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Preclinical characterization and clinical development of ILARIS® (canakinumab) for the treatment of autoinflammatory diseases

Hermann Gram

Interleukin-1beta (IL-1β) is a pro-inflammatory cytokine which is part of the first line innate response in vertebrates and is induced in injury, infection, and immunity. While temporally limited induction of IL-1β is believed to protect the organisms against traumatic or infectious insults, its aberrant expression in chronic inflammation is detrimental. Therefore, pharmacological neutralization of IL-1β in chronic inflammatory diseases is a meaningful strategy to treat inflammation and to alleviate respective clinical symptoms in man. Canakinumab is a high-affinity human monoclonal antibody designed to target human IL-1β in inflammatory diseases. Indeed, canakinumab has shown excellent efficacy in rare genetic autoinflammatory diseases or pathological conditions associated with aberrant production of IL-1β. This review focuses on the molecular and clinical mode of action and pharmaceutical development of canakinumab in (auto)inflammatory diseases.

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Introduction

Muckle Wells Syndrome (MWS) and Familial Cold Autoinflammatory Syndrome (FCAS) are autosomal dominant hereditary diseases, and they belong together with the non-hereditary Neonatal Onset Multisystem Inflammatory Disease/Chronic Infantile Neurologic Cutaneous and Articular syndrome (NOMID/CINCA) to the family of Cryopyrin Associated Periodic Syndrome (CAPS). CAPS is an extremely rare disease with an estimated prevalence of about 1 per 1,000,000 subjects [1]. The clinical symptoms in CAPS can be variable; they include severe fatigue, periodic fever, influenza-like myalgia, anemia and inflammation of the skin, eyes, bones, joints and meninges [2]. In 2001, the genetic cause of MWS was traced to mutations in the CIAS gene [3,4]. The CIAS1 gene codes for a protein previously termed NALP3, now NLRP3 [3–5].

At about the same time as the discovery of mutations in the NLRP3 protein in CAPS, the inflammasome, a regulatory protein complex for the production of mature and bioactive IL-1β was described [6]. Different inflammasomes with distinct regulatory subunits exist, which all converge on caspase I which cleaves pro-IL-1β [7] to generate its active and secreted form. NLRP3 is a regulatory subunit of the NLRP3 inflammasome, and mutations in the NLRP3 gene identified in CAPS patients render NLRP3 constitutively active (Figure 1). As a consequence, these NLRP3 mutants are associated with increased and pathological secretion of IL-1β from peripheral blood monocytes [8,9].

