PEGylated Protein Drugs: Basic Science and Clinical Applications
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Certolizumab pegol: a PEGylated anti-tumour necrosis factor alpha biological agent

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Abstract

Tumour necrosis factor (TNF)α is a proinflammatory cytokine involved in systemic inflammation that mediates chronic inflammatory diseases such as rheumatoid arthritis (RA), Crohn's disease (CD) and psoriasis. Recognition of TNFα as a primary mediator of inflammatory disease has driven the development of monoclonal antibodies (mAbs) against TNFα as potential novel therapies for these disorders. Certolizumab pegol is a novel, polyethylene glycol (PEG)-conjugated, humanised, antigen-binding fragment (Fab') of an anti-TNFα mAb that does not mediate apoptosis or neutrophil degranulation. Preclinical studies have shown excellent bioavailability, with preferential distribution and retention in inflamed tissue, which could be due to the low diffusion rate of PEGylated molecules and/or the lack of an Fc, which prevents FcRn-mediated transport. Pharmacokinetics are linear and predictable. Certolizumab pegol is a potentially valuable new treatment option for several inflammatory diseases. It has shown promising efficacy and tolerability results in Phase II and III trials for RA, CD and psoriasis.

History of anti-tumour necrosis factor agents in autoimmune inflammatory disease states

Tumour necrosis factor α, its structure and function and biological roles

Tumour necrosis factor (TNF)α is a proinflammatory cytokine involved in systemic inflammation that is known to be a mediator of chronic inflammatory diseases such as Crohn's disease (CD), rheumatoid arthritis (RA) and psoriasis [1]. The existence of lymphotoxin (LT), a cytotoxic factor produced by lymphocytes, was first described at the University of California in 1968 [2]. TNF itself was subsequently isolated by researchers at the Memorial Sloan-Kettering Cancer Center in New York from macrophages in 1975 [3].

Recognition of the sequential and functional homology of TNF and LT led to the renaming of these two compounds as TNFα and TNFβ, respectively. TNFα was then recognised as having a key role in cachexia and as a principal mediator of septic shock in patients with infection [4, 5]. This molecule was ultimately found to be the prototype for the large family of TNF cytokines whose members are involved in the control of cell differentiation, proliferation

and apoptosis, most notably in the immune and haematopoietic systems. Human TNFα is a nonglycosyated protein consisting of 157 amino acids that exists in both soluble and membrane-bound forms and is secreted by a variety of cell types, including macrophages, monocytes, neutrophils and T cells [6].

TNFα binds to two receptors, the 55 kDa TNFR1 (CD120a or p55, widely expressed on virtually all nucleated cell types) and the 75 kDa TNFR2 (CD120b or p75, expressed mainly by activated white blood cells and endothelial cells) [7]. The extracellular domains of TNFR1 and TNFR2 bind to the cleft between the subunits of the TNFα molecule, which initiates signalling [8]. The presence of two receptors allows for a wide diversity of signalling functions. Activation of TNFR1 can have a number of outcomes, depending on the availability of accessory proteins in differing cell types: the cytoplasmic domain of TNFR1 includes a death domain motif that initiates apoptosis after activation of caspases 3 and 8 (Fig. 1). Alternatively, TNF receptor–associated factor 2 (TRAF2) can recruit cellular inhibitors of apoptosis and activate pathways leading to nuclear translocation of antiapoptotic transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) (Fig. 1).

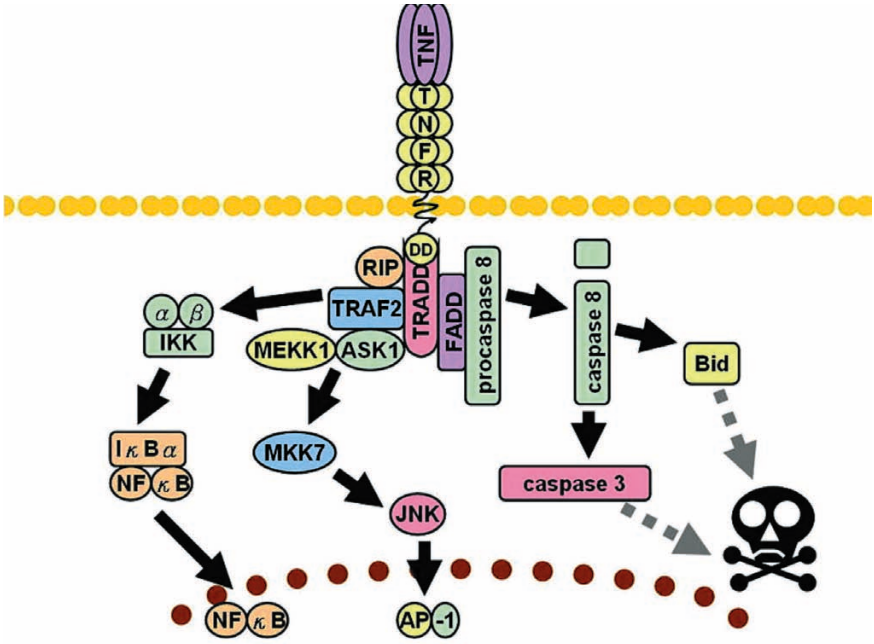

Figure 1. Simplified signaling pathways of TNFα. Dashed lines represent multiple steps. AP-1, activator protein-1; ASK1, apoptosis signal-regulating kinase 1; FADD, Fas-associated death domain; IKK, I κB kinase; JNK, c-Jun N-terminal kinase; MEK, mitogen-activated protein kinase kinase; MEKK1, MEK kinase 1; MKK, mitogen-activated protein kinase kinase 7; NF, nuclear factor-κB; RIP, receptor-interacting protein; TNFR, TNF receptor; TRADD, TNF receptor-associated death domain; TRAF2, TNF receptor-associated factor 2. Bid is a pro-apoptotic member of the Bcl-2 family.

These regulate the expression of genes blocking apoptosis, increasing cell proliferation and increasing expression of proinflammatory proteins [9]. Indeed, the NF-κB pathway alone activates more than 200 proinflammatory genes [10]. TNFR2 has specific signalling functions in T cells [11]. Although this receptor lacks the death domain found on TNFR1, it can mediate apoptosis via another, currently unknown, pathway [12]. TNFR2 can also activate NF-κB and AP-1.

TNFα in the pathogenesis of CD, RA and psoriasis

Normally, expression of TNFα and other proinflammatory cytokines is held in balance by anti-inflammatory factors, but this balance is shifted in inflammatory disease. Uncontrolled or excessive activity of TNFα leads among other effects to the chronic inflammation that characterises diseases such as CD, RA and psoriasis [13]. Through the pathways summarised above, TNFα upregulates adhesion molecules in endothelial tissue, stimulates fibroblast proliferation and recruits leukocytes into synovial fluid [14]. TNFα stimulates production of other cytokines and chemokines, reactive oxygen species, nitric oxide and prostaglandins, and increases rates of protease-mediated tissue remodelling [15, 16]. It promotes angiogenesis and osteoclast differentiation and activates bone-resorbing osteoclasts, which leads to joint erosion, particularly at marginal surfaces [17, 18]. TNFα also directly mediates pain, fever and cachexia.

