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**STUDY PROTOCOL**

# Characterization of Chemokine Receptor Expression in CD8+ T Lymphocytes in Bipolar Disorder

## SPERLIC

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*Caratterizzazione dell’espressione dei recettori per le chemochine dei linfociti CD8+ nel Disturbo Bipolare*

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# Study Protocol

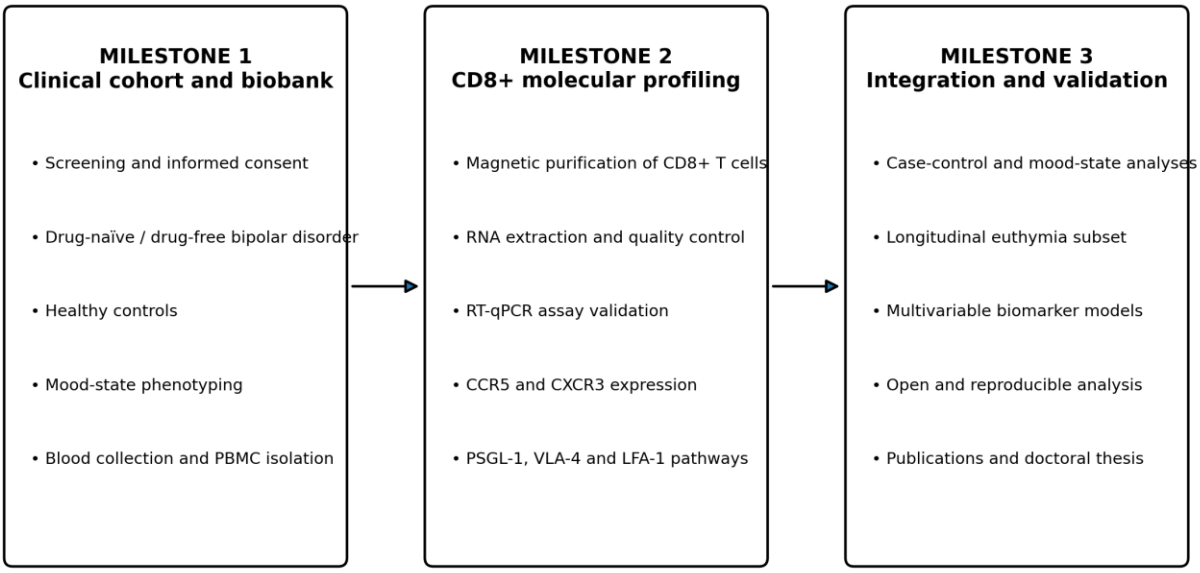
# Characterization of Chemokine Receptor Expression in CD8+ T Lymphocytes in Bipolar Disorder

*(SPERLIC study)*


## Project summary

Bipolar disorder is a severe and recurrent mood disorder whose biological mechanisms remain incompletely understood. A growing body of evidence indicates that immune dysregulation is not limited to circulating cytokines, but involves altered activation, differentiation and trafficking of lymphocyte subsets. In particular, CD8+ T cells have been linked to mood-state changes and to white-matter microstructural abnormalities. However, the molecular machinery that may permit activated CD8+ T cells to adhere to cerebral endothelium and respond to chemotactic gradients has not been systematically characterised in bipolar disorder. The SPERLIC project will test whether drug-naïve or drug-free patients with bipolar I or II disorder show a distinct CD8+ T-cell trafficking signature compared with healthy controls. The primary focus will be the expression of the chemokine receptors CCR5 and CXCR3. Secondary targets will include the adhesion-related pathways PSGL-1 (SELPLG), VLA-4 (ITGA4/ITGB1) and LFA-1 (ITGAL/ITGB2), together with CXCR3 transcript variants. The project will recruit 172 participants, equally divided between patients and controls, aged 25–35 years. Patients will be stratified according to current depressive or manic/hypomanic phase and characterised using standardised clinical scales. Peripheral blood mononuclear cells will be isolated by density-gradient centrifugation, CD8+ T cells purified by magnetic negative selection, and target transcripts quantified by SYBR-based one-step RT-qPCR. The analytical plan will combine prespecified case-control comparisons, mood-state analyses, adjustment for relevant confounders, and exploratory multivariable biomarker modelling. The expected output is a reproducible molecular and clinical framework for understanding CD8+ T-cell migration pathways in bipolar disorder. The project is designed to generate mechanistic evidence, candidate peripheral biomarkers and a platform for future studies integrating immunological profiles with neuroimaging and treatment response.

