

Working Title: *Targeted Anti-Interleukin-6 Monoclonal Antibody Therapy for Cancer: Rationale and Clinical Evidence*

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Key Messages (CNTO 328):

- No life-threatening side effects seen during therapy
- BE8 and CNTO 328 block interleukin-6 (IL-6) activity
- Virtually no immune response associated with anti-IL-6 antibodies
- Mixed clinical response in patients with end-stage multiple myeloma shows potential benefit of CNTO 328, indicating the need for further study
- Highlight use of mAb to IL-6 in solid tumors including renal cell carcinoma and prostate carcinoma

Manuscript Objectives:

The objectives of this manuscript are to give an overview of the important role of IL-6 in specific cancers, and to provide a detailed rationale for targeted anti-IL-6 therapies with monoclonal antibodies, and to review evidence from published clinical trials of anti-IL-6 monoclonal therapies, including BE-8 and CNTO 328.

Target Audience: Clinical oncologists and basic research scientists

Target Journal: JCO or Blood.

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3 **INTERLEUKIN-6 TARGETED MONOCLONAL ANTIBODY THERAPY FOR**
4 **CANCER :**
5 **A REVIEW OF THE RATIONALE AND CLINICAL EVIDENCE**
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1 **Abstract**

2 Interleukin-6 is a definitive pleiotropic cytokine with varied systemic function.
3 Implicated in a variety of disease states, interleukin-6 plays a major role in inflammatory
4 processes. It modulates the transcription of several liver-specific genes during acute
5 inflammatory states, particularly C-reactive protein (CRP), and controls the proliferation of
6 normal plasmablastic cells. In addition, interleukin-6 has been implicated in hematopoiesis as a
7 cofactor in stem cell amplification and differentiation, prompting the hypothesis that
8 immunomodulatory therapy given postoperatively may be beneficial in cancer patients. This
9 article is the first all-inclusive review of the decade-spanning clinical investigations of anti-
10 interleukin-6 mAb in the treatment of cancer and related lymphoproliferative disorders.

11 The preliminary clinical evidence from six structured clinical trials of monoclonal
12 antibodies to anti-interleukin-6 including BE8 and CNTO 328 in the treatment of cancer
13 including multiple myeloma, renal cell carcinoma, and B-lymphoproliferative disorders have
14 produced a number of interesting observations: in all cases, anti-interleukin-6 mAb treatment
15 had a substantial impact on CRP levels, and in most instances levels were reduced to below
16 detectable limits. Patients exhibited good tolerance and no toxic side effects were observed in the
17 vast majority of studies. The therapeutic impact of anti-interleukin-6 mAb on cancer-related
18 anorexia and cachexia may also be of clinical significance in a vast number of cancer patients.

1 Introduction

2 Interleukin-6 (IL-6) is a definitive pleiotropic cytokine, a member of a family of proteins
3 with varied systemic function. {Barton 1997 32109 /id} Secreted by a number of cell types, IL-6
4 is present in elevated levels in association with active disease and synthesis of other cytokines
5 stimulated by infection, trauma, and immunologic challenge. {Jones, Horiuchi, et al. 2001 36225
6 /id} {Kishimoto, Tanaka, et al. 1995 36243 /id} As testimony to the pleiotropic nature of IL-6,
7 the cytokine was initially assigned a variety of names based on function, including *interferon*
8 *beta-2 (IFN- β 2)*, *IL-1 inducible 26-kD protein*, *hepatocyte-stimulating factor*, *cytotoxic T-cell*
9 *differentiation factor*, and *B-cell stimulatory factor*. {Kishimoto, Taga, et al. 1989 36241 /id} As
10 the various attendant physiologic effects of the molecule became associated with a common
11 gene, the name *interleukin-6* was proposed. {Poupart, Vandenabeele, et al. 1987 36238 /id}

12 The physiologic activity of IL-6 is complex, producing both pro-inflammatory and anti-
13 inflammatory effects in the immune system (Figure 1). Interleukin-6 promotes inflammation by
14 contributing to the activation and proliferation of T cells, stimulating the differentiation of B
15 cells, and inducing the acute-phase reactants of the hepatocyte population. {Jones, Horiuchi, et al.
16 2001 36225 /id} In contrast, IL-6 also inhibits aspects of the inflammatory cascade. Both of the
17 two primary inflammatory cytokines, tumor necrosis factor alpha (TNF- α) and IL-1, stimulate
18 the production of prostaglandins, nitric oxide, and matrix metalloproteinases. Interleukin-6, on
19 the other hand, does not promote the production of these inflammatory mediators, and it is
20 hypothesized that IL-6 may play a role in regulating or turning off the *in vivo* synthesis of TNF-
21 α and IL-1. {Barton 1997 32109 /id} Despite these functions, IL-6 modulates the transcription of
22 several liver-specific genes during acute inflammatory states, particularly C-reactive protein
23 (CRP), and controls the proliferation of normal plasmablastic cells, as demonstrated in reactive

1 plasmacytosis by using monoclonal antibody (mAb) directed against IL-6 {Gavarotti,
2 Boccadoro, et al. 1985 38022 /id} In addition, IL-6 has been shown to be an activator or an
3 inhibitor of T-cell responses, depending on the target and the system used in vitro. This intricate
4 interaction of pro-inflammatory and anti-inflammatory activities hints at the critical role IL-6
5 potentially plays in regulating the physiologic response to disease.

6 Increased production of IL-6 has been implicated in a variety of disease processes,
7 including neoplasia, Alzheimer's disease, autoimmunity (e.g., rheumatoid arthritis),
8 inflammation, myocardial infarction, aging, Paget's disease, osteoporosis, neoplasia (renal cell
9 carcinoma [RCC], prostatic and bladder cancers, certain neurologic cancers), B-cell
10 malignancies (e.g., Castleman's disease), some lymphoma subtypes, and, particularly, multiple
11 myeloma (MM) {Keller, Wanagat, et al. 1996 36226 /id} {Simpson, Hammacher, et al. 1997
12 35743 /id} {Tupitsyn, Kadagidze, et al. 1998 36232 /id}. In addition, IL-6 is implicated in
13 proliferation pathways as a central proliferation factor or acting in cooperation with other factors,
14 such as heparin-binding epithelial growth factor and hepatocyte growth factor (Oncogene 2002,
15 21:460; Cancer Res 2001, 61: 383; {Wang, De Vos, et al. 2002 38024 /id} This reinforces the
16 hypothesis that blocking IL-6 may have significant benefit in a large variety of pathologic
17 situations. In the following discussion we review the role of IL-6 in the etiology and
18 pathogenesis of cancer, as well as a comprehensive review of clinical trials of targeted cancer
19 therapy using mAb to IL-6.

20 **Interleukin-6/Interleukin-6 Receptor Interaction**

21 Interleukin-6 is a multifunctional cytokine that binds to a specific IL-6 receptor (α chain,
22 IL-6R, or CD126) on target cells. This IL-6/IL6R complex associates with two molecules of the
23 ubiquitously expressed gp130 (β chain, CD130), the second chain of the receptor, resulting in the

1 formation of high-avidity IL-6 binding receptors {Kishimoto, Akira, et al. 1992 38003 /id};
2 {Ward, Howlett, et al. 1994 38023 /id} The gp130 functions as an affinity converter, since the
3 resulting affinity of IL-6 for the ternary complex is around 10^{-11} M, instead of 10^{-9} M for IL-6R.
4 Whereas gp80 binds specifically to IL-6, gp130 is a common signal-transducing receptor for a
5 subfamily of cytokines, including IL-6, IL-11, leukemia-inhibiting factor (LIF), ciliary
6 neurotrophic factor (CNTF), oncostatin M (OM), and cardiotropin 1 (CT-1), named the gp130
7 cytokine family. After binding to their specific receptors, all these cytokines induce
8 homodimerization of gp130 or its heterodimerization with the LIF receptor (LIFR), which
9 initiates cell signaling {Kishimoto, Akira, et al. 1992 38003 /id}. In contrast with the wide
10 distribution of gp130, gp80 is limited to hepatocytes and specialized subsets of leukocytes,
11 including monocytes, neutrophils, T cells, and B cells (Jones et al 2001). Stimulation of gp130 is
12 essential for hematopoiesis in vivo.

