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Single-Cell Transcriptomics in Rheumatoid Arthritis: A Comprehensive Review of Cellular Heterogeneity and Therapeutic Targets

Zhipeng Tai[1], Qiang Zan[2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
[2]The Orthopedic and Joint Department of the Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, 712000, Shaanxi, China
Correspondence Author

Abstract: ***Rheumatoid arthritis is a chronic inflammatory joint disease in which persistent synovitis can progress to cartilage destruction, bone erosion, pain, and disability despite expanding therapeutic options. Clinically similar disease presentations may arise from distinct cellular and molecular programs within synovial tissue, limiting the effectiveness of uniform immunomodulation. This review evaluates how single-cell RNA sequencing and single-nucleus RNA sequencing have reshaped understanding of rheumatoid arthritis by resolving stromal and immune heterogeneity, defining context-dependent cell states, and linking intercellular signaling to therapeutic opportunities. We synthesize recurring cell states across synovial tissue, synovial fluid, and peripheral blood and assess how experimental and computational choices influence state identification and cross-cohort comparability. Across studies, synovial fibroblast-like synoviocytes consistently organize along a spatial lining versus sublining axis, with inflammation-induced activation layered onto positional identity. Perivascular niches, including endothelial-stromal signaling, appear to instruct fibroblast programs and help explain gradients in stromal phenotypes. Immune profiling repeatedly identifies peripheral helper T cell states that support lymphoid organization and B cell help, alongside clonally expanded cytotoxic T cell programs whose interpretation is complicated by overlap between activation and inhibitory receptor expression. Myeloid diversity is best captured as continuous transitions shaped by tissue niche and inflammatory modules rather than binary polarization, with evidence for protective barrier-like macrophage programs and recruited inflammatory macrophage states. B cell and plasma cell heterogeneity reflects antigen experience, class switching, and association with ectopic lymphoid structures, while emerging work suggests metabolic dependencies across stromal and antibody-secreting compartments. We conclude that a Cell State–Niche–Target framework can reconcile heterogeneous atlases by prioritizing program-level concordance and explicitly conditioning therapeutic hypotheses on niche context. This review is synovium-centric; synovial fluid and peripheral blood are discussed primarily as bridging compartments for biomarker hypotheses rather than as independently comprehensive atlases. Progress toward precision medicine will require standardized sampling and analysis, spatial validation, and longitudinal studies that connect state shifts to treatment response.***

Keywords: Single-Cell Transcriptomics, Rheumatoid Arthritis, Cellular Heterogeneity, Therapeutic Targets.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease in which persistent synovitis may culminate in cartilage destruction, bone erosion, pain, and disability despite expanding therapeutic options, including conventional synthetic disease-modifying antirheumatic drugs (DMARDs) and biologic DMARDs. A central translational challenge is that clinically similar RA phenotypes may arise from divergent cellular and molecular programs within synovial tissue, suggesting that “one-size-fits-all” immunomodulation may be insufficient for many patients. Single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) have enabled cell-resolved characterization of pathogenic and regulatory programs across stromal and immune compartments, offering a plausible route to more mechanism-informed therapeutic stratification and target discovery [1].

Conceptually, single-cell transcriptomics in RA aims to deconvolute the synovial microenvironment into (i) cell types (e.g., fibroblast-like synoviocytes (FLS), macrophages, T and B lymphocytes), (ii) context-dependent cell states, and (iii) intercellular communication networks that may sustain inflammation or tissue damage. Early RA synovial scRNA-seq studies already suggested substantial intra-compartment heterogeneity. However, key research challenges include patient-to-patient variability, differences in tissue processing and cell recovery, and analytic choices that may shift cluster boundaries and marker attribution [2].

More recent studies have expanded the scale and depth of RA atlases, yet cross-study synthesis remains non-trivial because sampling strategies vary (synovial tissue versus synovial fluid versus peripheral blood), disease phases differ (treatment-naïve versus established disease), and platforms and preprocessing pipelines are heterogeneous. Several large-scale synovial analyses suggest that clinically relevant “pathotypes” may be underpinned by structured variation in stromal–immune composition and signaling programs [Evidence: MODERATE] (boundary conditions: human synovial biopsies analyzed primarily with droplet-based scRNA-seq and computational integration). In parallel, mechanistic work indicates that microenvironmental cues such as endothelial–stromal signaling (e.g., Notch-related programs) may shape fibroblast identity and spatial organization [Evidence: MODERATE] (boundary conditions: human synovium cross-sectional profiling complemented by murine arthritis models and perturbation experiments) [3,4].

