# RHEUMATOLOGY

## Understanding drug resistance to biologic therapy

# A personalized medicine approach to biologic treatment of rheumatoid arthritis: a preliminary treatment algorithm

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## Abstract

RA is a syndrome consisting of different pathogenetic subsets in which distinct molecular mechanisms may drive common final pathways. Recent work has provided proof of principle that biomarkers may be identified predictive of the response to targeted therapy. Based on new insights, an initial treatment algorithm is presented that may be used to guide treatment decisions in patients who have failed one TNF inhibitor. Key questions in this algorithm relate to the question whether the patient is a primary *vs* a secondary non-responder to TNF blockade and whether the patient is RF and/or anti-citrullinated peptide antibody positive. This preliminary algorithm may contribute to more cost-effective treatment of RA, and provides the basis for more extensive algorithms when additional data become available.

**Key words:** rheumatoid arthritis, treatment, biomarkers, biologics.

## RA is a syndrome rather than a disease entity

RA is a chronic inflammatory disease affecting synovial tissue in multiple joints. The diagnosis is based on signs and symptoms, like the number of affected joints, the pattern of joint involvement as well as the presence of elevated levels of acute-phase reactants, autoantibodies and erosions on radiographs. Interestingly, patients with identical clinical signs and symptoms may have very dissimilar patterns of leucocyte infiltration [1, 2] and cytokine expression [3, 4] in their synovium. In addition to marked heterogeneity between RA patients with regard to synovial leucocyte infiltration and activation of genes associated with inflammation [5, 6], there is also evidence of variability in genes associated with stromal cells such as fibroblast-like synoviocytes [7, 8].

There is not only inter-individual heterogeneity with respect to the gene signature in the synovial tissue, but also in the peripheral blood. For instance, elevated expression levels of IFN type I regulated genes, consistent with the activation of a pathogen-response programme, have been observed in the peripheral blood of about half of the RA patients [9, 10]. The notion that RA should be viewed as a syndrome consisting of more than one pathogenetic entity is strongly supported by the differences between patients with detectable anti-citrullinated peptide antibodies (ACPA) and those who are ACPA negative. ACPA-positive disease is associated with unfavourable outcome [11, 12], and there is an association between ACPA positivity and the presence of the specific genotype encoding the shared epitope [13], smoking [14, 15] and periodontitis [14]. It appears likely that the ACPA-negative RA group may be further subdivided into different groups based on molecular mechanisms underlying the disease process.
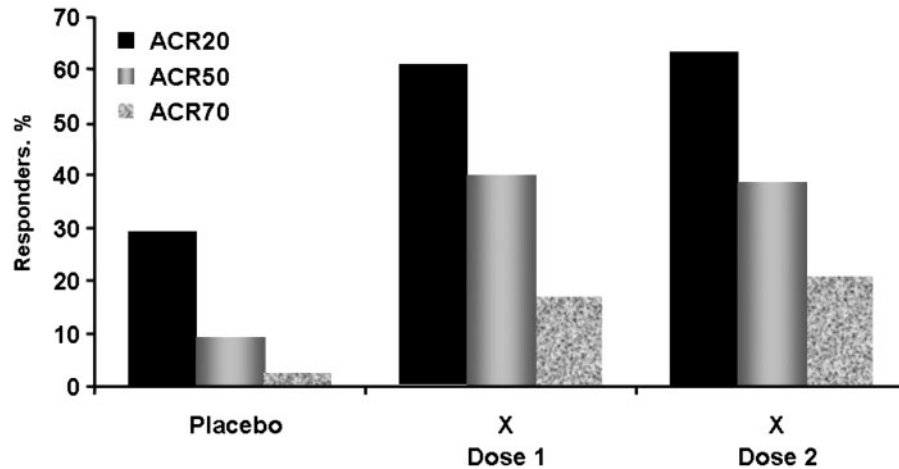
Finally, the variable clinical response to targeted therapies, including TNF blockade [16–18] and treatment with rituximab [19], abatacept [20] and tocilizumab [21], strongly highlights the variability of RA. The typical response to any targeted therapy in RA patients who have failed MTX is shown in Fig. 1. Of note, such ACR responses may be seen on the group level for any of the above-mentioned targeted therapies, but patients who fail one targeted drug are not necessarily the same failing a different mechanism of action. Collectively, the data

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Fig. 1 ACR responses typically observed 24 weeks after initiation of treatment with any of the biologics used for the treatment of patients with RA who have failed MTX. Although the results on the group level may be comparable, individual patients who fail one mechanism of action are not necessarily the same as those failing a different mechanism of action.



