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Review

Novel approaches to develop biomarkers predicting treatment responses to TNF-blockers.

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ABSTRACT

Introduction

Chronic inflammatory diseases (CIDs) cause significant morbidity and are a considerable burden for the patients in terms of pain, impaired function and diminished quality of life. Important progress in CID treatment has been obtained with biological therapies, such as Tumor-Necrosis-Factor blockers. However, more than a third of the patients fails to respond to these inhibitors, and are exposed to the side effects of treatment, without the benefits. Therefore, there is a strong interest to develop tools to predict response of patients to biologics.

Areas covered

We searched PubMed for recent studies on biomarkers for disease assessment and prediction of therapeutic responses, focusing on the effect of TNF blockers on immune responses in Spondyloarthritis (SpA), and other CID, in particular rheumatoid arthritis and inflammatory bowel disease. Conclusions will be drawn about the possible development of predictive biomarkers for response to treatment.

Expert opinion

No validated biomarker is currently available to predict treatment response in CID. New insight could be generated through the development of new bioinformatic modelling approaches to combine multidimensional biomarkers that explain the different genetic, immunological and environmental determinants of therapeutic responses.

KEYWORDS: spondyloarthritis, ankylosing spondylitis, chronic inflammatory disease, rheumatoid arthritis, inflammatory bowel disease, anti-TNF therapy, biomarkers, treatment response, prediction, transcriptome, immune responses

HIGHLIGHTS

- Anti-TNF therapy has strong effects on several immune response pathways, modulating gene expression, cell population frequencies and serum protein levels.
- Several biomarkers for disease progression in patients undergoing anti-TNF treatment have been identified, however no biomarker has been validated for clinical use to predict response to treatment at baseline.
- Genetic biomarkers based on single nucleotide polymorphisms have demonstrated limited power to predict response to treatment.
- The combination of several biomarkers may improve the prediction power of statistical models of response to anti-TNF therapy. In particular, the inclusion of different types of biomarkers (genetic,

transcriptional, protein, cellular) may be necessary to capture the biological complexity of response to treatment.

- New bioinformatic tools, including machine learning approaches, are necessary to handle the complexity of the large data sets being currently explored.

1. Introduction

Chronic inflammatory diseases (CID), such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), spondyloarthritis (SpA), psoriatic arthritis (PsA), and psoriasis (Pso) are a leading cause of morbidity. These conditions are associated with chronic pain and important functional impairment that have a large impact on productivity and quality of life [1,2].

In this review we will focus mainly on Spondyloarthritis (SpA), but also draw parallels with the larger literature on RA and IBD. SpA is one of the most common chronic inflammatory rheumatic diseases, with a prevalence ranging from 0.5% to 1.9% [3]. In addition to the disabling rheumatic manifestations, some SpA patients develop severe extra-articular manifestations such as inflammatory bowel disease (IBD), uveitis and psoriasis (Pso) [4]. SpA mainly affects young adults and the functional consequences of inadequately controlled disease alter both their quality of life and their professional capacity with direct impact on healthcare costs [5]. Improved control of the disease for affected individuals is thus an important public health issue. The introduction of biological therapies, such as strategies targeting the proinflammatory cytokine TNF, has revolutionized the treatment of CID. However, 30 to 40% of the patients fail to respond or acquire resistance to TNF-blockers, and it is currently not possible to predict response of patients to anti-TNF therapy [6–8]**.** Recently, anti-IL-17A drugs have been approved as a biologic alternative for axial SpA (axSpA) patients with active disease that do not respond to TNF inhibitors (TNFi), but this treatment also fails in about a third of the patients [9].

As a consequence, in current clinical practice treatment failures are observed for a substantial number of patients, who will not be appropriately treated for several months, while being exposed to the potential side effects of the drugs. There is therefore an urgent medical need to develop tools to guide treatment decisions for patients affected by SpA and other CID. The ability to predict the response to biologics, and to optimize the treatment will be the challenge for the next decade in CID.

Thus far, clinicians do not know, prior to treatment initiation, if a patient will respond or not to the treatment. Despite many efforts to identify predictive biomarkers of anti-TNF treatment response, there is still an unmet need for approaches that permit a pretreatment stratification of patients resulting in better patient healthcare and significant socio-economic benefits [9].

In this review we will explore recent work on the mechanistic action of TNF blockade in SpA, and comment on the latest approaches to identify predictive biomarkers. Comparison will be drawn with other CID (IBD and RA) for which a rich literature is available.

We performed an extensive literature search using PubMed. Our search mainly focused on papers published between 2015 and December 2020, but a few previous papers relevant to our subject were also included. We used keywords such as "Spondylarthritis", "Rheumatoid Arthritis", "Inflammatory Bowel Disease", "Anti-TNFα"(and single anti-TNF drugs), "Biomarkers", "Outcome", "Response" and "Prediction". Since the literature on predictive biomarkers is still limited, we also included papers investigating biomarkers associated with disease activity. Only English written papers were considered for our review.

2. Effects of anti-TNF therapy on the immune system in patients – a brief summary

The introduction of anti-TNF therapy has been a major breakthrough for the treatment of several chronic inflammatory diseases, in particular rheumatoid arthritis [10] (reviewed in [11]), inflammatory bowel disease [12,13], ankylosing spondylitis [14](reviewed in [15]), and psoriatic arthritis and psoriasis [16,17]. Early mechanistic studies revealed a strong reduction of inflammatory cytokines and acute phase proteins, such as IL-1, IL-6 and CRP within hours after injection of a TNF inhibitor (TNFi) [18]. Furthermore, levels of inflammatory chemokines and VEGF were also reduced, causing reduced granulocyte recruitment and angiogenesis in arthritic joints [19,20] (reviewed in [21]).

RNA sequencing technologies have become relatively affordable and permit the assessment, on a genome-wide level, of biomarkers within immune cells that might not be detectable in serum. mRNA profiling in SpA patients before and after treatment onset, may help identify molecular pathways associated with response to therapy. Using RNA sequencing, a gene expression analysis was performed on nineteen AS patients, to profile the transcriptomes of peripheral blood cells (PBMCs), and identified 656 genes differentially expressed before and after anti-TNF treatment. Analysis of signaling pathways using KEGG (Kyoto Encyclopedia of Genes and Genomes) revealed an enrichment of several immune and inflammation regulatory pathways, as well as infection metabolism-associated pathways in genes affected by anti-TNF therapy [22]. Among the differentially expressed genes were also genes associated to AS in GWAS studies (*IL6R*, *NOTCH1*, *IL10*, *CXCR2* and *TNFRSF1A*), highlighting pathogenic pathways that may be affected by TNF signaling. The altered expression of components of the NOTCH signaling pathway found in AS may be involved in osteoblast differentiation and ossification processes [23]. *TNFRSF1A* encodes an important receptor for TNF, and SNPs in the locus of the anti-inflammatory cytokine IL-10 have shown modest correlation with disease severity [24]. Interestingly, the study showed a distinct gene expression profile between male and female patients. Besides genes linked to sex chromosome, the only differentially expressed gene was *IL17RC*, which encodes an IL-17 receptor that binds IL-17A and IL-17F. This

proinflammatory pathway may play an important role in SpA [25]. An interesting hypothesis is that higher expression of *IL17RC* may be involved in the differences in disease severity and treatment response between the genders [22].

It is important to note that most transcriptomic studies are performed in PBMCs, and the mixture of different cell populations may hide biologically important features specific to a cell type. Peripheral blood cells may also not be representative of local inflammatory processes [26], however, biomarkers that can be identified in peripheral blood are worth investigating, since they are more easily applicable in large scale studies and in the clinics.

Menegatti et al. have recently investigated the global impact of TNFi on immune responses to microbial or pathway-specific stimuli in axSpA patients. The motivation for this study was that the effects of TNF-blockers had been studied mostly on resting immune cells but not in the setting of an ongoing immune response. The goals were to enhance the understanding of the molecular mechanisms of action of TNF-blockers in SpA patients and to identify immunological correlates of response to TNFi. To minimize sources of pre-analytical variability the authors used standardized whole-blood stimulation assays ("TruCulture" assays) [27] and a highly sensitive and robust pipeline to assess immune functions in patients [28]. Proteins in supernatants of stimulated whole-blood cultures were measured in a CLIA-certified laboratory and gene expression was measured using nCounter assays for immune genes, a technology not requiring enzymatic reactions and PCR amplification, already used in the clinics for diagnostic purposes [29]. Up to 300 genes (depending on the stimulation) were affected by anti-TNF therapy, revealing that TNFi induce profound changes in patients' innate immune responses. Stimulation of whole blood amplified the observable differences between samples from patients before and after TNF-blocker treatment, indicating that TNFi act primarily when the immune system is challenged but less in its resting state. The effects of TNFi on activated immune cells were detectable after a single injection of a TNF-blocker and persisted for 3 months of follow-up of the patients.

To understand the molecular basis of anti-TNF therapy action on immune responses, pathway analysis was performed on the differentially expressed genes. Since nCounter technology does not allow a genome-wide gene expression analysis, the Quantitative Set Analysis for Gene Expression (QuSAGE) method was employed [30]. QuSAGE quantifies gene module activity as a shift in the mean differential expression of the individual genes included in the module and is compatible with a limited number of genes. Similar to other GSEA approaches, QuSAGE allows reducing the number of variables by collapsing sets of coordinately expressed genes into gene modules, facilitating functional interpretation of the results and improving robustness of biomarker signatures. Bancherau et al. have previously applied QuSAGE to functionally interpret gene lists associated with specific phenotypes in Lupus patients [31] and Latis et al. used this algorithm to compare and interpret T cell gene expression profiles in recipients after allogeneic hematopoietic stem cell transplantation and their sibling donors [32]. Menegatti et al. designed gene

modules by grouping genes belonging to specific signaling pathways, associated with particular cellular phenotypes, or with specific cellular functions, according to Molecular Signatures Database (MSigDB) annotations [33] and based on current knowledge in the literature. Since genes can play roles in distinct signaling pathways, a single gene could be present in several modules. The minimum size of one module was set to 3 genes. **Fig. 1** gives an example for the influenza stimulus. The TNF module groups TNF and several molecules associated with its receptor. TNF itself and many of the genes in this gene module were downregulated after one week of anti-TNF therapy. This is similar for the IL-1 module. In this study, *IL1A* was among the genes most strongly targeted by anti-TNF therapy. As for Toll-like-receptors (TLRs), some were downregulated, whereas the expression of others actually increased. Signaling through many of these receptors activates NF-kB regulatory kinases that phosphorylate the NF-kB inhibitor IkB and thereby activate NF-kB. NF-kB transcription factors, IkB and many NF-kB target genes were among the genes that were most profoundly downregulated by TNF blockers (**Fig. 1** and **2**). Since TNF itself, IL-1 and many other proinflammatory molecules are NF-kB target genes, one of the mechanisms by which TNFi control inflammation may be the disruption of an autoregulatory loop driven mainly by NF-kB [28].

Only few gene modules showed an increased pathway activity, such as the "cytotoxic molecules" module and the "NK cells" (**Fig. 3A**). An increased fraction of CD8+ T cells following anti-TNF treatment has also been recently described in RA patients [34]. The analysis also indicated that TNF-blockers may skew monocyte/macrophage polarization towards an M2 regulatory phenotype (**Fig. 3B**). M2-polarized macrophages are implicated in the resolution of inflammation, have immunoregulatory functions and orchestrate tissue repair and remodeling [35,36]. On the other hand, shifting the balance from a M1-like to a more M2-like profile may contribute to the increased risk of *Mycobacterium tuberculosis* reactivation in patients treated with TNFi [37–39], as M1 macrophages are important for granuloma formation and *M. tuberculosis* protection [40].

Anti-TNF treatment did not affect the Th1 or Th17 arms of the patients' immune response, nor IL-6 production in cells from axSpA patients, which contrasts previous results reported for RA [18]. These data seem consistent with the limited therapeutic efficacy of IL-6-blockade in SpA [41] and may suggest that IL-6 is more relevant to RA, but less to SpA pathogenesis.

