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# Therapeutic Antibody-Based Drugs in the Treatment of Human Inflammatory Disorders

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Additional information is available at the end of the chapter

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## Abstract

Inflammation causes debilitating human conditions and older treatments rely on global immunosuppression that non-specifically alleviates symptoms. Currently, several monoclonal antibodies (mAbs) are available that specifically block pro-inflammatory cytokines. These include mAbs specific to tumour necrosis factor (TNF), interleukin (IL)-1 $\beta$ , IL-6, IL-17 and IL-12/IL-23. The chapter summarises the key elements in human inflammatory disease conditions, including various forms of arthritis, psoriasis, Crohn's disease and ulcerative colitis, plus pyrin-associated inflammatory syndromes and periodic fevers, to explain the benefit of cytokine neutralisation through mAb-type reagents. The chapter reviews the efficacy and safety of the current repertoire of anti-cytokine/cytokine receptor mAbs. It also discusses the known side effects and adverse events that are sometimes associated with systemic blockade of cytokines *in vivo*, and concludes that the accumulating knowledge of treatment failures can reveal unappreciated aspects of cytokine biology and even new treatment opportunities. The chapter includes mention of the rapidly expanding cohort of biosimilar mAbs and the mAbs to IL-4, IL-5 and IL-13 that are now emerging, in addition to the need for treatments for disorders that remain refractory to the current repertoire of anti-cytokine mAbs and conventional treatments. Thus, here we summarise the current status of anti-cytokine mAbs for human inflammatory diseases.

**Keywords:** arthritis, asthma, crohn's disease, cytokines, biosimilar, inflammation, interleukin (IL), IL-1 $\beta$ , IL-4, IL-5, IL-6, IL-13, IL-12, IL-17, IL-23, monoclonal antibodies (mAbs), periodic fevers, psoriasis, pyrin, tumour necrosis factor (TNF), and ulcerative colitis

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## 1. Introduction

Human inflammatory diseases are among some of the most debilitating conditions described and they inflict varying degrees of functional impairment and may be long-lasting, causing chronic pain. Some examples of inflammatory conditions include rheumatoid arthritis (RA) and other related or non-related arthritides, skin diseases such as psoriasis, or intestinal conditions such as Crohn's disease (CD) and ulcerative colitis (UC), as well as pyrin-associated inflammatory syndromes and periodic fevers. These conditions generally present as acute bouts of inflammation, but are in most cases active as chronic conditions with periods of worsening or 'flares'. Intense research, over many decades, has revealed important details regarding the mechanisms that contribute to the pathology of these conditions, although in many situations the initial trigger continues to remain undefined. This knowledge led to the use of broad-acting anti-inflammatory agents that exert benefit due to global immune suppression. Thus, drugs such as corticosteroids became the mainstream treatment option. Over time, however, as knowledge of the underlying pathobiology deepened, so the role of individual cytokines emerged as critical drivers of the *in vivo* inflammatory processes. Eventually, as it became known that microbes, especially viruses, encode and express cytokine-receptor mimics that block the biological effects of specific cytokines, and inhibit cytokine-mediated inflammation [1, 2], thus it became obvious to trial soluble receptor proteins as inhibitors of pro-inflammatory cytokines to treat human inflammatory diseases. Although the microbial products themselves are potent neutralising reagents, they were viral in origin—not human—and therefore immunogenic (not suitable for long-term human use). The use of neutralising cytokine-specific monoclonal antibodies (mAbs), and/or recombinant forms of soluble cytokine receptors, however, efficiently solved this problem, because these recombinant Ig-based molecules are essentially identical copies of endogenous human protein—purified monoclonal Ig. Thus was born the era of cytokine-neutralising mAb-based therapeutic reagents for the treatment of human inflammatory diseases.

## 2. Clinical presentation and processes of inflammatory diseases amenable for treatment with cytokines targeted neutralising mAbs

Inflammation is a natural and spontaneous process that occurs in response to an insult causing tissue damage. It involves the activation of innate and adaptive immune system components, including both vascular and cellular responses. Essentially, there are four signs that represent the clinical manifestations of inflammation: redness (Latin: *rubor*), warmth/heat (*calor*), swelling (*tumour*) and pain (*dolor*), and when unresolved, inflammation frequently results in the loss of physiological function (*function laesa*). Systemic symptoms such as fever also frequently occur. Together, these are the universal or classical hallmarks of inflammation in mammals.

The magnitude of the response is initially directly proportional to the severity of the insult, but reactivation of inflammation can be triggered, either by a reoccurrence of the same or similar event, or sometimes via an unrelated event. During times of exacerbation, the severity of symptoms escalates dramatically, and this is often referred to as an inflammation 'flare'. In either instance, the physiological events that follow are becoming increasingly well understood at a molecular level and this detailed mechanistic understanding has revealed a number of

opportunities for therapeutic blockage of specific mediators of inflammation. In many cases, the results of these specific interventions have been truly remarkable, such that previously debilitating disease conditions are now entirely manageable or, in some cases, almost unnoticeable. The following sections provide a summary of current knowledge of the molecular basis of the events that occur in several human inflammatory disorders together with a description of the mAbs and recombinant protein-based reagents that can be applied to successfully ameliorate inflammation.

### 2.1. Inflammatory cytokines in the pathology of arthritides: rheumatoid arthritis (RA), idiopathic juvenile arthritis (IJA) and ankylosing spondylitis (AS)

Rheumatoid arthritis (RA) is an autoimmune disease that comprises both systemic and tissue-specific inflammation, primarily inflammation of joint synovium, leading ultimately to erosion of the joint tissue. The initial trigger of the inflammation is usually unknown. Once present, however, it usually progresses and is characterised by episodes of greater intensity or flares. The systemic nature of the condition is exemplified by the fact that diverse tissues may be involved, including skin and kidneys. It is generally believed that there are three main phases of pathology in RA: (i) an initial induction phase of non-specific tissue inflammation, (ii) an expansion phase involving T lymphocyte (T cell) responses and (iii) a chronic systemic inflammation phase mediated by the production of cytokines such as interleukin (IL)-1 $\beta$ , tumour necrosis factor (TNF) and IL-6 [3] and the production of citrullinated fibrinogen among other substrates. The 'unnatural' citrullinated proteins are frequently the targets of rheumatoid factor IgM and IgG autoantibodies [4]. Although the systemic phase is debilitating in its own right, the inflammatory destruction of joint synovium results in immobile and dysfunctional joints, and this is often amplified by the involvement of multiple affected joints, that is, polyarthritis (**Figure 1**); for most patients, the painful chronic synovitis ultimately results in irreparable joint destruction.

RA occurs not only in adults but also in children [5]. There are many presentations of juvenile arthritis and most are idiopathic in nature, and include polyarticular and/or systemic arthritis, as well as fever, skin rash, anaemia, spleen, liver and sometimes even cardiac tissue inflammation [6, 7]. The inflammation is thought to be due to activation of macrophages and other immune cells (monocytes, dendritic cells, T cells, etc.), which may explain the different subtypes of juvenile idiopathic arthritis (JIA) [8], and in all cases there is inflammation mediated primarily



**Figure 1.** Clinical presentation of rheumatoid and psoriatic arthritis. (A) Long-standing RA characterised by ulnar deviation, metacarpal phalangeal joint subluxation and boutonniere deformity, and (B) Psoriatic arthritis with boutonniere deformity. (Images generously provided by Prof. Manolios, Westmead Hospital, Sydney, Australia).

by the production of soluble mediators—especially pro-inflammatory cytokines such as TNF [9, 10]. Systemic JIA (SJIA) is thus considered a multifactorial auto-inflammatory disease [6].

Previous treatments for SJIA have traditionally comprised non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids in more severe cases, but these drugs can have significant side effects and are often poorly tolerated in children, sometimes causing lifelong sequelae such as sterility. Fortunately, most anti-TNF-based mAbs have yielded promising results, although efficacy rates and treatment retention rates decrease with treatment time [11]. With the emergence of IL-6 neutralising mAbs, such as tocilizumab, other effective treatment options also now exist [12]. However, IL-1 $\beta$ -blocking agents such as anakinra or canakinumab also show significant efficacy in JIA [13, 14]. Taken together, these findings support the current dogma that the pathobiology of JIA is not entirely identical to adult RA even when joint arthropathy is the primary common lesion. Secondly, these results suggest that there exists an inflammation hierarchy among the contributing cytokines—some being critical to the production of other cytokines or inflammatory mediator substances, and others less significant, that is, some are ‘non-drivers’ of the pathology but elevated nonetheless [9]. Furthermore, the presence of TNF, IL-6 and IL-1 $\beta$  strongly points to the involvement of macrophages—particularly type-I macrophages (M1 M $\emptyset$ ) which, when activated, produce this combination of cytokines. Interestingly, a polymorphism in macrophage migration-inhibitory factor (MIF) has been found to be associated with SJIA [15, 16]. Indeed, the diverse presentation of juvenile arthritis suggests that there is still much more to learn about the aetiology of arthritis in children.

**Ankylosing spondylitis (AS)** is another type of inflammatory arthritis that usually involves the sacroiliac joint and spine [17]. As this disease worsens, shoulders can also be affected. The predominant symptoms are joint stiffness and pain caused by a chronic low-grade inflammation [17]. In advanced cases, vertebra can actually fuse and remain in a fixed and immobile position, explaining why many AS patients frequently present with a classical ‘forward-leaning’ posture or limited flexion in the lumbar spine and inter-vertebral calcification (**Figure 2A** and **B**). Despite a long-known association to HLA-B27, and other immune gene loci [18], and an increased prevalence in males, the trigger for this condition remains unknown [17]. The disease can be either undifferentiated or more specific in its presentation, for example, presenting in a more defined manner such as with reactive arthritis, psoriatic arthritis (see **Figure 1**) or more dispersed symptoms such as arthritis with an associated inflammatory



**Figure 2.** Clinical presentation of ankylosing spondylitis (AS) and psoriasis. (A) AS in a 30-year-old male with limited flexion of lumbar spine, (B) AS involving cervical spine; X-ray features show calcification of anterior longitudinal ligament, (C) psoriatic erosions involving proximal interphalangeal joints and second distal interphalangeal joint and (D) psoriatic skin lesion characterised by flaking and silver scales. (Images generously provided by Prof. Manolios, Westmead Hospital, Sydney, Australia).

bowel disease (IBD) condition. The link with IBD is intriguing, and although this has long been a rather poorly understood AS disease association (or presentation), recent evidence suggests a potential role of IL-17-family cytokines.

Early treatments for AS have been focused primarily on relieving pain, for example, through non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen or voltaren and so on. Cox-2 inhibitors have also been used. As these are broad inhibitors of inflammation and pain-reducing mimetics, they do not specifically target the specific factors that are critical to the underlying aetiology of the condition. Similarly, drugs, such as sulfasalazine, methotrexate or corticosteroids, while offering some degree of efficacy in the treatment of AS, are, again, broad-acting immune suppressants. As knowledge of the molecular aetiology of this disease has increased, it was found that TNF-neutralising drugs etanercept, adalimumab, certolizumab pegol, infliximab or golimumab can be effective [19]. Yet, precisely how anti-TNF mAbs provide benefit in AS patients, however, is still not entirely clear, due essentially to the gaps in knowledge surrounding this disease; the ability of the anti-TNF agents to prevent new bone formation, for example, is still controversial and poorly explained through existing knowledge. Moreover, anti-TNF mAbs are not beneficial in all AS patients. Thus, most clinicians conclude that while TNF may be produced in certain circumstances in AS pathology, it may or may not be the driving factor in AS disease pathology [20].

There is currently much excitement surrounding the role for cytokines IL-17 and IL-23 in AS. Indeed, the demonstrated efficacy of IL-17 and IL-23 neutralising mAbs in clinical trials has recently cemented these cytokines as central mediators of AS inflammation. Several previously unexpected immune cells are now therefore strongly implicated as being critical components of the pathobiology of AS, specifically Th-17 cells and lineage-negative innate-like immune cells (ILC) type 3 [21]. The different subsets of ILC3 cells typically produce not only IL-17-type cytokines but also other cytokines such as IL-6, TNF and IFN $\gamma$  (thus explaining the partial benefits of treatment with anti-TNF mAbs, and global immune-suppressive treatments). These ILCs are interesting in AS because they are exposed to bacteria and microbial products as they are found in skin and in gut and recognised for their role in preserving barrier function. Moreover, the detection of these innate cell types in the blood of AS patients [22] thus provides a mechanistic link with the AS arthritis and the inflammatory bowel disease-type symptoms that occurs in many AS patients. Moreover, both TNF and IL-17 have long been implicated in the structural bone damage and remodelling that is evident in AS [23, 24]. More research will be required to define the precise pathogenic mechanisms of IL-17-producing innate immune cells in AS.

## 2.2. Inflammatory cytokines in psoriasis (and psoriatic-type arthritis)

Psoriasis is an autoimmune skin condition where patches of scaly skin accumulate. The locations of these patches are usually elbows, knees or scalp, although the location is not a diagnostic feature per se, and the psoriatic skin lesions can occur almost anywhere on the body. A proportion of people with chronic skin psoriasis will also develop a type of psoriatic arthritis of joints (**Figure 1**). Like RA, this can result in significant joint erosion (**Figure 2**) but this type of arthritis is rheumatoid factor negative, and thus distinct from RA [25]. Psoriasis is also different from eczema, in that there is a thickening of the epidermis and the condition almost always persists, whereas eczema often fades spontaneously, for example, as children grow older. In fact, there are

various forms of psoriasis, including the most common form—plaque psoriasis, comprising an accumulation of dead skin cells building up, forming a cracked ‘plaque’ skin lesion (**Figure 2**). Some patients, however, develop smooth, shiny skin lesions; these usually being on the knee or under the arm. In addition, guttate psoriasis is a form of the psoriatic disease that sometimes form after *Streptococcal* sp. bacterial infections. Erythrodermic psoriasis is the most severe form of the disease, and in this condition large areas of skin eventually slough off.

In psoriasis treatment, a number of systemic immunosuppressive agents have been used, for example, cyclosporine or methotrexate. Nevertheless, the dysfunction of cytokines, especially IL-13, IL-17 and IL-23, appears to be integral to the pathology of all forms of psoriasis—consistent with the broad benefits of cyclosporin in psoriatic pathologies [26]. Benefit has long been established with mAb-based reagents that neutralise TNF [27], and more recently, new IL-23-neutralising mAbs are demonstrating considerable efficacy [28, 29]. The IL-17A-neutralising mAbs secukinumab and ixekizumab, and IL-17R-blocking mAbs brodalumab are also showing significant efficacy in ameliorating psoriatic-based skin conditions [30–32]. This is consistent with the observations of elevated IL-17A within psoriatic plaques (skin lesions), being produced from many immune cell types [33], as well as IL-13 [34]. In fact, it has also been recently demonstrated that IL-17 is intimately linked to IL-13 biology, whereby IL-13 regulates IL-17A production in Th17 cells [35, 36]. These findings are also consistent with the observation that transgenic IL-17A expression mice develop psoriatic-type skin lesions that resembles human psoriasis [37]. The striking efficacy of anti-IL-17 mAbs indicates that IL-17-producing cells, such as Th-17 cells, are integral to the pathobiology of many forms of psoriasis. Recently, however, IL-17-producing ILC3s have been shown to be present in psoriatic tissues [38, 39]. It has also been shown that CD1a-restricted IL-17-producing lipid antigen recognising T cells are present both in skin and in blood of psoriasis patients [40]. Hence, the dramatic success of these new mAbs not only brings psoriasis patients the promise of relief of their symptoms but also simultaneously reveals new and otherwise unappreciated knowledge of the critical aspects of the disease mechanisms at play in psoriasis.

Other interesting recent developments are new oral treatments for psoriasis [41]. For example, a small molecule phosphodiesterase-4 inhibitor (apremilast) works by preventing cAMP activation in immune cells, thereby limiting pro-inflammatory cytokine production [42–45]. It should be noted, however, that initial clinical trials were discontinued due to unexpected side effects such as diarrhoea, headache and nausea, although careful re-examination of dosing regimens and/or new molecular modifications may still be possible. Nonetheless, phosphodiesterase-4 has itself been found to be elevated in psoriatic lesion inflammatory cells [44], and thus the amelioration of symptoms correlates perfectly with its potent inhibition *in vivo*. In summary, these findings again strongly substantiate the involvement of inflammatory cytokines, especially IL-17 and IL-23, in the aetiopathology of human psoriasis. It is no exaggeration to conclude that newly developed mAbs blocking IL-17 and IL-23 pathways have completely revolutionised the treatment of chronic psoriasis— they now already comprise the ‘standard of care’ in plaque psoriasis treatments [46]. Even so, there is much more to learn about this complex condition, such as the roles of IL-12 versus IL-23, for example, in limiting IL-17 production, and the role of IL-17-producing skin  $\gamma\delta$  T cells [47].

### **2.3. Cytokines in the pathology of inflammatory bowel disease (IBD): Crohn's disease (CD) and ulcerative colitis (UC)**

There are several autoimmune-based chronic inflammatory bowel diseases (IBD) involving the gastrointestinal tract (GIT) and these most frequently include Crohn's disease (CD) and ulcerative colitis (UC). Generically speaking, CD is considered to involve the distal junction of the small intestine and thus primarily involves inflammation in the large intestine, whereas UC inflammation can occur anywhere within the entire GIT. These conditions are both progressive and characterised by relapsing inflammation [48]. CD lesions usually involve only the superficial mucosal tissue layers, whereas UC inflammation is often more extensive, even presenting through the full-tissue thickness of the intestine. A less-well-known feature of CD is that the inflammation may involve non-GIT mucosa, for example, skin, eyes or joints, and even liver can be affected. The pathological processes in CD and UC are complex, with a deep and interconnecting interplay between inflammation and fibrosis as there is often a constant need for tissue healing [49]. For both CD and UC, the differential diagnosis is usually confirmed through endoscopy, as this procedure permits the delineation of the anatomical location that is affected (site of the inflammation). Importantly, the endoscopy also provides the opportunity for the grading of lesion severity.