CD

CD is mediated by T cells and is characterised by relapsing inflammation of the gut with extraintestinal manifestations typically involving the skin, eyes and joints. The causative factors remain unknown, although bacteria appear to play a major role in the inflammatory process [19]. The significance of the disease in terms of healthcare resource consumption is underlined by a prevalence of around 0.1% across the developed world [20] and the presence of complications such as perianal fistulae (which may require surgery) in as many as 43% of patients [21].

An imbalance in cytokine expression is central to the pathogenesis of CD. Inflammation of the gut is marked by mucosal infiltration by neutrophils and macrophages (Fig. 2), which in turn activates T cells [22]. T-helper (Th) type 1 cells subsequently stimulate the production of proinflammatory cytokines, which amplify the immune response and promote tissue destruction [23]. Indeed, the immunopathogenesis of CD involves interactions between a number of cytokines which include not only TNFα but also a number of interleukins (ILs) (Fig. 2), as shown by studies in lamina propria mononuclear cells isolated from colonic biopsies from patients with untreated inflammatory bowel disease [23]. These cells were noted to produce IL-1β and IL-6 as well as TNFα. In addition, lamina propria T cells have been reported to resist apoptosis in patients with inflammatory bowel disease [24, 25].

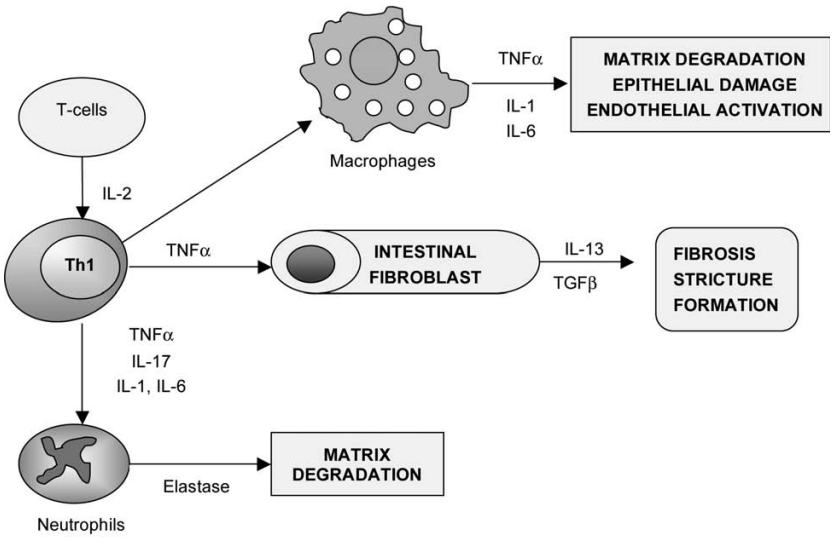

Figure 2. Extracellular molecular mediators of CD. IL, interleukin; Th1, T-helper cell type 1; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α.

This resistance is one of the best described disturbances to the immune system described in patients with this type of disorder; the beneficial effect of sulfasalazine in patients with CD appears to be at least partially attributable to the drug's proapoptotic effects in lamina propria T cells [26].

RA

As with CD, the precise aetiology of RA is unknown. However, it has become clear that the key drivers of inflammation in this disorder include most notably TNFα, in addition to IL-1 and IL-6 (Fig. 3) [14]. As in CD, Th1 cells stimulate the production of a variety of proinflammatory cytokines and destructive proteinases. This is seen after the activation of Th1 cells by antigen-presenting cells (APCs) and costimulatory pathways, and their infiltration of the synovium [14].

Cartilage explants treated with recombinant human TNFα show signs of tissue destruction, as demonstrated by enhanced resorption and inhibition of proteoglycan synthesis in experiments carried out in the 1980s [27]. Further experiments showed clinical and histologic changes indicative of RA in genetically engineered mice constitutively expressing TNFα [28]. Confirmation of these effects has come from results from three laboratories that show reduction of disease activity by anti-TNF antibodies as indicated by a standard collagen-induced arthritis model [29–31].

Psoriasis

Psoriasis, a chronic inflammatory disease of uncertain origin that affects approximately 2% of the population [32], can cause debilitating arthropathy in

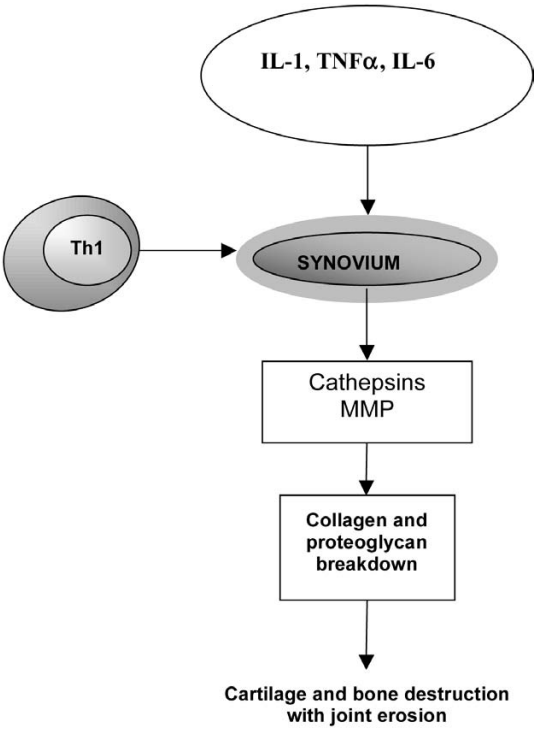

Figure 3. Extracellular molecular mediators of RA. IL, interleukin; MMP, matrix metalloproteinase; Th1, T-helper cell type 1; TNFα, tumour necrosis factor α.

as many as 30% of patients [33], and is characterised by the growth of scaly erythematous plaques. Similarly to CD and RA, psoriasis and psoriatic arthritis are mediated by T cells, which are found in abundance together with increased vascularity in skin plaques and inflamed synovial tissue. The inflammatory cascade is believed to be triggered by activation of CD4+ T cells, which generate in turn a number of proinflammatory cytokines that include TNFα. These cytokines activate CD8+ T cells, the main effectors in this disorder [34]. The centrality of the role of TNFα has been demonstrated by high concentrations of this factor in psoriatic skin lesions and the synovium of affected joints, and by its apparent importance in the perpetuation of inflammation in addition to the stimulation of angiogenesis and proliferation of keratinocytes [35].

Definitive work showing the key role played by TNFα in the pathogenesis of psoriasis has been carried out using a model in which human pre-psoriatic skin was grafted on to immunodeficient mice [36]. Approximately 6–8 weeks after engraftment, clinical and histologic features of psoriasis appeared, with increased expression of Ki-67 protein, major histocompatibility complex (MHC) Class II antigen, TNFα, IL-12, keratin 16, intracellular adhesion mol-

ecule (ICAM)-1 and platelet/endothelial cell adhesion molecule (PECAM)-1, which are all associated with inflammation. Neutralisation of TNFα significantly reduced indices of papillomatosis and acanthosis, and was linked to reduced numbers of T cells in grafts. This implies that the development of psoriasis and associated proliferation of T cells depends on the expression of TNFα.

