

Review

Targeted Anti-Interleukin-6 Monoclonal Antibody Therapy for Cancer: A Review of the Rationale and Clinical Evidence

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Abstract

Interleukin (IL)-6, a pleiotropic cytokine with varied systemic functions, plays a major role in inflammatory processes. It modulates the transcription of several liver-specific genes during acute inflammatory states, particularly C-reactive protein, and controls the survival of normal plasmablastic cells. In addition, IL-6 has been implicated in hematopoiesis as a cofactor in stem cell amplification and differentiation. This article is the first review of clinical studies in the 1990s with anti-IL-6 monoclonal antibodies (mAbs) in the treatment of patients with cancer and related lymphoproliferative disorders. In six clinical studies of mAbs to IL-6 with BE-8 or CNTO 328 in patients with multiple myeloma, renal cell carcinoma, and B-lymphoproliferative disorders, anti-IL-6 mAb treatment decreased C-reactive protein levels in all patients. In most patients, levels decreased below detectable limits. The antibodies were well tolerated, and no serious adverse effects were observed in the vast majority of studies. The fact that anti-IL-6 mAb therapy decreased the incidence of cancer-related anorexia and cachexia may also be useful in the treatment of cancer patients.

Introduction

Physiological Roles of IL-6. IL-6² is a pleiotropic cytokine with varied systemic functions (1). Because of its pleiotropic

pic nature, IL-6 was initially assigned a variety of names based on function, including IFN- β 2, IL-1 inducible 26-kDa protein, hepatocyte-stimulating factor, cytotoxic T-cell differentiation factor, and B-cell stimulatory factor (2). As the molecule's various attendant physiological effects became associated with a common gene, the name *IL-6* was proposed. IL-6 is secreted by a number of different cell types, and IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection, trauma, and immunological challenge (3, 4).

The physiological activity of IL-6 is complex, producing both pro-inflammatory and anti-inflammatory effects in the immune system (Fig. 1). IL-6 modulates the transcription of several liver-specific genes during acute inflammatory states, particularly CRP, and controls the survival of normal plasmablastic cells, as demonstrated in reactive plasmacytosis using mAbs directed against IL-6 (5, 6). In addition, IL-6 is an activator or an inhibitor of T-cell responses, depending on the target and the system used *in vitro*. This interaction of pro-inflammatory and anti-inflammatory activities suggests that IL-6 may play a role in regulating the physiological response to disease.

Increased production of IL-6 has been implicated in various disease processes, including Alzheimer's disease, autoimmunity (*e.g.*, rheumatoid arthritis), inflammation, myocardial infarction, Paget's disease, osteoporosis, solid tumors (RCC), prostatic and bladder cancers, certain neurological cancers, B-cell malignancies [*i.e.*, Castleman's disease, some lymphoma subtypes, CLL, and, in particular, MM (7–10)]. In some instances, IL-6 is implicated in proliferation pathways because it acts with other factors, such as heparin-binding epithelial growth factor and hepatocyte growth factor (11–13). Blocking IL-6 may thus be of benefit in many pathological situations. This article discusses the role of IL-6 in the etiology and pathogenesis of cancer and reviews clinical trials of targeted cancer therapy using mAbs to IL-6.

IL-6/IL-6R Interaction. IL-6 is a multifunctional cytokine that binds to a specific IL-6R (α chain, IL-6R, or CD126) on target cells. This IL-6/IL-6R complex associates with two molecules of the ubiquitously expressed gp130 (β chain, CD130), the second chain of the receptor, resulting in the formation of high-avidity IL-6 binding receptors (14, 15). The gp130 functions as an affinity converter because the resulting affinity of IL-6 for the ternary complex is approximately 10^{-11} M, instead of 10^{-9} M for IL-6R. Whereas gp80 binds specifically to IL-6, gp130 is a common signal-transducing receptor for a subfamily of cytokines, including IL-6, IL-11, LIF, ciliary neurotrophic factor, oncostatin M, cardiotropin-1, and neurotrophin-1, named the gp130 cytokine family. After binding to their specific receptors, all these cytokines induce homodimerization of gp130 or its heterodimerization with the LIF receptor, which initiates cell signaling (14). In contrast to the wide distribution of gp130, gp80 is limited to hepatocytes and specialized subsets

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² The abbreviations used are: IL, interleukin; CRP, C-reactive protein; RCC, renal cell carcinoma; MM, multiple myeloma; IL-6R, IL-6 receptor; LIF, leukemia-inhibiting factor; sIL-6R, soluble IL-6R; FLT-3, *fms*-related tyrosine kinase 3; SCF, stem cell factor; PB, peripheral blood; PCL, plasma cell leukemia; DXM, dexamethasone; BLPD, B-lymphoproliferative disorder; mAb, monoclonal antibody; CLL, chronic lymphocytic leukemia; TNF, tumor necrosis factor; B-CLL, B-cell CLL; 2-CdA, cladribine; Ab, antibody; HAMA, human antimouse antibody; cmAb, chimeric mAb.