In both CD and UC the immune system is highly activated, explaining the clinical benefits experienced from treatments that induce global immune suppression. Cytokine-specific mAb-based treatments are also effective at blocking and preventing IBD inflammation. It has become increasingly evident that environmental triggers are both constitutive and exacerbating during times of inflammatory flares, and hence the systemic presence of therapeutic mAbs provides a long-lasting inhibition towards the chronic inflammation. There is also a growing appreciation of the role of the gut microbiota in IBD [50]. Although the intestinal (mucosal) immune system is meant to remain unresponsive to commensal microorganisms, just as it is to food-based antigens, it retains a capacity to respond to intestinal pathogens. The current theory, however, is that there is an inappropriate, and potentially constitutive, activation of innate immune cells within the bowel and these activated cells constitute the basis of chronic IBD inflammation [48]. Theoretically, IBD inflammation may involve almost any innate immune cell residing within the GIT mucosa, but Th1- and/or Th17-type pro-inflammatory cytokines appear to be involved—and these cells produce both TNF and/or IL-17 plus IFN $\gamma$  [48]. Also, there is currently a high level of interest in the ILC3 cells in acting as the initial triggers of IBD inflammation [51, 52]. However, changes in commensal gut microflora are also now in focus, and especially the ability of bacteriophage viruses, due to their capacity to lyse bacteria and thereby alter the GIT microbiome diversity [53]. Thus, both a dysbiosis and inflammation-mediated disruption of the GIT epithelial barriers are currently thought to be integral to both UC and CD conditions. Fortunately, there are already several neutralising mAb-based therapies for IBD patients, especially for those who are refractory to traditional treatment of aminosalicylates and corticosteroids. These include the anti-TNF mAbs (infliximab, adalimumab, golimumab and certolizumab pegol) and two anti-integrin-blocking mAbs (natalizumab and vedolizumab). In contrast to the benefit evident in neutralising TNF, a contributing role for IL-17 in IBD is still uncertain, and IL-12/IL-23 are likely not the driver cytokines as there is only marginal efficacy from

ustekinumab (anti-IL-12/IL-23) in CD patients, and no benefit was evident in initial trials with briakinumab (anti-IL-12/IL-23 p40-neutralising mAbs) [54]. Indeed, brodalumab (anti-IL-17RA-neutralising mAb) caused worsening symptoms in CD [55]. Clearly, further investigation into the complex interactions between the normal and altered microbiome, and the endogenous intestinal cells, including resident innate and adaptive immune cells, is required to better understand these IBD pathologies.

#### **2.4. Autoinflammatory diseases: TNF-receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndrome (CAPS) and Muckle-Wells syndrome**

One of the clearest cases of the mechanistic role of cytokines in the aetiology of human inflammation concerns the hereditary periodic fever conditions. Here, an autoinflammatory trigger (or triggers) involves genes that are embedded within the innate immune system, but the response occurs in the absence of demonstrable infection—although there still remains the possibility that a subclinical and undetectable infection is present [56]. For example, patients with TNFR1 mutations are usually classified as TNF-Receptor-Associated Periodic Syndrome (TRAPS) [57]. TRAPS fevers typically last more than a week and exhibit a range of symptoms, such as myalgia, arthritis, fasciitis, abdominal pain, skin rashes and patches (**Figure 3**), or periorbital oedema, and even amyloidosis in severe cases [58, 59]. The precise mechanism(s) of pathology resulting in TRAPS has continued to mature over time, as TRAPS mutant TNFRs have been successively thought to result in altered activation of a key transcription factor within the immune system (NF- $\kappa$ B), an inability to bind to TNF, reduced surface expression of TRAPS TNFRs, the incorrect folding of the receptors leading to an ‘unfolded protein response’ which appears to activate the inflammasome and lead to mitochondrial reactive oxygen species, and ultimately to inflammation [56, 60]. Despite the varied presentations, a unifying presentation in TRAPS patients is the elevated levels of serum TNF, IL-1 $\beta$  and IL-6 cytokines. TRAPS treatment options vary but broad immunosuppression, such as with colchicine, is no longer generally recommended, as it is accepted that there is significant benefit in treating patients only at the times of inflammation, that is, during disease flares, and potentially monitored via levels of serum S100 proteins, IL-18, serum amyloid A, and even miRNA molecules [61, 62]. With the number of inflammatory cytokines that are elevated, the treatment options range from generic immune suppressants (e.g. colchicine) to the use of specific cytokine-neutralising mAbs. Unexpectedly perhaps, anti-TNF mAbs have largely proven ineffective in TRAPS, and they may even unexpectedly sometimes provoke a cytokine storm via the activation of the cRel (a component of the NF- $\kappa$ B system), and thereby escalating the inflammation [63]. Interestingly, the current standard treatment for TRAPS and the majority of hereditary autoinflammatory diseases is the neutralisation of IL-1 $\beta$ , and either recombinant IL-1 receptor antagonist (anakinra) or human IgG1 anti-IL-1 $\beta$  mAb (canakinumab) alleviates inflammation in TRAPS [64, 65]. Hence, it appears that targeting only IL-1 $\beta$  is beneficial in TRAPS. This, again, implies that there exists a hierarchy of inflammatory cytokines, such that blocking one cytokine has a broader effect of reducing the production of others. In fact, the administration of recombinant human TNF in human clinical trials for cancer and sepsis clearly demonstrated this principle: the administration of TNF induced elevated IL-1 $\beta$  and IL-6 [66, 67] (recently reviewed in Ref. [68]).





**Figure 3.** Clinical presentation of auto-inflammatory syndrome skin rashes and pseudo-gout inflammation. (A) TRAPS skin rash (from [69]), (B) Muckle-Wells syndrome / CAPS rash (image from [autoinflammatory.org](http://autoinflammatory.org)) and (C) joint and tissue inflammation due to pseudo-gout flair after total knee arthroplasty of right knee, both before (left) and after (right) antibiotics for potential culture-negative post-operative infection (Images used with permission).

Other autoinflammatory syndromes include Muckle-Wells syndrome (MWS), which presents with periodic episodes of skin rashes (**Figure 3**), sensorineural deafness, hives, episodal fever, joint pain and/or amyloidosis and other symptoms. These conditions are collectively known as cryopyrin-associated periodic syndrome (CAPS) and they are all universally associated with activation of pro-caspase-1 [70, 71] and thus also with mutations in NLRP3/CIAS1 and LNRC4 genes [72, 73] (see [www.autoinflammatory-search/diseases](http://www.autoinflammatory-search/diseases)). The central mechanism of pathogenesis of CAPS-type diseases is the elevated production of IL-1 $\beta$ , usually from activated monocytes/macrophages, and because of the involvement of caspase-1, there is usually a concomitant elevated production of IL-18. Thus, the neutralisation of IL-1 $\beta$  as the fundamental driver of the inflammation is proving to be beneficial in these conditions, that is, either with mAb canakinumab or with recombinant IL-1Ra protein (anakinra). Even deafness in Muckle-Wells syndrome patient was alleviated by neutralising IL-1 $\beta$  [74]. Finally, NLRP3 activation also results in elevated IL-1 $\beta$  in other unrelated sterile inflammatory conditions such as those involving monosodium urate (gout) and calcium pyrophosphate dihydrate (CPPD) (pseudo-gout) crystalline-induced arthritis (**Figure 3**) [75, 76]. Thus, neutralising IL-1 $\beta$  is effective in nearly all CAPS-type autoinflammatory conditions [60].

### 3. Biological therapeutics for inflammation

There are currently more than 20 recombinant cytokine receptor- and mAb- based protein drugs that have been developed and widely approved for the treatment of human inflammation (see **Boxes 1–5**). These can be classified as recombinant cytokine receptor-based proteins, or cytokine- or cytokine receptor-specific-neutralising mAbs (**Figure 4**).

#### 3.1. Recombinant cytokine receptors and receptor-Ig fusion proteins

Etanercept (trade name Enbrel; [www.enbrel.com](http://www.enbrel.com)) was the first human cytokine-receptor immunoglobulin chimeric fusion protein approved for the treatment of human diseases. Etanercept comprises the extracellular region of human TNFR2 and the Fc region of human IgG1, and is produced in Chinese hamster ovary (CHO) cells. As a TNFR2-based-Ig protein, it has properties of both a human cytokine receptor and human Ig protein: the TNFR2 component binds to TNF and lymphotoxin- $\alpha$ , whereas the human IgG1 portion confers serum longevity and Ig Fc receptor (FcR)-binding capacity. Etanercept is thus a TNF inhibitor capable of neutralising soluble

**Box 1.** Current therapeutic TNF and TNF-receptor-specific inhibitory agents.

TNF-receptor Ig fusion proteins and anti-tumour necrosis factor (TNF)				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Etanercept</b> Recombinant fusion protein: Human TNFR2:IgG1-Fc	Enbrel®	s.c. injection	Rheumatoid arthritis Polyarticular juvenile idiopathic arthritis (PJIA) Psoriatic arthritis Ankylosing spondylitis Plaque psoriasis	Cognitive impairment (peri-spinal delivery)?
<b>Infliximab</b> Humanised (chimeric) IgG1κ	Remicade®	i.v. infusion	Rheumatoid arthritis* Psoriatic arthritis* Ankylosing spondylitis Plaque psoriasis Crohn's disease Paediatric RA Paediatric Crohn's disease	
<b>Adalimumab</b> Human IgG1κ	Humira®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Plaque psoriasis Active ankylosing spondylitis Crohn's disease Juvenile idiopathic arthritis Ulcerative colitis	
<b>Golimumab</b> Human IgG1κ	Simponi®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Plaque psoriasis Ulcerative colitis	
<b>Certolizumab Pegol</b> Pegylated-Fab' of humanised IgG1κ	Cimzia®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Ankylosing spondylitis Crohn's disease	
Biosimilars: (Among others)				
<b>Erelzi</b> TNFR2-IgG1 Etanercept biosimilar	etanercept-szszs® (Sandoz)	i.v. infusion	Same indications as per etanercept	
<b>Brenzys (SB4)</b> TNFR2-IgG1 Etanercept biosimilar	(Samsung Bioepis; Merck and Biogen)	i.v. infusion	Same indications as per etanercept	
<b>CTP-13</b> Humanised IgG1κ Infliximab biosimilar	Remsima® (Infliximab) Inflectra® (Hospira)	i.v. infusion	Same as per infliximab	
<b>BOW015</b> Human IgG1κ Infliximab biosimilar	Infimab® (Reliance Life Sciences)	i.v. infusion	Same as per infliximab	
<b>SB2</b> Human IgG1κ Infliximab biosimilar	(Samsung Bioepis; Merck and Biogen)	i.v. infusion	Same as per infliximab	

TNF-receptor Ig fusion proteins and anti-tumour necrosis factor (TNF)				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Adalimumab-atto</b> Human IgG1κ Adalimumab biosimilar	Amjevita® (AMGEN)	s.c. injection	Same as per adalimumab	
<b>Adalimumab</b> (India) Human IgG1κ Adalimumab biosimilar	Adfrar® (Torrent Pharma)	s.c. injection	Same as per adalimumab	
<b>SB5</b> Human IgG1κ Adalimumab biosimilar	(Samsung Bioepis; Merck and Biogen)	s.c. injection	Same as per adalimumab	

Note: \*These agents can be used alone or in combination with methotrexate or other non-biologic disease-modifying anti-rheumatic drugs.

**Box 2.** Current therapeutic IL-1β-specific mAb, or IL-1-receptor antagonist, inhibitory agents.

Anti-interleukin-1β or IL-1-receptor-antagonist				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Anakinra</b> Recombinant human IL-1Rα ( <i>E. coli</i> -derived protein; non-mAb)	Kineret® (AMGEN/Biovitrum)	s.c. injection	Adult rheumatoid arthritis (moderate-to-severe, monotherapy or with DMARDS)	Lupus nephritis Inflammatory joint diseases: psoriatic arthritis, spondyloarthritis, osteoarthritis, etc. Periodic fevers Gout Asbestosis Epilepsy Stroke
<b>Rilonacept</b> Recombinant IL-1R accessory protein ( <i>E. coli</i> -derived)	Arcalyst® (Regenron Pharmaceuticals)	s.c. injection	Cryopyrin-associated periodic syndromes (CAPS), including familial cold auto-inflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS)	
<b>Canakinumab</b> Humanised anti-IL-1β IgG1κ	Ilaris™ (ACZ885) (Novartis)	s.c. injection	Cryopyrin-associated periodic syndrome (CAPS) Familial cold auto-inflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS) Systemic juvenile idiopathic arthritis (SJIA)	Rheumatoid arthritis Chronic obstructive pulmonary disease Coronary artery disease Gout Schizophrenia
<b>Gerokizumab</b> Humanised mouse anti-human IL-1β IgG2κ (Fab)	Eyeguard™ (XOMA Corp.)		No approved medical indications at present	Behçets Uveitis Non-infectious uveitis Pyoderma gangrenosum

**Box 3.** Current therapeutic IL-6 and IL-6-receptor-specific inhibitory agents.

Anti-interleukin-6 and IL-6R $\alpha$				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Tocilizumab</b> Humanised mouse anti-IL-6R IgG1 $\kappa$	Actemra® (Hoffmann–La Roche)	i.v. infusion (monthly) or more usually s.c. injection	Rheumatoid arthritis Systemic juvenile idiopathic arthritis (SJIA) Crohn's disease (moderate/severe) Castleman's disease	Neuromyelitis Optica (Devic's disease) GVHD? TRAPS?
<b>Sarilumab</b> Human anti-IL-6R IgG1 $\kappa$	VelocImmune® (Sanofi & Regeneron)	s.c. injection	Rheumatoid arthritis (with methotrexate) Plaque psoriasis (moderate/severe)	AS? (**failed trials)
<b>Sirukumab</b> Human mAb IgG1 $\kappa$	(GlaxoSmithKline)	s.c. injection	Rheumatoid arthritis (with or without methotrexate)	Giant cell arteritis (vasculitis) Non-eosinophilic asthma

**Box 4.** Current therapeutic IL-17 and IL-17-receptor-specific inhibitory agents.

Anti-interleukin-17 and IL-17R				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Brodalumab</b> Human anti-IL-17R IgG2 $\kappa$	(KHK4827, AMG827) (Valeant Pharmaceutical & Kyowa Hakko Kirin)	s.c. injection	Psoriasis (severe) Psoriatic arthritis Rheumatoid arthritis Asthma Crohn's disease (moderate/severe)	None yet known
<b>Ixekizumab</b> Humanised anti-IL-17A and anti-IL-17A/F IgG4	Taltz® (LY2439821) Eli Lilly & Co).	s.c. injection	Plaque psoriasis (moderate/severe)	None yet known
<b>Secukinumab</b> Human anti-17A IgG1 $\kappa$	Cosentyx® (Novartis Pharma AG)	s.c. injection	Plaque psoriasis (moderate/severe) Psoriatic arthritis Ankylosing spondylitis	None yet known

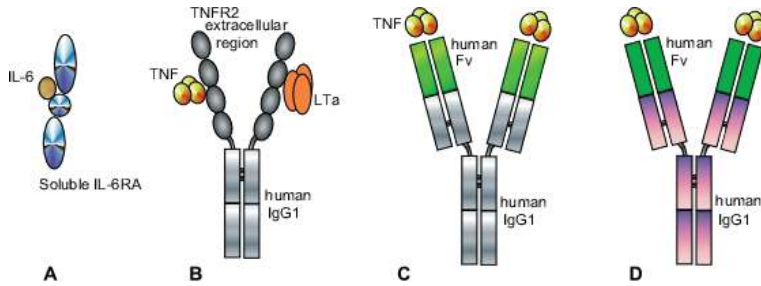
serum TNF and LT $\alpha$ , engaging with cytokine-expressing cells (i.e. membrane-bound TNF), and simultaneously also in engaging with FcR-expressing cells and henceforth of triggering FcR-mediated cell signalling (for a recent review, see [68]). An analogous TNFR1 p55-IgG1 Fc fusion protein (Lenercept) was similarly produced and tested in a double-blind placebo-controlled clinical trial for multiple sclerosis (MS). This disease choice was based on the fact that TNF is produced in MS and has demonstrable cytotoxic activity against oligodendrocytes—the cells that are destroyed by the immune system in MS—and because TNF neutralisation had been shown to be beneficial in mice with experimental autoimmune encephalitis (a murine model for MS-like disease). However, MS patients reported no benefits from the Lenercept treatment and

**Box 5.** Current therapeutic IL-12/IL-23 and common receptor-specific inhibitory agents.

Anti-interleukin-12 and interleukin-23 (IL-12 and IL-23)				
Drug name and structure	Brand name	Usual delivery	Approved disease indications	Additional potential uses
<b>Ustekinumab</b> Humanised mAb anti-IL-12/IL-23 p40 IgG1κ	Stelara® (CNTO 1275) (Centocor & Jassen-Cilag)	s.c. injection	Plaque psoriasis (moderate/severe)	RA AS CD Systemic lupus erythematosus Ankylosing spondylitis
<b>Briakinuman</b> Human mAb anti-IL-12/IL-23 p40 IgG1κ	ABT-874 (Abbott)	s.c. injection	Plaque psoriasis Psoriatic arthritis	RA, CD? MS?
<b>Tildrakizumab</b> Humanised mAb Anti-IL-23 p19 IgG1κ	(Merck; and now Sun Pharma)	s.c. injection	Plaque psoriasis (moderate/severe)	CD?
<b>Guselkumab</b> Humanised mAb Anti-IL-23 p19 IgG1κ	(Janssen Research & Development)	s.c. injection	Plaque psoriasis (moderate/severe)	CD?
<b>AMG139</b> Human mAb anti-IL-12/IL-23 p40 IgG1κ	(Amgen)		In Phase II trial for CD	
<b>BI655066</b> Human mAb anti-IL-12/IL-23 p40 IgG1	(Boehringer Ingelheim Pharmaceuticals)		In Phase II trial for psoriasis	

unfortunately many trial patients experienced an unexpected worsening of their disease [77]. Lenercept also failed clinical trials for sepsis [78]. The reasons for this failure, especially in the face of the success of etanercept, were enigmatic at the time and remain incompletely explained even today; it is not clear whether ligand-binding differences, or even minor differences in the Ig component, explain the divergence in *in vivo* behaviour and therapeutic efficacy. Onercept, a TNFR1-extracellular region without an FcR component was also created by molecular biology engineering. Onercept neutralised TNF *in vitro*, but it failed in clinical trials for psoriasis [79]. In fact, several other human TNF-inhibitory TNFR-based reagents have also been developed, such as pegsunercept (a pegylated recombinant soluble TNFR1 protein), but these were not licensed for various reasons, primarily a lack of efficacy for the disease situations in which they were tested (reviewed in Ref. [68]).