**SPERLIC doctoral project: from clinical phenotyping to a CD8+ T-cell trafficking signature**




Main output: a mechanistically informed and clinically characterised peripheral immune biomarker framework for bipolar disorder

# Project structure

1. Background and rationale
2. Overall hypothesis, aims and innovation
3. Milestone 1: clinical cohort, deep phenotyping and biobanking
4. Milestone 2: molecular profiling of CD8+ T-cell trafficking pathways
5. Milestone 3: longitudinal validation and integrated biomarker modelling
6. Statistical analysis and reproducibility framework
7. Ethics, data protection and dissemination
8. Three-year work plan, risks and expected outputs
9. Bibliography
10. Appendix: RT-qPCR targets and primer sequences

# Background and rationale

Bipolar disorder (BD) is a chronic and highly disabling psychiatric condition characterised by recurrent manic, hypomanic and depressive episodes, often separated by periods of partial or full remission [1,2]. Beyond its symptomatic burden, BD is associated with reduced life expectancy, high medical comorbidity and substantial suicide risk [3,4]. Although genetic, neurodevelopmental, neurochemical and environmental mechanisms have all been implicated, no single model fully explains the episodic course, clinical heterogeneity and systemic manifestations of the disorder [2,5].

Immune and inflammatory abnormalities are increasingly considered part of the pathophysiological architecture of BD. Studies have described altered monocyte and lymphocyte activation, shifts in T-cell differentiation, changes in circulating cytokines and transcriptomic signatures involving immune-response pathways [6–10]. Importantly, these changes may not be static. They may vary across mood phases, illness stages and treatment exposure, which makes the study of carefully phenotyped drug-naïve or drug-free patients particularly informative.

## Why focus on CD8+ T cells?

CD8+ T lymphocytes are cytotoxic and immunoregulatory cells capable of entering inflamed tissues after a coordinated sequence of chemokine sensing, endothelial rolling, firm adhesion and transendothelial migration. In BD, terminally differentiated CD8+ effector T-cell depletion in peripheral blood has been associated with white-matter microstructural alterations during mania, suggesting either redistribution, altered differentiation or abnormal tissue recruitment [7]. Single-cell transcriptomic work has also reported disease-related changes within peripheral CD8+ T-cell populations in mood disorders [9]. These observations provide a direct rationale for studying the molecular receptors that regulate CD8+ T-cell trafficking rather than measuring only nonspecific inflammatory markers.

## Chemokine receptors and adhesion molecules as a mechanistic pathway

CCR5 and CXCR3 are preferentially expressed by activated type-1 effector T cells and respond to inflammatory chemokines such as CCL3/CCL4/CCL5 and CXCL9/CXCL10/CXCL11. Their activation promotes directional migration and can cooperate with integrins to support firm adhesion and tissue entry [11,12]. In a previous BD study, the proportion of CD4+ lymphocytes expressing CCR5 and CXCR3 was reduced, while differences in CD8+ receptor-positive cells were not detected by flow cytometry [8]. This negative finding does not exclude altered transcript abundance, receptor isoform distribution, activation state or coordinated changes across the broader trafficking pathway.

The adhesion cascade is also biologically relevant. PSGL-1, encoded by SELPLG, mediates selectin-dependent leukocyte rolling. VLA-4, formed by the α4 and β1 integrin subunits encoded by ITGA4 and ITGB1, supports adhesion to VCAM-1 and contributes to T-cell entry into the central nervous system. LFA-1, formed by the αL and β2 subunits encoded by ITGAL and ITGB2, binds ICAM molecules and stabilises arrest and transmigration. These pathways are central to immune-cell trafficking in multiple sclerosis and other neuroinflammatory conditions, but have not been comprehensively profiled in purified CD8+ T cells from clinically characterised BD patients.