The development of anti-TNFα agents

The monoclonal antibodies: infliximab (Remicade®) and adalimumab (Humira®)

Evolving understanding of the role of TNFα as the primary mediator of the inflammatory diseases, together with advances in recombinant gene technology, has driven the development of monoclonal antibodies (mAbs) against TNFα as potential novel therapies for CD, RA and psoriasis. The first steps in mAb therapy were taken in the mid-1980s with the development of a murine mAb (muromonab-CD3) that recognised the CD3 antigen found on human T cells, and this proved useful in the management of renal transplant rejection. This compound was suitable for short-term use only, however, because of the development of an immune response in most patients [37, 38], and further research was needed to reduce the immunogenicity of these antibodies [39]. Further progress in this field resulted in the introduction of two mAbs against TNFα for use in patients with immunologic inflammatory disease; these are the chimeric mAb infliximab, and, more recently, the human mAb adalimumab.

Pharmacology

In the chimeric mAb infliximab (Tab. 1), the first anti-TNFα agent to be introduced for the management of CD, RA and psoriasis, the human regions consist of immunoglobulin G1κ (IgG1κ) constant regions, while the variable regions are murine. As reviewed by Wong et al. [40], infliximab binds to both soluble and membrane-bound TNFα with high affinity and specificity, but does not bind to TNFβ. Its binding to transmembrane TNFα can mediate apoptosis, and its specificity limits potential for unwanted effects on other biological pathways. However, the presence of the murine variable region can lead to the production of human anti-chimeric antibodies, which may limit the therapeutic applications of infliximab [41].

Administration is by intravenous infusion, typically at a dose of 3–5 mg/kg every eight weeks, increasing dosing to 10 mg/kg or increasing the dosing interval frequency to every 4–5 weeks, if efficacy weakens [42].

Adalimumab is a humanized IgG1 anti-TNFα mAb generated by antibody guided selection using phage display technology. Similarly to infliximab, it binds soluble and transmembrane TNFα with high affinity, but unlike infliximab is not chimeric and has entirely human amino acid sequences.

Table 1. Comparative characteristics and pharmacology of anti-TNFα agents [96, 42, 97]

Characteristics and parameters	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
Structure	Chimeric mouse-human IgG1 mAb	Recombinant human IgG1 mAb	PEGylated humanized anti-Fab' fragment of an anti-TNFα mAb No Fc region
Conjugate	None	None	PEG2*: 2 × 20 kDa chains
Route of administration	Intravenous	Subcutaneous	Subcutaneous
Dose	3–5 mg/kg at weeks 0, 2 and 6, then 8-weekly. Can be increased to 10 mg/kg or frequency increased	40 mg every second week (or weekly if necessary) (RA). An induction of up to 160 mg followed by 80 mg 2 weeks later is required for CD. An 80-mg initial dose followed by 40 mg 1 week later is required for psoriasis	400 mg every 2 weeks for first 4 weeks, then every 4 weeks
Half-life (days)	7.7–9.5	10–20	14
Major biomarkers affected	CRP, inflammatory cytokines, anti-CCP, RF, MMPs, bone and cartilage markers, regulatory T cells	CRP, inflammatory cytokines, anti-CCP, RF, MMPs	CRP

CCP, cyclic citrullinated peptide; CD, Crohn's disease; CRP, C-reactive protein; Ig, immunoglobulin; mAb, monoclonal antibodies; MMPs, matrix metalloproteinases; PEG, polyethylene glycol; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor.
* PEG2 is linked to the Fab cysteine thiol group.

Nevertheless, immune responses to adalimumab in patients are observed, and have been found to weaken the clinical response to treatment [43, 44]. The formation of antibodies to infliximab and adalimumab can be attenuated by the use of immunosuppressants such as methotrexate (MTX) or azathioprine/6-MP. In contrast to infliximab, adalimumab is given subcutaneously at a dose of 40 mg every two weeks, although this can be escalated to 40 mg once a week if necessary (Tab. 1).

Clinical development

The efficacy of infliximab in CD has been documented in randomised and controlled clinical studies. An early trial in 108 patients with moderate to severe disease showed an 81% response to a single dose of 5 mg/kg; this was compared with a 17% response rate in patients receiving placebo. Clinical remission was achieved by half of all patients on active treatment [45]. In the later ACCENT I study in 573 patients with a score of at least 220 on the Crohn's

Disease Activity Index (CDAI), 58% of patients responded to a single infusion of infliximab within two weeks [46]. Patients who responded to an initial dose of infliximab were found to be more likely to be in remission after 30 and 54 weeks, to discontinue corticosteroids and to maintain their response for longer periods, if active therapy was maintained every eight weeks.

Infliximab has also been shown to be effective for the management of fistulising disease, and for long-term therapy in patients with active CD not responding to conventional treatments [47, 48]. Approval for infliximab maintenance therapy in patients with fistulising CD was based on the results of the ACCENT II study in 306 patients [49]. The time to loss of response was significantly longer for patients who received infliximab maintenance therapy than for those who took placebo (>40 weeks *versus* 14 weeks; $P < 0.001$). At week 54, 19% of patients in the placebo group and 36% of those taking infliximab had complete absence of draining fistulae ($P = 0.009$).

Infliximab is approved for the treatment of active RA in patients failing disease-modifying therapy, and for severe progressive RA in previously untreated patients. In the ATTRACT trial in 428 patients taking MTX, patients given infliximab showed significant improvements in all indices of response [50]. Median changes from baseline to week 102 in the total radiographic score were 4.25 for MTX plus placebo and 0.50 for MTX plus infliximab. Quality of life was improved for up to two years in the MTX plus infliximab group. Benefit of early aggressive therapy for RA with infliximab was shown in the ASPIRE trial in patients not previously treated with MTX [51]. In this trial in 1049 randomised patients, infliximab plus MTX virtually halted radiographic progression of disease, with improvement noted in all American College of Rheumatology (ACR) indices.

Infliximab was approved for the management of psoriasis on the basis of two randomised and controlled trials. In the SPIRIT study in 249 patients with severe plaque psoriasis, 88% of patients treated with infliximab 5 mg/kg and 72% of those receiving 3 mg/kg achieved a 75% or greater improvement from baseline at week 10 in Psoriasis Area and Severity Index (PASI) score, compared with 6% of patients receiving placebo [52]. These changes were accompanied by improvements in health-related quality of life. In the subsequent EXPRESS study in 378 patients with moderate to severe disease [53], 80% of patients treated with infliximab achieved an improvement from baseline of at least 75% in PASI score, compared with 3% of placebo patients. High percentages of patients who received infliximab maintained their PASI responses for one year.

