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# Erythropoietin has an anti-myeloma effect – a hypothesis based on a clinical observation supported by animal studies

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Abstract: Recombinant human erythropoietin (rHuEpo) was introduced into clinical practice more than a decade ago, and has been found to be effective in the treatment of several types of anemia, including anemia of end-stage renal failure and cancer-related anemia. No study has suggested that Epo might have an effect on the biology of the disease, nor any survival advantage to cancer patients treated with Epo for anemia has been reported. Here we report six patients with advanced multiple myeloma (MM) with very poor prognostic features, whose expected survival was $<6$ months. All six patients were treated with rHuEpo for their anemia, either without any chemotherapy or very mild chemotherapy for a short time. Yet, surprisingly they lived for 45–133 months totally from MM diagnosis and 38–94 months with rHuEpo (with a good quality of life). In fact, one patient, is still alive and well, more than 8 yr after chemotherapy was discontinued because of a resistant aggressive disease. The course in these six MM patients led us to hypothesize that Epo might have an antineoplastic or antimyeloma effect. We proceeded and tested that hypothesis in mouse models of myeloma (Mittelman M *et al*., Proc Natl Acad Sci USA 98:5181,2001). In these models we confirmed that rHuEpo induced tumor regression in about 50% of the BALB/c mice inoculated with MOPC-315 myeloma cells. We then presented evidence that the mechanism is a new immune-mediated phenomenon, via activation of CD8+ T cells. Furthermore, evidence from the literature supports the antineoplastic effect of Epo. Epo might be used as an adjunct immune treatment in various malignant diseases, in addition to the current regimens and chemotherapeutic protocols. Fututre trials should determine the role of Epo in myeloma and cancer treatment, besides clarifying concerns about the presence of Epo receptors on some tumor cells.

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Erythropoietin (Epo) is a hematopoietic growth factor that is produced by the kidney and regulates red blood cell (RBC) production in response to hypoxia (1). The cloning of the Epo gene (2) led to the introduction of recombinant human Epo (rHuEpo) into clinical practice in the treatment of several types of anemia, including anemia of end-stage renal disease (3), and cancer-related anemia (4–12).

Multiple myeloma (MM) is characterized by clonal proliferation of bone marrow (BM) plasma cells (PC) secreting a paraprotein (M-protein), that can be detected in the serum and/or urine (13, 14). Most patients die of MM or its complications with or without chemotherapy with a median survival range of 15–48 months (14) and probably closer to the range of 25–31 months (15). About 60–90% of MM patients develop anemia, which adversely affects their quality of life (QoL) (13,14,16). The demonstration that inadequate Epo production is a major reason for cancer-related anemia (17) was the rationale for clinical trials applying rHuEpo in the

treatment of MM-associated anemia (18–23). rHu-Epo has been shown to be effective, resulting in an erythroid response rate of 70–80%. No study has tested the effect of Epo on the course or the biology of the disease.

In a clinical trial conducted in our center (22), 17 patients with MM and anemia were treated with rHuEpo. The initial dose was 150 U/kg s.c. three times per week, and in non-responders the dose was doubled. The study was designed for 12 wk. Thirteen of the 17 patients responded to rHuEpo with an erythroid response, i.e. an increase in hemoglobin (Hb), hematocrit (Hct), and the number of RBC, a reduction in the blood transfusion requirements (BTR) and an improved QoL. The focus, as in other trials, was treatment of anemia and survival was not an endpoint. However, six of the 13 responding patients continued Epo for longer periods. Four of them, and later on two additional patients (M.B. and K.M.), all six with poor prognostic features of MM, and whose life expectancy was less than 6 months, not only demonstrated an erythroid response, but have also lived longer than expected, for 38–94+ months later. In fact, while writing this report, one of the patients is still alive and well, now 102 months from her diagnosis, being mainly on Epo (for 94 months).

As these patients were mostly on Epo, without or with minimal chemotherapy, the longer survival than expected, based on the clinical and laboratory parameters, led us to hypothesize that in addition to the known erythroid effect, Epo might be affecting the biology and the course of the disease as well. This hypothesis was further tested and the results reported (24). Epo induced tumor regression in 50% of BALB/c mice inoculated with MOPC-315 myeloma cells. This was associated with longer survival among responders and a decrease in the pathologic protein in the serum. Further analysis revealed that the antimyeloma effect of Epo is an immune-mediated phenomenon. Here, we report in detail the course of the above-mentioned six MM patients (Tables 1–4).

