

Interleukin-6 as a Therapeutic Target

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Abstract

Human IL6 is a cytokine produced by many cell types that has pleiotropic effects. In agreement, anti-IL6 therapy reduces inflammation, hepatic acute phase proteins, and anemia and has antiangiogenic effects. Blocking IL6 has demonstrated therapeutic efficacy with drug registration in Castleman disease and inflammatory diseases (rheumatoid arthritis) without major toxicity. Interestingly, the inhibition of C-reactive protein (CRP) production is a trustworthy surrogate marker of anti-IL6 therapy efficacy. Clinically registered IL6 inhibitors include siltuximab, an anti-IL6 mAb, and tocilizumab, an anti-IL6R mAb. In various cancers, in particular plasma cell cancers, large randomized trials showed no efficacy of IL6 inhibitors, despite

a full inhibition of CRP production in treated patients *in vivo*, the numerous data showing an involvement of IL6 in these diseases, and initial short-term treatments demonstrating a dramatic inhibition of cancer cell proliferation *in vivo*. A likely explanation is the plasticity of cancer cells, with the presence of various subclones, making the outgrowth of cancer subclones possible using growth factors other than IL6. In addition, current therapeutic strategies used in these cancers already target IL6 activity. Thus, anti-IL6 therapeutics are able to neutralize IL6 production *in vivo* and are safe and useful in inflammatory diseases and Castleman disease. *Clin Cancer Res*; 21(6):1248–57. ©2015 AACR.

Introduction

IL6 was first described as a cytokine inducing B lymphocytes to produce immunoglobulin or stimulating hepatocytes and it was named B-stimulating factor-2 or hepatocyte growth factor (HGF; ref. 1; Figs. 1 and 2). IL6 is an inflammatory cytokine involved in various biologic processes, including dysimmune diseases and cancers (2, 3). It is produced by many cell lineages, including stromal cells, hematopoietic cells, epithelial cells, or muscle cells. IL6 binds to a membrane receptor (IL6R or CD126) or to its soluble form (sIL6R) with a 10^{-9} Kd (4). Then, the complexes IL6/IL6R or IL6/sIL6R bind to the gp130 IL6 transducer (CD130) with a low Kd (10^{-11} M). These bindings result in gp130 dimerization, phosphorylation, and activation of receptor-associated kinases (JAK1, JAK2, and Tyk2; ref. 4). Gp130 is a common transducing chain used by the seven members of the gp130 cytokine family and by IL27 (5).

Anti-IL6 therapies have been developed for the treatment of dysimmune diseases and in cancers. Current IL6 inhibitors include mAbs to IL6 (siltuximab) or IL6 receptor (tocilizumab), and other inhibitors are being investigated in clinical trials. This review updates the data on the clinical efficacy of IL6 inhibitors.

Lessons from Anti-IL6 Murine Monoclonal Antibodies

In the early 1990s, our group initiated the first clinical trials with anti-IL6 murine mAbs in patients with multiple myeloma or metastatic renal cell carcinoma (MRCC).

Clinical observations

The first treated patients had extramedullary multiple myeloma disease, in particular plasma cell leukemia, and the inhibition of malignant plasma cell proliferation within a few days of treatment confirmed that IL6 was a growth factor for malignant plasma cells *in vivo* (6, 7), as shown using *in vitro* models (8, 9). These first anti-IL6 treatments used anti-IL6 murine mAbs, elsilimomab (BE-8), and/or BE-4, or mAb8, and have shown that the C-reactive protein (CRP) production by human hepatocytes was completely under the control of IL6 *in vivo*, as evidenced by the loss of circulating CRP throughout anti-IL6 treatment and a quick loss reversal at treatment discontinuation (7). Anti-IL6 therapy also inhibited fever with body temperature normalization (7) in line with IL6 pyrogenic activity in rats (10). No major toxicity was evidenced, except a transient and mild (25%) decrease in platelet count (7, 11–13), which is expected given the role of IL6 to drive megakaryocyte maturation (14). Objective response with a decrease of the monoclonal component was observed in some patients, including refractory patients (11). We also treated 18 patients with MRCC with BE-8 (elsilimomab) and 4 patients received both BE-8 and IFN α -2a, resulting in 2 partial responses, 2 minor responses, one stable disease (12). Patients' conditions improved, in particular with an increase in hemoglobin level, analgesia, and a lack of flu-like syndrome despite IFN coadministration in some patients (12). Serum CRP was no more detectable within 2 days after start of anti-IL6 treatment, making it an easy marker of treatment efficacy (7, 11, 12). Other acute phase proteins also decreased rapidly (serum amyloid A protein, α 1 anti-trypsin) and serum albumin increased 20 days after the anti-IL6 therapy initiation in agreement with the major role of IL6 to control acute phase protein and albumin production by

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doi: 10.1158/1078-0432.CCR-14-2291