13 The system is complicated by the presence of soluble forms of both gp80 and gp130.
14 These circulating compounds are cleaved from the cell membrane molecule or translated from an
15 alternative spliced mRNA, yielding a protein that differs at its COOH-terminus by 14 amino-acid
16 residues {Mullberg, Schooltink, et al. 1993 38008 /id} {Horiuchi, Koyanagi, et al. 1994 38030
17 /id}. Cleavage of transmembrane proteins can be done by a transmembrane metalloproteinase,
18 distinct from matrix-type metalloproteases, that belongs to the family domains containing
19 metalloproteinases (ADAM) {Wolfsberg & White 1996 37992 /id}. Soluble (s) IL-6R or gp55
20 retains its capability to bind IL-6, and the complexes formed are able to activate the gp130
21 transducer receptor. Therefore, in contrast with other soluble cytokine receptors, which are
22 generally antagonists, sIL-6R is an agonist molecule, promoting IL-6 activity. This capability
23 may explain a possible activation of gp130 despite the lack of gp80, if sIL-6R molecules

1 circulate in great quantity, as demonstrated in certain pathologic situations. Cells that do not
2 express specific receptors for IL-6, IL-11, or CNTF are not able to respond to these cytokines.
3 The presence of sIL-6R leads to responsiveness of these cells, and this process has been named
4 transsignaling. Sera from healthy individuals contain sIL-6R (mean value, 89 ng/mL; range, 17–
5 300 ng/mL). Serum sIL-6 was increased by 116% in overt MM (mean value, 193 ng/mL)
6 {Gaillard, Bataille, et al. 1993 38029 /id}. Soluble gp130 (sgp130) has been observed in human
7 plasma and may bind soluble and membrane-anchored IL-6/IL-6R complexes, thus appearing as
8 an endogenous IL-6 antagonist. In MM, the serum level of sgp130 is above 800 ng/mL.

9 **Interleukin-6, A Major Cytokine Implicated in Self-Renewal and Differentiation of Early** 10 **Progenitor Cells**

11 Interleukin-6 plays a major role in inflammatory processes. In addition, IL-6 has been
12 implicated in hematopoiesis as a cofactor in the amplification and differentiation of stem cells.
13 Early hematopoietic stem cells express low levels of FLT-3 and c-kit receptors as well as gp130
14 receptor, but do not express IL-6R (Kollet O et al., Blood 1999, 94: 923). Therefore, IL-6/IL-6R
15 complexes are efficient in amplifying and maintaining early progenitor cells as well as other
16 cytokines, including SCF and FLT-3 or, to a lesser extent, IL-1. Primitive CD34-positive
17 progenitors provide a soluble positive-feedback signal that induces cytokine production by
18 stromal cells, including IL-6 (Gupta P, and al., Blood 1998, 91: 3724). Serum sIL-6R levels
19 reflect proliferative kinetics of the stem cells after mobilization (Omura H, et al., Leuk
20 Lymphoma 2002, 43: 623). The differentiation of various cells, including mast cells and
21 cardiomyocytes, is also under the control of IL-6 and the gp130-family, in addition to other
22 cytokines (Tsuruda T et al., Circ Res 2002, 90: 128).

1 Recently, it was shown that self-renewal of embryonic stem cells required sustained signaling by
2 gp130 cytokines, particularly LIF and also IL-6, in a concentration-dependent manner, with
3 thresholds in ligand-receptor signaling that modulate control of stem cell differentiation
4 (Viswanathan S, et al., Stem Cells 2002, 20: 119).

5 On the other hand, IL-6/IL-6R complexes and the gp130 cytokine family were shown to
6 be implicated in neural cell differentiation (Edoff K, Jerregard H J Neurosci Res 2002, 67: 255)
7 and in osteoclast differentiation. The gp130 family and, particularly, LIF regulate
8 osteoprogenitor differentiation in different models (Malaval L et al., J Bone Miner Res 1998, 13:
9 175).

10 **Interleukin-6, Possible Role in Cancer**

11 Comparable to the role of IL-6 in inflammation, inhibition and stimulation of cancer cell
12 proliferation are also functions of this cytokine, depending on the cell type and the presence or
13 absence of IL-6R. {Keller, Wanagat, et al. 1996 36226 /id} In addition to modulating the
14 antitumor activity of macrophages, IL-6 takes part in the production of lymphokine-activated
15 killer (LAK) cells and protects neutrophils from apoptosis, increasing their cytotoxic effect on
16 tumor cells. Also, through the stimulation of increased synthesis of CRP, IL-6 indirectly
17 influences the binding of this protein to phospholipids on tumor cells, activating C1q of the
18 complement system, which leads to tumor cell lysis in certain cases.

19 During the later stages of tumor growth, tumoral expression is associated with an increase
20 in the levels of IL-1, IL-6, and acute-phase proteins. Table 1 lists investigations that define the
21 role of IL-6 in a number of neoplastic diseases. In the majority of studies, active disease is
22 associated with elevated serum levels of IL-6, which are related to disease severity and outcome.

1 In many of these cancers, increased IL-6R expression was also detected, {Jones, Horiuchi, et al.
2 2001 36225 /id} and a proliferative mechanism has been suggested.

3 ***Interleukin-6 Is Implicated in Proliferation Pathways for B-Cell Malignancies, Particularly***
4 ***MM***

5 The function of IL-6 in the pathogenesis of MM is well documented (Klein B Blood
6 1995, 85:863). Multiple myeloma is a plasma cell dyscrasia characterized by the malignant
7 proliferation of bone marrow plasma cells. Interleukin-6 is the central factor of proliferation,
8 even though few of the plasma cells may secrete IL-6, particularly when cells are cultured in
9 vitro under specific conditions. The major potential mechanism of the proliferative expression of
10 IL-6 in MM is attributed to a paracrine direct cell-to-cell interaction between MM cells and the
11 bone marrow stromal cells, including cell-cell contact and also the production of diverse
12 molecules by tumoral cells, such as IL-1 (Costes V et al Br J Haematol 1998, 103: 1152). A
13 certain number of IL-6–dependent plasma cell lines have been generated, with some of them
14 sensitive to other gp130 cytokine family members, depending on the presence of other receptors
15 in this family and the action of IL-10 (Klein B et al., Leuk Lymphoma 1999, 34: 63, Gu ZJ et al.,
16 Blood 1996, 88: 3972). Serum IL-6 levels are correlated with prognosis in different B-cell
17 malignancies, including chronic B-lymphocytic leukemia (Fayad L et al., Blood 2001, 97: 256)
18 and lymphoma (Legouffe E et al., Leuk Lymphoma 1998, 31:351).

19 In addition, sIL-6 plays a role in the pathogenesis of the disease, via the formation of
20 complexes with IL-6 producing a 10-fold increase in the sensitivity of human IL-6–dependent
21 cell lines (Gaillard JP et al., Eur J Immunol 1993, 23: 820). The presence of high levels of sIL-
22 6R in the serum of patients with MM, independent of tumor cell mass and status of the disease,
23 suggests an important functional role of this circulating protein in the pathogenesis of

1 monoclonal gammopathies (Gaillard JP 1993). Clinical investigations have shown that serum IL-
2 6 and CRP levels are correlated, and are indicative of disease severity and progression (Bataille
3 R Bocadoro Blood).