## Patients

### Patient 1 – J.S.

A 59-yr-old male immigrated to Israel in October 1992 and was rushed to the hospital for evaluation of weakness and general deterioration. He looked sick and pale. His routine laboratory profile was as follows: erythrocyte sedimentation rate (ESR) 80 mm Westergren; Hb 10.6 g/dL; WBC $3.8 \times 10^9$/L; platelet count $116 \times 10^9$/L; potassium 6.3 mEq/L; calcium 9.7 mg/dL; phosphorus 6.5 mg/dL; total proteins 7.9 g/dL; globulins 3.3 g/dL; urea 174 mg/dL; creatinine 4.7 mg/dL and uric acid 10.2 mg/dL. Serum protein electrophoresis demonstrated a monoclonal peak of λ-light chain (LC) at a concentration of 1.84 g/dL. The urinary protein concentration was 0.66 g/24 h, with a positive test for Bence-Jones (BJ) protein. Urinary protein electrophoresis detected a λ-LC M-protein too. BM analysis demonstrated > 70% PC, many of them young immature. Skeletal X-ray survey showed an increased bone resorption with lytic lesions in the skull. MM, of the λ-LC type, with renal failure (RF), was diagnosed.

Treatment with monthly oral cycles of melphalan (alkeran) and prednisone (MP) was initiated (the patient refused more aggressive treatment). Two months later Hb dropped to 7.3 g/dL. During the following months the patient's condition deteriorated. He required 2–3 RBC units per month and developed severe (NYHA class IV) right and left congestive heart failure (CHF).

Table 1. Patient characteristics: demographic data and clinical presentation

| Patient initials | J.S. | O.G. | L.B. | T.S. | M.B. | M.K. |
|---|---|---|---|---|---|---|
| Age at diagnosis | 59 | 44 | 68 | 73 | 55 | 47 |
| Gender | M | F | F | F | M | M |
| Presentation | Weakness, general deterioration | Weakness, weight loss | LBP/W | LBP/weakness | LBP | LBP |
| Date of diagnosis | 10/1992 | 1/1995 | 8/1991 | 1/1994 | 6/1989 | 7/1994 |
| MM type | LC-λ | IgG-κ | IgG-κ | IgG-λ | IgG-λ | IgA-λ |
| Anti-MM therapy | MP | VMCP ×2<br>VBAP ×2<br>VAD ×4<br>HDC ×2 | MP, HD dexa, local RT | MP, plasmapheresis | VMCP, IFN, MP, RT, VAD | VAD, VMCP, ABMT, AlBMT, DexCycloAlk |
| Misc. | CHF | Local RT | DM, HT, hypothy, Parkinson | RF, HT, several surgeries | | |

MM, multiple myeloma; LBP, low back pain; W, walking difficulties; MP, melphalan-prednisone; VMCP, vincristine-melphalan-cyclophosphamide-prednisone; VBAP, vinblastine-BCNU-adriamycin-prednisone; VAD, vincristine-adriamycin-dexamethasone; HDC, high-dose cyclophosphamide; RT, radiation therapy; DM, diabetes mellitus; HT, hypertension; RF, renal failure; CHF, congestive heart failure; ABMT, autologous bone marrow transplantation; AlBMT, allogeneic bone marrow transplantation; LC, light chain; IFN, interferon.