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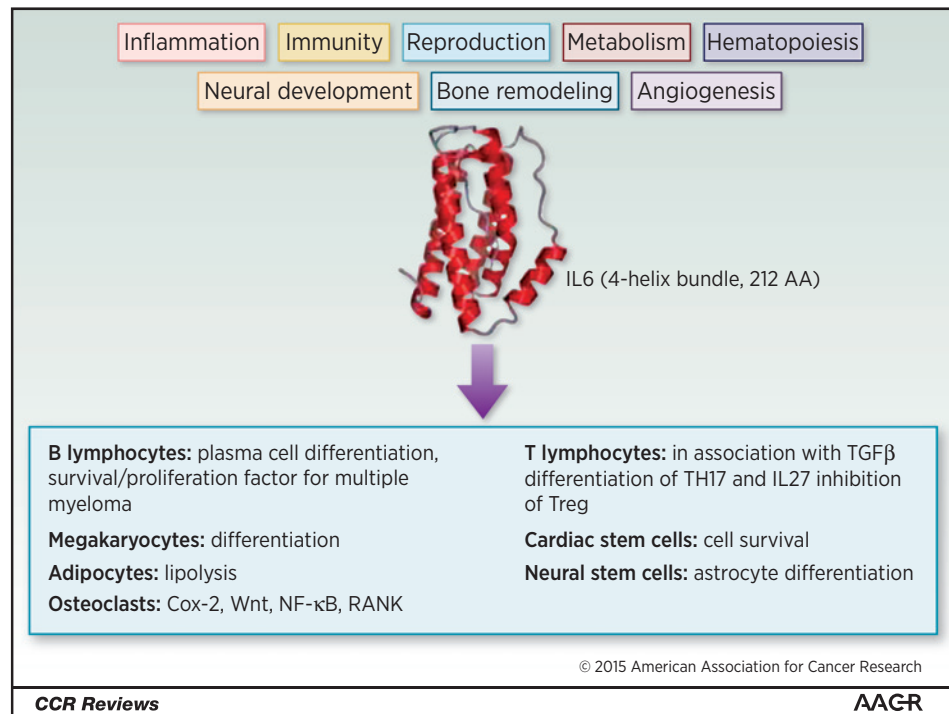


Figure 1. IL6 is a pleiotropic cytokine. gp130, glycoprotein 130; Treg, regulatory T cell.

hepatocytes (15). Hemoglobin level slightly increased by 1 to 1.5 g/dL due to inflammation control and no major change was seen in circulating T cells, natural killer cells, or B lymphocytes and in complement molecules. Overall, these first clinical trials with

murine anti-IL6 mAb have shown the lack of toxicity of anti-IL6 therapy, identified CRP as an easy and quick surrogate marker of IL6 bioactivity, and shown antitumor effects in some patients with multiple myeloma or MRCC. In addition, they made it possible to

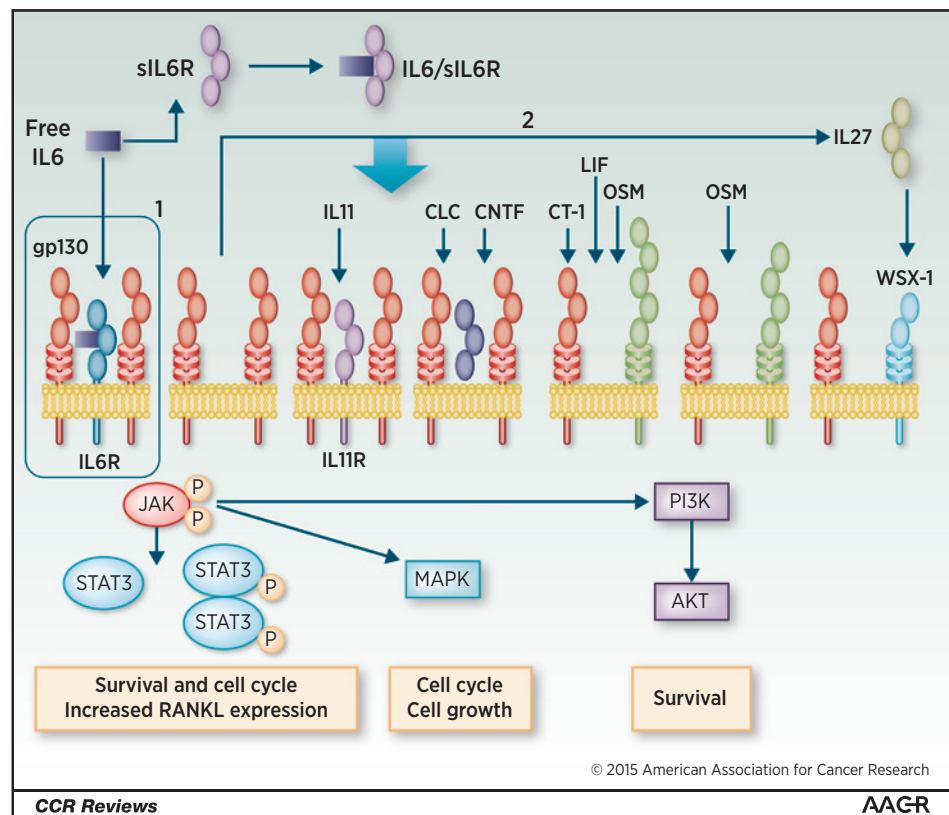


Figure 2. The IL6 receptor and glycoprotein (gp) 130 transducer cytokine family. Part 1 illustrates usual signaling. Part 2 shows *trans*-signaling. AKT, protein kinase B; CLC, cardiotrophin-like cytokine; CNTF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; gp, glycoprotein; LIF, leukemia inhibitory factor; OSM, oncostatin M; WSX-1, IL27 receptor subunit α .