Collectively, the analysis by Menegatti et al. revealed that TNFi target several distinct signaling pathways that cooperate to control inflammation. Some of these pathways may be of particular relevance for specific diseases. *PTGS2* (COX2) downregulation by TNFi (**Fig. 3C**) targets PGE_2 biosynthesis and is of particular relevance for enthesitis, a critical early pathogenic feature of SpA [42], while shifting the balance of macrophages from a pro-inflammatory (M1-like) phenotype to a pro-resolving (M2-like) phenotype is important for the resolution of synovitis, a key feature of RA. Expression of the PGE_2 receptor EP4 (encoded by *PTGER4*) was also downregulated by TNFi. Signaling through the EP4 receptor upregulates IL-23R expression and promotes human Th17 cell development [43].

3. The search for biomarkers of response

Anti-TNF therapy is not effective in 30-40% of patients affected by chronic inflammatory diseases, but the mechanisms underlying primary non-response to treatment are not known. Many studies have been performed to identify molecules that may serve as biomarkers to predict therapeutic responses to TNF-blockers, in particular in RA, IBD, and SpA (reviewed in [6,7,44,45]), however, no validated biomarker has yet emerged. In the following, we will summarize recent studies aiming at the identification of biomarkers for response to treatment, in particular in SpA, RA and IBD.

3.1 Transcriptomic biomarkers

To determine if therapeutic responses to TNFi were correlated with immune responses from SpA patients, Menegatti et al. have analyzed gene expression in whole-blood cultures stimulated with LPS or SEB from 80 SpA patients, before initiation of anti-TNF therapy [28]. The response to therapy was evaluated as changes in the "Ankylosing Spondylitis Disease Activity Score" (ASDAS) at 3 months after treatment initiation [46,47]. Gene expression analysis of these cultures revealed that 55 genes that were differentially expressed between responders and non-responders (**Fig. 4)** [28]. 15 of these genes were associated with leukocyte migration and invasion, such as the genes encoding urokinase (*PLAU*) and its receptor (*PLAUR*). The importance of leukocyte recirculation as a determinant of treatment responses to TNFi was supported by the finding that several chemokines and their receptors were differentially expressed between responders and primary non-responders. Genes encoding the receptors for the pro-inflammatory cytokines TNF, IL-6 and IL-1 were also expressed at higher levels in cultures from responders, as was expression of *NLRP3*, the gene encoding the intracellular sensor NOD-, LRR- and pyrin domain-containing protein 3, which plays an important role in the control of caspase-1-dependent processing of pro-IL-1β and IL-18 into active cytokines [48]. A polymorphism in the *NLRP3* gene (rs4612666) was associated with primary response to anti-TNF therapy in 2 independent cohorts of Ulcerative Colitis (UC) and IBD patients. This polymorphism is associated with reduced *NLRP3* expression [49]. Only 7 differentially expressed genes were expressed at higher levels in non-responders, including *CXCL9* (encoding a chemoattractant for CXCR3-expressing Th1 and other cytotoxic cells) and *IFNG*, encoding the signature cytokine of Th1, $CD8^+$ T and NK cells. Bank et al. previously reported that a polymorphism in the *IFNG* gene (rs2430561) was significantly associated with effective anti-TNF primary response in Crohn's disease (CD) patients but not in ulcerative colitis (UC) patients. This variant is associated with decreased IFN-γ level [50]. This result was confirmed in another study in UC patients, where, at baseline, responders had lower mucosal mRNA expression of IFN-γ than non-responders [51]. In contrast, Rismo et al. reported that high mRNA expression of mucosal IFN-γ and IL-17A in biopsies obtained before anti-TNF therapy was associated with

successful therapy response in UC patients [52], suggesting that the role of IFN-γ in this context needs further evaluation.

IL7R, the receptor for the homeostatic cytokine IL-7 was expressed at higher levels in stimulated immune cells from axSpA patients not-responding to TNFi [28](**Fig. 4**). A 20 genes signature of IL-7/IL-7R signaling has recently been analyzed in colon mucosal biopsies from previously published cohorts of IBD patients [53]. This study revealed that the IL-7R signaling signature is reproducibly altered in inflamed mucosa, with a significant accumulation of *IL7R* transcripts that may contribute to the maintenance of chronic inflammation. More interestingly, it also demonstrated that a strong colonic IL-7R signaling gene signature (and in particular *IL7R*), before initiation of therapy, is significantly and reproducibly associated with the absence of response to anti-TNF therapy both in UC and CD patients, identifying refractory IBD patients. The author showed that this is not the case for mucosal ileal biopsies, suggesting a specific association of the IL-7/IL-7R pathway with colonic but not ileal IBD inflammation. A combination of 10 genes (*IL7R, IL2RG, JAK1, PIK3CA, LCK, PTK2B, EP300, NMI, CRLF2, and TSLP*) from the IL-7R signaling signature was sufficient to discriminate anti-TNF non-responders from responders, constituting a potential new predictive biomarker to identify refractory patients [53]. A more recent IBD study developed a test to measure IL-7, together with 13 additional proteins in blood (ANG1, ANG2, CRP, SAA1, IL-7, EMMPRIN, MMP1, MMP2, MMP3, MMP9, TGFA, CEACAM1, and VCAM1). This signature was termed the "endoscopic healing index" (EHI). The EHI was used to score endoscopic disease activity in patients with CD. The EHI scores range from 0 to 100 units, with higher scores indicating more severe CD activity, based on endoscopy findings. Outcome prediction based on EHI was comparable to measurement of FC (fecal calprotectin) and higher than measurement of serum CRP [54].

Transcriptomic biomarkers have also been investigated in RA, with some limited results [55]. In this respect, the combined analysis of gene expression and genetic data in large patient cohorts may be a more promising approach. Aterido et al. defined gene co-expression modules in RA synovial tissue and analyzed their association with response to anti-TNF treatment at the clinical and genetic level, using set-based genetic association analysis. The analysis resulted in the identification of an 18-gene module expressed in synovial tissue, and significantly associated at the genetic level with response to adalimumab [56]. Pathway analysis showed that the module is enriched for genes involved in nucleotide metabolism, which plays an essential role in cell proliferation and in the synthesis of signaling molecules such as adenosine. Notably, adenosine signaling in macrophages has been shown to induce a switch from a pro-inflammatory M1 phenotype to an M2 regulatory phenotype [57,58].

Cherlin et al. applied the PrediXcan algorithm on genetic data from the MATURA consortium [59] to identify genes that are associated with changes in the erythrocyte sedimentation rate (ESR) in patients treated with TNF blockers. The prediction model identified the *IL18RAP* gene as a predictor of changes in ESR after treatment [60] . In a replication cohort, the expression of *IL18RAP* in whole blood at baseline

correlated with changes in ESR at 6-month follow-up, and a correlation was observed between *IL18RAP* expression in blood and in synovial tissue [60]. IL18RAP is important for IL-18 signaling, a pathway implicated in inflammation, and a potential treatment target in RA [61]. The association of *IL18RAP* with treatment outcome is not specific to one drug type, making it an interesting biomarker to explore for treatment responses in RA.

In IBD patients, analyses of gene expression in inflamed tissue and immune cells also proved useful in the understanding of the immunopathogenesis of IBD and prediction of therapy outcome. In a study by Arijs et al. baseline gene expression levels in mucosal biopsies could distinguish responders from non-responders to infliximab. The microarray analysis identified a set of 5 genes (*TNFAIP6*, *S100A8*, *IL11*, *G0S2*, and *S100A9*) that could be used to predict the response to anti-TNF treatment with 100% accuracy [62]. A subsequent genetic study demonstrated that haplotype differences in the loci of these 5 genes differentiated responders from non-responders to infliximab [63]. The same group identified also a panel of 5 genes that can be used to predict response to anti-TNF treatment in UC patient (*OPG*, *STC1*, *PTGS2*, *IL13RA2*, *IL11*) [64].

3.2 Genetic biomarkers

Explorative studies of genetic biomarkers to predict anti-TNF therapeutic outcome use genotyping technologies in order to associate genotype with good or bad response to treatment. Genotyping has been performed either on a genome-wide scale (GWAS), or on a narrower scale, by targeting a specific set of single nucleotide polymorphisms (SNPs), selected for their previous association with disease susceptibility or with proposed correlation with anti-TNF response. Results from GWAS performed on rheumatic diseases suggest that disease susceptibility and treatment response are influenced by the action of many genetic variants with modest effects, rather than a few variants with large effects. Thus, polygenic risk scores, based on the combination of several potentially predictive gene variants are expected to be more efficient in predicting treatment response than individual risk variants [65,66].

Overall, genetic biomarkers are particularly interesting as early predictive biomarkers: they are invariable, they precede disease onset and use affordable technologies. Pharmacogenomic profiling has shown important results in predicting treatment outcome in cancer biotherapy treated patients [67,68] and has also been considered for chronic rheumatic diseases such as SpA. In CIDs, the absence of common SNPs with a strong effect, and non-genetic sources of patients' heterogeneity suggest that studies with very large numbers of patients would be necessary to obtain a solid correlation of genetic variation with clinical outcome [69,70].

To date, only a few studies have suggested an association between anti-TNF responses in SpA patients and genetic variants, in particular in the TNF and TLR pathways, and downstream NF-kB signaling (**Table 1**). A number of these variants and genes have also been associated with clinical parameters of remission or low disease in RA (**Table 2**), supporting the importance of these molecular pathways in the

mechanisms of action of TNF-blockers. However, the reduced number of studies, the limited size of the cohorts analyzed, and the lack of robust replicated results limit the exploitation of these findings in a clinical setting.

Table 1. Response biomarkers in Spondyloarthritis (SpA).

GENETIC					
Seitz (2007) [71]	AS (22) RA (54) PsA (10)	IFX, ADA, ETN	TNFα - 308 G/G	TNF/TNFR	Associates with good response (DAS28 or BASDAI at 24weeks)
Liu J (2016) [72]	SpA, IBD, Ps, PsA. (1016)	TNFi	TNFα – 308 A/G TNFα – 238 A/G	TNF/TNFR	G/G predicts good response to TNFi (ASAS20, ASAS40, BASDAI20,BASDAI50 at 12 weeks)
Fabris M (2016) [73]	SpA (187)	ADA, IFX, GOL, ETN	TNFα -308 A/G IL6-174 C/G	TNF/TNFR IL-6 signaling	G/G predicts survival of the first TNFi (BASDAI, DAS28-EULAR)
Aita A (2018) [74]	SpA (137)	IFX, ADA, ETN, GOL	*TNFRSF1A* c.625+10 G	TNF/TNFR	Associates with late response to TNFi (BASDAI < 4)
Murdaca G (2014) [75]	57 (PsA)	ETN ADA IFX	TNFα + 489 A/G	TNF/TNFR	A allele associates with the response to ETN. (PASI, ACR criteria, DAS28, and HAQ at baseline, 3 and 6 months)
Liu J (2019) [76]	AS (92)	ETN	*MYOM2*-rs2294066		Associates with response (ASASAS40)
Borda (2019) [77]	SpA (118)	IFX, ADA, ETN, GOL	*CHUK* rs11591741 *MAPKAPK2* rs4240847 *TLR10* rs11096957 *IRAK3* rs11541076	NFκB TLR4 TLR10 NFKB/TLR4	Associates with non-response (BASDAI, BASFI and DAS28-CRP)
EPIGENETIC					
Ovejero-Benito (2018) [78]	Ps (39)	ADA	H3 and H4 acetylation, H3K4 and H3K27 methylation		Changes in H3K4 were found between Rs and NR (PASI75 at 3 and 6 months)
Ciechomska M (2018) [79]	AS (13) RA (10)	TNFi	Serum miRNA-5196	BCR signaling MHC-I antigen processing.	Changes in miRNA-5196 can be used as predictive marker of reduced disease activity. Better correlation with changes in DAS20 and ASDAS than CRP.
PROTEINS					
Arends (2011) [80]	AS (92)	ETN	MMP-3	MMPs	MMP-3 decreases with TNFi. Change in MMP-3 serum levels are not useful for predicting response to ETN. (ASAS20 and ASA40 at 3 and 12 months)
Wagner C (2012) [81]	AS (100)	GOL	92 Proteins profiling	acute inflammation, bone metabolism, coagulation, metabolic factors	Baseline and change in combination of biomarkers demonstrated stronger prediction for clinical efficacy than CRP. (ASAS20 at week 14)
Ademowo OS (2016) [82]	PsA (25)	ADA	57 proteins panel	Acute inflammation, tissue repair, coagulation	New biomarker panel that can be measured at baseline to predict PsA patients' response to biologics. (DAS28 at 12 weeks)
Turina (2014) [83]	SpA (78)	IFX, ETN	Calprotectin MMP3, hsCRP, IL-6, pentraxin-3, Alpha-2-macroglobulin, VEGF	Acute phase reactants IL-6 signaling TLR MMPs	Calprotectin and hs-CRP are good biomarkers with high sensitivity to change upon treatment.
Østgård RD (2017) [84]	AS (30)	ADA	fecal Calprotectin	TLR	Elevated baseline fecal calprotectin may predict better treatment response. (ASDAS at weeks 12,20 and 52)

