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Chimaeric anti-interleukin 6 monoclonal antibodies in the treatment of advanced multiple myeloma: a phase I dose-escalating study

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Summary. Interleukin 6 plays a key role in the pathogenesis of multiple myeloma (MM). Therefore we conducted a phase I dose-escalating study with chimaeric monoclonal anti-IL6 antibodies (cMab) in MM patients resistant to second-line chemotherapy. The cMab (CLB IL6/8; K_d $6{\cdot}25\times10^{-12}$ M) was given in two cycles of 14 daily infusions, starting on day 1 and day 28, repectively, with a daily dose of 5 mg in patients 1–3, 10 mg in patients 4–6, 20 mg in patients 7–9 and 40 mg in patients 10–12 (total dose 140 mg, 280 mg, 560 mg and 1120 mg of anti-IL6, respectively). 11/12 patients had elevated pretreatment IL6 levels.

Except for transient thrombocytopenia in two patients there was no toxicity. There were no changes in haemoglobin levels, granulocyte count, liver enzymes or renal function. No human anti-chimaeric antibodies were induced. This was also reflected in a long half-life time of the cMab (median 17·8 d), resulting in accumulation of the anti-IL6 cMab and high levels of circulating IL6. However, this was in the form of biologically inactive IL6/cMab complexes and did not result in acceleration of the disease. Although C-reactive protein (CRP) levels were decreased to below detection level in 11/12 patients, indicating effective IL6 blocking, none of the patients achieved a response according to the standard criteria. We conclude that this chimaeric anti-IL6 Mab has a low toxicity, low immunogenicity and a long $T_{1/2}$. A dose of 40 mg/d for 14 d can safely be used in future phase II studies.

Keywords: chimaeric, anti-interleukin 6, multiple myeloma.

Interleukin-6 (IL6) is a cytokine with multiple biological activities. It has been shown to be involved in such diverse processes as T-cell activation, induction of acute-phase proteins, and stimulation of haemopoietic precursor cell growth and differentiation (Heinrich *et al*, 1990; Kishimoto, 1989). In the last decade *in vitro* and *in vivo* observations have suggested a major role of IL6 in the pathogenesis of multiple myeloma (MM) (Anderson *et al*, 1989; Hilbert *et al*, 1995; Kawano *et al*, 1988; Klein *et al*, 1989; Lokhorst *et al*, 1994; Nordan *et al*, 1987; van Oers *et al*, 1993; Zhang *et al*, 1989). Especially in patients with active and/or terminal disease, serum IL6 levels have been found to be elevated (Bataille *et al*, 1989; Klein *et al*, 1990).

Murine anti-IL6 Mab have been used (in rather heterogenous schedules) in the treatment of myeloma patients (Bataille *et al*, 1995; Klein *et al*, 1991). No major side-effects have been observed, but antibodies to mouse immunoglobulin (HAMA) were frequently detected about 2 weeks after starting the treatment. This resulted in rapid Mab clearance and diminished efficacy of this treatment. These HAMAs are frequently directed against the Fc part of the mouse immunoglobulin (Hoffman, 1990), and may also induce anaphylactic reactions.

In order to reduce the risk of induction of HAMAs and thus to increase efficacy of the treatment, we produced a chimaeric anti-IL6 antibody (cMab). With this cMab we performed a phase I dose-escalating study in MM patients who were resistant to second-line chemotherapy. Here we report the results of this phase I study.

PATIENTS AND METHODS

All patients had MM according to the criteria of Durie & Salmon (1975), and had relapsed after, or were resistant to, second-line chemotherapy (VAD (vincristine, adriamycin

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Table I. Patients' characteristics.

Pt	M/F	Age (yr)	M-protein	Stage	CRP	IL6	β2M
1	M	53	IgGκ	IIIa	8	50	1·9
2	F	70	λ	IIIa	<3	3	4·2
3	F	58	IgGκ	IIIa	3	7	4·4
4	F	71	IgGκ	IIIa	12	22	7·7
5	F	74	IgAκ	IIIa	6	17	5·4
6	F	63	IgGκ	IIIa	4	13	1·5
7	F	60	IgAλ	IIIa	6	10	3·3
8	F	58	IgGκ	IIa	<3	41	2·4
9	F	54	IgGκ	IIIa	4	7	4·0
10	F	53	IgGλ	IIIa	<3	5	2·9
11	M	69	IgAκ	IIIa	6	33	2·8
12	F	64	*	IIIa	76	9	10·2
Median		61·5			5	11·5	3·7

CRP, C-reactive protein (normal value <5 mg/l); IL6, interleukin-6 (normal value <3 pg/ml); β2M, beta-2 microglobulin (normal value 1·1–2·4 mg/l).