### Knowledge gap

Most peripheral biomarker studies in BD have used serum, plasma, whole blood or mixed peripheral blood mononuclear cells. Such approaches are valuable but vulnerable to shifts in cellular composition and may conceal cell-specific molecular changes. The SPERLIC project addresses this limitation by purifying CD8+ T cells and quantifying a prespecified, mechanistically connected panel of chemokine receptors and adhesion molecules. The use of drug-naïve or drug-free participants, a narrow age range, mood-state stratification and a longitudinal stabilisation subset is intended to reduce major sources of biological and clinical heterogeneity.

## Overall hypothesis, aims and innovation

**Central hypothesis.** Bipolar disorder is associated with an altered CD8+ T-cell trafficking programme, reflected by coordinated changes in CCR5/CXCR3 chemotactic signalling and PSGL-1/VLA-4/LFA-1 adhesion pathways. Part of this signature will vary with the current mood state, while a more stable component may persist after clinical remission.

**Primary aim.** To compare normalised CCR5 and total CXCR3 mRNA expression in purified peripheral CD8+ T cells between patients with bipolar disorder and healthy controls.

**Secondary aims.**

- To compare CXCR3-A and CXCR3-B transcript expression between bipolar disorder and healthy control groups.
- To quantify expression of SELPLG, ITGA4, ITGB1, ITGAL and ITGB2 as components of the PSGL-1, VLA-4 and LFA-1 trafficking pathways.
- To compare molecular profiles between patients in a depressive episode and those in a manic or hypomanic episode.
- To examine associations between gene expression and symptom severity measured with the HAM-D, Mania Rating Scale and SCL-90.
- To determine, in a longitudinal subset, which molecular alterations normalise after clinical stabilisation and which remain relatively stable.
- To construct an exploratory multivariable trafficking signature capable of discriminating patients from controls and mood-state subgroups.

**Innovation.** The project combines cell-specific purification, a mechanistically coherent migration panel, strict clinical phenotyping and state-versus-trait analyses. It moves beyond generic inflammation and tests a specific biological route by which peripheral immune cells may interact with the neurovascular and central nervous systems.

## Milestone 1: clinical cohort, deep phenotyping and biobanking

### Background and rationale

The interpretation of immune biomarkers in psychiatry is strongly influenced by age, sex, menstrual phase, smoking, medication exposure, infection, metabolic disease and current symptom state. Milestone 1 therefore establishes a rigorously characterised cohort and a standardised biospecimen pipeline. This phase reproduces the core SPERLIC protocol while adding a prespecified screening log and quality-control procedures.

### Study design and setting

The core study is a case-control, non-pharmacological clinical investigation conducted at the U.O.C. Clinica Psichiatrica, A.O.U. Policlinico “G. Rodolico–San Marco”, Catania. Patients will be recruited from ordinary inpatient care, Day Service and Day Hospital pathways. Laboratory procedures will be performed at the cellular and molecular biology laboratories of the Section of Biology and Genetics, Department of Biomedical and Biotechnological Sciences, University of Catania.

## Participants

| Group | Main inclusion criteria | Main exclusion criteria |
|---|---|---|
| Bipolar disorder | Age 25–35 years; male or female; DSM-5-TR-compatible bipolar I or II disorder; first diagnosis and drug-naïve, or no psychotropic treatment for at least 6 months; current depressive, manic or hypomanic episode; written informed consent. | Current corticosteroid, immunosuppressive, antibiotic or hormone-replacement therapy; clinically relevant autoimmune, infectious or internal-medicine disease; major psychiatric comorbidity; psychotropic treatment ongoing or discontinued less than 6 months earlier. |
| Healthy controls | Age 25–35 years; male or female; no current psychotropic treatment; no clinical evidence of bipolar I or II disorder; HAM-D <8; Mania Rating Scale <11; no major stressful life event during the previous 6 months; written informed consent. | Current or past major psychiatric disorder; relevant medical, autoimmune or infectious disease; current psychotropic or immunomodulatory treatment; conditions likely to alter immune measurements. |

For women of reproductive age, blood sampling will be scheduled in the mid-luteal phase whenever feasible, as specified in the protocol, and cycle information will be recorded. Recent vaccination, acute infection, body mass index and smoking status will also be documented as potential covariates.

