the patients with acute IM have to be admitted to a hospital with acute complications. These include upper airway obstruction, difficulty swallowing and dehydration (Dunmire et al. 2015). Other complications include meningoencephalitis, haemolytic anaemia, thrombocytopaenia, neutropaenia, haemophagocytic syndrome, myocarditis, hepatitis, pancreatitis, pericarditis, pneumonitis, conjunctivitis, splenic rupture, Guillain-Barré syndrome, cranial neuritis, transverse myelitis, brachial neuritis and chronic fatigue syndrome (CFS) (Dunmire et al. 2015). Children typically miss 1 to 3 weeks of school due to IM and its sequelae; the reported average number of days off school or work is 20 days (De Paor et al. 2016). However, many students experience lingering fatigue for several weeks after the initial infection, which negatively impacts their school performance.

Participation in contact sports is not recommended for at least three weeks after IM due to the potential risk of splenic rupture. Infectious mononucleosis affects approximately 25% of university students who acquire primary EBV infection and is associated with significant academic disruption, including impaired concentration, class absenteeism, and, for a minority of students, withdrawal from or repetition of an academic year. From a socioeconomic perspective, treating acute IM could be considered an unmet medical need.

IM, which presents as acute purulent pharyngitis, fever, lymphadenopathy and fatigue, is frequently misdiagnosed and inappropriately treated with antibiotics (Dunmire et al., 2015). A Barts Health clinical audit of acute IM diagnosed in the Department of Virology from January 2008 to August 2014 showed that 60% of 63 subjects with serologically confirmed IM were inappropriately prescribed antibiotics in their acute care at the RLH. Antibiotics were prescribed to patients with acute IM before serological confirmation of the diagnosis to cover the possibility that purulent pharyngitis was due to group A streptococcus (S. pyogenes) or another bacterial infection.

5.2 Preclinical data

Not applicable

5.3 Clinical data

Molecular mimicry, GAS and ARF, Latent-lytic cycling

Immunologists currently favour molecular mimicry to explain how EBV may cause MS. Arguably, the most well-documented infectious agent to trigger autoimmunity is group A beta-haemolytic streptococcus (GAS), or *Streptococcus pyogenes*. It is the established cause of acute rheumatic fever (ARF), Sydenham's chorea, glomerulonephritis, arthritis and vasculitis (Cunningham 2016). The first attack of MS is asymptomatic in most people who develop MS and is likely to occur relatively soon after primary EBV exposure. MS manifests later, when a subsequent attack or lesion forms in a pathway that results in neurological symptoms. This explains why most people who develop MS have pre-existing old white matter lesions on their brain MRI when presenting with their first clinical attack. EBV differs from GAS because it establishes a latent infection that reactivates intermittently. This intermittent asymptomatic reactivation of EBV (latent-lytic cycling) likely drives MS disease activity (Giovannoni et al. 2025).

Why IM may be causally related to MS

In contrast, GAS tends to cause repeated symptomatic infections. However, asymptomatic colonisation with GAS is associated with elevated anti-streptolysin (ASO) titres and other serological markers of GAS infection. Similarly, people with MS have increased titres and responsiveness to a broader array of EBV epitopes in their antibody and T-cell receptor repertoires compared to healthy controls (Giovannoni et al. 2025), supporting the latent-lytic cycling hypothesis as the driver of MS disease activity (Giovannoni et al. 2025).

Current practice/guidelines including the use of Antivirals, Vaccines, Famciclovir/ why existing evidence is insufficient

The emergence of antibiotics to treat GAS pharyngitis was associated with a rapid

decline in the incidence of ARF. Observational data, rather than randomised controlled trial data, demonstrated the primary link between GAS infection and ARF. Randomised controlled trials were only conducted much later to prove that secondary antibiotic prophylaxis in patients with a prior episode of ARF could prevent recurrent attacks and the chronic sequelae of RF.

If EBV-related immune activation contributes to the biological processes associated with subsequent MS risk, modifying viral replication during acute IM could provide insight into whether EBV viral load and associated immunological responses are modifiable. Findings from a recent review showed near-complete absence of rigorous clinical research evaluating famciclovir specifically for EBV-associated infectious mononucleosis. Despite decades of famciclovir availability and the high prevalence of IM, no randomised controlled trials, prospective cohort studies, or even case series have been published on this indication.

Interventions that modify EBV replication during acute IM could provide an opportunity to investigate the relationship between viral activity and the associated immune response (Dunmire et al., 2018; Giovannoni et al., 2025). In this study, famciclovir is therefore used as an experimental intervention to explore whether modification of EBV viral load is accompanied by changes in immunological biomarkers, including B-cell and T-cell repertoire characteristics. The study is not designed to determine the clinical efficacy or overall effectiveness of famciclovir in treating IM or EBV infection, nor to determine whether treatment prevents MS. These questions would require appropriately designed future clinical studies.

Existing Evidence for Famciclovir in EBV Infection

Famciclovir is an oral prodrug of penciclovir with established activity against herpesviruses and favourable oral bioavailability. After conversion to penciclovir triphosphate, it inhibits viral DNA polymerase and suppresses viral replication. Despite these pharmacological properties, evidence supporting its use in EBV-associated infectious mononucleosis (IM) remains limited (Bacon, 1996; Field & Anthony Vere Hodge, 2013; Poole & James, 2018).

The only direct evidence of famciclovir treatment in acute EBV-associated IM comes from a case report by Goldani (2002), in which a patient with severe IM experienced rapid defervescence and regression of lymphadenopathy, tonsillar enlargement and hepatosplenomegaly within 72 hours of receiving famciclovir 500 mg three times daily. The authors concluded that the potential therapeutic role of famciclovir in severe IM warranted further investigation. However, as this was a single uncontrolled case report, the findings are insufficient to establish efficacy.

Additional evidence comes from phase II open-label studies conducted in patients with multiple sclerosis and chronic EBV infection (Dobson et al., 2023). In these studies, famciclovir 500 mg twice daily for 12 weeks did not significantly reduce salivary EBV shedding or EBV DNA copy number, although the investigators acknowledged that small treatment effects could not be excluded because of the limited sample size. While these studies did not evaluate acute IM, they provide important information regarding the potential virological activity of famciclovir against EBV.

Our recent systematic review identified a near-complete absence of rigorous clinical research evaluating famciclovir specifically for EBV-associated IM. Despite decades of availability and the high prevalence of IM, no randomised controlled trials, prospective cohort studies or case series have been published in this indication. Consequently, substantial uncertainty remains regarding the virological and immunological effects of famciclovir when administered during acute EBV-associated IM.

Infectious mononucleosis represents a distinct biological stage of EBV infection characterised by high viral loads, marked immune activation and extensive perturbation of B-cell biology. As a result, antiviral intervention during acute IM may have different virological and immunological effects from those observed in chronic infection. The present study will extend previous work by examining the effect of a short course of famciclovir administered during acute IM and by undertaking longitudinal assessment of EBV viral loads, immune responses and memory B-cell repertoires using serial blood and saliva samples collected over a 24-month period. This approach will provide mechanistic information on whether short-term famciclovir administration is associated with changes in EBV viral load and immunological biomarkers during and following acute IM.

Diagnostic pathway

Contrary to our audit findings, some studies suggest that Group A streptococcal infection increases EBV salivary shedding (Ueda et al. 2014). However, as people with EBV-associated IM universally have detectable virus in their blood, a positive blood EBV RT-PCR in the presence of acute pharyngitis and an IM-syndrome implies that the subject has acute IM (Dunmire et al. 2018). Serological tests will support the latter. However, as serology is only positive approximately 3 days after symptom onset, it is feasible that we will be able to diagnose rare cases of PCR-positive/serology-negative IM. The monospot or Paul Bunnell screen for heterophile antibodies is no longer used in the diagnosis of IM due to its poor positive and negative predictive values (Wang et al. 2022).

Study cohorts

The study will comprise two sequential cohorts involving a total of 80 participants aged 16 years and above. Participants will be recruited into four groups across two cohorts to investigate EBV-associated immune responses, the natural course of IM, and the potential effect of famciclovir on EBV viral load and immune responses.

