Chapter 7

Cytokine inhibition in myocardial infarction and chronic heart failure

Submitted

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ABSTRACT

A plethora of cytokines is currently under investigation as potential targets of treatment to improve cardiac function and outcome in the setting of acute myocardial infarction (MI) or chronic heart failure (HF). Here we aim to provide a translational overview of cytokine inhibiting therapies tested or under evaluation in experimental models and clinical studies in MI and HF setting. In various experimental studies inhibition of interleukin-1 (IL-1), -6 (IL-6), -8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), CC- and CXC chemokines, and tumor necrosis factor-α (TNF-α) had beneficial effects on cardiac function and outcome. On the other hand, neutral or even detrimental results have been reported for some of these cytokines (IL-1, IL-6, IL-8, and MCP-1). Ambivalence of cytokine function, differences in study designs, treatment regimes and chosen endpoints hamper the translation of experimental research into clinical practice. Human studies are currently limited to IL-1 receptor antagonists (IL-1RA), IL-6 receptor antagonists (IL-6RA) or TNF inhibition. Despite favorable effects on cardiovascular events observed in retrospective cohort studies of rheumatoid arthritis patients treated with TNF inhibition or IL-1RA, prospective studies reported disappointing results. In both MI and HF, findings were inconsistent. Smaller studies ($n<100$) generally reported favorable results of anticytokine therapy on cardiac function, but larger studies ($n>100$) presented neutral results. In conclusion, of the 10 anticytokine therapies tested in animals models beneficial effects have been reported in at least one setting. Only TNF inhibition, IL-1RA and IL-6RA have been tested in larger clinical studies and finding were unsatisfactory. Many anticytokine therapies with promising animal experimental data continue to require further evaluation in humans.
INTRODUCTION

Acute myocardial infarction (MI) and chronic heart failure (HF) are associated with decreased quality of life and unfavorable long-term outcome\(^1\)\(^-\)\(^3\) and novel therapeutic strategies are still needed to improve clinical outcome. After successful introduction of antiplatelet inhibitors, beta-blockers, statins and renin-angiotensin-aldosterone inhibitors, more recently there is increasing interest to target inflammation more specifically by immunomodulation or specific anticytokine treatment.

Cardiac remodeling is one of the major contributors to progression of MI to HF and considered to be importantly mediated by inflammation\(^4\). Epidemiological studies suggest that circulating concentrations of inflammatory markers, such as C-reactive protein (CRP), are associated with subsequent risk of atherosclerosis formation, coronary heart disease (CHD) and cardiac remodeling\(^5\). In the setting of acute MI elevated CRP levels are associated with impaired myocardial reperfusion\(^6\). In principle, the inflammatory response is a protective mechanism short-term but may lead to chronic overcompensatory failure. It is a complex conjunction between innate (quick and non-specific) and adaptive (slow and specific) immune systems\(^4,\)\(^7\). Upon tissue damage or stress endothelial cells, cardiomyocytes, leukocytes and platelets can release various inflammatory cytokines attracting antigen presenting cells. Antigen presenting cells such as dendritic cells, monocytes and macrophages from the innate immune system will recognize released self-antigens or danger signals and start to interact with B and T cells from the adaptive immune system\(^8,\)\(^9\). This interaction may be caused by the formation of receptor complexes and via cytokine production further activates and amplifies the instigated inflammatory response. Cytokines, such as interleukin-1 (IL-1), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α) were previously found to be elevated in MI and HF\(^10,\)\(^11\). They are known to promote cell death of cardiomyocytes and cell hypertrophy by induction of intracellular signaling cascades such as NF-κB, JAK/STAT and PI3K pathways in leukocytes. Some cytokines may even function as biomarker while the extent of elevation has been associated with outcome and degree of cardiac injury\(^12,\)\(^13\). Anticytokine therapy targeting inflammation is widely used and successful in rheumatoid arthritis\(^14\) and is currently an active field of investigation for treatment of MI and HF. Since their fluctuations during the process of cardiovascular remodeling and observed associations with clinical outcome, cytokines have attracted attention as potential therapeutic targets. The aim of this review is to provide a contemporary and translational overview of potential effects of cytokine inhibition on cardiac function and outcome in the setting of acute MI and chronic HF.
Outline of this review

For the selection of clinical studies, in total 534 articles were screened after which 206 articles, based on article type and drug therapy, were selected for further review (Supplements Methods). Irrelevant articles based on study design, patient population and drug therapy were excluded. In total 22 articles including 13 randomized clinical trials (RCTs) were reviewed thoroughly. For selection of animal experimental studies, we included search results and references of the clinical search and initially reviewed 56 articles of which 50 articles were considered relevant and are discussed. Since there are many pro-inflammatory cytokines being studied in the experimental field, we mainly focused on those that are currently under investigation in clinical setting. An introductory overview of cytokine levels and their mechanisms in cardiovascular disease has been given in the Supplements.

Anticytokine therapy in experimental MI models

IL-1 inhibition in small animals

A variety of experimental MI models evaluated the effect of IL-1 inhibition. Pre-treatment with IL-1 receptor antagonist (IL-1RA) showed positive effects on left ventricular ejection fraction (LVEF) and infarct size in a murine ischemia reperfusion model (Figure 1)\(^1\). IL-1 receptor 1 knockout mice (IL-1R1, one of the receptors of the IL-1R superfamily) undergoing permanent coronary artery ligation had larger infarct size compared to controls\(^2\). This aligns well with the observation that genetically engineered rat overexpressing IL-1RA in an ischemia reperfusion model had reduced infarct size and apoptosis\(^3\). IL-1RA overexpression in mice undergoing permanent coronary artery ligation had an equivalent effect on cardiac function and in the infarct-remote zone collagen expression was reduced, suggesting involvement of IL-1 in cardiac fibrosis\(^4\). Both pre- and post-treatment with IL-1RA have been reported to exert beneficial effects on cardiac function, dimensions and infarct size after permanent coronary artery ligation and ischemia reperfusion in mice and rats. Anakinra (recombinant human IL-1RA inhibitor) treatment initiated in the first weeks after permanent coronary artery ligation also resulted in improved left ventricular (LV) dimensions and fractional shortening (FS)\(^5\). These findings have been replicated in a comparable study with immediate and delayed treatment of Anakinra causing a reduction in infarct size\(^6\). IL-1 inhibition with IL-1 trap, also known as Rilonacept, a long-acting IL-1 inhibiting agent, was likewise successful in a chronic MI model. Using different dosages, less apoptosis and smaller infarct size was observed and LV dimensions and FS were attenuated\(^7\). Moreover, specific IL-1β inhibition after permanent coronary artery ligation led to less LV dilatation and increased FS\(^8\). Interestingly, in a larger chronic MI study detrimental effects have been reported with mice having larger infarct size, lower collagen gene expression and more ventricular ruptures after treatment with a similar dose of IL-1β antibody\(^9\). Taken together, there
appears to be evidence for both beneficial as well as detrimental effects of IL-1 inhibition on cardiac function and infarct size in experimental MI.