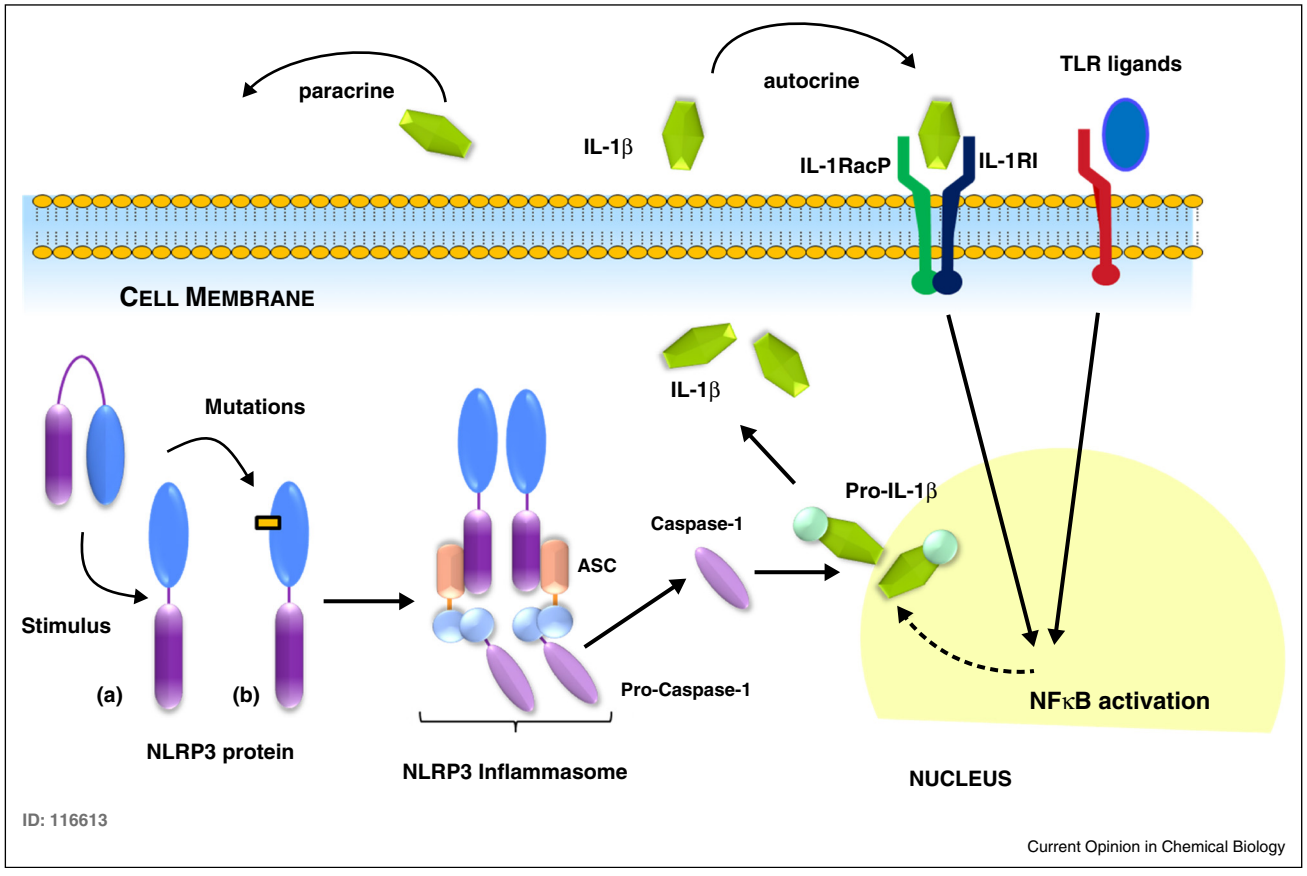
IL-1β signals through a heterodimeric receptor composed of IL-1 receptor 1 (IL-1RI) and IL-1 receptor associated protein (IL-1RacP). Pharmacological treatment of CAPS with recombinant IL-1 receptor antagonist (IL-Ra), a competitive antagonist of IL-1 for receptor binding, led to a rapid and complete clinical and serological response in a case study with 2 and 3 MWS patients, respectively [9,10].

Preclinical development of canakinumab

Novartis Pharma AG licensed the HuMab-Mouse™ technology from Medarex in the late 1990s and started a program aimed at the generation of therapeutic human antibodies targeting human IL-1β. HuMab mice have part of the human antibody repertoire integrated into their genome, while their endogenous immunoglobulin repertoire is inactivated by targeted genetic disruption [11]. Such mice produce human IgG1 antibodies upon immunization with antigen, and human monoclonal antibodies can be derived from such mice by conventional hybridoma technology [12]. Anti-human IL-1β monoclonal antibodies were generated from HuMab mice immunized with human IL-1β antigen. Several different monoclonal antibodies capable of neutralizing the bioactivity of human IL-1β emerged from this endeavor, and two of them were progressed into preclinical development. The more potent antibody, termed ACZ885, and later canakinumab, a human IgG1/κ antibody, entered full clinical development.

Canakinumab interacts with human IL-1β with an equilibrium binding constant of about 40 pM [13] and