Adalimumab has been shown to be effective in the induction and maintenance of clinical efficacy in CD in the CLASSIC [54, 55], CHARM [56] and GAIN [57] double-blind, placebo-controlled trials. In CLASSIC I [54], 299 patients with moderate to severe CD received at weeks 0 and 2 subcutaneous injections of adalimumab 40 mg/20 mg, 80 mg/40 mg or 160/80 mg or placebo. Rates of remission at week 4 ranged from 18–36% in the adalimumab groups, compared with 12% in the placebo group. Maintenance of clinical remission for up to 56 weeks with adalimumab was shown in the subsequent

CLASSIC II trial [55]. In the CHARM trial [56], open-label induction therapy with adalimumab 80 mg was followed by 40 mg at week 2; randomisation stratified by response took place at week 4. Percentages of randomised responders in remission were significantly ($P < 0.001$) greater with adalimumab 40 mg every other week or 40 mg weekly than with placebo at week 26 (40%, 47% and 17%, respectively) and at week 56 (36%, 41% and 12%, respectively). In the four-week, placebo-controlled GAIN trial, 325 adult patients with moderate to severe CD who had symptoms despite infliximab therapy or who could not take infliximab because of intolerance were randomly assigned to receive induction doses of adalimumab, 160 mg and 80 mg, at weeks 0 and 2, respectively, or placebo at the same time points. 21% of patients in the adalimumab group *versus* 7% of those in the placebo group achieved remission at week 4 [57].

Adalimumab has been assessed in more than 2000 patients with RA [42, 58, 59]. In study DE019, 63, 39 and 21% of patients receiving adalimumab 40 mg every other week plus MTX achieved ACR20, 50 and 70 responses respectively at week 24 [59]. All response rates were statistically significantly better than those in patients receiving placebo plus MTX ($P < 0.001$). Recent data from a 52-week study in 1,212 patients have shown efficacy of adalimumab in moderate to severe psoriasis [60]. At week 16, an improvement of at least 75% in PASI score was seen in 71% of patients receiving adalimumab 40 mg every other week and in 7% of placebo recipients. Continuing treatment with adalimumab was associated with greater proportions of patients maintaining response for up to a year than with placebo.

Infliximab and adalimumab are both licensed to treat adults with moderate to severely active CD, whilst infliximab, the older of the two mAbs, is also indicated for the treatment of paediatric CD patients and in adults with moderately to severely active ulcerative colitis. Both agents are also approved to treat moderately to severely active RA, psoriatic arthritis, ankylosing spondylitis as well as severe plaque psoriasis. Adalimumab is further indicted in juvenile idiopathic arthritis.

Certolizumab pegol (CIMZIA®): a PEGylated antigen-binding fragment of a TNFα monoclonal antibody

Certolizumab pegol represents a further technological advance in the development of therapeutic mAbs by combining the science of pharmaceutical PEGylation with recombinant gene technology.

Certolizumab pegol is a polyethylene glycol (PEG)-conjugated, humanised, antigen-binding fragment (Fab') of an anti-TNFα mAb that is suitable for subcutaneous administration (Tab. 1). This agent has been developed to bind to and neutralise both soluble and membrane-bound forms of TNFα, and is formed by grafting the short hypervariable complementarity-determining regions derived from the murine mAb HTNF40 into the framework of a human Ig Fab' fragment.

The pharmacokinetic properties of Fab' fragments *in vivo* usually result in a short half-life, but attachment of a 40 kDa PEG moiety markedly increases the half-life of the molecule to approximately 14 days, making it suitable for every-other-week, or monthly, dosing. Engineering of the Fab' fragment with a single free cysteine residue in the hinge region enables site-specific attachment of PEG without affecting the ability of the Fab' fragment to bind and neutralize TNFα [61]. Importantly, certolizumab pegol is devoid of the Fc region, therefore, unlike infliximab and adalimumab, there is no complement fixation and cell lysis is not observed (Fig. 4) [62]. In addition, there is no neonatal Fc

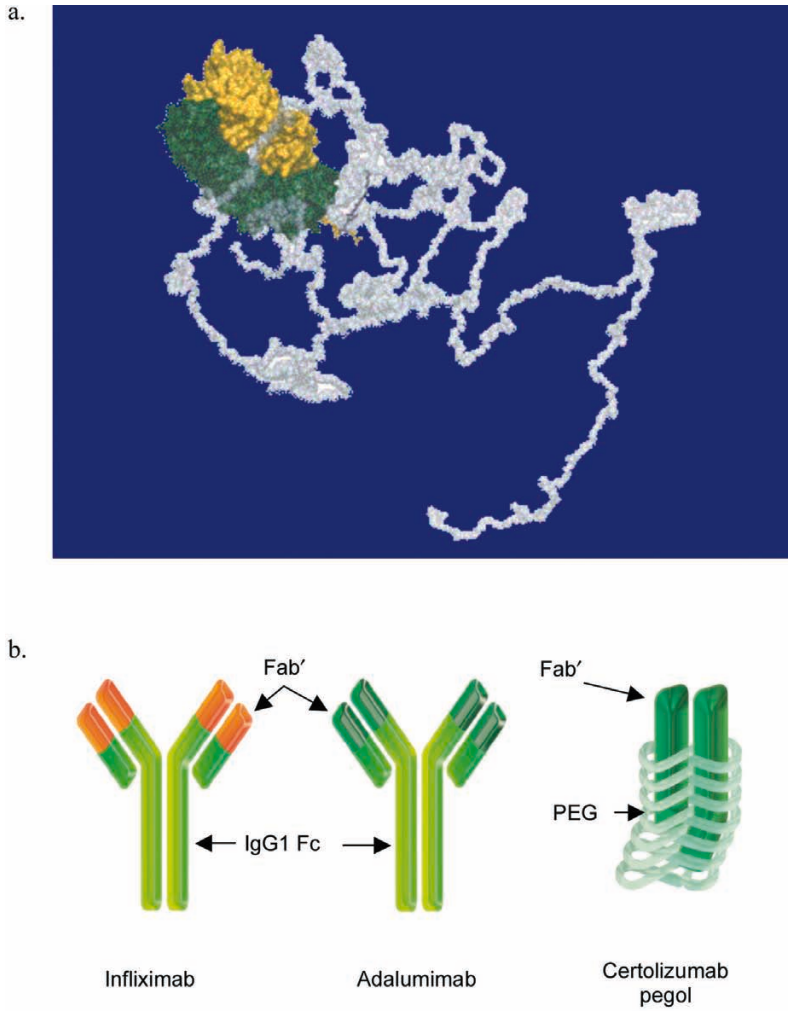

Figure 4. a. Molecular structure of certolizumab pegol. The PEG moiety is shown in grey. b. Comparative schematic representations of the three anti-TNFα monoclonal antibodies.

receptor(FcRn)-mediated transport, which could be the reason for preferential distribution and retention in inflamed tissue [63].

The FcRn molecule is involved in protecting Ig from catabolism and transporting it around the body as well as mediating transport across the placenta to provide immunity for the foetus. Certolizumab pegol is also distinguished within anti-TNFα mAbs by its valency: this compound is univalent, thus reducing the potential for large immune complex formation [64] whereas infliximab and adalumimab are divalent.

