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Targeting Fibroblast-like Synoviocytes in Rheumatoid Arthritis

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Abstract

Fibroblast-like synoviocytes (FLS) are mesenchymal-derived cells that play an important role in the physiology of the synovium by producing certain components of the synovial fluid and articular cartilage. In rheumatoid arthritis (RA), however, fibroblasts become a key driver of synovial inflammation and joint damage. Because of this, there has been recent interest in FLS as a therapeutic target in RA to avoid side effects such as systemic immune suppression associated with many existing RA treatments. In this review, we describe how approved treatments for RA affect FLS signaling and function and discuss the effects of investigational FLS-targeted drugs for RA.

Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease affecting 0.5–1% of the population worldwide[1]. The most prominent clinical feature of RA is destructive polyarthritis of diarthrodial joints characterized by synovial inflammation and hypertrophy, leading to eventual erosion of cartilage and periarticular bone[2]. RA causes significant morbidity in affected individuals and can cause chronic pain, disability, and increased mortality. RA was historically managed with synthetic disease-modifying anti-rheumatic drugs (DMARDs) prior to the era of targeted therapies. In the last three decades, an explosion of biologic agents led to a revolution in its treatment[3]. Despite the wealth of new treatments, many patients still fail to achieve remission[4], suggesting the need for new drug targets.

Many currently available biologic therapies for RA target systemic mediators of inflammation such as TNF or IL-6[5]. Recently, to avoid some of the systemic immune suppressive effects of current therapies, there has been renewed interest in the mesenchymal elements involved in the pathophysiology of RA, especially in synovium[6]. RA results in numerous alterations in the synovium, including infiltration with adaptive immune cells. However, expansion of fibroblast-like synoviocytes (FLS) and changes in their behavior are among the most significant differences. These mesenchymal cells reside in the synovial

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intimal lining in contact with macrophage-like synoviocytes[7]. Fibroblasts with a variety of phenotypes also populate the sublining regions (see below).

FLS maintain synovial fluid and extracellular matrix homeostasis in healthy joints through production of articular and synovial fluid components such as lubricin and hyaluronic acid[8]. In RA, FLS are imprinted by their environment and display an aggressive phenotype[9]. FLS then play a key role in the development of the RA pannus, a mass of inflammatory, invasive synovial tissue responsible for the erosive damage of advanced RA[10]. FLS within pannus produce matrix metalloproteinases (MMPs) that digest a variety of proteins in the cartilage and support structures, enabling further expansion and invasion by pannus[11]. FLS also have additional pro-inflammatory effects through production of cytokines such as interleukin-6 (IL-6) and Granulocyte macrophage colony-stimulating factor (GM-CSF) that activate B and T cell cells[12,13], chemotactic factors such as C-C Motif Chemokine Ligand 2 (CCL2) and IL-8 (CXCL8) that recruit myeloid cells, Receptor activator of nuclear factor kappa-B ligand (RANKL) that promotes osteoclast formation[7] and Dickkopf-related protein 1 that suppresses osteoblast-mediated bone repair[14].

The phenotypes of CD90+ fibroblasts within the synovium form a gradient, with FLS in the synovial lining expressing more proteoglycan 4 (PRG4) and podoplanin, while those within the sublining near blood vessels express the surface marker FAPα[15,16]. This transition is established, in part, through FLS interactions with endothelial cell-derived NOTCH within the sublining. In a mouse model of inflammatory arthritis, NOTCH3 blockade by an anti-NOTCH3 antibody reduced arthritis severity[15]. These results, along with studies showing that cadherin-11 directed therapies ameliorate pre-clinical models of arthritis, suggest that targeting FLS or other fibroblasts is a promising avenue for potential new RA treatments. In this review, we will discuss how current DMARDs and biologic therapies affect FLS and speculate on targeted therapies that could target FLS.

Effect of Conventional Synthetic DMARDs on FLS

The class of drugs known as conventional synthetic DMARDs (csDMARDs) includes methotrexate, hydroxychloroquine, sulfasalazine, and leflunomide. Methotrexate is an inhibitor of dihydrofolate reductase, decreases purine synthesis in rapidly dividing cells, and increases local adenosine concentrations[17,18]. Methotrexate remains a mainstay of the treatment of RA[19] and has multiple effects on FLS, including decreased MMP1 expression due to activation of adenosine A2b receptors[20]. A recent study of the cultured FLS transcriptome identified candidate genes that were differentially expressed after exposure to methotrexate. Notable changes included decreased transcription of MMP1 and MMP13, decreased production of the proinflammatory cytokines IL-6 and IL-7, and decrease in COL14A1 and CYTL1 which regulate cartilage homeostasis[21]. The in vitro growth rate of FLS is also reduced by methotrexate because it interferes with purines required for DNA synthesis[22]. FLS exposed to methotrexate also have decreased expression of RANKL, which would limit osteoclast formation and bone damage. Of interest, co-cultures of FLS and peripheral blood mononuclear cells (PBMCs) exposed to methotrexate resulted in decreased osteoclast differentiation[23].

Methotrexate.

Methotrexate also influences FLS autophagy, which is a survival strategy for stressed cells and protects tumor cells from chemotherapy[24]. For example, co-treatment with methotrexate and theaflavin-3,3'-digallate restored the apoptosis-autophagy balance in RA FLS[25]. In addition HMGB1-induced autophagy might be a marker of methotrexate resistance in RA patients[26]. Finally, methotrexate alters microRNA expression in FLS, with increased expression of the microRNA miR-877–3p leading to decreased production of CCL3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), both of which are associated with increased RA activity[27].