In an analogous manner, a recombinant bio-therapeutic IL-1β inhibitor comprising a purified recombinant IL-1 receptor antagonist protein, anakinra (trade name Kineret; [www.kineretrx.com](http://www.kineretrx.com)), has been developed and approved for the treatment of adult RA, usually administered as a weekly subcutaneous (s.c.) injection. Moreover, another IL-1RA (accessory) protein, rilonacept (trade name Arcalyst; [www.arcalyst.com](http://www.arcalyst.com)), is a dimeric fusion protein comprising



**Figure 4.** Examples of recombinant protein mAb-based drugs. (A) Soluble (extracellular region) cytokine receptor, (B) soluble (extracellular region) cytokine receptor–Ig Fc fusion protein, (C) humanised or fully human mAb and (D) biosimilar human or humanised mAb.

IL-1R1, and an IL-1RA linked to IgG1-Fc. It is approved for the treatment of Cryopyrin-Associated Periodic Syndromes, including Muckle-Wells syndrome in adults and in children of 12 and older. It should be noted that these IL-1 $\beta$  receptor-based inhibitors are specifically contraindicated for simultaneous use with anti-TNF agents due to a dramatically increased risk of infection (see below for a full list of contraindications).

### 3.2. Cytokine-neutralising mAbs

Infliximab (trade name Remicade; [www.remicade.com](http://www.remicade.com)) was the first anti-human cytokine mAb to be approved for therapeutic use. Infliximab binds to both soluble and membrane-bound human TNF, and this interaction prevents TNF from binding to either of its receptors TNFR1 or TNFR2. Since antibodies are high-affinity reagents, infliximab is thus a potent inhibitor of TNF's biological activities. Infliximab is administered by intravenous (i.v.) infusion, usually 5 mg/kg, every 8 weeks (see **Box 1**). Other human TNF-specific therapeutic mAbs now also exist. Adalimumab (trade name Humira; [www.humira.com](http://www.humira.com)) and golimumab (trade name Simponi; [www.simponi.com](http://www.simponi.com)) are both human and humanised anti-human TNF IgG1 mAbs. These mAbs are generally administered by s.c. injection, every 1–2 weeks (see **Box 1**). Certolizumab pegol (trade name Cimzia; [www.cimzia.com](http://www.cimzia.com)) is a pegylated human immunoglobulin Fab' fragment of an anti-TNF IgG1 mAb. It is also administered by s.c. injection, usually monthly. These agents are all approved for use in a broad array of arthritic- and psoriatic-related human inflammatory conditions (see **Box 1**). In the USA, Adalimumab has also recently been approved for hidradenitis suppurativa (apocrine acne). This is a chronic inflammatory condition that affects apocrine gland-bearing skin, such as that found in the axillae and groin, where recurrent boil-like nodules develop and fail to heal.

More recently, neutralising IL-1 $\beta$ -specific mAbs have also emerged, canakinumab (trade name Ilaris; [www.ilaris.com](http://www.ilaris.com)) and gerokizumab (trade name Eyeguard). These are approved for CAPS-type auto-inflammatory conditions, including MWS, as well as systemic JIA (see **Box 2**). Similarly, blocking mAbs specific to IL-6R, tocilizumab (trade name Actemra; [www.actemra.com](http://www.actemra.com)), sarilumab and sirukumab, have also been developed (**Box 3**). Sarilumab has recently successfully completed a phase III clinical trials in combination with methotrexate for RA, and

its approval appears to be imminent in the USA. These anti-IL-6 mAbs are being used in combination with methotrexate to slow RA and JIA progression in patients who do not benefit from anti-TNF agents, or especially when methotrexate monotherapy is less efficacious than expected. Tocilizumab is additionally approved for the B cell tumour Castleman's disease [80], and there is preliminary evidence that it might be effective against treating the refractory neuromyelitis known as Devic's disease [81].

Other recent additions to the repertoire of human cytokine-neutralising mAbs are those that inhibit IL-17 and IL-23 which are showing efficacy in the treatment of psoriasis and psoriatic-related conditions (see **Box 4**). Brodalumab, an IL-17RA-specific mAb, is one such reagent that acts by preventing IL-17-family cytokines from binding to the IL-17 receptor (**Box 4**). Recent Brodalumab data, derived from phase II and III clinical trials, have demonstrated effectiveness in the treatment of psoriasis [32], and reportedly with superior skin clearance than the anti-IL-12/IL-23 mAb ustekinumab [55, 82]. These are long-awaited treatment for a skin condition that has previously proven to be difficult to treat. However, the clinical trials with Brodalumab were unpredictable, in that trial-related adverse events apparently included suicidal ideation with trial-related harmful behaviours in some patients even suicide [83]. This unexpected outcome may translate to limitations with its use and has necessitated restrictive labelling and specific cautions in its use. On the other hand, ixekizumab (trade name Taltz; [www.taltz.com](http://www.taltz.com)), an IL-17A cytokine-neutralising mAb, is already approved for plaque psoriasis without any noted psychological symptoms or unfavourable behavioural side effects [30]. These IL-17-family cytokine-neutralising drugs represent a major breakthrough in psoriasis treatment.

Finally, the most recent addition to cytokine-neutralising mAb-based reagents are those that neutralise IL-12 and IL-23 (**Box 5**), which act, for example, by binding to the shared p40 subunit of these cytokines. Ustekinumab (trade name Stelera; [www.stelerainfo.com](http://www.stelerainfo.com)) is an IL-12- and IL-23-neutralising mAb, and as mentioned, it is now approved for the treatment of moderate-to-severe plaque psoriasis, psoriatic arthritis and moderately active CD [46]. Ustekinumab offers improved efficacy over anti-TNFs agents in CD patients, and, moreover, requires only tri-monthly administration (after an initial monthly dosing induction). Briakinuman, guselkumab and tildrakizumab also all block IL-23; briakinuman is a human IgG anti-IL-23p40 mAb, and tildrakizumab is a humanised IgG1 $\kappa$  anti-IL-23p19 mAb and both are effective and approved for psoriasis [46, 84]. Finally, guselkumab, an IgG1 $\lambda$  anti-IL-23p19 mAb, is reported to be safe in early-stage trials, and is also intended for use in psoriasis [85], where it outcompeted the anti-TNF mAb adalimumab in phase II trials [86]. As these are recently developed mAbs, their safety profiles will require ongoing monitoring, although early data suggest that they do not represent an increased risk of infection [87].

#### 4. New 'biosimilars' antibody reagents—biosimilars and interchangeables

It is now well over a decade since the first anti-cytokine mAbs have been used internally to treat human inflammatory conditions and already the next generation of reagents are emerging. These are the 'copy' reagents and they are generally known as 'biosimilar' reagents [88]. As the initial cohorts of biologics are all now nearing the end of their patent protection, many

pharmaceutical companies currently dedicate a large effort towards producing their new generation of mAbs. This is not just of benefit to the pharmaceutical companies that produce these drugs, but potentially hugely advantageous for mankind. The greater the competition in the marketplace the more downward pressure on the current high costs of cytokine-neutralising mAbs and protein biologics [89]; in other words, the development of biosimilar mAbs should ultimately translate into significant savings for the patient/consumer. The production of biosimilar reagents should therefore quickly provide access to these drugs for a much larger proportion of patients who might not otherwise be able to afford them. Already, the estimates of the monetary savings are being generated and they are in the order of over Euro 20M within the first 3 years, which equates to at least an additional estimated 1200–1800 patients [90].

A '*biosimilar*' reagent is defined by the US Federal Drug Administration (FDA) as a biological product that is approved on the basis that it has highly similar physical and functional properties to an existing FDA-approved biological product—known as the '*reference*' product. The US FDA guidelines for biosimilars and other drugs are available online (<http://www.fda.gov/>) and a review of the current guidelines for the production of biosimilar reagents has recently been published [91]. Theoretically, there are no clinically meaningful differences between a biosimilar reagent and its reference product in terms of either safety or efficacy. While this is essentially true in reality, it is important to note, however, that a biosimilar and reference product may not be entirely identical; minor differences in clinically inactive components are allowable in a biosimilar product [88]. Another term that is used in this field is that of an *interchangeable* product. This is a biosimilar that meets additional standards, that is, that it produces essentially the same clinical results as the referenced product within an identical patient cohort. This was achieved, for example, with Remsima (infliximab biosimilar), both in RA and in SA patient cohorts [92, 93]. An interchangeable biological can therefore be substituted for the reference product by a clinician or a pharmacist with essentially no discernable impact.

The establishment of the degree of similarity of a given candidate biosimilar is determined through extensive physical, chemical and functional characterisation—directly comparing the biosimilar product against the original reference product [94–96]. This includes a formal demonstration of the similarity of the primary, secondary and tertiary structure of the biosimilar, and examination of the similarity of the structural motifs that determine its mechanism of action. Firstly, the affinity of a given mAb for its cognate antigen needs to be identical, or closely similar, to that of the reference product, and analytical techniques such as surface plasmon resonance (SPR) are used to provide real-time-binding kinetic assessments (on- and off-rates) of the biosimilar and reference mAbs. Secondly, the biosimilar mAb must possess inherent properties integral on the reagent as a whole, for example, the capacity of the mAb to induce immune effector functions such as antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) [97], and the class of the mAb Ig is therefore an essential aspect that must be matched in the biosimilar; if the original reference mAb is an IgG1, then the biosimilar must also be an IgG1. Thirdly, glycosylation patterns are being increasingly recognised as critically important, as differences in sugars can interfere with an Ig's biological activity [98]. Taken together, the similarity of the biosimilar mAb is essential as it ensures identical interactions with (i) antigen, (ii) FcRs and (iii) its *in vivo* half-life. Often, more than 30 analytical methods may be required to establish a new product as a bone fide biosimilar [99].



#### 4.1. Approval processes for biosimilar mAbs

The US FDA recommends a step-wise approach for approving a biosimilar [100]. (Note: biosimilars are not generic drugs, and their development and licensing do not fall under the same regulatory pathways as generics.) The first step is the assessment of the critical quality attributes (CQAs) of the molecule, that is, those that are relevant to the clinical outcomes. Factors thought to be affecting the identity, purity and potency of a biosimilar molecule constitute its CQA. The FDA also suggests that CGQs should be classified into three tiers and there is a statistical approach for assessing CQAs, namely an equivalence testing for Tier 1, a quality-range approach for Tier 2 and descriptive testing (raw data and graphical comparison) for Tier 3. The processes are relatively similar worldwide, although there are differences in how biosimilars are assessed in different countries or regions throughout the world with respect to the need for *in vivo* toxicity testing [101]. There is also a need to provide evidence that all batches of the biosimilar will fall within the established range. This challenge occurs because recombinant mAbs are usually produced using a variety of host cell types and the newly generated recombinant biosimilar protein may be associated with production impurities including host cell proteins that co-purify with the biosimilar [102]—these are best identified by mass spectrometry-type approaches [103]. Additionally, a number of post-translational modifications, including glycosylation, oxidation, deamidation, pyroglutamation and formylation, can be introduced into a mAb during its production. Thus, the biochemical and biophysical profiles of a biosimilar molecule must closely match the reference product and any differences need to be investigated to understand the nature of the divergence between the biosimilar and the reference product and the potential effect(s) on safety, toxicity and biological function [88].

There are generally four phases of clinical research that are required for a new drug to be developed and approved for human use: A phase I study to establish an initial safe dose range and identify potential side effects, a phase II further assessing the efficacy and safety, followed by a phase III study that confirms the drugs' efficacy in comparison to a current treatment and further establishes its safety versus the severity of any detectable side effects. Sometimes, a phase IV study is additionally performed for further assessment of the drugs' efficacy in different populations and/or a better assessment the extent of side effects, for example, issues associated with long-term drug use, or its use within a different population. By contrast, the benefit of the biosimilar agent is its abbreviated assessment process. This is justified because of the existing breadth of understanding of the reference product and its mechanisms of efficacy, which have already been extensively demonstrated via the original, the reference product assessment [104]. This permits the approval process for biosimilars to be focused mostly on the analytical demonstration of similarity to the original reference product, and only two phases of clinical studies are required for a full approval of a biosimilar. First, a phase I study to demonstrate a similar pharmacokinetic and pharmacodynamic profile. This is generally followed by a pivotal phase III-type study that demonstrates similar efficacy, safety and immunogenicity—usually comparing against the reference product. The first biosimilar (Resima) for infliximab was assessed in RA and AS patients in exactly this manner [92, 93]. Importantly, it is assumed that the biosimilar product will be delivered by the same route and at the same dosage as the reference product. This process assumes that any newly produced biosimilar mAb reagent is therefore unlikely to reveal any new adverse drug responses that

have not already been documented in the original reagent. This process permits attention to be focused on testing the immunogenic potential of the biosimilar, for example, via close attention to the production processes, the mAb's physical similarity (glycosylation, etc.), and the presence (and quantity) of any co-purifying entities. Only time will determine if there are any subtle differences in the new-generation biosimilar mAbs, that is, compared to original product, and, henceforth, whether specific prescription guidelines need to be developed.

#### 4.2. New cytokine-neutralising biosimilar reagents and mAbs

Two etanercept (Enbrel®) TNFR-IgFc biosimilar reagents have been approved to date: Erelzi (*etanercept-szzs*) was approved in the US in mid-2016 and Brenzys (also known as SB4) was approved in Korea in 2015. SB4 is also now approved in Europe, Australia and Canada (See **Box 1**). Furthermore, there are several biosimilar anti-TNF mAbs that are either approved or in development (see **Box 1**). For example, CTP-13 (trade name Remsima) was the world's first registered biosimilar anti-TNF infliximab (Remicade®) mAb therapeutic, first registered in Europe and Korea in 2013. Additionally, inflectra (infliximab-dyyb) was approved in the US in early 2016 and Infimab is produced in India. Similarly, Adalimumab-atto (trade name Amjevita) and SB5 are other adalimumab (Humira®) biosimilars. For approval, Remsima was extensively evaluated in comparison to infliximab. It was found to have (i) virtually identical primary and higher-order structures, (ii) similar monomer and aggregate content, (iii) some less basic variants due to C-terminal lysine amino acid residues (but these appear to be rapidly removed in serum) and (iv) highly similar glycosylation patterns, to infliximab [105]. Nevertheless, the situation at present is that these new biosimilar drugs exist, but they are not commercially available because the original US patent for anti-human TNF mAb does not expire until late 2018. In fact, it has been estimated that there may already be as many as 20 anti-TNF biosimilar mAbs and mAb-based reagents in development, or under clinical assessment. It is expected that these drugs will be marketed for the treatment of RA, JIA, AS and psoriatic arthritis, that is, the indications as their reference drug(s) [106]. It is expected that eventually biosimilars will be produced for all of the anti-IL-1 $\beta$ -, IL-6-, IL-17- and IL-12/23-therapeutic mAbs (see **Box 1–5**).

Arguably, the most pressing issue with respect to the use of biosimilars and interchangeables is *when* and *how* to use them. Since there appears to be equivalent efficacy between these first- and second-generation drugs, then it can be assumed that either the original or the new-generation reagent can provide immediate benefit to treatment-naïve patients. Furthermore, initial studies also suggest that it is safe to switch to a biosimilar drug in anti-drug antibody-naïve patient [107]. However, a recent study has demonstrated that virtually all patients who developed anti-infliximab antibodies react to both inflectra and remsima—the infliximab biosimilar mAbs [108]. This suggests that epitopes that are present in infliximab that elicit the drug-specific antibodies are also present in the biosimilar mAbs [108]. It is also possible that new epitopes are present in the biosimilar, and, similarly, that unique drug epitopes can be present in the reference product. Data also exist showing that adalimumab-treated patient serum does not show cross-reactivity with either infliximab or its biosimilar remsima [109]. Thus, the cross-reactivity appears to be drug specific.

## 5. mAb and biosimilar Ig effector functions

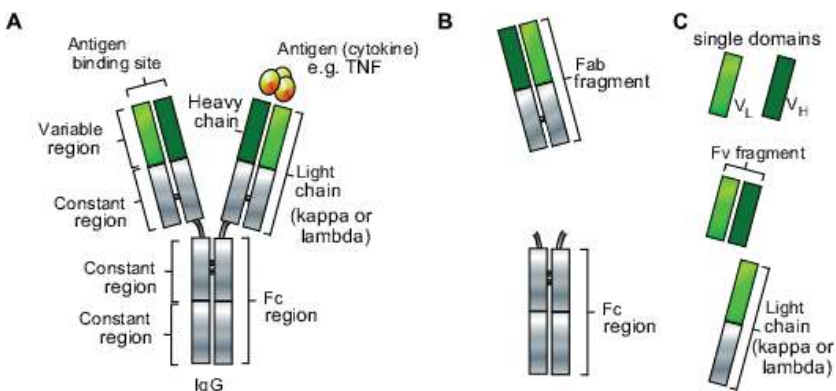
Igs are complex tetrameric molecules comprising two glycosylated heavy chains and two light-chain polypeptide molecules, bound together by disulphide bonds. The structure has different domains, termed 'constant' (C) and 'variable' (V) domains (**Figure 5**). The domains are encoded by different gene segments: C gene segments, plus a unique combination of V, plus 'diversity' (D) and 'joining' (J) gene segments conferring the antigen-binding site specificity.

### 5.1. mAb-antigen specificity and neutralisation

mAbs are highly specific reagents due to their extremely high affinity to their cognate Ag. Biochemically, the reactivity is generally nanomolar to picomolar ( $10^8$ – $10^{11}$   $K_D$ ). When antibodies bind to epitopes that block the antigen's normal Ag reactivity, that is, to their naturally occurring ligand, their on- and off-rates define them as blocking reagents. Thus, mAb reagents that are specific to cytokines or cytokine receptors can be strong inhibitors of cytokine biology *in vivo*. Therapeutic mAbs are long lasting (approximately 15 days) due primarily to the normal longevity of Ig in human plasma. Thus, the high affinity, neutralising capacity and longevity of mAbs make them ideal therapeutic reagents.