Table 2. Laboratory profile at presentation

| Patient initials | J.S. | O.G. | L.B. | T.S. | M.B. | M.K. |
|---|---|---|---|---|---|---|
| ESR (mm West) | 80 | 90 | 57/84 | 95/123 | 120/130 | |
| Hb (g/dL) | 10.6 | 8.0 | 12.3 | 8.9 | 9.5 | 13.6 |
| Serum Ca (mg/dL) | 9.7 | 8.5 | 13.7 | 9.4 | 8.7 | N |
| Creatinine | 4.7 | 0.7 | 0.9 | 1.4 | 1.0 | N |
| Serum total proteins (g/dL) | 7.9 | 13 | 9.3 | 9.1 | 11.6 | |
| Serum total globulins (g/dL) | 3.3 | 10 | 4.7 | 5.1 | 8.2 | |
| Ser IgG/A (g/dL) | 0.43 | | | 3.2 | | 3.52 |
| M-spike | | | | | | |
| Type | λ-LC | IgG-κ | IgG-κ | IgG-λ | IgG-λ | IgA-λ |
| Level | 1.84 g/dL | 6.33 g/dL | 4.2 g/dL | 2.3 g/dL | 4.56 g/dL | |
| Urinalysis | 0.6 g/24 h λ-LC | Negative | κ-LC | λ-LC | Negative | λ-LC |
| BM PC (%) | >70% | >90% | 30–40% | 40% | 27% (80)* | 70% |
| X-ray (lytic lesions) | ++ B. resorp | + | ++/+++ | Negative | +++ OP, Open | +++ Fractures |
| Poor prognostic features | RF, CHF, amyloidosis | Very high protein | High Ca | RF | High prot NLL | NLL high β2M |
| Misc. | K 6.3, P 6.5 UA 10.2 | β2M 2.5 mg/dL | | | Many fract | β2M 1.8 |

ESR, erythrocyte sedimentation rate (mm Westergren); Hb, hemoglobin in g/dL; BM PC, bone marrow plasma cells in %; CHF, congestive heart failure; UA, uric acid; NLL, numerous lytic lesions; N, normal; RF, renal failure; Open, osteopenia; OP, osteoporosis.
*80% when M.B. was transferred to our service.
Lytic lesions: + a few; ++ several to many; +++ numerous.

Table 3. rHuEpo treatment

| Patient initials | J.S. | O.G. | L.B. | T.S. | M.B. | M.K |
|---|---|---|---|---|---|---|
| Serum Epo (U/mL) | 63 | 120 | 117 | 20 | | |
| Hb (pre-Epo) (g/dL) | 8.8 | 8.5 | 9.9 | 6.8 | 6.8 | 9.9 |
| Hb (on Epo) (g/dL) | 15.2 | 11.6–12.5 | 14 | 11–12 | 13.2 | 12.3 |
| BTR (pre-Epo) RBC U/month | 2–3 | 3 | 2 | 3 | 4 | 2 |
| BTR (on Epo) | 0 | 0 | 0 | 0 | 0 | 0 |
| PS (pre-Epo) | 4 | 2–3 | 2 | 3 | 4 | 4 |
| PS (on Epo) | 0 | 0 | 0 | 1 | 2 | 4 |
| Epo load dose (U/kg) | 150–300 × 3/wk | 150 × 3/wk | 150 × 3/wk | 150–300 × 3/wk | 150 × 3/wk | 150 × 3/wk |
| Epo maintenance dose (U/kg) | 50 × 2/wk | 150 × 1–2/wk | 150 × 1–2/wk | 150 × 1/wk | 150 × 2/wk | 150 × 1–2/wk |
| Concurrent chemo type | MP | No | Mod MP | MP | No | |
| Concurrent chemo duration | 3 months | 0 | MP × 10 | 2 months | 0 | Cyc Alk x2 |
| Misc. | CHF resolved | | | | | |

Epo, erythropoietin; RBC, red blood cells; BTR, blood transfusion requirements (no. of units per month); PS, performance status, interpreted as 0–4 grade, according to the WHO scale; Mod, modified; MP, melphalan-prednisone (no. of monthly cycles).

Table 4. rHuEpo treatment – duration, survival, and outcome

| Patient initials | J.S. | O.G. | L.B. | T.S. | M.B. | M.K. |
|---|---|---|---|---|---|---|
| Disease duration pre-Epo (months) | 14 | 8 | 40 | 3 | 86 | 26 |
| Epo Rx duration | 77 | 94+ | 67 | 42 | 44 | 38 |
| Overall survival from MM diagnosis | 91 | 102+ | 107 | 45 | 130 | 64 |
| Date of death | May 11, 2000 | Alive | July 15, 2000 | October 30, 1997 | April 26, 2000 | November 2000 |
| Cause of death | Ischemia, arrhythmia | | MM, sepsis Hip fracture | Bacterial sepsis | Cardiac arrest (?) at home | Infection |
| Misc. | Dialysis sMDS Amyloid | | Breast cancer sMDS | | Heart disease Amyloid | sMDS |

Epo Rx, erythropoietin therapy; MM, multiple myeloma; sMDS, secondary myelodysplastic syndrome.