Cohort 1 will comprise of 60 participants divided into three groups of 20 participants each.

Group 1: will comprise participants with a confirmed diagnosis of acute EBV-associated IM. Participants will be identified through the Royal London Hospital (RLH) diagnostic pathway following clinical assessment and appropriate laboratory investigations. They will undergo baseline and longitudinal assessments to characterise EBV viral load, associated immune responses, clinical symptoms and recovery over time. Participants in this group will not receive famciclovir as part of the study.

Group 2: This group will comprise participants with laboratory-confirmed streptococcal bacterial pharyngitis, identified through the RLH diagnostic pathway. They will undergo the same specified baseline assessments and longitudinal sample collection to investigate immune responses associated with bacterial pharyngeal

infection and provide a symptomatic comparison group for participants with EBV-associated IM. Participants in this group will not receive famciclovir.
Group 3: This group will comprise healthy volunteers without current symptomatic pharyngitis or clinical evidence of acute infectious mononucleosis. They will undergo baseline and longitudinal assessments to provide a reference for EBV serostatus, viral detection and immune responses in individuals without acute symptomatic infection. EBV serostatus will be assessed to establish previous exposure to EBV and contextualise the immunological findings.

Cohort 2: The second cohort will comprise 20 participants with a confirmed diagnosis of acute EBV-associated infectious mononucleosis. These participants will be recruited after completion of recruitment into all three groups in Cohort 1.
Following confirmation of eligibility, informed consent and baseline sample collection, participants will receive oral famciclovir 500 mg twice daily for seven days. Famciclovir is an established antiviral medication licensed for the treatment of infections caused by herpes simplex virus (HSV) and varicella-zoster virus (VZV). Although it has antiviral activity against herpesviruses, it is not licensed for the treatment of IM, and its clinical effectiveness in this indication has not been established.
Participants in Cohort 2 will undergo the same baseline assessments and longitudinal follow-up procedures as participants in the observational IM group in Cohort 1. This will allow comparison of EBV viral load, immune responses and clinical recovery between participants receiving famciclovir and those followed without the study antiviral intervention.

5.4 Rationale

Patients with IM experience an "abnormal" immune response to EBV that is actually an overreaction of their own immune system (Dunmire et al. 2018). The symptoms of IM are not caused by B-cell lysis, but the T-cell response against infected B cells (Dunmire et al. 2018). In a typical minor EBV infection, characteristic of asymptomatic seroconversion, NK cells and T cells expand moderately to clear the virus (Dunmire et al. 2018). In IM, the cytotoxic T lymphocytes (CTLs) can grow to account for 50% or more of all white blood cells (Dunmire, Verghese, and Balfour 2018); usually, these cells constitute only about 5-10% of the white blood cell population. These CTLs release large amounts of inflammatory cytokines. This "cytokine storm" causes fever, fatigue, and body aches. The swollen lymph nodes and spleen, part of the IM syndrome, are packed with these rapidly dividing T cells.

In parallel, EBV infects naive B cells, leading to their proliferation and production of germline heterophile antibodies (Brigden et al. 1999). Many of these EBV-infected naive B-cells enter the germinal centre (GC). EBV "hacks" the GC to ensure its own long-term survival. Usually, the germinal centre is highly selective in educating and selecting B cells. B-cells compete to produce the best antibodies, and those that fail die by apoptosis. EBV subverts this process by providing prosurvival signals and blocking regular quality-control checkpoints (Dai et al. 2024). This allows EBV-infected B cells to survive without producing high-quality antibodies and to differentiate into long-lived memory B cells without completing affinity maturation.

These infected B cells then serve as a permanent reservoir for the virus and are potentially autoreactive. To survive the germinal centre reaction, a normal B cell needs two specific signals. EBV provides counterfeit versions of both these signals, allowing infected cells to bypass natural selection. The viral protein LMP1 (Latent Membrane Protein 1) acts as a constantly active CD40 receptor. It tricks the B cell into thinking it has received T-cell help, activating the NF-kappa pathway to drive proliferation and survival, even if the B cell hasn't actually interacted with a T cell (Mancao et al. 2005). B cells must also bind to an antigen via their B-cell Receptor (BCR) to survive. The EBV viral protein LMP2A contains signalling motifs that mimic a tonic (low-level) BCR signal. This "rescue signal" prevents the B cell from undergoing apoptosis even if its natural BCR is nonfunctional or "crippled" (Mancao et al. 2005).

The GC also relies on a delicate balance of transcription factors, primarily BCL6 (which keeps cells proliferating in the GC) and BLIMP1 (which forces them to exit and become plasma cells). EBV disrupts this axis. The EBV protein EBNA3C physically binds to BCL6 and targets it for degradation (Mancao et al. 2005; Pei et al. 2017). By lowering BCL6 levels, EBV helps the infected cell "exit" the germinal centre reaction so it can become a memory B cell. If the memory B-cell became a plasma cell, the virus would likely be forced into lytic replication (active viral production), which would kill the cell and alert the immune system (Mohamed et al. 2014). EBV (via LMP1) suppresses BLIMP1, the master regulator of plasma cells. This prevents the cell from terminally differentiating, keeping it in a state that favours the formation of memory B cells instead.

EBV also directly prevents cell death during the harsh environment of the GC. EBNA3A regulates cellular proteins like MCL-1 and BFL-1 (Price et al. 2017). These are anti-apoptotic proteins that protect the mitochondria, ensuring the cell survives the rigorous selection pressure of the germinal centre.

Despite increasing understanding of these biological mechanisms, it remains unclear how acute infectious mononucleosis influences longitudinal changes in EBV viral loads, immune responses and B-cell repertoire diversity, and whether antiviral intervention can modify these processes.

In the second phase, we will explore whether administration of famciclovir during acute IM is associated with changes in EBV viral load, peripheral immune activation and B-cell repertoire characteristics compared with untreated IM. These analyses are mechanistic and exploratory and are not intended to establish the clinical efficacy of famciclovir for the treatment of IM.

We hypothesise that B-cell and T-cell repertoire characteristics will differ between participants with IM and those with streptococcal pharyngitis or healthy controls.

5.5 Risks/benefits

5.5.1 Benefits

Participants are not expected to receive direct clinical benefit from taking part in this study. Participants in both cohorts may value contributing to research that improves understanding of EBV-associated IM and its immunological effects. Participants in

Cohort 2 will receive famciclovir; however, its clinical effectiveness for treating IM has not been established, and no direct therapeutic benefit can be assured. The findings may contribute to the design of future studies evaluating interventions for IM and EBV-associated disease. Participants may also elect to receive a lay summary of the study findings.

5.5.2 **Risks**

There are small risks associated with venepuncture, which is the primary method of sample collection. These include haematoma formation (bruising), infection, excessive bleeding, pain and allergy to adhesive tape used after venepuncture. There is also a risk of needlestick injury to the person performing the test. However, these risks are rare if the procedure is performed competently. Venepuncture will be performed by an appropriately trained healthcare professional or a person holding an NHS-recognised phlebotomy qualification. Saliva and throat-swab samples will be collected by appropriately trained study personnel in accordance with local procedures.

Subjects with IM participating in cohort 2 or the experimental medicine phase of the study may experience side effects from taking Famciclovir (Famciclovir 500mg film-coated tablets ("Famciclovir 500mg Film-Coated Tablets," n.d.). These include headache and nausea, which are generally mild to moderate and occurred at a similar rate in subjects who received placebo in clinical trials of famciclovir (MHRA Famciclovir SmPC, 01-06-2018). All other adverse reactions were identified during post-marketing and were generally rare. These included thrombocytopenia, confusional state and somnolence (predominantly in the elderly), hallucinations, dizziness, palpitations, nausea, vomiting, abdominal pain, diarrhoea, abnormal liver function tests, cholestatic jaundice, rash, pruritus, angioedema (e.g. face oedema, eyelid oedema, periorbital oedema, pharyngeal oedema) and urticaria. Importantly, anaphylactic shock, anaphylactic reactions and severe skin reactions (e.g. erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, hypersensitivity vasculitis) have not been reported.