## Clinical assessment

Trained physicians will confirm eligibility and collect demographic, clinical and treatment variables. The minimum dataset will include:

- Age, sex, educational attainment, employment, marital status, children and smoking or electronic-cigarette use.
- Bipolar subtype, current mood phase, age at onset, illness duration, number of previous hospitalisations and substance use.
- Medical comorbidity, recent infection or inflammatory condition, body mass index and concomitant non-psychiatric medication.
- Hamilton Depression Rating Scale (HAM-D), Mania Rating Scale and Symptom Checklist-90 (SCL-90).
- For the longitudinal extension: standard treatment initiated after baseline, dose equivalents, adherence and clinical outcome at follow-up.

## Biospecimen collection and biobank procedures

After informed consent, peripheral blood will be collected during routine clinical investigations. A portion of the sample will be processed for peripheral blood mononuclear cell isolation. Time from venepuncture to processing, sample volume, processing operator, cell yield, storage conditions and deviations from the standard operating procedure will be recorded. Remaining viable cells or RNA aliquots will be stored, where consent and infrastructure permit, to support repeat analyses and future validation.

## Ethical and regulatory considerations

The study will follow the Declaration of Helsinki, European and Italian data-protection requirements and the approved ethics protocol. Participation will not alter standard clinical treatment. Samples will be pseudonymised with a study code, and the re-identification key will be stored separately under restricted access. The proposed longitudinal extension and any additional storage or assays will be implemented only after the required ethics amendment and updated consent.

## Expected outputs

- A fully documented SPERLIC clinical and biological database with a screening log and standardised operating procedures.
- A protocol or methods publication describing the clinically controlled CD8+ T-cell biomarker platform.

# Milestone 2: molecular profiling of CD8+ T-cell trafficking pathways

## Background and rationale

The central laboratory objective is to quantify a coordinated set of transcripts that regulate chemotaxis, rolling, adhesion and transendothelial migration. Purification of CD8+ T cells is essential because whole-blood or PBMC transcript levels may reflect changes in cell proportions rather than changes within the cell type of interest.

## Laboratory workflow

11. Isolation of peripheral blood mononuclear cells by Ficoll-Isopaque density-gradient centrifugation.
12. Washing in phosphate-buffered saline to remove residual density-gradient medium and plasma components.
13. Negative magnetic selection of CD8+ T cells using the Miltenyi Biotec T-cell isolation system and separation columns.
14. Assessment of viable cell yield and, in a validation subset, CD8+ purity by flow cytometry or an equivalent cell-characterisation method.
15. Total RNA extraction using TRIzol, spectrophotometric quantification and documentation of A260/A280 ratio; samples with inadequate yield or purity will be repeated or excluded according to a prespecified rule.
16. One-step SYBR-based real-time RT-qPCR on the QuantStudio 7 Flex platform, including technical replicates, no-template controls, melt-curve analysis and amplification-efficiency assessment.

## Molecular targets

| Pathway | Gene/transcript | Encoded molecule and rationale |
|---|---|---|
| Chemotaxis | CCR5 | Chemokine receptor for CCL3, CCL4 and CCL5; marker and mediator of activated type-1 T-cell migration. |
| Chemotaxis | CXCR3 total, CXCR3-A, CXCR3-B | Receptor system for CXCL9, CXCL10 and CXCL11; transcript variants may differ in signalling and cellular effects. |
| Rolling | SELPLG | Encodes PSGL-1, a ligand involved in selectin-dependent leukocyte tethering and rolling. |
| Firm adhesion | ITGA4 and ITGB1 | Encode the α4 and β1 subunits of VLA-4, which mediates adhesion to VCAM-1 and supports CNS immune-cell entry. |
| Firm adhesion and migration | ITGAL and ITGB2 | Encode the αL and β2 subunits of LFA-1, which binds ICAM molecules and supports arrest and transmigration. |