In December 1993 he was re-admitted with shortness of breath and generalized bone pain. Physical examination detected severe cachexia and findings consistent with severe CHF, including prominent jugular venous congestion, S3 gallop heart rhythm, bilateral pleural effusions, a

significant abdominal ascites and +4 pitting edema in both lower extremities. Acute ischemia was excluded. The Hb level was 8.8 g/dL, urea 188 mg/dL and creatinine 5.35 mg/dL. X-rays detected new lytic lesions in both femurs. Chest X-ray showed an enlarged heart and bilateral pleural effusions. Echocardiography demonstrated findings consistent with cardiomyopathy, due to amyloidosis. The patient discontinued chemotherapy after 10 cycles of MP. Treatment included high-dose morphine, furosemide (500–1000 mg q.d.) and spironolactone (50 mg, q.d.) with angiotensin-converting enzyme inhibitors and RBC transfusions. He refused chemotherapy, dialysis or plasmapheresis. His expected survival at that point was estimated as a few days or weeks. Having an endogenous serum Epo level of 63 mU/mL, rHuEpo, 150 U/kg (about 10 000 U), three times weekly, was initiated. The aim was to improve his QoL, performance status and cardiac symptoms, and decrease his BTR. Four weeks later, his Hb level was 9.6 g/dL, and the dose was doubled. Hb measured during the following weeks were 10.0, 12.6, 12.9 and 15.2 g/dL, by weeks 6, 8, 10 and 12, respectively.

By week 12 of rHuEpo, J.S. became transfusion-independent, and all his CHF symptoms completely disappeared within the next couple of months. Jugular venous congestion disappeared, the heart sounds were normal (no gallop), the lungs were clear to auscultation, the ascites as well as edema of the extremities were all absent. He required neither diuretics nor morphine. His WHO PS score declined from grade 4 (fully bedridden) to 0 (fully active). Chest X-ray demonstrated normal heart size and echocardiography failed to show any evidence of amyloidosis. rHuEpo dose was tapered and his Hb was maintained on 12–13.5 g/dL level with a maintenance dose of 10 000 U weekly shot, 1/6 of the initial loading dose and then even 4000U × 2 weekly (about 50 U/kg two times per week). Serum creatinine level at that time was 4.7 mg/dL. Upon the patient's request, combination chemotherapy with oral MP was resumed for another three monthly cycle, along with rHuEpo. This resulted in a stabilization of Hb, a significant reduction of the urinary proteins (traces only), and a decreased serum creatinine (2.8 mg/dL).

From May 1994 through the next 3 yr, J.S was on rHuEpo only (8000–10 000 U per week), without any chemotherapy, enjoying an excellent QoL with a stable disease. His serum protein level remained stable with no proteinuria.

In May 1997, serum creatinine rose, but the patient was still asymptomatic. In July 1997, while creatinine rose to 7.5 mg/dL, hemodialysis was initiated.

In January 1998, carpal tunnel syndrome, related to amyloidosis was diagnosed, requiring orthopedic surgery. He also suffered from chronic temporomandibular joint pain and macroglossia, related to amyloidosis too.

In March 1999, intravenous pamidronate (Aredia) was added to the treatment (dialysis, analgesics, rHuEpo, no chemotherapy), because of bone pain related to new lytic lesions in the cervical column and the skull.

In April 1999, BM analysis detected 20–30% PC in the aspirate and more than 15% PC in the biopsy. In addition, the aspirate had some dysplastic features, consistent with early signs of secondary myelodysplastic syndrome (sMDS).

In September 1999, J.S. complained of typical anginal chest pain. ECG revealed old (silent) anterior wall ischemia. Tallium cardiac scan failed to detect active ischemia.

In October 1999, he was hospitalized with acute anterolateral wall acute myocardial infarction, complicated by CHF evolving later into pulmonary edema, ventricular tachycardia and ventricular fibrillation. The patient refused cardiac catheterization. From March through May 2000, J.S. was re-admitted several times with unstable anginal syndrome and acute ischemic episodes. On May 5, 2000, he underwent a new episode of acute ischemia, complicated by paroxysmal atrial fibrillation, ventricular tachycardia, sinus bradycardia and pneumonia. He was treated with appropriate medications, antibiotics, cardiac pacemaker and mechanical ventilation. His condition deteriorated and he developed cardiac and septic shock. On May 11, 2000, J.S. died.

