Pharmacology

Immune Mediator Pharmacogenomics: *TCL1A* SNPs and Estrogen-Dependent Regulation of Inflammation

Ming-Fen Ho* and Richard M. Weinshilboum

Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

This review describes the important functional implications of *TCL1A* single nucleotide polymorphisms (SNPs) discovered during pharmacogenomic studies of aromatase inhibitor-induced musculoskeletal adverse events that were subsequently shown to influence the expression of cytokines, chemokines, toll-like receptors (TLR), and NF-κB in a SNP and estrogen-dependent fashion. Functional genomic studies of these SNPs led to the discovery of novel mechanisms that may contribute to disease pathophysiology and which may also increase our understanding of pharmacogenomic aspects of regulation of the expression of inflammatory mediators. Specifically, TCL1A expression was induced by estrogens in a SNP-dependent fashion, resulting in downstream effects on the expression of immune mediators that included IL17RA, IL17A, CCR6, CCL20 TLR2, TLR7, TLR9, TLR10 and NF-κB. These observations have potential implications for inflammatory diseases such as rheumatoid arthritis—a disease for which two thirds of patients are women. Strikingly, this genomic phenomenon could be "reversed" by estrogen receptor antagonist treatment—once again in a SNP-dependent, i.e., in a pharmacogenomic fashion. Specifically, differential SNP-dependent effects on estrogen receptor binding to estrogen response elements before and after estrogen receptor blockade might be associated with mechanisms underlying the SNP genotype and estrogen-dependent regulation of TCL1A and the expression of downstream immune mediators. Furthermore, this SNP and estrogen-dependent phenotypic response could be "reversed" by SERM treatment. These observations could potentially open the way to understand, predict and even pharmacologically manipulate the expression of selected immune mediators in a SNP-dependent fashion.

TCL1A | pharmacogenomics | aromatase inhibitor therapy | SNPs | estrogens | inflammation | immune mediators | toll-like receptors | cytokines | chemokines | NF-κB

Introduction

Aromatase inhibitor (AI) therapy blocks estrogen synthesis and can decrease the recurrence of estrogen receptor positive breast cancer by approximately 50% (1). However, musculoskeletal side effects are among the most important adverse events observed in women treated with AIs (2) and often result in the discontinuation of AI therapy (2). We previously performed a genome-wide association study (GWAS) using DNA from women with estrogen receptor (ER) positive breast cancer who were enrolled in the MA.27 adjuvant AI clinical trial. MA.27 is one of the largest adjuvant AI clinical trials ever conducted, with over 7000 patients enrolled (3). We observed a single nucleotide polymorphism (SNP) during the GWAS that mapped near the 3'-terminus of the *TCL1A* gene (rs11849538) (see **Figure 1A**), and imputation identified two additional SNPs (rs7359033 and rs7160302). All three SNPs were in tight linkage disequilibrium (LD) and were associated with AI-induced musculoskeletal adverse events (4). The minor allele frequency for the *TCL1A* SNP (rs11849538) is approximately 19% in European, African, Asian and Caucasian-Americans populations according to the 1000 Genomes Project (5). Subsequent functional genomic studies of these SNPs identified a novel genetic mechanism for estrogen-dependent regulation of the expression of a series of immune mediators (4, 6). TCL1A is expressed in activated T and B lymphocytes as well as thymocytes, and alterations in circulating estrogen concentrations have previously been associated with a variety of musculoskeletal symptoms (7).

Our functional genomic studies were performed with a panel of lymphoblastoid cell lines (LCLs) that express a wide range of immune genes. We found that the SNPs near *TCL1A* that we had identified during the GWAS were associated with variation in estrogen-dependent TCL1A expression (see **Figure 1B**), and downstream the expression of a series of cytokines, chemokines (8) and toll-like receptors (TLRs) (9) as well as the transcriptional activity of NF-κB (6). Specifically, the top hit SNP (rs11849538) from our GWAS created a functional estrogen response element (ERE) (4). We also observed that rs7359033 and rs7160302 were each at a distance from ERE motifs that were separate from the ERE created by the variant sequence for the top hit SNP, rs11849538, as shown graphically in **Figure 1A.** All three of these SNPs appeared to act in concert to influence the estrogen-dependent induction of TCL1A (**Figure 1B**) with downstream effects on the expression of a series of pro-inflammatory cytokines, chemokines and TLRs in LCLs (4, 6, 8, 9). These findings served to identify a molecular mechanism that may play a role in the complex interplay among estrogens, inflammatory mediators and disease phenotypes.