## Assay validation and normalisation

Primer specificity will be evaluated through in-silico checks, melt curves and, where required, amplicon verification. Standard curves will be used to assess amplification efficiency. Candidate reference genes will be evaluated for stability in purified CD8+ T cells; the final normalisation strategy will use the most stable reference gene or the geometric mean of multiple reference genes. Experimental reporting will follow the MIQE principles [15]. Laboratory personnel will be blinded to diagnostic group whenever operationally feasible, and samples will be distributed across plates to minimise group-by-batch confounding.

## Outcomes and sample size

**Co-primary molecular outcomes.** Normalised ΔCt values for CCR5 and total CXCR3 in purified CD8+ T cells, compared between bipolar disorder and healthy control groups.

**Key secondary outcomes.** Normalised expression of CXCR3-A, CXCR3-B, SELPLG, ITGA4, ITGB1, ITGAL and ITGB2; relative fold change calculated with the $2^{-\Delta\Delta Ct}$ method; pathway-level composite scores; associations with mood state and symptom severity.

The planned sample comprises 172 participants, with 86 patients and 86 controls. The original calculation assumes a two-sided independent-samples t test, Cohen's d=0.50, α=0.05 and 90% power. This sample also retains more than 80% power when a stricter significance threshold is applied across the two co-primary markers. Subgroup and interaction analyses will be treated as secondary and interpreted using confidence intervals rather than significance alone.

### Expected outputs

- A validated RT-qPCR panel for CD8+ T-cell chemokine receptors and adhesion molecules.
- A primary case-control manuscript on CCR5/CXCR3 expression in bipolar disorder.
- A second manuscript addressing the broader PSGL-1/VLA-4/LFA-1 trafficking signature and mood-state differences.
- Training in cell separation, RNA handling, quantitative PCR, laboratory quality control and reproducible molecular analysis.

## Milestone 3: longitudinal validation and integrated biomarker modelling

### Background and rationale

A cross-sectional difference can represent a stable vulnerability marker, an acute mood-state effect, a consequence of recent behaviour or an unmeasured confounder. The third milestone therefore adds a longitudinal component to the core protocol. Patients enrolled during an acute episode will be invited for repeat clinical assessment and blood sampling after approximately five months, provided that they have achieved clinical stabilisation or euthymia. This extension is observational: treatment will be determined exclusively by the treating clinician.

### Longitudinal procedures

At follow-up, the baseline clinical and laboratory procedures will be repeated. Mood status, symptom scales, medication exposure, dose equivalents, smoking, body mass index, intercurrent infection and relevant medical events will be recorded. A minimum target of approximately 34 paired observations will provide 80% power to detect a medium within-subject effect under a two-sided paired comparison; recruitment will continue beyond this minimum where feasible to offset attrition and enable covariate-adjusted models.

### Integrated analyses

- State-versus-trait analysis: identify transcripts that differ between acute mood phases and those that remain altered after stabilisation.
- Pathway analysis: derive chemotaxis, rolling and integrin-adhesion scores from standardised expression measures.
- Clinical association analysis: relate molecular profiles to HAM-D, Mania Rating Scale and SCL-90 scores using regression and repeated-measures models.
- Exploratory classification: evaluate penalised logistic regression models with nested cross-validation, reporting optimism-corrected discrimination and calibration rather than a single apparent accuracy value.
- Sensitivity analyses: exclude participants with protocol deviations, recent infection, low RNA quality or poor CD8+ purity; assess the influence of sex, smoking and treatment exposure.

### Expected outputs

- A longitudinal manuscript separating mood-state-dependent from persistent CD8+ trafficking abnormalities.
- An integrated and openly documented statistical model of the SPERLIC molecular signature.
- A foundation for future studies combining the molecular signature with diffusion MRI, vascular biomarkers or treatment-response outcomes.