### Effective anti-rheumatic treatments affect common final pathways

The importance of collecting data on the primary site of inflammation, the synovium, to understand the effects of anti-rheumatic treatment is illustrated by the observation that clinical arthritis activity is accompanied by persistent histologic signs of synovitis after treatment with humanized anti-cluster of differentiation 52 (CD52) antibodies or chimeric anti-CD4 antibodies, despite profound depletion of peripheral blood lymphocytes [22, 23]. Similarly, it has for instance been shown that B-lineage cells may persist in the synovium in some RA patients after treatment with rituximab, in spite of marked depletion of peripheral blood B cells in nearly all patients [24, 25].

Successful treatment with DMARDs, such as gold [26], MTX [27–29], LEF [29] and CSs [30–32], has consistently been associated with decreased mononuclear cell infiltration in the synovium. Similarly, successful treatment of RA patients with infliximab [33–38], anakinra [39] and rituximab [24, 25, 40–42] results in reduced synovial inflammation.

In one study, patients were randomly assigned to treatment with prednisolone according to the Combinatietherapie Bij Reumatoide Artritis (COBRA) regimen or placebo for 2 weeks [32]. This study identified synovial sublining macrophages as the best biomarker associated with the clinical response to CSs. Next, the utility of macrophages in the synovial sublining as a candidate biomarker was tested across discrete interventions and kinetics [43, 44]. The consistent relationship between the decrease in synovial macrophages and clinical improvement after anti-rheumatic treatment was also confirmed by other studies [45–48]. Taken together, these studies indicate that successful treatment of RA results in decreased accumulation of synovial macrophages associated with clinical improvement, independent of the specific mechanism of action (Fig. 2).

### Understanding resistance to anti-rheumatic biologic treatment

The development of biologic agents that selectively block the effects of pro-inflammatory cytokines, interfere with co-stimulatory signals, or deplete immune cells has provided a major advance in the treatment of RA. However, not all patients respond to a given treatment, and therefore there is a clear need for the identification of clinical, radiological and molecular biomarkers that can help us understand the variable response to targeted therapy. There are as yet no known predictive biomarkers of response to abatacept and tocilizumab; hence, I will focus on biomarkers measured at baseline that might be predictive of response to anti-TNF or rituximab treatment.

#### The primary response to anti-TNF treatment

Blockade of TNF using systemic administration of soluble receptors or mAbs (all parenterally administered protein therapeutics or biologicals) has improved the treatment of RA considerably. These anti-TNF therapies have shown clinical efficacy in 60–70% of the RA patients with persistent disease activity in spite of conventional

Fɪɢ. 2 Clinical signs and symptoms of RA are associated with macrophage infiltration and activation. Different patho-genetic mechanisms may drive this common final pathway. Successful treatment of RA results in decreased accumulation of synovial macrophages associated with clinical improvement, independent of the specific mechanism of action. Mø: macrophage; FLS: fibroblast-like synoviocyte.
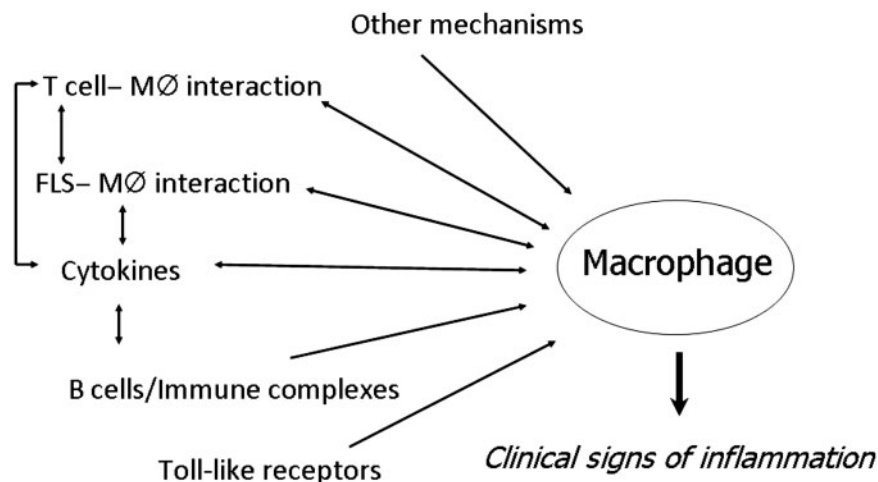
DMARD treatment. Of importance, TNF antagonists as well as other approved biologics, do not only improve clinical signs and symptoms of arthritis activity, but in most patients there is also protection against progression of joint destruction, reduced disability and a beneficial effect in terms of quality of life. To date, three TNF-targeting agents have dominated the biologic management of RA: adalimumab, a fully human mAb; etanercept, a soluble receptor construct; and infliximab, a chimeric mAb. Two other TNF antagonists, certolizumab and golimumab, have more recently also been shown to be effective in RA.