In this review, we will also describe our recent findings that SNPs at a distance from an ERE can have a profound effect on ERα binding and downstream phenotypes. Even more striking, selective estrogen receptor modulators (SERMs) such as tamoxifen can "**reverse**" this SNP genotype-dependent ERα binding (**Figure 1C-1E**) and downstream transcription—raising the possibility of the pharmacologic manipulation of ER binding to EREs and of subsequent phenotypes. Specifically, if the SNP genotypes are known, the phenotypes—in this case the downstream expression of cytokines, chemokines and TLRs—might be altered by estrogens and/or estrogen receptor blockade (6, 8, 9).

Reversal of SNP and drug induced transcriptional regulation

The "Human Variation Panel" LCL model system used in our studies has been utilized repeatedly to generate and test genomic and pharmacogenomic hypotheses and has proven to be a powerful tool because it allows the testing of many common genetic variants present in human populations (4, 6, 10-14).

Conflict of Interest: No conflicts declared.

*Correspondence and reprint request to Ming-Fen Ho, Ph.D.
Mayo Clinic 200 First Street S.W., Rochester, MN 55905, USA.
Email: ho.mingfen@mayo.edu

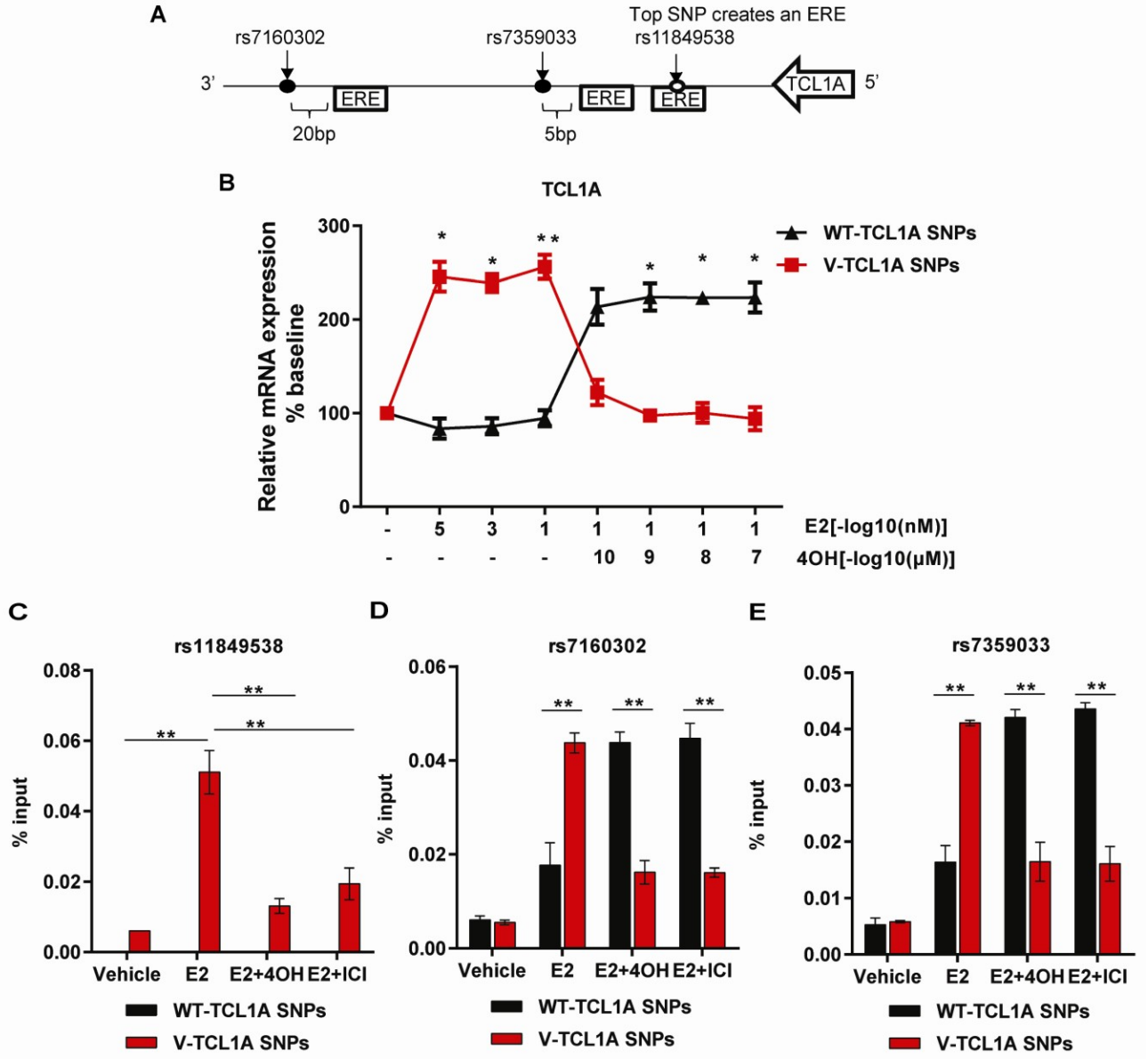

Figure 1. (**A**) Schematic diagram of two SNPs, rs7359033 and rs7160302, that are in tight LD with rs11849538—the "top hit" signal from the MA.27 musculoskeletal adverse event GWAS. Locations of estrogen response elements (EREs) are shown as boxes. All three of these SNPs map near the 3'-terminus of *TCL1A*. (**B**) SNP and estrogen-related variation in TCL1A mRNA expression in lymphoblastoid cell lines with known *TCL1A* SNP genotypes after