### 5.2. mAb-FcR binding

Ig molecules bind to their antigens, and also to Fc receptor (FcR) proteins that are typically expressed on many cells in the hematopoietic system, especially myeloid-lineage cells. Fc $\gamma$ R1 is a high-affinity receptor (typically  $K_A > 10^7$ M), whereas Fc $\gamma$ RIIA/B/C (CD32) and Fc $\gamma$ RIIIA/B (CD16) are low-affinity receptors (typically  $K_A < 10^7$ M) for human IgG1 [110] (See **Table 1**) and this difference means that low-affinity FcRs generally exist unbound by high-plasma circulating Ig [111]. The Ig affinity difference of FcRs also explains why Fc $\gamma$ R1 can bind to monomeric IgG, whereas Fc $\gamma$ RII and III tend to bind to IgG complexes.



**Figure 5.** Immunoglobulin (Ig) and antibody fragments. (A) Soluble intact mAb, (B) Fab and Fc fragments and (C) single (light-chain) domain antibodies (Dabs), mAb Fv antigen-binding fragment and intact whole light-chain (kappa,  $\kappa$  or lambda,  $\lambda$ ).

	Ag presentation	Ig type	Fc receptor type and function	LIR type	Refs.
1	T-independent	IgM	Polymeric IgR Fc $\alpha$ / $\mu$ R Fc $\mu$ R	?	[118] [111, 119]
2	T-dependent	IgG1	All Fc $\gamma$ Rs	LIR-1/2	[120]
3	T-independent and carbohydrate Ag's	IgG2	Fc $\gamma$ RIIA H131 (high affinity) Fc $\gamma$ RIIA R131 + V158 (low affinity)		
4	T-dependent	IgG3	All Fc $\gamma$ Rs	?	[121]
5	Chronic Ag and allergic responses	IgG4	*Fc $\gamma$ RI (CD64)—high affinity Fc $\gamma$ RIIA (CD32)—low affinity Fc $\gamma$ RIIB Fc $\gamma$ RIIC *Fc $\gamma$ RIIIA V158 (CD16)	LIR-1/2	
6		IgG (all isotypes)	Fc $\gamma$ RIIB (CD16) low affinity inhibitory receptor; GPI-linked	?	
7		IgA	Fc $\alpha$ R1 (inhibitory and activating) Fc $\alpha$ / $\mu$ R	?	[121–124]

Notes: (1) High affinity Ig receptor (\*) [120].

(2) Fc $\gamma$ RIIA and Fc $\gamma$ RIIC are single-domain-activating receptors [120].

(3) Fc $\gamma$ RIIB is a single-chain inhibitory receptor [120].

(4) Other human variants:

Fc $\gamma$ RIIA: two alleles H131 (low responder) and R131 (high responder).

Fc $\gamma$ RIIIA: two variants—V158 and F155.

Fc $\gamma$ RIIB: two variants at four positions—R36, N65, D82 and V106; S36, S65, N82 and I106.

Plus point mutant A78D (SH) [120].

**Table 1.** Human immunoglobulin interactions with FcR and LIRs.

FcR binding to Igs can be activating to the cells that express them (e.g. typically Fc $\gamma$ RIIA or Fc $\gamma$ RIIIA) or, alternatively, Ig binding of FcRs can trigger inhibitory signals (e.g. Fc $\gamma$ RIIB and Fc $\gamma$ RIIIB). This is due to FcR-activating receptors containing intracellular immunoreceptor tyrosine-based activation motifs (ITAM) defined as YXXL/I(X6-8)YXXL/I amino acids, while inhibitory FcRs contain cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) defined as (S/I/V/LxYxxI/V/L). The capacity to trigger activation or inhibitory FcR signalling also explains why circulating monomeric IgGs are generally not as stimulatory to immune cells as compared to Igs when they exist as immune complexes. Although therapeutic mAbs were initially designed to bind and neutralise cytokines, it is clear that these mAbs also bind to FcRs, and that this property is required for ADCC or CDC. However, FcRs are themselves associated with and regulated by additional proteins such as the immunoglobulin-like receptor (LIRs) [112]. LIRs fall into two basic categories: those that contain ITAMs (defined above), for example, LIR-6 and LIR-7, and those that contain inhibitory ITIMs, for example, LIR-1, -2, -3, -5 and -8. Some LIRs also contain asparagine (NxYxxL/V) or a serine residue (SxYxxL/V) [113, 114]. LIR-1, LIR-6 (a and b) and LIR-7 associate with the  $\gamma$ -chain of FcRs for human IgG, IgA and IgE (see **Table 1**) [112, 115]. The co-association of these molecules results in the LIR's intracellular region being physically close to the FcR, and

this permits the LIRs ITIM to dampen the FcR-signalling capacity [112]. Thus, interactions between Igs with an FcR are influenced by FcR-adjacent LIRs.

Since the LIRs themselves have not been extensively studied, the potential function(s) of LIRs with respect to mAb therapeutics is only now emerging. Nevertheless, the interactions of mAbs with FcRs, as well as the FcR-associated LIR molecules, are becoming increasingly appreciated as vitally important in understanding and predicting mAb effector function [116]. It is therefore equally important to consider the expression of both FcRs and LIRs in various disease settings. It is known, for example, that LIRs are expressed in the synovium of RA or AO patients [117]; however, how they modulate autoantibody-dominated diseases is only now emerging.

## 6. Adverse events related to cytokine-neutralising and biosimilar mAbs

### 6.1. Antigenicity of anti-cytokine mAbs and development of drug-immune complexes

mAb-based therapeutics and related agents represent some of the most biologically complex drugs currently available. The most common bio-manufacturing process involves the production of cell culture-expressed Ig proteins, most frequently a Chinese hamster ovary cell lines engineered to express the human, humanised or chimeric Ig-type mAbs. Unlike classic small-molecule drugs, these intact Ig-type drugs are large multicomponent proteins that are essentially similar to natural molecules: mAb, or unique molecules generated by recombinant technology, for example, fusion proteins comprising two (or more) naturally encoded proteins such as cytokine receptor proteins with or without an Ig Fc. Nevertheless, these mAb-based agents and their biosimilar counterparts can vary in numerous ways from the naturally existing component (see Section 4.1). This includes alterations in post-translational modifications of proteins as well as contamination by host cell proteins [103]. This, in part, explains why factors intrinsic to the drug production can contribute to the immunogenicity of the drug, even though mAbs (and biosimilar mAbs) are highly similar to human endogenously produced Ig proteins.

The formation of therapeutic mAb-type drug reagent-immune complexes can be significant to the patient for a variety of reasons. Drug-immune complexes can alter the activation threshold for FcRs; note that high-affinity Fc $\gamma$ RII and Fc $\gamma$ RIII preferentially bind to immune-complexed Ig [120] and the activation threshold for FcR signalling is lowered when engaging with higher-ordered complexes—meaning that smaller or mid-sized immune-complexed mAb drugs can have extended *in vivo* half-lives and engage with what would otherwise normally be low-affinity FcRs. This explains, at least in theory, the potential for mAb-based reagents to sometimes induce inflammatory reactions despite the fact that they are otherwise virtually identical to naturally produced endogenous Igs. (There is decreasing identity to endogenous host Ig for whole mAb, then Mab fragments, recombinant soluble receptor proteins, and finally receptor-IgG Fc proteins.) Moreover, tissue deposition of immune-complexed mAbs can lead to vascular thrombosis, neutrophil recruitment or tissue monocyte/macrophage cell activation resulting in the release of inflammatory and chemotactic molecules, cytokines and chemokines—in this case exactly the opposite to what the

anti-cytokine or cytokine-receptor mAb is designed to achieve. Ultimately, the immune-complexed-mAb drug can eventually elicit the anti-drug antibodies (ADAs) and cross-link B cell receptors, amplifying immune activation.

The production of anti-drug antibodies has long been debated as being either harmful or irrelevant. For example, the presence of anti-drug Igs might decrease the half-life of the mAb drug (when bound to the mAb drug), or the anti-drug Ig could bind to an epitope located within the Fv region of the mAb such that it naturally competes with its antigen specificity of the mAb drug, thereby rendering the drug incapable of neutralising (blocking) antigen binding. It is generally considered that there are two types of antibodies-drug mAb reactions: (i) mAb interactions with natural antibodies (usually IgM isotypes) and (ii) mAb interactions with matured, isotype class-switched IgG effector Igs. Natural antibodies exist in most individuals and are usually low-affinity IgM antibodies with broad specificity, secreted by CD5<sup>+</sup> B1 lymphocytes. Because they are IgM, they have an innate propensity to form immune complexes. On the other hand, immune-complexed mAb drugs can be taken up by antigen-processing cells, such as marginal zone macrophages, and presented to naïve B cells, eventually resulting in the production of high-affinity IgG. This type of 'mature' anti-drug Ig can ultimately involve the activation of T cells and thus also to drug-based T cell-mediated inflammation. In clinical practice, there is little evidence of Ig-based adverse drug reactions to therapeutic mAbs, thus the anti-mAb-based drug Abs, even when present, are often not pathological per se—although they may block the mAbs capacity to bind and neutralise cytokines thus rendering the mAb drug ineffective.

The evidence of mAb immune complexes, and B- and T-cell reactivity, is arguably best considered with respect to the anti-TNF-neutralising antibodies, as these agents have now been used for well over a decade and in various disease populations. Thus with time it has become clear that mAb-specific Igs are (i) not infrequent (they occur in as many as 14% of patients taking anti-TNF mAb-type drugs) [125], (ii) capable of immune clearance of the mAb drug, (iii) can alter the pharmacokinetic profile of the mAb (e.g. drug half-life), (iv) capable of inducing immune cross-recognition to the endogenously arising protein—particularly, a cytokine-receptor component of the drug and (v) capable of inducing an array of adverse events spanning less significant infusion-type reactions to severe hypersensitivity reactions. It is evident, therefore, that any patient with a history of prior sensitisation to mAb-type reagents should carefully consider the safety of using another mAb-type drug.

## 6.2. Adverse events related to cytokine neutralisation

The vast majority of conditions requiring cytokine blockade by neutralising mAbs are chronic conditions. This raises the important issue of what happens when the normal function of the cytokine is being blocked *in vivo*. Indeed, most of the cytokines highlighted here are central to inflammation that is beneficial to the host, especially that which is central to an efficient antiviral and/or antibacterial immune response, such as IL-1 $\beta$ , IL-6 and TNF—all of which are produced during the normal response to infection. This is because IL-1 $\beta$  helps initiate immune responses during infected-related inflammation (since RIG-I activates NF- $\kappa$ B and the inflammasome, and thus contributes to the aetiopathology of viral arthritis

[126, 127]), IL-6 and TNF are produced by activated macrophages *in vivo* [128], and TNF and IFN $\gamma$  are produced at virtually all stages of infection where they have potent antiviral effector functions [129]. IL-6 also aids Ig development, especially IgA at mucosal sites [130]. Neutralising these cytokines therefore necessarily significantly compromises the host's natural ability to effectively combat infections. This explains why anti-TNF therapy recipients are at serious risk of more severe acute virus infections [131], and reactivation of chronic viral or bacterial infection, especially tuberculosis [132]. This explains why there are numerous reports of reactivation of chronic virus infections such as varicella zoster virus ('shingles') in patients using anti-TNF mAbs [133]. It also explains why all therapeutic mAbs that neutralise IL-1 $\beta$ , IL-6 and TNF are naturally contraindicated for the use during times of active acute infection (**Boxes 6, 7 & 8**).

There is also evidence, albeit less convincing, that long-term use of anti-TNF therapeutics might be associated with an increased risk of certain cancers, especially lymphomas [134]. However, many of the chronic inflammatory conditions that triggered the use of anti-cytokine mAbs occur in older patients, and these are people who might also naturally be at risk of certain cancers. Thus, without this type of clinical trial data health professionals

**Box 6.** Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-tumour necrosis factor (TNF)		
Drug name and reagent	Known adverse event	Specific contraindication
<b>Etanercept</b> (Human TNFR2:IgG1-Fc) <b>Infliximab</b> (Humanised mouse IgG1 $\kappa$ ) <b>Adalimumab</b> (Human IgG1 $\kappa$ ) <b>Golimumab</b> (Human IgG1 $\kappa$ ) <b>Certolizumab Pegol</b> (Pegylated-Fab' IgG1 $\kappa$ )	<ul style="list-style-type: none"> <li>• <b>Common side effects and cautions:</b> Injection-site reactions (redness) Upper respiratory infections (sinus) Headache.</li> <li>• <b>Serious side effects:</b> Infection (new) infections, especially Tuberculosis, histoplasmosis, influenza and other viral infections, e.g. chickenpox and Hepatitis B (reactivation) Nervous system demyelination Blood pressure Heart failure Psoriasis Lupus-like syndrome Lymphoma and other cancers Autoimmune hepatitis</li> </ul>	<ul style="list-style-type: none"> <li>• Existing (chronic) infections, especially Tuberculosis, HIV, Hepatitis B but also varicella (chickenpox) and influenza or other respiratory infections</li> <li>• Vaccination with live microorganisms</li> <li>• Co-use of certain other immunosuppressant agents, e.g. anti-IL1<math>\beta</math> agents, e.g. anakinra (Kineret®), anti-CLTA4 mAbs, e.g. abatacept (Orencia®), or Cyclophosphamide</li> <li>• Multiple sclerosis</li> <li>• Guillain-Barré syndrome</li> <li>• Pregnancy</li> <li>• Confirmed drug hypersensitivity</li> </ul>
<b>BIOSIMILARS:</b> <b>CTP-13</b> (humanised mouse IgG1 $\kappa$ ) <b>Adalimumab biosimilar</b> (human IgG1 $\kappa$ ) <b>Infimab</b> (human IgG1 $\kappa$ )	Expected to be similar to those listed above	Expected to be similar to those listed above

**Box 7.** Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-1β or IL-1-receptor-α		
Drug name and reagent type	Known adverse event	Specific contraindication
<b>Anakinra</b> (Recombinant IL-1R)	<ul style="list-style-type: none"> <li>• <b>Common side effects and cautions:</b> Injection-site reactions (redness) Upper respiratory infections (sinus) Headache Latex allergy (needle cover contains latex)</li> <li>• <b>Serious side effects:</b> Infection (new) infections Vertigo Nasopharyngitis/respiratory tract infection, especially Tuberculosis</li> </ul>	<ul style="list-style-type: none"> <li>• Existing infections, especially Tuberculosis, HIV, Hepatitis B but also varicella, influenza or other respiratory infections</li> <li>• Vaccination with live microorganisms</li> <li>• Co-use of TNF- inhibitory agents: e.g. anakinra (Kineret®)</li> <li>• Pregnancy and breastfeeding</li> <li>• Confirmed drug hypersensitivity</li> </ul>
<b>Rilonacept</b> (Recombinant IL-1R)		
<b>Canakinumab</b> (Humanised mouse IgG1κ)		
<b>Gerokizumab</b> (Humanised mouse IgG2κ)		

**Box 8.** Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-6 or IL-6-receptor		
Drug name and reagent type	Drug name and reagent type	Drug name and reagent type
<b>Tocilizumab</b> (Human IgG1κ)	<ul style="list-style-type: none"> <li>• <b>Common side effects and cautions:</b> Injection-site reactions (redness) Upper respiratory infections (sinus perforations of stomach or intestines/prior diverticulitis, especially if taking other NSAID, corticosteroids or methotrexate Changes in blood tests (platelet and neutrophil count, LFTs, increased cholesterol)</li> <li>• <b>Serious side effects:</b> Infection (new) infections Nasopharyngitis/respiratory tract infection especially Tuberculosis</li> </ul>	<ul style="list-style-type: none"> <li>• Existing infections, especially Tuberculosis, HIV, Hepatitis B but also varicella and influenza or other respiratory infections</li> <li>• Vaccination with live microorganisms</li> <li>• Co-use of TNF- inhibitory agents, for example: Etanercept (Enbrel®), Adalimumab (Humira®), Infliximab (Remicade®), Golimumab (Simponi®) or Certolizumab (Cimzia®)</li> <li>• Co-use of B cell suppressive agents, e.g. rituximab (Rituxan®)</li> <li>• Co-use of T cell suppressive agents, e.g. anti-CTLA4 abatacept (Orencia®)</li> <li>• Pregnancy and breastfeeding</li> <li>• Confirmed hypersensitivity</li> </ul>
<b>Sarilumab</b> (Human IgG1κ)		
<b>Sirukumab</b> (Human IgG1κ)		

and epidemiologists only have access to patient data that are predominantly anecdotal in nature. Arguably, the lack of overwhelming evidence of increased tumour incidence in patients using anti-TNF mAbs-type drugs is consistent with the fact that clinical trials with

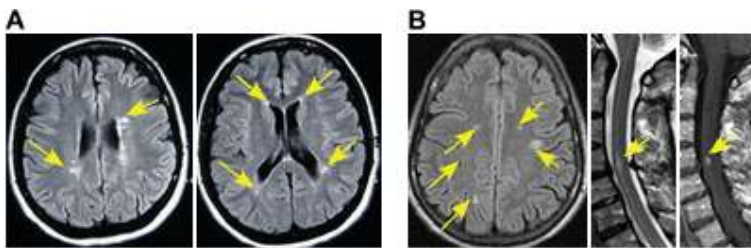


TNF as an anticancer agent induced systemic inflammation rather than controlling the tumour [135]. Yet, this is countered by clear *in vivo* evidence that TNF is tumouricidal [136]. It would seem wise, therefore, for patients to remain vigilant to the potential risks where practicable.

### 6.3. Unexpected anti-cytokine mAb adverse events—negative neurological events

Evidence comprising over a decade of use of anti-TNF-blocking reagents (TNFR-IgFc fusion proteins and anti-TNF mAbs) has substantiated that in some patients there is the unpredictable adverse event of developing demyelinating lesions in brain white matter (**Figure 6**). The spectrum of clinical presentation of demyelinating events includes optic neuritis, MS-like symptoms of paralysis, demyelinating neuropathies, or Guillain-Barre syndrome (for a recent review, see [137]). The incidence of these conditions in the general populations is normally quite low, but it is accepted that some patients develop these conditions within a few months of starting anti-TNF therapies [138, 139]. In fact, MS as an existing condition is strongly contraindicated for the use of anti-TNF therapeutics, and, as expected, cessation of anti-TNF drugs is mandated if demyelinating symptoms occur [140]. Alternative MS treatments such as glatiramer acetate (an undefined mixture of decoy CNS substrates) or interferon- $\beta$  are recommended in these patients. For the most part, demyelination events are transitory, however, in a small subset of patients the neurological symptoms persist.