**IL-6 inhibition in mice and rats**
Few studies investigated the effect of activation and inhibition of interleukin-6 (IL-6) and its receptor. One study evaluated IL-6 receptor antagonist (MR16-1) or placebo treatment after permanent coronary artery ligation in mice. FS increased, left ventricular end-diastolic diameter (LVEDD) was smaller and the survival rate was higher than controls. In an opposed model using gp130 knockout mice, the IL-6 binding common receptor, increased IL-6 and STAT3 expression, LV dilatation, LV rupture and mortality was seen.
compared to the wild-type\textsuperscript{25}. The effect was attenuated with an additional genetic reduction of STAT3, suggesting the destructive mechanism behind gp130 impaired signaling is STAT3 dependent. Contradictory with previous studies, infarct size increased and LVEF decreased in a different study with mice treated with IL-6 monoclonal antibody prior to permanent coronary artery ligation. Neutrophil infiltration was reduced in the treatment group suggesting the inflammatory response initiated by IL-6 also has functional and cardioprotective properties\textsuperscript{26}. In addition, IL-6/soluble IL-6 receptor (sIL-6R) complex suppletion has reduced cardiomyocyte apoptosis and lowered the infarct area vs. area at risk percentage in an ischemia reperfusion model\textsuperscript{27}. In conclusion, with contradictory findings on its inhibition illustrated by impaired cardiac function observed after pre-treatment and opposite effects after post-treatment, IL-6 appears a difficult target for therapy.

**TNF-α inhibition in small and large mammals**

In various ischemia reperfusion models with TNF-α inhibitor post-treatment reduced MI size\textsuperscript{28-30}. In an ex vivo study, treatment with monoclonal TNF-α antibodies after a period of ischemia in isolated rat hearts showed positive effects on LV pressure, coronary flow and oxygen consumption\textsuperscript{31}. However, TNF-α blockade had no effect when tested in adiponectin knockout mice, whereas adiponectin supplementation did, suggesting the effect of TNF-α inhibition is adiponectin dependent\textsuperscript{29}. Adiponectin itself has been shown to be cardioprotective in ischemia reperfusion and adiponectin blocks the pro-inflammatory effects of TNF-α, while elevated TNF-α can inhibit adiponectin production\textsuperscript{29}. To the contrary, administration of low-dose TNF prior to ischemia reperfusion in isolated murine hearts resulted in reduced infarct size, suggestive of a potential preconditioning effect\textsuperscript{32}. One chronic MI rat model receiving TNF-α inhibition post-treatment showed better LV pressures and diastolic function compared to controls\textsuperscript{33}. On top of that, less leukocyte infiltration and increased thickness of the LV free wall in the infarct area was seen. The effects of TNF-α antagonists have also been evaluated in larger mammals, including rabbits, swine and dogs. In one rabbit study, two groups received anti-murine TNF-α sheep antibodies pre-treatment and in one group this was combined with short periods of coronary artery occlusion (ischemic preconditioning) before the main coronary artery procedure\textsuperscript{34}. Infarct size was reduced in all treatment groups compared to controls. The concentration of circulating TNF-α correlated with infarct size. The authors suggested that ischemic preconditioning was as effective as anti-TNF-α administration in reducing infarct size. Anti-TNF-α treatment was again tested before permanent coronary artery ligation in another rabbit study and led to less necrosis, less circulating endothelial cells and neutralized levels of TNF-α compared to controls\textsuperscript{35}. In a permanent coronary artery balloon occlusion model in swine inducing ventricular fibrillation, treatment with TNF-α inhibition resulted in survival rates almost twice as high\textsuperscript{36}. In a similar study in
swine Infliximab treatment after induction of ventricular fibrillation and concomitant resuscitation was associated with higher mean arterial pressure and stroke work. Dog ischemia reperfusion models TNF-α inhibition after coronary artery balloon occlusion, or prior to coronary artery ligation, demonstrated attenuation of infarct size. Pre- and post-treatment with TNF-α antagonists in smaller and larger mammals reduced infarct size in both ischemia reperfusion and permanent myocardial ischemia models. Only two studies reported data on cardiac function after treatment with TNF-α antagonists and showed improvement of LV pressures.