In spite of the improvement observed in most patients after anti-TNF treatment, some patients do not respond. So far no factors have been identified that fully explain or predict the heterogeneous primary response, as determined 12–16 weeks after initiation of treatment. However, a small pilot study suggested that pre-treatment TNF level in the synovium might be related to clinical efficacy, where TNF-blocking therapy could be most effective in patients with high pre-treatment TNF levels [35]. Next, a study of 143 RA patients demonstrated increased TNF expression levels in the intimal lining layer and synovial sublining of patients responding at Week 16 compared with non-responding to infliximab treatment [49]. In line with these findings, there was increased infiltration by macrophages, including CD163^{+} resident tissue macrophages and myeloid-related protein 8^{+} (MRP8^{+}) and MRP14^{+} infiltrating macrophages, as well as T cells in responders compared with non-responders; these cells are the main source of TNF in the synovium of patients with RA. Consistent with the clinical experience that the response to TNF blockade is not a dichotomous phenomenon [50], there was no distinct threshold value in TNF expression in the synovium of patients with RA. Multivariate logistic regression analysis

of synovial markers showed that TNF expression in the synovial sublining at baseline could explain ~10% of the variance in response to therapy [49]. After adjusting for disease activity at baseline this further increased to 17%. Hence, the predictive value of synovial TNF expression is statistically significant, but overall limited. In other words, this study provided proof of principle confirming that biomarkers predictive of the response to anti-TNF therapy might be identified, but there is no role for measurement of synovial TNF levels in isolation in the context of personalized health care. In line with these findings, another study demonstrated that the primary response to anti-TNF treatment is related to higher TNF bioactivity in the peripheral blood [51].

A subsequent study revealed a highly significant relationship between the presence of synovial lymphocyte aggregates at baseline in the synovium and the primary clinical response to infliximab treatment defined at 16 weeks [52]. When the presence of synovial lymphocyte aggregates was added into a combined prediction model with synovial TNF expression, the 28-joint DAS (DAS-28) at baseline, and the presence of ACPA, the presence of lymphocyte aggregates increased the prediction of response from 19 to 29%. The positive predictive value of the model was 85% and the negative predictive value was 53% [52]. Other studies using gene array analysis of synovial tissue samples aimed at the identification of biomarkers predictive of response to anti-TNF treatment have generally suggested that patients with a more inflammatory gene profile are more likely to respond to TNF blockade [53, 54], although results have been somewhat variable [55]. These studies have also shown that it is pivotal to account for the microarchitecture and infiltrating cell populations when studying gene expression patterns in rheumatoid synovial tissue [54].

Transcription profiling using purified peripheral blood monocytes from RA patients identified CD11c as a biomarker that was capable of distinguishing at baseline between patients who would be responders to adalimumab treatment and those who would be non-responders [56]. CD11c levels significantly correlated with the ACR response. However, CD11c was not predictive of the response to adalimumab treatment in patients who used concomitant MTX therapy, limiting its use as biomarkers predictive of the response to anti-TNF treatment, as this is mostly used in combination [56].

Several studies have investigated the relationship between the presence of RFs and ACPA on the one hand and the primary response to anti-TNF treatment on the other, with conflicting results [57–60]. Together, it appears unlikely that autoantibody status can be used to predict the response to anti-TNF treatment in individualized health care. Other work has focused on different factors ranging from, for example, genetic factors [61, 62] to body weight [61] as predictive biomarkers of response. Many of these studies deepen our insight into the mechanisms involved in the primary response to anti-TNF therapy. Although proof of principle has been obtained that predictive biomarkers can be identified, none of these has at present sufficient positive predictive value and in particular negative predictive value to be used for treatment decisions at baseline in the individual patient. For future research, the combination of multiple markers bears most promise to improve the performance of a biomarker-guided approach, as it could reduce the extensive overlap in individual marker levels that exists between responders and non-responders.