Affinities for TNFα and neutralisation properties of the anti-TNFα mAbs are essentially similar [62], but PEGylation does confer distinct characteristics. PEG is a branched polyether of variable chain length that is heavily hydrated, which increases the haemodynamic radius of proteins. PEGylated proteins tend not to diffuse well and are therefore retained with high bioavailability in the bloodstream [65]. The detailed structure of certolizumab pegol has been determined by x-ray crystallography [66], and the molecule found to be highly flexible and asymmetrical (Fig. 4). The PEG moiety is attached to Fab' at a specific point at the Fab' C-terminus, but it does not interact directly with the surface of the Fab', which remains unaffected by any interaction, rearrangement or modification by the PEG moiety.

Mechanism of action

The mechanism of action of certolizumab pegol has been investigated and contrasted with those of infliximab and adalimumab in a series of *in vitro* studies examining affinity for and neutralisation of TNFα; induction of antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), apoptosis, degranulation of neutrophils and inhibition of lipopolysaccharide (LPS)-induced cytokine production [62]. All agents tested neutralised soluble TNFα and bound to and neutralised membrane-bound TNFα. Neutralisation of membrane-bound TNFα signalling was found to be mediated via both p55 and p75 receptors [67]. Affinity of certolizumab pegol for TNFα is high: measurement of the relative affinities of infliximab, adalimumab and certolizumab pegol was carried out using surface plasmon resonance. The affinity of certolizumab pegol for soluble TNFα was higher than that of either of the other two agents (Tab. 2) [62].

Resistance of cells such as T cells to apoptosis has been proposed to play an important role in inflammatory bowel disease [68] for example, and TNFα is a known survival factor in certain cell types. When compared with control human IgG1, treatment with infliximab and adalimumab caused CDC and ADCC of a TNFα transfected cell line with high levels of TNFα on the cell surface. As expected, certolizumab pegol did not mediate cell killing either directly through fixation of complement or via recruitment of effector cells; this is attributable to its lack of an Fc region. Additional experiments illustrated that certolizumab pegol did not mediate apoptosis of activated lymphocytes and monocytes by signalling through membrane TNFα *in vitro*, whereas infliximab and adalimumab both mediated this effect [62]. Therefore, direct

Table 2. Affinities of infliximab, adalimumab, and certolizumab pegol for soluble TNFα [62]

Parameter	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
Association rate constant, k_a (M^{-1} s^{-1})	$1.01 \pm 0.06 \times 10^6$	$0.724 \pm 0.30 \times 10^6$	$1.22 \pm 0.09 \times 10^6$
Dissociation rate constant, k_d (s^{-1})	$2.30 \pm 0.34 \times 10^{-4}$	$1.14 \pm 0.12 \times 10^{-4}$	$1.09 \pm 0.13 \times 10^{-4}$
Equilibrium constant, K_D (k_d/k_a, pM)	227.2	157.4	89.3

Data are means ± SD.

induction of apoptosis via binding of anti-TNFα agents to membrane-bound TNFα may not necessarily be required for clinical efficacy in autoimmune inflammatory disease. The reason why certolizumab pegol does not induce apoptosis in cells bearing membrane-bound TNFα is not clear; one hypothesis is that this agent binds to a different epitope to the other mAbs, which may lead to a different signalling pattern inside the cell. Indeed, it has recently been shown that all three clinically effective anti-TNFα agents are able to induce *in vitro* apoptosis in co-cultivated CD4+/CD14+ cells cultivated from lamina propria mononuclear cells derived from gut specimens of patients with CD. However, this is believed to be due to an indirect mechanism which is not related to signalling through membrane-bound TNFα [69].

Neutrophils are commonly found at sites of inflammation and are able to produce TNFα upon stimulation [70]. Incubation with certolizumab pegol *in vitro* did not result in changes to the integrity of polymorphonuclear neutrophilic leukocytes (PMNs), whereas degranulation and loss of cell membrane integrity were markedly increased after incubation with infliximab or adalimumab. Thus, infliximab and adalimumab induce loss of cell viability and release of intracellular granular material into the surrounding medium, whereas certolizumab pegol does not [62].

Preincubation of human monocytes with certolizumab pegol at concentrations of 1 μg/mL and above completely inhibited production of IL-1β in response to stimulation with LPS. Although all three agents caused complete inhibition of this cytokine at concentrations of 1 μg/mL, certolizumab pegol appeared to be the most potent compound in this respect, showing a slower titration of the attenuation of IL-1β expression at concentrations below 1 μg/mL [62]. LPS stimulation of cells of the immune system represents a powerful signalling mechanism, which suggests that the inhibition mediated by anti-TNFα agents may be an important anti-inflammatory process.

Stoichiometry and complex formation

The univalent nature of certolizumab pegol may be at least in part responsible

for some of the differences in mechanistic behaviour of this molecule when compared with infliximab and adalumimab. Binding of biological agents to TNFα can result in the formation of immune complexes, which can have unwanted destructive effects such as the PMN degranulation and superoxide production. Dynamic light-scattering studies have shown that, at an anti-TNFα:TNFα molar ratio of 1:1, adalimumab and infliximab form very large complexes of diameter exceeding 30 nm. In contrast, certolizumab pegol does not form these large complexes (Tab. 3). Isothermal titration calorimetry data show also that certolizumab pegol binds to 2.9 monomers in a TNFα trimer at saturation compared with 2.63 and 2.44 monomers in a TNFα trimer for infliximab and adalimumab, respectively [64]. Taken together, the dynamic light scattering and isothermal titration calorimetry data show that infliximab and adalimumab are able to cross-link TNFα trimers by binding to monomers in different trimers (Fig. 5). Certolizumab pegol cannot cross-link in this way due to its univalent structure. The large complexes produced by infliximab and adalimumab were noted to have proinflammatory effects on PMNs *in vitro* (see previous), which caused these cells to degranulate and produce superoxide ions [64].

Table 3. Approximate molecular weights (kDa) of anti-TNFα/TNFα immune complexes at differing molar ratios [64]

Parameter	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
TNFα alone	48	33	34
Antibody alone	289	286	417
Antibody:TNFα ratio 1:1	2727	2304	613
Antibody:TNFα ratio 4:1	559	277	581

PEGylation and tissue penetration

PEGylated molecules tend to diffuse slowly from blood because of the haemodynamic properties of PEG [65]. This gives rise to the possibility that such molecules might have distribution patterns that differ from intact IgG1 in terms of their exposure times in inflamed and non-inflamed tissues. The disposition of certolizumab pegol, infliximab and adalumimab has therefore been investigated using a biofluorescence method in healthy and inflamed murine tissue in two experiments [63]. All agents were conjugated with Alexa680, a low molecular weight fluorescent dye, and administered intravenously to healthy DBA/1 mice and to DBA/1 mice with collagen-induced arthritis. Levels of agents in hind paws were then measured.