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understand the main biologic mechanisms involved in anti-IL6 therapy.

Initial investigations of the biologic mechanisms involved in anti-IL6 treatment

At the stop of the anti-IL6 mAb treatment, a quick recovery of CRP production, fever, and cancer disease was observed (7). The reason is that the anti-IL6 mAb binds IL6, prevents its consumption by cells and its renal clearance, and accumulates large levels of circulating IL6 in the form of stable monomeric IL6/anti-IL6 complexes (16). The half-life of circulating IL6 increased from a few minutes in untreated individuals to several days, actually the same half-life of the free mAb, in anti-IL6-treated patients (16). Using this observation, we calculated the *in vivo* daily production of IL6 by integrating pharmacologic and affinities parameters and showed a high variation in daily IL6 production *in vivo*, from several $\mu\text{g/day}$ to mg/day , and in patients with the lower IL6 production, a complete blockade of CRP production and objective response (17). In a patient developing acute *Escherichia coli* septicemia during anti-IL6 treatment, a production of IL6 over 7 mg/day was calculated (18). Using CRP serum levels as a surrogate marker for IL6 bioactivity *in vivo*, we have shown that the anti-IL6 mAb was able to fully block IL6 activity in patients producing less than 18 μg IL6 per day, in association with an antitumor effect (11, 17). This finding indicated that the dose of anti-IL6 mAb injected could be several 100-fold too low to neutralize a huge IL6 production *in vivo* and to control CRP production and tumor growth in some patients.

In addition, at the end of anti-IL6 mAb treatment, we have shown that the ratio of the concentrations of free anti-IL6 mAbs to IL6/anti-IL6 mAb complexes decreased, making it possible for soluble or cell membrane IL6 receptors to disrupt IL6/anti-IL6 mAb complexes and use this large amount of circulating IL6 to trigger cell activation. This mechanism explains the quick recovery of CRP production, fever, and cancer cell progression occurring at the end of anti-IL6 treatment in some patients (11). A possibility to avoid this rebound effect is to use a combination of two or three anti-IL6 mAbs recognizing different epitopes. This would result in the formation of polymeric IL6/IL6 mAb complexes, which are captured mainly by hepatocytes leading to a rapid clearance, as we demonstrated in a murine model (19).

The Anti-IL6 Drugs

Several anti-IL6 mAbs have been developed. The CNTO328 chimeric anti-IL6 mAb (siltuximab) was used in clinical trials for multiple myeloma (20, 21), MRCC (22), and prostate cancer (23) and was recently registered for treating patients with Castleman disease (24). Sirukumab, a humanized anti-IL6 mAb, has been assayed in healthy subjects to determine PK/PD and safety. This drug has a half-life ranging from 18.5 to 29.6 days with no serious adverse events (25). Anti-IL6R mAb, particularly atlizumab (also called tocilizumab), has been assayed in dysimmune diseases (26). Recently, CytomX Therapeutics Inc. developed targeted "masked" antibodies, which are activated by disease-associated proteases (27). Sant7, a potent antagonist of the IL6 receptor, was engineered through targeted amino acid substitutions in key residues of the human IL6 molecule (28). Sant7 shows higher affinity than IL6 for the gp80 receptor subunit, but completely lacks binding capacity to the gp130 receptor signaling subunit. Other inhibitors have been developed (Tables 1 and 2).

Basis for Pharmacologic Effects of Antagonists

In the initial clinical trials with the BE-8 anti-IL6 murine mAb, a full inhibition of CRP production was achieved only in patients producing less than 18 $\mu\text{g/day}$ of IL6 (11). Considering the molecular weights of IL6 and BE-8 anti-IL6 mAb, the Kd of binding of BE-8 to IL6 (10^{-11} M), and the concentration of circulating BE-8 in patients (mean concentration of 10 $\mu\text{g/mL}$), a production of IL6 less than 18 $\mu\text{g/day}$ *in vivo* meant a 100-fold molar excess of BE-8 mAb to IL6 (Fig. 3). In patients with multiple myeloma treated with the siltuximab humanized anti-IL6 mAb, a siltuximab concentration of 5 $\mu\text{g/mL}$ was proposed to achieve a 300-fold molar excess of siltuximab to IL6, which is considered necessary to block IL6 activity and tumor growth with that mAb (29). In a phase I study in patients with MRCC, the administration of 6 mg/kg of siltuximab every 2 weeks efficiently suppressed serum CRP in patients who had a baseline CRP level ≤ 30 mg/L (30). These data fit well with the pharmacokinetics parameters of siltuximab (30). Indeed, clinically relevant schedules of siltuximab were simulated predicting a dosage of 6 mg/kg every 2 weeks or 9 mg/kg every 3 weeks would decrease CRP to below the lower limit of quantification (30).