Having shown that on the group level, primary non-responders to a first TNF antagonist have less TNF- and less inflammation-dependent disease, the clinical question arises whether the primary non-responders are less likely to respond to a second TNF inhibitor after having failed a first one compared with RA patients who are anti-TNF naïve. Twenty-eight uncontrolled studies observed clinical improvement after switching to adalimumab, etanercept or infliximab in patients who had discontinued at least one previous TNF inhibitor [63]. A limitation is that most studies do not distinguish between the primary response (primary non-response could be defined as lack of clinical improvement determined 12–16 weeks after initiation of treatment) and secondary response (secondary non-response here defined as initial clinical improvement followed by loss of response usually at least 6 months after initiation of treatment), which appears very relevant as the mechanisms for failure may be completely different. In addition, patients who discontinued anti-TNF therapy because of adverse events are often also grouped among the TNF inadequate responders. On the group level, clinical response to a second TNF inhibitor is lower than the response in anti-TNF-naïve patients [64–66]. Clinical response to a second TNF inhibitor is, however, not decreased in patients who switched because of secondary non-response associated with the development of anti-drug antibodies (see below) [65, 67].

Importantly, patients who failed adalimumab treatment without anti-drug antibodies had a diminished response after switching to etanercept treatment compared with patients who were TNF naïve [67]. These data are consistent with other studies, showing that the clinical response to a second or third TNF antagonist is decreased in primary non-responders to a first TNF inhibitor [68, 69]. Taken together, primary non-responders to anti-TNF treatment have less TNF-dependent disease and are less likely to respond to a second TNF inhibitor. Therefore, one might preferably prescribe a biologic with a different mechanism of action in primary non-responders to a first TNF blocker.

## The secondary response to anti-TNF treatment

In an environment where biologics with different mechanisms of action are available, many RA patients treated with TNF inhibitors discontinue their treatment over time due to lack of efficacy, adverse events as well as other reasons [70]; after 5 years, the cumulative drug survival is ~50%. Loss of response may be explained in part by the development of anti-drug antibodies. Antibodies against infliximab were detected in 22 (43%) RA patients during the first year after initiation of treatment with 3 mg/kg infliximab every 8 weeks, although 86% were receiving concomitant MTX [71]. Patients without detectable anti-infliximab antibodies were significantly more often classified as responders compared with patients with detectable anti-infliximab antibodies after 1 year [71]. The development of anti-drug antibodies is not limited to chimeric antibodies. In another cohort study, anti-adalimumab antibodies were detected in 21 (17%) patients during 28 weeks of adalimumab treatment; 79% used concomitant MTX [71]. Clinical non-responders at Week 28 had anti-adalimumab antibodies significantly more often than good responders [71]. After 3 years, the development of anti-adalimumab antibodies was associated with lower drug concentrations and lower likelihood of low disease activity or remission [72]. Thus, one of the mechanisms underlying secondary non-response to anti-TNF treatment is completely unrelated to the molecular mechanisms promoting synovial inflammation. Therefore, one could postulate that secondary non-responders may still respond to treatment with a second TNF inhibitor, at least when non-response is explained by the presence of anti-drug antibodies. Clinical improvement after treatment with a second TNF inhibitor is indeed apparently not diminished in patients who switched because of secondary non-response associated with the development of anti-drug antibodies [65, 67]. Thus, one could consider trying a second TNF antagonist in patients who initially responded to a first TNF inhibitor, but who lost response over time.

## The response to rituximab treatment

As the clinical response to rituximab treatment can be variable, we studied the relationship between changes in synovial cell populations during the first 16 weeks after rituximab treatment and the clinical response determined