Anti-malarial drugs.

Hydroxychloroquine is an antimalarial drug with anti-rheumatic effects through poorly defined mechanisms, although the agent appears to inhibit MHC class II expression, interfere with Toll-like receptor signaling, and block of cytokine production[28]. Hydroxychloroquine can potentially increase apoptosis in FLS through its effects on the pro-apoptotic enzyme caspase-3 and enhances apoptosis mediated by CD95[29]. This process is potentiated by decreased FLS expression of FLIP, which acts as an inhibitor against CD95-induced apoptosis[29]. Hydroxychloroquine also decreases the expression of 15-hydroxyprostaglandin dehydrogenase (HPGD) in FLS, resulting in decreased release of the pro-inflammatory prostaglandin E2 through a MAP-kinase dependent pathway[30]. Sulfasalazine, another csDMARD frequently used in combination therapy with methotrexate and hydroxychloroquine, decreases HPGD expression and osteoclast formation in a similar way to methotrexate and hydroxychloroquine[23,30].

Leflunomide.

This agent is an inhibitor of dihydroorotate dehydrogenase and exerts its anti-inflammatory effects through suppression of pyrimidine synthesis, primarily targeting rapidly dividing cells such as lymphocytes[31]. In FLS, leflunomide and its active metabolite teriflunomide (A77 1726) also inhibit production of prostaglandin E2, MMP1, and IL-6[32,33]. Leflunomide also decreases hyaluronic acid production by FLS[34], which could contribute to RA activity. In a mouse model of inflammatory arthritis, leflunomide and methotrexate co-treatment reduced FLS viability and expression of osteoclastic genes to a greater extent that monotherapy with either drug[35].

Effect of Biologic Agents on FLS

There are currently five classes of biologics approved for the treatment of RA in the United States: 1) TNF inhibitors, 2) IL-6 inhibitors, 3) IL-1 inhibitors, 4) T cell CD28-CD80/86 blockers and 5) a CD20-expressing B cell depletor[36].

TNF blockers.

The TNF inhibitors include infliximab, etanercept, adalimumab, golimumab, and certolizumab, exert their effects by blocking the interaction of TNF with its receptor on a variety of cell types. Dysregulated TNF signaling in FLS contributes to a robust inflammatory response[37]. Thus, blocking TNF receptor ligation would interfere with the

effects of TNF on FLS, including cytokine, MMP and prostanoid production. Infliximab treatment is also associated with decreased levels of ID-1, which is a transcription factor expressed by FLS that regulates FLS proliferation and cytokine secretion[38]. Infliximab and etanercept decrease FLS production of CCL20, which activates CCR6 on pro-inflammatory Th17 cells and directs their migration to sites of inflammation[39]. Infliximab, adalimumab, and etanercept can potentially induce apoptosis in FLS, although etanercept might be the most active[40]. This might be because infliximab and adalimumab activate a survival signal through their Fab regions, while etanercept is an Fc-soluble TNF receptor fusion protein and lacks this region[40]. Like methotrexate, infliximab decreases RANKL expression and reduces osteoclast formation[23,41]. Finally, increased expression by FLS of genes associated with cell division and cytokine expression might be associated with clinical response to adalimumab[42].

IL-6 blockers.

IL-6 responses and the IL-6 pathway are influenced by a wide variety of epigenetic changes in RA FLS, underscoring their importance in the pathogenesis of RA[43]. The IL-6 receptor inhibitors currently approved for RA in the United States include tocilizumab and sarilumab and, like TNF blockers, can affect FLS function. For example, tocilizumab blocks RANKL production in FLS in vitro[44]. Treatment with tocilizumab reduced FLS production of CCL20 like TNF inhibitors, although this likely occurs through an indirect process since stimulation of FLS with IL-6 does not increase CCL20 production[39]. Finally, expression of the chemokine Monocyte Chemoattractant Protein-1 (MCP-1) in FLS-PBMC co-cultures was significantly decreased by tocilizumab[45]. MCP-1 is expressed by FLS in RA synovium and plays an important role recruiting mononuclear cells to the joint, suggesting that this process is disrupted by tocilizumab.

Other biologics.

Anakinra is a natural IL-1 inhibitor that has been approved for use in RA, although it is less effective than other approved biologics[46]. Treatment with anakinra did not affect MCP-1 production by RA FLS, although it blocks in vitro functions of IL-1 like MMP and cytokine expression[45]. The CD80/86 inhibitor abatacept could affect FLS function like MMP expression, albeit indirectly because fibroblasts do not express CD80 or CD86[47], [48]. No data are available on the effects of rituximab on FLS, but direct effects are also unlikely because they do not express CD20.

Effect of Targeted Synthetic DMARDs on FLS

The three currently FDA-approved targeted synthetic DMARDs (tsDMARDs) for RA are tofacitinib, baricitinib, and upadacitinib. All three inhibit Janus kinas 1 (JAK1); tofacitinib and baricitinib also inhibit JAK2 while the former inhibits JAK3 to some extent[49]. JAK1 is likely the most important JAK for RA, as it lies downstream of IL-6, and interferon-gamma signaling pathways[50]. The IL-6 JAK-STAT signaling pathway in particular has distinctive epigenetic marks in RA FLS and is thought to be critical to FLS activity[51]. A recent study showed that tofacitinib decreased IL-6 and interferon-gamma pathway signaling in FLS[52], as well as IL-6 and MMP production[53] and downstream chemokine expression

in that pathway[54]. Tofacitinib also suppresses FLS autophagy but does not cause FLS apoptosis[55]. Baricitinib suppresses the interferon-gamma pathway[56] with a resultant decrease in IL-6 and MCP-1 production[57].