Regarding tocilizumab, an administration of 8 mg/kg once every 2 weeks resulted in a marked increase in serum sIL6R in the form of sIL6R/tocilizumab complexes and reached a steady state at day 42 of treatment (31). A decrease of CRP level was found as long as the concentration of free tocilizumab not engaged in sIL6R/tocilizumab complexes and able to inhibit binding of IL6 to membrane or soluble IL6R remained above 1 $\mu\text{g/mL}$ in serum (31).

Diseases Improved by Anti-IL6 Therapies

Dysimmune diseases

In the early phases of inflammation, IL6 is produced by monocytes and macrophages, in particular through the stimulation of Toll-like receptors. A deregulated and persistent IL6 production has been observed in various chronic inflammatory and/or autoimmune diseases, including in animal models. IL6 blockade by means of gene-knockout or administration of anti-IL6 or anti-IL6R antibody can suppress such disease development either preventively or therapeutically. The anti-IL6R mAb tocilizumab has demonstrated efficacy either as a monotherapy or in combination with disease-modifying antirheumatic drugs for adult patients with moderate to severe rheumatoid arthritis (for review refs. 26, 31). A Cochrane database systematic review concluded that tocilizumab-treated patients were four times more likely to achieve American College of Rheumatology 50% improvement (38.8% vs. 9.6%) and 11 times more likely to achieve Disease Activity Score remission as compared with control patients (30.5% vs. 2.7%; ref. 32). Thus, the anti-IL6R mAb tocilizumab is now approved for the treatment of rheumatoid arthritis in more than 90 countries. The outstanding results obtained with tocilizumab in rheumatoid arthritis led to a change in the treatment objective from protection against joint destruction to prolongation of life expectancy with normal activities in daily life. Safety has been reported from six studies performed in Japan. The incidence of adverse events (AE), including abnormal laboratory test results, was 465.1 per 100 patient-years, with infection being the most common AE

Table 1. Anti-IL6 antagonists in clinical trials

| Drug (company) | Clinical trial (disease/type) | ClinicalTrials.gov |
|--|---|-----------------------------|
| Elsilimomab or BE-8 | Lymphoma, MM, MRCC Phase I/II | |
| Siltuximab, chimeric (CNTO328) | Mab 1339, fully human from BE-8 OPI EUSA/Vaccinex/GSK | |
| | MM | |
| | Phase I | NCT01309412 |
| | Phase II multicentric CNTO 328 + Dex | NCT00402181 |
| | Randomized phase II, multicentric CNTO328 + Bor | |
| | First-line phase Ib/II Len/Bor/Dex/CNTO328 | NCT01531998 |
| | MGUS, SMM, indolent MM | |
| | Phase I (effect on heart) | NCT01219010 |
| | SMM high-risk | |
| | Phase II randomized | NCT01484275 |
| | Myelodysplastic syndrome | |
| | Randomized phase II | |
| CNTO328+BSC vs. placebo+BSC | NCT015133317 | |
| Solid cancers | Phase I/II, ovarian, pancreatic, colon, head and neck, lung cancers | NCT00841191 |
| | MRCC | |
| | Phase I/II | |
| | Prostate cancer metastatic, hormone-refractory | NCT00385827 |
| | Mitoxantrone + prednisone + CNTO328 phase II | |
| | Prostate cancer not responding to hormone therapy | NCT00433446 |
| | Multicentric Castleman disease | NCT0400503 |
| | Randomized phase II (long-term evaluation) | |
| | Active RA | |
| | Under methotrexate therapy | NCT00718718 |
| Humanized anti-IL6 <i>ALD518</i> or <i>BMS-945429</i> | Cancer | |
| | Phase I | |
| Anti-IL6, humanized | NSCLC | |
| | Phase I/II (fatigue and cachexia) multicentric | |
| Alder Biopharm. Inc./BMS | RA | |
| | Phase II | NCT00867516 |
| | Crohn disease | |
| Phase II (discontinued) | NCT01545050 | |
| Olokizumab (CDP6038) | RA | |
| | Humanized anti-IL6 | |
| UCB Group/Russia's R-Pharm | Anti-TNF failure phase II | NCT01533714 and NCT01463059 |

Abbreviations: Bor, bortezomib; BSC, best supportive care; Carbo, carboplatin; Dex, dexamethasone; Doxo, doxorubicin; Len, lenalidomide; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NSCLC, non-small cell lung carcinoma; RA, rheumatoid arthritis; SMM, smouldering multiple myeloma.

with 6.22 per 100 patient-years (32). As expected, increases in liver function and lipid parameters were observed. Tocilizumab is also a promising drug for systemic lupus erythematosus, systemic sclerosis, polymyositis, Takayasu and giant cell arteritis, Crohn disease, relapsing polychondritis, multiple sclerosis, Still disease, and Behçet disease (26, 31).