4 ***Interleukin-6 Is Also Implicated in Proliferation Pathways for Other Solid Cancers***

5 The increased levels of IL-6 seen in progressive disease are also associated with other
6 malignant conditions, including breast cancer and RCC (Table 1). Interleukin-6 is produced by
7 some RCC cell lines and tumor cells in vivo (Takenawa J, et al., J Natl Cancer Inst 1991, 83:
8 1668-72; Walther McM J Urol 1998). Proliferative expression of IL-6 in some RCC cell lines
9 was attributed to an autocrine mechanism (Miki S et al., FEBS Lett 1989, 250: 607-10), with
10 stat3 activation and p53 modulation (Horiguchi A et al., Kidney Int 2002, Angelo LS et al.,
11 Cancer Res 2002). We demonstrate that IL-6R is present on tumor cells, in correlation with
12 circulating IL-6 and disease aggressiveness (Costes V, et al., J Clin Pathol 1997). We and others
13 have demonstrated that circulating IL-6 level correlated with serum CRP level and that both are
14 prognostic factors, with a serum CRP level of 50 mg/L statistically discriminant (Blay JY Cancer
15 Res 1992, Walther MM J Urol 1998; Ljungberg B et al., Eur J Cancer 1997).

16 ***Interleukin-6 Is a Cooperation Factor for Alternative Tumour Promoting Mechanisms***

17 Recently, IL-6 was shown to be linked to drug-resistance mechanisms, including glutathione S-
18 transferase (GST) through demonstration of the sensitization of human RCC cell lines to
19 cisplatin by blocking IL-6 (Mizutani Y et al., Cancer Res 1995, 55: 590) or multidrug resistance
20 (MDR) in breast cancer. In different conditions, including breast cancer, prostatic cancer, and
21 MM, IL-6 amplifies the proliferation linked to epithelial growth factor (EGF)/ErbB molecules or
22 hepatocyte growth factor (HGF) (Oncogene 2002, 21:460; Cancer Res 2001, 61:383; Wang YD
23 et al., Oncogene 2002, 21: 2584).

1 ***Interleukin-6 Is Implicated in Paraneoplastic Syndrome***

2 Cancer-related anorexia and cachexia are serious complications associated with these malignant
3 conditions and affect as many as 87% of patients. {Loprinzi, Ellison, et al. 1990 36290 /id}
4 Although the etiology of cachexia is complex and multifactorial, IL-6 has been implicated in the
5 progressive wasting, with most of the evidence that points to IL-6 as a cachectic agent having
6 been obtained from animal studies. {Tisdale 1997 36231 /id} In RCC as well as in other cancers,
7 IL-6 and CRP serum levels are correlated with Stauffer's syndrome, particularly neoplastic fever,
8 body-weight loss, performance status (Blay JY Rossi JF et al. Int J Cancer 1997), depression
9 (Musselmann Am J Psychiatr 2001, 158: 1252), anemia, leukocytosis, thrombocytosis,
10 hypoalbuminemia, hypercalcemia, and other biologic symptoms.

11 ***The Role of Interleukin-6 in Renal Cell Carcinoma***

12 Interleukin-6 is expressed by the majority of RCCs and is known to have an essential role
13 in the proliferation of RCC cell lines. {Miki, Iwano, et al. 1989 36237 /id} {Takenawa, Kaneko, et
14 al. 1991 36289 /id} The exact mechanism of enhanced production of IL-6 in renal cell tumors is
15 unknown, but p53 mutations have been detected in up to 30% of primary kidney tumors and in
16 the majority of metastatic tumors (70% to 80%). {Haitel, Wiener, et al. 2000 36278 /id} {Oda,
17 Nakatsuru, et al. 1995 36242 /id} {Angelo, Talpaz, et al. 2002 36218 /id} {Uhlman, Nguyen, et
18 al. 1994 36390 /id} {Reiter, Anglard, et al. 1993 36389 /id} Findings were presented that
19 confirmed that p53 mutations can result in overexpression of IL-6, and that wild-type (wt) p53
20 represses IL-6 expression by inhibiting transcription factor binding to the IL-6 promoter.

21 Analyzing sera and tissue samples from 38 patients with primary RCC, Costes and
22 associates (1997) {Costes, Liautard, et al. 1997 35780 /id} found significant correlations between
23 level of IL-6 and disease state. Serum IL-6 levels correlated with tumor size and stage. Tissue

1 samples stained positive for IL-6R expression in 10 instances. The presence of IL-6R in tumors
2 was significantly associated with tumor stage, nuclear grade, proliferation index, and serum level
3 of IL-6. Study of this subgroup of patients with the most aggressive disease allowed the authors
4 to identify patients in whom IL-6 and IL-6R played a critical role in tumor progression or
5 proliferation and, thus, a group who may benefit from anti-IL-6 strategies. {Costes, Liautard, et
6 al. 1997 35780 /id} In a larger study that enrolled 122 patients with RCC, Yoshida and associates
7 (2002){Yoshida, Ikemoto, et al. 2002 35741 /id} confirmed serum levels of IL-6 in stage IV
8 patients that were significantly higher than those in patients with disease of less severe grades or
9 in the control group and concluded that serum IL-6 level and TNF- α may be a useful measure in
10 the early diagnosis of RCC.

11 Metastatic RCC is also associated with a high incidence of paraneoplastic syndrome, a
12 condition characterized by fever and elevated levels of acute-phase markers (e.g., CRP), a
13 decrease in serum albumin, thrombocytosis, and anemia, {Papac & Poo-Hwu 1999 35742 /id}
14 which appears to be the consequence of abnormal cytokine production or immunogenic
15 mechanisms. Blay and associates (1997){Blay, Rossi, et al. 1997 35779 /id} measured serum
16 levels of IL-6 in RCC patients with paraneoplastic fever and weight loss that were significantly
17 higher than those in patients with RCC who did not have paraneoplastic symptoms, thus
18 confirming the role of IL-6 in the syndrome. Three patients with paraneoplastic syndrome were
19 enrolled in a 21-day, phase II trial of mAb to IL-6 therapy (murine B-E8, IgG1). All three
20 patients who received mAb to IL-6 showed a reduction in levels of CRP, haptoglobin, and serum
21 alkaline phosphatases during the 21-day test period. After therapy was stopped, the serum levels
22 of these factors increased to pre-treatment levels or beyond, {Blay, Rossi, et al. 1997 35779 /id}

1 demonstrating the significance of IL-6 in paraneoplastic syndrome as well as the potential of
2 targeted anti-IL-6 therapy.

3 Humoral hypercalcemia is a complication of malignancy related to paraneoplastic
4 syndrome that is linked to the tumor's production of substances that stimulate osteoclastic
5 activity. Data from a study by Weissglas and colleagues (1995){Weissglas, Schamhart, et al.
6 1995 36234 /id} indicate the possible role of IL-6 overexpression by RCC cells in
7 hypercalcemia, most probably through its action on parathyroid hormone-related peptide, which
8 highlights a potential supportive therapeutic role for anti-IL-6 treatment.

9 Another possible use of targeted anti-IL-6 therapy in RCC is as an adjuvant to *cis*-
10 diamminedichloroplatinum (CDDP). Renal cell tumors are recalcitrant to CDDP therapy, and
11 they could be a prime target of combination therapy. Mizutani and colleagues (1995){Mizutani,
12 Bonavida, et al. 1995 33152 /id} reported the effects of mAb to IL-6 and anti-IL-6R mAb on the
13 sensitivity of human RCC cells to CDDP. In vitro anti-IL-6 or anti-IL-6R mAb enhanced the
14 susceptibility of RCC cells to CDDP, implying clinical benefit with combined therapy.