**CC and CXC chemokine inhibition in mice**
Evasin-3 is a chemokine binding protein discovered in tick saliva. Evasin-3 binds CXCL1, CXCL8, and macrophage inflammatory protein-2 and inhibits neutrophil cell recruitment and has been tested in experimental MI models. In a model of in vivo and ex vivo coronary artery ligation lasting 30 minutes, Evasin-3 post-treatment reduced infarct size and reactive oxygen species levels. The beneficial effects of post-treatment in a mouse ischemia reperfusion model were attributed to the prevention of neutrophil infiltration, which is induced by CXC chemokines. In a subsequent study, Evasin-3 and Evasin-4, chemokine binding proteins inhibiting CC chemokines (including CCL5 and CCL11), administration after inducing permanent coronary artery ligation in mice was associated with lower levels of CXCL1 and CCL2, less leukocyte infiltration and smaller infarct size. The effect of Evasin-4 on circulating chemokine levels was accelerated and survival after infarction improved compared to Evasin-3. Cardiac function did not differ between Evasin-3 and -4 groups and controls. Interestingly, direct inhibition of CXCL1 and receptor CXCR2 in several regimes was not successful in mice. Only anti-CXCR2 antibody improved LVEF and decreased infarct size when administered for a longer period up to 3 weeks. Inhibition of the CXCR2 and CXCR4 binding cytokine, macrophage migration inhibitory factor (MIF), was further tested in a genetic ischemia reperfusion model with chimeric mice lacking CXCR2 and wildtype mice. Pre-treatment with anti-MIF resulted in larger infarct size and impaired LVEF in wildtype mice and in the mice generated with CXCR2 lacking bone marrow derived inflammatory cells. In contrast, chimeric mice with CXCR2 lacking cardiomyocytes receiving anti-MIF treatment showed an improved LVEF compared to chimeric mice with control antibody treatment. Blocking MIF has detrimental effects presumably via CXCR2 cardiomyocytes as opposed to cardioprotective effects in CXCR2-deficient cardiomyocytes. Miller et al. investigated effects of genetic deletion of MIF (MIF−/−) in an ischemia reperfusion mouse model and observed larger infarct size compared to wildtype mice. In conclusion, Evasin-3 and -4 post-treatment, targeting CC and CXC chemokines, were associated with smaller infarct size. CXCR2 inhibition during a longer period attenuated cardiac function in experimental MI whereas contradictory findings are reported regarding inhibition of its ligand MIF.
MCP-1 (CCL2) inhibition in mice

Anti-MCP-1 treatment in mice, administered before and after permanent coronary artery ligation, resulted in improved survival and reduced LVEDD and improved FS\textsuperscript{46}. Extracellular matrix protein 9 concentration, involved in collagen degradation and thereby remodeling, was lower in the anti-MCP-1 treated group. In contrast, in transgenic MCP-1 overexpressing mice infarct size was reduced in ischemia reperfusion experiments but not in permanent coronary artery ligation\textsuperscript{47}. Likewise, in an ischemia reperfusion model with isolated hearts of MCP-1 overexpressing transgenic mice, improved LV pressures were observed\textsuperscript{48}. Taken altogether, the effects of MCP-1 inhibition and overexpression in experimental MI are ambiguous.

CCL5 (RANTES) inhibition in mice

Several pharmacological and genetic knock-out studies have been undertaken to determine the effect of CCL5 inhibition. Mice treated with anti-CCL5 after permanent coronary artery ligation or ligation for 30 minutes resulted in improved LVEF and smaller infarct size\textsuperscript{49}. A decline in infarct size was also observed in another ischemia reperfusion model in which mice received CCL5 antagonist prior to reperfusion\textsuperscript{50}. On the other hand, blocking CCR5, a receptor binding CCL5 and others, in a knockout model had detrimental effects\textsuperscript{51}. Though, CCL5 was not induced, which corresponds with the previous study suggesting CCL5 inhibition may exert its effects via CCR1 and not via de CCR5 receptor\textsuperscript{50}. The preliminary effects of CCL5 inhibition are promising but the mechanism or receptor of action needs to be elucidated.

IL-8 inhibition in rabbits and rats

Both genetic overexpression and inhibition of interleukin-8 (IL-8) have been studied. In an ischemia reperfusion model rabbits received a monoclonal antibody against IL-8 prior to coronary artery ligation which was associated with reduced infarct size\textsuperscript{52}. In a chronic MI model with rats, treatment with endothelial cell transfusion overexpressing IL-8 receptors at several hours after permanent coronary artery ligation decreased infarct size, inflammatory cells and improved LVEF was observed\textsuperscript{53}. Thus far, experimental MI studies are contradictory on the effect of IL-8 inhibition and overexpression.

Anticytokine therapy in MI in humans

IL-1 inhibition

The recombinant human IL-1RA (Anakinra) is currently registered for the treatment of rheumatoid arthritis. Two pilot studies and a phase 2 study with Anakinra have been performed in ST-segment elevation myocardial infarction (STEMI) patients (Table 1). In the pilot study VCU-ART 10 patients received Anakinra 100mg/day and showed a decrease in LV end-diastolic and end-systolic volume indices, compared to placebo\textsuperscript{54}. 
Details on study design and results can be found in Table 1. In a consecutive RCT, 30 STEMI patients undergoing percutaneous coronary intervention (PCI) were treated with Anakinra 100mg/day or placebo during the first two weeks and the primary end point, left ventricular end-systolic volume index, did not differ\textsuperscript{55}. A meta-analysis combining the data with the previous pilot study, VCU-ART, showed a lower incidence of HF symptoms in the Anakinra treated group\textsuperscript{54}. In a further analysis including extended follow-up data of these patients treatment was associated with decreased incidence of new-onset HF diagnoses and death\textsuperscript{56}. In the MRC ILA Heart Study, 182 acute non-ST-segment elevation myocardial infarction (NSTEMI) patients were randomized to Anakinra 100mg/day or placebo for a period of two weeks\textsuperscript{57}. No difference was found in levels of high sensitive C-reactive protein (hsCRP), Troponin and von Willebrandfactor one week after MI. In a later publication of the same author it was stated that the primary endpoint, hsCRP area under the curve over first 7 days, was significantly lower\textsuperscript{58}. Unfortunately, also more major adverse cardiac events (MACE) occurred in the IL-1RA treatment group during 1-year follow-up. The double-blind RCT VCU-ART3 in STEMI patients is currently ongoing, evaluating IL-1RA treatment on CRP (Table 4).