Here, we synthesize synovium-anchored RA cell states and highlight methodological factors shaping cross-cohort interpretability.

To reconcile cross-study heterogeneity, we organize evidence using the Cell State–Niche–Target (CSNT) framework, which links (i) cell states, (ii) the local niches that maintain them (cytokines, spatial context, cell–cell interactions), and (iii) candidate therapeutic targets implied by these relationships. This review aims to:

(1) Synthesize synovium-anchored cell states, using synovial fluid and peripheral blood primarily as bridging compartments for biomarker hypotheses, and summarize commonly used marker genes and state-defining programs.

(2) Compare key experimental and computational design choices (including QC, PCA/UMAP embedding, and DEG calling) that may influence cell-state identification and cross-cohort integration.

(3) Map proposed cell–cell communication and niche signals onto candidate therapeutic targets, explicitly tagging major claims by evidence tier (STRONG, MODERATE, or HYPOTHESIS-GENERATING) and stating boundary conditions.

(4) Identify concrete translational and methodological gaps (e.g., sampling bias, treatment confounding, and validation needs) and propose near-term research priorities for RA precision medicine [5].

2. Review Scope and Approach

Evidence is organized thematically by major compartments (stromal, myeloid, lymphoid), then by recurring cell states linked to inflammation, tissue remodeling, and therapeutic response, followed by an integrative section mapping candidate targets to cellular niches using a CSNT-oriented logic. We additionally highlight points of concordance and conflict attributable to sampling depth, batch correction, and annotation strategies [1].

In this review, “cell type” denotes a relatively stable lineage-defined identity (e.g., macrophages, FLS), whereas “cell state” denotes a context-dependent transcriptional program within a cell type (e.g., activated lining FLS). scRNA-seq refers to whole-cell RNA sequencing; snRNA-seq refers to nucleus-derived RNA profiling, often used for difficult-to-dissociate tissues. “DEG” denotes differentially expressed gene; “QC” denotes quality control; and “CSNT” denotes the Cell State–Niche–Target framework used here to connect states to actionable pathways.

scRNA-seq versus snRNA-seq. snRNA-seq can partially mitigate dissociation-associated loss and stress responses, which is particularly relevant for fragile lining-associated populations (e.g., lining FLS, lining macrophages) and rare stromal/endothelial subsets. However, nuclear RNA profiles may shift transcript representation and cell-type recovery, complicating direct comparisons with whole-cell scRNA-seq. Throughout this review, we treat snRNA-seq as a complementary view of the same state space, and we flag potential modality-sensitive rows in the CSNT table using technical caveats and validation tags.

Evidence counting and grading: N = independent human cohorts (not datasets from the same cohort). Independence = distinct patient populations from different centers/timepoints. Evidence levels: STRONG (typically N≥3 with orthogonal validation; exceptionally N=2 with strong causal perturbation, explicitly stated per CSNT row); MODERATE (N=2 without causal validation, or N≥3 transcriptomic-only/discordant definitions); HYPOTHESIS-GENERATING (N≤1 or inferential only). Animal studies noted as +mouse_support tags; N values in CSNT table, main text reports evidence levels only. Evidence tiers in this review quantify reproducibility of the state–niche axis across independent human cohorts; therapeutic targets are conditional hypotheses whose readiness is inferred only via validation tags (e.g., +spatial, +perturbation, +organoid, +clinical, +computational) and explicit boundary conditions.

3. Thematic Synthesis

3.1 Synovial Fibroblast Heterogeneity as a Spatially Encoded Disease Engine

Fibroblast heterogeneity in RA is best interpreted as positional identity combined with inflammation-induced activation [Evidence: STRONG]. PRG4+/CD55+ programs map to the synovial lining, whereas THY1+/CD34+ programs localize to sublining and perivascular regions [2,3,6] extended this into a quantitative gradient linking THY1/PRG4 ratios to vascular distance, while Smith et al. [1] showed that activation overlays onto positional scaffolds rather than replacing them.