Castleman disease

Castleman disease is a lymphoproliferative disease characterized by benign hyperplastic lymph nodes, follicular hyperplasia with polyclonal plasmablastic proliferation and capillary proliferation associated with vascular hyperplasia and high IL6 activity, mainly due to viral IL6 (33, 34). Viral IL6 is produced by cells infected by Kaposi sarcoma-associated herpes virus (KSHV) and directly binds and stimulates gp130 IL6 transducer in the absence of IL6R (33, 34). All of the HIV-positive and half of the HIV-negative patients with multicentric Castleman disease were infected with KSHV (33). The rationale for developing anti-IL6 therapy was first that IL6 is a main growth factor for plasmablasts (35) and that it has pleiotropic activities, which may explain vascular hyperplasia in particular. In particular, transgenic mice bearing an IL6 transgene driven by the immunoglobulin E μ enhancer develop massive polyclonal plasmacytoses and rapidly die (36).

The BE-8 anti-IL6 murine mAb was first used for a patient with Castleman disease, who showed obvious disease improvement after treatment (37). A clinical trial with the anti-IL6R mAb tocilizumab confirmed this benefit and led to its approval in Japan as an orphan drug for this disease in 2005. Recently, the anti-IL6 mAb siltuximab was also proven to provide major clinical benefit in a randomized phase II study enrolling 79 patients, and it has been approved for treatment of this disease (24).

Diseases with No Demonstrated Benefit of Anti-IL6 Therapies

Multiple myeloma

Before reviewing the efficacy of randomized anti-IL6 trials in patients with multiple myeloma, it is worthwhile to update the current knowledge about the place of IL6 as a growth factor for multiple myeloma cells (MMC). IL6 was recognized in the late 1980s as an important growth factor for MMCs, being produced mainly by the tumor environment (6, 7) and also by MMCs (8). Although most studies confirmed these findings (38), current data indicate that insulin-like growth factor-1 (IGF-1) is the major growth factor for MMCs, and that IL6 is active mainly in CD45⁺ MMCs (39, 40). The phosphatase CD45 dephosphorylates IGF-1R in MMCs and weakens IGF-1R signaling, making

Table 2. Anti-IL6 receptor antagonists in clinical trials

| Drug (company) | Clinical trial (disease/type) | ClinicalTrials.gov |
|---|--|--------------------|
| Tocilizumab | 208 studies including | |
| | Ovarian cancer | NCT01637532 |
| | Phase I/II (Carbo/pegylated liposomal doxorubicin hydrochloride or Carbo/Doxo) + tocilizumab/Peginterferon alfa-2b | |
| | Erdheim-Chester disease (histiocytose) | NCT01727206 |
| | Phase II | |
| | Hematophagocytic lymphohistiocytosis | NCT02007239 |
| Sarilumab IL6R Sanofi/Regeneron VX30 (Vaccinex) | Phase II | |
| | Graft-versus-host disease | NCT02174263 |
| | Phase II | |
| | Steroid-refractory | NCT01475162 |
| | Castleman disease KSHV ± Antiviral drugs | NCT01441063 |
| | Continuing in responding patients | NCT01183598 |
| ARGX-109 (arGEN-X) | 14 studies: 12 in RA, including a phase III trial, 1 in noninfectious uveitis, and 1 in ankylosing spondylitis | |
| FM101 (Formatech) | | |
| Sant7 (receptor superantagonist) | | |

Abbreviations: Bor, bortezomib; BSC, best supportive care; Carbo, carboplatin; Dex, dexamethasone; Doxo, doxorubicin; Len, lenalidomide; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NSCLC, non-small cell lung carcinoma; RA, rheumatoid arthritis; SMM, smouldering multiple myeloma.

MMCs more dependent on IL6 (39). Besides IGF-1 and IL6, other MMC growth factors have been described, including mainly BAFF/APRIL, produced by osteoclasts (41), and members of the EGF family, such as HGF and VEGF (see ref. 42 for review). In addition, CRP, whose production is under IL6 control, also increases IL6 production in MMCs, enhances their

proliferation under stress conditions, and protects them from chemotherapy (43).