\textit{TNF-α inhibition}

TNF-α antagonists (Infliximab, Etanercept and Adalimumab) are commonly used anti-inflammatory agents and inhibit TNF-α signaling by binding to its soluble receptors \textit{sTNFR1} and \textit{sTNFR2}. Only one double-blind RCT evaluated the effect of Etanercept 10mg or placebo treatment in 26 acute NSTEMI patients\textsuperscript{59}. Etanercept reduced neutrophil and

<table>
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<tr>
<th>Targeted cytokine</th>
<th>Main findings</th>
<th>N, age</th>
<th>Treatment</th>
<th>Follow-up period</th>
<th>Author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double blind RCT on IL-1RA after STEMI</td>
<td>+ stable LV function, lower incidence of heart failure</td>
<td>30 Mean age 58.7</td>
<td>Anakinra 100mg/day SC during 14 days compared to placebo treatment</td>
<td>14 weeks</td>
<td>Abbate A., 2013\textsuperscript{55}</td>
</tr>
<tr>
<td>Double blind RCT on IL-1RA in NSTEMI</td>
<td>- no difference in levels of hsCRP, IL-6 levels lower in placebo</td>
<td>182 Mean age 61</td>
<td>Anakinra 100mg/day SC during 14 days compared to placebo treatment</td>
<td>1 year</td>
<td>Morton AC, Foley C, 2011\textsuperscript{57}</td>
</tr>
<tr>
<td>Double blind RCT on IL-1RA after STEMI</td>
<td>+ safe and favorably affects LV remodeling (higher LVESVI)</td>
<td>10 Mean age 47.8</td>
<td>Anakinra 100mg/day SC during 14 days compared to placebo treatment</td>
<td>14 weeks</td>
<td>Abbate A, Kontos MC, 2010\textsuperscript{54}</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; RCT, randomized clinical trial; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; LV, left ventricular; mg, milligram; SC, subcutaneous; vWF, von Willebrandfactor; hsCRP, high-sensitive C-reactive protein; LVESVI, left ventricular end-systolic volume index.
IL-6 levels, although an increase in platelet-monocyte aggregation was seen. Cardiac function and infarct size were not assessed in this study, prohibiting in making a hard conclusion on the effects of Etanercept. An open label RCT testing Etanercept treatment in STEMI patients treated with PCI is currently ongoing (Table 4).

IL-6 inhibition
Promising results have recently been reported on the effect of a single gift of Tocilizumab on the primary endpoint hsCRP levels in NSTEMI patients (Table 1)\(^6^0\). In this double-blind trial, Area under the curves of hsCRP and Troponin T were higher in the placebo group, suggesting the inflammatory response can be attenuated by Tocilizumab. Echocardiography at 6 months follow-up showed no difference in cardiac function between the groups, although the trial was not primarily powered for this endpoint. Another trial focusing on the MACE incidence in patients with rheumatoid arthritis treated with Etanercept and Tocilizumab is ongoing (Table 4).

Anticytokine therapy in experimental HF models
IL-1 inhibition in mice
Only limited data is available on IL-1 inhibition in HF. One experimental model induced HF by injecting IL-1β (3µg/kg) causing a significant reduction in FS. When IL-1RA Anakinra was administered prior to this injection, LVEF and stroke volume improved in Anakinra treated mice\(^6^1\).

TNF-α inhibition in rats and dogs
TNF-α inhibition has been evaluated in experimental HF induced by Isoproterenol or chronic pacing or related to an animal constitution with hypertension or diabetes. In one of these experimental HF models, spontaneously hypertensive and healthy rats underwent treatment with Etanercept (TNF-α inhibitor) or placebo during 12 weeks\(^6^2\). Spontaneously hypertensive rats were suggested to display an early stage of HF with increased relative wall thickness and heart weight. After 12 weeks, FS did not differ, although relative wall thickness decreased and cardiac reserve increased compared to controls. Furthermore, TNF-α expression was not affected and blood pressure was increased only in the Etanercept treatment group. In healthy rats, Etanercept resulted in increased levels of β-1-adrenergic receptor mRNA expression, suggesting a positive inotropic effect. These findings indicate that anti-TNF-α treatment is ineffective and may even aggravate HF. In a different HF model with diabetic rats associated with enlarged thinned left ventricles with impaired LV function, no long-term beneficial effects within the context of cardiac function and remodeling were seen with Etanercept\(^6^3\). Etanercept treatment was studied yet in another HF model with Isoproterenol\(^6^4\). A single injection of Isoproterenol, a systemic β-adrenergic receptor agonist, is associated with myocardial
Cytokine inhibition in MI and chronic heart failure

Damage and numerous other characteristics resembling HF\textsuperscript{65}. FS and LV dilatation was indeed ameliorated in rats receiving Etanercept. Noteworthy, not TNF-\(\alpha\) levels, but IL-1\(\beta\) levels in the left ventricle were lower in the Etanercept group. In a distinct HF model dogs were paced chronically for four weeks and received placebo or Etanercept treatment twice a week\textsuperscript{66}. The chronic pacing resulted in reduced LVEF and LV dilatation. LV dilatation was less severe and the LVEF partially restored after Etanercept treatment. Also, mitochondrial respiratory chain enzyme complexes II and V in the Etanercept group were completely or partially restored. DNA fragments, Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells and Aldehyde levels were normalized in the Etanercept group whereby an increase was seen in the placebo group. This suggested that apoptosis and mitochondrial dysfunction in this HF model was attenuated by Etanercept. Due to the variety of HF models used to test the effect of anti-TNF-\(\alpha\), a general conclusion is hard to make.

**Anticytokine therapy in HF in humans**

**IL-1 inhibition in human**

Few RCTs, observational and cohort studies have evaluated the effect of TNF and IL-1 inhibition in relation to HF (Table 2 and 3). In one study, 14-day treatment with IL-1 receptor antagonist Anakinra in 7 HF patients improved median peak oxygen consumption\textsuperscript{61}. A similar treatment protocol was followed in the D-HART study, including 12 HF patients with preserved ejection fraction (HFpEF). Anakinra treatment led to improvement in peak oxygen consumption and decrease in C-reactive protein (CRP) levels\textsuperscript{67}. In a double-blind cross-over trial, 46 rheumatoid arthritis patients received 150mg Anakinra daily treatment for 30 days\textsuperscript{68}. The echocardiographic index of LV diastolic filling pressure, longitudinal strain measurements and LVEF improved after treatment with Anakinra. However, these patients were not primarily diagnosed with HF; LVEF at baseline was within normal range according to current guidelines\textsuperscript{69} and for the remaining echocardiographic parameters normal values are not yet available. One study reported a substantial improvement of cardiac function measured a few hours after single Anakinra injection in 80 coronary artery disease patients with on average decreased LVEF at baseline\textsuperscript{70}. In conclusion, IL-1 inhibition thus far seems a successful therapy in HF. Few HF studies are being performed investigating the effect of IL-1 inhibition by Anakinra and Canakinumab (Table 4).