* Non-secretor.

and dexamethasone) or VAD-like regimens, intermediate to high doses of melphalan; 70–140 mg/m^2 with or without autologous bone marrow or peripheral stem cell support).

Exclusion criteria were; age <18 or >75 years, life expectancy <3 months, Karnofsky score <60, diabetes mellitus, hypercalcaemia requiring treatment, recent allogeneic bone marrow transplantation, creatinine level >150 μmol/l, co-existing malignancies and active infection.

Pretreatment characteristics of these 12 patients are shown in Table I.

Chimaeric monoclonal anti-IL6 antibody. A murine-human chimaeric anti-IL6 monoclonal antibody (chimaeric CLB IL6/8) was developed, containing the antigen-binding variable region of the murine anti-IL6 antibody (CLB IL6/8) (Brakenhoff *et al*, 1990) and the constant region of a human IgG1 kappa immunoglobulin. The neutralizing Mab CLB IL6/8 blocks binding of IL6 to the IL6 receptor (CD 126) (Ehlers *et al*, 1994) and has a high affinity for recombinant as well as native IL6 ($K_d = 6{\cdot}25 \times 10^{-12}$ M) (Brakenhoff *et al*, 1990; van Zaanen *et al*, 1996). The chimaeric Mab was manufactured by Centocor, Leiden, The Netherlands.

Treatment schedule. After obtaining written informed consent according to the guidelines of the participating institutes, each patient received two cycles of treatment with cMab. Both cycles (starting at day 1 and 28 respectively) consisted of 14 daily 2 h i.v. infusions of the cMab (see Fig 1). This schedule was chosen to study the possible occurrence and response to re-treatment of a rebound phenomenon (i.e. acceleration of disease activity following cessation of the antibody administration) as has been described in patients treated with murine anti-IL6 Mab at the moment therapy was stopped (Klein *et al*, 1991). Before each cycle a test dose (10 μg) was given by slow i.v. push over 5 min. As none of the 12 patients developed an immediate hypersensitivity reaction, in all cases treatment was started 15 min later. The first three patients received a daily dose of 5 mg of the cMab (total dose 140 mg), the next three patients received 10 mg/d (total dose 280 mg), patients 7–9 received 20 mg/d (total dose 560 mg) and the last three patients received 40 mg/d (total dose 1120 mg).

Levels of IL6 and anti-IL6 antibodies. During treatment with the chimaeric CLB IL6/8 almost all the IL6 in the plasma circulated as a complex with the antibody (van Zaanen *et al*, 1996). IL6 levels were determined with the B9 bioassay as described before (Aarden *et al*, 1987; van Zaanen *et al*, 1996). One unit of B9-stimulating activity was defined as the amount inducing half-maximal proliferation and corresponded to 1 pg of IL6. To determine the total IL6 level (i.e. free IL6 plus IL6 complexed to cMab), an excess (10 μg/ml) of CLB IL6/14 was added to each well in order to displace IL6 from its binding to the *in vivo* administered neutralizing chimaeric CLB IL6/8. CLB IL6/14 and CLB IL6/8 Mab recognize partly overlapping sites of IL6. However, CLB IL6/14 is not capable of inhibiting IL6 activity in the B9 bioassay (Aarden, 1991). During treatment with the cMab actual free IL6 levels cannot be measured, because the dilution of the samples necessary for testing in the B9 bioassay or ELISA immediately influences the equilibrium between IL6–cMab complex, free IL6 and free cMab. Therefore free IL6 levels were calculated using the Henderson-Hasselbalch equation, with the K_d, the daily serum cMab levels and the total IL6 levels as known parameters.

Levels of the chimaeric CLB IL6/8 monoclonal antibody were determined using a radioimmunoassay as described in detail before (van Zaanen *et al*, 1996). The threshold of this assay is 0·5 ng/ml of antibody.