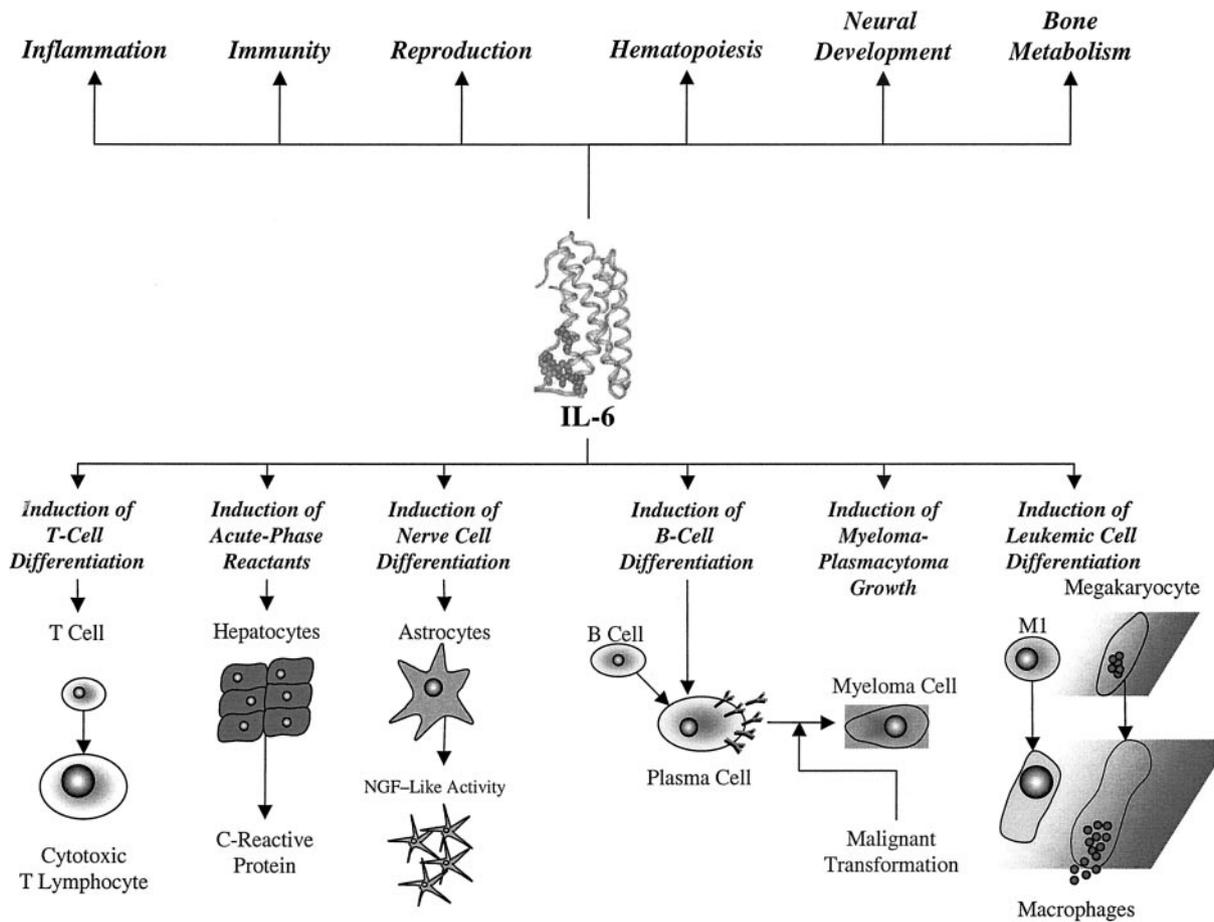


Fig. 1 Schematic representation of the physiological activity of IL-6.

of leukocytes, including monocytes, neutrophils, T cells, and B cells (3). Stimulation of gp130 is essential for hematopoiesis *in vivo*.

The system is complicated by the presence of soluble forms of both gp80 and gp130. These circulating compounds are cleaved from the cell membrane molecule or translated from an alternative spliced mRNA, yielding a protein that differs at its COOH terminus by 14 amino acid residues (16–18). Cleavage of transmembrane proteins can be done by a transmembrane metalloproteinase, distinct from matrix-type metalloproteinases (ADAM; Ref. 19). sIL-6R or gp55 retains its capability to bind IL-6, and the complexes formed are able to activate the gp130 transducer receptor. Therefore, unlike other soluble cytokine receptors, which are generally antagonists, sIL-6R is an agonist molecule, promoting IL-6 activity. This capability may explain a possible activation of gp130 despite the lack of gp80, if sIL-6R molecules circulate in great quantity, as demonstrated in certain pathological states. Cells that do not express specific receptors for IL-6, IL-11, or ciliary neurotrophic factor cannot respond to these cytokines. The presence of sIL-6R makes these cells responsive, a process called trans-signaling. Sera from healthy individuals contain sIL-6R (mean value, 89 ng/ml; range, 17–300 ng/ml). Soluble gp130 has been observed in

human plasma and may bind soluble and membrane-anchored IL-6/IL-6R complexes, thus appearing as an endogenous IL-6 antagonist.

Implications of IL-6 in Self-Renewal and Differentiation of Stem Cells and Committed Progenitors. IL-6 plays a major role in inflammatory processes and has been implicated in hematopoiesis as a cofactor in the amplification and differentiation of stem cells. Early hematopoietic stem cells express low levels of FLT-3 and c-kit receptors as well as gp130 receptor but do not express IL-6R (20). Therefore, IL-6/IL-6R complexes are efficient in amplifying and maintaining early progenitor cells as well as other cytokines, including SCF and FLT-3 or, to a lesser extent, IL-1. Primitive CD34-positive progenitors provide a soluble positive-feedback signal that induces cytokine production by stromal cells, including IL-6 (21). Serum sIL-6R levels reflect proliferative kinetics of the stem cells after mobilization (22). The differentiation of various cells, including mast cells and cardiomyocytes, is also under the control of IL-6 and the gp130 family, in addition to other cytokines (23).

Recently, it was shown that self-renewal of embryonic stem cells required sustained signaling by gp130 cytokines, particularly LIF and also IL-6, in a concentration-dependent manner, with thresholds in ligand-receptor signaling that mod-

ulate control of stem cell differentiation (24). On the other hand, IL-6/IL-6R complexes and the gp130 cytokine family were shown to be implicated in neural cell (25) and osteoclast (26) differentiation. The gp130 family, particularly LIF, regulates osteoprogenitor differentiation in different models (27).

IL-6 and Cancer

Possible Role. During the late stages of tumor growth, tumor expression is associated with an increase in the levels of IL-1, IL-6, and acute-phase proteins. Table 1 lists investigations that define the role of IL-6 in a number of neoplastic diseases or conditions (28–65). In the majority of studies, active disease is associated with elevated serum levels of IL-6, which are related to disease severity and outcome. Evaluating the prognostic significance of serum IL-6 in patients with prostate cancer, Nakashima *et al.* (55) demonstrated that elevated levels of serum IL-6 are significant indicators of poor prognosis. Under-scoring the potential value of targeted anti-IL-6 therapy in prostate cancer, a recent investigation showed that anti-IL-6 mAb induced prostate tumor apoptosis and regression of xenografted human cancer cells in a nude mouse model (66). In many of these cancers, increased IL-6R expression was also detected (3). Comparable to the role of IL-6 in inflammation, inhibition and stimulation of cancer cell proliferation are also functions of this cytokine, depending on the cell type and the presence or absence of IL-6R (8). Regarding its antitumor activity, IL-6 promotes the antitumor activity of macrophages, helps produce lymphokine-activated killer cells, and protects neutrophils from apoptosis, which may increase their cytotoxic effect on tumor cells. Also, through the stimulation of increased synthesis of CRP, IL-6 indirectly influences the binding of this protein to phospholipids on tumor cells, activating C1q of the complement system, which may sometimes lead to tumor cell lysis. In most cancers, however, IL-6 likely favors tumor growth *in vivo* as described below.