6. Study objectives

6.1 Primary objective

To compare deep immunophenotyping and EBV viral loads between subjects with infectious mononucleosis (IM), subjects with antibiotic-treated streptococcal pharyngitis, and healthy subjects who are either EBV-seronegative or EBV-seropositive following asymptomatic EBV exposure in the past.

Hypothesis: Participants with IM will demonstrate distinct CD8+ T-cell immunophenotypes, NK-cell function and B-cell repertoire characteristics, together with higher EBV viral loads, compared with participants with streptococcal pharyngitis and healthy controls.

6.2 Secondary objective

To explore the effects of oral famciclovir (500 mg twice daily for 7 days) on EBV viral load, viral shedding and immunological biomarkers, including B-cell repertoire and immune-cell characteristics, in participants with IM.

Hypothesis: Participants with IM who receive famciclovir will demonstrate lower EBV viral loads and differences in CD8+ T-cell immunophenotypes, NK-cell function and B-cell repertoire characteristics compared with participants with untreated IM.

6.3 Primary endpoint

Longitudinal changes in EBV viral load and deep immunophenotyping measures, including CD8+ T-cell immunophenotypes, NK-cell function and B-cell repertoire characteristics, in participants with IM, streptococcal pharyngitis, healthy EBV-seronegative controls and healthy EBV-seropositive controls.

6.4 Secondary endpoint

Longitudinal changes in EBV viral load, viral shedding and deep immunophenotyping measures, including CD8+ T-cell immunophenotypes, NK-cell function and B-cell repertoire characteristics, in untreated IM compared with famciclovir-treated IM.

7. Study population

This study will recruit 80 participants aged 16 years and over into two sequential cohorts to investigate the virological and immunological features of Epstein-Barr virus (EBV) infection and the potential effects of famciclovir on viral load and immune responses. The study population will comprise individuals with confirmed EBV-associated infectious mononucleosis, individuals with confirmed streptococcal pharyngitis, and healthy volunteers.

Cohort 1 will consist of three participant groups: 20 individuals with confirmed EBV-associated infectious mononucleosis, 20 individuals with confirmed streptococcal pharyngitis, and 20 healthy volunteers. Participants in this cohort will undergo clinical assessment, questionnaire completion, and biological sample collection but will not receive famciclovir. Cohort 2 will consist of 20 individuals with confirmed EBV-associated infectious mononucleosis who will receive a seven-day course of oral famciclovir in addition to the study assessments and biological sampling schedule. Recruitment to Cohort 2 will not commence until recruitment to Cohort 1 has been completed.

Participants with suspected infectious mononucleosis or streptococcal pharyngitis will be identified during routine clinical care in the Emergency Department (ED) and Urgent Treatment Centre (UTC) at Barts Health NHS Trust. Members of the direct clinical care team will undertake the initial identification and screening of potentially eligible patients. Where a patient appears to meet the preliminary screening criteria, the treating clinician will introduce the study and provide the appropriate Participant Information Sheet. The clinician will seek the patient's permission for their contact details and relevant clinical information to be shared with the research team for the purpose of assessing eligibility and discussing potential participation. Only patients who provide permission for research contact will be approached by a delegated member of the research team.

Following confirmation of eligibility, a member of the research team will contact the patient to discuss the study in more detail, answer questions and confirm ongoing interest in participation. Potential participants will be given a minimum of 24 hours to consider the study information before informed consent is sought. Written informed consent will be obtained by an appropriately trained and delegated member of the research team before any study-specific procedures are undertaken.

Healthy volunteers will be recruited from staff, students and the families of staff of Barts Health NHS Trust and Queen Mary University of London through locally approved advertisements, posters, electronic study notices and word-of-mouth referral. Interested individuals will contact the research team directly and will be provided with a Participant Information Sheet before eligibility assessment and consent procedures are completed.

7.1 Inclusion criteria

Inclusion criteria for IM (cohort 1):

- Molecular and/or serological diagnosis of IM with symptom onset within the previous 14 days
- Aged 16 years or over
- Able to provide Informed consent

Inclusion criteria for acute streptococcal pharyngitis:

- Microbiologically-confirmed (culture-positive) diagnosis of acute streptococcal pharyngitis
- Age above 16 years
- Able to provide informed consent

Inclusion criteria for healthy subjects:

- No previous history of IM
- Age above 16 years
- Able to provide informed consent

Inclusion criteria for IM (cohort 2):

- Molecular and/or serological diagnosis of IM with symptom onset in the last 14 days
- Age above 16 years
- Able to provide Informed consent
- Willing to take a course of oral famciclovir 500mg BD for 7 days.

7.2 Exclusion criteria

Exclusion criteria for study subjects:

- Pre-existing autoimmune diseases, including but not limited to type I diabetes mellitus, autoimmune thyroid disease, inflammatory bowel disease, psoriasis, etc.
- Unable to comply with study requirements
- Unable to give informed consent to participate

Additional exclusion criteria for subjects with IM in cohort 2:

- Unwilling to take a 7-day course of famciclovir
- Concomitant treatment with probenicid and raloxifene

- Known contraindication, allergy or hypersensitivity to famciclovir, penciclovir or any component of the formulation
- Pregnancy (self-declaration of pregnancy status and LMP more than 4 weeks or urine HCG test when pregnancy cannot be reasonably excluded)

7.3 Vulnerable participant considerations

(i) Employees and students: Healthy controls may be recruited from staff and students of Barts Health NHS Trust and Queen Mary University of London. Recruitment materials will make clear that participation is entirely voluntary and that deciding not to participate will have no effect on employment, education or access to services.

(ii) Acutely unwell participants: Potential participants with IM or streptococcal pharyngitis may be identified during an acute illness episode. Their clinical care will take priority over research activities, and they will only be approached for consent when appropriately able to consider the study information and make an informed decision regarding participation.

8. Study design

This is a prospective longitudinal study involving serial collection and analysis of peripheral blood, throat swab and saliva samples from three groups of participants in the first cohort (individuals with IM, acute streptococcal pharyngitis and healthy controls), followed by a second cohort comprising participants with IM only. The second cohort of participants will be recruited after recruitment of the first cohort has been accomplished. The second cohort will receive open-label oral famciclovir for 7 days to explore its effects on EBV viral load and immunological biomarkers. Participants with IM and streptococcal pharyngitis will be identified through clinical and laboratory pathways across Barts Health NHS Trust hospitals Tower Hamlets urgent treatment care. Identification will involve review of Emergency Department and Urgent Treatment Centre attendances, Live Emergency Patient Boards, diagnostic requests and results held by the Departments of Virology and Microbiology, laboratory information systems (WinPath), and electronic patient records (Cerner Millennium or equivalent local systems). Identification will be undertaken by appropriately authorised members of the clinical care and research teams operating under agreements with the participating NHS organisations.

EBV serostatus in controls will be assessed. Healthy controls, including staff, students and family members of staff at Barts Health NHS Trust and Queen Mary University of London, will be recruited through internal email newsletters, social media, student bulletins and bulletin boards. Interested individuals will contact the study team through the study email account. Participants with IM or streptococcal pharyngitis may also be provided with study information to share with partners or friends who may be interested in volunteering as healthy controls. Interested volunteers will be provided with the healthy volunteer participant information sheet and, if they remain interested in participating, an appointment will be arranged for informed consent and baseline study procedures.

Appropriately authorised members of the clinical care team and/or research team will provide potential participants with information about the study and ask whether they may be contacted subsequently. Potentially eligible individuals who agree to further

contact may then be contacted by telephone, text or email. The relevant participant information sheet will be provided, and potential participants will have an opportunity to consider participation and ask questions before providing consent. Participants will have at least 24 hours to consider participation and may discuss the study with family members, friends, your GP, or other healthcare professionals if you wish.

Informed consent and baseline study procedures will be scheduled as soon as practicable after the potential participant has received the study information, had the opportunity to ask questions and indicated that they wish to take part. Written informed consent will be obtained before any study-specific procedures are performed.