Human anti-chimaeric antibody (HACA) levels were determined using an ELISA. Briefly, the cMab (CLB IL6/8, IgG1-kappa) was coated overnight at room temperature (2 μg/ml in 100 μl well) on flat-bottomed microtitre plates. The plates were washed four times with PBS/Tween solution. Patients

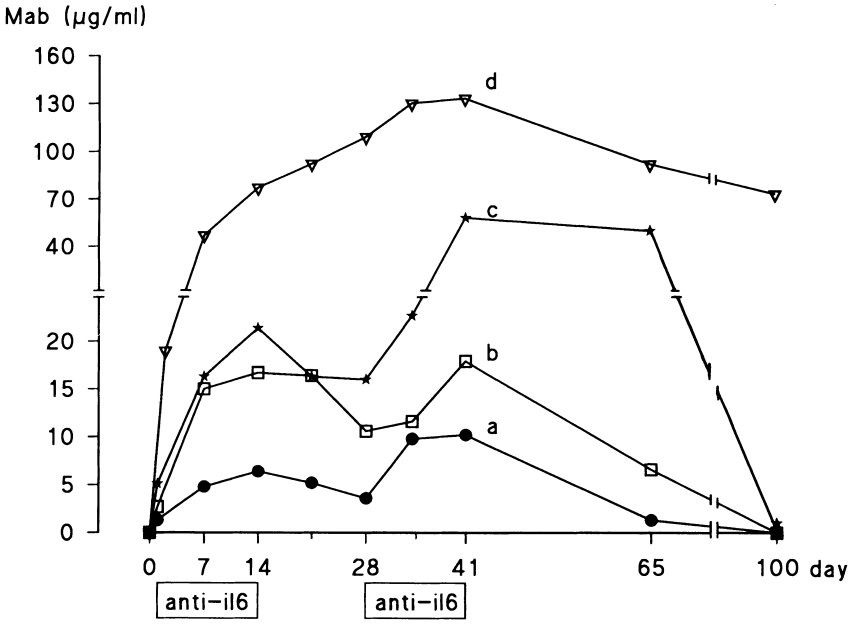

Fig 1. Mean serum levels of chimaeric anti-IL6 Mab in the different dosage groups. Patients 1–3 (a) = 5 mg/d; patients 4–6 (b) = 10 mg/d; patients 7–9 (c) = 20 mg/d; patients 10–12 (d) = 40 mg/d. Patient 12 received treatment on days 1–4 and 16–29. Day 65: $n = 9$, day 100: $n = 5$.

sera were added in different dilutions (1/50 up to 1/800) in HPE-buffer (CLB, Biotechnology, Amsterdam, The Netherlands) and incubated for 2 h at room temperature. After washing, the plates were incubated with HRP-conjugated monoclonal mouse anti-human lambda light chain (KH29, CLB, Amsterdam, The Netherlands) in 100 µl HPE buffer for 1 h at room temperature. Subsequently, after washing, the bound peroxidase was detected at 450 nm in a Titertek Multiskan.

C-reactive protein (CRP) was determined by nephelometry (Behring, Germany); detection level 3 mg/l; normal value <5 mg/l.

Beta-2 microglobulin (β2M) was determined by a microparticle enzyme immunoassay (Abbott Laboratories, U.S.A.); normal value 1·1–2·4 mg/l.

Paraprotein (M-protein) levels were determined once weekly by immunonephalometry (Behring Nephalometer 100 Analyzer; Behring Diagnostics, Amsterdam, The Netherlands).

Levels of IL6 and the cMab were determined daily from day 0 until day 14 and day 28 until day 41 (samples were drawn before starting infusion of anti-IL6), and on days 17, 21, 44, 48, 56 and 100.

Response to treatment was defined as a decrease in M-protein level of >50% or a decrease in plasmacytoma size in patient 12.

RESULTS

Dosage of chimaeric anti-IL6

Twelve patients (three in each dosage group) completed at least one treatment cycle of 14 d. Eight of them also completed a second 14 d treatment cycle. In the other four patients anti-IL6 treatment was stopped because they fulfilled a predefined stopping criterion: patient 1 required radiotherapy for neurological complications at day 30. Patient 5 had fever and an urinary tract infection when admitted for the second treatment cycle on day 28. In patient 9 anti-IL6 was stopped at day 34 because of pneumonia and septicaemia. In patient 12 (receiving anti-IL6 until day 5) treatment was interrupted because of fever. With antibiotic treatment her clinical condition improved and anti-IL6 was given from day 16 to day 29 (one treatment cycle of 14 d). A second cycle was not given at the patient's request.