All three agents penetrated inflamed tissue more effectively than non-inflamed tissue, but certolizumab pegol penetrated inflamed arthritic paws

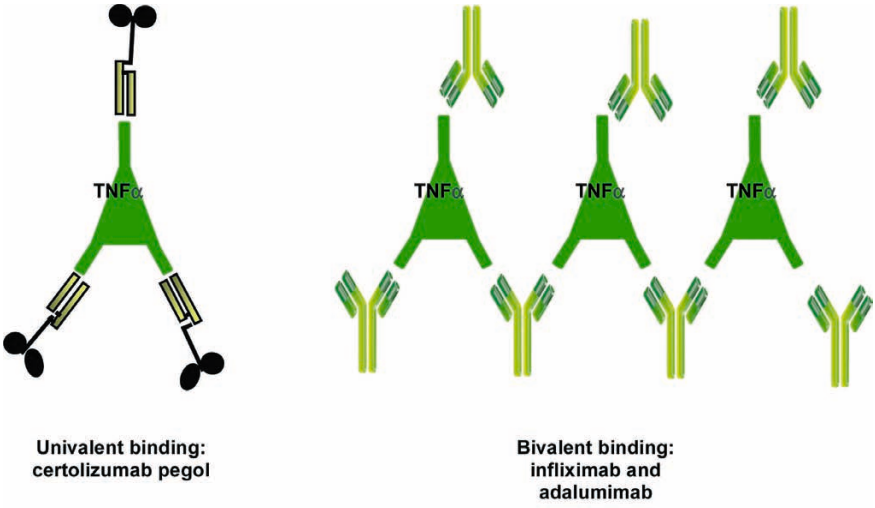

Figure 5. Formation of immune complexes via TNFα trimer cross-linking [64].

most effectively and was retained for longer than infliximab or adalimumab. The longer retention time could be a result of the low diffusion rate of PEGylated molecules and/or the lack of an Fc, which leads to a lack of recycling by the FcRn. Therefore, it is possible that, due to PEGylation, certolizumab pegol will have a higher inflamed to normal tissue ratio than a mAb. Indeed, macromolecules such as PEG have been suggested as possible vehicles for delivery of drugs to rheumatoid joints because of this very property [71].

As discussed earlier, certolizumab pegol has no Fc region. Adalimumab and infliximab are whole IgG1 molecules and possess an Fc region, which potentially allows passage across the placenta via specific neonatal FcRn receptors [72]. In an experiment to test for the likelihood of placental transfer of PEGylated Fab's [73], rats were injected during gestation with either a complete chimeric anti-murine TNFα antibody (cTN3 IgG1) or a PEGylated Fab' fragment (cTN3 PF). In foetal samples from five rats given cTN3 PF while pregnant, PEGylated Fab' was found at very low concentrations in only two of the samples and was entirely undetectable in the others. This suggests that the PEGylated fragment does not undergo FcRn-mediated placental transfer, but that there may be a low level of passive transfer as with all proteins. However, biologically relevant levels of intact IgG1 were found in rats given whole immunoglobulin, which indicates that this molecule had crossed the placenta. Concentrations in milk of PEGylated Fab' were also much lower than those of IgG1 eight days postpartum.

Thus, on the basis of these findings, certolizumab pegol, as an Fc-free molecule, would not be expected to cross the placenta via FcRn receptors. As the

number of pregnant women whom have been exposed to these drugs is low, this area of study remains unresolved and requires further research.

In vivo *pharmacology*

In vivo pharmacological testing of certolizumab pegol was originally carried out in animal models, with initial research focusing on the fate of the PEG moiety after administration. Radiolabelled PEG is not suitable for quantification because it is unstable [74]. Therefore, distribution and elimination studies have been carried out using ^{1}H nuclear magnetic resonance spectroscopy (NMR) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

In rat studies [75], nine animals were given one subcutaneous injection of certolizumab pegol at a dose of 400 mg/kg, with daily urine and faeces samples being collected for 84 days; a further group of rats received 100 mg/kg subcutaneously of either certolizumab pegol or cTN3 PF (cTN3 PF is a Fab' conjugated with 40 kDa PEG, which acts as a homologous reagent binding to and neutralising rat TNFα and was used to show any tissue targeting due to TNFα binding). These rats were killed at prespecified intervals in groups of four, and their major organs removed for NMR examination. In an additional study, certolizumab pegol was injected into the tail veins of male Lewis rats at doses of 10 or 100 mg/kg, and urine collected over 28 days for analysis by SDS-PAGE [75]. The data showed overall that PEG derived from certolizumab pegol is distributed to major organs (excluding the brain), it has a plasma half-life of approximately three weeks, it is not influenced by target site binding and it is excreted largely unchanged by the kidneys with first-order kinetics. Moreover, urinary data showed the rate of excretion essentially to match the rate of administration, with no evidence of accumulation.

NMR analysis showed PEG to be detectable in urine for up to 84 days after administration, with the peak concentration of 198 μg/mL being observed on day 4. PEG concentrations then declined in a first-order manner to 14 μg/mL during week 12. Mean daily excretion reached 1.9% on day 6 and declined to 0.21% during week 12. The mean cumulative dose excreted in urine was 65% after 84 days. Extrapolation to infinity projected a total urinary excretion of 73%, with a half-life for elimination of the PEG component of 23.4 days, much longer than the two-day plasma elimination half-life of certolizumab pegol. Total faecal excretion in rats was 18%, with possible ingestion of a portion of the PEG dose from the injection site and some urinary contamination accounting for approximately 11% of faecal recovery. Published murine data [76] suggest that approximately 1% of the dose of 40 kDa PEG might be excreted via the biliary route, although this remains unconfirmed. Overall, NMR analysis indicated the mean urinary and faecal excretion of PEG in rats to be 83% of an administered dose after 84 days, with extrapolation to a final total of >90% [75]. There was no evidence of accumulation in any major organ, with the spleen having the highest level of exposure, and the highest

proportion of the dose being found in the liver (the largest organ). Distribution of certolizumab pegol was found to be similar to that for cTN3 PF, which implies that TNFα binding is not a significant mediator of PEG distribution in tissue [75]. SDS-PAGE analysis showed that the PEG excreted in urine was 40 kDa, suggesting that after the Fab' was cleaved from the PEG, the PEG was cleared by the kidney with no further metabolism [75].

Clinical development

The safety profile and pharmacological properties of certolizumab pegol have been assessed in primates and in healthy human volunteers. Several clinical trials have also been conducted to examine the efficacy and tolerability of certolizumab pegol in patients with CD, RA and psoriasis.

Pharmacology in primates and humans

The disposition of certolizumab pegol has been characterised over a range of doses in primates and in healthy human volunteers [77]. Human males aged 18–50 years were recruited in two studies: a double-blind, placebo-controlled, ascending single-dose trial in 16 patients of certolizumab pegol 0.3–10.0 mg/kg given by intravenous infusion over 60 min, and a double-blind, double-dummy, ascending single-dose study in 24 patients who received a subcutaneous bolus of 20, 60 or 200 mg or an intravenous infusion of 1 mg/kg. In a study in non-human primates, 20 cynomolgus monkeys were divided into five groups and given certolizumab pegol by intravenous infusion over 60 min (50, 100 or 400 mg) or by subcutaneous bolus injection (3.0 or 31 mg/kg). Plasma concentrations of certolizumab pegol were determined using an enzyme-linked immunosorbent assay (ELISA).