Regarding signaling pathways, IL6 triggered the JAK/STAT3 pathway, which drives an antiapoptotic response in MMCs, mainly through upregulation of the MCL1 antiapoptotic protein (44)). It also triggers the MAP kinase ERK1/2, activating the cell

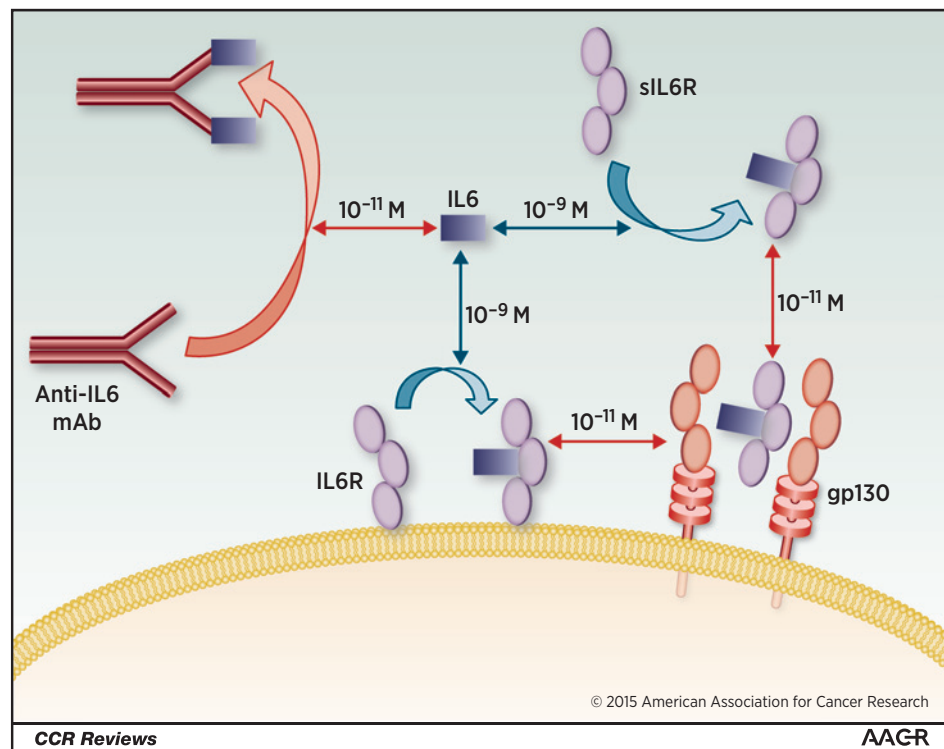


Figure 3. Basis for pharmacologic effects of IL6 antagonists. gp, glycoprotein.

cycle (41). The PI3K and AKT signaling pathway is also important to promote MMC growth. The AKT kinase is activated in MMCs (45). But, in our opinion, IL6 does not activate this pathway, unlike IGF-1 or other MMC growth factors (44). This may explain the additive effects between IL6 and other growth factors to support MMC growth.

Two randomized trials were recently published showing a lack of effect of anti-IL6 therapy. In one trial, the anti-IL6 mAb siltuximab was given to transplant-ineligible patients in association with a regimen of bortezomib–melphalan–prednisone compared with bortezomib–melphalan–prednisone and then as a maintenance therapy for 18 months or until relapse (20). In another trial, siltuximab was given in relapsed/refractory multiple myeloma in association with bortezomib compared with bortezomib alone and then as maintenance therapy until progression (21). These two trials showed no benefit for event-free or overall survivals. In the first study, an improvement of response was found. The initial anti-IL6 treatments performed by our group did not investigate event or overall survival, but showed a quick blockade of tumor proliferation and reduction in tumor mass in patients with fulminant disease, mainly with extramedullary proliferation (7, 11). In a series of 24 newly diagnosed patients, the BE-8 anti-IL6 mAb was given together with 140 mg/m² of melphalan and autologous stem cell transplantation for 21 days (13). This combination was shown to be as active as 200 mg/m² of melphalan to decrease the tumor mass, based on historical comparison, and without impairment on the hematopoietic recovery (13).

How can we explain this lack of benefit in randomized trials with anti-IL6 mAb despite the major role of IL6 to control healthy plasmablast proliferation (35, 36) and despite the blockage of myeloma cell proliferation by anti-IL6 mAb in patients with fulminant disease (7, 11)? In these randomized trials, siltuximab was able to block CRP production, indicating its neutralization of IL6 bioactivity in the liver. Thus, a lack of antimyeloma effect of siltuximab was not due to an inability to block a too large IL6 production. In line with this, median CRP levels are not higher in patients with multiple myeloma compared with those with rheumatoid arthritis for whom anti-IL6 therapy had benefit (Table 3). Several mechanisms could explain this lack of effect of anti-IL6 therapy. First of all, the efficacy of anti-IL6 therapy was compared with drug combination (bortezomib or bortezomib–melphalan–prednisone), which already reduces IL6 production and inhibits CRP production (20). Second, as summed up above, other factors could stimulate MMC growth in the absence of IL6, in particular IGF1 in CD45^{−/low} MMCs, and favor the emergence of MMC subclones independent of IL6 (39, 40). This emergence of IL6-independent subclones could not be investigated in our initial short-term treatments in patients with fulminant disease. Third, other cytokines of the IL6 family can trigger the gp130 IL6 transducer and also the growth of MMCs and could substitute for IL6 to promote MMC survival and growth *in vivo* (46).