Pretreatment values of IL6, CRP and B2M are shown in Table I. In all but one patient pretreatment IL6 levels were elevated. 6/12 patients had elevated CRP levels. There was no correlation between CRP and IL6 levels ($r = -0{\cdot}13$) in these patients. Serum β2M was increased in nine patients. Patient 12 had a non-secreting myeloma, with large plasmacytomas on the chest wall and the right upper arm. The plasmacytoma on the chest wall was measured bidimensionally, before and daily during anti-IL6 treatment. Before treatment it had a diameter of 14×16 cm and a growth rate of 1·5 cm/week in two perpendicular directions.

Levels of cMab and IL6

Data regarding the pharmacokinetics have been published previously (van Zaanen *et al*, 1996). The median half-life time of this cMab was 17·8 d (range 7·8–39·7), with a median distribution volume of 6·0 litres (range 3·0–9·7). Peak serum levels of the cMab ranged between 6·7 µg/ml (patient 1) and 288 µg/ml (patient 11). No HACAs were found in any of the patients during the study period of 100 d. During treatment, accumulation of the cMab occurred due to its long half-life time (Fig 1). This resulted in high total serum IL6 levels, complexed with the cMab (van Zaanen *et al*, 1996). Calculated free IL6 levels decreased to <0·5 pg/ml during treatment in all patients (Fig 2).

Toxicity

During and after administration of the cMab, no changes in

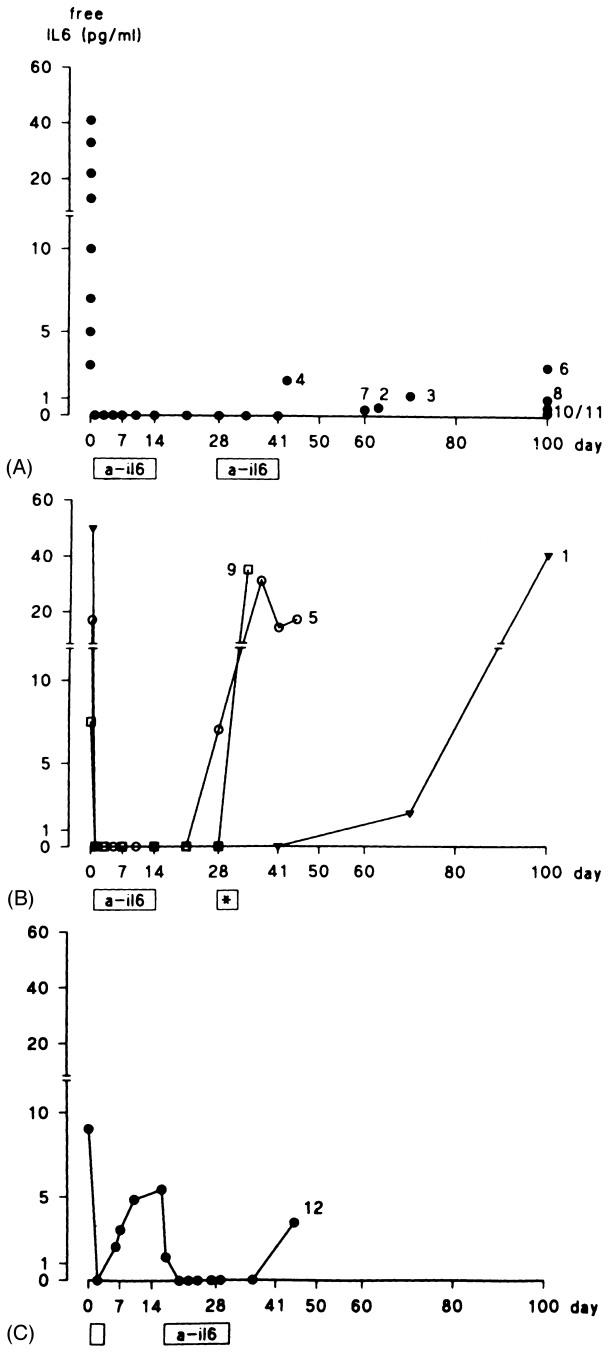

Fig 2. Free IL6 levels. (A) The eight patients who received two complete treatment cycles. Free IL6 levels <0·3 pg/ml during treatment in all patients. (B) The three patients who completed one treatment cycle of 14 d (see Results). Patient 1: anti-IL6 until day 30; patient 5: anti-IL6 until day 14; patient 9: anti-IL6 until day 34. (C) Patient 12 received anti-IL6 on days 1–4 and 16–29. Because of fever of unknown origin, anti-IL6 treatment was interrupted at day 5.