Hu H (2019) [85]	AS (262)	TNFi NSAID DMARD	Serum Calprotectin	TLR	Change of calprotectin during first month could predict patients achieving ASAS 40 with AUC of 0.691.
Jarlborg M (2020) [86]	AxSpA(451) RA(969) PsA(237)	TNFi	Serum Calprotectin	TLR	Calprotectin serum levels associate with disease activity in RA, SpA but not in PsA. (SJC, DAS, HAQ, joint radiographs, USPD, BASDAI, ASDAS, SJC, DAPSA)
Baraliakos X (2019) [87]	AS (867)	ETN	C-reactive protein CRP	IL-6 signaling Complement triggering	Very high baseline CRP is a predictor for week-12 outcomes (ASAS20, ASAS50, ASDAS-CRP at week 12)
Hokstad I (2019) [88]	SpA (51)	TNFi	Complement activation (sC5b-9 serum levels)	Complement pathway	Decrease from baseline to 6 weeks of TNFi
Chimenti MS (2012) [89]	PsA (55)	ETN ADA	Plasma complement C3, C4, and B	Complement pathway	High baseline C3 levels predict non-response. (DAS28 and EULAR at baseline and 22 weeks)
TRANSCRIPTS					
Wang XB (2017) [22]	AS (19) (PBMC)	TNFi	656 DEG : *CXCR2*, *NOTCH1*, *TNFRSF1A*, *IL6R*, *IL10*	Chemokines NOTCH signaling TNF/TNFR IL-6 signaling IL-10	It was not possible to develop a predictive algorithm for TNFi response (BASDAI, CRP and ESR)
Dolcino M (2017) [90]	AS (10) (PBMC)	ADA	Assessed 14500 DEG in AS patients before and after ADA treatment	TLR signaling, TNF signaling, IFN Type I Wnt signaling,	ADA treatment modified 44% of DEGs in Rs compared to 12% in NR. (BASDAI6)
IMMUNE CELL POPULATIONS					
Enginar AU (2019) [91]	AS (203) RA (68)	TNFi	NLR PLR	Neutrophils Lymphocytes Platelets	NLR and PLR are strongly correlated with disease activity, ESR and CRP and decrease with anti-TNF treatment. (BASDAI/DAS28 at 3 and 6 months)
Miyagawa I (2019) [92]	PsA (26)	ADA IFX UST SEC	Th1 type Th17 type Th1/Th17 high Th1/Th17 low	T cells	bDMARD therapy selected strategically based on the results of peripheral blood lymphocyte phenotyping. (CRP, ESR, SDAI, DAS28 and PASI at baseline and 6 months)
Schulte-Wrede U (2018) [93]	AS (31)	ADA, ETN	NK CD8+ cells	NK cells	Composition of NK cell compartment predicts therapeutic outcome. (BASDAI50 between baseline and 1 to 6 months)
Dulic S(2018) [94]	AS (22)	TNFi	T cell repertoire	T cells	Increase in CD4HLADR, CD8HLADR and CD4CD25 cells in Rs. (ASAS)
Xueyi L (2013) [95]	AS (222)	TNFi	Th17 and Treg	T cells	Higher baseline Th17 in AS vs controls. Th17 significantly decreased in Rs, increased in NR. Treg increased in Rs and decreased in NR. (ASAS at 6 months)
Andersen T (2019) [96]	SpA (30)	TNFi	Th17 and Th22	T cells	Associates with good clinical response to TNFi at 1 year. (ΔASDAS, ΔBASDAI from 0 to 12 months, MRI)
Yang M (2020) [97]	AS (177)	ETN	28 T lymphocyte and 12 B lymphocyte subsets	T cells B cells	Baseline: imbalance of T and B cell subsets in AS patients. After TNFi: decreased naïve CD4+ cells, increased Treg and B10 cells, Treg increase positively correlated with CRP decrease. (CRP, ASDAS, BASDAI at 12 weeks)

Abbreviations: SpA, Spondyloarthritis; AS, Ankylosing Spondylitis; RA, Rheumatoid Arthritis; PsA, Psoriatic Arthritis; IBD, Inflammatory Bowel Disease; ADA, adalimumab; ETN, etanercept; GOL, golimumab; IFX, infliximab; TNFi, UST, ustekinumab; SEC, secukinumab; TNF-inhibitors; HC, healthy controls; Rs, responders; NR, non-responders; MiRNA, Micro RNA; BCR, B cell receptor; NLR, neutrophils/lymphocytes

ratio; PLR: platelet/lymphocyte ratio, CRP, C-reactive protein; hsCRP, High sensitivity C-reactive protein; SDAI, simplified disease activity index; VEGF, vascular endothelial growth factor; ESR, erythrocyte sedimentation rate; DAS28, disease activity score 28; ASDAS, Ankylosing Spondylitis Disease Activity Score; MMPs, Matrix Metalloproteinases; TLR, Toll like receptor; DEG, differentially expressed genes; SJC, swollen joint count; DAS, Disease Activity Score; HAQ, Health Assessment questionnaire; USPD, ultrasound power Doppler; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; DAPSA, Disease Activity Index for Psoriatic Arthritis.

Table 2. Biomarkers in Rheumathoid Arthritis (RA).

GENETIC					
Pavy S (2010) [98]	RA (1721, 12 cohorts) meta-analysis	TNFi	G-308A TNFα	TNF	No association with treatment response (deltaDAS28 at 12 weeks)
Zeng Z (2013) [99]	RA (2127, 15 cohorts) meta-analysis	TNFi	G-308A TNFα	TNF	Patients with the G allele respond better to treatment (DAS28/ACR)
Cui J (2013) [100]	RA (2706)	IFX ADA ETN	rs6427528 *CD84*	SLAM family	Association with response to ETN (EULAR criteria at 3-12 months)
	RA (390)	ETN			
Swierkot J (2013) [101]	RA (280)	IFX ADA ETN	*TNFR1A* G36A	TNF	Associated with remission or low disease activity (EULAR criteria at 6 months)
			TNFA C-857T		Associated with DAS28 at 6 months treatment
Ferreiro-Iglesias A (2016) [102]	RA (755)	IFX ADA ETN	rs10919563 PTPRC (CD45) rs1800896 *IL10* rs11591741 *CHUK*	CD45 IL10 NFKB	associated with response to treatment (EULAR criteria at 3-6 months)
Honne K (2016) [103]	RA (487)	IFX ADA ETN	rs284511 *MAP3K7*	TGFbeta, BMP	associated with treatment response (deltaDAS28-CRP at 3-6 months)
Julià A (2016) [104]	RA (372)	IFX ADA ETN	rs11387825 *MED15*	PC2, transcriptional activation	Association with response to ETN (EULAR criteria at 3 months)
	RA (245)		rs11387825 *MED15* rs6065221 *MAFB*	transcriptional activation	Association with response to ETN Association with response to ETN IFX
Cui J (2017) [105]	RA (1094)	IFX ADA ETN	*NFKBIA* *AICDA* *CDK6*	NFkB, somatic hypermutation class-switch recombination cell cycle	No association with response at genome wide significance. Three genes with individual *P* values of <0.01 (EULAR criteria at 3-6 months)
Marwa OS (2017) [106]	RA (108) HC (202)	ETN IFX	rs763780 *IL17F* 7488 A/G rs2397084 *IL17F* 7383 A/G	IL-17	Association with response. Serum levels of IL-17 higher in RA versus HC (DAS28)
Sieberts SK (2016) [107]	RA (2706 training) RA (591 validation)	TNFi	Published SNP data predicting response to TNFi Meta-analysis		no significant genetic contribution to prediction accuracy of response (deltaDAS28 at 3-12 months)
Cherlin S (2018) [108]	RA (1819)	TNFi	GWAS data from Matura consortium		poor prediction of response using 11 different statistical methods (delta CRP, delta ESR, delta SJC28 at 3-6 months)
	RA (657)	MTX			
Guan (2019) [109]	RA (2706 training) RA (591 validation)	TNFi	rs1990099 *MAGI2* rs10833455 *NELL1* rs10833456 *NELL1*	small GTPase signalling osteochondro-genesis	clinical and SNP data modelled to predict treatment response. Genetic information only marginally improves the prediction (deltaDAS28 at 3-12 months)
Ferreiro-Iglesias A (2019) [110]	RA (3978,	IFX ETN ADA	rs2378945 *NUBPL*	NADH dehydrogenase	association with response to ETN (EULAR criteria at 3 months)

	755 replication)		rs12142623 rs4651370 *PLA2G4A*	arachidonic acid pathway	
Bai M (2019) [111]	RA (507) HC (499)		rs969129 *IL7R* rs6451231 *IL7R*	IL-7	Association with RA risk
EPIGENETIC					
Castro-Villegas C (2015) [112]	RA (95)	IFX ADA ETN	Serum microRNAs signature (miR-23 and miR-223)	Putative targets: CHUK, IL6R, IRAK2, BMPR2	miR-23 and miR-223 baseline levels correlate with treatment response (EULAR criteria at 6 months)
Krintel SB (2015) [113]	RA (180)	ADA	microRNA in pre-treatment whole blood	ER alpha extracellular matrix (Cyr61) Chemokines (CXCL12)	low expression of miR-22 and high expression of miR-886.3p is associated with response (EULAR criteria at 3 months)
TRANSCRIPTS					
Oswald M (2015) [114]	RA (240)	IFX ADA ETN GOL CTZ	gene modules for plasmacells, B cells, MHC/ribosome proteins, T cells	B cells T cells	increased expression after TNFi in responders. No baseline difference between Rs and NRs (EULAR criteria at 14 weeks)
Huang Q-I (2017) [115]	RA (384, 8 cohorts) meta-analysis	IFX	*FKBP1A* *FGF12* *ANO1* *LRRC31* *AKR1D1*	regulation of trans-membrane transport	Logistic regression model of the 5 genes predicts IFX response. (EULAR/ACR criteria at week 6, 14, 22)
Byng-Maddick R (2017) [116]	RA (37) HC (13)	IFX ETN ADA	TNF-inducible genes	TNF	TNFi do not affect TNF-inducible gene expression at the site of acute immune challenge (tuberculin test). TNF-dependent gene expression decreased in blood sample from treated patients
PROTEIN					
Shi R (2018) [117]	RA (69)	ETN	IL-6 survivin	IL-6 apoptosis	Baseline serum IL-6 increased in Rs to ETN, baseline survivin decreased. Only survivin and CRP are independent predictive factors
BK Han (2016) [118]	RA (29)	TNFi	Serum CXCL10 and CXCL13	chemokines	Rs have higher baseline levels of CXCL10 and CXCL13 compared to NRs (EULAR criteria at week 14)
Haschka J (2016) [119]	RA in DAS28 remission (101)	conventional and/or biological DMARD, randomized to tapering	anticitrullinated protein antibodies (ACPA)		ACPA positivity independently associated with relapse
Curtis JR (2012) [120]	RA (230) RA (45) (treatment response)	MTX anti-TNFa	Multi-biomarker disease activity (MBDA) score : EGF, VEGF-A, leptin, IL-6, SAA, CRP, VCAM-1, MMP-1, MMP-3, TNFRI, YKL-40, and resistin	EGF TNF IL-6 VEGF Integrins MMPs Apolipo-proteins	significant association with DAS28-CRP. Changes in MBDA score discriminates clinical Rs from NRs (ACR20 week 6-12, deltaDAS28-CRP week 6-12)
Centola M (2013) [121]	RA (676, 5 cohorts)	Conventional and biological DMARDs	MBDA	MBDA pathways	Discriminates patients with low vs. moderate/high clinical disease activity. Tracks changes in DAS28-CRP
Hirata S (2015) [122]	RA (84)	ADA ETN IFX	MBDA	MBDA pathways	MBDA scores correlate with disease activity. Tracks response to treatment (deltaDAS28-CRP, deltaDAS28-ESR, at 24 and 52 weeks)
Hirata S (2016) [123]	RA (83)	ADA ETN IFX	MBDA	MBDA pathways	MBDA score and DAS28 at week 24 are significant predictors of radiographic progression at week 52
Li W (2016) [124]	RA (163)		MBDA	MBDA pathways	MBDA score is independently associated with radiographic progression.