at Week 24 [41]. No baseline characteristics of the synovium could significantly predict clinical response to treatment, although there was perhaps a minor trend towards more B cells at baseline in responders compared with non-responders. Importantly, linear regression analysis identified a statistically significant, positive correlation between the change in intimal macrophages and plasma cells between 4 and 16 weeks on the one hand and the decrease in disease activity after 24 weeks on the other [41]. The decrease in plasma cells between 4 and 16 weeks was predictive for the decrease in DAS-28. The change in plasma cell numbers was also correlated with a decrease in the serum levels of ACPA. Consistent with these results, the change in plasma cells differed significantly between responders and non-responders [41]. Other studies have confirmed the importance of the (indirect) effect of rituximab treatment in RA on B-lineage cells associated with autoantibody production for the ultimate clinical effectiveness [73, 74]. The notion that clinical improvement after rituximab treatment can only be achieved if numbers of autoantibody-producing plasma cells after treatment are low is supported by the association between a high number of CD20-negative pre-plasma cells before treatment with rituximab, incomplete B-lineage cell depletion and worse clinical response [74]. Other biomarkers of response recently identified include the presence of a Type I IFN signature in peripheral blood mononuclear cells at baseline that was negatively correlated with the response to rituximab [75]. Among various effects, Type I IFNs may stimulate the production of a proliferation-induced ligand (APRIL) and B-lymphocyte stimulator (BLyS) and directly enhance B-cell survival.

Although B cells may have different roles, including antigen presentation, stimulation of T cells and cytokine production, the studies discussed above suggest that in particular the role of autoantibodies produced by plasma cells may be important in RA [76]. Consistent with this hypothesis, RA patients who test positive for RFs and/or ACPA are more likely to respond to rituximab treatment than autoantibody-negative patients [77–79]. Therefore, the recommendation is to preferably prescribe a biologic other than rituximab in RA patients who are both RF and ACPA negative. If an RF and ACPA-double-negative patient has failed all other mechanisms of action, rituximab could still be tried in light of the possibility that the patient may have autoantibodies other than those detected by the currently used tests for RF and ACPA.

A next question is whether a patient should receive a second course of rituximab treatment if there was no clinical response to a first course of rituximab treatment. As discussed above, in autoantibody-positive RA patients, lack of response may be related to persistence of B-lineage cells. Conceivably, more intense dosing regimens might result in clinical improvement in these patients. Although results have been somewhat variable, it appears unlikely that re-treatment of non-responders to a first rituximab course with the currently approved dosing regimens will result in robust clinical improvement [74, 80, 81]. Of particular interest is a randomized placebo-controlled trial showing that patients who did not achieve a clinical response to the first course were no more likely to achieve response to a second course ($n = 126$) than to placebo ($n = 60$) [81]. In light of the availability of other therapeutic options, for individual patients who are rituximab non-responders, other treatment options should be considered [82].

## A preliminary treatment algorithm

In RA patients who have failed conventional therapy with DMARDs, biologic treatment may be indicated. At present, in most cases, anti-TNF therapy will be employed, consistent with the recommendations of the European League Against Rheumatism (EULAR) [83]. It should be noted, however, that tocilizumab and abatacept have also been approved for the treatment of MTX-inadequate responders. Depending on the features of the individual patient, one may consider the prescription of a biologic with a mechanism of action other than TNF blockade. In any case, the objective at any time is to achieve remission, or at least a state of low disease activity [83].

If a TNF inhibitor is initiated and there is subsequent lack of clinical response, one could consider a second TNF inhibitor [84], rituximab [19], tocilizumab [20] or abatacept [20]. First, it should be noted that a decision may be influenced by patient-specific characteristics such as the presence of systemic manifestations like anaemia, comorbidity, the need to prescribe biologic treatment without concomitant treatment with conventional DMARDs, or the risk of specific infections like tuberculosis. Secondly, there are at present no published data on reliable biomarkers predictive of the response to tocilizumab or abatacept. Thus, at present these biologics can be considered in all TNF-inadequate responders.

Apart from these considerations, the first question that should be asked is: is the patient a primary non-responder or a secondary non-responder to the first TNF antagonist (see above)? If the patient is a primary non-responder, the patient is more likely to have less TNF-dependent disease and a lower likelihood of robust clinical improvement to a second TNF antagonist (Fig. 3). If the patient exhibited initial clinical improvement, but lost response over time (secondary non-responder), then a second TNF inhibitor may be considered, as the lack of response may be related to the development of anti-drug antibodies rather than TNF-independent disease.

The next question to ask is if the patient is RF positive and/or ACPA positive. If both RF and ACPA are negative, the likelihood of clinical improvement after rituximab treatment is diminished and biologics other than rituximab should be considered (Fig. 3).

If an RA patient has been treated with rituximab, but there was no clinical response to the first course, then the likelihood of a good response to a second course according to the current dosing schedule appears low. Thus, biologic treatment other than rituximab should be initiated (Fig. 3).