Invasive and destructive phenotypes concentrate in activated sublining states combining chemokine, protease, and osteoclastogenic programs [Evidence: STRONG]. Mizoguchi et al. (2018) demonstrated that CD34−THY1+ subsets differ in invasion and RANKL bioavailability, and Smith et al. [1] showed that TNF+IFN-γ stimulation approximates activated sublining signatures whereas TNF+IFN-γ+IL-1β aligns with activated lining programs.

Metabolic determinants may stabilize pathogenic FLS phenotypes [Evidence: MODERATE]. Torres et al. [7] highlighted HK2 as a candidate regulator, and Ahmed et al. [8] supported targeting glycolysis/glutaminolysis, though niche-specific mapping remains incomplete.

Therapeutic implications: CSNT-guided interventions targeting activated sublining FLS warrant investigation in early RA and ACPA+ disease. State–niche axis (NOTCH3/DLL4 relay): [Evidence: STRONG] [+spatial, +perturbation] [3,9]. Target hypotheses (HK2, metabolic inhibitors): [Evidence: MODERATE] but translational readiness is constrained by systemic toxicity and limited niche-specific validation [8].

Section summary: Lining–sublining positional identity scaffolds FLS activation and niche-linked targets. [Evidence: STRONG] (N in CSNT table)

3.2 T Cell Functional States Connecting Antigen Experience to Lymphoid Organization

CXCL13+ Tph programs are the most reproducible T cell

signature in RA [Evidence: STRONG]. Stephenson et al. [2] first identified CXCL13+ CD4+ Tph populations. Argyriou et al. [5] distinguished CXCL13^high and CXCL13^low Tph states with TCR sequencing, linking them to clonality patterns and markers including PDCD1, PRDM1, and GPR56. Wu et al. [10] showed that synovial compartments contain distinct mixtures of memory, interferon-activated, and cytotoxic modules. Fonseka et al. [11] provided orthogonal evidence that discrete immune states track with clinical phenotypes.

Cytotoxic programs recur in both CD8+ and CD4+ compartments but are more context- and serostatus-dependent [Evidence: MODERATE]. Moon et al. [12] demonstrated citrullinated antigen-responsive CD8+ T cells with clonal expansion in ACPA+ RA. Argyriou et al. [5] reported cytotoxic CD4+ T cells (NKG7+/GZMH+/PRF1+) linked to anti-CCP titers. Jonsson et al. [13] described GZMK+ CD8 T cells as activated tissue-resident cells without classical exhaustion signatures.

The exhaustion versus activation distinction remains interpretively challenging [Evidence: MODERATE]. PDCD1/TIGIT/LAG3 expression may reflect activation-induced checkpoint upregulation rather than epigenetic exhaustion, with dissociation protocols potentially inflating immediate-early signatures [10,13].

Therapeutic implications: State–niche axis (CXCL13+ Tph targeting): most relevant to ELS-rich seropositive synovitis [Evidence: STRONG] [+TCR-seq]. Target hypotheses (checkpoint modulation, antigen-specific tolerance): carry nontrivial risks; checkpoint manipulation can precipitate inflammatory syndromes [14]; antigen-specific tolerance most plausible in ACPA+ patients with demonstrable reactivity [12] [Evidence: HYPOTHESIS-GENERATING].

Section summary: CXCL13+ Tph programs link antigen experience to ELS-supporting niches. [Evidence: STRONG] Cytotoxic T cell contributions to tissue damage remain serostatus-dependent. [Evidence: MODERATE] (N in CSNT table)

3.3 Myeloid Cell Diversity: from Barrier Macrophages to Inflammatory Recruitment

Myeloid heterogeneity in RA reflects lineage origin, niche positioning, and inflammatory modules rather than M1/M2 polarization [Evidence: MODERATE] (+mouse_support). Culemann et al. [15] showed that CX3CR1+ lining macrophages form a barrier-like layer in mice. Xia et al. [16] added temporal resolution demonstrating dynamic barrier disruption–restoration. In humans, dissociation bias means barrier macrophages may be under-sampled.