Participants will also be asked to consent to repeated sampling over 24 months after the first visit. Samples will be collected at baseline and at five follow-up time points: Day 7, Week 4, Month 6, Month 12 and Month 24. At each visit, approximately 60 mL of venous blood will be collected, with a throat swab and a saliva sample. During the follow-up visits, participants will also complete the study questionnaire as detailed in the Schedule of Activities (see Appendix 1 for laboratory samples). The questionnaire will take approximately 10-15 minutes to complete and will be completed during study visits. No special login will be required. Study follow-up visits will take place at Ward 11D (Day Case Unit), Royal London Hospital, Whitechapel, London, or another designated outpatient facility at Barts Health NHS Trust.

Participants will be informed that their decision whether or not to participate in the research will have no effect on their current or future treatment or clinical care.

They will be asked to complete a brief questionnaire regarding their demographics, current medications, and prior medical history. Study samples and research data will be pseudonymised using a unique participant study ID. The participant identification log linking the study ID to identifiable participant information will be stored separately on an access-controlled Barts Health NHS Trust system and will be accessible only to authorised members of the study team. Samples transferred to the Blizard Institute for analysis will be labelled using the study ID and will not contain directly identifying information.

For subjects with IM, IM severity will be assessed using the validated Katz IM Severity Scale (Katz et al. 2019) (Appendix 2). As this is a dynamic scale, it will be updated at visits on days 7, week 4, and month 6. In addition, subjects with IM will be asked whether they experience post-viral fatigue; if so, the severity will be assessed using the short-form DePaul Symptom Questionnaire (SF-DSQ) (Sunnquist et al. 2019) (see Appendix 2 for the questionnaires). These clinical assessments will be exploratory and descriptive and are not intended to assess the clinical efficacy or overall effectiveness of famciclovir in treating IM.

9. Study procedures

9.1 Informed Consent

Written informed consent will be obtained by an appropriately trained and delegated member of the study team before any study-specific procedure is performed.

9.2 Screening and recruitment

Participants with infectious mononucleosis (IM) and bacterial pharyngitis will be identified through routine clinical care pathways at Barts Health NHS Trust and urgent treatment care, Tower Hamlets. Potentially eligible patients may be identified by members of the direct clinical care team through clinical assessment, diagnostic investigations, and laboratory test requests and results processed by the Departments of Virology and Microbiology and associated laboratory information systems. Where a patient appears to meet the preliminary eligibility criteria, a member of the clinical care team will introduce the study, provide the appropriate Participant Information Sheet (PIS), and seek permission for their contact details and relevant clinical information to be shared with the research team for eligibility assessment and discussion of potential participation.

Patients who provide permission for further contact will subsequently be contacted by a delegated member of the research team, who will discuss the study in detail, answer any questions, and confirm eligibility.

Healthy controls, including employees, students and family members of staff at Queen Mary University of London (QMUL) and Barts Health NHS Trust, will be recruited through approved recruitment materials, including internal email newsletters, student bulletins, social media advertisements and notice boards. Participants with IM or bacterial pharyngitis may also be provided with study information to share with friends, partners or family members who may be interested in volunteering as healthy controls. Interested individuals will contact the study team directly and will be provided with the Healthy Volunteer Participant Information Sheet. Eligible volunteers who wish to participate will be invited to attend a study visit for informed consent and baseline assessments.

All potential participants will be given a minimum of 24 hours to consider participation and discuss the study with others if they wish before written informed consent is obtained.

9.3 Randomisation procedures (blinding, unblinding, etc.)

N/A

9.4 Study interventions

Participants in Cohort 2 will receive oral famciclovir 500 mg twice daily for 7 days. Famciclovir is being used as an experimental intervention to explore effects on EBV viral load and immunological biomarkers and not to assess its clinical efficacy for the treatment of IM.

Study drugs

A course of oral famciclovir 500mg twice daily for 7 days.

9.5 Criteria for discontinuation

• The participant develops an adverse reaction or intolerance for which the responsible clinician considers discontinuation of famciclovir clinically appropriate.

• The participant develops a clinical condition or contraindication for which continued administration of famciclovir is considered inappropriate.

• The participant chooses to discontinue famciclovir.

• The participant withdraws consent for participation in the study.

9.6 Procedure for collecting data

9.6.1 Screening and recruitment

Potential study participants with IM and bacterial pharyngitis will be identified across Barts Health NHS Trust hospitals and collaborating NHS organisations within the Northeast London region Trust laboratory records (WinPath) and electronic patient records (Cerner Millennium). Potentially eligible individuals will be contacted in accordance with the recruitment procedures described in Section 9.2 to explain the study and determine their interest in participating. Interested individuals will be provided with the relevant participant information sheet and given an opportunity to consider participation and ask questions before providing written informed consent.

9.6.2 Informed consent

Written informed consent and baseline sample collection for participants with IM and bacterial pharyngitis will be scheduled as soon as practicable after the participant has had sufficient opportunity to consider the study information and ask questions. Consent will include consent for linkage to relevant electronic health records held by participating NHS organisations, where applicable. Healthy volunteers will provide written informed consent before any study-specific procedures are performed. Informed consent and sample collection will take place the outpatient departments of participating hospitals.

9.7 Procedures

Once written consent is obtained, participants will be asked to complete a questionnaire and to have a 60 mL blood sample, a throat swab, saliva samples collected. A trained health care professional will take the blood sample with experience in venepuncture. Pseudonymised blood samples will be transported to the Blizard Institute by hand. Samples will then undergo processing and storage, followed by sorting and analysis by flow cytometry, real-time PCR, and similar methods. Participants with IM in Cohort 2 will provide written informed consent using the Cohort 2 consent form and will be supplied with sufficient famciclovir to administer 500 mg orally twice daily for 7 days. Participants will be provided with instructions on how to take the medication.

9.8 Laboratory assessments

Blood samples will be processed according to a rigorous protocol to isolate peripheral blood mononuclear cells (PBMCs). PBMCs will be stained using antibodies and analysed using Fluorescence-Activated Cell Sorting (FACS). T and B cell populations will be quantified using specific antibodies to stain surface antigens. Saliva samples will be processed using standard techniques to extract DNA.

9.8.1 EBV detection and viral loads

PBMCs will be used to isolate aliquots of total B-cells and memory B-cells using FACS-sorted cells. DNA will be extracted from saliva, PBMCs, total B-cells, and sorted memory B-cells for EBV detection using a standard in-house quantitative RT-PCR assay (Holden et al. 2018) and a new-generation micro-drop PCR method (Shuto et al. 2019).

9.8.2 Plasma and Serum

Aliquots of plasma and serum will be prepared and stored for subsequent biomarker analyses. PBMCs will be cryopreserved and stored for subsequent analyses. RNA isolated from sorted B cells will also be stored for subsequent analysis.

9.8.3 B-cell and T-cell repertoire analysis

Antibody genes will be amplified by nested PCR following a published protocol (King et al. 2021). Antibody genes will be sequenced using high-throughput Illumina NextSeq (300 bp paired-end reads).

9.9 Follow-up procedures

Study participants will be followed up at predefined intervals according to the Schedule of Activities (Appendix 3) at Day 7, Week 4, Month 6, Month 12 and Month 24. At each follow-up visit, repeat venous blood, throat swab and saliva samples will be collected, together with the applicable study assessments.

9.10 Laboratory assessments (see more in the laboratories section)

Laboratory assessments will be performed as described in Section 9.8 and its subsections.