					Significantly discriminates patients with low CRP and high risk of progression.
Fleischmann R (2016) [125]	RA (241) RA (251)	ABC ADA	MBDA	MBDA pathways	MBDA does not reflect the status of radiographic progression, nor disease activity
Hambardzumyan K (2016) [126]	RA (205)	MTX+IFX, conventional DMARDs	MBDA	MBDA pathways	MBDA score at baseline, month 3 and year 1 is correlated with subsequent rapid radiographic progression
Rech J (2016) [127]	RA (94)	conventional and/or biological DMARD (tapering)	MBDA	MBDA pathways	Higher baseline MBDA scores significantly associated with relapse. ACPA positivity and MBDA are independent predictors of relapse.
Hambardzumyan K (2017) [128]	RA (157)	MTX+IFX, conventional DMARDs	MBDA	MBDA pathways	MBDA score is associated with response to second-line therapy and with response to IFX (EULAR criteria at 3 and 12 months)
Krabbe S (2017) [129]	RA (52)	ADA	MBDA	MBDA pathways	High MBDA in patients with structural progression
Bouman CAM (2017) [130]	RA (171)	ADA ETN randomized discontinuation	MBDA	MBDA pathways	Baseline MBDA score is not predictive for clinical outcome in the taper group, but predicts major flares in the usual care group. Radiographic progression was minimal and not predicted by MDBA score.
Ghiti-Moghadam M (2018) [131]	RA (439) Low disease activity	TNFi randomized discontinuation	MBDA	MBDA pathways	Low MDBA score independently associated with successful discontinuation
Bechman K (2018) [132]	RA (152)	conventional and/or biological DMARD	MBDA	MBDA pathways	Baseline MBDA score not predictive of flares
Curtis JR (2019) [133]	RA (929, 5 cohorts) Meta-analysis	MTX, ADA, IFX, ABA	MBDA	MBDA pathways	High negative predictive value of MBDA score for radiographic progression
Brahe CH (2019) [133]	early RA (180)	MTX MTX+ADA	MBDA	MBDA pathways	Baseline MBDA was associated with radiographic progression at 1 year, delta MBDA (baseline to 3 months) with clinical remission at 6 months (DAS28-CRP≤2.6)
Cuppen B (2018) [134]	RA (65 training) RA (185 validation)	IFX ADA ETN GOL CTZ	CCL3, CCL17, CCL19, CCL22, IL-4, IL-6, IL-7, IL-15, sCD14, sCD74, sIL-1R1, sTNFRII	Chemokines IL6 IL1 TNF	The protein score marginally improves prediction of DAS28 at 3 months after treatment, compared to clinical scores
Choi IY (2015) [135]	RA (170)	ADA IFX RIX	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Baseline calprotectin serum levels are higher in responders. Levels in Rs decrease after treatment (EULAR criteria at 16 weeks)
Nair SC (2016) [136]	RA (170)	ADA IFX RIX	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Logistic regression model of baseline calprotectin serum levels predicts response (EULAR criteria at 16 weeks)
Nordal HH (2016) [137]	RA (39)	IFX	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Weak association of baseline calprotectin levels with radiographic progression. No predictive power for outcome.
Smith SL (2017) [138]	RA (236)	ETN	Serum S100A9	TLR	No association with response, nor clinical parameters of disease activity (EULAR criteria at 6 months)
Inciarte-Mundo J (2018) [139]	RA (47) PsA (56)	IFX ADA ETN	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Baseline calprotectin serum independently predicts relapse

Tweehuysen L (2018) [140]	RA (125) RA (102)	ADA ETN starting tapering	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Baseline calprotectin levels predictive of response only in one cohort. No predictive advantage over clinical factors (EULAR criteria at 6 months)
Yunchun L (2018) [141]	RA (180)	rhTNFR-Fc	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Rapid decrease of calprotectin levels is associated with a positive clinical response (ACR20). Levels decrease during treatment.
De Moel EC (2019) [142]	RA (104) RA (57)	tapering DMARDs or biologics	Serum calprotectin (MRP8/14, *S100A8/A9*)	TLR	Higher calprotectin is associated with increased risk of relapse after TNFi tapering
IMMUNE CELL POPULATIONS					
Daien CI (2014) [143]	RA (96) HC (31)	TNFi	memory B cells in peripheral blood	B cells	$CD27^+$ cells produced three times more TNFα than did naïve B cells. Higher proportion of CD27+ memory B cells at baseline is associated with response to TNFi (EULAR criteria at 3 months)
Citro A (2015) [144]	RA (16)	ETN	CD8+ T cells	CD8+ T cells	Percentage apoptotic epitope-specific effector $CD8^+$ T cells is more elevated in Rs (EULAR criteria at 6 months)
Hull DN (2016) [145]	RA (25)	ADA ETN	Th17 cells at baseline	Th17	Higher frequencies of baseline Th17 cells is associated with worse response defined by ultrasonography (synovitis). Th17 frequency increases with TNFi therapy
Salomon S (2017) [146]	RA (31) HC (17)	TNFi ABT TCZ	CD24hiCD27+ Breg Th17 cells	B cells Th17	Higher levels of Breg at baseline is associated with DAS28 remission at 6 months (ABT) Lower baseline Th17 is associated with a good response at 6 months. RA have reduced Breg, Th17
Khanniche A (2018) [34]	RA (49) HC (65)	GOL, MTX	Increased effector memory and decreased central memory CD8+ and CD4+ T cells after TNFi	CD8, CD4 T cells	Increased effector CD8+ T cells responses to viral antigens in GOL treated patients
Lee HN (2019) [147]	RA (82) HC (328)	ADA ETN IFX	Neutrophil/ lymphocyte ratio (NLR) Platelet/ lymphocyte ratio (PLR)	Neutrophils Lymphocytes platelets	High NLR and PLR at baseline correlated with treatment response at 12, but not at 24 weeks (EULAR criteria, DAS28-ESR)
Cianciotti BC (2020) [148]	RA (27) HC (20)	ETN	CD45RA+CD62L+CD96+	Tscm IL-17	Expansion of citrullinated vimentin specific Tscm in RA, reduction after TNFi. Expansion of Th17 and IL-17+ Tscm in RA, reduction after TNFi
Rodriguez-Martin E (2020) [149]	RA (98)	TNFi MTX, PREDN	CD19+CD27-	Naïve B cells	Modest positive correlation of B/T cell ratio with clinical outcome (DAS28≤2.6 at 6 months)
MULTIDIMENSIONAL PARAMETERS					
IMMUNE CELLS and PROTEINS					
Bystrom J (2017) [150]	RA (97)	ADA ETN GOL CTZ, MTX	GM-CSF, IL-1beta	GM-CSF, IL1	GM-CSF mostly produced by T cells. GM-CSF+ T cells are TNFα positive and IL-17 negative. High pre-treatment blood levels of GM-CSF are predictive of response to TNFi (EULAR criteria at 3 months)
IMMUNE CELLS and TRANSCRIPTS					
Lewis MJ (2019) [26]	RA (90)	Conventional DMARDs	Synovial pathotypes determined by immunohisto-	Type I IFN signature, chemokine modules,	Synovial gene modules correlate with DAS28-CRP, and with delta DAS28-CRP at 6 months

			chemistry and synovial gene expression	monocyte, DC and B cell modules	
Lliso-Ribera G (2019) [151]	early RA (200)	Conventional DMARDs	Synovial pathotypes determined by immunohisto-chemistry and synovial gene expression	Neutrophils lymphocytes	Baseline lympho-myeloid pathotype is significantly associated with requirement for biologics at 12 months. These patients have higher expression of genes regulating B and T cell differentiation/ activation, *MMP1*, *TIMP1*, *TNFA*
GENETIC and TRANSCRIPTS					
Aterido A (2019) [56]	RA (11) RA (384 exploration) RA (2706 validation)	IFX ADA ETN	synovial gene coexpression module (18 genes)	Nucleotide metabolism IL-7	The gene coexpression module is associated with response to ADA (EULAR criteria at week 14)
Cherlin S (2020) [60]	RA (4741) RA (90 replication)	IFX ETN ADA GOL CTZ	*IL18RAP*	IL-18	Transcript levels in blood correlate with ESR changes after treatment
GENETIC and EPIGENETIC					
Massey J (2018) [152]	RA (1752)	IFX ADA ETN	rs7195994 *FTO* rs2540767 rs11599217 *DOCK1* rs10739537 *BRINP1* rs948138 *MMP20/MMP27* rs2187874 *ZNF595,ZNF718*	Phagocytosis, cell migration, FoxP3, Treg MMPs	Association with response to TNFi (delta DAS28-ESR at 3-6 months)
TRANSCRIPT and PROTEIN					
Farutin V (2019) [153]	RA (40) RA (36)	ADA IFX	increased innate cell signature at baseline in Rs, increased adaptive cell signature in NRs	Myeloid cells	Downregulation of myeloid and platelets genes, upregulation of T and B cell markers after TNFi. Increased NLR at baseline correlates with increased probability of response (EULAR criteria at 3 months)
	RA (1962)		NLR		
TRANSCRIPTS and EPIGENETICS					
Tao W (2020) [154]	RA (89)	ADA, ETN	Gene expression and DNA methylation profiling		Random Forest model based on differentially expressed genes or DNA methylation predicts response to ADA or ETN (DAS28 at 6 months)
GENETIC , EPIGENETICS, TRANSCRIPTS					
Spiliopoulou A (2019) [155]	RA (2938)	ADA ETN CTZ	CD39 CD40	CD39/CD73 pathway T cell costimulation	RA risk score at the CD40 locus, and expression score for CD39 on CD4 T cells are associated with response to TNFi (delta SJC, delta ESR at 6 months)
GENETIC , TRANSCRIPTS, PROTEINS					
Folkersen L (2016) [156]	RA (185, of which 59 with TNFi) HC (61)	MTX, TNFi	*SORBS3, AKAP9, CYP4F12, MUSTN*, *CX3CR1*, *SLC2A3*, *C21orf58,TBC1D8* sICAM1, CXCL13 rs6028945 rs7305646 lncRNA	ICAM1 chemokines	The combined variables explain half of the variation in ΔDAS28-CRP in the TNFi group. Most of the prediction comes from sICAM1, CXCL13, *CX3CR1* and *SLC2A3* (EULAR criteria, deltaDAS28-CRP at 3 months)
TRANSCRIPTS PROTEINS, IMMUNE CELLS					
Tasaki S (2018) [157]	RA (45) HC (35)	MTX IFX TCZ	whole blood transcriptome, serum proteome, cell counts	Neutrophils, NK complement (C3)	After treatment: decreased neutrophils, increased NK (gene expression and cell counts), normalization of complement pathway proteins

Abbreviations: ABA, abatacept; ADA, adalimumab; CTZ, certolizumab; ETN, etanercept; GOL, golimumab; IFX, infliximab; MTX, methotrexate; DMARD, disease-modifying antirheumatic drugs; TNFi, TNF-inhibitors; HC, healthy controls; Rs, responders; NRs, non-responders; NLR, neutrophils/lymphocyte ratio; PLR:

platelet/lymphocyte ratio, CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, disease activity score 28; SJC, swollen joint counts, EULAR, European League Against Rheumatism.

A recent community-based effort has assessed the use of available SNP data to predict clinical outcome of TNF inhibition in RA patients, using machine learning algorithms [107]. Despite a significant estimate of heritability of the treatment response, none of the proposed models could demonstrate a significant contribution of genetic variants to response prediction, over the conventional clinical parameters [107]. A useful strategy to improve the power of the predictive models could be the combination of different types of biomarkers that better capture the non-genetic components of response to therapy.