Figure 1

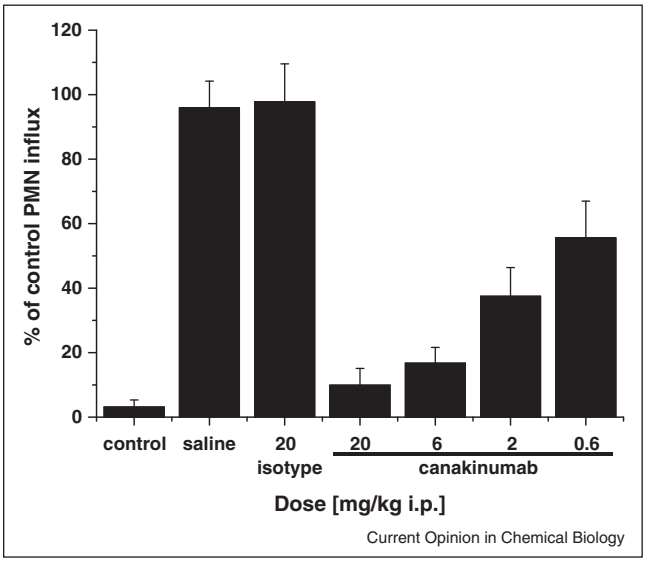


Production of mature IL-1β is regulated by the inflammasome. IL-1β production requires two steps: (i) 'priming', that is induction of mRNA for pro-IL-1β via NFκB by for example, toll-like receptor (TLR) signaling and (ii) activation of the inflammasome by the regulatory subunit NLRP3 by a physiological stimulus **(a)**, or by gain-of-function mutations in CAPS patients **(b)** [8]. NLRP3 activation leads to multimerization and recruitment of the adapter protein ASC which enables pro-caspase I to bind to this complex. Autocatalytic cleavage to proteolytically active caspase I is required for the proteolytic processing of the inactive pro-IL-1β and the secretion of mature and active form of IL-1β [53].

neutralizes the biological activity of IL-1β *in vitro* with an IC_{50} of about 43 pM [14]. Canakinumab has high selectivity towards human IL-1β, it does not bind to other members of the IL-1 family, including the most related members IL-1α and IL-1Ra, which both bind also to the IL-1RI receptor chain. Demonstration of pharmacodynamic action *in vivo* was conducted in preclinical mouse models. To this end, mouse NIH 3T3 cells engineered to secrete human IL-1β (3T3-hIL-1β) were employed to induce local inflammatory pathology in the mouse. For example, neutrophil migration into subcutaneous air-pouches was initiated by injecting the 3T3-hIL-1β cells into the airpouch (Dawson *et al.*, unpublished). Systemic application of canakinumab by intraperitoneal injection inhibited the neutrophil invasion into the pouch in a dose dependent manner, demonstrating *in vivo* potency of this antibody (Figure 2).

In order to elucidate the molecular mechanism by which canakinumab interferes with the bioactivity of IL-1β, the crystal structure of canakinumab in complex with IL-1β was determined [14]. The structural model based on X-ray diffraction data revealed a large interaction surface of 957 $Å^2$ for canakinumab, which recognizes an extended, discontinuous epitope on human IL-1β. All six complementarity-determining regions are involved in antigen binding, and 19 residues of canakinumab, 7 contributed by the light-chain and 12 by the heavy-chain, make contacts with IL-1β, while 20 residues of IL-1β are in contact with the antibody. Overlay of the canakinumab:IL-1β structure with the structure of the IL-1β:IL-1RI:IL-1RacP complex [15,16] revealed that a complex of canakinumab and IL-1β cannot interact with the IL-1 receptor due to a steric interference between canakinumab V_H domain and the D1 domain of IL-1RI. Canakinumab does not seem to interfere with the binding of IL-1RacP, the second component of the IL-1 receptor, to the IL-1β::IL-1RI complex, as there is no steric overlap with the respective interaction sites (Figure 3). Therefore, canakinumab blocks the first step of the assembly of

Figure 2



Inhibition of polymorphonuclear leucocyte (PMN) influx. Subcutaneous airpouches were formed by injection of air on the back of OF-1 female mice, and antibodies or controls were injected intraperitoneally (i.p.) six days later. The following day, recombinant NIH3T3 cells expressing human IL-1β were injected into the airpouch, and 24 h later, PMN numbers were determined in lavage fluid from the airpouch. Data represent the mean ± standard error of the mean from 5 animals per group (Dawson *et al.*, unpublished data). Controls were animals which did not receive NIH3T3-IL-1β cells (control), vehicle (saline) and isotype-matched irrelevant antibody as treatment controls.

the active IL-1β receptor complex, binding of IL-1β to IL-1RI. This structure-based prediction is confirmed by demonstration of competitive binding to IL-1β between canakinumab and recombinant soluble IL-1RI and IL-1RII [14].

Canakinumab exhibits also a very high degree of species specificity, as it does not bind to mouse, rat or rabbit IL-1β. Not even the highly related IL-1β from rhesus or cynomolgus monkeys (96% sequence identity) is recognized by canakinumab. The X-ray structure of canakinumab in complex with IL-1β revealed that Glu64 in human IL-1β is a critical residue for antibody:antigen interaction. This residue forms multiple strong interactions with both heavy and light chain residues of canakinumab, but is not conserved in macaque monkeys, rodents, canines, and many other mammalian species [14].