Promotion of tumor growth can occur through an “autocrine” mechanism in which cancer cells begin to express a growth factor (or receptor for the growth factor) that is not expressed normally (67, 68). There is some evidence that cytokines such as IL-6 may be involved in the regulation of solid tumor growth in a paracrine manner (69, 70), but early-stage clinical trials have demonstrated significant hematological changes and flu-like symptoms. A Phase I clinical trial was conducted in 13 patients with RCC treated simultaneously with granulocyte macrophage-colony stimulating factor and escalating doses of IL-6 (71). The most common side effects were fever, fatigue, and arthralgias. In patients receiving IL-6, dose-limiting toxicity included thrombocytosis and hyperbilirubinemia. In patients receiving combination IL-6 and granulocyte macrophage-colony stimulating factor, the common side effects were leukocytosis and thrombocytosis, platelet activation, and increases in peripheral blood progenitors. The side effect profiles of two other concurrent Phase I trials were reported by Sosman *et al.* (72). Sixty-nine patients (1-h trial, $n = 40$; 120-h trial, $n = 29$) were enrolled, including 27 patients with renal cancer and 16 patients with melanoma. The most commonly reported side effects included fever (97%), anemia (78%), fatigue (56%), nausea or vomiting (49%), and elevated serum

transaminase levels (42%). Twenty-three patients (33%) developed transient hypotension. There were three deaths during the study due to progressive disease and/or infection. Primary dose-limiting toxicities included atrial fibrillation (one episode in the 1-h trial and four episodes in the 120-h trial) and neurological toxicities (three episodes in the 1-h trial and four episodes in the 120-h trial) and confusion, slurred speech, blurred vision, proximal leg weakness, paraparesis, and ataxia. Discontinuation of IL-6 treatment resulted in disappearance of these side effects.

IL-6 has also been shown to differentially impact melanoma cell proliferation based on tumor cell stage of development (73). Addition of nanogram quantities of recombinant human IL-6 *in vitro* to early-stage melanoma cells resulted in the inhibition of tumor cell proliferation. Although >50% of advanced-stage tumor cell lines produced IL-6 (74), these cells were resistant to the inhibitory effect of IL-6. Thus, IL-6 is “bifunctional” in that it can switch from behaving as a paracrine growth inhibitor to an autocrine growth stimulator with the same cells during malignant tumor cell proliferation. This resistance to IL-6-mediated tumor growth is believed to be due to a down-regulation of IL-6R α chain (75). In patients with metastatic malignant melanomas, elevated levels of IL-6 correlate with tumor burden and failure of therapy. Mouawad *et al.* (48) demonstrated that before biochemotherapy treatment 50% of the melanoma cells and 32% of CD3⁺ cells expressed IL-6R. After treatment, the percentage of cells expressing functional IL-6R decreased significantly in comparison with CD3⁺IL-6R⁺ cells ($P = 0.0256$).

Leukemia and Lymphoma. B-CLL is the most common leukemia, characterized by the clonal proliferation and accumulation of B lymphocytes (76, 77). These leukemic cells in B-CLL can express and secrete IL-6 (78) as determined by Northern blot analysis. This cytokine functions as a B-cell-stimulatory factor and mediates B-cell differentiation and growth of B-cell lymphoid malignancies (79). In addition, IL-6 has also been shown to inhibit TNF- α -induced proliferation of B-cells from CLL patients (80). In CLL patients, IL-6 plasma levels increased in a stage-dependent manner, suggesting that IL-6 may be a useful marker of disease prognosis (81). Increasing levels of IL-6 significantly correlated with patient age, Rai stage, and white cell count. Median IL-6 plasma levels in CLL patients were not different statistically from those of normal patients ($P = 0.38$), but patients with Rai stage III/IV had significantly higher median IL-6 plasma levels than normal controls ($P < 0.05$). Cox regression hazard models show that elevated IL-6 plasma levels (>3 pg/ml) correlate with shorter survival rates ($P = 0.0001$). Studies conducted by Hulkkonen (82) in B-CLL patients were able to confirm elevated serum IL-6 concentrations in comparison with healthy subjects ($P < 0.005$). Analysis of IL-6 allelic frequencies showed that IL-6 plasma levels were dependent on exogenous growth factors rather than an allelic imbalance of IL-6 genes.

In addition to IL-6, the prognostic value of sIL-6R serum levels has been demonstrated by Robak *et al.* (38). Measurements of serum IL-6 and sIL-6R concentrations were conducted in 63 patients with B-CLL and 17 healthy volunteers. Simultaneous measurements were also made in 25 untreated patients and 38 patients treated with 2-CdA. The results indicate correlation of IL-6 serum levels with Rai's clinical disease stage and

Table 1 Cancers associated with abnormal IL-6 production

Cancer type or related disorder	Serum IL-6 levels (pg/ml) ^a	Study findings	Reference(s)
Breast	6.0	Median serum IL-6 level ~10 times higher in patients with metastatic disease than in those with localized disease	Benoy <i>et al.</i> (28)
	6.9	Significantly higher serum IL-6 levels in patients with >1 metastatic site	Zhang and Adachi (29)
	86.0	IL-6 and -8 levels higher in patients with progressive disease	Yokoe <i>et al.</i> (30)
Cachexia	9.83	Decrease in IL-6 serum levels associated with subjective improvement after therapy	Yamashita and Ogawa (31)
		High serum levels of IL-1, IL-6, and TNF- α in advanced-stage cancer patients, particularly those with cachexia	Mantovani <i>et al.</i> (32)
Gastrointestinal	12.53	Serum IL-6 levels were elevated in advanced-stage gastrointestinal cancer patients and correlated with overall survival	De Vita <i>et al.</i> (33)
	35.7	Serum IL-6 levels indicative of tumor proliferative activity in colorectal cancer patients	Kinoshita <i>et al.</i> (34)
	1272.6	High serum levels of IL-6 mark patients with cholangiocarcinoma and correlate with tumor burden	Goydos <i>et al.</i> (35)
	79.6	Serum levels of IL-1 β , IL-6, and TNF- α elevated in patients with squamous cell carcinoma of the oral cavity	Jablonska <i>et al.</i> (36)
	10.0	Mean serum levels of IL-6 higher in patients with gastric cancer	Wu <i>et al.</i> (37)
Leukemia		Serum levels of IL-6 in chronic lymphocytic leukemia patients not significantly different from that of control subjects, IL-6 levels in patients treated with 2-CdA lower, especially in patients who achieved remission	Robak <i>et al.</i> (38)
Lymphoma	≥ 2 considered elevated	Elevated serum IL-6 levels seen in 25% of indolent non-Hodgkin's lymphomas, predictive of poor outcome	Fayad <i>et al.</i> (39)
	2.7	IL-6 serum levels frequently elevated in patients with Hodgkin's disease; these normalize with remission	Seymour <i>et al.</i> (40)
	4.6 median	Serum IL-6 levels elevated in patients with diffuse large cell lymphoma	Preti <i>et al.</i> (41)
Lung		IL-6 and -8 and TNF- α found in higher concentrations in malignant pleural effusion than in serum	Alexandrakis <i>et al.</i> (42)
	32.2	Serum IL-6 levels higher in patients with extensive small cell lung cancer than in patients with limited-stage disease	Dowlati <i>et al.</i> (43)
	104.2	Increased IL-6 level related to extensive disease, impaired performance status, and enhanced acute-phase response	Martin <i>et al.</i> (44)
	10.2	Mean IL-6 concentrations significantly higher in non-small cell lung cancer patients than in control subjects	De Vita <i>et al.</i> (45)
	28.7 vs. 6.3	Serum concentration of IL-6 significantly higher in mesothelioma than in lung adenocarcinoma	Nakano <i>et al.</i> (46)
Melanoma	19.4	Significantly higher serum IL-6 and IL-12 levels observed in patients with localized and metastatic melanoma	Moretti <i>et al.</i> (47)
	8.01	Baseline serum IL-6 level significantly higher in patients with metastatic malignant melanoma	Mouawad <i>et al.</i> (48)
Multiple myeloma	35.2	Increased proportion of T cells producing IL-6 in MM patients with active disease	Frassanito <i>et al.</i> (49)
	16.6	Significantly increased serum concentration of IL-6 in MM patients	Urbanska-Rys <i>et al.</i> (50)
	13.2	Serum levels of IL-6 significantly higher in MM patients, highest levels seen in patients with progressive disease	Wierzbowska <i>et al.</i> (51)
	3.2	Serum levels of IL-6 and of IL-6R significantly higher in patients with MM who died within 3 years than in those who survived	Pulkki <i>et al.</i> (52)
Ovarian	55.6 median	Median serum levels of IL-6 significantly elevated in ovarian cancer patients	Tempfer <i>et al.</i> (53)
Pancreatic		Increased serum levels of IL-6 detected in 54.5% of pancreatic cancer patients; significantly among the patients with weight loss	Okada <i>et al.</i> (54)