Another unexpected concern is that with the use of the IL-17-inhibiting reagent, soluble IL-17RA (Brodalumab), there have been unexpected reports of an increased incidence of depression and suicidal ideation-type behaviours in some trial patients (<https://www.aad.org/eposters/Submissions/getFile.aspx?id=1146&type=sub>) (**Box 9**). These unfortunate adverse events resulted in a decision by Amgen and AstraZeneca to offload the drug to another pharma company, the Canadian-based multinational Valeant Pharmaceuticals and Kyowa Hakko Kirin Company in Japan [83]. Nevertheless, the lack of any negative psychological symptoms when using Ixekizumab (an IL-17A-neutralising mAb) indicates that IL-17A itself



**Figure 6.** Two patients showing MRIs of demyelinating CNS lesions associated with anti-TNF agents. (A) A 46-year-old Caucasian female taking etanercept for 4 years for psoriatic arthritis developed multiple periventricular and subcortical lesions (arrows), and (B) a 57-year-old Caucasian female with AS treated with etanercept for 6 years developed multiple periventricular and subcortical frontal, parietal and temporal lobe lesions and level a C4–C5 cervical spine lesion (arrow). (Images adapted from [138] in compliance with copyright).

**Box 9.** Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-17 and IL-17R $\alpha$		
Drug name and structure	Known adverse events	Specific contraindications
<b>Brodalumab**</b> (Human IgG2 $\kappa$ )	<b>Common side effects and cautions:</b> Injection-site reactions (redness)	<ul style="list-style-type: none"> <li>• Patients suffering from psoriasis are sometimes afflicted with co-morbidities including psychiatric conditions (depression, anxiety, suicidality**). Patients with these conditions are not excluded; however, depression (PHQ-8) and suicidality (eC-SSRS) test monitoring are recommended</li> <li>• Drug hypersensitivity</li> <li>• Infection</li> </ul>
<b>Ixekizumab</b> (Human IgG4)	Upper respiratory infections and/or nasopharyngitis	
<b>Secukinumab</b> (Human IgG1 $\kappa$ )	Headache Arthralgia <b>Serious side effects:</b> Major cardiovascular events (including myocardial infarction) Cholelithiasis Suicidal ideation and behaviour**	

is not the culprit per se. Thus, other IL-17-related cytokines, or other IL-17R-binding partners (but not IL-17A), may be necessarily required for the development of negative emotions, especially those related to depression and suicide. This unexpected trial outcome, although highly unfortunate, may have simultaneously inadvertently illustrated a previously unappreciated role for the IL-17/IL-17R axis in depression and suicidality. Although the mechanism is currently unknown, it has been reported that inflammatory cytokines IL-1 $\beta$  and IL-6 are elevated in blood of suicide victims [141], and recombinant interferon- $\alpha$  therapy has been associated with depression in chronic hepatitis patients [142, 143]. There is growing evidence that cytokines such as IFN- $\alpha$  drive neuroinflammation via triggering the tryptophan pathway [144], and high levels of the downstream tryptophan metabolite, quinolinic acid, has been linked to microglia expression in suicide victims [145, 146]. Furthermore, one might hypothesise that blocking IL-17, but not IFNs, might still leave type-I IFN levels high, and promoting depression and suicidality by mechanisms described above. However, another possibility could be that IL-17R agonistic mAbs, or IL-17 small-molecule agonists, might have value in potentially preventing depression, suicide and other negative emotions. It is currently unknown, for example, whether the brodalumab-IL-17RA interactions completely block all IL-17-related cytokines, prevent IL-17RA from interactions with one or more of its potential hetero-complexed IL-17 receptors, for example, IL-17 receptor RB, IL-17RC or IL-17R. Nevertheless, it is clear that the clinical use of brodalumab must likely only occur with a clear 'suicide-risk' warning for those who choose to use it to ameliorate inflammatory conditions such as psoriasis. Additionally, it remains a plausible possibility that altering the IL-17R mAb epitope may generate a non-'suicide-risk' next-generation reagent, that retains its anti-inflammatory properties. Even more intriguing, the current FDA submissions claim that the latest clinical data do not replicate the initial finding of an increased risk of suicidal ideation. Further investigation will be needed to determine the broader and

usual spectrum of adverse events of brodalumab. No adverse events are known yet for IL-12/IL-23 neutralising mAbs.

## 7. Expanding treatment indications for existing cytokine-neutralising and biosimilar mAbs—current realities and exciting futures

mAb-type drug development procedures in the US and Europe typically involve small-scale clinical trials demonstrating safety followed by trials showing efficacy relative to a specific disease(s) indication. These so-called landing indications are often followed by fast-tracked priority review. The expanded use may include a different disease indication or a different use of the mAb, such as the delivery of a radio-isotope conjugated to the mAb drug, such as was the case for the anti-CD20 mAb rituximab. The fast track and priority review is justified primarily because of the availability of existing safety and toxicity data.

With existing safety data in place, there is the ability to file for expanded use of mAb-based drugs. This is particularly the case for cytokine- and cytokine-receptor-specific mAbs, as the target cytokine/cytokine receptor may be elevated and involved in additional pathologies, apart from the disease indication directly assessed in the original clinical trials. (In some cases, a mAb drug has even failed in the original trial, but has been successful in subsequent trials, e.g., the TNF-neutralising mAb infliximab failed in clinical trials of sepsis, but is successful when used in RA and Crohn's disease patients, etc.; see **Box 1**). Most often, the expanded use label is related to diseases or conditions that are similar in terms of aetiopathology. For example, anti-TNF mAb-based reagents Enbrel, infliximab and adalimumab are recommended for a spectrum of arthritis and tissue-related inflammatory diseases: RA, psoriatic arthritis, plaque psoriasis, AS, JIA, CD and UC.

### 7.1. Anti-TNF mAb-based reagents in neuroinflammation and cognition

Etanercept (a TNFR2-Ig Fc) has additionally been used in off-label situations, most notably, in the treatment of cognitive decline after brain injury or Alzheimer's disease, and also in stroke. These uses are consistent with evidence that activated microglia produce TNF and with the idea that TNF is important in modulating neuronal synaptic function and neuropathic pain. In fact, there is an extensive literature base demonstrating important roles for TNF in the development and homeostasis of neurological systems [147]. One unifying hypothesis is that TNF causes glutamate excitotoxicity in neurones in a number of neurodegenerative diseases, and it is sobering to consider that cerebral TNF is elevated in degenerative CNS conditions, traumatic brain injury and even situations of post-operative delirium with cognitive decline [148]. So too, the levels of neuronal and microglial glutamate are important in these diseases, but it is also known that either TNF or IL-1 $\beta$  induces high level of neuronal glutamate and neurotoxicity [149]. Despite the growing body of evidence implicating TNF in neuroinflammation, there is still debate about the effectiveness and strategy of neutralising TNF in neurodegenerative

disorders. One of the reasons for this likely surrounds the difficulties in delivering the TNF-neutralising mAb-based reagents to the brain, although it appears that this can be successfully achieved by peri-spinal administration [150]. Moreover, the recent discovery of the brain lymphatics [151] provides an avenue for drug removal away from brain tissue.

Another off-label use of mAbs that neutralise cytokines in inflammation is stroke and traumatic brain injury. The main focus of treatment in stroke is thrombolytic therapy with an emphasis to reduce stroke size and reverse localised ischaemia. Nevertheless, there is evidence that the stroke penumbra region evokes or experiences an inflammatory response that comprises microglial TNF production and subsequent neurotoxicity. Peri-spinal-delivered etanercept appears to ameliorate this inflammation, even years after the neurological injury [152, 153]. Moreover, even a single injection of etanercept has been reported to alleviate symptoms of aphasia, speech apraxia, a hemiparesis in a patient with non-recent traumatic acute brain injury [154]. In animal models, traumatic brain injury induces both microglial and astrocytic activation with increasing production of TNF that can be neutralised by etanercept [155]. In humans, there is also strong evidence of elevated pro-inflammatory cytokine  $\text{IFN}\gamma$ , TNF and  $\text{IL-1}\beta$  and IL-6 which is associated with poorer cognitive outcomes [156]. This is an area of increasing investigation and current models suggest a key role for reactive oxygen species, matrix metalloproteases, angiogenic factor, inflammatory cytokine and leukocyte adhesions such that in early stages neuroprotection may be mediated by neurotrophic factors such as brain-derived neurotrophic factor, nerve growth factor and vascular endothelial growth factor, plus cytokines  $\text{TGF}\beta$ , IL-1Ra, IL-4 and IL-10, among others, with a switch to neurodegenerative changes in chronic inflammation involving cytokines TNF,  $\text{IL-1}\beta$  and IL-6 [157]. Hence, brain microglia are essential for both neurorestoration and neurorecovery, but prolonged activation is more likely to be disadvantageous, that is, to have pathological sequelae [158]. With the apparent efficacy of etanercept treatment to neutralise TNF, even years after the initial insult or injury, it remains plausible that the administration of IL-1Ra might also be beneficial in early stages, that is, to block inflammation by  $\text{IL-1}\beta$ , with subsequent administration of mAb-based neutralisation of TNF,  $\text{IL-1}\beta$  and IL-6 in later stages. This is consistent with documented TNF immune reactivity in brain tissues from early times, extending to 18 days or more after ischaemic stroke in humans [159]. A greater understanding of the processes that regulate microglial activation and function will critically inform the potential standardised use of anti-cytokine treatments to neutralise inflammation-mediated tissue injury after TBI and stroke.

One of the most intriguing uses of anti-TNF mAbs has been in the treatment of cognitive impairment, a concept already introduced above. In infectious situations, prolonged activation of the transcription factor  $\text{NF-}\kappa\text{B}$  and the sustained expression of TNF have been linked to AIDS-related dementia complex [160]. In particular, the regional location of TNF-producing cells correlated with HIV gp41-reactive cells, and correlated with increasing cognitive impairment and dementia [161]. In animal models, increased TNF is associated with cognitive decline that is linked to non-enzymatic glycation of proteins, for example, modification by D-glucose [162]. Similarly, exposure to certain anaesthetics is associated with the potential for post-surgery delirium and with later cognitive dysfunction [163], and this is especially apparent in the elderly [164]. Surgery-associated cognitive dysfunction has suggested to be linked to the production of pro-inflammatory cytokines [165], the activation of caspases, and to the increased synthesis and accumulation of  $\beta$ -amyloid ( $\text{A}\beta$ ) protein, and thus to the induction of

hyperphosphorylation of tau [166, 167], although contradictory studies also exist [168]. Recent studies further suggest that TNF and IL-6 are components of the pro-inflammatory response [169]. Furthermore, another recent study has even suggested that high IL-6 prior to surgery is a risk factor for post-operative delirium onset in the elderly [170]. Therefore, there is a potential use for TNF- and/or IL-6-neutralising mAbs in these conditions, although they are not currently a component of the standard treatment. At present, one can only surmise that these drugs might be beneficial to elderly patients, especially long term, particularly because of the possibility that post-operative delirium is associated with subsequent cognitive impairment [171] or indeed, potentially even, as a possible trigger for subsequent neurodegenerative pathologies.

## **7.2. IL-17- and IL-17R-related mAbs and negative emotions: anxiety and suicidal ideation**

A recent and unexpected complication of IL-17 cytokine blockade via IL-17R-specific mAbs was a report of self-harm ideation and suicidality, as noted above (**Box 9**). This appears specific to IL-17R blockade, rather than IL-17A neutralisation alone, although a recent re-evaluation of the phase II and III trial data, literature and expert opinion has refuted these findings [172], and others interpret the data to imply accidental findings, rather than being suggestive of a direct suicidal causation [173]. Nevertheless, further investigation will clearly be required, and close monitoring of its use, in a broader population, will be required to confirm a role for IL-17-related cytokines, or other IL-17R-interacting molecules, in the propagation of negative emotions, especially depression and anxiety. In this regard, it is nevertheless intriguing that anxiety has previously been negatively correlated with serum levels of TGF- $\beta$ 1 and IL-17 [174], whereas others have reported increased TNF and IL-17 in individuals with generalised anxiety disorder [175]. Moreover, increased levels of dopamine-induced glucocorticoid-resistant Th-17 cells are reported in multiple sclerosis—a condition where depression is a frequent co-morbidity [176]. Although these intriguing observations clearly warrant further investigation, it remains possible, although quite controversial, that this represents a new opportunity: to target IL-17R in individuals experiencing suicidal ideation.

## **8. Inflammatory conditions still requiring new treatments**

### **8.1. Other inflammatory diseases amenable to mAb cytokine blockade: anti-IL-4, IL-5 and IL-13 in asthma, allergy and atopic dermatitis**

Asthma is a chronic disease of airways where pre-exposure and complement result in cytokine- and allergen-triggered inflammation that is characterised by the dysregulation of IL-4, IL-5 and/or IL-13. Mepolizumab (Nucala) and reslizumab (Cinquinair) are IL-5-specific-neutralising mAbs that have recently been demonstrated to be capable of preventing and controlling moderate to severe asthma [177, 178]. Since eosinophilia is a feature of this condition, mepolizumab is also indicated for other hyper-eosinophilic conditions, such as eosinophilic airway inflammation, allergic rhinitis, atopic dermatitis, and eosinophilic oesophagitis [179]. Similarly, benralizumab is an IL-5R $\alpha$ -neutralising mAb currently in development [180]. However, it is clear that asthma is more accurately defined as a heterogeneous syndrome, which explains why many patients do not respond well to older, more conventional asthma therapies. Apart from targeting IL-5, mAbs that target and neutralise IL-13 (e.g. tralokinumab,

produced by LEO Pharma) are also emerging as effective reagents in clinical trials for atopic dermatitis, and are additionally being considered for conventionally unresponsive asthma patients. Lebrikizumab neutralises IL-4 and IL-13 and prevents airway inflammation, mucous secretion and airway remodelling that occurs in chronic asthma [181, 182]. As with the other inflammatory conditions discussed in this chapter, the challenge for clinicians is to determine which of these recently developed anti-IL-4, -IL-5 and -IL-13 cytokine and IL-5R $\alpha$  cytokine-receptor-neutralising reagents are optimal for a given disease condition. Comorbidities may be highly informative in this regard, and already it has been suggested that the lebrikizumab is most effective in patients with serum periostin, a potential predictor of airway eosinophilia [183, 184] and a correlate for IL-13 bioactivity *in vivo* [185]. Moreover, recent studies indicate that in the context of asthma, allergy and atopic dermatitis, Th-2 cytokines producing ILC2 cells play an important role in modulating IL-3, IL-5 and IL-13 functions at the lung mucosa or skin [186, 187]. ILC2 subsets may vary considerably accordingly to the anatomical location. For example, lung-resident IL-33R+ ILC2s produce IL-5 and IL-13, whereas skin ILC2s express thymic stromal lymphopoietin (TSLP) and IL-4 [188]. Indeed, vaccine adjuvants such as IL-13Ra2 or IL-4R antagonist can significantly alter ILC2 function at vaccination sites, acting within the first 24 h after administration [189, 190]. Thus, designing drugs that target the different ILC2 subsets at the lung mucosa or skin has high potential to provide the next-generation therapeutics for asthma, allergy and atopic dermatitis. So, too, the therapeutic value of the current Th-2 cytokine-neutralising antibodies will become clearer with time, and a current challenge is the paucity of treatments available for asthma patients who present with little or no evidence of Th-2 cytokine-based inflammation.

## 8.2. Remaining challenges including neurological inflammation

Still, there are several conditions or situations where treatments remain suboptimal, or difficult, and where treatment failure is inexplicably common. For example, despite advances in the current understanding of SJIA, up to 50% of cases experience a chronic disease and many patients appear to be refractory to existing treatments—including cytokine-specific mAbs [6]. This reality may again highlight the possibility that there exists a spectrum of aetiologies, some of which are not sufficiently affected by existing treatments. Alternatively, it is possible that the mechanisms that regulate checkpoints and exert inhibition of the immune system require additional specific enhancement. Other classic autoimmune diseases such as Scleroderma, although uncommon, involves systemic immune attack of tissues, including vascular endothelium, that remains extremely challenging to treat and can even require in-limb amputation by end-stages, in extreme cases. Even today, there remains no durable effective treatment for scleroderma. Other rare immune-destructive conditions such as myasthenia gravis, involving autoantibody blockade of neuromuscular junctions, urgently require better treatments rather than global B-cell immune suppression.

Remaining high on the list of current clinical challenges in neuroinflammatory conditions is multiple sclerosis, especially the chronic progressive forms of multiple sclerosis where patients experience progressive worsening with each disease flare. Also, there are other neurodegenerative conditions, such as amyotrophic lateral sclerosis (also known as Lou Gehrig's disease, or motor neurone disease) that present with elements of neuroinflammation, even if inflammation

is not necessarily the primary driver of neuronal loss. The recent intriguing success of anti-TNF therapies in stroke and brain injury [152–154] highlighted above suggests that the cytokine/cytokine-receptor blockage in the brain is possible. Innovation for easier brain-specific delivery methods, and a considerably deeper knowledge of immune cells with their extensive tissue- and cell-specific interactions in the brain, in both normal and disease settings, should accelerate the development of new treatment options and address this opportunity and serious clinical need.

Food intolerances and food-related atopy also remain a clinical challenge. Ranging from peanut allergies to raw meat intolerance that arises after tick-bite, the current treatments remain global in nature and need to better embrace the microbiome, including dysbiosis exerted by viruses (especially bacteriophages) rather than just the diversity in bacteria communities. Hence, there remains a critical need for more information, that is, a more detailed mechanistic understanding of these immunological diseases and food allergies. Despite the successes of mAb-based biotherapeutics for human inflammatory diseases, a challenge for the global pharmaceutical companies who have benefited from these biotechnology successes is thus to direct more funding into these research areas.

## 9. Summary

Nearly 20 years have passed since the first cytokine-specific biological reagent, Enbrel (etanercept), was FDA approved in 1997, and, as reviewed here, there are already now more than 20 cytokine- or cytokine-specific mAbs and recombinant soluble cytokine receptor proteins in clinical use, or on the verge of approval, for inflammatory diseases. Thus, the treatment of human inflammatory diseases has experienced a watershed era. Arguably, three challenges now remain. The first is to address the less common but nevertheless devastating conditions for which there are no cures or effective treatments—irrespective of the number of people affected by them. The second is to determine how to better stratify the existing treatments for optimum use in selected subconditions. Thirdly, the overwhelming concern is that these treatment breakthroughs remain out of reach for millions of people worldwide; there are still, undoubtedly, millions of patients who cannot afford them. With the era of biosimilars upon us, there is an opportunity to provide cheaper mAb-based therapeutics to affected people. Yes, the opportunity is there, but whether it will change the unsustainable appetite for large financial gains and reduce costs in developed countries remains to be seen.