This unusually high species selectivity posed a problem for the preclinical development of canakinumab, as the commonly used macaque non-human primates, cynomolgus or rhesus monkeys, were not acceptable for toxicological evaluation due to the lack of target binding. Marmoset monkeys (*Callithrix jacchus*) belong to the group of non-human primates, and their physiology is fairly well characterized [17]. Furthermore, breeding colonies for pharmacological testing under good laboratory practice are available. IL-1β from marmoset shares 96% identity with human IL-1β and, most interestingly for the preclinical development of canakinumab, marmoset IL-1β has Glu64 like human IL-1β. Canakinumab exhibits full crossreactivity to marmoset IL-1β, and the bioactivity of marmoset IL-1β is effectively neutralized by canakinumab [14]. Therefore, marmoset monkeys fulfilled the criteria of a relevant species for toxicological examination of canakinumab, and the required toxicological program for clinical development of canakinumab could successfully be conducted in this species.

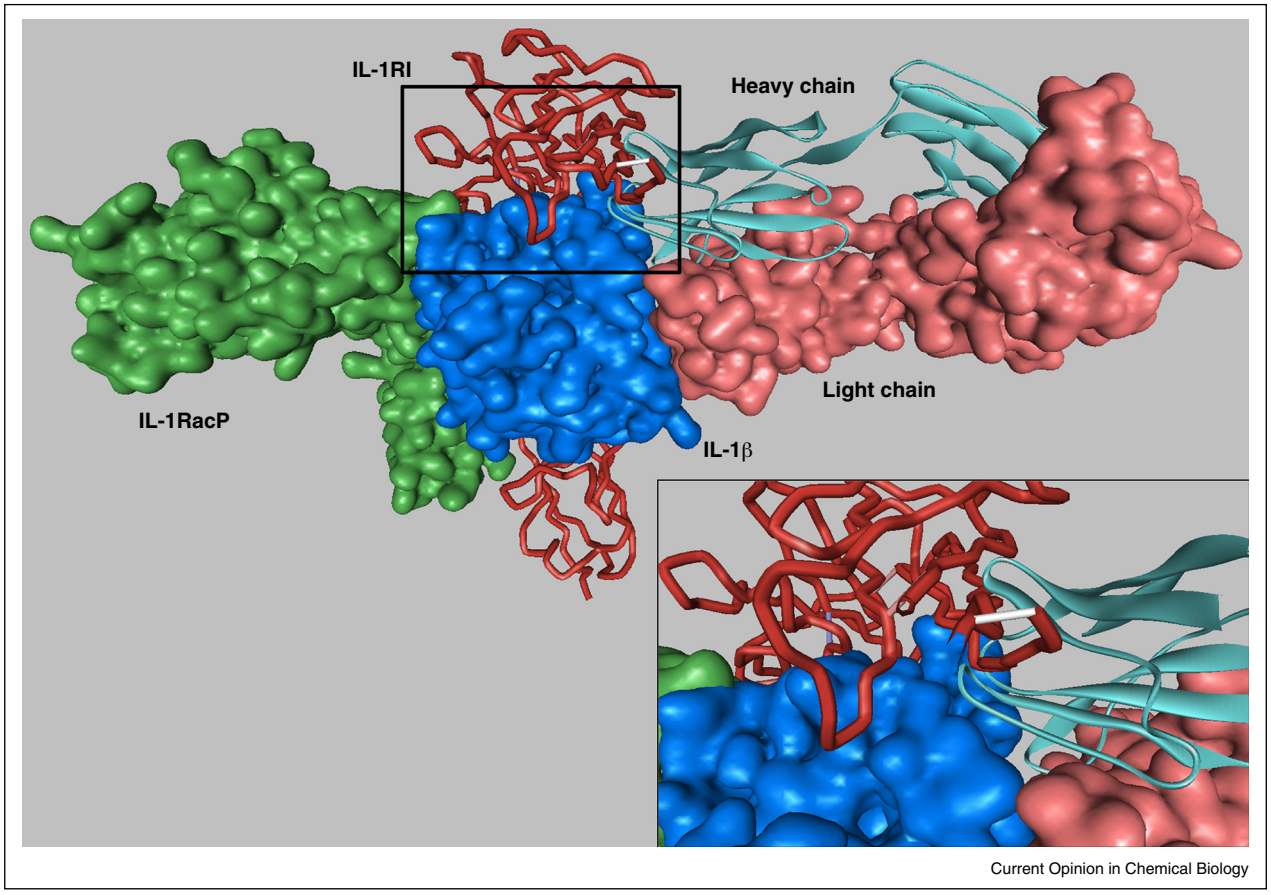
Clinical development

The molecular understanding of the affected cellular pathway by mutations observed in CAPS patients allows for the application of a targeted medicine paradigm, that is specifically and exclusively targeting IL-1β, a highly potent inflammatory mediator closely downstream of the causative defect in NLRP3 and the inflammasome. CAPS is therefore a highly relevant model disease best suited to unequivocally demonstrate the pharmacokinetic/pharmacodynamic (PK/PD) relationship and clinical utility of canakinumab.