Totally J.S. lived 91 months from MM diagnosis, and 77 months with rHuEpo.

### Patient 2 – O.G.

A 44-yr-old lady, was admitted in January 1995, for evaluation of generalized weakness and weight loss. She looked sick and pale. Laboratory evaluation revealed anemia (Hb 8.0 g/dL), hyperproteinemia (13 g/dL) and hyperglobulinemia (10 g/dL). The remaining routine laboratory profile, including urinalysis, was within normal limits. Serum electrophoresis demonstrated IgG-$\kappa$ M-spike (6.33 g/dL). The BM was packed (>90%) with immature PC. Several lytic lesions were seen in the skull. Serum β-2 microglobulin was 0.25 mg/dL (normal range 0–0.22 mg/dL).

Stage III MM-IgG-$\kappa$ was diagnosed and chemotherapy was initiated. Two cycles of vincristine-melphalan-cyclophosphamide-prednisone (VMCP), alternating with two cycles of vinblastine-BCNU-adriamycin-prednisone (VBAP) were administered.

In April 1995, we switched to vincristine-adriamycin-dexamethasone (VAD). Again, four cycles failed to reduce the M-protein level. The same holds for two cycles of high-dose cyclophosphamide. Since September 1995 the patient has been off chemotherapy. However, she suffered from the complications of chemotherapy. She was introduced to several bone marrow transplantation (BMT) programs, but was rejected on the basis of having a chemoresistant disease, with no chances to survive or benefit.

Since presentation, O.G. was RBC transfusion-dependent, receiving 3 units per month (on average). In August 1995, she suffered from limited physical activity (WHO PS 2–3), with Hb 8.5 g/dL. Her endogenous serum Epo level was 120 mU/mL. rHuEpo (150 U/kg three times per week), was initiated. After 6–8 wk Hb rose to 11.6–12.5 g/dL, with a complete abolition of her BTR, and a significant improvement of her QoL and PS score, which declined to 0 (fully active). The rHuEpo dose was then tapered to a maintenance dose of 150 U/kg one to two times per week, adjusted according to the Hb level.

In February 1999, monthly intravenous pamidronate was added. In May 2001, thalidomide was added, but was discontinued after 3 months of a low dose (50–100 mg) because of side effects. In February 2002, a new pathologic fracture in the sixth right anterior rib was detected and radiation therapy was applied. Disease evaluation showed no change in the protein pattern, negative urinalysis for BJ proteins and more than 90% BM PC. In April 2002, pamidronate was switched to clodronate.

Currently (July 2003) the serum protein profile as well as the BM PC content remain stable and the bone disease is slowly progressive. O.G., being on rHuEpo only (+ bisphosphonates, but no chemotherapy), continues to be minimally symptomatic (bone pain which responds to mild pain killers, no narcotics), fully active, acting as a director of a bank branch and works full time.

Totally, so far, O.G. has lived 102 months since diagnosis, and 94 months with Epo.

### Patient 3 – L.B.

A 68-yr-old lady complained in August 1991 of nausea, vomiting, low back (LBP) and pelvic pain, with difficulties in walking. Her past medical history had been remarkable for hypertension, diabetes mellitus, hypothyroidism, and Parkinson's disease. Laboratory work up was as follows: ESR 57/84 mm Westergren, Hb 12.3 g/dL, WBC $3.4 \times 10^9$/L, serum calcium 13.7 mg/dL, creatinine 0.9 mg/dL, total proteins 9.3 g/dL, and globulins 4.7 g/dL. Serum protein electrophoresis detected IgG-$\kappa$ M-spike, with a concentration of 4.2 g/dL. Urinary electrophoresis was also positive for the same paraprotein. About 30–40% PC were found in the BM, and skeletal X-ray survey demonstrated many lytic lesions in the skull, lumbar spinal column, arms and ribs.