Table 1 Continued

Cancer type or related disorder	Serum IL-6 levels (pg/ml) ^a	Study findings	Reference(s)
Prostate	7.2	Serum IL-6 levels significantly correlated with clinical stage of prostate cancer	Nakashima <i>et al.</i> (55)
	20.9	Serum levels of IL-4, -6, and -10 significantly elevated in hormone-refractory prostate cancer	Wise <i>et al.</i> (56)
	93.15	Levels of IL-6 and transforming growth factor correlate with tumor burden and clinically evident metastases	Adler <i>et al.</i> (57)
	5.7	Serum IL-6 level significantly elevated in hormone-refractory prostate cancer	Drachenberg <i>et al.</i> (58)
	21.8	Serum levels of IL-6 related to the metastatic burden to osseous tissue in patients with prostate cancer	Akimoto <i>et al.</i> (59)
Renal cell	67.0 with short survival times Range: 0.35–120	Serum IL-6 levels before surgery were higher in renal cell cancer patients with short survival	Kallio <i>et al.</i> (60)
	Median: 4.35	Levels of serum IL-6 and basic fibroblast growth factor were significantly higher in renal cell cancer patients with malignant cysts	Hayakawa <i>et al.</i> (61)
	Median: 14	56% of patients with metastatic renal cell carcinoma had detectable serum levels of IL-6	Walther <i>et al.</i> (62)
	62.0	Serum IL-6 levels were significantly higher in renal cell cancer patients with paraneoplastic fever and weight loss	Blay <i>et al.</i> (63)
	28.1	There was a significant difference in survival among renal cell carcinoma patients with detectable levels of IL-6	Costes <i>et al.</i> (64)
		Survival time was significantly shorter for renal cell carcinoma patients with serum IL-6 levels above the median level for all patients studied	Ljungberg <i>et al.</i> (65)

^a Mean values unless otherwise indicated.

response to 2-CdA treatment. IL-6 was measurable in 62 of 63 (98.4%) untreated patients and 14 of 38 (37.8%) treated patients. IL-6 serum levels in untreated patients did not differ significantly when compared with the control group. In patients treated with 2-CdA, concentrations of IL-6 were statistically different ($P < 0.02$), with the lowest levels achieved in patients with complete remission (median, 1.4 pg/ml; $P < 0.02$). The concentrations of sIL-6R were significantly higher in untreated (median, 61.8 ng/ml) and treated (median, 50.1 ng/ml) CLL patients in comparison with normal persons (median, 61.8 ng/ml; $P = 0.04$ and $P < 0.001$, respectively). There was no difference in sIL-6R levels between patients with complete remission and the healthy controls. sIL-6R levels in nonresponders were statistically similar to those of untreated patients. Significant correlations were also made between levels of sIL-6R and lymphocyte counts in CLL patients ($P = 0.423$; $P < 0.001$). Hence, serum concentrations of either IL-6 or sIL-6R can act as useful indicators of CLL activity. CLL cells are frozen in G₀-G₁ phase and have extended life spans *in vivo*. However, in culture, PB B-CLL cells undergo rapid and spontaneous apoptosis. Studies indicate that IL-6 dimers in the conditioned medium are potent factors for the inhibition of B-CLL apoptosis (83).

Serum concentrations of IL-6 can also be used as a prognostic indicator for complete response and failure-free survival in patients diagnosed with diffuse large cell lymphoma. Preti *et al.* (41) demonstrated that serum levels of IL-6 were higher (median, 4.6 pg/ml) in 118 patients diagnosed with diffuse large cell lymphoma than in 45 healthy volunteers ($P = 0.009$). The high IL-6 concentration (>4.3 pg/ml) was associated with lower complete response rate ($P = 0.013$) and an inferior failure-free

survival rate ($P = 0.004$). Failure-free and overall survival of patients (as determined by International Prognostic Index Score) could also be correlated to IL-6 serum concentrations ($P \leq 0.03$). It appears that IL-6 levels are more elevated in the more aggressive lymphomas and that serum IL-6 levels could identify patients with evolving forms of lymphoma, even though morphological evidence of transformation is not yet evident.

Several other reports demonstrate that serum IL-6 levels are elevated in patients with aggressive non-Hodgkin's lymphoma (84–86), indolent lymphomas (39), and adult T-cell leukemia lymphoma (87) and correlate with shorter failure-free survival (85, 88). Serum IL-6 levels show significant correlation with overall survival. For patients with elevated serum IL-6 concentrations, the 3-year survival rate was 51% (95% confidence interval, 72–91%). In comparison, for the patients with normal serum IL-6 levels, the 3-year survival rate was 81% ($P = 0.0004$).

MM. The function of IL-6 in the pathogenesis of MM is well documented (89). MM is a plasma cell dyscrasia characterized by the accumulation of a clone of malignant plasma cells in the bone marrow. IL-6 is overproduced by MM patients' bone marrow stromal cells (7) through interaction with MM cells, including cell-cell contact and the production of diverse molecules. In particular, IL-6 production is induced mostly by IL-1 β produced by the tumor environment and myeloma cells.