Four MWS patients treated by intravenous (i.v.) infusion of canakinumab at 10 mg/kg showed a fast and complete clinical, serological, and biochemical response with a medium duration of 185 days [18]. Complete clinical responses were subsequently observed in the same patients upon i.v. infusion of a 10-fold lower dose of 1 mg/kg canakinumab or subcutaneous (s.c.) injection of 150 mg canakinumab when clinical signs reappeared. The long duration of clinical remission in CAPS patients upon administration of a single dose is fully explained by the pharmacokinetics and potency of canakinumab, which has a serum half-life of 26 days in CAPS patients [19]. Clinical development of drugs requires a proper description of the dose and dose interval to be applied in the respective patient population. A traditional parallel arm, placebo-controlled dose finding phase IIb study was not possible due to the extremely rare prevalence of CAPS. Therefore, a mathematical model describing the relationship between pharmacokinetics (PK), pharmacodynamics (PD), target binding (KD), and the production rate of IL-1β was developed from data collected in this first clinical phase I/II study in Muckle Wells patients [18].

Pharmacodynamic activity of canakinumab in patients can be traced by measuring circulating IL-1β in complex to canakinumab in the serum. The elimination rate of free IL-1β from blood is very high [20], and steady state concentrations in blood are therefore extremely low and hardly measurable in the serum of healthy individuals or patients [18]. IL-1β in complex to canakinumab has a much lower rate of elimination, and steady state serum concentrations of the complex between IL-1β and