Certolizumab pegol showed linear pharmacokinetics over the dose ranges studied in both species, with biexponential disposition after intravenous dosing and monoexponential disposition after subcutaneous injection (Tab. 4). Volumes of distribution were equivalent in terms of species-relevant plasma volumes, and subcutaneous absorption was sustained, with low clearances and extended plasma elimination half-lives (approximately two weeks).

Table 4. Pharmacokinetic parameters (modeled estimates with 95% CIs) of certolizumab pegol in humans and primates (cynomolgus monkeys) after intravenous or subcutaneous administration [77]

Parameter	Humans	Primates
Central volume of distribution, V_1 (mL/kg)	45 (44–47)	46 (44–48)
Total body clearance, CL (mL/h/kg)	0.17 (0.16–0.18)	0.29 (0.27–0.31)
Peripheral volume of distribution, V_2 (mL/kg)	29 (25–33)	31 (26–37)
Intercompartmental clearance, CLd (mL/h/kg)	0.69 (0.52–0.90)	1.1 (0.8–1.5)
Elimination half-life, $t_{1/2}$ (h)	313 (284–345)	192 (174–212)
Bioavailability, F	1 (0.9–1.1)	1.1 (1.0–1.3)
Absorption rate constant, Ka (h^{-1})	0.018 (0.015–0.020)	0.11 (0.08–0.16)

Pharmacokinetics were shown to be predictable, with extended or equivalent exposure relative to anti-TNFα agents already available for clinical use. Certolizumab pegol had bioavailability of approximately 80% (ranging from 76–88%) following subcutaneous administration in humans [78].

Evaluation of 10,275 plasma concentration *versus* time records from 1,580 persons (80% with CD and 15% with RA, and 5% of whom were healthy individuals) showed the clearance of certolizumab pegol to be typically 0.428 L/d, with volume of distribution 4.0 L in a 70 kg person [79]. The disposition of certolizumab pegol was found to be unaffected by age, gender, creatinine clearance, leukocyte count and concomitant use of drugs such as corticosteroids, aminosalicylic acid and its analogues and anti-infectives. Of other covariates tested, only the presence of antibodies to certolizumab pegol had an effect of more than 30% on peak plasma concentrations and areas under curves of plasma concentration *versus* time, but such antibodies were found in only 8% of individuals tested [79]. A doubling in body weight was found to be the second most influential covariate. Overall, however, none of the covariates tested had a clinically relevant effect. Notably, other recent data show that the PEG component of certolizumab pegol is not immunogenic and therefore does not provoke the production of antibodies [80]. A small proportion of patients have been noted to produce antibodies to the protein part of certolizumab pegol, but these antibodies have been shown not to cross-react with other anti-TNFα mAbs [81].

CD

The efficacy and safety of subcutaneously administered certolizumab pegol was examined in two Phase II studies in patients with moderate to severely active CD [82, 83]. In the first, 92 patients with moderate to severely active disease were randomised to a single intravenous infusion of certolizumab pegol 1.25 mg/kg, 5 mg/kg, 10 mg/kg or 20 mg/kg or placebo. Although the primary endpoint of this study, a difference in the rate of clinical response or remission at week 4 between either of the active treatment arms and placebo, was not attained, a significantly different remission rate at week 2 was observed for the 10 mg/kg dose compared with placebo [82]. Certolizumab pegol was well tolerated by the patients in this study, with the majority of adverse events being of mild to moderate intensity and with no infusion reactions being reported.

In a larger, Phase II dose-ranging study, patients received subcutaneous injections of certolizumab pegol 100 mg (n = 74), 200 mg (n = 72), 400 mg (n = 72) or placebo (n = 73) at weeks 0, 4 and 8 with no induction dose, and efficacy assessments were made every two weeks until week 12. The highest subcutaneous dose of 400 mg was the most effective in inducing clinical response and remission relative to all time points [83]. Although the primary efficacy endpoint of clinical response at week 12 was not met, statistically significant differences between certolizumab pegol 400 mg and placebo were noted in favour of the active drug as early as week 2, and also at week 4, week

8 and week 12. Certolizumab pegol 400 mg had a favourable safety profile, with a similar percentage of patients reporting adverse events as in the placebo group over a 12-week period.

Data from these Phase II studies facilitated the design of the two Phase III trials (PRECiSE 1 and 2), which were the next stage in the development of certolizumab pegol for the treatment of patients with moderate to severely active CD. Patients received an induction dose consisting of certolizumab pegol 400 mg at weeks 0, 2 and 4 followed by a four-weekly 400-mg maintenance dose. To further investigate influence of CRP, patients in PRECiSE 1 and 2 were stratified according to baseline CRP levels ≥10 mg/L or <10 mg/L. In the PRECiSE 1 trial, 662 patients were randomised to double-blind induction and maintenance treatment with certolizumab pegol or placebo for 26 weeks [84]. In the 26-week PRECiSE 2 trial, 668 patients received open-label induction with certolizumab pegol and those (n = 428; 64%) with a clinical response (≥100 points decrease in baseline CDAI score) at week 6 were stratified according to CRP level and randomised into the double-blind, placebo-controlled maintenance phase [85].

The efficacy endpoints in PRECiSE 1, of clinical response at week 6 and clinical response at both week 6 and week 26, were met both in the primary population of patients with CRP levels ≥10 mg/L and in the overall patient population [84]. Similarly, CRP levels also had no influence over the efficacy outcomes in the PRECiSE 2 study [85].

Patients who completed PRECiSE 1 and 2 were eligible for entry into the long-term open-label extension study, PRECiSE 3, where the long-term efficacy and safety outcomes of scheduled certolizumab pegol treatment can be assessed. Patients withdrawing from PRECiSE 1 or 2 due to exacerbation of symptoms of CD were eligible for entry into the open-label study, PRECiSE 4, designed to evaluate the efficacy and safety of reinduction. Interim results from these two studies have shown that remission rates after 18 months of continued treatment in PRECiSE 3, and after 12 months of treatment following reinduction in PRECiSE 4, have remained stable. Furthermore, no new safety signals were identified following re-exposure of placebo patients from PRECiSE 2 to certolizumab pegol [86].

Certolizumab pegol has a good tolerability profile in patients with CD. Safety data for 1,328 patients who took part in the PRECiSE 1 and 2 studies illustrated that the majority of adverse events were rated as mild to moderate in intensity, with headache, nasopharyngitis, abdominal pain and cough being the most common adverse events in the certolizumab pegol treatment groups [87]. Local injection reactions were rare with certolizumab pegol (<3%) and autoantibody seroconversion rates were low (anti-nuclear: 8.3% or less and anti-double-stranded DNA: 1.4% or less) [87].

The clinical development programme of certolizumab pegol in CD continues with Phase IIIb studies which have been designed to examine aspects such as the efficacy of certolizumab pegol in infliximab-refractory patients (WELCOME) and in other measures of efficacy such as mucosal healing (MUSIC).