15 Experimental and clinical findings supporting the role of IL-6 in active cancers provide a
16 rationale for targeted therapeutic investigations. A variety of therapeutic agents have a mode of
17 action that has an impact on IL-6 production. Known inhibitors of IL-6 include corticosteroids,
18 nonsteroidal anti-inflammatory agents, estrogens, and cytokines, such as IL-4. {Lauta 2001
19 33087 /id} Dexamethasone has also been shown to inhibit both IL-6 and IL-6R gene expression
20 in myeloma cell lines. Targeted biologic therapies include toxic IL-6 and MAbs directed against
21 IL-6 and IL-6R. {Brochier, Gaillard, et al. 1998 36221 /id} {Lauta 2001 33087 /id} Toxic IL-6
22 therapy is based on fusing the IL-6 gene to *Pseudomonas* exotoxin or diphtheria toxin genes,
23 although the potential viability of this approach is questionable, as many normal cells express IL-

1 6R, most notably hepatocytes. {Lauta 2001 33087 /id} The remainder of this review will focus on
2 the clinical experience with mAb therapy for cancer.

3 *Clinical Experience with mAb to IL-6*

4 The chronology of clinical investigations of mAb to IL-6 in the treatment of cancer and
5 related lymphoproliferative disorders spans roughly a decade, beginning with the first report of a
6 single patient with plasma cell leukemia (PCL) who was treated with mAb to IL-6 in
7 1991. {Klein, Wijdenes, et al. 1991 33100 /id} The most recent effort was detailed in an article
8 describing a phase I-II clinical trial that evaluated mAb therapy for B-lymphoproliferative
9 disorder in recipients of organ transplants. {Haddad, Paczesny, et al. 2001 36223 /id} The general
10 characteristics of and clinical results from each of the investigations, in chronologic order, are
11 summarized in Table 2. Initial investigations discussed below were conducted with mouse mAb
12 to IL-6 (murine mAbs B-E4 and B-E8) and more recently, a humanized mouse mAb to IL-6
13 (human-mouse chimeric mAb to IL-6) with the investigational name CNTO 328 is current
14 undergoing extensive clinical investigation for the treatment of MM, renal cell carcinoma and
15 solid tumors. CNTO 328 contains the variable antigen-binding region of the murine anti-IL-6
16 antibody and the constant region of the human IgG1 kappa immunoglobulin {van Zaanen,
17 Lokhorst, et al. 1998 32875 /id}.

18 A patient with primary PCL that was recalcitrant to chemotherapy was the first reported
19 recipient of mAb to IL-6 therapy. {Klein, Wijdenes, et al. 1991 33100 /id} The patient was a 61-
20 year-old male with primary PCL, bone lesions, hypercalcemia, renal deficiency, anemia,
21 leukocytosis, bone marrow invasion by malignant plasma cells, and 25% myeloma cells in the
22 peripheral blood. After informed consent was obtained, the patient received daily intravenous
23 infusions of mAb to IL-6 (murine B-E4, and murine B-E8) in the following dosing regimen: days

1 0–5, 40 mg B-E4; day 6, 120 mg B-E4; days 7–10, 8 mg B-E8; days 11–14, 4 mg B-E8; days
2 15–16, 20 mg B-E4; days 17–23, no injection; days 24–59, 8 mg B-E8; days 60–63, no injection;
3 days 64–67, 16 mg B-E8; and after day 68, no injection.

4 Clinical observations regarding treatment noted the blocking of myeloma cell
5 proliferation in the bone marrow, along with a reduction in serum levels of calcium, monoclonal
6 IgG, and CRP. Levels of CRP were reduced to undetectable, and overall no major side effects
7 were noted. This study demonstrated the feasibility and potential of mAb to IL-6 therapy,
8 resulting in a transient tumor cytostasis and reduction in toxicities from IL-6. {Klein, Wijdenes,
9 et al. 1991 33100 /id} This same patient developed a case of *Escherichia coli* sepsis during
10 therapy, and serum levels of IL-6 in the form of monomeric complexes of IL-6/mAb to IL-6
11 remained very high for 20 days after sepsis, indicating the persistence of increased production of
12 IL-6. {Lu, Brailly, et al. 1993 36228 /id} This technique for measuring the IL-6/anti-IL-6
13 complex provides a means of estimating overall IL-6 production as well as a method for
14 estimating the levels of mAb to IL-6 necessary for effective neutralization of IL-6. {Lu, Brailly,
15 et al. 1993 36228 /id} {Lu, Brailly, et al. 1995 33151 /id}

16 Emilie (1994) {Emilie, Wijdenes, et al. 1994 32969 /id} also reported the results of an
17 open, multicenter clinical trial of mAb to IL-6 (murine B-E8) for the treatment of patients
18 positive for human immunodeficiency virus type 1 (HIV-1) who had immunoblastic or
19 polymorphic large cell lymphoma. {Emilie, Wijdenes, et al. 1994 32969 /id} Anti-IL-6 mAb
20 (10–40 mg/day) was administered intravenously for a study period of 21 days. Clinical
21 suppression of IL-6 activity was assessed by measurement of serum levels of CRP.

22 At total of 11 HIV-1–positive patients with lymphoma entered the study and were
23 evaluated. Anti-IL-6 mAb therapy was observed to suppress the spontaneous growth of the

1 lymphoma in 6 of the 9 patients, with detectable neutralization of endogenous IL-6. Five of these
2 patients were classified as clinically stabilized, and one achieved partial remission. {Emilie,
3 Wijdenes, et al. 1994 32969 /id} For the patients with disease stabilization, follow-up
4 assessments indicated that stabilization was sustained for 8 to 28 weeks. Overall, the antitumor
5 activity was limited and inconsistent. {Emilie, Wijdenes, et al. 1994 32969 /id}. Further, all
6 patients developed an immune response to the murine mAb BE8 {Legouffe, Liautard, et al. 1994
7 36227 /id} Side effects seen during therapy included consistent thrombocytopenia and,
8 occasionally, decreases in neutrophil counts. The authors note that the most clear-cut effect
9 associated with the BE8 therapy was its alleviation of the systemic symptoms, including fever,
10 sweats, and cachexia. The authors conclude that in some cases IL-6–dependent growth of
11 malignant lymphomas occurs, and that effective neutralization of endogenous IL-6 with targeted
12 mAb therapy alleviates clinical symptoms. {Emilie, Wijdenes, et al. 1994 32969 /id}

13 A subsequent study by Bataille and colleagues (1995){Bataille, Barlogie, et al. 1995
14 33014 /id} reported the results of mAb to IL-6 therapy in the treatment of MM. A total of 9 of
15 the 10 patients who had advanced and progressive MM with mainly primary or secondary PCL
16 received intravenous mAb to IL-6 (B-E8) therapy (20 mg/day) for at least 4 days and for as long
17 as 68 days. One patient with pleural effusion received local intrapleural administration of mAb-
18 8, 20 mg/day for 3 days.

