ONLINE SUPPLEMENTARY MATERIAL

Background

For the purpose of this publication, it may be important to note that in brief, IL-6 is on the one hand produced by a plethora of cells and on the other hand affects numerous cell populations. Among all cytokines, it is the one most abundantly present in the circulation and thus has particularly prominent endocrine activities, in addition to its para- and autocrine actions. Indeed, for example, adrenal androgens have anti-inflammatory potential and their synthesis is inhibited by IL-6: a study has shown that neutralization of IL-6 leads to an increase of serum adrenal androgens in RA patients, which might convey an additional anti-inflammatory stimulus.[1;2]

IL-6 serves as major stimulator of hepatic acute phase reactants (APR) including hepcidin production; is an anti-apoptotic cytokine for hepatocytes and thus hepatoprotective; activates B-cells and fosters growth of myeloma/plasmocytoma cells; is an important inducer of T-cell function including upregulation of IL-2 and the IL-2 receptor, and particularly induces Th17 cells,[3] a T-cell population pivotally involved in chronic inflammation; activates hematopoetic stems cells and megakaryocytes; leads to activation of osteoclasts, primarily via activation of the pivotal osteoclastogenic cytokine, receptor activator of nuclear factor κB ligand (RANKL); inhibits anabolic functions of chondrocytes; induces proliferation of synovial fibroblasts; and activates expression of adhesion molecules on endothelial cells.[4-11] While the action of IL-6 on fibroblasts may be controversial,[12] there are published data suggesting that IL-6 also stimulates synovial and dermal fibroblast proliferation.[13;14] In addition IL6 acts as a chemokine on monocytic cells by inducing integrin activation, cell adhesion, chemotaxis and cell migration.[15] Thus, IL-6 is one of the most profound and most effective proinflammatory and immunostimulatory molecules which by itself can be responsible for most of the abnormalities found in the course of inflammation, such as increased acute phase reactant [APR] levels, anemia (of chronic disease via hepcidin upregulation), thrombocytosis, increase in

immunoglobulin production (hypergammaglobulinemia, including autoantibody secretion), fatigue, mood changes and others. TNF and IL-1 induce IL-6 and mediate many of their effects via this cytokine. In reverse IL-6 can activate IL-1 and thus further amplify the inflammatory response.[16;17]

The IL-6 receptor complex comprises two polypeptide chains: (i) the actual IL-6R, also named IL-6R α-chain, which has a molecular size of 80kD and lacks a signaling moiety; and (ii) a signal transducing co-receptor molecule of 130kD size; gp130 also serves as signal transducer for other cytokines of the IL-6 family.[6;18] The IL-6R exists as a membrane bound form (m) and as a soluble form (s). Once IL-6 engages mIL-6R, an association with gp130 is triggered and the actual signaling complex involves a hexamer consisting of two gp130, two IL-6R and two IL-6 molecules. Of particular importance is the IL6-gp130 trans-signaling mechanism that mediates most of the chronic vascular inflammation, thus contributing to the pathogenesis of atherosclerosis and whose inhibition has recently been shown to dramatically inhibit the progression of atherosclerosis in experimental models.[19] IL-6R can be released from the cell membrane or produced by alternative mRNA splicing as a soluble receptor (sIL-6R). However, sIL-6R does not antagonize IL-6 as is usually seen with other soluble cytokine receptors, but in the presence of IL-6 forms a stimulating complex that can engage gp130 and likewise activate signaling, an event termed trans-signaling.[6;18] Importantly, gp130 is present abundantly on most nucleated cells of the body; since IL-6 and sIL-6R can circulate in high concentrations, their complexes can form readily and bind to cellular gp130, thus activating many cell populations, even those cells that do not express mIL-6R. Targeting the IL-6R, therefore, interferes with both traditional signaling and trans-signaling.

Contraindications

Hypersensitivity to TCZ is a clear contraindication. While this is unlikely to exist before the first application, sensitization can occur at any time point, especially with lower doses.[20] Initially, the signs

and symptoms may even be mild, but this should warn of the risk of more severe subsequent hypersensitivity reactions and therapy not be continued.

Chronic, active infections are also contraindications for use of TCZ.

(Regarding the use of IL-6 pathway inhibition in relation to past malignancy and pregnancy, see the sections on screening and adverse events.)

