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Review

Clinical Cancer Research

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.

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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 IFNα-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, α1 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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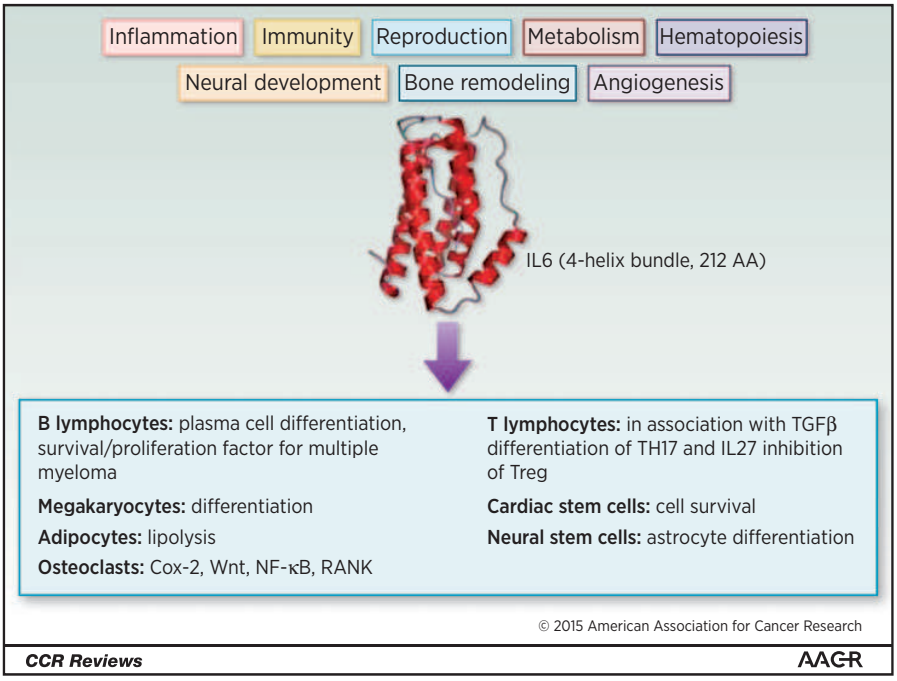

Figure 1.
IL6 is a pleiotropic cytokine. gp130, glycoprotein 130; Treg, regulatory T cell.