19 Three of the treated patients succumbed to the disease after less than 1 week of therapy,
20 including the patient who received intrapleural treatment. Two of these patients with evaluable
21 data showed marked inhibition of plasmablastic proliferation. The seven remaining patients
22 received intravenous therapy for at least 1 week, with three exhibiting an objective
23 antiproliferative effect as measured by myeloma cell labeling in the bone marrow, and one of

1 these three patients showed a 30% regression of tumor mass. However, it is important to note
2 that none of the patients in the study achieved remission or improvement as assessed by standard
3 clinical criteria. Although the data were not reported, the authors noted that a beneficial effect of
4 mAb to IL-6 therapy was the resolution of fever and hypercalcemia. The data suggest that mAb
5 to IL-6 therapy could prove beneficial in patients with early-stage disease. {Bataille, Barlogie, et
6 al. 1995 33014 /id}

7 A phase I study of mAb to IL-6 therapy for MM has been presented by van Zaanen:
8 reporting a dose-escalation analysis in patients with MM resistant to second-line
9 chemotherapy. {van Zaanen, Koopmans, et al. 1996 36233 /id} {van Zaanen, Lokhorst, et al.
10 1998 32875 /id} The first report concerns the treatment of patients with end-stage, progressive
11 MM, diagnosed according to the criteria of Durie and Salmon (1975). {Durie & Salmon 1975
12 36339 /id} Nine patients entered the study and received human-mouse chimeric mAb to IL-6
13 (murine-human chimeric mAb [cMAb]) in two cycles of 14 daily intravenous infusions, starting
14 on day 1 and day 28 of therapy. The dosing regimen was as follows: 5 mg/day in patients 1 to 3
15 (total dose, 140 mg); 10 mg/day in patients 4 to 6 (total dose, 280 mg); and 20 mg/day in patients
16 7 to 9 (total dose, 560 mg). This antibody is now known as CNTO 328.

17 Based on a one-compartment open pharmacokinetic model, the investigators were able to
18 calculate the day-to-day endogenous production of IL-6 using the measured values of free IL-6
19 and the binding characteristics of the anti-IL-6 cMAb. The median half-life of the CNTO 328
20 was 17.6 days, and no human anti- CNTO 328 antibodies were produced in the treated patients.

21 Clinically, 8 of 9 patients had stabilization of their disease; however, clinical response,
22 indicated by a decrease in levels of M protein of more than 50%, was not achieved by any of the
23 patients treated. The significant clinical results from the study included the development of a

1 methodology for calculating endogenous IL-6 production, and the finding that CNTO 328
2 therapy normalizes endogenous IL-6 production but does not have an impact on the IL-6
3 production associated with infection. These investigations suggest that CNTO 328 was able to
4 block IL-6-dependent processes in vivo. {van Zaanen, Koopmans, et al. 1996 36233 /id}

5 The second paper based on this dose-escalation study by van Zaanen and colleagues, was
6 published in 1998 {van Zaanen, Lokhorst, et al. 1998 32875 /id}. Twelve patients entered the
7 study and received chimeric mAb to IL-6 (murine-human chimeric mAb) in two cycles of 14
8 daily intravenous infusions starting on day 1 and day 28 of therapy. The dosing regimen was as
9 follows: 5 mg/day in patients 1 to 3 (total dose, 140 mg); 10 mg/day in patients 4 to 6 (total dose,
10 280 mg); 20 mg/day in patients 7 to 9 (total dose, 560 mg); and 40 mg/day in patients 10 to 12
11 (total dose, 1120 mg).

12 A total of 11 of 12 patients exhibited clinical stabilization of disease, and the twelfth
13 patient with progressive disease responded to the second course of treatment. There were no
14 toxic or allergic reactions to CNTO 328 therapy, although there was transient thrombocytopenia
15 (2 patients) and granulocytopenia (6 patients). Although stabilization of disease was evident, no
16 clinically significant response was seen, as none of the patients achieved a reduction in the level
17 of M protein greater than 50%. As noted in the previous investigation, no immune response to
18 anti-IL-6 cMAB occurred. Levels of CRP were also reduced to undetectable in 11 of 12
19 patients. {van Zaanen, Lokhorst, et al. 1998 32875 /id}

20 The study concluded that there were no life-threatening side effects associated with anti-
21 IL-6 cMAB therapy, and pharmacokinetic measurements indicated that the cMAB had a long
22 half-life (17.8 days). Although none of the patients achieved remission, the authors hypothesized
23 that the absence of a reduction in the level of M protein of more than 50% in treated patients who

1 exhibited a profound reduction in CRP levels may be related to the presence of immature and
2 mature myeloma cells. The immature myeloma cells are highly proliferative, whereas the mature
3 cells have low levels of proliferative activity. The mature cells are also responsible for synthesis
4 of M protein, implying that there is a population of IL-6-independent myeloma cells in end-stage
5 MM{van Zaanen, Lokhorst, et al. 1998 32875 /id} not targeted by mAb to IL-6.

6 More recently, Moreau and associates (2000){Moreau, Harousseau, et al. 2000 32876
7 /id} investigated the potential of combination therapy including murine mAb to IL-6 (B-E8),
8 dexamethasone (DXM), and high-dose melphalan (HDM220) 220 mg/m², followed by
9 autologous stem cell transplantation (ASCT), in the treatment of advanced MM. A total of 16
10 patients received treatment. Of these, at the time of enrollment, 2 were resistant to all
11 chemotherapy and 14 patients had relapsed. A dose of 250 mg of B-E8 was infused over 4 days
12 in combination with DXM 49 mg/day on days 1 to 4, followed by HDM220 infused over 30
13 minutes on day 5, and ASCT on day 7. {Moreau, Harousseau, et al. 2000 32876 /id}

14 In general, IL-6 activity was strongly inhibited, as indicated by reduced CRP levels.
15 Overall, 13 of 16 patients (81.2%) exhibited a response, with a complete response seen in 6
16 (37.5%). There were no toxic or allergic reactions reported, but the incidence of
17 thrombocytopenia and neutropenia was increased.

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

1 In addition, tumoral cells are induced to apoptosis. If IL-6 is not controlled, apoptosis
2 could be reversed with clonal selection in a period with immune deficiency. This period is also
3 an optimal period for this strategy because of the synergism between melphalan and anti-IL-6. In
4 a control group, we observed that CRP/IL-6 was enhanced after HDM administration,
5 particularly during hematologic recovery. In other words, this strategy is a very promising if the
6 blocking of IL-6 is total and lasting.

7 For all of these reasons, in a recent trial involving 34 patients with MM treated with
8 melphalan 140 mg/m² plus B-E8, a murine mAb, we carefully analyzed the biologic data by
9 applying our mathematical model for calculating the daily IL-6 production and the efficacy of
10 blocking, and we are waiting to provide longer follow-up data on this study (personal data,
11 submitted to publication). As previously shown, the injection of mAb to IL-6 in a patient with
12 MM induced the circulation of high amounts of IL-6 in the form of IL-6/anti-IL-6 monomeric
13 complexes. Estimations of the daily production of IL-6 were given for some patients, including
14 one who developed *Escherichia coli* sepsis during mAb to IL-6 treatment. The range of
15 production may exceed 7 mg/day in this particular situation (Lu ZY et al., Cytokine 1993,
16 5:578).

17 In addition, we demonstrated that the use of a unique mAb to IL-6 *in vivo* was unable to
18 efficiently block daily production of IL-6 at a level greater than 18µg/day (Lu ZY Blood 1995,
19 86: 3123). In this particular study, we observed that there was an inverse correlation between
20 clinical response and daily production of IL-6 evaluated during the treatment if production
21 exceeded this cut-off value. This confirms the importance of calculating this particular parameter
22 for optimising anti-IL-6 dosing strategy. In addition, a certain number of patients presented
23 delayed CRP/IL-6 production that was also associated with a lack of clinical efficacy. This

1 means that efficient blocking of in vivo IL-6 production has to be complete and lasting. In
2 addition, mAb to IL-6 had an effect on patient quality of life, as demonstrated by a reduction in
3 mucositis episodes and in disease aggressiveness, a decrease in the number of red blood cell
4 transfusions, with no difference in hematologic recovery and no increase in the infectious risk
5 (personal data). However, in all the studies using mouse BE8, several problems were observed,
6 including the necessity of having low daily IL-6 production, below 18 $\mu\text{g}/\text{day}$, and a shorter half-
7 life when compared to the chimeric antibody CNTO 328. Therefore a chimeric antibody may
8 permit chronic administration of mAb.