MM-IgG-$\kappa$ was diagnosed, and therapy, including hydration, diuretics, radiation therapy to L2-L3 and monthly oral MP chemotherapy was initiated. In June 1992, a clinical improvement was noted, along with a 55% reduction in the paraprotein concentration (to the level of 1.890 g/dL), and MP was stopped.

In mid-1994, generalized bone pain occurred with a rise in the serum M-protein. Resumption of oral MP failed to achieve a clinical or laboratory response. Oral monthly high-dose dexamethasone was prescribed. In October 1994, L.B. was admitted with fever, lethargy, clinical deterioration, and disorientation, rapidly evolving into coma. A combined infection, including systemic bacterial sepsis, candidemia and herpes encephalitis was diagnosed. Treatment included combination intravenous antibiotics, amphotericin B, and acyclovir along with intensive supportive therapy. After 2 months the patient was discharged from the hospital with no neurologic sequelae. Dexamethasone was discontinued.

In December 1994, because of symptomatic anemia (Hb 9.9 g/dL), rHuEpo (150U/kg three times per week) was initiated. Hb rose to 14 g/dL with a significant improvement in her QoL. Epo dose was then tapered to a maintenance of about half the loading dose. Her BTR dropped from 2 per month to 0.

In April 1997, infiltrating ductal adenocarcinoma of the left breast, grade 3, was diagnosed, and left radical modified mastectomy was performed. All 14 axillary lymph nodes were positive. Oral tamoxifen was prescribed.

In April 1998, monthly pamidronate was added because of bone disease. Although BM analysis revealed about 8% PC, the paraproteinemia has shown a mild steady rise since early 1998. Thus, a very mild MP dose (3 d of melphalan 6 mg q.d. and prednisone 20 mg q.d., every 6–8 wk) was added.

BM analysis also showed dysplastic changes, especially in the erythroid series. These findings, together with leukopenia ($2.5–3 \times 10^9$/L), thrombocytopenia ($100 \times 10^9$/L), macrocytosis, and high serum ferritin level, suggested sMDS.

Till late December 1999, L.B., while on oral modified MP, monthly i.v. pamidronate and rHuEpo (10 000 U × 2–3/wk), continued to be relatively well and minimally symptomatic. The rising

M-protein level and some new lytic lesions suggested a relatively stable or slowly progressive MM.

On December 27, 1999, she slipped and a pathologic supracondylar fracture of the left femur was detected. X-ray demonstrated a lytic lesion in the fracture site. This required a major orthopedic operation with intramedullary nailing and distal interlocking. Unfortunately, rehabilitation failed, and she remained bedridden with several episodes of infections during the following months. In June 2000, firm right axillary lymphadenopathy was noted, representing apparently breast carcinoma, but further work up was not performed. In July 2000, the patient was hospitalized with bacterial sepsis, complicated by ventricular tachycardia, pulmonary edema and acute renal failure. On July 15, 2000, L.B. died.

Totally, L.B. lived 107 months from MM diagnosis and 67 months with rHuEpo. Till the last few months she enjoyed reasonably good QoL.

### Patient 4 – T.S.

A 73-yr-old lady presented in January 1994 with LBP and weakness. Her past medical history had been remarkable for hypertension, thyroidectomy, cholecystectomy, hysterectomy and surgical vagotomy for peptic ulcer. Her laboratory evaluation showed ESR 95/123 mm Westergren, Hb 8.9 g/dL, creatinine 1.4 mg/dL, total proteins 9.1 g/dL, globulins 5.1 g/dL and IgG 3.2 g/dL. Serum protein electrophoresis detected an IgG-λ M-spike of 2.3 g/dL. Urinalysis confirmed the presence of the same LC. About 40% PC were seen in the BM, but skeletal X-ray survey was negative. MM, of the IgG-λ type was diagnosed, and oral MP was initiated.

In March 1994, Hb dropped to 6.8 g/dL, and serum creatinine rose to 3.96 mg/dL. Urinary protein content increased to 0.6 g/24 h. Renal biopsy diagnosed a myeloma kidney. Treatment included RBC transfusions, plasmapheresis and oral MP. This intervention resulted in a decrease in the serum creatinine to 1.8 mg/dL. Given the anemia and the low serum endogenous Epo level (20 mU/mL), rHuEpo (150 U/kg three times per week) was initiated. The dose was then doubled. In May 1994, oral MP was discontinued after five monthly courses.