Experimental Therapies That Directly Target FLS

Two biologic agents intended to target FLS directly have completed Phase 2 human trials to date. RG6125 is a humanized monoclonal antibody that binds cadherin 11, which is an adhesion molecule expressed by FLS and responsible for aggregation in the synovial intimal lining. Pre-clinical models suggest that cadherin 11 is involved in articular cartilage erosion[58]. However, a Phase 2 trial RG6125 in RA failed to show benefit, including clinical disease activity scores and MRI synovitis scores[59]. ASP5094 is a monoclonal antibody directed against integrin alpha-9, which is highly expressed by RA FLS and also contributes to cell adhesion and inflammation[60]. ASP5094 failed in a Phase 2a trial, where it did not show any difference in disease activity scores compared to placebo[61].

Despite these disappointing results, several other therapeutic candidates are being explored in human trials. Seliciclib is a cyclin-dependent kinase (CDK) inhibitor initially developed for treatment of non-small cell lung cancer[62]. In RA FLS, endogenous CDK inhibitors such as p21 are lower than in osteoarthritis FLS, which results in increased proliferation, IL-6 and MMP production[63]. Seliciclib (previously known as roscovitine) blocks CDK directly[64] and increases expression of p21[65]. A phase 1b trial of seliciclib in RA patients refractory to TNF inhibitors had a good safety profile with a trend towards decreased disease activity. A phase 2a study is in progress to assess clinical efficacy[66].

FLS also express several Toll-like receptors which signal through the interleukin-1 receptor-associated kinase 4 (IRAK4) and increase production of IL-6 and MMPs[67]. PF-06650833 is a small-molecule inhibitor of IRAK4 that decreased the release of MMPs and cytokines in-vitro and attenuated arthritis severity in a rat model[68]. The potential benefit of this compound is now being explored in Phase 2 trials, and interim data at week 12 suggests a greater clinical response rate than placebo[69]. Numerous other candidate drugs are currently being studied in preclinical studies[15,70–75] (see Table 2).

Conclusion

FLS play a key role in the pathogenesis of RA through MMP and cytokine production, osteoclast activation, and immune cell recruitment. These pro-inflammatory mechanisms are modulated by currently existing RA therapies. The effects of current treatments on FLS can be direct and indirect with various agents modulating FLS signaling, metabolism, apoptosis, and cytokine production. None of the current treatments are specific for FLS, however, and to date no FLS-targeted therapy demonstrated efficacy. One potential advantage of FLS-targeted therapy might be clinical benefit without systemic immune suppression associated with current RA treatments. For that reason several FLS-targeted drugs are being developed, despite some clinical trial failures so far. As we learn more about the functions of various fibroblast phenotypes and subsets in RA, new opportunities for safer and more targeted

treatment options may become apparent, with the potential to place more RA patients into remission.

References

1. Hunter TM, Boytsov NN, Zhang X, Schroeder K, Michaud K, Araujo AB: Prevalence of rheumatoid arthritis in the United States adult population in healthcare claims databases, 2004–2014. Rheumatol Int 2017, 37:1551–1557. [PubMed: 28455559]
2. Smolen JS, Aletaha D, McInnes IB: Rheumatoid arthritis. Lancet 2016, 388:2023–2038. [PubMed: 27156434]
3. Demoruelle MK, Deane KD: Treatment Strategies in Early Rheumatoid Arthritis and Prevention of Rheumatoid Arthritis. Curr Rheumatol Rep 2012, 14:472–480. [PubMed: 22773387]
4. Feldmann M, Maini R: Perspectives From Masters in Rheumatology and Autoimmunity: Can We Get Closer to a Cure for Rheumatoid Arthritis? Arthritis Rheumatol 2015, 67.
5. Aletaha D, Smolen JS: Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA 2018, 320:1360–1372. [PubMed: 30285183]
6. Svensson MND, Zoccheddu M, Yang S, Nygaard G, Secchi C, Doody KM, Slowikowski K, Mizoguchi F, Humby F, Hands R, et al. : Synoviocyte-targeted therapy synergizes with TNF inhibition in arthritis reversal. Sci Adv 2020, 6:eaba4353. [PubMed: 32637608]
7. Bartok B, Firestein GS: Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev 2010, 233:233–255. [PubMed: 20193003]
8. Jay GD, Britt DE, Cha CJ: Lubricin is a product of megakaryocyte stimulating factor gene expression by human synovial fibroblasts. J Rheumatol 2000, 27:594–600. [PubMed: 10743795]
9. Bottini N, Firestein GS: Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. Nat Rev Rheumatol 2013, 9:24–33. [PubMed: 23147896]
10. Bustamante MF, Garcia-Carbonell R, Whisenant KD, Guma M: Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. Arthritis Res Ther 2017, 19:110. [PubMed: 28569176]
11. Sabeh F, Fox D, Weiss SJ: Membrane-type I matrix metalloproteinase-dependent regulation of rheumatoid arthritis synoviocyte function. J Immunol 2010, 184:6396–6406. [PubMed: 20483788]
12. Yamamura Y, Gupta R, Morita Y, He X, Pai R, Endres J, Freiberg A, Chung K, Fox DA: Effector function of resting T cells: activation of synovial fibroblasts. J Immunol 2001, 166:2270–2275. [PubMed: 11160281]
13. Reparon-Schuijt C, van Esch W, C van K, Bc R, Ew L, Fc B, Cl V: Regulation of synovial B cell survival in rheumatoid arthritis by vascular cell adhesion molecule 1 (CD106) expressed on fibroblast-like synoviocytes. Arthritis Rheumatol 2000, 43.
14. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, Korb A, Smolen J, Hoffmann M, Scheinecker C, et al. : Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007, 13:156–163. [PubMed: 17237793]
15. Wei K, Korsunsky I, Marshall JL, Gao A, Watts GFM, Major T, Croft AP, Watts J, Blazar P, Lange J, et al. : Notch signaling drives synovial fibroblast identity and arthritis pathology. Nature 2020, 582:259–264. [PubMed: 32499639]
16. Croft AP, Campos J, Jansen K, Turner JD, Marshall J, Attar M, Savary L, Wehmeyer C, Naylor AJ, Kemble S, et al. : Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature 2019, 570:246–251. [PubMed: 31142839]
17. Rajagopalan PTR, Zhang Z, McCourt L, Dwyer M, Benkovic SJ, Hammes GG: Interaction of dihydrofolate reductase with methotrexate: Ensemble and single-molecule kinetics. Proc Natl Acad Sci U S A 2002, 99:13481–13486. [PubMed: 12359872]
18. Friedman B, Cronstein B: Methotrexate Mechanism in Treatment of Rheumatoid Arthritis. Joint Bone Spine 2019, 86:301–307. [PubMed: 30081197]
19. Fraenkel L, Bathon JM, England BR, St Clair EW, Arayssi T, Carandang K, Deane KD, Genovese M, Huston KK, Kerr G, et al. : 2021 American College of Rheumatology Guideline for the