9 Alternative methods have been developed by using humanized anti-IL-6R mAb. One
10 method is PM1, currently tested in Phase I-II trials in MM and in rheumatoid arthritis (Nishimoto
11 N et al., Blood 2000, 95: 56; Yoshizaki K et al., Springer Sem Immunopathol 1998, 20: 247-
12 259). Other methods include combinations of three anti-IL-6 or anti-IL-6R MAbs that shorten
13 the half-life of the IL-6/IL-6R complexes (from 4 days to less than 20 minutes) in vivo, in
14 addition to the formation of polymeric complexes instead of monomeric complexes, a situation
15 compatible with increased clearance of these IL-6/IL-6R complexes (Klein B, Brailly H
16 Immunol Today 1995, 16:216; Brochier J Current trends in Immunol 1998, 1:105; Brochier J et
17 al., Eur J Immunol 2001, 31:259).

18 The most recent Phase I-II clinical trial that evaluated anti-IL-6 therapy investigated the
19 treatment of B-lymphoproliferative disorder (BLPD). {Haddad, Paczesny, et al. 2001 36223 /id}
20 The open, multicenter trial examined the effect of murine mAb to IL-6 (B-E8) in 12 transplant
21 recipients whose conditions were refractory to reduction of immunosuppression, in whom BLPD
22 subsequently developed. A total of 5 of the 12 patients received a dose of 0.4 mg/kg per day, and
23 the remaining 7 patients received 0.8 mg/kg per day for a scheduled treatment period of 15 days.

1 Treatment was completed in 10 patients; therapy was discontinued in 2 patients, owing to disease
2 progression. The patients tolerated treatment with no major side effects.

3 The study observed mAb to IL-6 therapy to be effective in 8 of the treated patients, with
4 complete remission achieved by 5 patients 4 months after treatment was initiated. A partial
5 remission was seen in 3 patients. At the time of the report, 7 patients were alive and
6 well. {Haddad, Paczesny, et al. 2001 36223 /id} This preliminary investigation suggests that
7 mAb to IL-6 therapy is a potential option in the treatment of BLPD and should be explored
8 further.

9 *Anti-Interleukin-6 Monoclonal Antibodies in Metastatic Renal Cell Carcinoma*

10 To our knowledge, there is only one study that used such a strategy in metastatic RCC,
11 conducted by one of these authors and partly published (personal data and Blay et al. {Blay,
12 Rossi, et al. 1997 35779 /id}). Eighteen patients were included in this trial. Fourteen patients had
13 progressive disease under IFN- α and/or IL-2 therapy, and 4 additional patients were not
14 previously treated for their metastatic disease but had contraindications for immune therapy. The
15 mAb was delivered at 20 mg/day for 21 consecutive days by the intravenous route. No toxicity
16 was observed. All of the 18 patients had an increase in their performance status associated with
17 analgesic effect, including 3 of 4 patients who stopped morphinic intake. In five patients who
18 presented with specific fever, temperature normalization was correlated with the inhibition of
19 CRP production. Conversely, in 1 patient with fever, temperature did not normalize, and this was
20 associated with partial CRP inhibition. One patient who presented with hypercalcemia had a
21 transient reduction in the serum calcium level. Two patients were inevaluable for response
22 because the period of treatment was too short, being 4 and 6 days, respectively. A total of 3 of 16
23 patients presented a minor response (<50% reduction). All the patients were immunized, but this

1 immunization did not affect the ability of the BE8 to block CRP production. All three of these
2 patients had not been previously treated, and their mean CRP serum level was 24 ± 11 mg/L, as
3 opposed to the 5 patients with stable disease (mean CRP serum level, 42 ± 47 mg/L), and the 8
4 patients with progressive disease (mean CRP serum level, 134 ± 53 mg/L). Two additional
5 patients presented a dissociated response, on the liver and lung. Maximal reductions of serum
6 levels of acute-phase proteins were observed at day 7 for CRP ($96 \pm 6\%$ decrease), and at days 16,
7 17, and 19 for fibrin ($55 \pm 8\%$ decrease), haptoglobin ($66 \pm 17\%$ decrease), and orosomucoid
8 ($46 \pm 12\%$ decrease), respectively. An increase in albumin level ($+17\%$) was noted at day 20.
9 White blood cells decreased by 34%, transiently at day 3. Four patients had an increase in
10 hemoglobin level ($+1.1 \pm 0.2$ g/dayL). All the patients had a decrease in the platelet count by 45%
11 at day 17, with normal bone marrow aspirates done in 3 patients. No changes in the levels of
12 bleeding factors were observed, or in serum immunoglobulin level, and creatinine and liver
13 enzymes. Slight decreases in IL-1 and TNF- α were observed in the serum or plasma from the 6
14 patients analyzed. No change in CD3, CD4, CD8, CD19, CD19DR+, CD56, CD3DR+, and
15 CD14 positive cells were observed in peripheral blood using flow cytometry technique. Such
16 therapeutic strategy may constitute a new way of treating RCC. It may be combined with
17 standard immune therapy, as tested in 7 patients with combinations of IFN- α and 1 patient with
18 IL-2 and IFN- α , demonstrating the reduction in toxicity and the maintenance of response for 1
19 previously untreated patient (JFR, personal data).

20 ***Daily Whole-Body Production of Interleukin-6***

21 Recently, there have been developed cytokine-binding proteins (CBPs)—for example,
22 monoclonal antibodies (MAbs) to cytokines or soluble cytokine receptors—in order to neutralize
23 cytokine activity in vivo. A major difficulty in the evaluation of the efficacy of cytokine

1 antagonists in vivo is the lack of data on whole-body production of a cytokine in normal and
 2 pathologic conditions. Indeed, such information is necessary for predicting the amount of CBP
 3 needed to neutralize the target cytokine in vivo. Two elements are of importance in such targeted
 4 therapy. First, in some pathologic circumstances, the quantity of the cytokine present in vivo
 5 may be largely beyond the blocking ability of the CBPs. Second, the half-life of IL-6, in the form
 6 of IL-6/anti-IL-6 complexes, was increased 200 fold {Lu, Brochier, et al. 1992 38028 /id}. In
 7 order to predict the efficacy of anti-IL-6 treatments, whole-body IL-6 production has been
 8 estimated by our group (Lu ZY and al. Blood 1995, 86: 3123). The daily whole-body production
 9 of IL-6 was first estimated in 13 patients with MM or metastatic RCC who were treated with
 10 mAb to IL-6. Recently, this mathematical formula was applied to 34 additional patients with
 11 MM. As CRP production by hepatocytes is induced by the different cytokines that activate the
 12 gp130, the measurement of serum levels of CRP may serve as a pharmacodynamic marker of
 13 efficacy. Different parameters were also measured, including the diverse fractions of IL-6, free
 14 IL-6, and antibody bound IL-6 as well as the serum IL-6-like bioactivity, by using B9
 15 hybridoma assay (Lu ZY et al. Blood 1995, 86: 3123). The calculation of daily IL-6 production
 16 was determined with a mathematical procedure developed and previously described in detail (Lu
 17 ZY Blood 1995). In brief, there are two forms of circulating mAb (free mAb and IL-6/mAb
 18 immune complexes) and one form of circulating IL-6 (IL-6/mAb immune complexes) in patients
 19 receiving mAb to IL-6 treatment. The half-life of IL-6/mAb immune complexes was similar to
 20 that of free mAb. Hence, IL-6 production on day “n,” which can be calculated as shown below:

$$21 \quad "n" = 2 * M * b ([IL-6_n] - b * [IL-6_{n-1}]) / \{([M_n] - b * [M_{n-1}]) * (1 + b)\}$$

22 In the formula above, M, [IL-6_n], [IL-6_{n-1}], [M_n], and [M_{n-1}] represent the daily amount of
 23 injected mAb, the IL-6 concentration on day n, the IL-6 concentration a day before day n, the

1 mAb concentration on day n , and the mAb concentration one day before day n , respectively. The

2 “ b ” was a coefficient concerning the half-life of mAb

3 ($b = e^{-(Ln2)/(\text{half-life of mAb})}$).