Adverse Events

Adverse events may be due to the nature of the molecule, such as being a humanized antibody, or to the pharmacological effects of IL-6R inhibition. Generally, available evidence from above mentioned early phase trials on other agents inhibiting the actions of IL-6 suggests that the adverse event profile observed with TCZ is mostly not specific to the drug but rather to IL-6 blockade, since also antibodies to the cytokine itself elicit similar mode of action related adverse events.[21].

For details on adverse events and their frequencies, we refer to recent reviews and to the package insert,[22;23] but provide a brief summary here.

Infections

From a meta-analysis of phase III and long-term extension TCZ trials, the rates of serious infections have been reported as 3.5 per 100 patient-years in the control population, 3.5 per 100 patient-years at the 4mg/kg dose, and as 4.9 per 100 patient-years at the 8mg/kg dose; they were primarily pneumonia, urinary tract infections and gastroenteritis.[23] The rate of serious infections remained stable over time during these trials with prolonged TCZ treatment, and if considering registry data evaluating serious infection rates with TNF-inhibitors, these rates appear to be similar.[23] Risk factors for serious infections appear to be high body mass index, diabetes, a history of chronic lung disease, advanced age, concomitant glucocorticoid therapy, previous TNFi and possibly also previous rituximab as well as leflunomide use.[24;25] Disease duration longer than 10 years and Steinbrocker's

Class 3 and 4 are other risk factors identified in the Japanese post-marketing surveillance report,[23;26] where higher serious infection rates were observed (9.1/100 patient-years) in TCZ users. Opportunistic infections occurred more frequently within clinical trials among TCZ users at a rate of approximately 0.25 per 100 patient years and include mycobacterial and pneumocystis infections. Tuberculosis was observed in 7 of about 4700 patients.[27] Whether these were new cases of tuberculosis or cases in which the initial screening for latent tuberculosis was inadequate is unknown. Herpes Zoster occurred at a rate of 0.3 per 100 patient-years (control: 0.1).[23;28] Further, there is the possibility of EBV-reactivation; indeed, a case of fatal EBV reactivation has been observed in Japan.[29] It should be noted that the effects of TCZ on the acute phase response may mask signs and symptoms of infections, such as fever or CRP increase.

Hypersensitivity

Hypersensitivity can manifest as, among other symptoms, rash, urticaria, arthralgia, headache, diarrhea and can occur during or also several hours after the end of the infusion. Clinically significant hypersensitivity reactions were reported in about 1 of 100 patients.[23] The rate of anaphylactic reactions is several fold higher with the 4mg/kg than 8mg/kg dose,[20] in line with the higher frequency of anti-tocilizumab antibodies at low doses,[20] and they are usually observed between the 2nd and 5th infusion,[21] but can occur also at the first infusion or later;[20] fatal hypersensitivity events have been observed.[23] These reactions can occur at all doses, with mono- or combination therapy and without previous indications of such reactions. To mitigate the risk, appropriate treatment should be available for immediate use in the event of an anaphylactic reaction and TCZ stopped immediately and permanently discontinued.

The mere presence of antibodies to TCZ which occur in about 2-4% of the patients, does not predict such reactions, but more information is needed regarding the effects of interruptions of therapy such as during infections on immunogenicity and hypersensitivity reactions.

Malignancies

During clinical trials, the overall rate of solid malignancies appeared to be similar among the control and TCZ treated patients and there was no increase in malignancy rates, including lymphoma, with prolonged exposure of TCZ.[23;30] Interestingly, in experimental models IL-6 appears to have tumor promoting activity[23] and targeting IL-6 may be effective in some types of or some patients with cancers;[31;32] therefore, IL-6 blockade might even be beneficial in such situations. Nevertheless, long term observational studies are necessary to further evaluate the potential relationship between IL-6 pathway inhibition and malignancy.

Nervous system

Demyelinating disorders, previously seen with TNF-blockers, have been reported during TCZ therapy;[33;34] therefore, a relationship to this treatment cannot be excluded; until further clarification, presence of demyelination diseases should be viewed as a contraindication.