Inflammatory macrophage modules characterized by chemokines and interferon-stimulated genes show serostatus dependence [Evidence: MODERATE]. Wu et al. [10] reported CCL+ macrophage subsets with differential distributions across ACPA+ versus ACPA− RA. Zhang et al. [17] showed that IFN-γ and TNF-α drive CXCL10+/CCL2+ polarization signatures, supporting a cytokine-driven module view rather than M1/M2 categories.

Dendritic cells (LAMP3+/CXCL10+ DC signatures) appear important but variably captured [Evidence: MODERATE]. Wu et al. [18] identified migratory DC programs supporting antigen presentation and T cell activation.

Therapeutic implications: State–niche axis (CX3CR1+ barrier stabilization, CXCL10/CCL2 attenuation): supported by mouse lineage tracing [17] [Evidence: MODERATE] (+mouse_support). Target hypotheses (cell therapy restoring barrier function): preclinical support [19] but require validation in chronic human RA [Evidence: HYPOTHESIS-GENERATING].

Section summary: Myeloid states align with origin- and niche-linked programs that diversify beyond the M1/M2 dichotomy; barrier macrophage evidence is strongest in mouse models. [Evidence: MODERATE] (+mouse_support) (N in CSNT table)

3.4 B Cells and Plasma Cells: Local Maturation and Metabolic Control Points

B lineage diversity reflects antigen experience, class-switching, and local differentiation in association with ELS [Evidence: MODERATE]. Wu et al. [18] distinguished memory B and plasma cell compartments with class-switched trajectories. Stephenson et al. [20] observed plasma cells co-occurring with CXCL13+ Tph cells. Kongpachith et al. [21] demonstrated ACPA affinity maturation, supporting antigen-driven B cell evolution in local microenvironments.

Memory B cell heterogeneity is best interpreted by activation and antigen-presentation capacity [Evidence: MODERATE]. Wu et al. [18] described HLA-DR–associated memory B features implying enhanced antigen presentation. Discrepant reports likely reflect cohort composition and sampling compartment differences.

Plasma cell differentiation via local versus recruited routes remains challenging to distinguish transcriptomically [Evidence: HYPOTHESIS-GENERATING]. Metabolic regulation (lactylation, PD-1–associated phenotypes) is proposed but remains conceptual [22].

Therapeutic implications: State–niche axis (ELS-associated B-lineage maturation and Tph–B coupling): [Evidence: MODERATE] (N in CSNT table). Target readiness (CD20 B cell depletion): [+clinical]. Target hypotheses (dual Tph + plasma cell targeting, state-resolved biomarkers): may improve durable ELS control and patient selection [23] [Evidence: MODERATE].

Section summary: B lineage diversity reflects antigen experience and ELS-associated differentiation trajectories. [Evidence: MODERATE] (N in CSNT table)

3.5 Endothelial and Stromal Niches: Perivascular Instruction

Endothelial–stromal interactions define perivascular niches that instruct fibroblast identity [Evidence: MODERATE]. Wei et al. [3] quantified a fibroblast transcriptional gradient tied to vascular distance, linking perivascular positioning with

NOTCH activation. Smith et al. [1] demonstrated spatial constraint of cytokine responses using paired scRNA-seq and spatial transcriptomics. Reshef et al. [24] showed that neighborhood-based methods recover NOTCH gradients across samples.

Vascular heterogeneity shows endothelial activation consistent with leukocyte trafficking [Evidence: MODERATE]. Disagreements about endothelial cluster numbers likely reflect methodological differences in dissociation and QC thresholds.

Perivascular signaling couples endothelial activation to fibroblast phenotypes [Evidence: MODERATE]. Wei et al. [3] described a NOTCH3 relay from arterial endothelium through mural intermediates to sublining fibroblasts. Smith et al. [1] showed these niche effects coexist with cytokine-driven activation.

Therapeutic implications: State–niche axis (perivascular NOTCH3 relay): supported by spatial and organoid evidence [3] [Evidence: MODERATE] [+spatial, +organoid]. Target hypotheses (systemic NOTCH inhibition): carries toxicity concerns; niche-restricted delivery may be required [Evidence: HYPOTHESIS-GENERATING].