# Statistical analysis and reproducibility framework

## Primary analysis

Analyses will be performed on ΔCt values because they are closer to the scale on which measurement error is approximately symmetric. Group means will be compared using independent-samples tests when assumptions are met; otherwise, robust regression or suitable non-parametric methods will be used. Effect sizes will be reported with 95% confidence intervals. The two co-primary outcomes will be controlled using the Holm procedure. Secondary transcript-level analyses will use false-discovery-rate control.

## Covariate-adjusted and subgroup analyses

Linear models will adjust for prespecified variables that may influence immune expression: age, sex, smoking, body mass index, recent infection or vaccination, sampling batch and menstrual-cycle information where applicable. Mood phase, bipolar subtype and symptom severity will be examined through stratified and interaction models. Longitudinal outcomes will be analysed with paired tests and linear mixed-effects models including participant-level random intercepts.

## Missing data, outliers and quality control

Missing clinical data will be described by variable and group. Multiple imputation will be considered for covariates when the missing-at-random assumption is plausible; molecular outcomes will not be imputed when assay failure is the cause. Outliers will not be removed solely because they reduce statistical significance. Exclusion will require a documented pre-analytical or analytical reason, and analyses with and without influential observations will be reported when appropriate.

## Reproducibility and open science

The statistical analysis plan will be finalised before group labels are released for the definitive analysis. Data dictionaries, assay metadata and analysis scripts will be version controlled. De-identified data and code will be shared to the maximum extent permitted by participant consent, ethics approval and institutional policy. Reporting will follow relevant observational-study and RT-qPCR standards.

# Ethics, data protection and dissemination

The principal risks are those associated with venepuncture and the handling of sensitive psychiatric and biological data. No experimental drug or device is administered. Capacity to consent will be evaluated clinically, and patients unable to provide valid informed consent during an acute episode will not be enrolled until capacity is restored. Research data will be pseudonymised, access controlled and retained according to institutional rules.

Results will be disseminated through peer-reviewed publications, national and international psychiatric or neuroimmunology meetings. Aggregate findings may also be communicated to participants and clinical staff in accessible language. Negative and inconclusive results will be reported because they are essential for evaluating the proposed immune-trafficking model.

# Three-year work plan

| Activity | Year 1 | Year 2 | Year 3 |
|---|---|---|---|
| Ethics, SOPs, training and assay optimisation | Primary activity | Maintenance | — |
| Recruitment and clinical phenotyping | Start and pilot cohort | Main recruitment | Completion / replacement recruitment |
| PBMC and CD8+ processing | Pilot and ongoing | Ongoing at full capacity | Completion and repeat assays |
| RT-qPCR data generation | Assay validation | Main laboratory phase | Longitudinal samples and verification |

| | | | |
|---|---|---|---|
| Cross-sectional statistical analysis | Interim QC only | Primary analysis | Sensitivity and final analyses |
| Longitudinal follow-up | Protocol amendment and setup | Start follow-up | Completion and mixed models |
| Publications and conferences | Protocol/methods abstract | Cross-sectional manuscript | Longitudinal manuscript and thesis |

## Feasibility, risks and mitigation

| Risk | Potential consequence | Mitigation strategy |
|---|---|---|
| Slow recruitment due to narrow age range and drug-free criteria | Delayed sample-size completion and unbalanced mood-state groups | Maintain a screening log; coordinate inpatient, Day Service and Day Hospital pathways; consider ethically approved multicentre expansion; extend recruitment rather than relaxing core eligibility without justification. |
| Low RNA yield from purified CD8+ cells | Missing molecular outcomes | Standardise blood volume and time to processing; monitor cell yield; use validated low-input procedures; retain backup aliquots; repeat extraction when technically justified. |
| Variable CD8+ purity | Biased cell-specific expression estimates | Validate the magnetic protocol in a subset; document purity; perform sensitivity analyses using purity thresholds. |
| Batch effects in RT-qPCR | Spurious group differences | Randomise diagnostic groups across plates; include inter-plate calibrators; blind laboratory staff; include batch in statistical models. |
| Multiple testing | Inflated false-positive rate | Prespecify two co-primary outcomes; use Holm correction and false-discovery-rate control; emphasise effect sizes and replication. |
| Attrition in longitudinal follow-up | Reduced power for state-versus-trait inference | Schedule follow-up before discharge; use reminder systems permitted by consent; recruit beyond the minimum paired sample; analyse mixed models using all available repeated data. |
| Treatment heterogeneity after baseline | Confounding of longitudinal changes | Record all psychotropic treatments and dose equivalents; model treatment class and exposure; interpret follow-up as observational rather than causal treatment evidence. |