In the context of IBD, an association study highlighted polymorphisms in the *TLR2* gene that are associated with response to anti-TNF therapy. rs3804099 and rs1816702 were associated with beneficial response to treatment in CD patients, suggesting that genetically determined increased *TLR2* levels were associated with beneficial response among patients with CD [50]. However, the rs11938228 and rs4696480 polymorphism at the *TLR2* locus were associated with nonresponse to treatment in the UC patients [50]. Another recent study from Bank et al. showed that polymorphisms in genes involved in the regulation of the NFκB pathway (*TLR2*, *TLR4*, and *NFKBIA*), the TNF-α signaling pathway (*TNFRSF1A*), and other cytokine pathways (*NLRP3*, *IL1RN*, *IL18*, and i) were associated with primary response to anti-TNF therapy in IBD patients [49].

Another recent genetic association study in two independent large IBD cohorts successfully replicated 2 variants (rs116724455 in TNFSF4/18, rs2228416 in *PLIN2*), which may predict anti-TNF response in patients with IBD at genome-wide significance. The minor alleles of the two polymorphisms were associated with refractory response to anti-TNF agents [158].

3.3 Epigenetic biomarkers

Pharmacoepigenetic profiling may also add another layer of complexity to the relation between genetics and variations in drug responses [159]. Only a few studies in the literature have explored the epigenetic modifications of chromatin before and after anti-TNF treatment in order to identify epigenetic biomarkers that may predict treatment responses in patients affected by rheumatic diseases. To date, most of the epigenomic studies were designed to identify diagnosis biomarkers or new therapeutic targets, and only few were aimed at the identification of biomarkers predictive of anti-TNF outcome. In an attempt to identify epigenetic biomarkers that could predict response to biological drugs, including adalimumab, in psoriasis and psoriatic arthritis patients, Ovejero-Benito et al. assessed 4 histone modifications (H3 and H4 acetylation, H3K4 and H3K27methylation) in PBMC of 39 psoriasis patients using an ELISA technique, before and after treatment initiation. Significant changes in histone H3 and H4 acetylation and H3K4 methylation were observed between psoriasis patients and controls, however, no changes in histone

modification were seen upon treatment, except for a decrease in H3K27 methylation in responders compared to non-responders [78]. Although this study presents many limitations, including the fact that the small number of patients were treated with different biologicals and that a mixture of immune cell populations was analyzed, it is one of very few studies that tried to identify epigenetic biomarkers to predict treatment outcome in rheumatic diseases, and it set the basis for other similar studies on larger cohorts.

Most of the epigenetic studies performed on SpA have focused on miRNA, very few on DNA methylation and even fewer on histone modifications [160]. miRNAs are regulatory non-coding RNA molecules; their expression may be modified under pathophysiological stress conditions, disease or treatment [161]. In a small exploratory study, miRNA-5196 expression levels in serum were measured using real-time PCR. The analysis of samples from 10 RA patients and 13 AS patients, before and after anti-TNF-α therapy, revealed that changes in miRNA-5196 expression positively correlated with the decrease in disease activity scores in patients following anti-TNF-α therapy, suggesting that MiRNA-5196 may be an interesting biomarker to assess treatment response [79]. In silico analysis identify the Fra2 transcription factor, Matrix Metalloproteinase 15 (MMP-15) and the IL-1 receptor as possible targets of MiRNA-5196. The possible biological role of MiRNA-5196 in AS and RA, as well its utility as a clinical biomarker, need confirmative replication studies in larger cohorts.

Baseline serum or whole blood MiRNA signature associated with treatment responses have also been explored in RA patients treated with TNF-blockers. Among the putative targets of differentially expressed MiRNA were molecules of the IL6 and NF-kB pathways, chemokines, and extracellular matrix proteins [112,113]. Although these studies included a larger number of patients (Table 2), validation in a replication cohort is still lacking.

In a recent study, Tao et al. analyzed both gene expression and DNA methylation profiles in peripheral blood mononuclear cells, and in CD4+ T cells and monocytes isolated from RA patients before anti-TNF treatment [154]. The authors identified a large number of genes differentially expressed (DEG) between responders and non-responders at baseline, as well as differentially methylated regions (DMR), although the majority of DEG and DMR were not significant after correction for multiple testing, and the overlap of DEG with those identified previously was small [162]. Notably, both DEG and DMR were specific not only to the cell population studied, but also to the type of anti-TNF treatment (etanercept versus adalimumab). Among the DEG identified in this study were transcription factors (such as IRF1, FOXO3 and FOXO4), genes of the TNF and JAK/STAT signaling pathways, and IL18R1. The authors applied random forest-based algorithms with internal cross-validation, in order to construct predictive models for response to adalimumab or etanercept. The models were subsequently tested in a small replication cohort (9 patients) and in patients assigned to a second TNFi treatment. The prediction accuracy of the models were different for each cell population, treatment and type of data, and the model based on methylation data for response to adalimumab reached the highest accuracy of 88% [154]. Although these results require

replication in an independent patient cohort, this study underlines the importance of exploring several approaches to identify predictive biomarkers, and of analyzing patients treated with different drugs separately.

Spiliopoulos et al. also tested different parameters to construct predictive models for response to TNFi in RA patients [155]. The authors took advantage of the new GENOSCORE platform to create genetic scores for RA associated loci, for immune cell traits and for the expression or methylation of a set of genes whose expression levels were previously associated with treatment response. The genotypic scores for each type of intermediate trait was evaluated for its ability to improve predictive models when added to clinical parameters. Genotypic scores for RA risk improved response prediction, however explained less than 1% of the variance in phenotype, suggesting only a marginal overlap of the genetics of RA disease risk and of response to TNFi. The effect was driven mainly by regional score at the CD40 locus. Among the scores for immune cell traits, expression levels of CD39 on CD4+ T cells were associated with worse response. The inclusion of scores linked to expression (eQTL) quantitative trait loci also resulted in a small improvement of the prediction performance of the model, but further addition of methylation QTLs had no effect, suggesting that eQTLs and mQTLs provide overlapping information.

Overall, integrative approaches for prediction of anti-TNF treatment outcome are still poorly explored. A proof of concept for this strategy in RA patients was provided by Folkersen et al., who constructed prediction models based on selected SNP, transcripts or protein biomarkers which had been associated with response to therapy in the literature [156]. The model combining all three different type of biomarkers explained 51% of the variation in DAS28-CRP after TNFi therapy, and resulted in an improved AUC, compared to the model with protein biomarkers alone [156].

3.4 Cellular profiling

Explorative studies to identify cell-based biomarkers that can be used to predict response to TNF inhibitors have used flow cytometry-based approaches to identify qualitatively and/or quantitatively modified cell populations before and after treatment onset.

An interplay of several innate and adaptive immune cell populations may be involved in SpA pathogenesis [163], and it has been reported that TNFi impact T cell populations in SpA patients [94–97]. All these observations suggest that analyzing changes in immune cells populations in SpA patients undergoing treatment may be of interest to explore disease mechanisms and therapeutic correlations.

Ursula Schulte-Wrede et al [93] performed a deep profiling of peripheral leukocytes in 31 AS patients under adalimumab or etanercept, using an integrated, multi-parametric flow cytometric approach to analyze 50 different surface antigens. The authors then applied an automated cell clustering approach (immunoClust) to identify immunophenotypic signatures predictive of response. The baseline frequencies of CD8+ NK cell subsets turned out to carry the best predictive power for therapeutic outcome in AS patients, with pre-treatment frequency of CD8+ NK cells significantly higher in responders compared

with non-responders, in particular for etanercept-treated patients. Although further validation of NK cell subsets for clinical prediction of TNF inhibitor outcome is necessary, this study suggests that cellular response signatures can be identified in peripheral blood using extensive immunophenotyping approaches [93].

Increased effector responses after TNFi treatment has also been observed in RA patients (Table 2). Patients treated with golimumab showed an increase in the CD8+ T cell effector population, accompanied by increased responses to viral antigens, suggesting that TNF blockade, while broadly suppressing inflammation, does not induce generalized immunosuppression, but rather may "normalize" immune responses [34]. In agreement with this concept, anti-TNF treatment did not affect the expression of TNF-inducible genes in the site of acute inflammatory challenge (tuberculin injection), while decreasing the inducibility of these genes in blood samples [116].

Many studies have suggested a role for effector T cell populations such as Th1 and Th17 in SpA pathogenesis and disease activity [164]. In a targeted cellular profiling approach, some studies focused on specific effector cell subsets, such as Th1, Th17, and Treg cell populations. The evaluation of these cell subsets before and after anti-TNF treatment is so far rather inconclusive, perhaps due to the difficulties of reliably quantifying small cell populations by immunophenotyping large cohorts of patients. Xueyi et al [95] analysed Th17 and Treg populations in 222 AS patients at baseline and after anti-TNF treatment. At baseline, significantly higher Th17 frequencies and lower Treg frequencies were observed relative to healthy controls, independently of future response to treatment. Baseline frequencies of Th17 cells, and baseline levels of Th17-related cytokines were positively correlated with the BASDAI disease score. After anti-TNF therapy Th17 cell frequencies declined significantly only in responders, while increasing in non-responders. In the same study, the authors found that also serum levels of IL-6, IL-17 and IL-23 significantly decreased only in responders [95]. On the contrary, an increase in IL-17+ and in IL-22+ cells after adalimumab treatment was reported by Andersen et al. in 30 SpA patients [96].

An increase of Th17 frequency in patients' blood after TNF inhibition was also observed in a study on 25 RA patients. This study also reported that higher Th17 frequencies at baseline correlated with worse response to anti-TNF therapy, as defined by ultrasonographic measure of synovial thickening [145].

Alterations in blood cell counts may occur in inflammatory disorders, and ratios of circulating blood cell components can be used to assess inflammatory activity. Neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR) and platelet-lymphocyte ratio (PLR) have been found to correlate with the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, suggesting their use as markers of systemic inflammation for the prognosis of chronic inflammatory diseases [91,165]. Enginar and Kacar [91] retrospectively evaluated 68 RA patients and 203 AS patients on anti-TNF medication for at least 6 months. NLR and PLR correlated with disease activity in both RA and SpA patients. Other studies performed on RA and AS patients have also found similar results [166,167].

Despite growing evidence of the association of NLR and PLR with disease activity, the association of baseline NLR and PLR with response to treatment is less clear. In UC patients with moderate to severe disease, high baseline neutrophil to lymphocyte ratio (NLR), reflecting a high neutrophil count, is an indicator of loss of response to infliximab in patients with moderate-to-severe active UC [168].

Similarly, Han-Na Lee et al. found that, in 82 RA patients, high baseline NLR and PLR were independently associated with a higher risk of non-response to anti-TNF treatment at 12 weeks, but not at 24 weeks [147].

On the other hand, Farutin et al., in a combined whole blood transcriptome and plasma proteome analysis of RA patients, identified the downregulation of myeloid cells and platelet genes as a molecular signature of anti-TNF treatment. This signature was present in both responders and non-responders. However, at baseline, the top differentially expressed genes between responders and non-responders included markers of myeloid cells, which were higher in good responders. The association between higher NLR and good response was also confirmed by the analysis of the NLR in a cohort of 1962 RA patients [153]. The discrepancies observed in the relationship of the NLR with therapeutic responses may be due not only to the limited number of patients in some studies, but also to heterogeneity in the timing of the observation, and in the measures of clinical outcome. As cellular ratios are simple to measure, inexpensive, and easily accessible parameters, further exploring their significance in response prediction may still be a reasonable strategy.

A particularly informative approach may be the analysis of cell populations in the inflamed tissue, when obtaining tissue biopsies is possible. The combined immunohistochemical and gene expression analysis of synovial tissue from early RA patients showed that detection of a lympho-myeloid pathotype before treatment is significantly associated with disease progression requiring biologics at 12 months from the diagnosis [151]. Several synovial gene expression modules, including type I IFN and antiviral modules, B cells and dendritic cell modules, correlated with response to treatment [26].

In ileal Crohn's disease patients, a recent single cell analysis of the inflamed ileal tissue indicated that a subgroup of patients presented a distinct cellular pattern, called the GIMATS module, which included IgG+ plasma cells, inflammatory macrophages and activated dendritic cells, activated T cells, and stroma cells (activated fibroblasts, and endothelial cells). The enrichment of this cellular module before anti-TNF therapy was predictive of resistance to anti-TNF therapy [169].