RA

Several Phase II and III trials have assessed the safety and efficacy of certolizumab pegol in patients with RA. In a Phase II double-blind, randomised trial (n = 36), certolizumab pegol demonstrated significant efficacy in reducing inflammation and improving symptoms of RA [88]. In this trial, 75% of patients who received an intravenous infusion of certolizumab pegol at 5 or 20 mg/kg achieved ACR 20 scores after eight weeks of treatment.

In a Phase III trial, the efficacy and safety of subcutaneous certolizumab pegol monotherapy (400 mg every four weeks) was assessed in patients with active RA who had previously failed therapy with at least one disease-modifying antirheumatic drug (DMARD). At week 24, the ACR20 responder rate was 45.5% in the certolizumab pegol group (n = 111) compared with 9.3% in the placebo group (n = 109). The effect of certolizumab pegol treatment was evident early after treatment, with 80.6% of ACR20 responders having achieved this response by week 1 [89]. In addition, certolizumab pegol treatment provided rapid and sustained improvements in physical function and pain relief in these patients who had previously failed DMARD therapy [90].

The efficacy and safety of certolizumab pegol as add-on therapy to methotrexate (MTX) were evaluated in two Phase III double-blind, placebo-controlled, randomised trials (RAPID 1 and 2) [91, 92]. In these trials, adults with active RA (n = 982 and n = 619 in RAPID 1 and 2, respectively) were randomised 2:2:1 to receive subcutaneous certolizumab pegol, lyophilized formulation (RAPID 1) or liquid formulation (RAPID 2), 200 mg or 400 mg plus MTX, or placebo plus MTX, every two weeks for 52 weeks (RAPID 1) or 24 weeks (RAPID 2). In the 200-mg active treatment groups, a loading dose of 400 mg of certolizumab pegol at weeks 0, 2 and 4 was also implemented. Enrolled patients were maintained on a stable dose of MTX for the duration of the trial. Primary study endpoints were ACR20 response rate at week 24 (RAPID 1 and 2) and change from baseline in modified Total Sharp Score (mTSS) at week 52 (RAPID 1). Secondary endpoints included ACR50 and ACR70 response rates at weeks 24 and 52 in RAPID 1 and week 24 in RAPID 2. Patient-reported outcomes included physical function, health-related quality of life, pain, fatigue and RA-related work and household productivity.

In the RAPID 1 trial, the ACR20, ACR50 and ACR70 response rate for patients receiving certolizumab pegol plus MTX were significantly higher than that for patients receiving placebo plus MTX [91]. Comparable results for ACR20/50/70 response rates were observed in the RAPID 2 trial [92]. Certolizumab pegol had a fast onset of action, with statistically significant ACR20 and ACR50 improvements after the first dose [92].

In both trials, the radiographic progression of structural joint damage was significantly inhibited by certolizumab pegol plus MTX. In RAPID 1, after 52 weeks of therapy and RAPID 2, after 24 weeks of therapy, the changes from baseline in mTSS, erosion scores and joint space narrowing were significantly lower in patients receiving certolizumab pegol plus MTX compared with

patients receiving placebo plus MTX. In RAPID 1, the effects of certolizumab pegol plus MTX on the retardation of structural joint damage were observed as early as week 16 [93].

Results from RAPID 1 and 2 revealed that in addition to significantly improving the signs and symptoms of RA, certolizumab pegol plus MTX significantly improved all aspects of patients' quality of life, including physical function, SF-36 physical and mental component summary scores (a widely used health questionnaire containing 36 questions to assess quality of life), pain, fatigue and work and home productivity [94]. The rapid onset of action was also reflected in these patient reported outcomes, with improvements in physical function, pain and fatigue showing statistical significance as early as Week 1 [94].

Certolizumab pegol was well tolerated by RA patients, both when administered as monotherapy treatment and when administered as add-on therapy to MTX [90–92]. The majority of adverse events were mild to moderate, and discontinuation due to adverse events was low in all groups (<6%). In all studies there was a low incidence of injection-site pain or reactions. The incidence of infections was also minimal, and was comparable to that seen with other anti-TNFα agents [90–92].

These data further support a role for certolizumab pegol monotherapy, or combination therapy with MTX, as an effective and well-tolerated treatment option for patients with RA. Certolizumab pegol has an acceptable safety profile, significantly reduces the signs and symptoms of RA and inhibits progression of structural damage. Onset of benefit of certolizumab pegol treatment is rapid, and the combination of certolizumab pegol plus MTX causes sustained and long-term reduction in the signs and symptoms of RA.

Psoriasis

Results from the first study to evaluate the efficacy and safety of certolizumab pegol in patients with moderate to severe psoriasis illustrated that both the 200-mg and 400-mg liquid formulations of certolizumab pegol, given every two weeks over a period of 12 weeks, significantly reduced the redness, thickness and scaliness of lesions when compared with placebo treatment [95]. In this Phase II, randomised, double-blind, placebo-controlled dose-ranging study, 176 patients with moderate to severe chronic plaque psoriasis, who were candidates for systemic therapy and/or phototherapy or photochemotherapy, were randomised. 60 patients (34.1%) had previously used biologicals, including 41 (23.3%) with prior anti-TNFα use [95].

By week 12, 74.6% and 82.8% of patients in the certolizumab pegol 200- and 400-mg groups, respectively, achieved response rates on the PASI 75, compared with 6.8% in the placebo group ($P<0.001$). Furthermore, 52.5% and 72.4% of patients in the certolizumab pegol 200- and 400-mg groups, respectively, gave the rating 'clear' or 'almost clear' on the Psoriasis Global Assesment (PGA) scale, compared with 1.7% in the placebo group ($P < 0.001$) [95].

Certolizumab pegol was well tolerated in patients with psoriasis, with a safety profile consistent with what is expected from this class of therapy. The most common adverse events were headache, pruritus and cough. Administration of certolizumab pegol was well tolerated with <3% of the study population reporting injection-site reactions and <1% of patients reporting injection-site pain [95].

The results from this study suggest that certolizumab pegol has the potential for further development as a valuable new treatment option for this difficult-to-treat disease.

Closing statement

The involvement of the cytokine TNFα in immune-mediated inflammatory diseases was little known just two decades ago. In a short period, basic scientific researchers utilising cutting-edge advancements in recombinant gene technology developed mAbs-targeting TNFα, which, over the years, has been proven to be effective in bringing relief to thousands of patients with RA, CD, ulcerative colitis and psoriasis. Certolizumab pegol utilises the technique of PEGylation to enhance the pharmacokinetic properties of the antigen-binding fragment of an anti-TNFα mAb without detrimental effect to the pharmacodynamic properties. These advances have led to a compound that can be administered via subcutaneous injection every-other-week or once monthly, with a rapid onset of action, a low level of injection-site pain, preferential distribution in inflamed tissue, and, low levels of immunogenicity [84, 83]. Certolizumab pegol was approved for use in the United States in adults with moderate to severe Crohn's disease in April 2008. The continuation of the clinical development of CIMZIA® (certolizumab pegol) may lead to a significant advance in the treatment of CD, RA and psoriasis and improve the lives of patients with these diseases.

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