Section summary: Perivascular niches pattern stromal identity via NOTCH3 signaling relay. [Evidence: MODERATE] (N in CSNT table)

3.6 Treatment Response Signatures: State Plasticity under DMARDs

DMARD efficacy may reflect selective pressure on cell states rather than uniform inflammatory suppression [Evidence: MODERATE]. Wu et al. [25] showed TNF antagonist exposure alters FLS responsiveness, supporting state rewiring rather than simple cytokine blockade. Chen et al. [9] identified HBEGF+ fibroblast programs enriched in specific disease contexts, consistent with therapy-induced state composition shifts.

Biomarker candidates from blood-based inference of synovial states remain preliminary [Evidence: HYPOTHESIS - GENERATING]. Fonseka et al. [11] demonstrated single-cell association frameworks detecting disease-linked immune states, though blood-to-synovium mapping fidelity varies by cell type.

Emerging modalities Park et al. [26] reported MSC infusion signals without synovial readouts. Zachs et al. [27] showed ultrasound stimulation reducing arthritis severity via systemic immunoregulation.

Therapeutic implications: State–niche axis (state rewiring under TNFi/JAK): supported by computational integration [9,25] [Evidence: MODERATE] [+computational]. Target hypotheses (combination strategies targeting cytokine circuits, stromal niches, barrier programs): require prospective validation with standardized tissue processing and trial-integrated spatial sampling [Evidence: HYPOTHESIS - GENERATING].

Section summary: Therapy modulates state distributions within niches rather than erasing lineages. [Evidence: MODERATE] Blood-to-synovium biomarker translation remains preliminary. [Evidence: HYPOTHESIS - GENERATING] (N in CSNT table)

4. Cross-Study Synthesis

Across the reviewed literature, our synthesis indicates that synovial fibroblast-like synoviocytes (FLS) are best described along a spatially organized lining–sublining axis rather than discrete, fixed subsets. This conclusion is strongly supported by multiple independent human synovial cohorts spanning scRNA-seq and spatial profiling, including the initial RA synovium atlas of Stephenson et al. [2], the functionally annotated fibroblast subsets in Mizoguchi et al. [6], the positional gradient and NOTCH3 program described by Wei et al. [3], and the paired scRNA-seq/scATAC-seq framework in Smith et al. [1], with re-analytical support for inflammatory fibroblast programs in Chen et al. [9]. The agreement across platforms suggests that positional cues (vascular proximity, lining boundary) and cytokine combinations likely imprint transcriptomes, making “state” inseparable from niche in enzymatically dissociated human synovium [Evidence: STRONG] (N in CSNT table; +spatial/+multiome where available).

A second cross-study pattern is that RA myeloid heterogeneity recurrently resolves into inflammatory IL1B/S100A12-high programs and chemokine-enriched CCL+ programs, with additional antigen-presentation and tissue-repair signatures depending on tissue compartment. This conclusion is supported by multiple independent human synovial cohorts [2,10,25,28]. While these studies converge on shared axes, they diverge on the relative abundance of subsets, plausibly reflecting boundary conditions in sampling (synovial tissue versus synovial fluid), serostatus stratification, and treatment exposure, which may shift macrophage activation trajectories without eliminating core programs. [Evidence: MODERATE] (N in CSNT table; boundary conditions: tissue vs fluid, serostatus, treatment exposure).

Methodological heterogeneity appears to shape “what is seen” more than “what exists.” Our synthesis suggests that the detection of rare or transitional states is sensitive to feature selection, integration, and statistical modeling choices, whereas broad lineage structure (FLS axis; myeloid inflammatory versus reparative programs) is relatively stable across pipelines. Townsend et al. [29] highlighted that genetic-to-single-cell linking and integration strategies yield partially non-overlapping disease-associated states, and Reshef et al. [24] illustrated how neighborhood-based association testing can recover gradients missed by cluster-level tests. Ranjan et al. [30] further implies that upstream feature selection can systematically alter clustering granularity, offering a plausible mechanism for cross-study discrepancies when the same tissue yields different “numbers” of states.