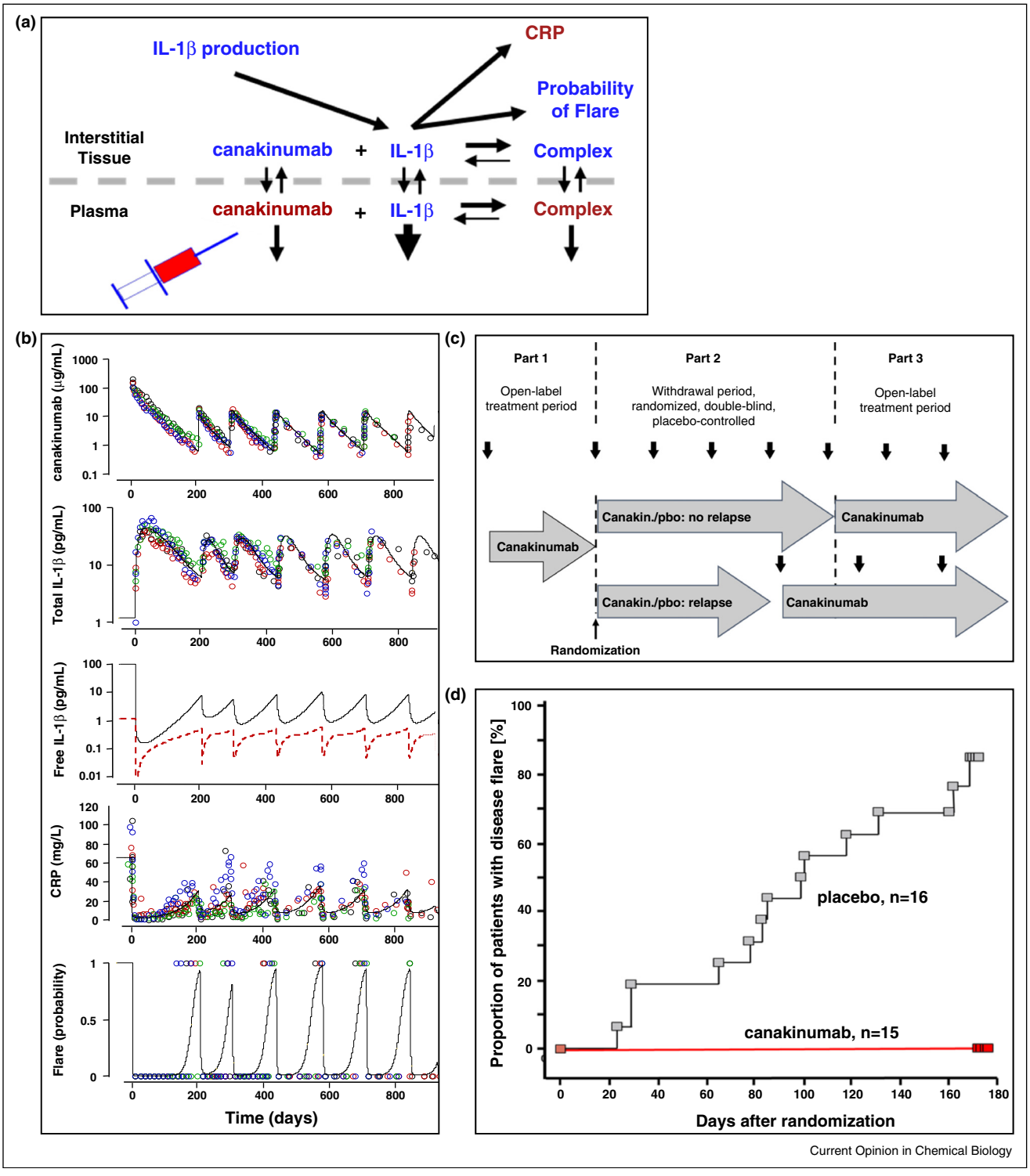
Figure 3



Overlay of the X-ray structures of the IL-1β receptor complex consisting of IL-1β (blue surface), IL-1RI (red C-atom backbone), IL-1RacP (green surface) and the Fab fragment of canakinumab (light chain, light red surface; heavy chain, blue green backbone). The magnified region (insert) shows the steric overlap between the heavy chain of canakinumab and the D1 domain of IL-1RI. Because of this steric exclusion, only free IL-1β can form the active receptor complex, and IL-1β complexed to canakinumab cannot bind to IL-1RI. The X-ray structure of the complex between the canakinumab Fab and IL-1β is described by Rondeau *et al.* [13].

canakinumab can be determined in specific ELISA-based assays. Measurements of the rate of complex formation and serum concentrations of canakinumab over time and elimination rates of the canakinumab-IL-1β complex allowed for the generation of a PK/PD binding model, describing antibody-target complex formation and the distribution of canakinumab into extravascular and serum compartments (Figure 4a). Further parameters, like the equilibrium binding constant (K_D) for binding to IL-1β, volume of distribution and steady state tissue concentration of canakinumab are used to generate a two-compartment PK/PD model [18]. Clinical information, such as the serum concentration of inflammatory markers, C-reactive protein (CRP) and the clinical assessment of disease activity in patients were combined with the PK/PD model. As shown in Figure 4b, clinical relapse or flare, that is recurrence of signs and symptoms of disease, is correlated with the inflammatory serum marker CRP, the model-predicted free IL-1β levels in serum and tissue, the formation of the complex, and ultimately with the pharmacokinetics of canakinumab. This model also predicts that the duration of clinical remission, that is, time to subsequent flare, is inversely correlated to the endogenous production rate of IL-1β in these patients. On the basis of these correlations, a simpler flare-probability model was created to link the pharmacokinetics of canakinumab to the probability of clinical relapse, that is a flare, in MW patients. Basis for such a model were clinical data obtained from seven MWS patients. These patients received different doses of canakinumab, ranging between 1 mg/kg and 10 mg/kg, applied intravenously or subcutaneously [18]. The duration of clinical response and the change in CRP over time was recorded, and patient received re-treatment when clinical signs of a relapse occurred. The resulting flare-probability model thereby connected the applied dose of canakinumab to the change in CRP and the probability of a clinical flare.