exposure to estradiol (E2) with or without 4-OH-TAM. (**C-E**) ChIP assays showing the effect of ERα binding to EREs in or near the SNPs (see **Figure 1A**) in response to E2 (0.1nM), E2 (0.1nM) plus 4-OH-TAM (10^{-7}μM) or E2 (0.1nM) plus ICI (10^{-7}μM) treatment of LCLs homozygous for either wildtype (WT) or variant (V) genotypes for the rs11849538, rs7359033 and rs7160302 SNPs (note that the WT sequence for the rs11849538 SNP did not encode an ERE, so there was no binding for the WT genotype, see panel **C**). Adapted and modified from **Figure 2** in Ho et al. Molecular Endocrinology 2016 (8).

This model system consists of three different ethnicities, 100 cell lines per ethnic group, for a total of 300 cell lines. Each of the cell lines has genome-wide mRNA expression, genome-wide SNP and CpG methylation data, all of which made it possible for us to perform functional genomic studies. We found that TCL1A expression was up-regulated by estradiol (E2) only in LCLs carrying the variant sequence for the SNP (rs11849538) that created an ERE (**Figure 1B**) (4). Furthermore, *TCL1A* induction was associated with variation in the expression of a series of pro-inflammatory cytokines and their receptors (IL17A, IL17RA, IL12, IL12RB2 and IL1R2) as well as NF-κB transcriptional activity (6). We also recently reported that the expression of additional immune mediators such as *CCR6* and its only known ligand, *CCL20*, could also be modulated by *TCL1A* in a SNP-estrogen-dependent fashion (8) as could the expression of a series of TLRs (9). However, these estrogen induced effects were pharmacologically reversible. In the presence of 4-hydroxytamoxifen (4-OH-TAM), an active metabolite of