9.11 Radiology assessments

N/A

9.12 Participant withdrawal

Study subjects may withdraw their consent at any time. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

10. Schedule of assessments

10.1 Cohort 1

Study activities	Screening	Baseline (D0)	Visit 1 (D7±3)	Visit 2 (D28±4)	Visit 3 (D183 ± 21)	Visit 4 (D365 ± 28)	Visit 5 (Day730 ± 28)
Initial contact	X						
Informed consent		X					
Eligibility confirmation		X					
Demographics		X					
Concomitant medications		X					

Medical History		X					
Katz IM severity scale		X	X	X	X		
SF-DSQ post-viral fatigue assessment (IM only, if applicable)		X	X	X	X		
Throat swab		X	X	X	X	X	X
Saliva collection		X	X	X	X	X	X
PBMC bloods		X	X	X	X	X	X
Plasma and serum bloods		X	X	X	X	X	X
B-cell and T-cell repertoire analysis		X	X	X	X	X	X

10.2 Cohort 2

Study activities	**Screening**	**Baseline** (D0)	**Visit 1** (D7±3)	**Visit 2** (D28±4)	**Visit 3** (D183 ± 21)	**Visit 4** (D365 ± 28)	**Visit 5** (Day730 ± 28)
Initial contact	X						
Informed consent		X					
Eligibility confirmation		X					
Demographics		X					
Concomitant medications		X					
Medical History		X					
IM anti-viral medication		X					

Katz IM severity scale		X	X	X	X		
SF-DSQ post-viral fatigue assessment (IM only, if applicable)		X	X	X	X		
Saliva collection		X	X	X	X	X	X
Throat swab		X	X	X	X	X	X
PBMC bloods		X	X	X	X	X	X
Plasma and serum bloods		X	X	X	X	X	X
B-cell and T-cell repertoire analysis		X	X	X	X	X	X

11. End of study

The planned recruitment target for Cohort 1 is 20 participants with EBV-associated infectious mononucleosis (IM), 20 participants with streptococcal pharyngitis, and 20 healthy control participants. The planned recruitment target for Cohort 2 is 20 participants with IM treated with famciclovir. These represent the minimum recruitment goals. Sufficient funding is secured to support the planned recruitment targets and complete participant follow-up over the full 36-month study duration. Additional participants may be recruited within the planned 12-month recruitment period, provided sufficient funding and resources remain available to support their participation and complete follow-up. Recruitment is planned to be completed within 12 months of study commencement, with each participant followed for 24 months after enrolment. The study will end when the last participant completes their final follow-up visit, no later than 36 months after study commencement.

12. Assessment and management of risk

The study involves the collection of blood, throat swab, and saliva samples from participants with infectious mononucleosis (IM), streptococcal pharyngitis and healthy controls. The principal risks associated with participation are those related to repeated venepuncture, throat swab collection, and administration of famciclovir in Cohort 2, confidentiality of personal information, and the potential discovery of clinically significant incidental findings. Data collection procedures and study assessments are otherwise considered low risk.

12.1 Risks associated with venepuncture, throat swab and saliva collection

Blood sampling (venepuncture) may cause discomfort, pain, bruising (haematoma formation), excessive bleeding, infection, vasovagal episodes or, rarely, allergic reactions to dressings or adhesive tapes. Participants will undergo repeated blood sampling throughout the study follow-up period. Throat swab collection is a routine, minimally invasive procedure but may cause temporary discomfort, gagging, coughing, throat irritation, watering of the eyes, or mild nausea. Rarely, the procedure may result in minor mucosal irritation or a transient vasovagal response in susceptible individuals. There are no known significant risks associated with saliva collection. Venepuncture and throat swab will be performed by suitably trained healthcare professionals or personnel holding an NHS-recognised training and competency certification. All procedures will be undertaken within a clinical environment in accordance with local infection prevention and control policies. Hypoallergenic dressings will be used where appropriate and participants will be monitored for adverse effects following blood sampling.

12.2 Risks associated with famciclovir administration

Participants enrolled in Cohort 2 will receive oral famciclovir 500 mg twice daily for seven days. Famciclovir is licensed for the treatment of herpes simplex virus and varicella zoster virus infections but is not licensed for the treatment of infectious mononucleosis. Potential adverse effects include headache, nausea, dizziness, diarrhoea, abdominal discomfort and skin rash. Rare adverse reactions include thrombocytopenia, confusion, somnolence, hallucinations, abnormal liver function tests, angioedema and urticaria. Participants will be provided with written and verbal information regarding the potential adverse effects of famciclovir prior to consent. A medical history and concomitant medication review will be undertaken before enrolment. Participants receiving probenecid or raloxifene, which may interfere with famciclovir metabolism, will be excluded from participation. Adverse events will be monitored and recorded throughout the study. Any serious adverse events will be managed and reported in accordance with the study pharmacovigilance procedures and sponsor requirements.

12.3 Risks associated with genetic and immunological analyses

The study includes immunological and genetic analyses of participant samples. There is a small possibility that clinically important incidental findings may be identified. Participants will provide specific consent for genetic analyses. Any clinically significant findings identified during the study will be reviewed by the study investigators and managed according to the protocol. Where appropriate, participants will be informed and referred for further assessment through NHS clinical services.

12.4 Risks to confidentiality and data security

The collection of personal, clinical and genetic information introduces a small risk of unauthorised disclosure of participant information. Participant information will be pseudonymised using a unique study identifier. Personal identifiers and research data will be stored separately on secure password-protected NHS and QMUL systems. Access will be restricted to authorised study personnel only. All study data will be handled in accordance with the UK General Data Protection Regulation (UK GDPR), the Data Protection Act 2018 and local information governance policies.

12.5 Psychological and emotional risks

Participants may experience minor inconvenience or anxiety associated with blood sampling, completion of questionnaires, or discussion of medical history and illness-related symptoms. The level of psychological burden is considered low. Participants will be informed that they may decline to answer any question they find uncomfortable and may withdraw from the study at any time without affecting their clinical care. Study staff will be available to address any concerns raised during study procedures.

12.6 Risks to researchers

Research staff performing venepuncture and handling biological specimens may be exposed to blood-borne pathogens and needle-stick injuries. All blood sampling and specimen processing will be conducted by appropriately trained personnel using standard infection prevention procedures, personal protective equipment, approved sharps disposal containers and local biosafety protocols. Staff will receive training in safe handling of biological materials and management of occupational exposures.

12.7 Radiation exposure

No ionising radiation procedures are undertaken as part of this study and therefore there are no radiation-related risks.

12.8 Financial risks

Participants Participants will not incur any study-related costs. Participation may require time and travel to attend study visits; however, no additional financial burden is anticipated.

12.9 Oversight and monitoring

Given the exploratory biomarker-focused nature of the study, the limited duration of famciclovir administration and the established safety profile of famciclovir, a separate Data Monitoring Committee is not considered necessary.

13. Statistical considerations

This is a longitudinal study with up to six repeated measurements per subject (baseline D0, and follow-up at D7, D28, D183, D365 and D730). Analyses must therefore account for within-subject correlation across repeated visits, permit valid inference in the presence of intermittent missing data, and support estimation of (a) within-group change from baseline to end of study, and (b) between-group differences in the trajectory of change. Cross-sectional group comparisons at individual timepoints (e.g. one-way ANOVA) are not appropriate as the primary analysis because they discard within-subject correlation, inflate the family-wise type I error rate through repeated testing, and exclude subjects with any single missing visit.

13.1 Sample size

Primary power calculation. For a comparison of EBV viral loads between two groups at Day 7 (subjects with untreated IM compared to subjects with famciclovir-treated IM), 13 subjects per group will have greater than 90% power to show an increase of the RT-PCR Ct value (an indicator of viral load) from 20 to 25, with a standard deviation of 4 and a significance level (alpha) of 0.05. Allowing for a dropout rate over the follow-up period, we propose enrolling 20 subjects per arm.

The longitudinal mixed-model analysis (see below) is expected to increase precision relative to a two-sample cross-sectional test at Day 7 by borrowing strength across the correlated follow-up visits. The above power calculation is therefore conservative for the primary between-group comparison. The other biomarker analyses are exploratory: similar immunological biomarker studies require at least 12 patients per group to achieve face validity, and aiming for 20 subjects per group will account for potential dropouts over the 24-month collection period.

Cohort 1 will comprise 20 subjects with IM, 20 with streptococcal pharyngitis and 20 healthy controls (n = 60). Cohort 2 will comprise 20 subjects with IM treated with famciclovir.

13.2 Method of analysis

13.2.1 Overview and analysis populations

Descriptive statistics will be used to characterise each cohort at baseline (demographics, IM severity by Katz score, SF-DSQ if applicable, concomitant medications). Continuous variables will be summarised as mean ± SD or median (IQR) as appropriate; categorical variables as n (%). Baseline comparability of groups will be described but not tested by hypothesis tests (per CONSORT guidance).
Two analysis populations are defined:

- All-participants population: all subjects with a baseline sample and at least one post-baseline measurement (primary analysis).
- Per-protocol population (cohort 2): all cohort-2 subjects who completed at least 5 of 7 famciclovir doses and provided the Day 7 sample within the ±3-day visit window (sensitivity analysis).