Figure 4



The dose regimen for canakinumab in CAPS is predicted by a modeling approach. **(a)** Basis of the PK/PD model. Measured parameters over time are depicted in brown, and model predicted parameters are represented in blue. The model also incorporates delineated PK parameters, such as elimination rates, volume of distribution in blood and tissue, equilibrium binding constants, and serum half-life. **(b)** Time dependent profiles for the concentration of canakinumab in serum, concentration of the IL-1β:canakinumab complex in plasma, predicted free IL-1β in the central (red) and peripheral compartment (black), plasma concentration of CRP, and flare probability. Model derived predictions are shown as lines, actual data from four patients are shown as circles. **(c)** Withdrawal design of the pivotal phase III study of canakinumab in CAPS. An open label part 1 is followed by a randomized and double-blind, placebo controlled phase. Patients who completed the part 2 without a clinical relapse and patients

Monte Carlo simulations were applied to this flare probability model, which predicted that a dose regimen of 150 mg s.c. every 8 weeks would keep the majority of CAPS patients in clinical remission [18].

The subcutaneous dose regimen of 150 mg every 8 weeks was tested in a subsequent phase III clinical trial in CAPS patients [21]. In this three-part, 48-week, double-blind, placebo-controlled, randomized withdrawal study, 35 patients received first 150 mg of canakinumab. Those patients who maintained a complete response for 8 weeks (part 1) entered part 2 and were randomly assigned to treatment with either canakinumab or placebo every 8 weeks for up to 24 weeks. After the completion of part 2 or at the time of relapse, patients progressed to part 3 and received canakinumab in an open label fashion (Figure 4c). 34 out of 35 patients enrolled into part 1 of the study had a complete clinical response to a single dose of canakinumab. This response was typically observed at day 8 or day 15 post treatment. 31 patients who maintained complete response during part 1 were randomized to either placebo or canakinumab, which were administered every 8 weeks. All 15 patients in the canakinumab group remained in clinical remission throughout part 2, while 13 out of 16 patients (81%, $p < 0.001$) who received placebo displayed signs of a clinical relapse (Figure 4d).

Results from the this phase III study were confirmed in a larger open label clinical trial study in which 166 patients with CAPS were enrolled [22]. Complete clinical response to canakinumab was observed in 78% of patients. Upward dose adjustment is sometimes required for patients with severe manifestation of disease within the CAPS spectrum [23]. Other clinical studies confirmed these initial findings, also reporting improvements in quality of life measures [24,25]. Canakinumab was well tolerated, and most adverse events were transient and mild in nature. Reported adverse events included a higher incidence of infections in children [22].

Other autoinflammatory diseases

Periodic Fever Syndromes such as Familial Mediterranean Fever (FMF), TNF Receptor Associated Periodic Syndrome (TRAPS), or Hyper IgD Syndrome (HIDS) are rare genetic disorders associated with increased production of IL-1β from peripheral blood immune cells. TRAPS and HIDS, like CAPS, are rare and are mostly confined to Western Europe. FMF is more common and prevalent around the Mediterranean Sea [26].

The genes and corresponding proteins identified as causal in these rare genetic disorders are not directly linked to the IL-1β secretion or signaling, except the pyrin protein in FMF, which interacts with components of the inflammasome [27]. TRAPS is caused by mutations in the TNF receptor I, leading to aberrant folding, intracellular accumulation, stress of the endoplasmic reticulum, and production of reactive oxygen which is believed to stimulate the inflammasome leading to hypersecretion of IL-1β from peripheral blood monocytes [28]. HIDS is an autosomal recessive disorder caused by mutations in mevalonate kinase. Deficiency in this enzyme leads to accumulation of mevalonate, and further downstream in the pathway to a shortage of isoprenoids [29]. Small GTPases require modification by isoprenoids for proper function, and lack of prenylation of the small GTPases Rac1 and RhoA leads to specific upregulation of IL-1β mRNA and hypersecretion of IL-1β protein by monocytic cells [30•,31].

Though the molecular defects in FMF, TRAPS and HIDS are not immediately related to inflammasome activation like in CAPS, the pathophysiological pathways in these diseases converge on mechanisms directly linked to dysregulation and overproduction of IL-1β. Proof of IL-1β as the causative mediator in these fever syndromes comes from the clinical use of IL-1-targeted therapy, including the use of canakinumab. Indeed, FMF, HIDS and TRAPS patients showed excellent clinical responses to treatment with canakinumab [32,33,34••,35–39,40•].