The IL-1 effect is mediated through induction of prostaglandin E₂ synthesis that subsequently triggers IL-6 production (90). Similar findings were observed in murine plasmacytomas (91). A certain number of IL-6-dependent plasma cell lines have been generated (92), some of which are sensitive to other gp130 cytokine family members (92), depending on the presence of

other receptors in this family and the action of IL-10 (93, 94). Increased serum IL-6 levels in MM patients are associated with a poor prognosis (95).

sIL-6R plays a role in the pathogenesis of MM by forming complexes with IL-6, thus producing a 10-fold increase in the sensitivity of human IL-6-dependent cell lines (96). The presence of high levels of sIL-6R in the serum of patients with MM, independent of tumor cell mass and disease status, suggests that this circulating protein has an important functional role in the pathogenesis of monoclonal gammopathy (96). Clinical investigations have shown that serum IL-6 and CRP levels are correlated and indicate disease severity and progression (97).

Recent studies have shown that IL-6 is a survival factor for myeloma cells. In particular, we found that among 10 antiapoptotic and proapoptotic proteins of the bcl-2 family, IL-6 promotes myeloma cell survival by inducing Mcl-1 gene and protein expression (98). Further studies have shown that blocking Mcl-1 induced myeloma cell apoptosis (99) and that Mcl-1 overexpression induced IL-6 independent survival of myeloma cells.³ In addition, using the model of overexpression of Mcl-1 in myeloma cells, it appears that IL-6 is primarily a survival factor and not a proliferation factor for myeloma cells (4). This information would be important in the combined use of IL-6 inhibitors and chemotherapy.

RCC. IL-6 is expressed by the majority of RCCs and is essential to the proliferation of RCC cell lines (68, 100). The exact mechanism of enhanced production of IL-6 in renal cell tumors is unknown, but p53 mutations have been detected in up to 30% of primary kidney tumors and in 70–80% of metastatic tumors (101–105). One study has confirmed that p53 mutations can result in overexpression of IL-6 and that wild-type p53 represses IL-6 expression by inhibiting transcription factor binding to the IL-6 promoter (103).

Analyzing sera and tissue samples from 38 patients with primary RCC, Costes *et al.* (64) found significant correlations between the level of IL-6 and disease state. Serum IL-6 levels correlated with tumor size and stage. Tissue samples stained positive for IL-6R expression in 10 instances. The presence of IL-6R in tumors was markedly associated with tumor stage, nuclear grade, proliferation index, and serum level of IL-6. Study of this subgroup of patients with the most aggressive disease allowed the authors to identify patients in whom IL-6 and IL-6R played a critical role in tumor progression or proliferation (64). In a study of 122 patients with RCC, Yoshida *et al.* (106) confirmed that serum levels of IL-6 in stage IV patients were markedly higher than those in patients with lower-stage disease or in the control group and concluded that serum IL-6 level and TNF- α may be a useful measure in the early diagnosis of RCC.

Metastatic RCC is also associated with a high incidence of paraneoplastic syndrome, a condition characterized by fever and elevated levels of acute-phase markers (*e.g.*, CRP), as well as by a decrease in serum albumin, thrombocytosis, and anemia (107), all of which appear to be due to abnormal cytokine production or immunogenic mechanisms. Blay *et al.* (63) found that serum

levels of IL-6 in RCC patients with paraneoplastic fever and weight loss were higher than those in RCC patients without paraneoplastic symptoms, thus suggesting a role for IL-6 in this syndrome. Three patients with paraneoplastic syndrome entering this study were treated with a mAb to IL-6 therapy, and all showed reduced levels of CRP, haptoglobin, and serum alkaline phosphatases over 21 days of treatment. After therapy was stopped, serum levels of these factors increased to pretreatment levels or beyond (63), demonstrating the significance of IL-6 in paraneoplastic syndrome as well as the potential of targeted anti-IL-6 therapy. Humoral hypercalcemia is a complication of malignancy related to paraneoplastic syndrome that is linked to tumor production of substances that stimulate osteoclastic activity. Weissglas *et al.* (108) found that RCC cells may overexpress IL-6 in hypercalcemia, probably by acting on parathyroid hormone-related peptide; this highlights a potential supportive therapeutic role for anti-IL-6 treatment. In RCC and other cancer patients, IL-6 and CRP serum levels are correlated with Stauffer's syndrome, particularly neoplastic fever, weight loss, performance status (63), depression (109), anemia, leukocytosis, thrombocytosis, hypoalbuminemia, hypercalcemia, and other biological symptoms.

Anorexia and Cachexia. Cancer-related anorexia and cachexia are serious complications associated with malignant disease, affecting up to 87% of patients (110). IL-6 has been implicated in the etiology of cachexia, based primarily on the results of preclinical animal studies (111).