In December 1995, serum creatinine rose to 6.4 mg/dL, and BM biopsy demonstrated PC infiltration (80%). Plasmapheresis with supportive therapy (including rHuEpo, but no chemotherapy) was again successful.

The next 2 yr were characterized by a stable course of the disease with excellent QoL. Hb level of 11–12 g/dL was maintained by a rHuEpo dose which was about a third of the loading dose (10 000 U once a week). Serum creatinine was relatively stable at the level of 2–3 mg/dL. Serum M-protein concentration was 2.0 g/dL. No significant change in the BM PC pattern was noted (relatively stable disease). During this period, the patient received Epo only but no chemotherapy. In October 1997, rising creatinine was noted. In the BM aspirate there were about 30–50% PC. For the first time lytic lesions were demonstrated in both femurs and the left tibia. T.S. was re-admitted for another attempt of plasmapheresis. However, she developed systemic bacterial sepsis and died on October 30, 1997.

Totally, T.S. lived 45 months from MM diagnosis and 42 months with rHuEpo.

### Patient 5 – M.B.

A 55-yr-old male presented to another hospital in mid-1989 with LBP. M-protein of the IgG-λ type was detected, along with 27% PC in the BM, and lytic lesions, in the skull and vertebrae. The serum IgG concentration at that time was 3.7 g/dL. A diagnosis of MM-IgG-λ was established. Combination chemotherapy with VMCP was initiated, along with supplemental palliative radiation therapy. In 1990 interferon was added. In January 1992 treatment was switched to monthly oral cycles of MP, resulting in a relative stabilization of the disease. In August 1992, compression fracture of L3 vertebra was noted. BM analysis showed massive PC infiltration. Another radiation course to the lumbar spinal column was administered. In November 1992, he underwent unsuccessful orthopedic surgery to fix a fracture of L2-L3. Since then he was unable to walk and bedridden.

In January 1993, M.B. was referred to our center because of progressive disease. He complained of weakness, fatigue and generalized bone pain. On physical examination he looked sick and cachectic. He was fully bedridden. (WHO PS score 4). Neurologic examination detected symmetric significant weakness in both lower extremities, marked muscular atrophy and bilateral paraparesis. His laboratory evaluation was as follows: ESR 120/130 mm Westergren, Hb 9.5 g/dL, WBC $3.7 \times 10^9$/L, and platelet count $191 \times 10^9$/L. Routine blood chemistry was normal except for total proteins 11.6 g/dL, globulins 8.2 g/dL, with an M-spike of 4.56 g/dL. Urinalysis was negative for proteinuria including BJ proteins. BM was heavily infiltrated by young PC. Skeletal X-ray survey revealed massive bone involvement, with numerous lytic lesions virtually in all the vertebrae along the spinal column, as well as the pelvis, hip, long bones,

and the skull. Significant osteoporosis and osteopenia were also noted.

Local radiation to painful bone lesions was administered. This supplemented combination chemotherapy with VAD. In May 1995 X-ray revealed many lytic lesions, some of them new.

In June 1995, staphylococcal sepsis was diagnosed, requiring hospitalization with intensive antibiotics (vancomycin) and supportive therapy. Hb dropped to 6.8 g/dL and he suffered from weight loss. His BTR was 4 RBC units per month. In December 1995, after 12 cycles of VAD, chemotherapy was discontinued because of neither clinical nor laboratory response. Another attempt with oral MP (three cycles) failed. The disease was defined as chemoresistant.

In June 1996, M.B. suffered from a pathologic fracture of the left femur, which was treated conservatively. At that time, he was not on chemotherapy but RBC transfusion-dependent, rHuEpo was initiated, (150 U/kg three times per week). Hb rose from 6.8 to 13.2 g/dL, with a significant improvement in his general condition. He became transfusion-independent and no longer suffered from weight loss. rHuEpo dose was reduced to a maintenance of 150 U/kg two times per week.

In early 1999, oral clodronate was added. In February 2000, M.B. was re-admitted with anginal syndrome and CHF. Hb was maintained by rHuEpo (150 U/kg two times per week) at a level of 12–13 g/dL. This time echocardiography showed a pattern consistent with amyloidosis. On April 26, 2000, M.B. died at home, apparently from cardiac arrest.