Treatment of Rheumatoid Arthritis. Arthritis Care Res (Hoboken) 2021, 73:924–939. [PubMed: 34101387]

20. Boyle DL, Han Z, Rutter JL, Brinckerhoff CE, Firestein GS: Posttranscriptional regulation of collagenase-1 gene expression in synoviocytes by adenosine receptor stimulation. Arthritis Rheumatol 1997, 40:1772–1779.
21. Shervington L, Darekar A, Shaikh M, Mathews R, Shervington A: Identifying Reliable Diagnostic/Predictive Biomarkers for Rheumatoid Arthritis. Biomark Insights 2018, 13:1177271918801005.
22. Lories R, Derese I, De Bari C, Luyten F: In vitro growth rate of fibroblast-like synovial cells is reduced by methotrexate treatment. Ann Rheum Dis 2003, 62:568–571. [PubMed: 12759297]
23. Lee C-K, Lee EY, Chung SM, Mun SH, Yoo B, Moon H-B: Effects of disease-modifying antirheumatic drugs and antiinflammatory cytokines on human osteoclastogenesis through interaction with receptor activator of nuclear factor kappaB, osteoprotegerin, and receptor activator of nuclear factor kappaB ligand. Arthritis Rheumatol 2004, 50:3831–3843.
24. Sato K, Tsuchihara K, Fujii S, Sugiyama M, Goya T, Atomi Y, Ueno T, Ochiai A, Esumi H: Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation. Cancer Res 2007, 67:9677–9684. [PubMed: 17942897]
25. Misra S, Bagchi A, Sarkar A, Niyogi S, Bhattacharjee D, Chatterjee S, Mondal S, Chattopadhyay A, Saha A, Chatterjee S, et al. : Methotrexate and theaflavin-3, 3'-digallate synergistically restore the balance between apoptosis and autophagy in synovial fibroblast of RA: an ex vivo approach with cultured human RA FLS. Inflammopharmacology 2021, 29:1427–1442. [PubMed: 34350509]
26. Xu K, Cai Y, Lu S-M, Li X-L, Liu L, Li Z, Liu H, Xu P: Autophagy induction contributes to the resistance to methotrexate treatment in rheumatoid arthritis fibroblast-like synovial cells through high mobility group box chromosomal protein 1. Arthritis Res Ther 2015, 17:374. [PubMed: 26702616]
27. Iwamoto N, Furukawa K, Endo Y, Shimizu T, Sumiyoshi R, Umeda M, Koga T, Kawashiri S, Igawa T, Ichinose K, et al. : Methotrexate Alters the Expression of microRNA in Fibroblast-like Synovial Cells in Rheumatoid Arthritis. Int J Mol Sci 2021, 22:11561. [PubMed: 34768991]
28. Schrezenmeier E, Dörner T: Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol 2020, 16:155–166. [PubMed: 32034323]
29. Kim W-U, Yoo S-A, Min S-Y, Park S-H, Koh H-S, Song S-W, Cho C-S: Hydroxychloroquine potentiates Fas-mediated apoptosis of rheumatoid synoviocytes. Clin Exp Immunol 2006, 144:503–511. [PubMed: 16734620]
30. Kim H-J, Lee S, Lee H-Y, Won H, Chang S-H, Nah S-S: 15-Hydroxyprostaglandin dehydrogenase is upregulated by hydroxychloroquine in rheumatoid arthritis fibroblast-like synoviocytes. Mol Med Rep 2015, 12:4141–4148. [PubMed: 26082314]
31. Teschner S, Burst V: Leflunomide: a drug with a potential beyond rheumatology. Immunotherapy 2010, 2:637–650. [PubMed: 20874647]
32. Burger D, Begué-Pastor N, Benavent S, Gruaz L, Kaufmann M-T, Chicheportiche R, Dayer J-M: The active metabolite of leflunomide, A77 1726, inhibits the production of prostaglandin E2, matrix metalloproteinase 1 and interleukin 6 in human fibroblast-like synoviocytes. Rheumatology (Oxford) 2003, 42:89–96. [PubMed: 12509619]
33. Vergne-Salle P, Léger DY, Bertin P, Trèves R, Beneytout J-L, Liagre B: Effects of the active metabolite of leflunomide, A77 1726, on cytokine release and the MAPK signalling pathway in human rheumatoid arthritis synoviocytes. Cytokine 2005, 31:335–348. [PubMed: 16099671]
34. Stuhlmeier KM: Effects of leflunomide on hyaluronan synthases (HAS): NF-kappa B-independent suppression of IL-1-induced HAS1 transcription by leflunomide. J Immunol 2005, 174:7376–7382. [PubMed: 15905585]
35. Yao Y, Ding C, Fang Y: Combination of MTX and LEF attenuates inflammatory bone erosion by down-regulation of receptor activator of NF-kB ligand and interleukin-17 in type II collagen-induced arthritis rats. Rheumatol Int 2013, 33:1845–1853. [PubMed: 23334376]
36. Ben Mrid R, Bouchmaa N, Ainani H, El Fatimy R, Malka G, Mazini L: Anti-rheumatoid drugs advancements: New insights into the molecular treatment of rheumatoid arthritis. Biomed Pharmacother 2022, 151:113126. [PubMed: 35643074]