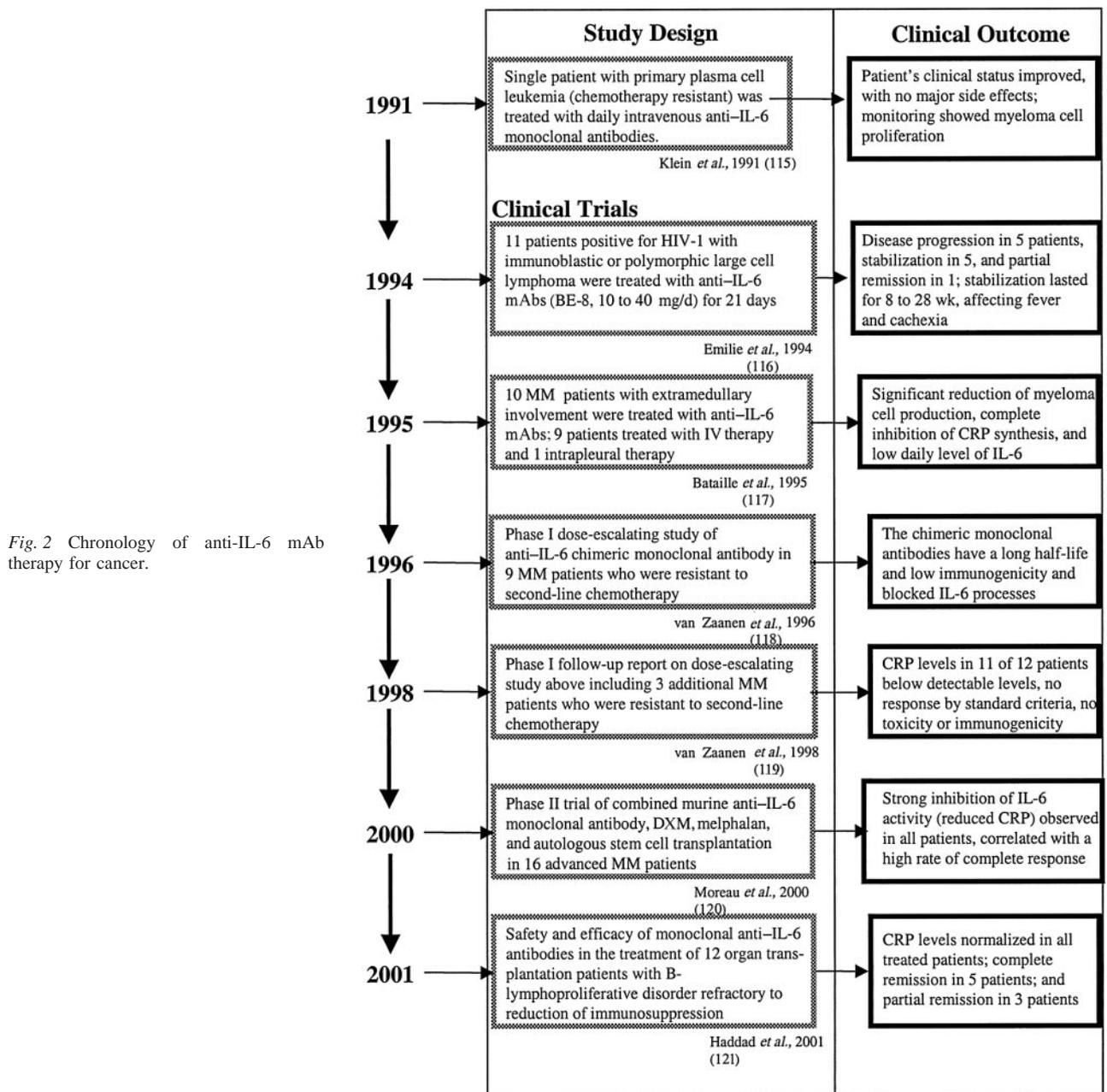
Drug Resistance of Tumor Cells. IL-6 is linked to drug resistance mechanisms, including glutathione *S*-transferase, through demonstration of the sensitization of human RCC cell lines to cisplatin by blocking IL-6 (112) or multidrug resistance in breast cancer. Renal cell tumors are refractory to cisplatin therapy, and anti-IL-6 or anti-IL-6R mAb enhanced the *in vitro* susceptibility of RCC cells to cisplatin, suggesting clinical benefit with combined therapy (112).

Use of mAbs to IL-6

Clinical Experience. Experimental and clinical findings support the role of IL-6 in active cancers and provide a rationale for targeted therapeutic investigations. Various therapeutic agents have a mode of action that affects IL-6 production. Known inhibitors of IL-6 include corticosteroids, nonsteroidal anti-inflammatory agents, estrogens, and cytokines [*e.g.*, IL-4 (113)]. DXM has also been shown to inhibit both IL-6 and IL-6R gene expression in myeloma cell lines. Targeted biological therapies include IL-6-conjugated toxins and mAbs directed against IL-6 and IL-6R (113, 114). IL-6-conjugated toxin therapy is based on fusing the IL-6 gene to *Pseudomonas* exotoxin or diphtheria toxin genes, although the potential viability of this approach is questionable because many normal cells express IL-6R, most notably hepatocytes (114).

The chronology of clinical investigations of mAbs to IL-6 in the treatment of cancer and related lymphoproliferative disorders spans a decade. Fig. 2 summarizes the general characteristics of and clinical results from the seven studies (115–121). Initial investigations were conducted with mouse mAb to IL-6 (murine mAbs BE-4 and BE-8). More recently, a chimerized mouse mAb to IL-6 (human-mouse cmAb to IL-6) with the

³ B. Klein, unpublished data.



investigational name CNTO 328 was used in a Phase I clinical trial in patients with MM. CNTO 328 contains the variable antigen-binding region of the murine anti-IL-6 Ab and the constant region of the human IgG1 κ immunoglobulin (119).

A patient with primary PCL that was resistant to chemotherapy was the first reported recipient of anti-IL-6 (115). The patient was a 61-year-old male with primary PCL, bone lesions, hypercalcemia, renal deficiency, anemia, leukocytosis, bone marrow invasion by malignant plasma cells, and 25% myeloma cells in the peripheral blood. After giving informed consent, the patient received daily i.v. infusions of mAb to IL-6 (murine BE-4 and murine BE-8) in the following dosing regimen: days

0–5, 40 mg of BE-4; day 6, 120 mg of BE-4; days 7–10, 8 mg of BE-8; days 11–14, 4 mg of BE-8; days 15 and 16, 20 mg of BE-4; days 17–23, no injection; days 24–59, 8 mg of BE-8; days 60–63, no injection; days 64–67, 16 mg of BE-8; and after day 68, no injection.

There was inhibition of myeloma cell proliferation in the bone marrow, along with decreased serum levels of calcium and monoclonal IgG. Levels of CRP became undetectable, and no serious side effects were noted. This study demonstrated the potential of mAb to IL-6 therapy, resulting in a transient tumor cytostasis and reduction in toxicities from IL-6 (115). A subsequent study by Bataille *et al.* (117) reported the results of mAb

to IL-6 therapy in treating MM patients. Of the 10 patients who had advanced and progressive MM with mainly primary or secondary PCL, 9 received i.v. mAb to IL-6 (BE-8) therapy (20 mg/day) for at least 4 days and for as long as 68 days. One patient with pleural effusion received local intrapleural administration of BE-8 (20 mg/day for 3 days).

Three of the treated patients died of MM after less than 1 week of therapy, including the patient who received intrapleural treatment. Two of these patients with assessable data showed marked inhibition of plasmablastic proliferation. The seven remaining patients received i.v. therapy for at least 1 week, with three exhibiting an objective antiproliferative effect as measured by myeloma cell labeling in the bone marrow; one of the three showed a 30% regression of tumor mass. However, no patient achieved remission or improvement as assessed by standard clinical criteria. Although the data were not reported, the authors noted that fever and hypercalcemia resolved with a mAb to IL-6 therapy. These data suggest that a mAb to IL-6 therapy could help patients with early-stage disease (117). One of these nine patients developed a case of *Escherichia coli* sepsis during therapy, and serum levels of IL-6 in the form of monomeric complexes of IL-6/mAb to IL-6 remained very high for 20 days after sepsis, indicating the persistence of increased production of IL-6 (122). This technique for measuring the IL-6/anti-IL-6 complex provided a way to estimate overall IL-6 production as well as the levels of Ab necessary for effective neutralization of IL-6 (122, 123).

Emilie *et al.* (116) also reported the results of an open-label, multicenter clinical trial of mAb to IL-6 (murine BE-8) to treat HIV-1-positive patients who had immunoblastic or polymorphic large cell lymphoma. BE-8 (10–40 mg/day) was administered i.v. for 21 days. Clinical suppression of IL-6 activity was assessed by measuring serum levels of CRP.