**TNF-\(\alpha\) inhibition in human**

Few small and larger cohort studies have focused on the potential beneficial effect of TNF inhibition on cardiac function and cardiovascular endpoints. In one of these cohort studies, 23 female rheumatoid arthritis patients without overt or latent history of HF underwent Infliximab treatment for 1 year and LVEF improved significantly\textsuperscript{71}. As mean
<table>
<thead>
<tr>
<th>Targeted cytokine</th>
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<th>Treatment</th>
<th>Follow-up period</th>
<th>Author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical trial on IL-1ra in HF patients</td>
<td>+/- improved median peak oxygen consumption</td>
<td>7</td>
<td>Anakinra 100mg/day SC during 14 days</td>
<td>2 weeks</td>
<td>Van Tassell BW, 201261.</td>
</tr>
<tr>
<td>Double blind RCT crossover in HFpEF patients</td>
<td>+ improved peak oxygen consumption, reduction in CRP</td>
<td>12</td>
<td>Mean age 48</td>
<td>2 weeks</td>
<td>Van Tassell BW, 201467.</td>
</tr>
<tr>
<td>Double blind (R)CT on IL-1ra in RA patients</td>
<td>+ increase in LV performance (E/Em, LongS and LongSRS)</td>
<td>46</td>
<td>Mean age 56</td>
<td>1 month</td>
<td>Ikonomidis I, 201168.</td>
</tr>
<tr>
<td>Double blind crossover IL-1ra trial in RA with CAD patients</td>
<td>+ increase in LVEF, improved LV myocardial deformation and twisting</td>
<td>60</td>
<td>Mean age 59.5</td>
<td>3 hours</td>
<td>Ikonomidis, 201470.</td>
</tr>
<tr>
<td>Double blind RCT on TNFi treatment in HF patients</td>
<td>- no benefit of Etanercept on death/ HF hospitalization</td>
<td>2048</td>
<td>Mean age 63.5</td>
<td>24 weeks</td>
<td>Mann DL, 200482. RENEWAL</td>
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<tr>
<td>Double blind RCT on TNFi treatment in HF patients</td>
<td>- no improvement, possible CHF worsening, LVEF improved with 5mg/kg</td>
<td>150</td>
<td>Mean age 61.4</td>
<td>28 weeks</td>
<td>Chung ES, 200381. ATTACH</td>
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<tr>
<td>Double blind RCT on TNFi treatment in HF patients</td>
<td>+ significant dose-dependent improvement in LV structure and function</td>
<td>47</td>
<td>Mean age 55.0</td>
<td>3 months</td>
<td>Bozkurt B, 200179.</td>
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<tr>
<td>Controlled clinical trial on TNFi in CHF patients</td>
<td>+ improved systemic endothelial vasodilator capacity</td>
<td>18</td>
<td>Mean age 53.6</td>
<td>1 week</td>
<td>Fichtlscher-er S, 200187.</td>
</tr>
<tr>
<td>Double blind RCT on TNFi treatment in HF patients</td>
<td>+ significant improvement of LVEF in 4 or 10mg/m² group</td>
<td>18</td>
<td>Mean age 63.3</td>
<td>2 weeks</td>
<td>Deswal A, 199986.</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; RCT, randomized clinical trial; HF, heart failure; RA, rheumatoid arthritis; CAD, coronary artery disease; CHF, congestive heart failure; TNFi, TNF inhibitors; LV, left ventricular; mg, milligram; m², square meters; kg, kilogram; SC, subcutaneous; IV, intravenously; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; Em, early diastolic mitral annulus velocity; E, early transmitial flow velocity; LongS, longitudinal end-systolic strain; LongSRS, longitudinal peak systolic strain rate.
### Table 3. Cytokine inhibition and CVD – Cohort and retrospective studies