blood pressure, pulse rate or temperature were observed. These parameters were measured every 15 min for 2 h immediately after the administration of anti-IL6. No changes in liver function were found. Creatinine levels fluctuated in patients 2, 5, 10 and 12 but remained <150 μmol/l; in the other eight patients no changes in renal function were found. Haemoglobin levels remained stable, while minor changes occurred in platelet and leucocyte counts. In patients 2 and 5 we observed a transient thrombocytopenia with a nadir of 24 and 58×10^9/l respectively (on days 56 and 14, respectively). Before anti-IL6 treatment both patients had hypocellular bone marrow aspirate and biopsy (with a decreased number of megakaryocytes). In 6/12 patients (patients 2, 4, 7, 8, 9 and 11) a mild transient granulocytopenia was documented after one dose of anti-IL6. The median decrease in the number of granulocytes was 32·5% (range 20–46%), with a median time to nadir of 1 d. The granulocytes returned to base-line levels within 2 d. No alterations in lymphocyte subsets were found.

Three patients developed infectious complications; patient 5 (with known recurrent urinary tract infections) had fever with low blood pressure due to a urinary tract infection with *Escherichia coli* on the day she was admitted for the second treatment cycle. Patient 9 died because of pneumonia and septicaemia with *Staphylococcus aureus*. Patient 12 developed fever of unknown origin on day 5. She was treated with antibiotics; all cultures remained negative.

Clinical effects

In all but one patient the M-protein level did not change during both cycles of anti-IL6 treatment. In eight patients (patients 1, 3, 4, 7–11) this indicated an unaffected disease course because they had similar M-protein levels at 2 months before anti-IL6 treatment (Figs 3A and 3B). In patient 2 disease activity remained progressive during the first treatment cycle; this was reflected by a rise of M-protein and β2M levels (77% and 145% increase in relation to day 0, respectively). However, during the second treatment cycle, M-protein and β2M levels remained stable (Fig 3B). After day 60 she received 70 mg/m^2 melphalan intravenously, resulting in a temporary decrease of M-protein level. In two patients (patients 5 and 6) a marked stabilization of the M-protein occurred during therapy (Fig 3B). This was also true for the growth rate of the plasmacytoma of patient 12. In two patients (patients 1 and 7) we observed a possible acceleration in the increase of the M-protein levels after stopping anti-IL6 (Fig 3B). Likewise, after the anti-IL6 treatment was stopped in patient 12, the growth rate of the plasmacytoma was increased when compared to the growth rate before therapy (1·5 cm/week versus 2 cm/week).

Immediately after starting the anti-IL6 treatment the CRP levels decreased to below detection level in all patients except for patient 12 in which CRP decreased from 76 to 10 mg/l. Other acute-phase proteins tested, pre-albumin, alpha-1 antitrypsin and alpha-1 acid glycoprotein, remained stable and within normal ranges.

Survival

Six patients were still alive 9 months after treatment with anti-IL6. Three patients died within the study period of 100 d (patients 5, 9 and 12). Patient 5 went off study because of an urinary tract infection on day 28. After appropriate