Methodological considerations affecting state detection: Dissociation, rare-cell capture, and integration choices can bias lining, endothelial/DC, and transitional states; interpret these CSNT rows with sensitivity checks and, where possible,

spatial/paired-sampling validation. Broad lineage structure is stable, but rare/transitional states and blood–synovium bridging remain pipeline-sensitive.

Cross-species comparisons suggest partial conservation of stromal and myeloid organizing principles, with important model-specific constraints. Notch-dependent fibroblast positioning and arthritis severity modulation in mice [3] parallels the niche-linked FLS states observed in human synovium, yet the quantitative contribution of each program may be model dependent. Similarly, resident synovial macrophage barrier dynamics in murine arthritis [15] align conceptually with lining-associated macrophage phenotypes, but translation to human disease likely depends on chronicity and treatment history, which are difficult to recapitulate in short-horizon models.

The field would benefit from study designs that more routinely co-measure state and niche—ideally through paired scRNA-seq and spatial profiling across synovial tissue regions and synovial fluid, with harmonized QC and metadata—because many apparent disagreements may reflect unmeasured boundary conditions rather than biology. Future studies might also consider longitudinal sampling around defined therapeutic perturbations to distinguish stable lineage programs from treatment-responsive state transitions, an approach that seems particularly pertinent given the treatment-associated state changes observed in synovial stromal cells under TNF blockade contexts [25].

5. Limitations and Unresolved Controversies

Single-cell transcriptomics has delineated RA synovial ecosystems, yet several field-level constraints temper inference. Tissue dissociation and viability-dependent capture can distort cell-state frequencies, particularly for lining macrophages and fibroblast-like synoviocytes, while stress programs and ambient RNA may be misread as pathogenic activation [2]. Current atlases are also unevenly distributed across compartments and populations, with relatively sparse coverage of treatment-naïve early RA, seronegative RA, and longitudinal sampling across flare–remission cycles; most studies remain cross-sectional snapshots of established disease obtained from surgical synovium.

6. Conclusion

This review contributes a CSNT-oriented synthesis that links compartment-resolved cell states to their plausible niche drivers and candidate intervention points, aiming to reconcile heterogeneous atlases into a clinically interpretable framework. We propose that prioritizing “program-level” concordance over cluster identity can serve as a practical bridge for cross-cohort integration and target nomination.

Perhaps most importantly, therapeutic inference from scRNA-seq is most actionable when state definitions are stable across cohorts and supported by convergent modalities, whereas niche-driven targets remain more tentative when based primarily on ligand–receptor inference. Additionally, integrating synovial tissue with synovial fluid and blood can sharpen biomarker hypotheses, though this appears most applicable when sampling is temporally aligned and treatment effects are accounted for. Finally, analytic transparency around QC, PCA/UMAP, and DEG choices is not merely technical; it conditions biological interpretation.

With continued growth in multimodal and spatially resolved single-cell profiling, RA transcriptomic atlases are likely to move from descriptive catalogs toward testable, patient-stratifying mechanistic models. Realizing this trajectory will depend on tighter coupling between state discovery and prospective clinical and experimental validation.

6.1 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6.2 Author Contributions

QZ contributed to conception and design of the study. ZPT wrote the first draft of the manuscript. QZ modified the manuscript. All authors read and approved the final manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

6.3 List of Abbreviations

ACPA= Anti-Citrullinated Protein Antibody; CSNT= Cell State–Niche–Target; DEG= Differentially Expressed Gene; DMARD= Disease-Modifying Antirheumatic Drug; ELS= Ectopic Lymphoid Structure; FLS= Fibroblast-Like Synoviocyte; JAK= Janus Kinase Inhibitor; PCA= Principal Component Analysis; QC= Quality Control; RA= Rheumatoid Arthritis; scRNA-seq= Single-Cell RNA Sequencing; snRNA-seq= Single-Nucleus RNA Sequencing; TCR= T Cell Receptor; TNFi= Tumor Necrosis Factor Inhibitor; UMAP= Uniform Manifold Approximation And Projection;

6.4 CSNT Framework Integration

Evidence tiers as defined in Review Scope; N = independent human cohorts; +mouse_support = animal causal validation. Target readiness tags: +spatial, +perturbation, +organoid, +clinical, +computational indicate validation modalities supporting target prioritization.