<table>
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<tr>
<th>Targeted cytokine</th>
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<th>Follow-up period</th>
<th>Author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort study on TNFi in RA patients</td>
<td>++ modest increase in LVEF and reduced biochemical HF markers</td>
<td>23</td>
<td>Mean age 51.3</td>
<td>Infliximab 3mg/kg body weight per infusion every 8 weeks</td>
<td>1 year</td>
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<tr>
<td>Cohort study on TNFi in RA patients and CV events</td>
<td>++ reduced risk of non-fatal and fatal cardiovascular events</td>
<td>10156</td>
<td>Mean age 59</td>
<td>TNFi treatment in person years of exposure</td>
<td>Mean 22.9 months</td>
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<tr>
<td>Cohort study on TNFi in RA patients and HF incidence</td>
<td>+ no increased risk of (worsening) HF</td>
<td>2757</td>
<td>Mean age 53.7</td>
<td>Conventional Infliximab, Etanercept, Adalimumab treatment compared to conventional DMARD treatment (n=1491)</td>
<td>3 years</td>
</tr>
<tr>
<td>Cohort study on TNFi in elderly RA patients and HF incidence</td>
<td>- increased risk HF hospitalization and exacerbation, TNFi treated patients had more severe RA</td>
<td>1002</td>
<td>Mean age 73/ 77 in HF</td>
<td>At least one prescription of Etanercept, Infliximab and Adalimumab compared to MTX using controls (n=5593)</td>
<td>Mean 1.6/ 1.7 years in HF</td>
</tr>
<tr>
<td>Cohort study on TNFi and MI incidence in RA patients</td>
<td>-/+ No difference in MI incidence, but reduced incidence of MI in responders</td>
<td>10829</td>
<td>Mean age 56.8</td>
<td>TNFi treatment of minimal 6 months (n=8659) compared to DMARD treatment (n=2170)</td>
<td>Mean 18 months</td>
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<td>Retrospective study TNFi and exacerbation of CHF</td>
<td>- no difference between CHF, but mortality rates non-significantly different</td>
<td>103</td>
<td>Mean age 58.7</td>
<td>At least one dose of Infliximab, Adalimumab or Etanercept compared to RA and non-RA controls (n=200)</td>
<td>Max. 4 years</td>
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<tr>
<td>Retrospective study on TNFi and prevalence of HF in RA/CD patients</td>
<td>-/+ no significant increase in HF incidence</td>
<td>4018</td>
<td>Mean age RA 40/ 38</td>
<td>At least three prescriptions for Etanercept or Infliximab compared to controls</td>
<td>15/ 21 months</td>
</tr>
<tr>
<td>Retrospective study on TNFi in RA patients and CVD risk</td>
<td>+ risk of CVD lower in TNF blocker treated RA patients</td>
<td>983</td>
<td>Mean age 58</td>
<td>TNFi treatment (n=531) compared to no TNFi treatment patients (n=543)</td>
<td>Max. 2 years</td>
</tr>
<tr>
<td>Cohort study on TNFi in RA patients and HF incidence</td>
<td>+ HF less common in TNFi treated RA patients</td>
<td>13171</td>
<td>Mean age 61</td>
<td>Infliximab or Etanercept treatment compared to no TNFi treated controls</td>
<td>2 years</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; TNFi, TNF inhibitors; RA, rheumatoid arthritis; CV, cardiovascular; RCT, randomized clinical trial; HF, heart failure; MI, myocardial infarction; CHF, congestive heart failure; CD, Crohn’s disease; CVD, cardiovascular disease; LVEF, left ventricular ejection fraction; mg, milligram; kg, kilogram; DMARD, disease-modifying antirheumatic drugs; MTX, Methotrexate.
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>N</th>
<th>status</th>
<th>Study objectives</th>
<th>Author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IL-1β Canakinumab</td>
<td>10120</td>
<td>Active, not recruiting</td>
<td>Cardiovascular Risk Reduction Study (CANTOS) testing Canakinumab treatment in patients with prior MI and elevated hsCRP to prevent recurrent cardiovascular events within 36 months.</td>
<td>Novartis Pharmaceuticals 2011100.</td>
</tr>
<tr>
<td>Anti-IL-1β Canakinumab</td>
<td>16</td>
<td>Active, not recruiting</td>
<td>A substudy of the CANTOS trial testing Canakinumab treatment in patients with prior MI and elevated hsCRP, reduced LVEF and symptomatic HF to improve peak oxygen consumption at 3 months.</td>
<td>Abbate A, 2015101.</td>
</tr>
<tr>
<td>IL-1RA Anakinra</td>
<td>30</td>
<td>Has been completed</td>
<td>A double-blind RCT Anakinra ADHF testing Anakinra treatment in patients with acute decompensated HF to dampen the inflammatory response measured by CRP AUC over 3 days.</td>
<td>Virginia Commonwealth University, 2015102.</td>
</tr>
<tr>
<td>IL-1RA Anakinra</td>
<td>99</td>
<td>Currently recruiting</td>
<td>A double-blind RCT VCU-ART3 testing Anakinra treatment in patients with STEMI to evaluate acute phase response measured by CRP AUC over 14 days.</td>
<td>Abbate A, 2015103.</td>
</tr>
<tr>
<td>IL-1RA Anakinra</td>
<td>60</td>
<td>Currently recruiting</td>
<td>A double-blind RCT RED-HART testing Anakinra treatment in patients with decompensated heart failure to evaluate safety and efficacy with peak oxygen consumption and ventilatory efficiency.</td>
<td>Abbate A, 2015104.</td>
</tr>
<tr>
<td>Anti-IL-1β Canakinumab</td>
<td>189</td>
<td>Has been completed</td>
<td>A double-blind RCT testing ACZ885 treatment in patients with (pre-)diabetes and atherosclerosis to evaluate effects on vascular function. Statistical analyses are not (yet) provided in the study results section.</td>
<td>Novartis Pharmaceuticals 2015105.</td>
</tr>
<tr>
<td>MCP-1 NOX-E36</td>
<td>72</td>
<td>Has been completed</td>
<td>A double-blind RCT first-in-human study NOX-E36 reported it was safe and well tolerated and showed a dose-dependent reduction in peripheral blood monocytes with CCR2 receptors.</td>
<td>Landgraf G, 2013106,107.</td>
</tr>
<tr>
<td>PF-04634817</td>
<td>27</td>
<td>Has been completed</td>
<td>A double-blind RCT testing PF-04634817 (CCR2 and CCR5 antagonist) treatment to evaluate safety and tolerability in healthy subjects.</td>
<td>Pfizer, 2010108.</td>
</tr>
<tr>
<td>IL-6RA Tocilizumab</td>
<td>120</td>
<td>Has been completed</td>
<td>A double-blind RCT testing Tocilizumab treatment to evaluate the inflammatory response by hsCRP AUC over 56 hours in NSTEMI patients.</td>
<td>Gullestad L, 2014109.</td>
</tr>
<tr>
<td>IL-6RA Tocilizumab</td>
<td>3146</td>
<td>Ongoing</td>
<td>An open-label RCT testing Etanercept and Tocilizumab treatment to evaluate incidence of MACE within 5 years in RA patients.</td>
<td>Hoffmann-La Roche, 2015110.</td>
</tr>
<tr>
<td>Anti-TNF-α Etanercept</td>
<td>200</td>
<td>Unknown</td>
<td>An open label RCT testing Etanercept treatment to evaluate incidence of MACE 30 days in STEMI patients treated with PCI.</td>
<td>Tao L., 2011111.</td>
</tr>
<tr>
<td>Biologic agents</td>
<td>90</td>
<td>Ongoing</td>
<td>A clinical study with Adalimumab, Etanercept, Infliximab and Ustekinumab treatment to evaluate risk of coronary artery disease as measured by coronary calcium score in psoriasis patients.</td>
<td>Hjuler KF, 2015112.</td>
</tr>
<tr>
<td>TNFi</td>
<td>100</td>
<td>Unknown</td>
<td>A clinical study testing TNFi treatment to evaluate change in cardiac function within 24 months in RA and Ankylosing Spondylitis patients.</td>
<td>Bonfá ESDO, 2013113.</td>
</tr>
</tbody>
</table>
LVEF was still within the normal range, it may be premature to extrapolate these results to clinical overt HF. In an observational study of 303 rheumatoid patients and controls no difference was found in mortality and the incidence or worsening of HF\textsuperscript{72}. Results from a larger observational study in 4018 rheumatoid arthritis and Crohn’s disease patients were also neutral. The risk for HF was non-significantly increased in patients receiving TNF inhibition compared to non-biologicals\textsuperscript{73}. In a similar neutral study 2757 rheumatoid arthritis patients receiving either Infliximab, Etanercept or Adalimumab were compared to 1491 patients receiving non-biological disease-modifying drugs (DMARDs) regarding the incidence or worsening of HF\textsuperscript{74}. When corrected for cardiovascular disease (CVD) risk factors, increased incidence of HF was mainly related to disease activity scores. There was no significant risk related to TNF inhibition. The authors suggested that anti-TNF-α treatment is rather beneficial than harmful in the context of risk on HF. One study reported negative effects of TNF inhibition on HF hospitalizations in 1002 rheumatoid arthritis elderly patients compared to 5593 Methotrexate users\textsuperscript{75}. Baseline characteristics of TNF inhibition vs. Methotrexate users showed that patients taking TNF inhibitory drugs had more severe rheumatoid arthritis, indicated by higher CRP levels, more co-medication or injections and more comorbidities, though statistical significance was not reported. In addition, the study was not randomized and selection bias in TNF inhibitory drug prescription reserved to patients with more severe disease could have influenced the results. Methotrexate may also be a suboptimal control treatment; it is reported to reduce incidence of cardiovascular events\textsuperscript{76}. In one of the larger cohort studies in 10156 rheumatoid arthritis patients, the risk of non-fatal (MI) and fatal cardiovascular events was lower in the TNF inhibition group compared to patients taking DMARDs\textsuperscript{77}. Positive effects were also observed in a study including 13171 rheumatoid arthritis and 2568 osteoarthritis patients showing a lower HF prevalence in TNF inhibitory drug treated patients\textsuperscript{78}. When pre-existing CVD was absent, there was a low risk of HF unrelated to TNF inhibitory therapy. However, age, sex and comorbidity differed significantly between