tamoxifen, or of fulvestrant (formerly ICI 182,780), an estrogen receptor antagonist, the SNP-estrogen-dependent induction of ERα binding was **"reversed"**, as determined by chromatin immunoprecipitation (ChIP) assays, and the expression of TCL1A responded in parallel (**Figure 1B-1E**) (8). Strikingly, the expression of cytokines, chemokines and TLRs "downstream" of TCL1A responded in a similar fashion—indicating that these estrogen receptor blocking drugs might be used to alter the expression of the inflammatory mediators in a SNP-dependent fashion—i.e., that "pharmacogenomics" could be used to predict and guide the use of drugs to alter the expression of known mediators of inflammation. It should be emphasized that the SNPs involved were located 3' of *TCL1A*. These SNPs were **not** in the genes encoding the pro-inflammatory cytokines, chemokines or TLRs. These observations could potentially open the way to make it possible to understand, predict and pharmacologically manipulate the expression of immune mediators in a SNP-dependent fashion by the administration of tamoxifen or fulvestrant.

As stated earlier, TLR2, TLR7, TLR9 and TLR10 expression, as well as that of MYD88, could be modulated by *TCL1A* in a SNP and estrogen-dependent fashion, and all of these effects could be reversed in the presence of SERMs (9). In addition, MYD88 inhibition blocked the *TCL1A* SNP and estrogen-dependent NF-κB activation. It is known that TLRs can regulate NF-kB signaling by a process that requires the MYD88 adaptor protein. These results served to confirm that *TCL1A* SNP and estrogen-dependent NF-κB activation may occur, at least in part, through a TLR-MYD88-dependent pathway. These functional genomic studies greatly extended our original observations from the clinical GWAS and highlighted a novel pharmacogenomic mechanism by which *TCL1A* can influence and modulate the immune response and inflammation (9).

Discussion and Conclusions

This series of functional genomic studies began with a GWAS performed using DNA from postmenopausal women receiving AIs to treat ER+ breast cancer to identify SNPs related to musculoskeletal adverse events that occur during AI therapy, as outlined in **Table 1**. Briefly, the SNP signal most highly associated with this phenotype included SNPs near *TCL1A*. Those SNPs could influence TCL1A estrogen-dependent gene expression in a SNP-dependent fashion, with downstream effects on the expression of a series of cytokines, chemokines, TLRs and NF-κB transcriptional activity. **Table 1** summarizes the major observations made in the series of studies that led to these conclusions. We also observed differential effects on ER binding to EREs before and after drug-induced ER blockade, with drug-induced "reversal" of both ER binding and downstream phenotypes that included the expression of inflammatory mediators. Because of the critical role of cytokines, chemokines and TLRs in inflammation, these observations have potential implications for the pathophysiology and treatment of rheumatologic disease. Female sex hormones are thought to play an important role in the etiology and pathophysiology of various rheumatic diseases (15). For example, rheumatoid arthritis (RA) not only shows a strong sex bias toward women, but also displays profound changes in incidence and severity during periods of change in estrogen levels. The incidence of RA and the risk of flares are increased during the postpartum period, a time of rapidly falling plasma estrogen levels (16). The highest risk for RA is observed during the menopausal years (17), while the use of estrogen-based contraceptives has been associated with a lower risk for the development of RA in some women with early undifferentiated arthritis (18). These observations display interesting parallels to the clinical impact of the pharmaceutical lowering of estrogen levels by drugs such as AIs.