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The authors declare that they have no conflicts of interest.

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## References

- [1] Upton C, Macen JL, Schreiber M, McFadden G. Myxoma virus expresses a secreted protein with homology to the tumor necrosis factor receptor gene family that contributes to viral virulence. *Virology*. 1991;184(1):370–82.
- [2] Upton C, Mossman K, McFadden G. Encoding of a homolog of the IFN-gamma receptor by myxoma virus. *Science*. 1992;258 (5086):1369–72.
- [3] Thomas R, Cope AP. Chapter 109: Pathogenesis of rheumatoid arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner editor. *Oxford Textbook of Rheumatology*: Oxford University Press; 2016.
- [4] England BR, Thiele GM, Mikuls TR. Anticitrullinated protein antibodies: origin and role in the pathogenesis of rheumatoid arthritis. *Curr Opin Rheumatol*. 2017;29(1):57–64. DOI: 10.1097/BOR.0000000000000356.
- [5] Baildam E. Chapter 116: Juvenile idiopathic arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. *Oxford Textbook of Rheumatology*: Oxford University Press; 2016.
- [6] Gurion R, Lehman TJA, L.N. M. Systemic arthritis in children: a review of clinical presentation and treatment. *Int J Inflamm*. 2012;2012:271569. doi: 10.1155/2012/271569.



- [7] Ramanan A.V., Akikusa JD. Chapter 14: The systemically unwell child. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner editor. Oxford Textbook of Rheumatology. 4th Edition (2016) ed: Oxford University Press; 2016.
- [8] Mellins ED, Macaubas C, Grom AA. Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions. *Nat Rev Rheumatol*. 2011;7(7): 416–26. doi: 10.1038/nrrheum.2011.68.
- [9] Macaubas C, Nguyen K, Milojevic D, Park JL, Mellins ED. Oligoarticular and polyarticular JIA: epidemiology and pathogenesis. *Nat Rev Rheumatol*. 2009;5(11):616–26. doi: 10.1038/nrrheum.2009.209.
- [10] Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine-directed therapies. *Annu Rev Med* 2. 2015;66:145–59. doi: 10.1146/annurev-med-061813-012806.
- [11] Mourão AF, Santos MJ, Melo Gomes JA, Martins FM, Mendonça SC, Oliveira Ramos F, Fernandes S, Salgado M, Guedes M, Carvalho S, Costa JA, Brito I, Duarte C, Furtado C, Lopes A, Rodrigues A, Sequeira G, Branco JC, Fonseca JE, Canhão H. Effectiveness and long-term retention of anti-tumour necrosis factor treatment in juvenile and adult patients with juvenile idiopathic arthritis: data from Reuma.pt. *Rheumatology (Oxford)*. 2016;55(4):697–703. DOI: 10.1093/rheumatology/kev398.
- [12] Turnier JL, Brunner HI. Tocilizumab for treating juvenile idiopathic arthritis. *Expert Opin Biol Ther*. 2016;16(4):559–66. DOI: 10.1517/14712598.2016.1150997.
- [13] Church LD, McDermott MF. Canakinumab: a human anti-IL-1 $\beta$  monoclonal antibody for the treatment of cryopyrin-associated periodic syndromes. *Expert Rev Clin Immunol*. 2010;6(6):831–41. DOI: 10.1586/eci.10.66.
- [14] Peitz J, Horneff G. Treatment of systemic-onset juvenile arthritis with canakinumab. *Open Access Rheumatol*. 2015;7:23–31. DOI: 10.2147/OARRR.S54215.
- [15] Donn R, Alourfi Z, Zeggini E, Lamb R, Jury F, Lunt M, Meazza C, De Benedetti F, Thomson W, Ray D, Group. BPRS. A functional promoter haplotype of macrophage migration inhibitory factor is linked and associated with juvenile idiopathic arthritis. *Arthritis Rheum*. 2004;50(5):1604–10. DOI: 10.1002/art.20178.
- [16] Hersh AO, Prahald S. Immunogenetics of juvenile idiopathic arthritis: A comprehensive review. *J Autoimmun* 2. 2015;64:113–24. DOI: 10.1016/j.jaut.2015.08.002.
- [17] Sieper A. Chapter 113: Axial spondyloarthropathies. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. Oxford Textbook of Rheumatology: Oxford University Press; 2016.
- [18] International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, Cremin K, Pryce K, Harris J, Lee S., Joo JB, Shim S-C, Weisman M, Ward M, Zhou X, Garchon H-J, Chiochia G, Nossent J, Lie BA,

- Førre Ø, Tuomilehto J, Laiho K, Jiang L, Liu Y, m, Wu X, Bradbury LA, Elewaut D, Burgos-Vargas R, Stebbings S, Appleton L, Farrah C, Lau J, Kenna TJ, Haroon N, Ferreira MA, Yang J, Mulero J, Fernandez-Sueiro JL, Gonzalez-Gay MA, Lopez-Larrea C, Deloukas P, Donnelly P, Australo-Anglo-American Spondyloarthritis Consortium (tASC), Groupe Française d'Etude Génétique des Spondylarthrites (GFeGS), Nord-Trøndelag Health Study (HUNT), Spondyloarthritis Research Consortium of Canada (SPARCC), Wellcome Trust Case Control Consortium 2 (ETCCC2), Bowness P, Gafney K, Gaston H, Gladman DD, Rahman P, Maksymowych WP, Xu H, Crusius JBA, van der Horst-Bruinsma IE, Chou C-T, Valle-Oñate R, Romero-Sánchez C, Hansen IM, Pimentel-Santos FM, Inman RD, Videm V, Martin J, Breban M, Reveille JD, Evans DM, Kim T-H, Wordsworth BP, Brown MA. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet.* 2013;45(7):730–8. doi: 10.1038/ng.2667.
- [19] Maxwell LJ, Zochling J, Boonen A, Singh JA, Veras MM, Tanjong Ghogomu E, Benkhalti Jandu M, Tugwell P, Wells GA. TNF- $\alpha$  inhibitors for ankylosing spondylitis. *Cochrane Database Syst Rev* 2015;4:CD005468. doi: 10.1002/14651858. CD005468.pub2.
- [20] Barr A, Keat A. Spondyloarthritis: evolving therapies. *Arthritis Res Ther.* 2010;12 (6):221. doi: 10.1186/ar3178.
- [21] Ciccia F, Guggino G, Rizzo A, Saieva L, Peralta S, Giardina A, Cannizzaro A, Sireci G, De Leo G, Alessandro R, Triolo G. Type 3 innate lymphoid cells producing IL-17 and IL-22 are expanded in the gut, in the peripheral blood, synovial fluid and bone marrow of patients with ankylosing spondylitis. *Ann Rheum Dis.* 2015;74(9):1739–47. doi: 10.1136/annrheumdis-2014-206323.
- [22] Triggianese P, Conigliaro P, Chimenti MS, Biancone L, Monteleone G, Perricone R, Monteleone I. Evidence of IL-17 producing innate lymphoid cells in peripheral blood from patients with enteropathic spondyloarthritis. *Clin Exp Rheumatol.* 2016;34(6): 1085–93.
- [23] Wendling D, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. *Joint Bone Spine.* 2017;74(3):304–5. DOI: 10.1016/j.jbspin.2006.11.005.
- [24] Barnabe C, Hanley DA. Effect of tumor necrosis factor alpha inhibition on bone density and turnover markers in patients with rheumatoid arthritis and spondyloarthropathy. *Semin Arthritis Rheum.* 2007;39(2):116–22. DOI: 10.1016/j.semarthrit.2008.04.004.
- [25] Coates LC, Helliwell PS. Chapter 114: Psoriatic arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. *Oxford Textbook of Rheumatology*: Oxford University Press; 2016.
- [26] Walling HW, Swick BL. Update on the management of chronic eczema: new approaches and emerging treatment options. *Clin Cosmet Investig Dermatol.* 2010; 3:99–117.
- [27] Campa M, Ryan C, Menter A. An overview of developing TNF- $\alpha$  targeted therapy for the treatment of psoriasis. *Expert Opin Investig Drugs.* 2015;24(10):1343–54. doi: 10.1517/13543784.2015.1076793.

- [28] Krueger JG, Ferris LK, Menter A, Wagner F, White A, Visvanathan S, Lalovic B, Aslanyan S, Wang EE, Hall D, Solinger A, Padula S, Scholl P. Anti-IL-23A mAb BI 655066 for treatment of moderate-to-severe psoriasis: Safety, efficacy, pharmacokinetics, and biomarker results of a single-rising-dose, randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol.* 2015;136(1):116–24.e7. doi: 10.1016/j.jaci.2015.01.018.
- [29] Sofen H, Smith S, Matheson RT, Leonardi CL, Calderon C, Brodmerkel C, Li K, Campbell K, Marciniak SJJ, Wasfi Y, Wang Y, Szapary P, Krueger JG. Guselkumab (an IL-23-specific mAb) demonstrates clinical and molecular response in patients with moderate-to-severe psoriasis. *J Allergy Clin Immunol.* 2014;133(4):1032–40. doi: 10.1016/j.jaci.2014.01.025.
- [30] Blauvelt A. Ixekizumab: a new anti-IL-17A monoclonal antibody therapy for moderate-to severe plaque psoriasis. *Expert Opin Biol Ther.* 2016;16(2):255–63. doi: 10.1517/14712598.2016.1132695.
- [31] Leonardi C, Matheson R, Zachariae C, Cameron G, Li L, Edson-Heredia E, Braun D, Banerjee S. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N Engl J Med.* 2012;366(13):1190–9. doi: 10.1056/NEJMoa1109997.
- [32] Papp KA, Leonardi C, Menter A, Ortonne JP, Krueger JG, Kricorian G, Aras G, Li J, Russell CB, Thompson EH, Baumgartner S. Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. *N Engl J Med.* 2012;366(13):1181–9. doi: 10.1056/NEJMoa1109017.
- [33] Girolomoni G, Mrowietz U, Paul C. Psoriasis: rationale for targeting interleukin-17. *Br J Dermatol.* 2012;167(4):717–24. doi: 10.1111/j.1365-2133.2012.11099.x.
- [34] Wongpiyabovorn J, Suto H, Ushio H, Izuhara K, Mitsuishi K, Ikeda S, Nakao A, Okumura K, Ogawa H. Up-regulation of interleukin-13 receptor alpha1 on human keratinocytes in the skin of psoriasis and atopic dermatitis. *J Dermatol Sci.* 2003;33(1):31–40.
- [35] Newcomb DC, Boswell MG, Zhou W, Huckabee MM, Goleniewska Sevin CM, Hershey GK, Kolls JK, Peebles RSJ. Human TH17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production. *J Allergy Clin Immunol.* 2011;127(4): 1006-13.e1-4. doi: 10.1016/j.jaci.2010.11.043.
- [36] Ravichandran J, Jackson RJ, Trivedi S, Ranasinghe C. IL-17A expression in HIV-specific CD8 T cells is regulated by IL-4/IL-13 following HIV-1 prime-boost immunization. *J Interferon Cytokine Res.* 2015;35(3):176–85. doi: 10.1089/jir.2014.0078.
- [37] Wohn C, Brand A, van Ettinger K, Brouwers-Haspels I, Waisman A, Laman JD, Clausen BE. Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A. *Cell Immunol.* 2016;308:57–65. doi: 10.1016/j.cellimm.2015.11.006.
- [38] Teunissen MB, Munneke JM, Bernink JH, Spuls PI, Res PC, Te Velde A, Cheuk S, Brouwer MW, Menting SP, Eidsmo L, Spits H, Hazenberg MD, Mjösberg J. Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. *J Invest Dermatol.* 2014;134(9): 2351–60. doi: 10.1038/jid.2014.146.
- [39] Villanova F, Flutter B, Tosi I, Grys K, Sreeneebus H, Perera GK, Chapman A, Smith CH, Di Meglio P, Nestle FO. Characterization of innate lymphoid cells in human skin and

- blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J Invest Dermatol.* 2014;134(4):984–91. doi: 10.1038/jid.2013.477.
- [40] Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, Hardman C, Xue L, Cerundolo V, Ogg G. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. *J Exp Med.* 2016; 213(11):2399–412. DOI: 10.1084/jem.20160258.
- [41] Mahmood T, Zaghi D, Menter A. Emerging oral drugs for psoriasis. *Expert Opin Emerg Drugs.* 2015;20(2):209–20. doi: 10.1517/14728214.2015.1010509.
- [42] Papp K, Cather JC, Rosoph L, Sofen H, Langley RG, Matheson RT, Hu C, Day RM. Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial. *Lancet.* 2012;380(9843):738–46. doi: 10.1016/S0140-6736(12)60642-4.
- [43] Papp KA, Kaufmann R, Thaçi D, Hu C, Sutherland D, Rohane P. Efficacy and safety of apremilast in subjects with moderate to severe plaque psoriasis: results from a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison study. *J Eur Acad Dermatol Venereol.* 2013;27(3):e376–83. doi: 10.1111/j.1468-3083.2012.04716.x.
- [44] Schafer PH, Truzzi F, Parton A, Wu L, Kosek J, Zhang LH, Horan G, Saltari A, Quadri M, Lotti R, Marconi A, Pincelli C. Phosphodiesterase 4 in inflammatory diseases: Effects of apremilast in psoriatic blood and in dermal myofibroblasts through the PDE4/CD271 complex. *Cell Signal.* 2016;28(7):753–63. doi: 10.1016/j.cellsig.2016.01.007.
- [45] Dong C, Virtucio C, Zemska O, Baltazar G, Zhou Y, Baia D, Jones-Iatauro S, Sexton H, Martin S, Dee J, Mak Y, Meewan M, Rock F, Akama T, Jarnagin K. Treatment of skin inflammation with benzoxaborole phosphodiesterase inhibitors: selectivity, cellular activity, and effect on cytokines associated with skin inflammation and skin architecture changes. *J Pharmacol Exp Ther.* 2016;358(3):413–22. doi: 10.1124/jpet.116.232819.
- [46] Campa M, Mansouri B, Warren R, Menter A. A review of biologic therapies targeting IL-23 and IL-17 for use in moderate-to-severe plaque psoriasis. *Dermatol Ther (Heidelb).* 2016;6(1):1–12. DOI: 10.1007/s13555-015-0092-3.
- [47] Kulig P, Musiol S, Freiberger SN, Schreiner B, Gyülveszi G, Russo G, Pantelyushin S, Kishihara K, Alessandrini F, Kündig T, Sallusto F, Hofbauer GF, Haak S, Becher B. IL-12 protects from psoriasiform skin inflammation. *Nat Comm.* 2016;7:13466. doi: 10.1038/ncomms13466.
- [48] Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun Rev.* 2014;13(1):3–10. doi: 10.1016/j.autrev.2013.06.004.
- [49] de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol.* 2016;13(1):13–27. DOI: 10.1038/nrgastro.2015.186.

- [50] Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. *Proc Natl Acad Sci U S A*. 2016;113(37):10400–5. DOI: 10.1073/pnas.1601060113.
- [51] Buela KA, Omenetti S, Pizarro TT. Cross-talk between type 3 innate lymphoid cells and the gut microbiota in inflammatory bowel disease. *Curr Opin Gastroenterol*. 2015;31(6):449–55. DOI: 10.1097/MOG.0000000000000217.
- [52] Goldberg R, Prescott N, Lord GM, MacDonald TT, Powell N. The unusual suspects--innate lymphoid cells as novel therapeutic targets in IBD. *Nat Rev Gastroenterol Hepatol*. 2016;12(5):271–83. DOI: 10.1038/nrgastro.2015.52.
- [53] Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. *Proc Natl Acad Sci U S A*. 2013;110(50):20236–41. doi: 10.1073/pnas.1319470110.
- [54] Khanna R, Preiss JC, MacDonald JK, Timmer A. Anti-IL-12/23p40 antibodies for induction of remission in Crohn's disease. *Cochrane Database Syst Rev*. 2015;5:CD007572. DOI: 10.1002/14651858.CD007572.pub2.
- [55] Targan SR, Feagan B, Vermeire S, Panaccione R, Melmed GY, Landers C, Li D, Russell C, Newmark R, Zhang N, Chon Y, Hsu YH, Lin SL, Klekotka P. A randomized, double-blind, placebo-controlled phase 2 study of brodalumab in patients with moderate-to-severe Crohn's disease. *Am J Gastroenterol*. 2016;111(11):1599–607. doi: 10.1038/ajg.2016.298.
- [56] Peckham D, Scambler T, Savic S, McDermott MF. The burgeoning field of innate immune-mediated disease and autoinflammation. *J Pathol*. 2017;241(2):123–39. doi: 10.1002/path.4812.
- [57] McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, Mansfield E, Gadina M, Karenko L, Pettersson T, McCarthy J, Frucht DM, Aringer M, Torosyan Y, Teppo AM, Wilson M, Karaarslan HM, Wan Y, Todd I, Wood G, Schlingens R, Kumarajeewa TR, Cooper SM, Vella JP, Kastner DL. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell*. 1999;97(1):133–44.
- [58] Hull KM, Shoham N, Chae JJ, Aksentijevich I, Kastner DL. The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. *Curr Opin Rheumatol*. 2003;15(1):61–9.
- [59] Hull KM, Drewe E, Aksentijevich I, Singh HK, Wong K, McDermott EM, Dean J, Powell RJ, Kastner DL. The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)*. 2002; 81:349–68.
- [60] Aksentijevich I, McDermott MF. Lessons from characterization and treatment of the autoinflammatory syndromes. *Curr Opin Rheumatol*. 2016. DOI:10.1097/BOR.0000000000000362.