Table 1: CSNT Module Mapping Table

State (Markers/Module)	Niche (Location/Context)	Target (Pathway/Dependency)	Evidence Level	Evidence (N + Validation)	Boundary Conditions	Technical Caveats
PRG4+/CD55+ lining FLS (positional identity)	Synovial lining barrier, cartilage interface	Lining barrier maintenance, lubricin/PRG4 axis	STRONG	N=4, human tissue scRNA-seq + spatial/functional validation (Stephenson 2018, Mizoguchi 2018, Wei 2020, Smith 2023)	Synovial tissue; early/established RA	10x/Smart-seq2; enzymatic dissociation may under-recover lining
THY1+/CD34+ sublining/perivascular FLS (positional scaffold; activation overlays)	Perivascular/sublining stroma	NOTCH3/DLL4 axis, cytokine circuits (TNF/IFN-γ responsiveness)	STRONG	N=4, spatial + perturbation validation (Stephenson 2018, Mizoguchi 2018, Wei 2020, Smith 2023)	Synovial tissue; established RA	Activation overlays on positional identity
FLS metabolic axis (HK2/glycolysis)	Activated FLS compartments	HK2, glutaminolysis pathways	MODERATE	N=2, ex vivo/preclinical validation (Torres 2023, Ahmed 2023)	Synovial tissue; systemic toxicity risk for clinical translation	Niche-specific mapping incomplete
CXCL13+ Tph (peripheral T helper)	ELS-like aggregates, synovial fluid	Tph-B cell collaboration, CXCL13 axis	STRONG	N=3, TCR-seq–supported (Stephenson 2018, Argyriou 2022, Wu 2021)	Synovial tissue/fluid; ACPA+ enriched	3' scRNA-seq may underrepresent TCR diversity
Cytotoxic CD4/CD8 (NKG7+/GZMH+/PRF1+)	Tissue-resident compartments, synovial fluid	Citrullinated antigen pathways, checkpoints	MODERATE	N=3, scRNA-seq + tetramer validation (Moon 2023, Argyriou 2022, Jonsson 2022)	Synovial tissue/fluid; ACPA+ associated	Functional validation needed; exhaustion vs activation ambiguity
CX3CR1+ lining macrophages (barrier)	Synovial lining barrier	Barrier restoration programs	MODERATE (+mouse_support)	N_human=2; +mouse_support (2 causal studies) (Culemann 2019, Xia 2025)	Synovial tissue; species asymmetry (mouse > human)	Digestion-sensitive; human recovery limited
CXCL10+/CCL2+ inflammatory macrophages	Sublining interstitial infiltrates	IFN/TNF combinatorial modules	MODERATE	N=3, multimodal (Zhang 2019, Wu 2021, Zhang 2021)	Synovial tissue; serostatus-dependent	Recruited vs resident origin unclear
Memory B cells, class-switched plasma cells	ELS structures	B cell depletion, Tph-B collaboration	MODERATE	N=3, BCR-seq + affinity maturation validated (Wu 2021, Kongpachith 2019, Stephenson 2018)	Synovial tissue; seropositive RA	Local vs systemic differentiation unclear
Plasma cell metabolic hypothesis	Hypoxic/lactate-rich niches	Lactylation, metabolic checkpoints	HYPOTHESIS-GENERATING	N=1, conceptual/computational (Fu 2024)	Limited direct validation in RA synovium	Functional causality not established
Arterial endothelium, perivascular mural cells	Vascular-distance gradient relay	NOTCH3, endothelial adhesion programs	MODERATE	N=2, spatial + organoid validation (Wei 2020, Smith 2023)	Synovial tissue; needs deeper sampling	Low endothelial recovery in scRNA-seq
HBEGF+ FLS (treatment-associated)	Cytokine-modulated microenvironment	HBEGF-EGFR axis	MODERATE	N=2, computational integration (Chen 2022, Wu 2022)	Synovial tissue; treatment-exposed	Cross-sectional design limits causal inference

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