3.5 Protein biomarkers

Studies relative to protein biomarkers for TNF inhibitors treatment in SpA have been largely focused on candidate proteins selected for their known role in inflammation and/or bone metabolism, such as acute phase proteins or cytokines. Many studies have shown that anti TNF treatments are effective in modulating acute inflammation proteins [83,170–172]. However, if the baseline serum levels of these proteins can be used to prospectively predict response to treatment needs to be confirmed. In addition,

these markers may lack specificity and be elevated in other inflammatory processes unrelated to SpA. To date, CRP is the most important marker of inflammation in rheumatology and is the only biomarker currently used in clinical practice to select SpA patients for treatment and to predict TNFi therapy outcome [173]. Using a large dataset of 867 AS patients treated with Etanercept, Baraliakos et al. found that categorization of CRP levels prior to treatment initiation is a highly significant predictor of treatment outcome at 12 weeks, with higher baseline CRP categories predictive of a greater proportion of responders, and decreases from very high to normal CRP levels after treatment start, predictive of the future course of TNFi treatment [87].

Many studies have shown that anti TNF treatments are effective in modulating acute inflammation proteins [83,170–172] and several studies attempted to correlate baseline serum levels of biomarkers in TNF naïve patients with treatment outcome [170]. However, at date there is no robust replicated evidence that permits to extrapolate the results to all SpA patients, in particular because baseline inflammatory protein levels fluctuate among patients and depend on disease activity status. An alternative approach is the use of short-term changes in serum biomarkers levels upon anti-TNF treatment to predict long term response to treatment. A common conclusion for both approaches is that combining markers yields a stronger predictive power than the use of an individual marker [65].

Wagner et al. tested serum samples collected at baseline, 4 and 14 weeks from 100 active AS patients randomized to receive golimumab. Selected inflammatory, bone and cartilage markers were analyzed, and profiling of 92 different proteins was performed [81]. The levels of many of these markers was affected by TNF inhibition, including a number of acute phase reactants, bone metabolism factors, coagulation factors (eg. C3), inflammatory chemokines (eg. RANTES and MIP1β), matrix metalloproteinase pathways (eg. MMP3). A robust logistic regression analysis associated baseline biomarkers levels of insulin, leptin, apolipoprotein C3, IL-6, osteocalcin, N-terminal propeptide of type 1 collagen (P1NP) with ASAS 20 response at week 14. The combination of baseline levels of insulin and P1NP was a stronger predictor of response than CRP levels [81].

Leptin, IL-6 and MMP3 are also included in the 12-proteins Multi-Biomarker Disease Activity score, or MBDA. The MBDA score was originally aimed to assess disease activity, and was developed through a multi-step approach of candidate biomarker prioritization and algorithm development [121]. Of the 130 putative protein biomarkers tested, 12 were retained in the final multi-biomarker statistical model. Eleven of these (tumor necrosis factor receptor I (TNF-RI), IL-6, vascular cell adhesion molecule 1 (VCAM-1), epidermal growth factor (EGF), VEGF-A, cartilage glycoprotein 39 (YKL40), matrix metalloproteinase 1 (MMP1), MMP3, serum amyloid A (SAA), leptin, and resistin) were modelled to predict disease activity scores, and then combined with CRP to produce the final MBDA score. These proteins are involved in a variety of innate and adaptive immune pathways, in systemic inflammation and bone/cartilage metabolism [121]. The MBDA score was significantly correlated with Disease Activity Score 28-CRP (DAS28-

CRP), as well as with additional measures of joint inflammation and structural progression by ultrasonography and radiography [122].

The potential of the MBDA score in predicting disease progression has also been extensively analyzed (Table 2), with low MBDA score, in particular, showing good negative predictive power for structural progression [133]. MBDA scores at baseline have been associated with patients' response to anti-TNF therapy (Table 2). On the other hand, a randomized prospective study did not find a correlation between MBDA score and different clinical scores in patients treated with adalimumab or abatacept [125], and the contribution of the MBDA score to personalized patient management is still under discussion [174–176]. The score may perhaps find its most informative application in the detection of subclinical inflammation in patients with low disease activity by conventional parameters, such as patients in DAS28-CRP remission [177], or low CRP [124], and may therefore play a complementary role to these measures.

Calprotectin is another inflammation marker particularly studied in CID such as SpA, RA, and IBD. Calprotectin may be a promising candidate biomarker to monitor disease activity, bone damage progression and predict response to anti-TNF therapy [178]. This protein is a heterodimeric complex of the S100A8 and S100A9 proteins (also called myeloid-related protein MRP8 and MRP14), and is a damage associated molecular pattern (DAMP) that triggers the innate immunity receptor TLR4, and the Receptor for Advanced Glycation Endproducts (RAGE) [179]. Calprotectin is produced by monocytes and neutrophils locally, suggesting it may be a good marker of tissue inflammation. Both serum and faecal calprotectin levels are increased in SpA patients compared to healthy controls and are associated with CRP, ESR, and disease scores in SpA [85]. Serum calprotectin levels decreased with anti TNF treatment and early changes in calprotectin levels could predict patients achieving ASAS40 with an AUC of 0.691 [85]. In a similar study on faecal calprotectin, Østgård et al. found that high faecal calprotectin levels accurately identified SpA patients with intestinal inflammation. This group of patients was more likely to respond better to Adalimumab treatment, as evaluated by changes in the ASDAS. Faecal calprotectin also associated with stronger inflammation in the sacroiliac joint, as shown by MRI [84]. Turina et al identified Calprotectin as the most promising biomarker of treatment response in SpA and PsA patients, compared to high-sensitive CRP (hsCRP), MMP3 and IL-6. With the highest sensitivity to change upon clinical effective treatment, calprotectin outperformed hsCRP, suggesting it may be a useful maker of disease in CRP-negative patients [83]. Whether baseline concentrations of calprotectin in serum, synovial fluid or faeces can predict treatment responses needs to be confirmed by replication studies on larger cohorts and using standardized methodologies.

Also in RA, serum levels of calprotectin correlate well with disease activity, and are potentially a predictive marker for response (Table 2). Higher baseline calprotectin levels were found to correlate with treatment response [135,136], with relapse after remission [139] and flares after tapering or discontinuation of TNF blockers [142]. However, inconsistencies for this marker have also been reported,

since some studies failed to find a correlation with clinical response [138], or obtained different results in different cohorts [140].

Matrix Metalloproteinases (MMPs), and MMP3 in particular, are reported biomarkers of synovial inflammation and cartilage turnover in inflammatory joint diseases, such as SpA and RA [180]. MMP3 baseline serum levels are elevated in SpA patients compared to healthy controls and correlate with disease activity [181–183]. In the Turina et al study [83], MMP3 showed a statistically significant decrease after two weeks of anti-TNF treatment, however, the effects of treatment were modest and less consistent across different subtypes of SpA. The impact of TNF inhibition on MMP3 levels is so far inconclusive with opposing results from different studies [184], raising the question of whether MMP3 can be used as a predictive biomarker for anti-TNF response. Some studies found a good predictive value for either baseline serum levels and/or reduction over time of MMP3 in SpA and PsA patients under anti-TNF [185,186], Arends et al. [80], however, showed that changes in MMP-3 levels after treatment correlated with disease activity, but baseline MMP3 levels had no predictive value for treatment response.

Tissue biomarkers are *OSM* (encoding the cytokine oncostatin-M) and its receptor *OSMR,* which were overexpressed in the intestinal mucosa of patients with active IBD (5 different cohorts, 227 patients) [187]. Neither *OSM* nor *OSMR* expression was correlated with standard clinical parameters, including CRP. However, *OSM* and *OSMR* expression was increased in patients with IBD who required surgery. High baseline *OSM* expression in the intestinal mucosa was reproducibly associated with resistance to anti-TNF therapy in IBD [187]. Two additional studies found that high plasma OSM was associated with non-response to anti-TNF treatment in CD patients [188,189]. Oncostatin M is a cytokine produced by T cells and innate immune cells, including monocytes and neutrophils. The OSM receptor is expressed on non-hematopoietic cells, such epithelial cells, fibroblasts and endothelial cells, and the OSM/OSMR axis may therefore be crucial for the cross-talk between stroma and immune cells [190].

4. Conclusions

The past years have been marked by a major increase of our understanding of the cellular and molecular mechanisms of TNF-blocker action in patients affected by chronic inflammatory diseases. However, despite substantial efforts, a validated biomarker predicting therapeutic responses of patients to TNFi has not yet been identified.

There are several reasons that may explain our limited success to identify validated biomarkers that can inform treatment choices. Many studies reported in the literature have been underpowered and patients have not been appropriately stratified. An important issue that is more difficult to tackle is that currently used clinical outcome measures, in particular for SpA, extensively rely on self-reporting. It will be of critical importance to move from self-reporting to objective and quantifiable outcome measures. Furthermore, it is now well appreciated that the biology of treatment failures and development of

resistance to treatment is highly complex, as it is influenced by multiple patient-intrinsic, drug-specific and environmental parameters. To account for this complexity, it may be necessary to develop multi-dimensional biomarkers. However, the general use of biomarkers in the clinics requires that they rely on robust and inexpensive, non-invasive procedures.

Several biomarker candidates to predict treatment responses have emerged recently, in particular OSM and IL7R, whose expression is negatively correlated with response to anti-TNF therapy in IBD. Further studies are needed to validate these candidates, and to test their potential relevance for other diseases.

5. Expert Opinion

The introduction of biologic therapies, such as TNF-blockers, has been a major breakthrough in the treatment of CID. However, these therapies are effective only in a sub-population of patients, and it is currently not possible to predict which patients will not respond to the treatment. In clinical practice, patients non-responsive to a TNF-blocker are treated with a different TNF inhibitor until an effective therapeutic agent is identified. This procedure is expensive and may take a long time, during which the patient is not appropriately treated and is exposed to side effects without clinical benefit. The negative impact of unsuccessful therapy on patients' quality of life, adds to the negative impact on society, due to the associated high costs of treatment.

A reason for the lack of a "personalized" approach to treatment is the insufficient understanding of what determines the individual predisposition to disease and the mechanisms associated with the response to a specific therapy. In the past years there have been multiple efforts to identify biomarkers that predict response to biological therapies in SpA, RA, IBD and other CID. The results have been, however, rather limited. Some of these biomarkers appear to be highly promising (for example Oncostatin M, in the case of IBD), however, none has been validated yet as a tool to inform treatment choices. The search for biomarkers that correlate with disease activity has been more successful: the Endoscopic Healing Index (EHI) Score has been developed commercially as a tool to monitor disease activity in Crohn's disease patients, and the MBDA blood test is commercialized as a biomarker for disease activity in RA, although their use is still considered investigational. These biomarkers have also been proposed to predict treatment outcome, but this application remains to be validated.

Several hurdles to the prediction of treatment responses have emerged, such as the limited size and the heterogeneity of cohorts, in terms of patients' characteristics and treatment (for example, patients treated with different anti-TNF compounds are often analyzed together). There is also a lack of adequate replication studies in different patient populations. In addition, a key issue is the suitability of the current clinical outcome measures, in particular for SpA. The BASDAI score is exclusively based on subjective parameters of self-reporting, and the ASDAS, is a composite of self-reporting and CRP levels. In the case of RA, the DAS28 may represent a more robust outcome measure, since it combines the subjective

measure of tender joint counts (TJC) with the objective observation of swollen joints (SJC). However, even the DAS28 may not constitute a reliable end-point measure [45]. In addition, these scores are usually determined at single time points: integration of longitudinal monitoring would be needed to smoothen score fluctuations.

Genetic biomarkers are attractive, because they are robust, do not vary and are easily measured. However, none of the proposed candidates have been validated to date: the identification may be hindered by the small effect size of common SNPs, which would require studies on very large cohorts to determine an extended panel of SNPs that, combined, have an impact on treatment outcome. Additional complications for the use of SNPs as response biomarkers are the effects of population ancestry, and the fact that they do not address the non-genetic contribution to disease and response to therapy.

Blood biomarkers are ideal for clinical applications, for the ease of sampling. This is especially true for pathologies (such as axSpA) where routine access to diseased tissues is difficult. To date, no robust blood response biomarker has been identified in SpA and in other CID, possibly because of the fluctuation of biomarker levels with time, and the fact that they may not quantitatively reflect pathological processes in tissue.