13.2.2 Primary analysis: linear mixed-effects model (MMRM)

For each continuous longitudinal outcome (log-transformed EBV viral load in copies/ml, immune-cell subset frequencies as proportions, repertoire diversity metrics,

etc.), the primary analysis will be a linear mixed-effects model — specifically a Mixed Model for Repeated Measures (MMRM) — implemented in SPSS via the MIXED procedure (or equivalently R lme4::lmer with lmerTest, or SAS PROC MIXED). The MMRM approach is the current regulatory standard for longitudinal continuous endpoints and is preferred over classical repeated-measures ANOVA because it uses all available observations from each subject, provides valid inference under the Missing At Random (MAR) assumption, and explicitly accounts for the correlation between repeated measurements within each subject.

Model specification (three-group cohort 1 comparison):

- Fixed effects: group (IM vs streptococcal vs healthy), visit (categorical: D0, D7, D28, D183, D365, D730), and the group × visit interaction. Pre-specified covariates: age at baseline and sex.
- Random effects: random intercept per subject (a random slope on time may be added if the model converges and improves fit by AIC/BIC).
- Residual covariance structure: unstructured by default. If the unstructured model fails to converge with the available sample size (n = 20 per group), a parsimonious alternative will be used — either first-order autoregressive AR(1), Toeplitz, or heterogeneous compound symmetry — selected by lowest AIC/BIC among converged models.
- Degrees of freedom: Kenward-Roger approximation.
- Baseline handling: constrained longitudinal data analysis (cLDA) — baseline (D0) is modelled jointly with follow-up visits as an outcome, with a constraint that group means are equal at baseline. This is preferred over ANCOVA-on-change-from-baseline for observational cohorts because it uses the baseline value efficiently and avoids regression-to-the-mean bias.

The primary omnibus test is the F-test of the group × visit interaction, which asks whether the mean trajectory differs across the three groups. Where the interaction is significant ($p < 0.05$), pre-specified between-group contrasts at each follow-up visit will be estimated using estimated marginal means (EMMs) with 95% confidence intervals. Within-group change from baseline at each visit will also be estimated from the EMMs, with 95% CIs.

13.2.3 Cohort 2 analysis (famciclovir-treated IM vs untreated cohort-1 IM)

The same MMRM specification will be applied, restricted to the two IM groups (n = 40). The primary between-group contrast at Day 7 — matching the power calculation — is estimated as a pre-specified linear combination of fixed-effect coefficients within the single model; a separate two-sample test is not required. Secondary comparisons at D28, D183, D365 and D730 will be reported with 95% CIs and interpreted as exploratory.

13.2.4 Non-continuous and specialised outcomes

Generalised linear mixed models (GLMMs) will be used for outcomes where a Gaussian model is inappropriate: negative-binomial GLMMs for count outcomes (e.g. absolute viral copy numbers analysed without log-transformation), and binomial GLMMs (logit link) for binary outcomes (e.g. proportion of subjects with detectable salivary EBV at each visit). Fixed and random effect structures mirror the MMRM.

B-cell and T-cell repertoire data will be reduced per subject per visit to interpretable summary metrics — Shannon diversity index, Gini clonality index, IgG/IgM class-switch fraction, VH-gene usage entropy, mean CDR3 length, and mutation frequency — and each summary metric will be analysed with the MMRM framework above. High-dimensional multivariate comparisons of repertoire composition will use PERMANOVA (adonis2) on Bray-Curtis or Jensen-Shannon distances, with permutations restricted within subjects to preserve the repeated-measures structure. Downstream analyses will follow the established bioinformatics pipeline (King et al. 2021).
Non-parametric mixed models (e.g., nparLD in R) will be used as a robustness check when residuals remain severely non-normal despite log-transformation.

13.2.5 Missing data

The MMRM approach yields valid inference under the Missing At Random (MAR) assumption — i.e. the probability of a missing value at a given visit may depend on observed values but not on unobserved values conditional on the observed. Missing data patterns and reasons for withdrawal will be tabulated and reported. Where dropout exceeds 20%, the following sensitivity analyses will be conducted:

- Multiple imputation (MI) with chained equations, generating 20 imputed datasets and pooling estimates via Rubin's rules.
- Pattern-mixture models under plausible Missing Not At Random (MNAR) assumptions (e.g. tipping-point / delta-adjustment analyses in which imputed values in the intervention arm are progressively worsened to identify the smallest shift that would overturn the primary conclusion).
- Complete-case analysis reported alongside the primary MMRM result.

13.2.6 Sensitivity and supportive analyses

- Generalised estimating equations (GEE) with an exchangeable or AR(1) working correlation and robust (sandwich) standard errors, providing population-averaged estimates that are robust to covariance-structure misspecification. Note that GEE requires the stronger MCAR assumption to be valid.
- Per-subject area-under-the-curve (AUC) of viral load across follow-up computed by the trapezoidal rule, then compared between groups by a single one-way ANOVA (parametric) or Kruskal-Wallis test (non-parametric), as a simple summary of cumulative exposure.
- Per-protocol re-analysis for cohort 2 (adherence-defined population, see above).
- Analyses stratified or adjusted by baseline IM severity (Katz score) and time from symptom onset to enrolment.

13.2.7 Multiple comparisons

The primary endpoint (EBV viral load) has a single pre-specified test per pairwise contrast; no correction is applied. Between-group contrasts across the multiple follow-up visits within a single MMRM model are protected by the omnibus interaction F-test. For the exploratory panel of immunological endpoints, the false discovery rate will be controlled at $q < 0.05$ using the Benjamini-Hochberg (BH) procedure applied across

the family of endpoints within each analysis question. Bonferroni-type family-wise adjustments are considered overly conservative for exploratory biomarker discovery in a study of this size.

13.2.8 Effect estimates and reporting

Results will be reported primarily as adjusted mean differences (or ratios, for log-transformed outcomes back-transformed to fold-change) with 95% confidence intervals at each follow-up visit, alongside the omnibus interaction p-value. P-values less than 0.05 will be described as statistically significant for the pre-specified primary contrasts. Reporting will follow the STROBE guideline for observational cohorts and adapt CONSORT-style presentation for the cohort-2 interventional component. Analyses will be pre-specified in a Statistical Analysis Plan (SAP) finalised prior to database lock.

13.2.9 Model diagnostics and assumption checks

- Residual plots (standardised residuals vs fitted values; residuals vs visit) to assess homoscedasticity.
- Q-Q plots of residuals and of empirical Bayes random-effect estimates to assess normality assumptions.
- Comparison of AIC/BIC across candidate residual covariance structures.
- Log-transformation of skewed continuous outcomes (viral load in copies/ml) verified by residual normality; alternative transformations (e.g. Box-Cox) considered if residuals remain non-normal.
- Influence diagnostics (Cook's distance analogue for mixed models) to identify high-leverage subjects.

14. Ethics

Ethical approval for this study will be sought from an appropriate UK Research Ethics Committee (REC) through the Integrated Research Application System (IRAS) prior to commencement of participant recruitment. The study will be conducted in accordance with the principles of the Declaration of Helsinki, the UK Policy Framework for Health and Social Care Research, Good Clinical Practice (GCP), the Data Protection Act 2018 and the UK General Data Protection Regulation (UK GDPR).

Informed consent

All participants will provide written informed consent before any study-specific procedures are performed. Participants will be provided with Participant Information Sheet and given adequate time to consider participation and ask questions. Participation is entirely voluntary, and participants may withdraw from the study at any time without affecting their current or future clinical care.

Recruitment

Participants with infectious mononucleosis (IM) and streptococcal pharyngitis will be identified through routine clinical and laboratory pathways within Barts Health NHS

Trust. Healthy controls will be recruited through advertisements and voluntary self-referral. Recruitment procedures have been designed to minimise coercion or undue influence. Participants will only be approached when clinically appropriate and able to make an informed decision regarding participation.