- [61] Holzinger D, Kessel C, Omenetti A, Gattorno M. From bench to bedside and back again: translational research in autoinflammation. *Nat Rev Rheumatol.* 2015;11(10): 573–85. doi: 10.1038/nrrheum.2015.79.
- [62] Lucherini OM, Obici L, Ferracin M, Fulci V, McDermott MF, Merlini G, Muscari I, Magnotti F, Dickie LJ, Galeazzi M, Negrini M, Baldari CT, Cimaz R, Cantarini L. First report of circulating microRNAs in tumour necrosis factor receptor-associated periodic syndrome (TRAPS). *PLoS One.* 2013;8(9):e73443. doi: 10.1371/journal.pone.0073443.
- [63] Nedjai B, Hitman GA, Quillinan N, Coughlan RJ, Church L, McDermott MF, Turner MD. Proinflammatory action of the antiinflammatory drug infliximab in tumor necrosis factor receptor-associated periodic syndrome. *Arthritis Rheum.* 2009;60(2): 619–25. doi: 10.1002/art.24294.
- [64] Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. *Nat Rev Rheumatol.* 2014;10(3):135–47. doi: 10.1038/nrrheum.2013.174.
- [65] Ter Haar NM, Oswald M, Jeyaratnam J, Anton J, Barron KS, Brogan PA, Cantarini L, Galeotti C, Grateau G, Hentgen V, Hofer M, Kallinich T, Kone-Paut I, Lachmann HJ, Ozdogan H, Ozen S, Russo R, Simon A, Uziel Y, Wouters C, Feldman BM, Vastert SJ, Wulffraat NM, Benseler SM, Frenkel J, Gattorno M, Kuemmerle-Deschner JB. Recommendations for the management of autoinflammatory diseases. *Ann Rheum Dis.* 2015;74(9):1636–44. doi: 10.1136/annrheumdis-2015-207546.
- [66] Brouckaert P, Spriggs DR, Demetri G, Kufe DW, Fiers W. Circulating interleukin-6 during a continuous infusion of tumor necrosis factor and interferon- $\gamma$ . *J Exp Med.* 1989;169:2257–62.
- [67] Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med.* 1989(169):333–8.
- [68] Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants—past, present and future. *Cytokine Growth Factor Rev.* 2014;25(4):453–72. doi: 10.1016/j.cytogfr.2014.07.016.
- [69] Federici S, Caorsi R, Gattorno M. The autoinflammatory diseases. *Swiss Med Weekly.* 2012; June 19: w13602. doi: 10.4414/smw.2012.13602.
- [70] Broderick L, De Nardo D, Franklin BS, Hoffman HM, Latz E. The inflammasomes and autoinflammatory syndromes. *Annu Rev Pathol.* 2015;10:395–424. doi: 10.1146/annurev-pathol-012414-040431.
- [71] Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature.* 2012;481(7381):278–86. doi: 10.1038/nature10759.
- [72] Canna SW, de Jesus AA, Gouni S, Brooks SR, Marrero B, Liu Y, DiMattia MA, Zaal KJ, Sanchez GA, Kim H, Chapelle D, Plass N, Huang Y, Villarino AV, Biancotto A, Fleisher

- TA, Duncan JA, O'Shea JJ, Benseler S, Grom A, Deng Z, Laxer RM, Goldbach-Mansky R. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet.* 2014;46(10):1140–6. doi: 10.1038/ng.3089.
- [73] Mortimer L, Moreau F, MacDonald JA, Chadee K. NLRP3 inflammasome inhibition is disrupted in a group of auto-inflammatory disease CAPS mutations. *Nat Immunol.* 2016;17(10):1176–86. doi: 10.1038/ni.3538.
- [74] Rynne M, Maclean C, Bybee A, McDermott MF, Emery P. Hearing improvement in a patient with variant Muckle-Wells syndrome in response to interleukin 1 receptor antagonism. *Ann Rheum Dis.* 2006;65(4):553–4. doi: 10.1136/ard.2005.038091.
- [75] Kingsbury SR, Conaghan PG, McDermott MF. The role of the NLRP3 inflammasome in gout. *J Inflamm Res.* 2011;4:39–49. doi: 10.2147/JIR.S11330.
- [76] Busso N, Ea HK. The mechanisms of inflammation in gout and pseudogout (CPP-induced arthritis). *Reumatismo.* 2012;63 (4):230–7. doi: 10.4081/reumatismo.2011.230.
- [77] Group TLMSSGaTUoBCMMA. TNF neutralization in MS. Results of a randomized, placebo-controlled multicenter study. *Neurology.* 1999;53(3):457–65.
- [78] Abraham E, Laterre PF, Garbino J, Pingleton S, Butler T, Dugernier T, Margolis B, Kudsk K, Zimmerli W, Anderson P, Reynaert M, Lew D, Lesslauer W, Passe S, Cooper P, Burdeska A, Modi M, Leighton A, Salgo M, Van der Auwera P, Group. LS. Lenercept (p55 tumor necrosis factor receptor fusion protein) in severe sepsis and early septic shock: a randomized, double-blind, placebo-controlled, multicenter phase III trial with 1,342 patients. *Crit Care Med.* 2001;29(3):503–10.
- [79] Papp K. Clinical development of onercept, a tumor necrosis factor binding protein, in psoriasis. *Curr Med Res Opin.* 2010;26(10):2287–300. doi: 10.1185/03007995.2010.507492.
- [80] Kawabata H, Kadowaki N, Nishikori M, Kitawaki T, Kondo T, Ishikawa T, Yoshifuji H, Yamakawa N, Imura Y, Mimori T, Matsumura Y, Miyachi Y, Matsubara T, Yanagita M, Haga H, Takaori-Kondo A. Clinical features and treatment of multicentric Castleman's disease : a retrospective study of 21 Japanese patients at a single institute. *J Clin Exp Hematop.* 2013;553(1):69–77.
- [81] Ringelstein M, Ayzenberg I, Harmel J, Lauenstein AS, Lensch E, Stögbauer F, Hellwig K, Ellrichmann G, Stettner M, Chan A, Hartung HP, Kieseier B, Gold R, Aktas O, Kleiter I. Long-term therapy with interleukin 6 receptor blockade in highly active neuromyelitis optica spectrum disorder. *JAMA Neurol.* 2015;72(7):756–63. doi: 10.1001/jamaneurol.2015.0533.
- [82] Lebwohl M, Strober B, Menter A, Gordon K, Weglowska J, Puig L, Papp K, Spelman L, Toth D, Kerdel F, Armstrong AW, Stingl G, Kimball AB, Bachelez H, Wu JJ, Crowley J, Langley RG, Blicharski T, Paul C, Lacour JP, Tyring S, Kircik L, Chimenti S, Callis Duffin K, Bagel J, Koo J, Aras G, Li J, Song W, Milmont CE, Shi Y, Erondun N, Klekotka P, Kotzin B, Nirula A. Phase 3 studies comparing brodalumab with ustekinumab in psoriasis. *N Engl J Med.* 2015;373(14):1318–28. doi: 10.1056/NEJMoa1503824.

- [83] Schmidt C. Suicidal thoughts end Amgen's blockbuster aspirations for psoriasis drug. *Nat Biotechnol.* 2015;33(9):894–5. doi: 10.1038/nbt0915-894b.
- [84] Papp KA, Sundaram M, Bao Y, Williams DA, Gu Y, Signorovitch JE, Wang Y, Valdes JM, Mulani PM. Effects of briakinumab treatment for moderate to severe psoriasis on health-related quality of life and work productivity and activity impairment: results from a randomized phase III study. *J Eur Acad Dermatol Venereol.* 2014;28(6):790–8. doi: 10.1111/jdv.12177.
- [85] Zhuang Y, Calderon C, Marciniak SJJ, Bouman-Thio E, Szapary P, Yang TY, Schantz A, Davis HM, Zhou H, Xu Z. First-in-human study to assess guselkumab (anti-IL-23 mAb) pharmacokinetics/safety in healthy subjects and patients with moderate-to-severe psoriasis. *Eur J Clin Pharmacol.* 2016;72(11):1303–10. DOI: 10.1007/s00228-016-2110-5.
- [86] Gordon KB, Duffin KC, Bissonnette R, Prinz JC, Wasfi Y, Li S, Shen YK, Szapary P, Randazzo B, Reich K. A Phase 2 Trial of Guselkumab versus Adalimumab for Plaque Psoriasis. *N Engl J Med.* 2015;373(2):136–44. doi: 10.1056/NEJMoa1501646.
- [87] Yiu ZZ, Exton LS, Jabbar-Lopez Z, Mohd Mustapa MF, Samarasekera EJ, Burden AD, Murphy R, Owen CM, Parslew R, Venning V, Ashcroft DM, Griffiths CE, Smith CH, Warren RB. Risk of serious infections in patients with psoriasis on biologic therapies: a systematic review and meta-analysis. *J Invest Dermatol.* 2016;136(81):1584–91. doi: 10.1016/j.jid.2016.03.035.
- [88] Müller-Ladner U, Hong S, Oh C, Taylor P. Scientific rationale behind the development and approval of biosimilar infliximab (CT-P13) in Europe. *Expert Rev Clin Immunol.* 2015;11(Supp.1):S5–14. doi: 10.1586/1744666X.2015.1090310.
- [89] Putrik P, Ramiro S, Kvien TK, Sokka T, Pavlova M, Uhlig T, Boonen A, Europe'. WGEiattorai. Inequities in access to biologic and synthetic DMARDs across 46 European countries. *Ann Rheum Dis.* 2014;73(2):198–206. doi: 10.1136/annrheumdis-2012-202603.
- [90] Isaacs JD, Cutolo M, Keystone EC, Park W, Braun J. Biosimilars in immune-mediated inflammatory diseases: initial lessons from the first approved biosimilar anti-tumour necrosis factor monoclonal antibody. *J Intern Med.* 2015;279(41–59). doi: 10.1111/joim. 12432.
- [91] Christl LA, Woodcock J, Kozlowski S. Biosimilars: The US Regulatory Framework. *Ann Rev Med.* 2. 2016; ePub. DOI: 10.1146/annurev-med-051215-031022.
- [92] Park W, Hrycaj P, Jeka S, Kovalenko V, Lysenko G, Miranda P, Mikazane H, Gutierrez-Ureña S, Lim M, Lee YA, Lee SJ, Kim H, Yoo DH, Braun J. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. *Ann Rheum Dis.* 2013;72(10):1605–12. doi: 10.1136/annrheumdis-2012-203091.
- [93] Yoo DH, Hrycaj P, Miranda P, Ramiterre E, Piotrowski M, Shevchuk S, Kovalenko V, Prodanovic N, Abello-Banfi M, Gutierrez-Ureña S, Morales-Olazabal L, Tee M, Jimenez R,



- Zamani O, Lee SJ, Kim H, Park W, Müller-Ladner U. A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study. *Ann Rheum Dis.* 2013;72(10):1613–20. doi: 10.1136/annrheumdis-2012-203090.
- [94] Fang J, Doneanu C, Alley WRJ, Yu YQ, Beck A, Chen W. Advanced assessment of the physicochemical characteristics of Remicade® and Inflectra® by sensitive LC/MS techniques. *MAbs.* 2016;8(6):1021–34. doi: 10.1080/19420862.2016.1193661.
- [95] Magnenat L, Palmese A, Fremaux C, D'Amici F, Terlizze M, Rossi M, Chevalet L. Demonstration of physicochemical and functional similarity between the proposed biosimilar adalimumab MSB11022 and Humira®. *MAbs.* 2016. doi: 10.1080/19420862.2016.1259046.
- [96] Cho IH, Lee N, Song D, Jung SY, Bou-Assaf G, Sobic Z, Zhang W, Lyubarskaya Y. Evaluation of the structural, physicochemical, and biological characteristics of SB4, a biosimilar of etanercept. *MAbs.* 2015;8(6):1136–55. doi: 10.1080/19420862.2016.1193659.
- [97] Velayudhan J, Chen YF, Rohrbach A, Pastula C, Maher G, Thomas H, Brown R, Born TL. Demonstration of functional similarity of proposed biosimilar ABP 501 to adalimumab. *BioDrugs.* 2016;30(4):339–51. doi: 10.1007/s40259-016-0185-2.
- [98] Raju TS. Terminal sugars of Fc glycans influence antibody effector functions of IgGs. *Curr Opin Immunol.* 2008;20(4):471–8. doi: 10.1016/j.coi.2008.06.007.
- [99] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), (CVM) CfVM. Guidance for Industry, Bioanalytical Method Validation. 2013. Available from: <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.
- [100] Olech E. Biosimilars: Rationale and current regulatory landscape. *Semin Arthritis Rheum.* 2016;45(5 Suppl.):S1–10. doi: 10.1016/j.semarthrit.2016.01.001.
- [101] Chapman K, Adjei A, Baldrick P, da Silva A, De Smet K, DiCicco R, Hong SS, Jones D, Leach MW, McBlane J, Ragan I, Reddy P, Stewart DI, Suitters A, Sims J. Waiving in vivo studies for monoclonal antibody biosimilar development: National and global challenges. *MAbs.* 2016;8(3):427–35. doi: 10.1080/19420862.2016.1145331.
- [102] Hogwood CE, Bracewell DG., Smales CM. Measurement and control of host cell proteins (HCPs) in CHO cell bioprocesses. *Curr Opin Biotechnol.* 2014;30:153–60. doi: 10.1016/j.copbio.2014.06.017.
- [103] Bracewell DG, Francis R, Smales CM. The future of host cell protein (HCP) identification during process development and manufacturing linked to a risk-based management for their control. *Biotechnol Bioeng.* 2015;112(9):1727–37. DOI: 10.1002/bit.25628.
- [104] Feldman SR. Inflammatory diseases: Integrating biosimilars into clinical practice. *Semin Arthritis Rheum.* 2015;44(6 Suppl.):S16–21. DOI:10.1016/j.semarthrit.2015.04.003.

- [105] Jung SK, Lee KH, Jeon JW, Lee JW, Kwon BO, Kim YJ, Bae JS, Kim DI, Lee SY, Chang SJ. Physicochemical characterization of Remsima. *MAbs*. 2014;6(5):1163–77. doi: 10.4161/mabs.32221.
- [106] Azevedo VF, Galli N, Kleinfelder A, D'Ippolito J, Urbano PCM. Etanercept biosimilars. *Rheumatol Int*. 2015;35:197–209. doi: 10.1007/s00296-014-3080-5.
- [107] Buer LC, Moum BA, Cvancarova M, Warren DJ, Medhus AW, Høivik ML. Switching from Remicade® to Remsima® is safe and feasible: a prospective, open-label study. *J Crohns Colitis* 2016;Sept 22(pii: jjw166.). doi: 10.1093/ecco-jcc/jjw166.
- [108] Ruiz-Argüello MB, Maguregui A, Ruiz Del Agua A, Pascual-Salcedo D, Martínez-Feito A, Jurado T, Plasencia C, Balsa A, Llinares-Tello F, Rosas J, Torres N, Martínez A, Nagore D. Antibodies to infliximab in Remicade-treated rheumatic patients show identical reactivity towards biosimilars. *Ann Rheum Dis*. 2016;75(9):1693–6. DOI: 10.1136/annrheumdis-2015-208684.
- [109] Ben-Horin S, Yavzori M, Benhar I, Fudim E, Picard O, Ungar B, Lee S, Kim S, Eliakim R, Chowers Y. Cross-immunogenicity: antibodies to infliximab in Remicade-treated patients with IBD similarly recognise the biosimilar Remsima. *Gut*. 2016;65(7):1132–8. DOI: 10.1136/gutjnl-2015-309290.
- [110] Daëron M. Fc receptor biology. *Annu Rev Immunol*. 1997;15:203–34. DOI: 10.1146/annurev.immunol.15.1.203.
- [111] Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY. Identity of the elusive IgM Fc receptor (FcmuR) in humans. *J Exp Med*. 2009;206(12):2779–93. DOI: 10.1084/jem.20091107.
- [112] Cosman D, Fanger N, Borges L, Kubin M, Chin W, Peterson L, Hsu ML. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity*. 1997;7:273–82.
- [113] Borges L, Hsu ML, Fanger N, Kubin M, Cosman D. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol*. 1997;159:5192–6.
- [114] Fanger NA, Borges L, Cosman D. The leukocyte immunoglobulin-like receptors (LIRs): a new family of immune regulators. *J Leukoc Biol*. 1999;66:231–6.
- [115] Nakajima H, Samaridis J, Angman L, Colonna M. Human myeloid cells express an activating ILT receptor (ILT1) that associates with Fc receptor gamma-chain. *J Immunol*. 1999;162:5–8.
- [116] Woof JM, Burton DR. Human antibody-Fc receptor interactions illuminated by crystal structures. *Nat Rev Immunol*. 2004;4:89–99. doi:10.1038/nri1266.
- [117] Tedla N, Gibson K, McNeil H.P., Cosman D, Borges L, Arm JP. The co-expression of activating and inhibitory leukocyte immunoglobulin-like receptors in rheumatoid synovium. *Am J Pathol*. 2002;160(2):425–31. DOI: 10.1016/S0002-9440(10)64861-4.