Immune biomarkers are also strongly impacted by many variables such as age and sex, or environmental factors (for example smoking or CMV infection), introducing another layer of variability that has to be taken into account in the study design, by providing adequate patient stratification.

For the years to come, a key issue will be the constitution of large and highly annotated longitudinal cohorts. These resources are now widely available in the cancer field (examples are The Cancer Genome Atlas, TCGA, and the International Cancer Genome Consortium, ICGC). Standardization of procedures for the collection and management of demographic and clinical data will facilitate comparison of biomarker studies performed in different clinical centers. In addition, the importance of the application of standardized and robust methodology for sample analysis cannot be overstated.

Finally, the combination of different biomarkers that capture the biological complexity of the disease and of the effect of treatment may be necessary to successfully stratify patients to the most appropriate treatment. In this respect, we believe that the combination of multi-dimensional biomarkers and the development of mathematical models (such as deep learning tools) to capture all the biological/environmental influences on treatment outcomes will be an important driver of progress in this area. Finally, it will be important to move towards “objective” outcome measures (including imaging technology) and molecular biomarkers to develop objective and reliable criteria to help clinical decision making.

Figure legends

Figure 1. Design of gene modules for Quantitative Set Analysis for Gene Expression (QuSAGE).

The design the 45 gene modules was based on the deconvolution of cell signaling pathways. The NF-kB signaling pathway was decomposed to create modules dedicated to the genes coding for transmembrane proteins forming the receptors and the genes encoding their ligands. Other modules group intermediate intracellular kinases (NF-kB regulators), which, once activated, phosphorylate IκBα (inhibitor of NF-κB α-subunit), and NF-kB inhibitors and transcription factors.

Figure 2. Analysis of signaling pathways reveals multiple mechanisms of TNF-blocker action in SpA.

Shown are the effects of anti-TNF therapy on the "pathway activity" of 45 gene modules generated from 594 immune-related genes. Peripheral blood samples were obtained from 17 axSpA patients before (D0) and 7 days (D7) after the first injection of a TNF-blocker. Whole-blood cultures were stimulated with *Candida albicans* and RNA was extracted after a 22-hour culture. Gene expression was measured using Immunology_v2 nCounter panel (Nanostring). For each gene module, the mean activity fold-change and 95% confidence interval are plotted and color-coded according to their FDR-corrected P-values (means compared to fold-change zero). Confidence intervals overlapping the horizontal dotted line indicate statistically significant increased or decreased module activity at D7 as compared to D0.

Figure 3. Detailed gene activity for 3 representative modules.

Shown is the "gene activity" for individual genes from 3 modules shown in **Fig. 2**. A, Cytotoxic molecules; B, M2 macrophages; and C, Pro-inflammatory molecules. Whole-blood cultures were stimulated with *Candida albicans* as in Fig. 2. The horizontal dashed blue line and the grey band indicate the mean differential expression of all genes in the module at D7 versus D0, and the 95% confidence interval.

Figure 4. A gene expression signature associated with therapeutic responses to anti-TNF therapy in axSpA.

Shown is a heat map representation of genes differentially expressed between axSpA patients with a major response to anti-TNF therapy (delta ASDAS ≥ 2, green squares) and non-responders (delta ASDAS < 1.1, red squares) in whole-blood cultures stimulated with LPS or SEB before initiation of therapy. The heatmap shows the levels of differentially secreted genes (red indicates higher and green lower levels of protein secretion, LIMMA analysis, adjusted p-value < 0.05). Gene-stimulus combinations were ranked by decreasing fold-change. Expression levels and fold-change values of the 58 gene-stimulus combinations (corresponding to 55 genes) that are the most differentially expressed between responders and non-responders are reported in [28].

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Declaration of interest

The authors declare no competing financial interest.

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** OSM and OSMR are overexpressed in the majority of active IBD mucosa, particularly in patients resistant to anti-TNF. OSM could therefore be a novel predictive biomarker for anti-TNF and therapeutic target for this clinically challenging population.

Figure 1

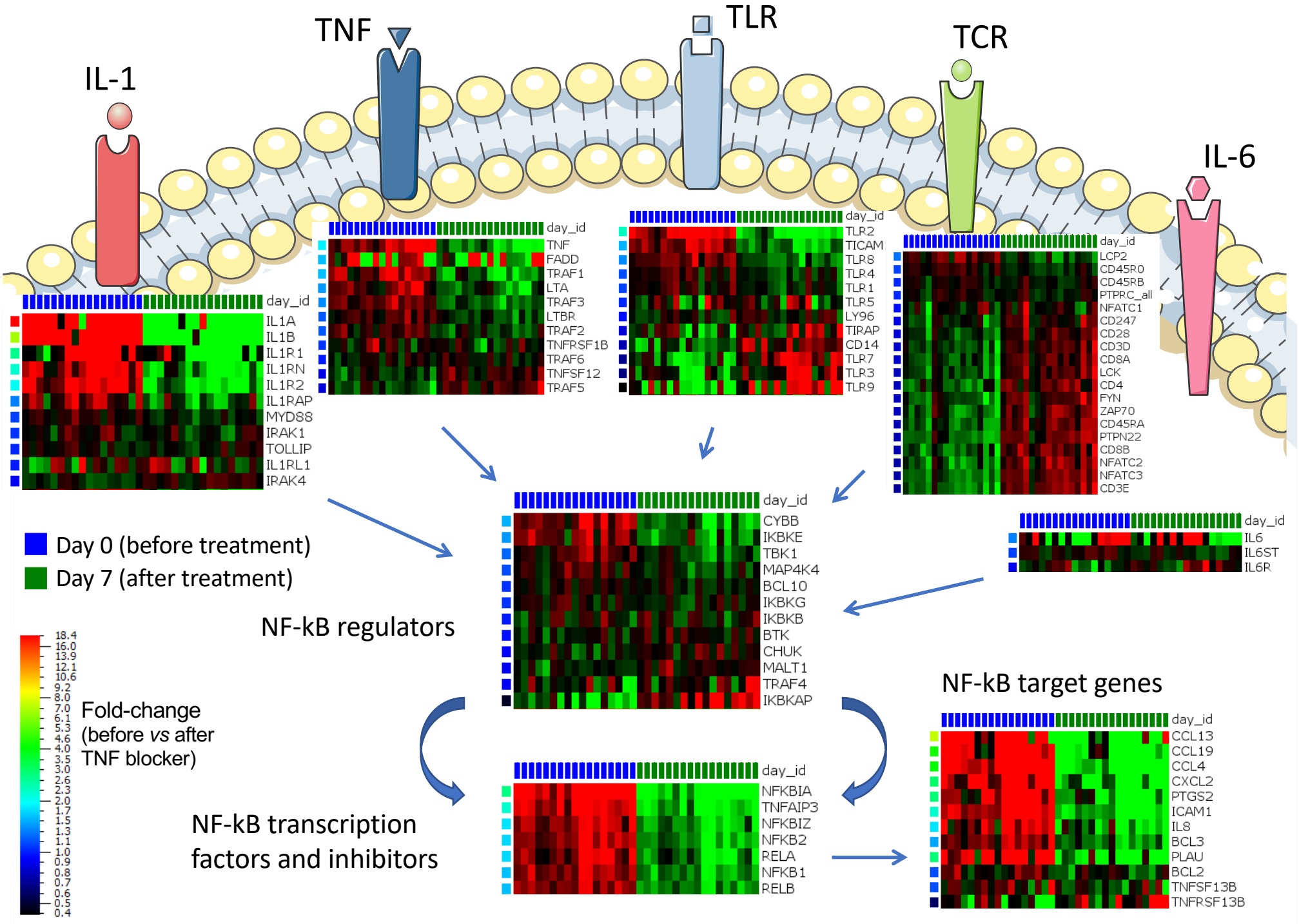

IL-1
TNF
TLR
TCR
IL-6
day_id
IL1A
IL1B
IL1R1
IL1RN
IL1R2
IL1RAP
MYD88
IRAK1
TOLLIP
IL1RL1
IRAK4
TNF
FADD
TRAF1
LTA
TRAF3
LTBR
TRAF2
TNFRSF1B
TRAF6
TNFSF12
TRAF5
TLR2
TICAM
TLR8
TLR4
TLR1
TLR5
LY96
TIRAP
CD14
TLR7
TLR3
TLR9
LCP2
CD45R0
CD45RB
PTPRC_all
NFATC1
CD247
CD28
CD3D
CD8A
LCK
CD4
FYN
ZAP70
CD45RA
PTPN22
CD8B
NFATC2
NFATC3
CD3E
Day 0 (before treatment)
Day 7 (after treatment)
NF-kB regulators
CYBB
IKBKE
TBK1
MAP4K4
BCL10
IKBKG
IKBKB
BTK
CHUK
MALT1
TRAF4
IKBKAP
IL6
IL6ST
IL6R
18.4
16.0
13.9
12.1
10.6
9.2
8.0
7.0
6.1
5.3
4.6
4.0
3.5
3.0
2.6
2.3
2.0
1.7
1.5
1.3
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Fold-change
(before vs after
TNF blocker)
NF-kB transcription
factors and inhibitors
NFKBIA
TNFAIP3
NFKBIZ
NFKB2
RELA
NFKB1
RELB
NF-kB target genes
CCL13
CCL19
CCL4
CXCL2
PTGS2
ICAM1
IL8
BCL3
PLAU
BCL2
TNFSF13B
TNFRSF13B