Table 1, Summary of key findings for TCL1A functional genomic studies

References	Title	Key findings
Ingle et al, 2010 (4)	Genome-Wide Associations and Functional Genomic Studies of Musculoskeletal Adverse Events in Women Receiving Aromatase Inhibitors	*TCL1A* SNP (rs11849538), the "top hit" SNP from the GWAS created an estrogen response element and was associated with musculoskeletal adverse events in women treated with aromatase inhibitors for early breast cancer.
Liu et al, 2012 (6)	Aromatase inhibitors, estrogens and musculoskeletal pain: estrogen-dependent T-cell leukemia 1A (TCL1A) gene-mediated regulation of cytokine expression	Estradiol induced SNP-dependent TCL1A expression, which, in turn, was associated with alterations in expression of IL-17, IL-17RA, IL-12, IL-12RB2, and IL-1R2 as well as NF-kB transcriptional activity.
Ho et al, 2016 (8)	Estrogen, SNP-Dependent Chemokine Expression and Selective Estrogen Receptor Modulator Regulation	Variation in TCL1A expression influenced the downstream expression of CCR6 and CCL20. Furthermore, SNPs at a distance from EREs could regulate ERα binding and ER antagonists could **reverse** phenotypes associated with those SNPs.
Ho et al, 2017 (9)	*TCL1A* SNPs and estrogen-mediated toll-like receptor-MYD88-dependent NF-kB activation: SNP and SERM-dependent modification of inflammation and immune response	TCL1A expression could influence the downstream expression of TLR2, TLR7, TLR9, TLR10, and MYD88. Inhibition of MYD88 resulted in the blockade of *TCL1A* SNP-dependent NF-kB activation, indicating that the TLR-MYD88–dependent NF-kB signaling pathway could contribute to *TCL1A* SNP- and estrogen-dependent effects.

Our results are compatible with a role for genetic variation near the *TCL1A* gene in the regulation of the estrogen-dependent induction of selected cytokines and chemokines (IL17RA, IL17A, CCR6, CCL20,) all of which have implications for clinical inflammatory diseases and all of which might potentially be manipulated by drug therapy, i.e. treatment with selective estrogen receptor modulators (SERMs) such as tamoxifen. CCR6 and CCL20 mediated migration of Th17 cells has been suggested as an important molecular mechanism in a variety of autoimmune diseases including RA as a result of the recruitment of inflammatory cells to synovial/tenosynovial structures (19). In addition, IL17 and IL17RA are drug targets for the treatment of autoimmune diseases such as RA—with significant differences in incidence between the sexes, which might suggest critical molecular mechanisms that involve interplay between estrogens and immune regulation. Our experimental results demonstrate that the expression of these immune mediators can be modulated by TCL1A in a SNP and estrogen-dependent fashion. Although anti-cytokine therapies have been used to treat rheumatic diseases, for many autoimmune and inflammatory diseases, therapeutic decisions are often made by using a trial-and-error approach. Estrogens can play an important role in inflammatory diseases such as RA, for which two thirds of patients are women. However, the effect of sex hormones on the immune response in rheumatologic diseases remains to be fully elucidated at the molecular level. Therefore, our observations with regard to *TCL1A* SNP-dependent transcriptional regulation of immune mediators before and after estrogen receptor blockade offer insight into pharmacogenomic aspects of the regulation of the expression of pro-inflammatory cytokines, chemokines and TLRs. If these observations can eventually be translated into the clinic, they might represent a novel mechanism by which drugs could be used to regulate the estrogen-dependent induction of inflammatory mediator expression.

Acknowledgements

This work was supported in part by NIH grants U19 GM61388 (The Pharmacogenomics Research Network), P50 CA116201 (Mayo Clinic Breast Cancer Specialized Program of Research Excellence), the Mayo Center for Individualized Medicine, the RIKEN Center for Genomic Medicine, the Breast Cancer Research Foundation, and by NIH grants R01 GM28157, R01 CA138461, R01 CA196648.