A total of 11 HIV-1-positive patients with lymphoma entered the study and were evaluated. BE-8 therapy suppressed the spontaneous growth of the lymphoma in 6 of the 11 patients, with detectable neutralization of endogenous IL-6. Five of these patients were classified as clinically stabilized; one achieved partial remission (116). Follow-up assessments in the five patients whose disease stabilized indicated that stabilization was sustained for 8–28 weeks. Overall, antitumor activity was limited and inconsistent (116). Side effects seen during therapy included a 25–40% reduction in platelet count. Because the most clear-cut benefit of BE-8 therapy was the alleviation of systemic symptoms (*i.e.*, fever, sweats, and cachexia), the authors concluded that IL-6-dependent growth of malignant lymphomas may occur in some cases and that effective neutralization of endogenous IL-6 with targeted mAb therapy may alleviate clinical symptoms (116).

Using a one-compartment model, the half-life of BE-8 mAb was estimated at 3–4 days (122). Human Abs to the BE-8 or BE-4 anti-IL-6 Ab (HAMAs) were detected in all patients. In 80% of patients, these HAMAs targeted the F(ab')₂ part of the murine mAb and did not result in *in vivo* clearance of the mAb. This finding explains why murine anti-IL-6 mAb could inhibit IL-6 activity for at least 2 months. In 20% of patients, HAMAs to the Fc part of the murine anti-IL-6 mAb were detected 7–10 days after the start of treatment. In these patients, the mAb was eliminated rapidly, and IL-6 activity could no longer be inhibited *in vivo* (124).

Anti-IL-6 mAb Dosing. van Zaanen *et al.* (118, 119) wrote two articles based on a dose-escalation analysis of a chimeric human-mouse Ab to IL-6 in MM patients resistant to second-line chemotherapy. The first article concerns the treatment of patients with end-stage, progressive MM, diagnosed according to the criteria of Durie and Salmon (125). Nine patients entered the study and received human-mouse cmAb to IL-6 (murine-human cmAb CCLB8; now called CNTO 328) in two cycles of 14 daily i.v. infusions, starting on days 1 and 28 of therapy. The dosing regimen was as follows: 5 mg/day in patients 1–3 (total dose, 140 mg); 10 mg/day in patients 4–6 (total dose, 280 mg); and 20 mg/day in patients 7–9 (total dose, 560 mg). The median half-life of the CNTO 328 was 17.6 days, and none of the treated patients produced human anti-CNTO 328 Abs.

Although the disease stabilized in eight of nine patients, no patient achieved a clinical response (*e.g.*, a decrease in levels of M protein of >50%). Data from the study enabled the development of a method for calculating endogenous IL-6 production and the finding that CNTO 328 therapy normalized endogenous IL-6 production but did not affect the IL-6 production associated with infection. These investigations suggest that CNTO 328 was able to block IL-6-dependent processes *in vivo* (118).

In the second report (119), three additional patients were enrolled in the dose-escalation study and received CNTO 328 in two cycles of 14 daily i.v. infusions starting on days 1 and 28 of therapy. The dosing regimen was 40 mg/day in these patients (patients 10–12; total dose, 1120 mg).

Disease stabilized in 11 of 12 patients; the twelfth patient with progressive disease responded to the second course of treatment. No patient had a toxic or allergic reaction to CNTO 328 therapy, although two patients developed transient thrombocytopenia, and six patients developed granulocytopenia. Despite stabilization of disease, no patient had a clinically significant response (*e.g.*, a reduction in the level of M protein greater than 50%). As in the previous investigation (118), no immune response to chimeric anti-IL-6 mAb occurred, and CRP levels became undetectable in 11 of 12 patients (119).

The study concluded that no life-threatening side effects were associated with CNTO 328 therapy, and pharmacokinetic measurements indicated that the cmAb had a long half-life (17.8 days). Although no patient achieved remission, the authors hypothesized that the level of M protein did not decrease more than 50% in treated patients who exhibited decreased CRP levels due to the presence of immature and mature myeloma cells. Immature myeloma cells are highly proliferative, but mature cells are not. Mature cells are also responsible for synthesizing M protein, implying that some IL-6-independent myeloma cells in end-stage MM (119) cannot be targeted by a mAb to IL-6.

Combination Therapy. More recently, Moreau *et al.* (120) investigated the potential of combination therapy, including a murine mAb to IL-6 (BE-8), DXM, and high-dose melphalan [220 mg/m² (HDM220)], followed by autologous stem cell transplantation, in the treatment of patients with advanced MM. A total of 16 patients received treatment. At enrollment, 2 were resistant to all chemotherapy, and 14 had relapsed. A dose of 250 mg of BE-8 was infused over 4 days in combination with DXM (49 mg/day) on days 1–4, followed by HDM220 infused over 30 min on day 5 and autologous stem cell transplantation on day 7 (120).

In general, IL-6 activity was strongly inhibited, as indi-

cated by reduced CRP levels. Overall, 13 of 16 patients (81.3%) exhibited a response, with a complete response seen in 6 patients (37.5%). No toxic or allergic reactions were reported, but the incidence of thrombocytopenia and neutropenia increased.

This study, however, did not precisely determine the biological parameters of the IL-6 blockade, particularly in terms of level and duration, a situation that could be associated with a possible and rapid regrowth of the disease. The treatment period associated with the major risk of triggering proliferation by IL-6 is the period just after the autograft because high levels of IL-6 could be produced during hematopoietic recovery.

Another recent trial used BE-8 before and after high-dose melphalan and graft of hematopoietic progenitors.⁴ The rationale was to increase the sensitivity of myeloma cells to melphalan, blocking their major survival factor, and then to prevent a rescue of melphalan-resistant cells that might be mediated by the large *in vivo* IL-6 production occurring after high-dose melphalan. This study involved 34 MM patients treated with BE-8 melphalan (140 mg/m²) and graft of hematopoietic progenitors. BE-8 therapy had an effect on quality of life, as demonstrated by a reduction in mucositis episodes and in disease aggressiveness, a decrease in the number of RBC transfusions, with no difference in hematological recovery and no increase in the infectious risk.⁴ As shown previously, the injection of BE-8 in patients with MM induced the circulation of high amounts of IL-6 in the form of IL-6/BE-8 complexes.

In addition, a study by Lu *et al.* (123) demonstrated that BE-8 could not efficiently block daily production of IL-6 at a level greater than 18 µg/day. This particular study observed an inverse correlation between clinical response and daily production of IL-6 during treatment if production exceeded 18 µg/day. This confirms the importance of calculating this particular parameter for optimizing an anti-IL-6 dosing strategy. In addition, some patients presented delayed CRP/IL-6 production that was also associated with lack of clinical efficacy. This suggests that efficient blockade of IL-6 production must be complete and lasting. One limitation is the amount of BE-8 that can be injected due to its short half-life (3–4 days) and the continuous production of IL-6 *in vivo*. Therefore, the chimeric Ab CNTO 328 with an 18-day half-life may be beneficial for chronic administration.