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

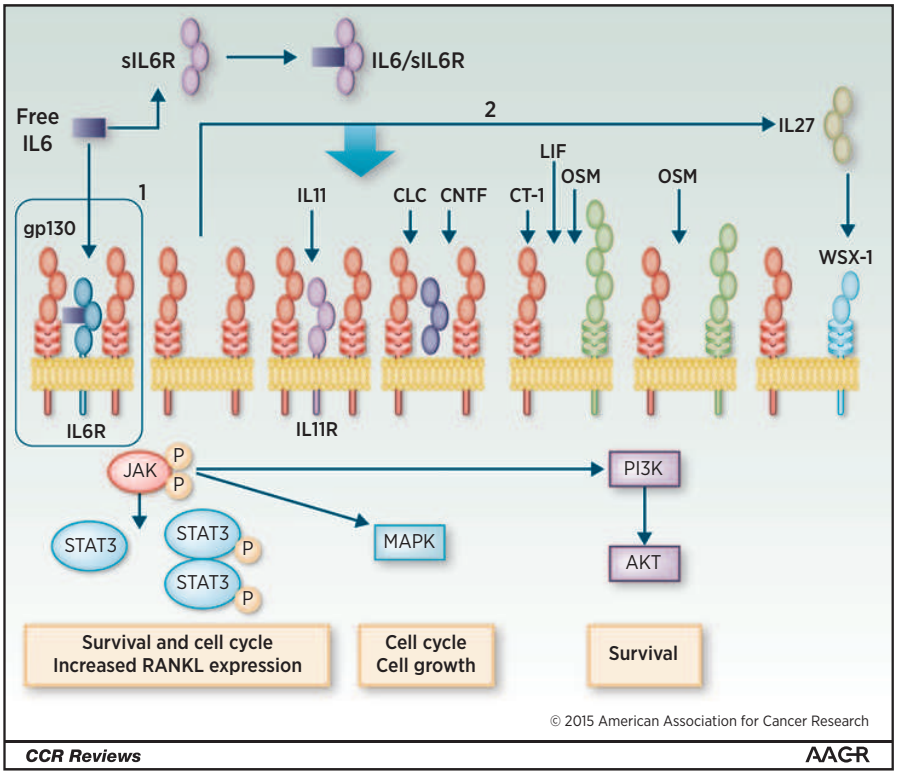

Figure 2.
The IL6 receptor and glycoprotein (gp) 130 transducer cytokine family. Part 1 illustrates usual signaling. Part 2 shows *trans*-signaling. AKT, protein kinase B; CLC, cardiotrophin-like cytokine; CNTF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; gp, glycoprotein; LIF, leukemia inhibitory 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 μg/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 μg/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 μg/mL), a production of IL6 less than 18 μg/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 μg/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 $\leq$ 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 biding of IL6 to membrane or soluble IL6R remained above 1 μg/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 though 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

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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	
	Mab 1339, fully human from BE-8 OPi EUSA/Vaccinex/GSK	
Siltuximab, chimeric (CNTO328)	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)	
Sirukumab, CNTO 136	Active RA	
Humanized anti-IL6	Under methotrexate therapy	NCT00718718
ALD518 or BMS-945429	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)		
Humanized anti-IL6	RA	
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 Eμ 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

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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
	Phase II	
	Graft-versus-host disease	NCT02174263
	Phase II	
	Steroid-refractory	NCT01475162
	Castleman disease KSHV	NCT01441063
	± Antiviral drugs	
	Continuing in responding patients	NCT01183598
Sarilumab IL6R	14 studies: 12 in RA, including a phase III trial,	
Sanofi/Regeneron	1 in noninfectious uveitis, and 1 in ankylosing spondylitis	
VX30 (Vaccinex)		
ARGX-109 (arGEN-X)		
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

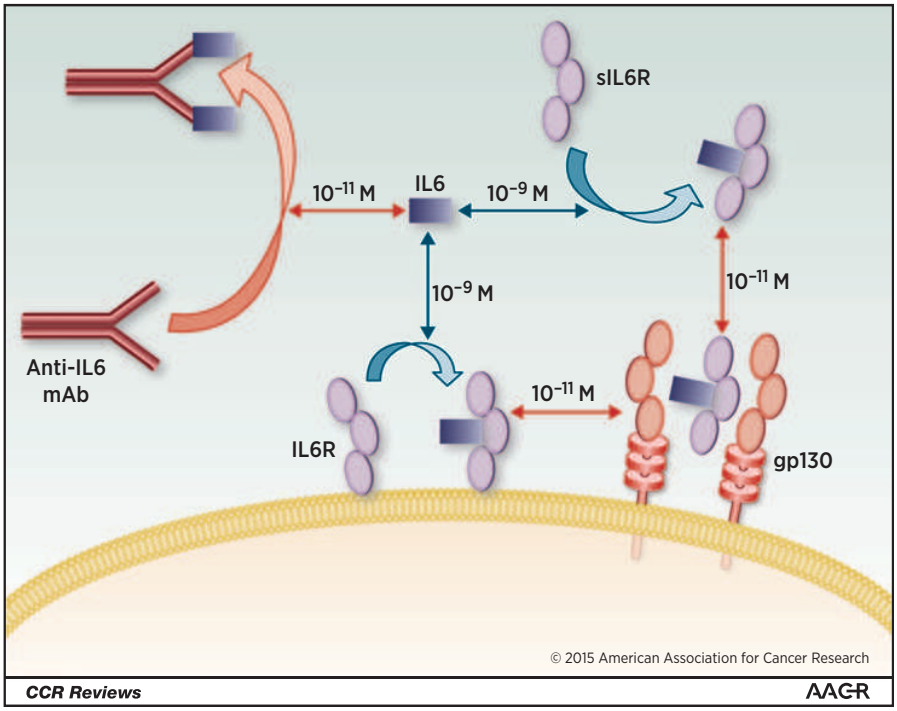

Figure 3.
Basis for pharmacologic effects of IL6 antagonists. gp, glycoprotein.

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