37. Lee A, Qiao Y, Grigoriev G, Chen J, Park-Min K-H, Park SH, Ivashkiv LB, Kalliolias GD: TNFα induces sustained signaling and a prolonged and unremitting inflammatory response in synovial fibroblasts. Arthritis Rheumatol 2013, 65:928–938.

38. Ohara RA, Edhayan G, Rasmussen SM, Isozaki T, Remmer HA, Lanigan TM, Campbell PL, Urquhart AG, Lawton JN, Chung KC, et al. : Citrullinated Inhibitor of DNA Binding 1 Is a Novel Autoantigen in Rheumatoid Arthritis. Arthritis Rheumatol 2019, 71:1241–1251. [PubMed: 30861322]

39. Kawashiri S-Y, Kawakami A, Iwamoto N, Fujikawa K, Aramaki T, Tamai M, Arima K, Kamachi M, Yamasaki S, Nakamura H, et al. : Proinflammatory Cytokines Synergistically Enhance the Production of Chemokine Ligand 20 (CCL20) from Rheumatoid Fibroblast-like Synovial Cells in vitro and Serum CCL20 Is Reduced in vivo by Biologic Disease-modifying Antirheumatic Drugs. J Rheumatol 2009, 36:2397–2402. [PubMed: 19797510]

40. Pattacini L, Boiardi L, Casali B, Salvarani C: Differential effects of anti-TNF-α drugs on fibroblast-like synoviocyte apoptosis. Rheumatology (Oxford) 2010, 49:480–489. [PubMed: 20040530]

41. Ziolkowska M, Kurowska M, Radzikowska A, Luszczykiewicz G, Wiland P, Dziewczopolski W, Filipowicz-Sosnowska A, Pazdur J, Szechinski J, Kowalczewski J, et al. : High levels of osteoprotegerin and soluble receptor activator of nuclear factor kappa B ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor alpha treatment. Arthritis Rheumatol 2002, 46:1744–1753.

42. Badot V, Galant C, Nzeusseu Toukap A, Theate I, Maudoux A-L, Van den Eynde BJ, Durez P, Houssiau FA, Lauwerys BR: Gene expression profiling in the synovium identifies a predictive signature of absence of response to adalimumab therapy in rheumatoid arthritis. Arthritis Res Ther 2009, 11:R57. [PubMed: 19389237]

43. Ai R, Hammaker D, Boyle DL, Morgan R, Walsh AM, Fan S, Firestein GS, Wang W: Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. Nat Commun 2016, 7:11849. [PubMed: 27282753]

44. Hashizume M, Hayakawa N, Mihara M: IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. Rheumatology (Oxford) 2008, 47:1635–1640. [PubMed: 18786965]

45. Nielsen MA, Lomholt S, Mellemkjaer A, Andersen MN, Buckley CD, Kragstrup TW: Responses to Cytokine Inhibitors Associated with Cellular Composition in Models of Immune-Mediated Inflammatory Arthritis. ACR Open Rheumatol 2020, 2:3–10. [PubMed: 31943973]

46. Mertens M, Singh JA: Anakinra for rheumatoid arthritis. Cochrane Database Syst Rev 2009, doi:10.1002/14651858.CD005121.pub3.

47. Zou Q-F, Li L, Han Q-R, Wang Y-J, Wang X-B: Abatacept alleviates rheumatoid arthritis development by inhibiting migration of fibroblast-like synoviocytes via MAPK pathway. Eur Rev Med Pharmacol Sci 2019, 23:3105–3111. [PubMed: 31002160]

48. Corrigall VM, Solau-Gervais E, Panayi GS: Lack of CD80 expression by fibroblast-like synoviocytes leading to anergy in T lymphocytes. Arthritis Rheumatol 2000, 43:1606–1615.