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<tr>
<td>TNFi NCT01548768</td>
<td>25</td>
<td>Currently recruiting</td>
<td>An open label clinical study (RHYTHM) testing TNFi treatment to evaluate change in cardiac function and structure in RA patients.</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; TNFi, TNF inhibitors; MI, myocardial infarction; hsCRP, high-sensitive C-reactive protein; LVEF, left ventricular ejection fraction; HF, heart failure; RCT, randomized clinical trial; CRP, C-reactive protein; area under the curve; CCR2, C-C chemokine receptor 2; CCR5, C-C chemokine receptor 5; NSTE-MI, non-ST-segment elevation myocardial infarction; MACE, major adverse cardiac/cardiovascular events; RA, rheumatoid arthritis; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention.
the TNF inhibitory drug and non-TNF inhibitory drug treated groups so it remains to be determined if TNF inhibition lowers the risk for HF in these patients.

In a RCT including 47 HF patients for safety and tolerability of Etanercept showed LVEF improvement in a dose-dependent manner\textsuperscript{79}. In a similar double-blind RCT including 18 HF patients, a single intravenous injection of Etanercept was associated with improvement of LVEF\textsuperscript{80}. In another RCT, 150 patients with HF NYHA class III-IV and LVEF≤35% received placebo or Infliximab treatment 5mg/kg or 10mg/kg at baseline, week 2 and week 6\textsuperscript{81}. LVEF increased in the 5mg/kg Infliximab group, but the primary endpoint, clinical functional status after two weeks, did not improve. Conversely, 10mg/kg Infliximab treatment was associated with increased risk for HF hospitalization or death of any cause at 28 weeks.

To evaluate the effect of Etanercept on HF hospitalization and death, data were combined of two RCTs; RECOVER and RENAISSANCE, including 1366 HF patients receiving placebo or Etanercept 25mg once, twice or three times a week\textsuperscript{82}. Both studies were prematurely stopped because of a lack of benefit. Etanercept treatment did not decline rate of death or HF hospitalizations. The results did not change after subgroup analyses for NYHA class or LVEF.

In smaller RCTs TNF-α antagonists ameliorated cardiac function. However, this positive effect is absent in previously mentioned large RCTs. In high dose, TNF-α antagonists seem to have adverse effects on clinical outcome. To summarize, TNF-α inhibition in HF patients did improve cardiac function in several smaller studies and is more likely to have a beneficial effect on cardiovascular events in patients with rheumatoid arthritis, but fails to show effect in large RCTs. There are currently no large studies ongoing that evaluate TNF-α inhibition treatment in HF.

DISCUSSION

In a wide range of experimental animal models of MI and in various HF models cytokine inhibition showed promising results. Few clinical studies investigated the effects of anticytokine therapy in MI and HF patients. Unfortunately, the larger clinical trials to date have failed to show an improvement on cardiac function and outcome. Several explanations are thinkable to explain this failure of translation from animal studies to human trials. First of all, the association of cytokines with MI and HF is not completely elucidated. The time course of cytokine activation and elevation may appear obvious at first glance, however it is not clear whether, for instance, the duration of ischemia and the degree of successful reperfusion (or co-medication) has an impact on the inter- and intraindividual biochemical curves of cytokine levels. The complex pathways underlying cytokine activation and interactions are still only partly understood. The presence of po-
tential negative feedback loops correcting hormone imbalances, such as SOCS3 inhibiting IL-6, are not yet clarified for most cytokines involved in MI and HF and its presence and function not readily translatable from animals to humans. Furthermore, inhibition of some cytokines may lead to increased expression of other cytokines. When looking at effects of anticytokine therapy, levels of related cytokines should therefore also be taken into account.

Another complexity is that many cytokines appear to have an ambivalent role. The function and effects of cytokine activation could be time-dependent, as is contemplated for instance for IL-6. Prolonged activation and excessive cytokine production may be detrimental. Pro-inflammatory effects are counterbalanced by anti-inflammatory downstream signaling, as in case of TNF-α, and selective inhibition of pro-inflammatory pathways is therefore challenging.