4 In addition, a numerical model of mAb to IL-6’s ability to neutralize IL-6 binding to its high-

5 affinity receptor was described and predicts the fraction of gp130 transducer activated by the IL-

6 6/IL-6R or IL-6/sIL-6R complexes as a function of IL-6 and mAb to IL-6 concentrations. The

7 affinities of the interactions of the different components were known and were integrated into the

8 model and can be used to predict the efficacy of a treatment using mAb to IL-6 [(IL-6.IL-6R ~

9 IL-6 + IL-6R (kD = 500 pmol/L) and IL-6/IL-6R/gp130 ~ IL-6/IL-6R = gp130 (kD = 5 pmol/L;

10 IL-6/sIL-6R ~ IL-6 + IL-6R (kD = 1 nmol/L) and IL-6/sIL-6R/sgp130 ~ IL-6/sIL-6R + sgp130

11 (kD = 10 nmol/L); IL-6/sIL-6R/gp130 ~ IL-6/sIL-6R + gp130 (kD =10 pmol/L)]. This

12 mathematical model was tested in a group of patients with MM and metastatic RCC, thus

13 confirming the validity of this mathematical modeling that predicted the range of efficacy of

14 anti-IL-6 MAbs.

15 **Conclusions**

16 The experimental evidence linking IL-6 to the pathogenesis of a variety of cancers

17 emphasizes the potential role of this cytokine in targeted biologic therapies. The evidence

18 presented in this review indicates a complex role for IL-6 in a variety of cancer disease states.

19 Interleukin-6 is implicated in proliferation pathways of B-cell malignancies especially MM, as

20 well as a cooperation factor for tumor promotion, and in proliferation pathways for solid tumors.

21 Evidence also supports a significant role for IL-6 in the etiology of renal cell carcinoma. The

22 mAbs B-E8 and CNTO 328 have shown promising results in preliminary clinical trials for the

1 treatment of B-lymphoproliferative disorders, plasma cell leukemia, lymphoma, multiple
2 myeloma, and renal cell carcinoma, and warrant further investigation in these disease states.

3 The preliminary evidence from six structured clinical trials of mAb to IL-6 in the
4 treatment of produced a number of interesting observations: in all cases, mAb to IL-6 therapy
5 had a substantial impact on CRP levels, and in most instances levels were reduced to below
6 detectable limits. Patients exhibited good tolerance, with no toxic side effects, and immunogenic
7 responses toward anti-IL-6 were not observed in the vast majority of studies. The advanced
8 cancer stages examined in the trials reviewed and the substantial impact of mAb to IL-6 on IL-6-
9 mediated activity indicate that there may be significant potential for mAb to IL-6 in the treatment
10 of cancers at an earlier stage. The therapeutic impact of mAb to IL-6 on paraneoplastic
11 syndromes including cancer-related anorexia and cachexia may also be of clinical significance in
12 a vast number of cancer patients with malignant disease.

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Table 1. Cancers Associated with Abnormal Interleukin-6 Production

Cancer Type	Serum IL 6 Levels	Study Findings	Reference(s)
Breast cancer	↑	Median serum IL-6 level ~ 10 times higher in patients with metastatic disease than in those with localized disease	Benoy et al., 2002
	↑	Significantly higher serum IL-6 levels in patients with more than 1 metastatic site	Zhang and Adachi, 1995
	↑	IL-6 and IL-8 levels significantly higher in patients with progressive disease	Yokoe et al., 1997
Cancer-related cachexia	...	Decrease in IL-6 serum levels associated with subjective improvement following therapy	Yamashita and Ogawa, 1998
	↑	High serum levels of IL-1, IL-6, and TNF- α in advanced stage cancer patients, particularly those with cachexia	Mantovani et al., 1998
Gastrointestinal cancer	↑	Serum IL-6 levels were elevated in advanced gastrointestinal cancer patients and correlated with overall survival	De Vita et al., 2001
	↑	Serum IL-6 levels indicative of tumor proliferative activity in colorectal cancer patients	Kinoshita et al., 1999
	↑	High serum levels of IL-6 mark patients with cholangiocarcinoma and correlate with tumor burden	Goydos et al., 1998
	↑	Serum levels of IL-1 β , IL-6, and TNF- α elevated in patients with squamous cell carcinoma of the oral cavity	Jablonska et al., 1997
	↑	Mean serum levels of IL-6 significantly higher in patients with gastric cancer	Wu et al., 1996
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 cladribine significantly lower, especially in patients who achieved remission	Robak et al., 1999
Lymphoma	↑	Elevated serum IL-6 levels seen in 25% of	Fayad et al., 1998

	↑	indolent non-Hodgkin's lymphomas, predictive of poor outcome	Seymour et al., 1997
	↑	IL-6 serum levels frequently elevated in patients with Hodgkin's disease; these normalize with remission	Preti et al., 1997
		Serum IL-6 levels elevated in patients with diffuse large cell lymphoma	
Lung cancer	...	IL-6, IL-8, and TNF- α found in higher concentrations in malignant pleural effusion than in serum	Alexandrakis et al., 2001
	↑	Serum IL-6 levels higher in patients with extensive small cell lung cancer than in patients with limited-stage disease	Dowlati et al., 1999
	↑	Increased IL-6 level related to extensive disease, impaired performance status, and enhanced acute-phase response	Martin et al., 1999
	↑	Mean IL-6 concentrations significantly higher in non-small cell lung cancer patients than in control subjects	De Vita et al., 1998
	↑	Serum concentration of IL-6 significantly higher in mesothelioma than in lung adenocarcinoma	Nakano et al., 1998
Melanoma	↑	Significantly higher serum IL-6 and IL-12 levels observed in patients with localized and metastatic melanoma	Moretti et al., 2001
	↑	Baseline serum IL-6 level significantly higher in patients with metastatic malignant melanoma	Mouawad et al., 1996
Multiple myeloma	↑	Increased proportion of T cells producing IL-6 in MM patients with active disease	Frassanito et al., 2001
	↑	Significantly increased serum concentration of IL-6 in MM patients	Urbanska-Rys et al., 2001
	↑	Serum levels of IL-6 significantly higher in MM patients, highest levels seen in patients with progressive disease	Wierzbowska et al., 1999
	↑		Pulkki et al., 1996

		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	
Ovarian cancer	↑	Median serum levels of IL-6 significantly elevated in ovarian cancer patients	Tempfer et al., 1997
Pancreatic cancer	↑	Increased serum levels of IL-6 detected in 54.5% of pancreatic cancer patients; significantly among the patients with weight loss	Okada et al., 1998
Prostate cancer	...	Serum IL-6 levels significantly correlated with clinical stage of prostate cancer	Nakashima et al., 2000
	↑	Serum levels of IL-4, IL-6, and IL-10 significantly elevated in hormone-refractory prostate cancer	Wise et al., 2000
	↑	Levels of IL-6 and transforming growth factor (TGF) correlate with tumor burden and clinically evident metastases	Adler et al., 1999
	↑	Serum IL-6 level significantly elevated in hormone-refractory prostate cancer	Drachenberg et al., 1995
	↑	Serum levels of IL-6 related to the metastatic burden to osseous tissue in patients with prostate cancer	Akimoto et al., 1998
Renal cell cancer	↑	Serum IL-6 levels prior to surgery were significantly higher in renal cell cancer patients with short survival	Kallio et al., 2001
	↑	Levels of serum IL-6 and basic fibroblast growth factor were significantly higher in renal cell cancer patients with malignant cysts	Hayakawa et al., 1998
	...	56% of patients with metastatic renal cell carcinoma had detectable serum levels of IL-6	Walther et al., 1998
	↑	Serum IL-6 levels were significantly higher in renal cell cancer patients with paraneoplastic fever and weight loss	Blay et al., 1997
	...		Costes et al., 1997
	↑	There was a significant difference in survival among renal cell carcinoma patients with detectable levels of IL-6	Ljungberg et al., 1997