49. Harrington R, Al Nokhatha SA, Conway R: JAK Inhibitors in Rheumatoid Arthritis: An Evidence-Based Review on the Emerging Clinical Data. J Inflamm Res 2020, 13:519–531. [PubMed: 32982367]

50. Choy EH: Clinical significance of Janus Kinase inhibitor selectivity. Rheumatology (Oxford) 2019, 58:953–962. [PubMed: 30508136]

51. Hammaker D, Nygaard G, Kuhs A, Ai R, Boyle DL, Wang W, Firestein GS: Joint Location–Specific JAK-STAT Signaling in Rheumatoid Arthritis Fibroblast-like Synoviocytes. ACR Open Rheumatol 2019, 1:640–648. [PubMed: 31872186]

52. Palasiewicz K, Umar S, Romay B, Zomorrodi RK, Shahrara S: Tofacitinib therapy intercepts macrophage metabolic reprogramming instigated by SARS-CoV-2 Spike protein. Eur J Immunol 2021, 51:2330–2340. [PubMed: 34107055]

53. Emori T, Kasahara M, Sugahara S, Hashimoto M, Ito H, Narumiya S, Higashi Y, Fujii Y: Role of JAK-STAT signaling in the pathogenic behavior of fibroblast-like synoviocytes in rheumatoid

arthritis: Effect of the novel JAK inhibitor peficitinib. Eur J Pharmacol 2020, 882:173238. [PubMed: 32561292]

54. Rosengren S, Corr M, Firestein GS, Boyle DL: The JAK inhibitor CP-690,550 (tofacitinib) inhibits TNF-induced chemokine expression in fibroblast-like synoviocytes: autocrine role of type I interferon. Ann Rheum Dis 2012, 71:440–447. [PubMed: 22121136]
55. Vomero M, Caliste M, Barbati C, Speziali M, Celia AI, Ucci F, Ciancarella C, Putro E, Colasanti T, Buoncuore G, et al. : Tofacitinib Decreases Autophagy of Fibroblast-Like Synoviocytes From Rheumatoid Arthritis Patients. Front Pharmacol 2022, 13:852802. [PubMed: 35308233]
56. Karonitsch T, Beckmann D, Dalwigk K, Niederreiter B, Studenic P, Byrne RA, Holinka J, Sevelda F, Korb-Pap A, Steiner G, et al. : Targeted inhibition of Janus kinases abates interfon gamma-induced invasive behaviour of fibroblast-like synoviocytes. Rheumatology (Oxford) 2018, 57:572–577. [PubMed: 29228301]
57. Weston S, Macdonald JL, Williams LM, Roussou E, Kang NV, Kiriakidis S, Taylor PC: The JAK inhibitor baricitinib inhibits oncostatin M induction of proinflammatory mediators in ex-vivo synovial derived cells. Clin Exp Rheumatol 2022, 40:1620–1628. [PubMed: 34665696]
58. Lee DM, Kiener HP, Agarwal SK, Noss EH, Watts GFM, Chisaka O, Takeichi M, Brenner MB: Cadherin-11 in synovial lining formation and pathology in arthritis. Science 2007, 315:1006–1010. [PubMed: 17255475]
59. Finch R, Sostelly A, Sue-Ling K, Blaeuer A, Duchateau-Nguyen G, Ukarma L, Petry C, Ravva P, Villiger P, Junker U: Op0224 Results of a Phase 2 Study of Rg6125, an Anti-Cadherin-11 Monoclonal Antibody, in Rheumatoid Arthritis Patients with an Inadequate Response to Anti-Tnfalpha Therapy. Ann Rheum Dis 2019, 78:189–189.
60. Emori T, Hirose J, Ise K, Yomoda J-I, Kasahara M, Shinkuma T, Yoshitomi H, Ito H, Hashimoto M, Sugahara S, et al. : Constitutive Activation of Integrin α9 Augments Self-Directed Hyperplastic and Proinflammatory Properties of Fibroblast-like Synoviocytes of Rheumatoid Arthritis. J Immunol 2017, 199:3427–3436. [PubMed: 29038247]
61. Takeuchi T, Tanaka Y, Erdman J, Kaneko Y, Saito M, Higashitani C, Smulders R, Lademacher C: ASP5094, a humanized monoclonal antibody against integrin alpha-9, did not show efficacy in patients with rheumatoid arthritis refractory to methotrexate: results from a phase 2a, randomized, double-blind, placebo-controlled trial. Arthritis Res Ther 2020, 22:252. [PubMed: 33087159]
62. Aldoss IT, Tashi T, Ganti AK: Seliciclib in malignancies. Expert Opin Investig Drugs 2009, 18:1957–1965.
63. Perlman H, Bradley K, Liu H, Cole S, Shamiyeh E, Smith RC, Walsh K, Fiore S, Koch AE, Firestein GS, et al. : IL-6 and matrix metalloproteinase-1 are regulated by the cyclin-dependent kinase inhibitor p21 in synovial fibroblasts. J Immunol 2003, 170:838–845. [PubMed: 12517948]
64. Alessi F, Quarta S, Savio M, Riva F, Rossi L, Stivala LA, Scovassi AI, Meijer L, Prosperi E: The Cyclin-Dependent Kinase Inhibitors Olomoucine and Roscovitine Arrest Human Fibroblasts in G1 Phase by Specific Inhibition of CDK2 Kinase Activity. Exp Cell Res 1998, 245:8–18. [PubMed: 9828096]
65. Park J, Schutzer W, Lindsley J, Bagby S, Oyama T, Anderson S, Weiss R: p21 is decreased in polycystic kidney disease and leads to increased epithelial cell cycle progression: roscovitine augments p21 levels. BMC Nephrol 2007, 8.
66. Pratt AG, Siebert S, Cole M, Stocken DD, Yap C, Kelly S, Shaikh M, Cranston A, Morton M, Walker J, et al. : Targeting synovial fibroblast proliferation in rheumatoid arthritis (TRAFIC): an open-label, dose-finding, phase 1b trial. The Lancet Rheumatology 2021, 3:e337–e346. [PubMed: 33928262]
67. Ospelt C, Brentano F, Rengel Y, Stanczyk J, Kolling C, Tak PP, Gay RE, Gay S, Kyburz D: Overexpression of toll-like receptors 3 and 4 in synovial tissue from patients with early rheumatoid arthritis: toll-like receptor expression in early and longstanding arthritis. Arthritis Rheumatol 2008, 58:3684–3692.
68. Winkler A, Sun W, De S, Jiao A, Sharif MN, Symanowicz PT, Athale S, Shin JH, Wang J, Jacobson BA, et al. : The Interleukin-1 Receptor–Associated Kinase 4 Inhibitor PF-06650833 Blocks Inflammation in Preclinical Models of Rheumatic Disease and in Humans Enrolled in a Randomized Clinical Trial. Arthritis Rheumatol 2021, 73:2206–2218. [PubMed: 34423919]