Haematological abnormalities

Neutropenia is more common in TCZ treated patients. This is presumably due to increased margination of neutrophils.[28;35;36] Decreases in neutrophils (>500-1000/mm^3, grade 3) occur in 4% of the patients treated with TCZ at 8mg/kg, while values <500/mm^3 are seen more rarely (0.6%).[37;38] With few exceptions, serious infectious events during clinical trials were not associated temporally with neutropenia.[23]

Thrombocytopenia (<100.000/mm^3) was seen in 1.7% of patients receiving 8mg/kg in the clinical trials.[23] In about 0.8% of the exposed patients, platelet counts can fall <50.000/mm^3, and there was one reported bleeding episode.[20] It is likely that a reduction from baseline in platelet count is frequently due to the decrease in inflammatory load after starting TCZ, since thrombocytosis is a consequence of inflammation and mediated by IL-6.

Anemia usually does not occur during TCZ therapy, in contrast, hemoglobin levels increase particularly early after start of therapy.[23]

Gastrointestinal perforations

Intestinal perforations have been reported to occur at a rate of about 0.3 per 100 patient years, the majority in patients with a history of diverticulitis[22] (not necessarily diverticular disease) and/or use of NSAIDs and glucocorticoids. It must be borne in mind that signs and symptoms of diverticulitis, such as fever or CRP increases, may be masked by IL-6 blockade. This is true for other infectious states as well.

Hepatic manifestations

Increases in transaminase levels occurred in the controlled phases of the trials in about one third of TCZ treated patients, and elevations of >3x upper limit of normal (ULN) occurred in approximately 6% of patients using TCZ at 8mg/kg plus MTX, 5% using 4mg/kg plus MTX, >3.5% on MTX monotherapy and <2% on TCZ monotherapy.[23] Overall, the rate can be around 10% if looking at any point in time during prolonged therapy. However, no clinically significant liver injury or hepatitis was observed. Among the 11 liver biopsies performed the majority had steatohepatitis, but these patients also had other risk factors such as obesity.[23;39] In a rare case, hepatocellular necrosis suggesting acute hepatotoxicity was reported on liver biopsy, although treatment was continued without further deterioration.[23]

Cardiovascular events

Within the clinical development program, the rates of myocardial infarctions and strokes were similar in the TCZ treatment groups as in the control group and did not increase over time.[40] While this is in line with indications that IL-6 is partly responsible for cardiovascular risk,[23] a finding reinforced by recent genetic data,[41-43] longer term outcome studies will be needed to assess the overall long-term effects of TCZ-induced lipid elevations on cardiovascular events. The ENTRACTE study is currently one such study and is currently enrolling patients.[44]

Other

An exacerbation of psoriasis in two patients with Still's disease and psoriasis has been reported.[45] Further, induction or exacerbation of interstitial lung disease has been described in few patients.[46]

Effects of uncertain clinical relevance

Lipid levels

Total cholesterol and LDL-cholesterol increase significantly and in a dose-dependent fashion on TCZ therapy, as does HDL-cholesterol, albeit to a lesser extent. Total and LDL-cholesterol increases occur early, stabilize and amount to about 10-20% increase on the group level, but can be much higher in individual patients, although individual responses can vary.[47] Lipid-lowering therapy should be commenced on the basis of relevant national cardiovascular risk scores and based on absolute risk of cardiovascular disease rather than lipid levels alone, as recommended in recent EULAR statement

Evidence and recommendation hierarchy

In the Consensus Statement, levels of evidence are indicated next to each recommendation, in line with published guidelines[48] (Supplementary Table S1). Table S1 provides the evidence and recommendation hierarchy that was used.

Table S1. Evidence and recommendation hierarchy[48]

Level of evidence*	**Type of study**
1a	Systematic reviews of RCTs *or ≥2 RCTs with homogeneity**
1b	Individual RCT
1c	All or none effects
2a	Systematic review (with homogeneity) of cohort studies
2b	Individual cohort study
2c	Outcomes research

3a	Systematic review of case-control studies
3b	Individual case-control study
4	Case series and poor quality cohort or case-control studies
5	Expert opinion based on physiology or "first principles"
Grade of recommendation	**Appraisal**
A	Consistent level 1 studies
B	Consistent level 2 or 3 studies or extrapolations from level 1 studies
C	Level 4 studies or extrapolations from level 2 or 3 studies
D	Level 5 evidence or troublingly inconsistent or inconclusive studies of any level

Oxford Centre for Evidence-based Medicine - Levels of evidence (March 2009).

* a slight modification, namely assignment of level 1a category upon availability of ≥ 2 randomised controlled trials (RCTs) with similar results, was agreed upon among the committee.

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