Inclusion and exclusion criteria

Eligibility criteria have been selected to ensure participant safety and scientific validity. Participants unable to provide informed consent will not be enrolled. Individuals with pre-existing autoimmune diseases and, for Cohort 2, participants receiving medications known to interact with famciclovir, will be excluded to reduce potential confounding and minimise risks associated with participation.

Study design

This is a prospective longitudinal study involving serial collection of blood, throat swab and saliva samples over a 24-month follow-up period. An additional cohort of participants with IM will receive oral famciclovir for seven days as an experimental intervention. The study is designed to investigate EBV viral load and immunological biomarkers and is not intended to establish the clinical efficacy, safety or overall effectiveness of famciclovir in the treatment of IM or EBV infection. Future studies would be required to address these questions.

Risks, burdens and benefits

The principal risks associated with participation relate to venepuncture, throat swab collection, administration of famciclovir in Cohort 2, confidentiality of personal information, and the possibility of identifying clinically significant incidental findings. Measures are in place to minimise these risks, including performance of procedures by appropriately trained personnel, adherence to infection prevention procedures, pharmacovigilance monitoring and secure data management.

Participants are unlikely to receive direct clinical benefit from participation. However, the knowledge generated may improve understanding of the role of EBV in infectious mononucleosis and immune dysregulation and may inform future studies of therapeutic interventions.

Confidentiality and data protection

Participant information will be pseudonymised using a unique study identifier. Personal identifiers and research data will be stored separately on secure password-protected NHS and Queen Mary University of London systems with access restricted to authorised study personnel. Data will be handled in accordance with UK GDPR, the Data Protection Act 2018 and local information governance policies.

Genetic and immunological analyses

The study includes genetic and immunological analyses of biological samples. Participants will provide specific consent for these analyses. We will carry out genetic tests and tests of your immune system on your samples for research purposes. These tests may occasionally identify an unexpected finding that could be important for your health. This is called an "incidental finding". If this happens, the study investigators will review the finding to assess its potential importance. If they consider that it should be shared with you, a member of the study team will contact you and explain what it may mean. Where appropriate, you may be referred to NHS services for further assessment.

Conflicts of interest

The investigators have no known financial or commercial conflicts of interest relating to the conduct of this study. The study is funded by the Horne Family Foundation, which will have no role in participant recruitment, data collection, data analysis, interpretation of results or publication of findings.

15. Public Involvement

As IM is a monophasic, usually self-limiting illness, we have not actively involved patients with acute IM in the design or management of the study. However, we have engaged the broader multiple sclerosis community on the importance of EBV and IM in the pathogenesis of MS and other autoimmune diseases and have received active feedback and support via our online communication channels (see https://gavingiovannoni.substack.com/). The grant supporting this study was obtained through one of our online public engagement campaigns focused on EBV and IM as preventive strategies for MS and other EBV-associated autoimmune diseases. After a public communication programme about this study, we completed an online survey of people with MS and received 64 responses. 98.4% supported the need for this study, and 76% of respondents did not think 5 study visits over 2 years would be an obstacle to participation. 86% stated that if they had acute IM, they would be prepared to take an antiviral medication to see its effects on EBV and the immune system response to EBV. Importantly, 86% said that if they had IM and were approached, they would volunteer to participate in the study.

Regarding dissemination of findings, when we obtain informed consent, we will ask each volunteer whether they would like to receive a lay summary of our findings at the end of the study. If this is the case, we will make a note of it and, upon conclusion of the study, send them a letter explaining the study outcome in lay terms. We will also post a summary of the results online and disseminate links to the results via other social media channels (BlueSky Social, LinkedIn and Facebook).

16. Data handling and record keeping

16.1 Data management

For subjects with IM and acute pharyngitis, demographic data and contact details will be accessed via secure NHS computers, and no identifiable information will be

transferred outside of these computers, i.e., name, date of birth, and address.
Participants (subjects with IM and bacterial pharyngitis and healthy controls) will be assigned a unique study identifier (pseudonymisation). The code-break to identify the participant from the unique study identifier will be stored in a password-protected spreadsheet on a secure NHS trust computer, accessible only to the study investigators. For healthy subjects, their names and contact details will be kept in the same spreadsheet stored on secure NHS trust computers, accessible only to the study investigators. This is to allow the research team to contact healthy study subjects for repeat sampling. Contact details for people with IM and acute pharyngitis will not be recorded on the spreadsheet, as this information is already available in the Barts Health NHS Trust electronic patient records.

Once data is pseudonymised using the unique study identifier, it will be transferred to QMUL university computers based in the Blizard Institute for data analysis. Data used on non-NHS systems, e.g. laboratory computers for data analysis, will be pseudonymised to protect confidentiality.
All NHS investigators undergo regular training on data confidentiality and information governance, in line with the trust's confidentiality policy. All investigators will be certified in Good Clinical Practice or the UK policy framework for health and social care research by the start date of the project. All laboratory data generated by the study will be stored securely on the Blizard Institute QMUL computers.

16.2 Source data

Participant questionnaires
Lab-generated data

16.3 Confidentiality

Participants will be identified from Barts Health NHS Trust, which is kept on the Cerner Millennium clinical record system. Patients will be required to provide informed consent for investigators to access their clinical records.
Healthy controls will be approached via email newsletters and internal advertising bulletin boards. They will contact us via a general study email account. People with IM and acute pharyngitis volunteering for the study will be asked if they have partners and friends who would be willing to volunteer for the study.
Participants will be pseudoanonymised by a unique code that will be used for their blood samples and any information collected about them. Only the study investigators will have access to the break code, which will be stored on a password-protected spreadsheet held on a secure NHS trust computer. No identifiable participant data will be taken off secure NHS computers.

16.4 Record Retention and Archiving

When the research trial is complete, as per the requirements of the UK policy framework for health and social care research and sponsor policy, the records will be kept for a further 25 years.

17. Laboratories

17.1 Central and local laboratories

Blizard Institute
Barts and the London School of Medicine and Dentistry
Queen Mary University of London
4 Newark St
London
E1 2AT

17.2 Sample collection and preparation

Blood sample collection will take place in Ward 11D, the daycase unit, at The Royal London Hospital. Patients will have been required to give written consent. Blood will be collected in appropriate vacutainer, throat swab and sputum collection tubes. The tubes will be labelled with the anonymised participant number. Samples will then be transported to the Blizard Institute by a member of the research team for processing and storage.

17.3 Laboratory procedures

Procedure	Pre-procedure storage	Timing of procedure
Mononuclear peripheral monocyte isolation and flow cytometry	N/A	On receipt of blood samples in the Blizard Institute
Real-time PCR - cell-free plasma, PBMCs, B-cells, memory B-cells, T-cells and saliva	-80ºC freezers in the Blizard Institute	This will be done in batches after processing several participants' samples

17.4 Sample storage and transfer

Samples will be stored in -80 °C freezers or, in the case of PBMCs in liquid nitrogen. At the end of the study, residual samples will be transferred into an HTA-licensed biobank.

18. Interventions and tools

18.1 Devices

Not applicable

18.2 Techniques and interventions

Venepuncture

18.3 Tools

- Katz Severity of Infectious Mononucleosis Scale, consists of 17 items (Katz et al. 2019)
- DePaul Symptom Questionnaire, consists of 14 items (SF-DSQ) (Sunnquist et al. 2019)

18.4 Medicinal product

Name: Famciclovir 500 mg film-coated tablets.

Indication: Famciclovir is licensed for the treatment of infections caused by herpes simplex virus (HSV) and varicella zoster virus (VZV). In this study, famciclovir will be used to investigate whether reducing Epstein-Barr virus (EBV) replication alters EBV viral shedding and the associated immune response in subjects with infectious mononucleosis (IM).

Licensing status: Famciclovir is a licensed medicinal product in the United Kingdom for the treatment of HSV and VZV infections. It is not licensed for the treatment of infectious mononucleosis and is therefore being used off-label in this study.