Other hematologic malignancies

IL6 and/or CRP are prognostic factors for several B-cell malignancies, particularly malignant lymphoma (47). IL6 is a biologic marker in other lymphoid malignancies, including Hodgkin disease, mantle-cell lymphoma, cutaneous CD30-positive lymphomas, and KSHV-associated malignancies (48–52). Polymorphisms of different cytokine genes, and particularly of IL6 gene (IL6-174GG genotype) are associated with treatment failure

in Hodgkin disease (53). The majority of B-cell tumors expressed the IL6 receptors, in particular mantle cell lymphoma (54). BE-8 was used in 11 patients with HIV-associated lymphoma and showed a clinical benefit, particularly on B symptoms (55), and in one patient with acute monoblastic leukemia (56). Therefore, IL6 and more generally gp130 IL6 transducer signaling could play an important role in the pathogenesis of certain B-cell neoplasias, and new clinical programs could be developed in such diseases.

Metastatic renal cell carcinoma

IL6 was shown to be an autocrine proliferation factor for tumor cell lines obtained from patients with MRCC (57). Mutations in the *TP53* gene could contribute to the overexpression of IL6 in renal cell carcinoma (58). We and others have demonstrated that CRP/IL6 is a prognostic factor in MRCC (12, 59, 60). Furthermore, we demonstrated a significant association between the presence of the IL6R in tumors and tumor stage, nuclear grade, proliferation index, and serum IL6 (61). Treatment of renal cell carcinoma cells with cisplatin (CDDP) in combination with anti-IL6 or anti-IL6R mAbs can overcome their CDDP resistance by downregulation of glutathione S-transferase π expression (62). Furthermore, in a phase I study, the use of IL6 and GM-CSF in patients with renal cell carcinoma was associated with inverse clinical effects (63). We treated 18 MRCC patients with BE-8 mAb and demonstrated a clinical benefit, including abrogation of fever, hypercalcemia, and inflammatory syndrome, reduction of anemia and morphine intake, and weight increase, with objective responses observed (2/18 patients with partial responses, one with a minor response, and one with stable disease; ref. 12). In a phase I/II study, siltuximab was shown to stabilize the disease in more than 50% of progressive MRCC patients, with one partial response and a favorable safety profile, a situation that could authorize further evaluation of dose-escalation strategies and/or combination therapy (22). Nevertheless, to our knowledge, no further studies have been performed.

Prostate cancer

IL6 acts as a paracrine and autocrine growth factor for both benign and cancer prostate cells. The levels of IL6 and IL6R are increased during prostate carcinogenesis and tumor progression (64). In addition, a correlation between increased serum IL6 and soluble IL6R levels with aggressiveness of the disease was reported (65). During the treatment of prostate cancer, castration resistance usually leads to the death of the patient. The signaling pathway mediated by IL6 represents an alternative pathway in the cancer-resistance phenotype acquisition and cancer progression (64, 65). During androgen deprivation therapy, a regulation loop may emerge between sex steroids and IL6, with a strong positive correlation with total-testosterone, androstenedione, and estradiol levels (66). Other gp130 cytokines have shown involvement in prostate tumor progression by promoting VEGF and urokinase plasminogen activator (67). A meta-analysis was recently performed to evaluate the association between the IL6 174GC genotype and susceptibility to prostate cancer with an increased risk observed in two cohort studies. Additional well-designed studies are needed to validate the role of IL6 genetic polymorphism in prostatic cancer risk (68).

All the above-mentioned data show the importance of the IL6/IL6R pathway in the regulation of growth and drug resistance of prostate cancer cells. In a phase I study, 20 patients scheduled

Table 3. Clinical effects of anti-IL6 therapies (monoclonal antibodies tocilizumab and siltuximab)

| | RA | MCCD | MM | MRCC | Lymphoma | Prostate cancer | Crohn disease |
|---|--------------|--|--------------|-------------------------------|--|-----------------|---------------|
| Median CRP levels (mg/L; range) | 5.6 (2-19.4) | 17.6 (0.1-181) | 3.75 (0-121) | 4.9 (0.3-183) | 29.2 (0-129) | 1.55 (0.8-130) | 5.8 (1.7-8.1) |
| Effects of IL6 inhibitors | ++ | ++ | ± | ± | Effect on lymphoma associated with HIV infection | - | - |
| CRP inhibition 90%-100% within 2-4 days | + | + | + | + | + | + | + |
| Other acute-phase proteins (ferritin 20% decrease; other, 40%-70% reduction in 2-4 weeks) | + | + | + | + | + | + | + |
| Albumin increase (20%-30% increase after 3-4 weeks) | + | + | + | + | + | NR | NR |
| Fever (B symptom) | NR | + | NR | + | + | NR | NR |
| Analgesia | ++ | + | NR | + | NR | NR | NR |
| Lymphocyte counts (peripheral blood) | NR | NR | NR | No major change | NR | NR | NR |
| Platelet decrease (20%-30% decrease) | + | + | + | + | + | + | + |
| Hemoglobin increase (1.1-1.5 g/dL) within 1-8 weeks | + | + | + | + | + | NR | + |
| Disease improvement | ++ | ++ | -effect HCa | Paraneop. syndrome effect HCa | Paraneop. syndrome | NR | + |
| Drug registration | Tocilizumab | Tocilizumab (J; situximab EU and U.S.) | No | No | No | No | Tocilizumab |