Schnitzler's syndrome is a chronic autoinflammatory disease with unknown etiology and manifestation of systemic and local inflammation. A genetic cause for this rare disease has not been identified, but targeted treatment by blockers of the IL-1 signaling resulted in remarkable clinical responses [41,42•]. A recent study by de Koning *et al.* [43••] demonstrates increased IL-1β production by isolated peripheral blood mononuclear cells from Schnitzler's patients in response to lipopolysaccharide, but low cytokine responses to TLR3 or TLR2/6 agonists. This finding may point to a selective pathogenic stimulation of the TLR4 receptor pathway, which is not only responsive to bacterial lipopolysaccharide, but also to a variety of endogenous damage-associated molecular pattern molecules (DAMPs). High serum levels of IL-6, a downstream cytokine to IL-1β, in symptomatic patients and normalization of IL-6 serum concentrations concomitant to a clinical response upon treatment with canakinumab clearly establish a pathogenic and pivotal role of IL-1β in Schnitzler's syndrome.

Systemic Juvenile Idiopathic Arthritis (sJIA) does not exhibit strong association with dominant or recessive

(Figure 4 Legend Continued) who had a clinical relapse in part 2 received canakinumab treatment. **(d)** Kaplan–Meier estimates of the proportions of patients with a disease flare in part 2 of the pivotal study. All patients who received canakinumab completed part 2 without a clinical relapse, while 13 of 16 patient on placebo had a clinical relapse in part 2 of the study. The difference is statistically significant ($p < 0.001$). Figure parts a and b were modified based on [18].

gene defects, but cellular and biochemical analysis suggest that sJIA is an autoinflammatory disease rather than an autoimmune disease [44]. IL-1 and IL-6 have been demonstrated to play a pivotal role in the pathogenesis of sJIA, as targeted treatment to either cytokine results in a dramatic clinical response in the vast majority of sJIA patients [45,46]. Two phase III trials enrolling patients with active sJIA were successfully conducted with canakinumab specifically targeting IL-1b. Both trials demonstrated excellent clinical response rates, good tolerability, and the ability to taper glucocorticoids in a substantial fraction of patients [47].

Future directions

Genetic analysis has led to recognition of further autoinflammatory hereditary fever syndromes, directly or indirectly involving inflammasome dysregulation. One of the latest examples is the identification of mutants in the NLRC4 inflammasome, leading to overproduction of IL-1β and autoinflammation, providing a solid rationale for treating such conditions by IL-1 blockade [48,49]. IL-1β has been found to be involved in a number of clinically relevant human pathologies, in particular inflammatory and vascular disease [50,51]. There are currently two more drugs approved for CAPS: Rilonacept, a recombinant soluble IL-1 receptor, and anakinra, the recombinant IL-1 receptor antagonist, which neutralize both IL-1α and IL-1β. Canakinumab is the most selective pharmaceutical agent targeting only IL-1β, and hence, provides the opportunity to specifically address the role of IL-1β in human pathology. Indeed, a phase III clinical testing the hypothesis that neutralization of IL-1β by canakinumab reduces the risk for subsequent cardiovascular events in post myocardial infarction patients is currently conducted [52]. The underlying pharmacological hypothesis for this trial is that IL-1β is a driver of atherosclerosis and plaque instability in patients with a high risk for cardiovascular events. It remains to bee seen if IL-1β is indeed a pivotal mediator of vascular inflammation, and whether neutralization of IL-1β results in a reduction of cardiovascular events and mortality.

Regulatory status

Canakinumab currently has orphan drug status for CAPS in the US. The US Food and Drug Agency (FDA) granted market authorization in June 2009 for the treatment of FCAS and MWS patients aged four years and older under the trade name Ilaris®. The European Medicines Agency (EMA) approved Ilaris® for the treatment of CAPS, including NOMID/CINCA in October 2009. Ilaris® is currently approved for the treatment of CAPS in about 73 countries worldwide. In addition, Ilaris® obtained approval for the treatment of gouty arthritis attacks in the EU, and it is approved for the treatment of systemic juvenile idiopathic arthritis (SJIA) in the US and in the EU.

Conflict of interest statement

Hermann Gram is a full time employee of Novartis Pharma AG, Basel.

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- • of special interest
- •• of outstanding interest

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