Figure 2

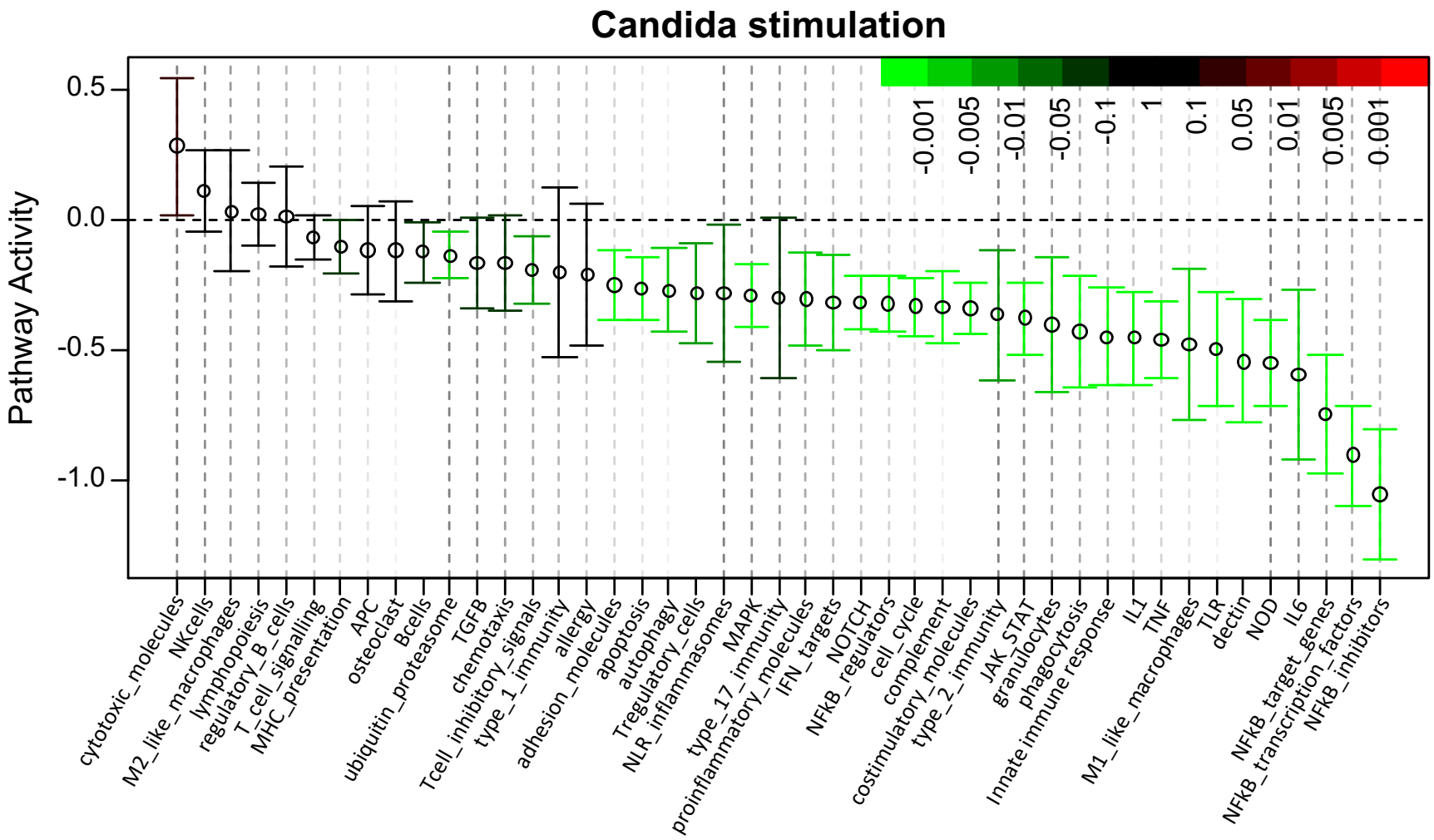

Candida stimulation
Pathway Activity
0.5
0.0
-0.5
-1.0
-0.001
-0.005
-0.01
-0.05
-0.1
1
0.1
0.05
0.01
0.005
0.001
cytotoxic_molecules
NKcells
M2_like_macrophages
lymphopoiesis
regulatory_B_cells
T_cell_signalling
MHC_presentation
APC
osteoclast
Bcells
ubiquitin_proteasome
TGFB
chemotaxis
Tcell_inhibitory_signals
type_1_immunity
allergy
adhesion_molecules
apoptosis
autophagy
Tregulatory_cells
NLR_inflammasomes
MAPK
type_17_immunity
proinflammatory_molecules
IFN_targets
NOTCH
NFkB_regulators
cell_cycle
complement
costimulatory_molecules
type_2_immunity
JAK_STAT
granulocytes
phagocytosis
Innate immune response
IL1
TNF
M1_like_macrophages
TLR
dectin
NOD
IL6
NFkB_target_genes
NFkB_transcription_factors
NFkB_inhibitors

Figure 3

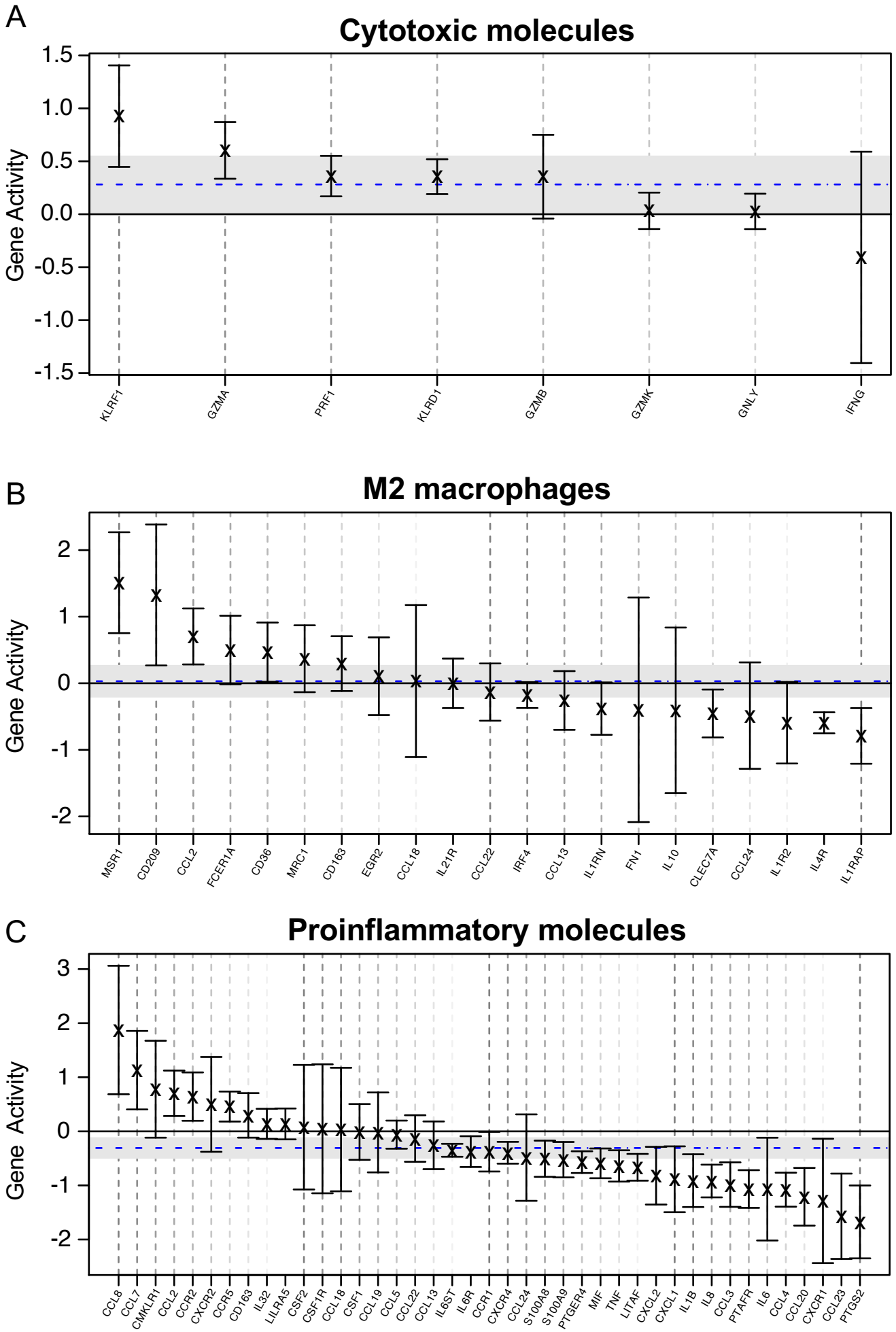
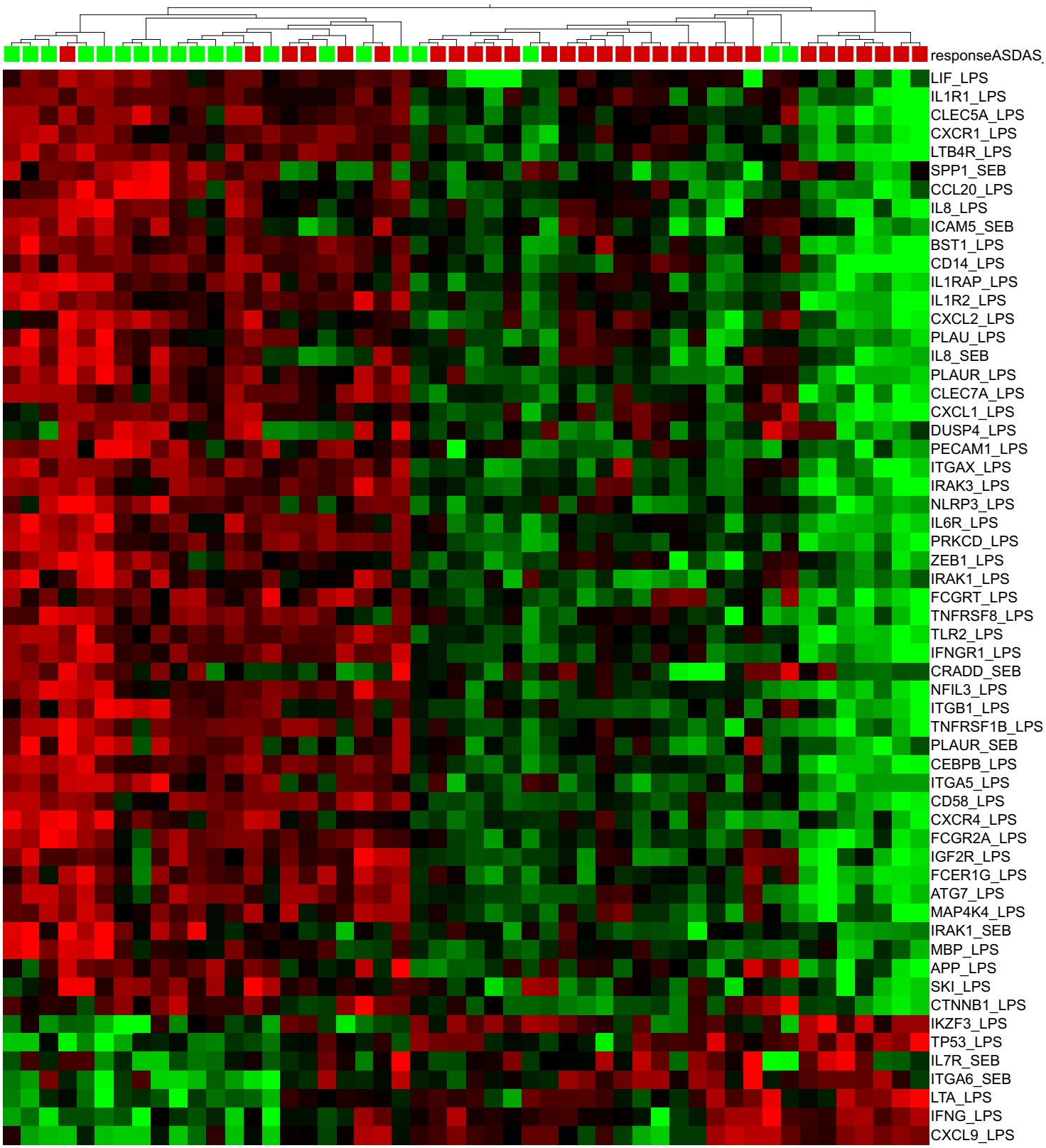

A
Cytotoxic molecules
Gene Activity
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
KLRF1
GZMA
PRF1
KLRD1
GZMB
GZMK
GNLY
IFNG
B
M2 macrophages
Gene Activity
2
1
0
-1
-2
MSR1
CD209
CCL2
FCER1A
CD36
MRC1
CD163
EGR2
CCL18
IL21R
CCL22
IRF4
CCL13
IL1RN
FN1
IL10
CLEC7A
CCL24
IL1R2
IL4R
IL1RAP
C
Proinflammatory molecules
Gene Activity
3
2
1
0
-1
-2
CCL8
CCL7
CMKLR1
CCL2
CCR2
CXCR2
CCR5
CD163
IL32
LILRA5
CSF2
CSF1R
CCL18
CSF1
CCL19
CCL5
CCL22
CCL13
IL6ST
IL6R
CCR1
CXCR4
CCL24
S100A8
S100A9
PTGER4
MIF
TNF
LITAF
CXCL2
CXCL1
IL1B
IL8
CCL3
PTAFR
IL6
CCL4
CCL20
CXCR1
CCL23
PTGS2

Figure 4


responseASDAS
LIF_LPS
IL1R1_LPS
CLEC5A_LPS
CXCR1_LPS
LTB4R_LPS
SPP1_SEB
CCL20_LPS
IL8_LPS
ICAM5_SEB
BST1_LPS
CD14_LPS
IL1RAP_LPS
IL1R2_LPS
CXCL2_LPS
PLAU_LPS
IL8_SEB
PLAUR_LPS
CLEC7A_LPS
CXCL1_LPS
DUSP4_LPS
PECAM1_LPS
ITGAX_LPS
IRAK3_LPS
NLRP3_LPS
IL6R_LPS
PRKCD_LPS
ZEB1_LPS
IRAK1_LPS
FCGRT_LPS
TNFRSF8_LPS
TLR2_LPS
IFNGR1_LPS
CRADD_SEB
NFIL3_LPS
ITGB1_LPS
TNFRSF1B_LPS
PLAUR_SEB
CEBPB_LPS
ITGA5_LPS
CD58_LPS
CXCR4_LPS
FCGR2A_LPS
IGF2R_LPS
FCER1G_LPS
ATG7_LPS
MAP4K4_LPS
IRAK1_SEB
MBP_LPS
APP_LPS
SKI_LPS
CTNNB1_LPS
IKZF3_LPS
TP53_LPS
IL7R_SEB
ITGA6_SEB
LTA_LPS
IFNG_LPS
CXCL9_LPS