FIG. 3 Treatment algorithm for patients with RA who have failed conventional treatment with DMARDs and treatment with one TNF inhibitor. Treatment decisions are influenced by distinguishing between primary non-responders (here defined as no clinical response 12–16 weeks after initiation of anti-TNF treatment) and secondary non-responders (here defined as initial clinical improvement followed by loss of response $\geqslant 24$ weeks after initiation of anti-TNF treatment) as well as positivity for RF and ACPAs. Switching to a different mechanism of action is recommended if a patient does not respond clinically to the first course of rituximab.
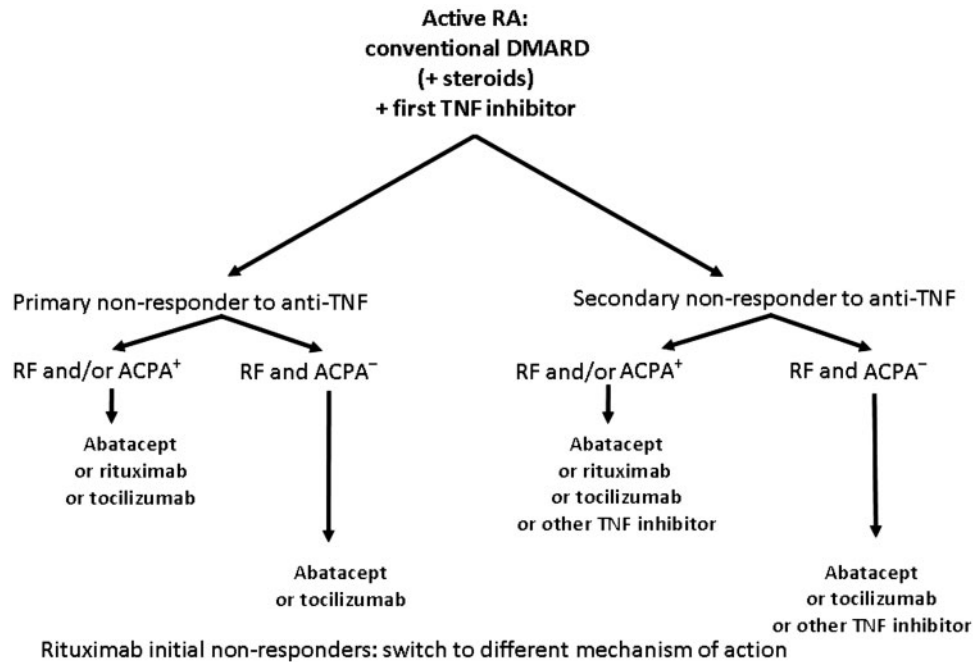


## Concluding remarks

As RA is a syndrome comprising different pathogenetic subsets, some patients may be more likely to respond to a specific therapeutic intervention than others. Recent work has provided proof of principle that biomarkers may be identified predictive of the response to targeted therapy. Most of these biomarkers increase the insight into the mechanisms promoting synovial inflammation and joint destruction as well as in the mechanism of action of the treatment. However, the use of biomarkers in the context of individualized health care is still limited. In addition to the need for identification of novel biomarkers, combination of multiple markers bears most promise to improve the performance of a biomarker-guided approach.

Based on recent insights, an initial algorithm can be made assisting in treatment decisions in patients who have failed one TNF inhibitor. Key questions in this algorithm relate to the question whether the patient is a primary vs a secondary non-responder to TNF blockade and whether the patient is autoantibody positive.

This preliminary algorithm may contribute to more cost-effective treatment of RA, and supports the rationale for the development of more extensive algorithms. For this purpose more data are needed, most importantly, the identification of biomarkers predictive of the response to tocilizumab and abatacept. Therefore, there is a need for well-designed clinical studies aimed at the identification of disease subgroups that would benefit from one mechanism of action over another. Future research should also focus on the reasons for secondary loss of response and the potential value of therapeutic drug monitoring. Together, these approaches may improve the ACR responses compared with those shown in Fig. 1. Finally, there is a need for the identification of biomarkers predictive of adverse events.

> **Rheumatology key messages**
> - RA is a syndrome rather than one disease entity.
> - Effective anti-rheumatic treatments affect common final pathways.
> - A therapeutic algorithm can be made for patients who have failed one TNF inhibitor.

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