Dosing schedule: Participants enrolled into Cohort 2 will receive famciclovir 500 mg orally twice daily for 7 days (14 doses in total). Treatment should commence as soon as possible following enrolment and consent.

Route of administration: oral administration

Source of Medicinal Product: Famciclovir will be obtained through standard NHS prescribing and dispensing arrangements or an approved NHS pharmacy supplier. The study medication will be prescribed by an appropriately qualified clinician and dispensed according to NHS procedures.

Storage and dispensing: Famciclovir tablets will be stored and dispensed in accordance with the manufacturer's recommendations and local pharmacy procedures. Study participants will be provided with a 7-day supply of medication and instructed on the correct administration, storage and handling of the medication. Participants will be advised to store the medication in its original packaging and keep it out of the reach of children.

Contraindications: Participants will not receive famciclovir if they:

- Are unwilling to take a 7-day course of famciclovir.
- Are receiving concomitant treatment with probenecid or raloxifene.
- Have any known contraindication, allergy or hypersensitivity to famciclovir, penciclovir, or any component of the formulation.

Side-effects: The most reported adverse effects of famciclovir are headache and nausea. These effects are generally mild to moderate in severity and have been reported at rates like those observed in participants receiving placebo in clinical studies. Other adverse effects that have been reported during routine clinical use are generally uncommon and include dizziness, vomiting, abdominal pain, diarrhoea, rash,

pruritus, palpitations, abnormal liver function tests, cholestatic jaundice, thrombocytopenia, confusional states, somnolence (predominantly in older adults), hallucinations, angioedema and urticaria. No cases of anaphylactic shock, anaphylactic reactions, Stevens-Johnson syndrome or toxic epidermal necrolysis have been reported in the referenced product information. Participants will be informed of the potential adverse effects of famciclovir during the consent process and will be monitored for adverse events throughout the study.

18.5 Other biological or chemical products

Not applicable

19. Safety reporting

19.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a participant to whom an intervention has been administered, including occurrences which are not necessarily caused by or related to that intervention. An AE can therefore be any unfavourable or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with study activities.
All Adverse Events (AEs) and Adverse Reactions (ARs) occurring during study participation will be recorded in the study documentation and reviewed by the study team. Particular attention will be paid to events related to study procedures, including venepuncture, saliva collection, and, for participants enrolled in Cohort 2, treatment with oral famciclovir.

19.2 Adverse Reaction (ARs)

An AR is any untoward and unintended response in a participant to an intervention. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to the intervention qualify as adverse reactions. The expression ‘reasonable causal relationship’ means in general that there is evidence or an argument to suggest a causal relationship.

19.3 Notification and reporting of Adverse Events and Reactions

If the AE is not defined as serious, the AE will be recorded in the study documents and the participant followed up by the research team. The AE will be documented in the participants’ source documents, the Case Report Form (CRF), and, where appropriate, medical records.

19.4 Serious Adverse Events (SAEs) or reactions

A serious adverse event (SAE) is defined as an untoward occurrence that:

- Results in death,
- Is life-threatening,
- Requires hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity,
- Consists of a congenital anomaly or birth defect, or
- Is otherwise considered medically significant by the investigator.

SARs will be reported to the REC where in the opinion of the CI the event was serious and:

- Related (it may have resulted from administration of any of the research interventions), and
- Unexpected (the type of event is not listed in the protocol or other Reference Safety Information as an expected occurrence).

19.5 Notification and reporting of Serious Adverse Events

Serious Adverse Events (SAEs) that are considered to be 'related' and 'unexpected' will be reported to the sponsor within 24 hours of learning of the event, and to the REC within 15 days in line with the required timeframe. The CI or delegate will be responsible for reporting of such events to the REC.
The treatment code for the participant will be broken when reporting an 'unexpected and related' SAE. The unblinding of individual participants by the PI / CI in the course of a clinical study will only be performed if necessary for the safety of the study participant.

19.6 Urgent Safety Measures

The CI will take urgent safety measures if necessary to ensure the safety and protection of the clinical study participant from immediate hazards to their health and safety. The measures will be taken immediately. The approval of the REC prior to implementing urgent safety measures is not required. However the CI will inform the sponsor and REC (via telephone) of this event immediately.

The CI will inform the REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office (JRMO)) will be sent a copy of the correspondence with regards to this matter.

19.7 Overview of the Safety Reporting responsibilities

The CI is the medical assessor on behalf on the sponsor and will review all events reported. The CI will ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

20. Monitoring and auditing

The sponsor or delegate retains the right to audit any study, study site, or central facility. Any part of the study may be audited by the funders, where applicable.

21. Trial committees

The research team and the CI will review the study and will undergo peer review in accordance with the requirements of QMUL and JRO. As this is an experimental biomarker, with a relatively safe drug that is now generic with well-established safety protocol, data monitoring and steering committees are not necessary

22. Finance and funding

A grant from the Horne Family Foundation funds this study.

23. Insurance and Indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study, as well as "No-Fault Compensation" for participants, which provides an indemnity for negligent and non-negligent harm.

24. Dissemination of research findings

- Patients will be asked at the time of consent whether they would like to be informed of the study outcome. If they request to be notified, they will be contacted by post with a lay summary of the trial findings when the study has been concluded.
- Depending on the study outcome, it is likely to be presented at internal, national, and international scientific meetings and published in a peer-reviewed scientific journal. In addition to scientific dissemination, the findings will be shared through several public engagement channels run by the study team (Annual Barts Health Research Day, and other social media channels).

Appendices
Appendix 1 - Laboratory samples

Appendix ??

Katz Severity of Infectious Mononucleosis Scale (Katz et al. 2019)

Severe symptoms[a]

_____ Pharyngitis to the point where can't swallow even liquids

_____ Headache so severe as to prompt an LP or neuroimaging

_____ Fever > 40ºC for < 2 weeks

_____ Fever > 40ºC for > 2 weeks

_____ Fever > 38.3ºC for > 5 weeks

_____ Not able to leave home during worst symptoms

_____ Reduced walking distance during worst symptoms

_____ Trouble breathing

_____ GI symptoms (anorexia, nausea, vomiting: NOT Diarrhea alone)

Severe Physical Examination findings[a]:

_____ Jaundice

_____ Bull neck (prominent, warm, tender bilateral cervical adenopathy with edema)

_____ Painfully enlarged spleen

_____ Painfully enlarged liver

_____ Palatal petechiae

Complications[b]:

_____ Cardiac (e.g., myocarditis)

_____ Hematological (e.g., thrombocytopenia (<150,000/mm3), neutropenia (<1000/mm3), lymphopenia (<2000/mm3), hemolytic anemia, hemophagocytic lymphohistiocytosis)

_____ Neurological (e.g., meningoencephalitis)

_____ Pulmonary (e.g., pleural effusion, pneumonitis)

Scoring

[a]Each sign, symptom or complication present scores a 1

[b]Do not count the same sign or symptom more than once (e.g., if trouble breathing due to pleural effusion, count one or the other but not both)

Appendix 2 - DePaul Symptom Questionnaire (SF-DSQ) (Sunnquist et al. 2019)

For the following 14 questions, please rate how often you have had each symptom (frequency) and how much each symptom has bothered you (severity) over the last 6 months.

	Symptom	Frequency	Severity
1	Fatigue	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
2	Next-day soreness after non-strenuous activities	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
3	Minimum exercise makes you physically tired	0: None of the time 1: A little of the time	0: Symptom not present

		2: About half of the time 3: Most of the time 4: All of the time	1: Mild 2: Moderate 3: Severe 4: Very severe
4	Unrefreshing sleep	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
5	Muscle pain	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
6	Bloating	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
7	Problems remembering things	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe

8	Difficulty paying attention for a long period of time	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
9	Irritable bowel problems	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
10	Feeling unsteady on your feet, like you might fall	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
11	Cold limbs	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
12	Feeling hot or cold for no reason	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe

13	Flu-like symptoms	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe
14	Some smells, foods, medications, or chemicals make you feel sick	0: None of the time 1: A little of the time 2: About half of the time 3: Most of the time 4: All of the time	0: Symptom not present 1: Mild 2: Moderate 3: Severe 4: Very severe

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