Additional Therapies. Alternative methods have been developed by using humanized anti-IL-6R mAb (rhPM-1, IgG1 class). One method is PM1, currently tested in Phase I-II trials in patients with MM (126) and rheumatoid arthritis (127). Other methods include using a mixture of three anti-IL-6 or anti-IL-6R mAbs that shorten the half-life of the IL-6/IL-6R complexes (from 4 days to less than 20 min) *in vivo*, in addition to the formation of polymeric complexes instead of monomeric complexes, a situation compatible with increased clearance of these IL-6/IL-6R complexes (114, 128, 129).

The most recent Phase I-II clinical trial that evaluated anti-IL-6 therapy investigated the treatment of BLPD (121). The open-label, multicenter trial examined the effect of a murine mAb to IL-6 (BE-8) in 12 transplant recipients whose conditions were refractory to reduction of immunosuppression, in whom

BLPD subsequently developed. Of the 12 patients, 5 received 0.4 mg/kg/day, and 7 received 0.8 mg/kg/day for a scheduled treatment period of 15 days. Ten patients completed treatment, and two patients discontinued treatment due to disease progression. The patients tolerated treatment with no major side effects, although all patients developed an immune response to BE-8.

The study found BE-8 therapy to be effective in 8 of the 10 treated patients; 5 patients achieved complete remission, and 3 achieved partial remission 4 months after treatment was initiated. At the time of the report, seven patients were alive and well (121). This preliminary investigation suggests that mAb to IL-6 therapy is a potential option in the treatment of BLPD and should be explored further.

Anti-IL-6 mAbs in Metastatic RCC. To our knowledge, only one study (63) has used mAb to IL-6 therapy in metastatic RCC. Of the 18 patients in this trial, 14 had progressive disease during IFN-α and/or IL-2 therapy, and 4 patients were not previously treated for their metastatic disease but had contraindications for immune therapy. BE-8 was delivered at 20 mg/day for 21 consecutive days *i.v.* No toxicity was observed. All 18 patients had an increase in performance status associated with decreased analgesic intake, including 3 of 4 patients who stopped taking morphine. In five patients who presented with fever, temperature normalization was correlated with the inhibition of CRP production. Conversely, in one patient with fever, temperature did not normalize, and this was associated with partial CRP inhibition. One patient who presented with hypercalcemia had a transient reduction in serum calcium level. Two patients could not be assessed for response because treatment was too short (4 and 6 days, respectively).

Of the 16 patients assessed for response, 3 had a minor response (<50% reduction). All treated patients developed an immune response to BE-8; this did not affect the ability of BE-8 to block CRP production. These three patients had not been treated previously, and their mean CRP serum level was 24 ± 11 mg/liter; it was 42 ± 47 mg/liter in the five patients with stable disease and 134 ± 53 mg/liter in the eight patients with progressive disease. Two additional patients experienced slight tumor mass reduction in the liver and lung, respectively. Maximal reductions of serum levels of acute-phase proteins were observed at day 7 for CRP (96 ± 6% decrease) and at days 16, 17, and 19 for fibrin (55 ± 8% decrease), haptoglobin (66 ± 17% decrease), and orosomucoid (46 ± 12% decrease), respectively. An increase in albumin level (+17%) was noted at day 20. WBC count decreased transiently by 34% at day 3. Four patients had an increase in hemoglobin level (+1.1 ± 0.2 g/day/liter).

All patients had a decrease in platelet count by 45% at day 17, with normal bone marrow aspirates done in three patients. No changes were observed in bleeding factor, serum immunoglobulin, or creatinine and liver enzyme levels. Slight decreases in IL-1 and TNF-α were observed in serum or plasma from the six patients analyzed. No change in CD3-, CD4-, CD8-, CD19-, CD19DR-, CD56-, CD3DR-, and CD14-positive cells was observed in peripheral blood using flow cytometry technique. This therapeutic strategy may constitute a new way of treating RCC. It may be combined with standard immunotherapy, as tested in seven patients with combinations of IFN-α and one patient with IL-2 and IFN-α, demonstrating reduced toxicity for the eight

⁴ J-F. Rossi, unpublished data.

patients treated with cytokines and a sustained response in one previously untreated patient.⁴

Clinical Study Limitations. A major difficulty in assessing the efficacy of cytokine antagonists *in vivo* is the lack of data on whole-body production of a cytokine under normal and pathological conditions, although such information is necessary for predicting the amount of cytokine-blocking proteins needed to neutralize the target cytokine. To predict the efficacy of anti-IL-6 treatments, whole-body IL-6 production has been estimated using a mathematical procedure developed by Lu *et al.* (123) and described previously in detail. This mathematical model was tested in a group of patients with MM and metastatic RCC, confirming its validity and predicting the range of efficacy of anti-IL-6 mAbs.

Conclusions

Data indicate that IL-6 is linked to the pathogenesis of various cancers and suggest that it has a complex role in a number of malignancies. IL-6 is implicated in survival pathways of B-cell malignancies, especially MM; in growth pathways for solid tumors; and as a possible cofactor for tumor promotion.

Analysis of data from six structured clinical trials of various mAbs to IL-6 in the treatment of cancer has shown a number of interesting observations. In most patients, mAb to IL-6 therapy reduced CRP levels substantially to below detectable limits. The drug was well tolerated and did exhibit a decrease in cancer-related symptoms (*i.e.*, fever, cachexia, and pain). A lack of clearance of the anti-IL-6 mAb was observed in the majority of studies. In studies using murine mAbs to IL-6, this might be explained by the occurrence of human Abs to the F(ab')₂ region, unlike the Fc region of the murine mAb.

The mAbs BE-8 and CNTO 328 have shown promising results in early-stage clinical trials for the treatment of BLPDs, PCL, lymphoma, MM, and RCC and warrant further investigation in these disease states. Because IL-6 is mainly a survival factor rather than a proliferation factor for human myeloma cells and blocks DXM-induced apoptosis, a main issue in MM will be to use anti-IL-6 mAb therapy to potentiate tumor killing by various drugs, including DXM or high-dose chemotherapy. Similar strategies might be useful in other IL-6-related cancers, including RCC and prostate cancer.

The selection of patients with advanced-stage cancer in the trials reviewed and the substantial impact of mAb to IL-6 on IL-6-mediated activity suggest that mAb to IL-6 therapy may be useful in treating cancer patients at an earlier stage of their disease. In addition, the therapeutic impact of mAb to IL-6 on paraneoplastic syndromes and cancer-related anorexia and cachexia may also be of clinical benefit in cancer patients with malignant disease.

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