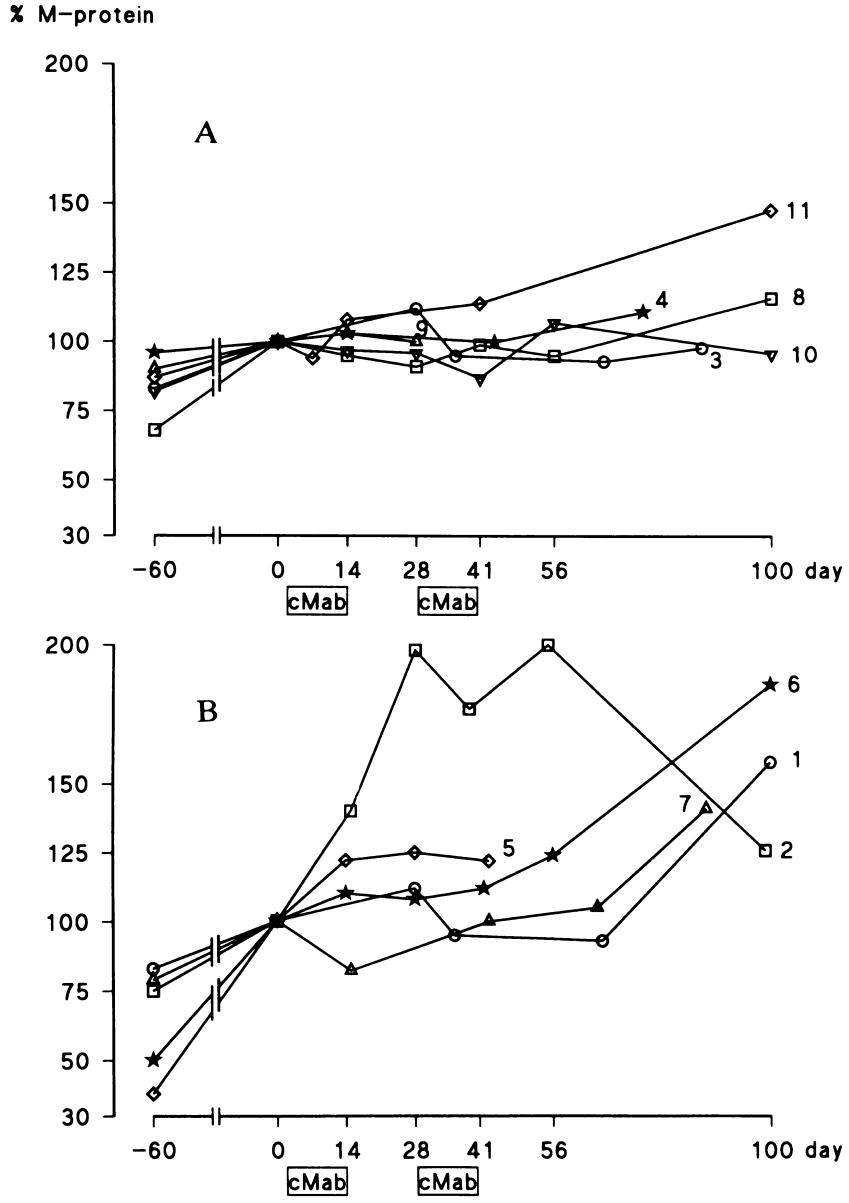

Fig 3. Percentage of M-protein levels before, during and after anti-IL6 treatment in relation to day 0 (day 0 = 100%). Anti-IL6 cMab was given in two cycles of 14 d (two boxes below abscissa). (A) Patients 3, 4, 8, 9, 10 and 11; (B) patients 1, 2, 5, 6 and 7. At day 60, patient 2 received melphalan i.v. (70 mg/m^2).

treatment she was discharged and died several weeks later at home from progressive disease. Patient 9 died on day 35 due to an irreversible septic shock. Although radiotherapy was a treatment option for the large plasmacytomas in patient 12, no further treatment was given according to the wish of the patient. She died shortly afterwards because of progressive disease with pleural effusion. Three patients died after day 100 because of refractory multiple myeloma.

DISCUSSION

From this phase I dose-escalating study with chimaeric anti-IL6 Mab in patients with advanced MM, two important conclusions can be drawn. First, the use of this cMab is safe. Despite high levels of circulating cMab, no toxicity was observed and no HACAs were induced. Second, accumulation of the high-affinity cMab resulted in high levels of circulating IL6. However, this was in the form of biologically inactive complexes and did not result in acceleration of the disease during anti-IL6 treatment.

Toxicity

Although IL6 is a multi-functional cytokine, blocking its biological activity by chimaeric anti-IL6 Mabs did not result in serious side-effects. Only a transient thrombocytopenia occurred in two patients who had hypocellular bone marrow before starting anti-IL6 treatment. The mechanism of this thrombocytopenia during anti-IL6 treatment is not completely understood. Although from studies in primates and humans it has become evident that IL6 is able to induce megakaryocyte maturation and thrombocytopoiesis (Asano *et al*, 1990; Stahl *et al*, 1991; Wickenhauser *et al*, 1995;

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