Abbreviations: EU, European Union; J, Japan; MCCD, Multicentric Castleman disease; MM, multiple myeloma; NR, not reported; RA, rheumatoid arthritis; Paraneop., paraneoplastic.

for radical prostatectomy received either no drug or siltuximab (6 mg/kg, 5 patients/group with administration once, twice, and three times, prior to surgery). A decrease in phosphorylation of Stat3 transcription factor and p44/p42 MAPKs, with a down-regulation of genes immediately downstream of the IL6 signaling pathway and key enzymes of the androgen signaling pathway, was observed (69). A phase II study designed by SWOG was completed in chemotherapy-pretreated patients with castration-resistant prostate cancer, showing stable disease in 7 patients (23%). An increase of IL6 was observed after treatment, likely due to the accumulation of stable IL6/anti-IL6 mAb complexes, as we demonstrated in other trials (16). This may explain the lack of clinical benefit (70). The combination of mitoxantrone/prednisone and siltuximab was not associated with a clinical improvement in a randomized phase II study, as compared with chemotherapy alone (23). Other molecules have been shown efficacious on tumorigenesis in prostate cancer, including polyphenols, by mediating a decrease in IL6 signaling (71). Because of a rise in IL6 both *in vitro* and *in vivo* in the presence of tyrosine kinase-resistant cells, IL6 may represent a biomarker for tyrosine kinase resistance (72).

Other Potential Indications

IL6 inhibition may have therapeutic indications in other cancers, including ovarian cancer. Recently, a correlation between thrombocytosis, IL6, and thrombopoietin was shown in 619 patients (73). Neutralizing IL6 significantly enhances the therapeutic efficacy of paclitaxel in mouse models of epithelial ovarian cancer, reducing tumor growth and angiogenesis (73, 74). Other studies have shown that IL6 is involved in the progression of squamous cell carcinoma, melanoma, hepatocellular carcinoma, and neuroblastoma (2, 75–78).

Conclusions

Several conclusions can be drawn from 25 years of experiments with anti-IL6 therapeutics.

1. The current clinical-grade anti-IL6 or anti-IL6R mAbs have been proven to efficiently neutralize IL6 in most patients *in vivo*. Our initial observation that CRP is a perfect surrogate marker for IL6 activity, its production by hepatocytes being fully dependent on IL6 *in vivo* (7, 11), has been confirmed in all studies. The daily production of IL6 ranges from several

µg/day in most individuals *in vivo* (16, 17) to several mg/day in cases of acute infection (18), and anti-IL6 mAbs are efficient in cases of daily production of IL6 below 20 µg/day.

2. Anti-IL6 or anti-IL6R mAbs have clinical efficacy in Castleman disease and in inflammatory diseases such as rheumatoid arthritis or Crohn disease.
3. Anti-IL6 or anti-IL6R mAbs have no demonstrated clinical efficacy in various types of cancer despite a great deal of evidence showing the involvement of IL6 in controlling the growth of malignant cells and of their healthy counterparts.
4. The lack of effect of IL6 inhibitors in cancer is not due to IL6 production that is too high in these cancers compared with inflammatory diseases. Indeed, as summarized in Table 3, the mean CRP levels are in the same range in these various diseases and anti-IL6 therapeutics efficiently neutralized CRP production by hepatocytes. In addition, the various biologic activities (fever normalization, analgesia, platelet decrease) associated with anti-IL6 therapeutics are observed in both IL6 responsive and unresponsive diseases (Table 3).
5. The lack of effect of IL6 inhibitors in cancer is most likely due to a plasticity of tumor cells, with the presence of various tumor clones in tumor samples *in vivo* (79). In cases of IL6 inhibition, the IL6-sensitive clones may disappear, creating free tumor environment niches and favoring clones, whose growth is triggered by other growth factors triggering signaling pathways similar to those triggered by IL6, in particular cytokines of the gp130 family. Such mechanisms may explain the lack of effect of long-term anti-IL6 therapeutics in patients with multiple myeloma, whereas initial short-term treatment in patients with fulminant multiple myeloma demonstrated a dramatic antiproliferative effect.

Disclosure of Potential Conflicts of Interest

J.-F. Rossi is a consultant/advisory board member for BETA-INNOV, the Castleman Disease Collaborative Network (CDCN), and LEO Pharma. No potential conflicts of interest were disclosed by the other authors.

Acknowledgments

The authors thank Dr. Vidal Benatar for manuscript corrections.

Received September 4, 2014; revised November 26, 2014; accepted December 15, 2014; published OnlineFirst January 15, 2015.

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