69. Danto SI, Shojaee N, Singh R, Manukyan Z, Mancuso J, Peeva E, Vincent M, Beebe J: Efficacy and Safety of the Selective Interleukin-1 Receptor Associated Kinase 4 Inhibitor, PF-06650833, in Patients with Active Rheumatoid Arthritis and Inadequate Response to Methotrexate [abstract]. Arthritis Rheumatol 2019, 71.

70. Park JK, Shon S, Yoo HJ, Suh D-H, Bae D, Shin J, Jun JH, Ha N, Song H, Choi YI, et al. : Inhibition of histone deacetylase 6 suppresses inflammatory responses and invasiveness of fibroblast-like-synoviocytes in inflammatory arthritis. Arthritis Res Ther 2021, 23:177. [PubMed: 34225810]

71. Grabiec AM, Korchynskyi O, Tak PP, Reedquist KA: Histone deacetylase inhibitors suppress rheumatoid arthritis fibroblast-like synoviocyte and macrophage IL-6 production by accelerating mRNA decay. Ann Rheum Dis 2011, 71:424–431. [PubMed: 21953341]

72. Angiolilli C, Kabala PA, Grabiec AM, Rossato M, Lai WS, Fossati G, Mascagni P, Steinkühler C, Blackshear PJ, Reedquist KA, et al. : Control of cytokine mRNA degradation by the histone deacetylase inhibitor ITF2357 in rheumatoid arthritis fibroblast-like synoviocytes: beyond transcriptional regulation. Arthritis Res Ther 2018, 20:148. [PubMed: 30029685]

73. Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P, Donà G, Fossati G, Sozzani S, Azam T, et al. : The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. Proc Natl Acad Sci U S A 2002, 99:2995–3000. [PubMed: 11867742]

74. Li X-F, Wu S, Yan Q, Wu Y-Y, Chen H, Yin S-Q, Chen X, Wang H, Li J: PTEN Methylation Promotes Inflammation and Activation of Fibroblast-Like Synoviocytes in Rheumatoid Arthritis. Front Pharmacol 2021, 12:700373. [PubMed: 34305608]

75. Dorst DN, Rijpkema M, Boss M, Walgreen B, Helsen MMA, Bos DL, Brom M, Klein C, Laverman P, van der Kraan PM, et al. : Targeted photodynamic therapy selectively kills activated fibroblasts in experimental arthritis. Rheumatology (Oxford) 2020, 59:3952–3960. [PubMed: 32734285]