- [118] Honda S, Kurita N, Miyamoto A, Cho Y, Usui K, Takeshita K, Takahashi S, Yasui T, Kikutani H, Kinoshita T, Fujita T, Tahara-Hanaoka S, Shibuya K, Shibuya A. Enhanced humoral immune responses against T-independent antigens in Fc alpha/muR-deficient mice. *Proc Natl Acad Sci U S A*. 2009;106(27):11230–5. doi: 10.1073/pnas.0809917106.
- [119] Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H. Identification of TOSO/FAIM3 as an Fc receptor for IgM. *Int Immunol*. 2010;22(3):149–56. DOI: 10.1093/intimm/dxp121.
- [120] Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieyus S, Daëron M. Specificity and affinity of human Fc- $\gamma$  receptors and their polymorphic variants for human IgG subclasses. *Blood*. 2009;113:3716–25. DOI: 10.1182/blood-2008-09-179754.
- [121] Monteiro RC, Van De Winkel JG. IgA Fc receptors. *Annu Rev Immunol*. 2001;21:177–204. DOI: 10.1146/annurev.immunol.21.120601.141011.
- [122] Aleyd E, Heineke MH, van Egmond M. The era of the immunoglobulin A Fc receptor Fc $\alpha$ RI; its function and potential as target in disease. *Immunol Rev*. 2015;268(1):268. doi: 10.1111/imr.12337.
- [123] Ben Mkaddem S, Rossato E, Heming N, Monteiro RC. Anti-inflammatory role of the IgA Fc receptor (CD89): from autoimmunity to therapeutic perspectives. *Autoimmun Rev*. 2013;12(6):666–9. doi: 10.1016/j.autrev.2012.10.011.
- [124] Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffié C, Hénin D, Benhamou M, Pretolani M, Blank U, Monteiro RC. Identification of Fc $\alpha$ RI as an inhibitory receptor that controls inflammation: dual role of FcR $\gamma$  ITAM. *Immunity*. 2005; 22(1):31–42. doi: 10.1016/j.immuni.2004.11.017.
- [125] Thomas SS, Borazan N, Barroso N, Duan L, Taroumian S, Kretzmann B, Bardales R, Elashoff D, Vangala S, Furst DE. Comparative immunogenicity of TNF inhibitors: impact on clinical efficacy and tolerability in the management of autoimmune diseases. A systematic review and meta-analysis. *BioDrugs*. 2015;29(4):241–58. doi: 10.1007/s40259-015-0134-5.
- [126] Suhrbier A, Mahalingam S. The immunobiology of viral arthritides. *Pharmacol Ther*. 2009;124(3):301–8. doi: 10.1016/j.pharmthera.2009.09.005.
- [127] Rathinam VA, Fitzgerald KA. Inflammasomes and anti-viral immunity. *J Clin Immunol*. 2010;30(5):632–7. doi: 10.1007/s10875-010-9431-4.
- [128] Shalaby MR, Waage A, Aarden L, Espevik T. Endotoxin, tumor necrosis factor and interleukin 1 induce interleukin 6 production in vivo. *Clin Immunol Immunopathol*. 1989;53:488–98.
- [129] Sedger LM, Shows DM, Blanton RA, Peschon JJ, Goodwin RG, Cosman D, Wiley SR. IFN- $\gamma$  mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression. *J Immunol*. 1999;163(2):920–6.
- [130] Ramsay AJ, Husband AJ, Ramshaw IA, Bao S, Matthaei KI, Koehler G, Kopf M. The role of interleukin-6 in mucosal IgA antibody responses in vivo. *Science*. 1994;264(5158):561–3.

- [131] Kim SY, Solomon DH. Tumor necrosis factor blockade and the risk of viral infection. *Nat Rev Rheumatol*. 2010;6:165–74. doi: 10.1038/nrrheum.2009.279.
- [132] Cantini F, Niccoli L, Goletti D. Adalimumab, etanercept, infliximab, and the risk of tuberculosis: data from clinical trials, national registries, and postmarketing surveillance. *J Rheumatol Suppl*. 2014;91:47–55. doi: 10.3899/jrheum.140102.
- [133] Tresch S, Trueb RM, Kamarachev J, French LE, Hofbauer GF. Disseminated herpes zoster mimicking rheumatoid vasculitis in a rheumatoid arthritis patient on etanercept. *Dermatology*. 2009;219:347–9. doi: 10.1159/000232389.
- [134] Wong AK, Kerkoutian S, Said J, Rashidi H, Pullarkat ST. Risk of lymphoma in patients receiving antitumor necrosis factor therapy: a meta-analysis of published randomized controlled studies. *Clin Rheumatol*. 2012;31:631–6. doi: 10.1007/s10067-011-1895-y.
- [135] Dinarello CA, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MAJ, O'Connor JV. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med*. 1986;164:1443–50.
- [136] Smyth MJ, Kelly JM, Baxter AG, Korner H, Sedgwick JD. An essential role for tumor necrosis factor in natural killer cell-mediated tumor rejection in the peritoneum. *J Exp Med*. 1998;188:1611–9.
- [137] Kaltsonoudis E, Voulgari PV, Konitsiotis S, Drosos AA. Demyelination and other neurological adverse events after anti-TNF therapy. *Autoimmun Rev*. 2014;13(1):54–8. doi: 10.1016/j.autrev.2013.09.002.
- [138] Adreadou E, Kemanetzoglou E, Brokalaki C, Evangelopoulos M, Kilidireas C, Rambos A, Stamboulis E. Demyelinating disease following anti-TNF $\alpha$  treatment: A causal or coincidental association? Report of four cases and review of the literature. *Case Reports Neurol Med*. 2013; ID67935. doi: 10.1155/2013/671935.
- [139] Kaltsonoudis E, Zikou AK, Voulgari PV, Konitsiotis S, Argyropoulou MI, Drosos AA. Neurological adverse events in patients receiving anti-TNF therapy: a prospective imaging and electrophysiological study. *Arthritis Res Ther*. 2014;16(3):R125. doi: 10.1186/ar4582.
- [140] Mohan N, Edwards ET, Cupps TR, Oliverio PJ, Sandberg G, Crayton H, Richert JR, Siegel JN. Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides. *Arthritis Rheum*. 2001;44(12):2862–9.
- [141] Black C, Miller BJ. Meta-analysis of cytokines and chemokines in suicidality: distinguishing suicidal versus nonsuicidal patients. *Biol Psychiatry*. 2015;78(1):28–37. doi: 10.1016/j.biopsych.2014.10.014.
- [142] Dieperink E, Ho SB, Thuras P, Willenbring ML. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. *Psychosomatics*. 2003;44(2):104–12.

- [143] Schaefer M, Schmidt F, Horn M, Schmid-Wendtner MH, Volkenandt M. Depression during treatment with interferon alpha. *Psychosomatics*. 2004;45(2):176. DOI: 10.1176/appi.psy.45.2.176.
- [144] Murakami Y, Ishibashi T, Tomita E, Imamura Y, Tashiro T, Watcharanurak K, Nishikawa M, Takahashi Y, Takakura Y, Mitani S, Fujigaki H, Ohta Y, Kubo H, Mamiya T, Nabeshima T, Kim HC, Yamamoto Y, Saito K. Depressive symptoms as a side effect of Interferon- $\alpha$  therapy induced by induction of indoleamine 2,3-dioxygenase 1. *Sci Rep*. 2016;6:29920. doi: 10.1038/srep29920.
- [145] Bay-Richter C, Linderholm KR, Lim CK, Samuelsson M, Träskman-Bendz L, Guillemin GJ, Erhardt S, Brundin L. A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. *Brain Behav Immun*. 2015;43:110–7. doi: 10.1016/j.bbi.2014.07.012.
- [146] Erhardt S, Lim CK, Linderholm KR, Janelidze S, Lindqvist D, Samuelsson M, Lundberg K, Postolache TT, Träskman-Bendz L, Guillemin GJ, Brundin L. Connecting inflammation with glutamate agonism in suicidality. *Neuropsychopharmacology*. 2013;38(5):743–52. doi: 10.1038/npp.2012.248.
- [147] Clark IA, Vissel B. A neurologist's guide to TNF biology and to the principles behind the therapeutic removal of excess TNF in disease. *Neural Plast*. 2015;2015:358263. DOI: 10.1155/2015/358263.
- [148] Clark IA, Vissel B. Excess cerebral TNF causing glutamate excitotoxicity rationalizes treatment of neurodegenerative diseases and neurogenic pain by anti-TNF agents. *J Neuroinflammation*. 2016;13(1):236. doi: 10.1186/s12974-016-0708-2.
- [149] Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, Qian G, Zheng JC. IL-1 $\beta$  and TNF- $\alpha$  induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. *J Neurochem*. 2013;125(6):897–908. DOI: 10.1111/jnc.12263.
- [150] Tobinick EL. Perispinal delivery of CNS drugs. *CNS Drugs*. 2016;30(6):469–80. doi: 10.1007/s40263-016-0339-2.
- [151] Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. *Nature*. 2015;523(7560):337–41. doi: 10.1038/nature14432.
- [152] Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. *CNS Drugs* 2011 Feb;25(2):145–55 2011;25(5):145-55. doi: 10.2165/11588400-000000000-00000.
- [153] Tobinick E, Kim NM, Reyzin G, Rodriguez-Romanacce H, DePuy V. Selective TNF inhibition for chronic stroke and traumatic brain injury: an observational study involving 629 consecutive patients treated with perispinal etanercept. *CNS Drugs* 2012;26(12):1051–70. doi: 10.1007/s40263-012-0013-2.

- [154] Tobinick E, Rodriguez-Romanacce H, Levine A, Ignatowski TA, Spengler RN. Immediate neurological recovery following perispinal etanercept years after brain injury. *Clin Drug Investig.* 2014;34(5):361–6. doi: 10.1007/s40261-014-0186-1.
- [155] Chio CC, Chang CH, Wang CC, Cheong CU, Chao CM, Cheng BC, Yang CZ, Chang CP. Etanercept attenuates traumatic brain injury in rats by reducing early microglial expression of tumor necrosis factor- $\alpha$ . *BMC Neurosci.* 2013;14:33. doi: 10.1186/1471-2202-14-33.
- [156] Licastro F, Hrelia S, Porcellini E, Malaguti M, Di Stefano C, Angeloni C, Carbone I, Simoncini L, Piperno R. Peripheral inflammatory markers and antioxidant response during the post-acute and chronic phase after severe traumatic brain injury. *Front Neurol.* 2016;7:189. DOI: 10.3389/fneur.2016.00189.
- [157] Tuttolomondo A, Pecoraro R, Pinto A. Studies of selective TNF inhibitors in the treatment of brain injury from stroke and trauma: a review of the evidence to date. *Drug Des Devel Ther.* 2014;8:2221–38. doi: 10.2147/DDDT.S67655.
- [158] Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Exp Neurol.* 2016 Jan;275 Pt 3:316–27. 2016;275:316–27. doi: 10.1016/j.expneurol.2015.08.018.
- [159] Sairanen T, Carpén O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, Lindsberg PJ. Evolution of cerebral tumor necrosis factor- $\alpha$  production during human ischemic stroke. *Stroke.* 2001;32(8):1750–8.
- [160] Rappaport J, Volsky DJ. Role of the macrophage in HIV-associated neurocognitive disorders and other comorbidities in patients on effective antiretroviral treatment. *J Neurovirol.* 2015;21(3):235–41. doi: 10.1007/s13365-015-0346-y.
- [161] Seilhean D, Kobayashi K, He Y, Uchihara T, Rosenblum O, Katlama C, Bricaire F, Duyckaerts C, Hauw JJ. Tumor necrosis factor- $\alpha$ , microglia and astrocytes in AIDS dementia complex. *Acta Neuropathol.* 1997;93(5):508–17.
- [162] Han C, Lu Y, Wei Y, Wu B, Liu Y, He R. D-ribosylation induces cognitive impairment through RAGE-dependent astrocytic inflammation. *Cell Death Dis.* 2014;5:e1117. doi: 10.1038/cddis.2014.89.
- [163] Mandal P, Schifilliti D, Mafrica F, Fodale V. Inhaled anesthesia and cognitive performance. *Drugs Today (Barc).* 2009;45(1):47–54. doi: 10.1358/dot.2009.45.1.1315075.
- [164] Fong TG, Davis D, Growdon ME, Albuquerque A, Inouye SK. The interface between delirium and dementia in elderly adults. *Lancet Neurol.* 2015;14(8):823–32. DOI: 10.1016/S1474-4422(15)00101-5.
- [165] Simone MJ, Tan ZS. The role of inflammation in the pathogenesis of delirium and dementia in older adults: a review. *CNS Neurosci Ther.* 2011;17(5):506–13. doi:10.1111/j.1755-5949.2010.00173.x.
- [166] Xie Z, Dong Y, Maeda U, Moir RD, Xia W, Culley DJ, Crosby G, Tanzi RE. The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-

- protein accumulation. *J Neurosci.* 2007;27(6):1247–54. doi: 10.1523/JNEUROSCI.5320-06.2007.
- [167] Zhang Y, Zhen Y, Dong Y, Xu Z, Yue Y, Golde TE, Tanzi RE, Moir RD, Xie Z. Anesthetic propofol attenuates the isoflurane-induced caspase-3 activation and A $\beta$  oligomerization. *PLoS One.* 2011;6(11):e27019. doi: 10.1371/journal.pone.0027019.
- [168] Jiang J, Jiang H. Effect of the inhaled anesthetics isoflurane, sevoflurane and desflurane on the neuropathogenesis of Alzheimer's disease (review). *Mol Med Rep.* 2015;12(1):3–12. doi: 10.3892/mmr.2015.3424.
- [169] Qiao Y, Feng H, Zhao T, Yan H, Zhang H, Zhao X. Postoperative cognitive dysfunction after inhalational anesthesia in elderly patients undergoing major surgery: the influence of anesthetic technique, cerebral injury and systemic inflammation. *BMC Anesthesiol.* 2015;15:154. doi: 10.1186/s12871-015-0130-9.
- [170] Capri M, Yani SL, Chattat R, Fortuna D, Bucci L, Lanzarini C, Morsiani C, Catena F, Ansaloni L, Adversi M, Melotti MR, Di Nino G, Franceschi C. Pre-operative, high-IL-6 blood level is a risk factor of post-operative delirium onset in old patients. *Front Endocrinol (Lausanne).* 2014;5:173. doi: 10.3389/fendo.2014.00173.
- [171] Androsova G, Krause R, Winterer G, Schneider R. Biomarkers of postoperative delirium and cognitive dysfunction. *Front Aging Neurosci* 2015;7:112. doi: 10.3389/fnagi.2015.00112.
- [172] Chiricozzi A, Romanelli M, Saraceno R, Torres T. No meaningful association between suicidal behavior and the use of IL-17A-neutralizing or IL-17RA-blocking agents. *Expert Opin Drug Saf.* 2016;5(12):1653–9. doi: 10.1080/14740338.2016.1228872.
- [173] Galluzzo M, D'adamio S, Bianchi L, Talamonti M. Brodalumab for the treatment of psoriasis. *Expert Rev Clin Immunol.* 2016;12(12):1255–71. doi: 10.1080/1744666X.2016.1246957.
- [174] Pallav P, Sagar R, Mehta M, Sharma S, Subramaniam A, Shamshi F, Sengupta U, Pandey RM, Mukhopadhyay AK. Serum cytokines and anxiety in adolescent depression patients: Gender effect. *Psychiatry Res.* 2015;229(1–2):374–80. doi: 10.1016/j.psychres.2015.06.036.
- [175] Vieira MM, Ferreira TB, Pacheco PA, Barros PO, Almeida CR, Araújo-Lima CF, Silva-Filho RG, Hygino J, Andrade RM, Linhares UC, Andrade AF, Bento CA. Enhanced Th17 phenotype in individuals with generalized anxiety disorder. *J Neuroimmunol.* 2010;229(1–2):212–8. doi: 10.1016/j.jneuroim.2010.07.018.
- [176] Ferreira TB, Barros PO, Teixeira B, Cassano T, Centurião N, Kasahara TM, Hygino J, Vasconcelos CC, Filho HA, Alvarenga R, Wing AC, Andrade RM, Andrade AF, Bento CA. Dopamine favors expansion of glucocorticoid-resistant IL-17-producing T cells in multiple sclerosis. *Brain Behav Immun.* 2014;41:182–90. doi: 10.1016/j.bbi.2014.05.013.
- [177] Menzella F, Lusuardi M, Galeone C, Taddei S, Zucchi L. Profile of anti-IL-5 mAb mepolizumab in the treatment of severe refractory asthma and hypereosinophilic diseases. *J Asthma Allergy.* 2015;8:105–14. doi: 10.2147/JAA.S40244.

- [178] Nixon J, Newbold P, Mustelin T, Anderson GP, Kolbeck R. Monoclonal antibody therapy for the treatment of asthma and chronic obstructive pulmonary disease with eosinophilic inflammation. *Pharmacol Ther.* 2016; pii: S0163-7258(16):30195-4. doi: 10.1016/j.pharmthera.2016.10.016.
- [179] Corren J. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. *Discov Med.* 2012;13(71):305-12.
- [180] Khorasanizadeh MH, Eskian M, Assa'as AH, Camargo CAJ, Rezaei N. Efficacy and safety of benralizumab, a monoclonal antibody against IL-5R $\alpha$ , in uncontrolled eosinophilic asthma. *Intl Rev Immunology.* 2016;35:294-311. doi: 10.3109/08830185.2015.1128901.
- [181] Walsh GM. Anti-IL-4/13 based therapy in asthma. *Expert Opin Emerg Drugs.* 2015;20(3):349-52. doi: 10.1517/14728214.2015.1050377.
- [182] Walsh GM. Biologics targeting IL-5, IL-4 or IL-13 for the treatment of asthma – an update. *Expert Rev Clin Immunol.* 2016;Aug 2:1-7. doi: 10.1080/1744666X.2016.1216316.
- [183] Matsumoto H. Serum periostin: a novel biomarker for asthma management. *Allergol Int.* 2014;63(2):153-60. doi: 10.2332/allergolint.13-RAI-0678.
- [184] Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, Shikotra A, Carter R, Audusseau S, Hamid Q, Bradding P, Fahy JV, Woodruff PG, Harris JM, Arron JR, Group. BERSoBiC-rABS. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol.* 2012;130(3):647-54. doi: 10.1016/j.jaci.2012.06.025.
- [185] Bujarski S, Parulekar AD, Hanania NA. Lebrikizumab in the treatment of asthma. *Expert Opin Biol Ther.* 2016:847-52. doi: 10.1080/14712598.2016.1182152.
- [186] Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, Flynn RJ, Sayers I, Hall IP, McKenzie AN. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol.* 2013;132(4):933-41. doi: 10.1016/j.jaci.2013.05.012.
- [187] Halim TY, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, McKenzie AN, Takei F. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity.* 2014;40(3): 425-35. doi: 10.1016/j.immuni.2014.01.011.
- [188] Kim BS, Artis D. Group 2 innate lymphoid cells in health and disease. *Cold Spring Harb Perspect Biol.* 2015;7(5): pii: a016337. doi: 10.1101/cshperspect.a016337.
- [189] Jackson RJ, Worley M, Trivedi S, Ranasinghe C. Novel HIV IL-4R antagonist vaccine strategy can induce both high avidity CD8 T and B cell immunity with greater protective efficacy. *Vaccine.* 2014;32(43):5703-14. doi: 10.1016/j.vaccine.2014.08.023.
- [190] Ranasinghe C, Trivedi S, Stambas J, Jackson RJ. Unique IL-13R $\alpha$ 2-based HIV-1 vaccine strategy to enhance mucosal immunity, CD8+ T-cell avidity and protective immunity. *Mucosal Immunol.* 2013;6(6):1068-80. doi: 10.1038/mi.2013.1.