		Survival time was significantly shorter for renal cell carcinoma patients with serum IL-6 levels above the median level for all patients studied	
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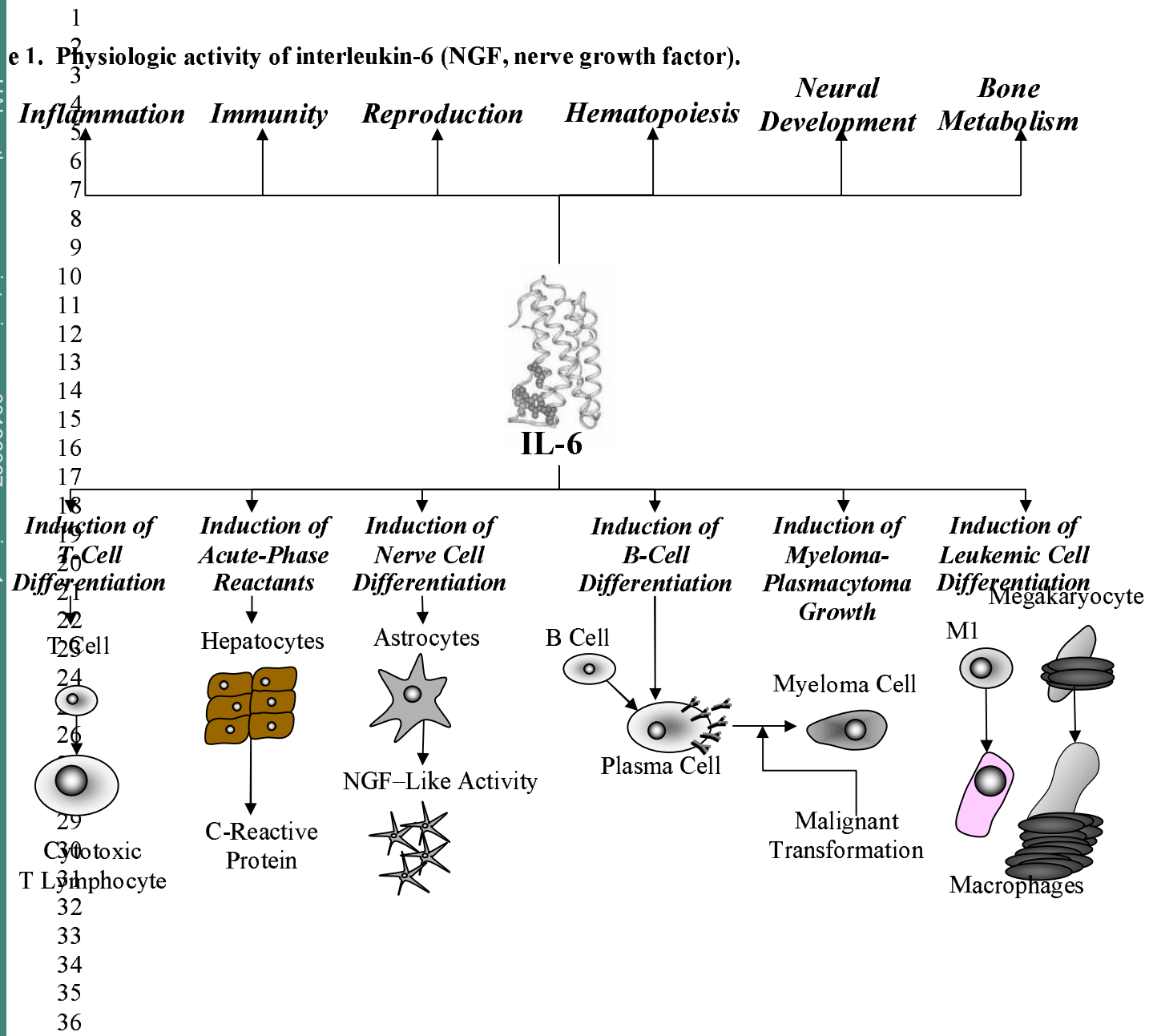


Table 2. Chronology of Anti-interleukin-6 Monoclonal Therapy for Cancer

	Study Design	Clinical Outcome
1 2 3 4 5 6 7 8 9	<p>1991</p> <p>Single patient with primary plasma cell leukemia (chemotherapy resistant) was treated with daily intravenous anti-IL-6 monoclonal antibodies.</p> <p>Klein et al., 1991</p>	<p>Patient's clinical status improved, with no major side effects; monitoring showed myeloma cell proliferation</p>
10 11 12 13 14 15 16 17	<p>1994</p> <p>11 patients positive for HIV-1 with immunoblastic or polymorphic large cell lymphoma were treated with anti-IL-6 monoclonal antibodies (B-E8; 10 to 40 mg/d) for 21 days.</p> <p>Emilie et al., 1994</p>	<p>Disease progression in 5 patients, stabilization in 5, and partial remission in 1; stabilization lasted for 8 to 28 wk, affecting fever and cachexia</p>
18 19 20 21 22 23	<p>1995</p> <p>10 MM patients with extramedullary involvement were treated with anti-IL-6 monoclonal antibodies; 9 patients treated with IV therapy and 1 intrathecal therapy.</p> <p>Battaille et al., 1996</p>	<p>Significant reduction of myeloma cell production, complete inhibition of CRP synthesis, and low daily level of IL-6</p>
24 25 26 27 28 29	<p>1996</p> <p>Phase I/II dose-escalating study of anti-IL-6 chimeric monoclonal antibody in 9 MM patients who were resistant to second-line chemotherapy.</p> <p>Van Zaanen et al., 1996</p>	<p>The chimeric monoclonal antibodies have a long half-life and low immunogenicity and blocked IL-6 processes</p>
30 31 32 33 34 35	<p>1998</p> <p>Phase I dose-escalating study of anti-IL-6 chimeric monoclonal antibody in 12 MM patients who were resistant to second-line chemotherapy.</p> <p>Van Zaanen et al., 1998</p>	<p>CRP levels in 11 of 12 patients below detectable levels, no response by standard criteria, no toxicity or immunogenicity</p>
36 37 38 39 40	<p>2000</p> <p>Phase II trial of combined murine anti-IL-6 monoclonal antibody, dexamethasone, melphalan, and autologous stem cell transplantation in 16 advanced MM patients.</p> <p>Moreau et al., 2000</p>	<p>Strong inhibition of IL-6 activity (reduced CRP) observed in all patients, correlated with a high rate of complete response</p>
41 42 43 44 45 46	<p>2001</p> <p>Safety and efficacy of monoclonal anti-IL-6 antibodies in the treatment of 12 organ trans-plantation patients with B-lymphoproliferative disorder refractory to reduction of immunosuppression.</p> <p>Haddad et al., 2001</p>	<p>CRP levels normalized in all treated patients; complete remission in 5 patients; and partial remission in 3 patients</p>

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