## Expected scientific and clinical impact

SPERLIC is not designed as an immediate diagnostic test. Its first contribution will be mechanistic: determining whether CD8+ T cells in BD show coordinated alterations in the receptors and integrins required for inflammatory trafficking. A reproducible signal would refine neuroimmune models of BD, identify molecular targets for larger validation studies and help explain previously observed relationships between peripheral CD8+ phenotypes and white-matter alterations. A null result would also be informative by narrowing the range of plausible trafficking mechanisms and preventing overinterpretation of nonspecific inflammatory findings.

The research project is feasible because the clinical recruitment pathway, laboratory collaboration, target panel and initial primer design are already established. Its added value lies in converting an approved clinical protocol into a rigorous three-year research programme with quality-controlled molecular assays, longitudinal validation and a transparent analytical framework.

## Planned publications

| Planned output | Provisional focus |
|---|---|
| Paper 1 | Protocol and laboratory framework for cell-specific immune-trafficking profiling in bipolar disorder. |
| Paper 2 | CCR5/CXCR3 and adhesion-molecule expression in purified CD8+ T cells: bipolar disorder versus healthy controls. |
| Paper 3 | Mood-state and longitudinal changes in the SPERLIC CD8+ trafficking signature. |
| Optional paper 4 | Exploratory integrated biomarker model and methodological lessons for peripheral immune studies in psychiatry. |
| Optional paper 5 | Clinical, molecular and longitudinal characterisation of CD8+ T-cell trafficking pathways in bipolar disorder. |

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## Appendix: RT-qPCR targets and primer sequences

The following oligonucleotides were provided in the SPERLIC laboratory order file. Sequences are shown 5′→3′. Final use remains conditional on assay-specific validation, amplification efficiency and specificity checks.

| Target | Primer | Sequence (5′→3′) |
|---|---|---|
| CCR5 | Forward | CAAAAAGAAGGTCTTCATTACACC |
| CCR5 | Reverse | CCTGTGCCTCTTCTTCTCATTTCG |
| CXCR3-A | Forward | ATGGTCCTTGAGGTGAGTGA |
| CXCR3-A | Reverse | CAGAAAGCCAGCCACCAG |
| CXCR3-B | Forward | GCCCTCACAGGTGAGTGA |
| CXCR3-B | Reverse | CAGAAAGCCAGCCACCAG |
| CXCR3 total | Forward | TTCGCCCACAGTCTGACTTC |
| CXCR3 total | Reverse | AATGGTGCAGTCCTCAGAGC |
| ITGA4 | Forward | TCAATCCCGGGGCGATTTAC |
| ITGA4 | Reverse | TGGAAAGTGTGACCCCCAAC |
| ITGAL | Forward | TGCTTATCATCATCACGGATGG |
| ITGAL | Reverse | CTCTCCTTGGTCTGAAAATGCT |
| ITGB1 | Forward | TGGTGTGGTTGCTGGAATTG |
| ITGB1 | Reverse | TTTTCACCCGTGTCCCATTTG |
| ITGB2 | Forward | TGCGTCCTCTCTCAGGAGTG |
| ITGB2 | Reverse | GGTCCATGATGTCGTCAGCC |
| SELPLG | Forward | GGCTGGGACCTTGTCACTAA |
| SELPLG | Reverse | AGGCTTTCTCGGCTTCATCT |