At times, effects of anticytokine therapy and cytokine overexpression were in disagreement. Both anti-MCP-1 treatment and MCP-1 overexpression showed positive effects on cardiac function. Ischemia reperfusion and permanent coronary artery ligation models might have different effects on cytokine signaling. Lower cytokine levels have been reported in mice undergoing ischemia reperfusion vs. permanent coronary artery ligation. Hypothetically, when reperfusion is achieved, the cytokine burst is interrupted together with the initial inflammatory response that is associated with beneficial effects on myocardial healing. Therefore, cytokine overexpression might have a place in ischemia reperfusion, whereas anticytokine treatment is more sensible in chronic MI models. Pre-treatment could also downregulate cytokine receptors and after a second stimulation caused by infarction cardiomyocytes may be less prone to cytokine activation.

In some of the studies described, inhibition of cytokines or its receptors had contradictory consequences and interacting pathways involved in inhibition of each cytokine are as yet not cleared up. This prohibits in making a general conclusion on the responsible targets and potential clinical use of anticytokine therapy. Different treatment regimes are practiced. In experimental MI mode, timing and duration of treatment vary widely. This is illustrated by a model were mice received anti-MCP-1 gene therapy 3 days before and 14 days after coronary artery ligation. Effective plasma concentrations of the MCP-1 receptor binding protein are reached for 14 days after the injection, explaining the choice for this treatment regime. Instead of long-lasting treatment, others focused on pre-treatment, or short-term post-treatment. After initial inflammatory response in MI a second cytokine burst has been observed after 8 days. Hence, the optimal time-point to interfere with pro-inflammatory effects of cytokines might also be after the first week, which is barely studied in the discussed experimental MI models.

Other explanations for observed discrepancies in MI models may be the different design, namely ischemia reperfusion vs. permanent coronary artery ligation. Ischemia
reperfusion is believed to trigger a more pronounced inflammatory response. For instance, contradictive findings have been reported for IL-6 and IL-8. In addition, anti-inflammatory properties have been ascribed to IL-8. Hypothetically, anti-inflammatory actions by IL-6R inhibition and IL-8 overexpression may have positive effects on cardiac function during chronic MI while the opposite is true during ischemia reperfusion. Again in experimental HF a wide variety of models was used. In one study, HF was induced by injecting IL-1β and at the same time IL-1RA was administered. As HF is a complicated disease with many underlying factors, it may be too simplistic to imitate and evaluate treatment in a model with addition and inhibition of a single cytokine. The dog model with chronic overpacing might be a good model for HF caused by atrium fibrillation, but may not account for other etiologies linked to HF. Recently, guidelines have been proposed to enhance similarity of experimental animal studies and human HF and might help in providing a structured approach for translation to humans.

Chosen endpoints in experimental MI and HF were also disparate. In experimental models, applied methods and timing of the evaluation of the inflammatory response and cardiac remodeling were very different. To illustrate, in an ischemia reperfusion model the inflammatory response was studied and TNF-α, IL-1β and IL-6 were markedly upregulated 6 hours after MI. The authors of this study endorsed the assessment of inflammatory mediators to be performed during the first three days. They also recommended that assessment of dilative remodeling should take place at least four weeks after MI. LV dilatation increased significantly between 1 and 4 weeks after MI reflecting progressive LV remodeling. In many of the previous experimental models these criteria for measuring LV dilatation at a later time point are not met.

Again, chosen endpoints in clinical MI and HF studies differ from each other and from experimental endpoints. This makes it even harder to judge if experimental findings can be readily translated to humans. Also, publication bias in experimental studies could play a part in the neutral results found in humans. In the European Society of Cardiology guidelines, LVEF is stated as an important prognostic parameter after MI. However, surrogate endpoints, including LVEF, might not be good representatives for long-term outcome. In the reviewed experimental animal studies regarding anticytokine therapy in MI endpoints vary widely. Apart from enzymatic and functional infarct size and LVEF, other parameters, such as LV dilatation, LV pressures, LV mass and stroke work, are used to evaluate treatment effects. In the two smaller clinical MI studies positive effects of IL-1RA were seen on NYHA class, LV dimensions, incidence of HF and death were seen. In one larger study they found no effects on Troponin levels one week after NSTEMI and the incidence of MACE after one-year follow-up did not differ. In HF, reduced LVEF is generally associated with worse outcome. In HFpEF, important predictors of HF hospitalization and cardiovascular death were LV hypertrophy, increased pulmonary artery and LV filling pressures. The reviewed experimental HF model evaluated effects of
IL-1RA and TNF inhibition on LVEF, FS, LV dilatation and stroke work. Human studies on anti-TNF-α inhibition mainly focused on LVEF, NYHA class, HF hospitalizations and death.

CONCLUSION AND FUTURE PERSPECTIVES

In this review we summarize the rapidly developing field of anticytokine therapy in cardiovascular disease and highlighted the contradictory findings in experimental MI and HF compared to the neutral results in clinical studies. In various experimental studies inhibition of IL-1, -6, -8, MCP-1, CC chemokines, CXC chemokines and TNF-α had profound beneficial effects on cardiac function and outcome. On the other hand, neutral or even detrimental results have been reported for some (IL-1, IL-6, IL-8, MCP-1) of these cytokines. Ambivalence of cytokine function, differences in study designs, species, treatment regimes and chosen endpoints appear to hamper the successful translation of experimental research into clinical practice. In clinical setting, only TNF-α inhibition, IL-1RA and IL-6RA have been tested so far. Promising results were seen in smaller studies, but up until now larger RCTs showed neutral results on cardiac function and outcome. Several studies on TNF inhibition and IL-1RA in MI and HF are currently ongoing, among them the CANTOS trial. Furthermore, a MCP-1 inhibitor and a CCR2/CCR5 antagonist are being tested in healthy individuals and diabetes patients. IL-6RA trials are ongoing in (N)STEMI and rheumatoid arthritis patients. Many other anticytokine therapies with encouraging animal experimental data require further evaluation in humans, but the first clinical studies suggest this translation can be troublesome.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.
REFERENCES