1. Early Breast Cancer Trialists' Collaborative G. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378(9793):771-84.
2. Crew KD, Greenlee H, Capodice J, Raptis G, Brafman L, Fuentes D, et al. Prevalence of Joint Symptoms in Postmenopausal Women Taking Aromatase Inhibitors for Early-Stage Breast Cancer. Journal of Clinical Oncology. 2007;25(25):3877-83.
3. Goss PE, Ingle JN, Pritchard KI, Ellis MJ, Sledge GW, Budd GT, et al. Exemestane Versus Anastrozole in Postmenopausal Women With Early Breast Cancer: NCIC CTG MA.27—A Randomized Controlled Phase III Trial. Journal of Clinical Oncology. 2013;31(11):1398-404.
4. Ingle JN, Schaid DJ, Goss PE, Liu M, Mushiroda T, Chapman J-AW, et al. Genome-Wide Associations and Functional Genomic Studies of Musculoskeletal Adverse Events in Women Receiving Aromatase Inhibitors. Journal of Clinical Oncology. 2010;28(31):4674-82.
5. The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
6. Liu M, Wang L, Bongartz T, Hawse J, Markovic S, Schaid D, et al. Aromatase inhibitors, estrogens and musculoskeletal pain: estrogen-dependent T-cell leukemia 1A (TCL1A) gene-mediated regulation of cytokine expression. Breast Cancer Research. 2012;14(2):R41.
7. Noguchi M, Ropars V, Roumestand C, Suizu F. Proto-oncogene TCL1: more than just a coactivator for Akt. The FASEB Journal. 2007;21(10):2273-84.
8. Ho M-F, Bongartz T, Liu M, Kalari KR, Goss PE, Shepherd LE, et al. Estrogen, SNP-Dependent Chemokine Expression and Selective Estrogen Receptor Modulator Regulation. Molecular Endocrinology. 2016;30(3):382-98.
9. Ho M-F, Ingle JN, Bongartz T, Kalari KR, Goss PE, Shepherd LE, et al. TCL1A SNPs and estrogen-mediated toll-like receptor-MYD88-dependent NF-kappaB activation: SNP and SERM-dependent modification of inflammation and immune response. Molecular Pharmacology. 2017;92(2):175-84.
10. Ingle JN, Liu M, Wickerham DL, Schaid DJ, Wang L, Mushiroda T, et al. Selective Estrogen Receptor Modulators and Pharmacogenomic Variation in ZNF423 Regulation of BRCA1 Expression: Individualized Breast Cancer Prevention. Cancer Discovery. 2013;3(7):812-25.
11. Ingle JN, Xie F, Ellis MJ, Goss PE, Shepherd LE, Chapman J-AW, et al. Genetic polymorphisms in the long noncoding RNA MIR2052HG offer a pharmacogenomic basis for the response of breast cancer patients to aromatase inhibitor therapy. Cancer Research. 2016;76(23):7012–23. PMCID: 5135610.
12. Li L, Fridley B, Kalari K, Jenkins G, Batzler A, Safgren S, et al. Gemcitabine and Cytosine Arabinoside Cytotoxicity: Association with Lymphoblastoid Cell Expression. Cancer Research. 2008;68(17):7050-8.
13. Liu M, Ingle JN, Fridley BL, Buzdar AU, Robson ME, Kubo M, et al. TSPYL5 SNPs: association with plasma estradiol concentrations and aromatase expression. Mol Endocrinol. 2013;27(4):657-70. PMCID: 3607698.
14. Niu N, Liu T, Cairns J, Ly RC, Tan X, Deng M, et al. Metformin pharmacogenomics: a genome-wide association study to identify genetic and epigenetic biomarkers involved in metformin anticancer response using human lymphoblastoid cell lines. Human Molecular Genetics. 2016;25(21):4819-34.
15. Cutolo M, Brizzolara R, Atzeni F, Capellino S, Straub RH, Puttini PCS. The immunomodulatory effects of estrogens. Annals of the New York Academy of Sciences. 2010;1193(1):36-42.
16. Peschken CA, Robinson DB, Hitchon CA, Irene S, Hart D, Bernstein CN, et al. Pregnancy and the Risk of Rheumatoid Arthritis in a Highly Predisposed North American Native Population. The Journal of Rheumatology. 2012;39(12):2253-60.
17. Goemaere S, Ackerman C, Goethals K, De Keyser F, Van der Straeten C, Verbruggen G, et al. Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. J Rheumatol. 1990;17(12):1620-2.
18. Salliot C, Bombardier C, Saraux A, Combe B, Dougados M. Hormonal replacement therapy may reduce the risk for RA in women with early arthritis who carry HLA-DRB1 *01 and/or *04 alleles by protecting against the production of anti-CCP: results from the ESPOIR cohort. Annals of the Rheumatic Diseases. 2010;69(9):1683-6.
19. Hirota K, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, et al. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. The Journal of Experimental Medicine. 2007;204(12):2803-12.