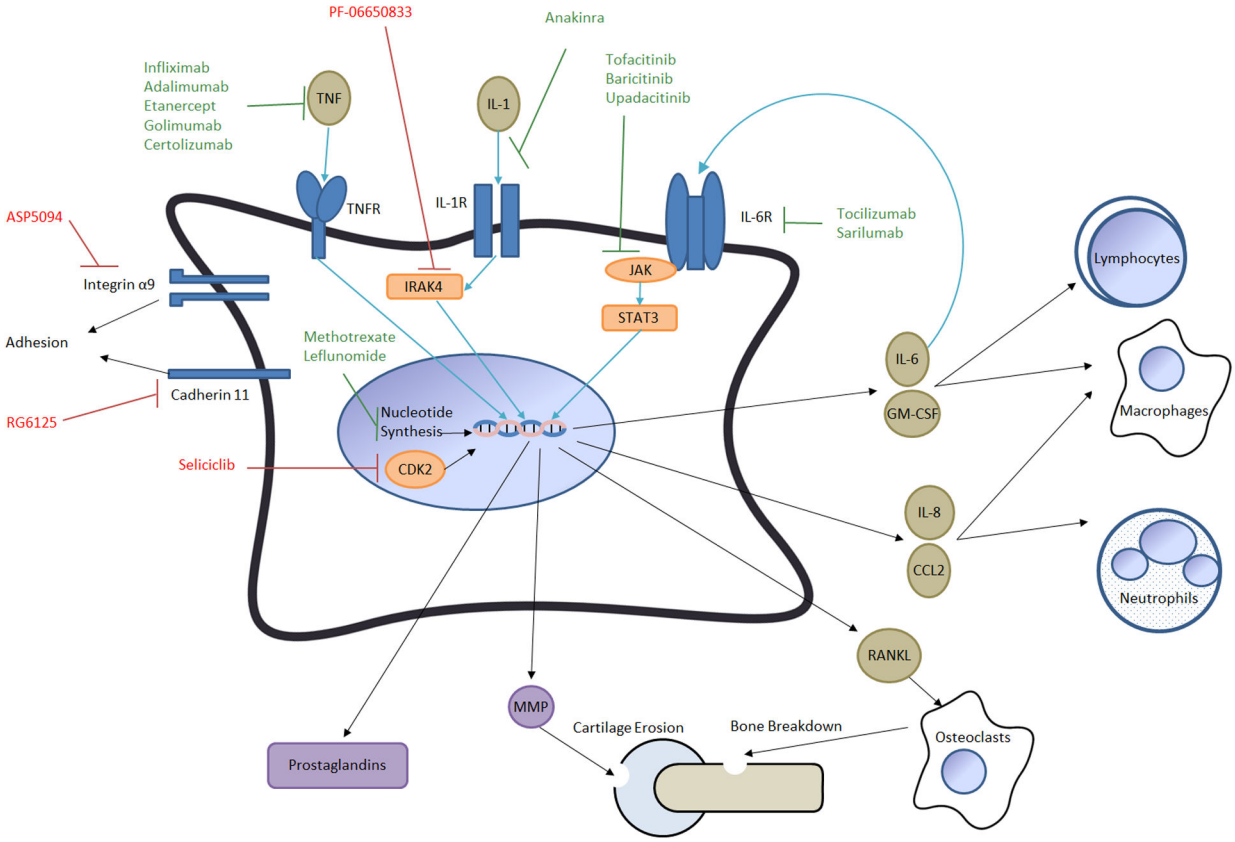

Figure 1.

FLS signaling and function in RA. FLS have numerous effects on the synovial immune environment through production of cytokines, chemokines, and prostaglandins, and directly erode cartilage using MMP. Some approved drugs (green) and investigational drugs (red) for RA inhibit several important FLS functional pathways.

Abbreviations: CCL2: C-C Motif Chemokine Ligand 2; CDK: cyclin-dependent kinase; GM-CSF: Granulocyte macrophage colony-stimulating factor; IL: interleukin; JAK: Janus kinase; IRAK4: interleukin-1 receptor-associated kinase 4; MMP: matrix metalloproteinase; RANKL: Receptor activator of nuclear factor kappa-B ligand; STAT: Signal transducer and activator of transcription; TNF: tumor necrosis factor;

Table 1:

Summary of currently approved drugs for rheumatoid arthritis and their effects on FLS

Drug Name	Target/Biologic effect	Effect on FLS
csDMARDs		
Methotrexate	Dihydrofolate reductase	↓MMP, IL-6, RANKL, growth, autophagy
Hydroxychloroquine	Possible effect on MHC Class II, Toll-like receptors	↑Apoptosis ↓Prostaglandins
Sulfasalazine	Multiple effects of metabolites sulfapyridine and 5-aminosalicylic acid	↓Prostaglandins, osteoclast formation
Leflunomide	Dihydroorotate dehydrogenase	↓MMP, IL-6, prostaglandins
Biologics		
Infliximab Etanercept Adalimumab Certolizumab Golimumab	TNF	↑Apoptosis ↓Proliferation, chemokine production, RANKL, osteoclast formation
Tocilizumab Sarilumab	IL-6 receptor	↓Chemokines, RANKL
tsDMARDs		
Tofacitinib Baricitinib Upadacitinib	JAK1, JAK2, and/or JAK3	↓IL-6, MMP, chemokines, autophagy

Abbreviations: csDMARDs: conventional synthetic disease modifying antirheumatic drugs; FLS: fibroblast-like synoviocytes; IL: interleukin; MHC: major histocompatibility complex; JAK: Janus kinase; MMP: matrix metalloproteinase; RANKL: Receptor activator of nuclear factor kappa-B ligand; TNF: tumor necrosis factor; tsDMARDs: targeted synthetic disease modifying antirheumatic drugs

Table 2:

Selected FLS-targeted therapies under investigation

Drug Name	Target	Status	Effect on FLS
Small Molecules			
Seliciclib	Cyclin-dependent kinase	Phase 2a (ongoing)	↓Proliferation, IL-6, MMP[66]
PF-06650833	IRAK4	Phase 2 (ongoing)	↓MMP, cytokines[69]
Trichostatin A Givinostat SAHA M808	Histone deacetylase	Preclinical *	↓IL-6, MMP, chemokines, synovitis[70–73]
5-azacytidine	DNA methyltransferase	Preclinical *	↓IL-6, chemokines, synovitis[74]
Biologics			
RG6125	Cadherin 11	Phase 2 (no efficacy)	↓Adhesion[59]
ASP5094	Integrin alpha-9	Phase 2a (no efficacy)	↓Adhesion[61]
Anti-NRR3	Notch3	Preclinical *	↓Synovitis, bone erosion[15]
28H1	Fibroblast activation protein-α	Preclinical *	↓Synovitis[75]

* Preclinical studies in rodent arthritis models

Abbreviations: IL: interleukin; IRAK4: interleukin-1 receptor-associated kinase 4; MMP: matrix metalloproteinase; NRR: negative regulatory region; SAHA: suberoylanilide hydroxamic acid;