Totally, M.B. lived 130 months from MM diagnosis and 44 months with rHuEpo. Most of the time, till the last few months, he enjoyed a reasonable QoL, being transfusion-independent and able to use a walker, with a relatively stable disease.

### Patient 6 – M.K.

A 47-yr-old male, a computer technician, presented in July 1994 with LBP and compression fractures of T12, L1 and the sacrum. His Hb at that time was 13.6 g/dL and serum protein electrophoresis detected an IgA monoclonal peak. Serum IgA at presentation was 3524 mg/dL.

He was initially treated with a single cycle of VAD, which failed to relieve his pain. He was then switched to VMCP for several cycles, supplemented with local radiation (2400r). This was followed by autologous BM transplantation (ABMT) in February 1995. The preparatory regimen for the ABMT consisted of thiotepa (60 mg/sq m), melphalan (60 mg/sq m) and etoposide (200 mg/sq m). He relapsed less than a year later, and was treated with moderate-dose cyclophosphamide (1 g/sq m) and LAK (lymphocyte activated killer) cells from an HLA-identical sibling. Following BMT the IgA level fell to 263 mg/dL (within normal limits).

In June 1996, the IgA level rose to 4320 mg/dL and VAD was readministered. He was given nine courses of VAD, and in July 1997, he was retransplanted with stem cells from his allogeneic matched sibling donor. Pretransplant, his IgA level had risen to 5260 mg/dL. Transplant conditioning included fludarabine (30 mg/sq m for 6 d), busulfan (4 mg/sq m for 2 d) and ATG (10 mg/kg for 4 d).

As there was no evidence of engraftment, autologous peripheral stem cells were returned to the patient on August 18, 1997. He was discharged in early September 1997 on recombinant erythropoietin (10 000 U three times per week). He was given no further chemotherapy for the next 36 months, only monthly courses of oral dexamethasone (40 mg/d for 4 d). He was also given two courses of local radiotherapy, to the scapula and thoracic spine, and later to the cervical spine. His blood counts did not tolerate the radiation. An attempt to prescribe thalidomide lasted less than a month and failed due to neurologic adverse effects.

Interestingly, follow-up of his laboratory data showed that during the period of Epo administration he had a gradual fall in his M-protein. During 1998, the IgA level fell from 4590 to 1900 mg/dL (February 1999). The paraprotein remained low until December 1999. During this time, he was on monthly oral dexamethasone (40 mg/d for 4 d), intravenous bisphosphonates, i.v. IgG and erythropoietin (10 000 U two to three times per week). He was able to work most of that period. He was using no or minor analgesia for bone pain.

In December 1999, his paraprotein began to rise, and he was given two courses of low dose chemotherapy: cyclophosphamide (1 g/sq m), melphalan (12 mg over 3 d), and prednisone. This treatment resulted in a drastic fall in blood counts.

BM analysis performed in June 2000 showed 50% PC as well as 50% abnormal BM precursors. Cytogenetic analysis detected del (20q), indicating that the patient had developed secondary MDS.

It was felt that he could not tolerate chemotherapy due to severe pancytopenia and rising paraprotein (6180 mg/dL in October 2000), and he remained on Epo, i.v. IgG and bisphosphonates, until he died in November 2000, of pneumonia.

## Discussion

The effect of Epo on various types of anemia has been well characterized (3–7). However, this

well-known effect has been isolated so far to the erythroid series only. This report, supported by animal experiments (24), and other reports suggest an additional unrecognized hitherto biologic effect, i.e. an anti-neoplastic agent.

Ludwig *et al.* (4) reported that patients with various malignancies, who had responded to Epo, lived longer than the control arm, although the two groups were not exactly comparable. Rubins (25) observed a single patient with metastatic renal cell cancer (mRCC), who responded to rHuEpo with a regression of the metastases. This was followed by Morere *et al.* (26) who administered rHuEpo to 20 patients with mRCC. They observed a single complete response, one partial response and additional two minor responses. Lissoni *et al.* (27) then treated patients with mRCC who had shown a progressive disease on IL-2. The addition of Epo to IL-2 resulted in several responses. Recently, Littlewood *et al.* and Littlewood and Mandelli (10, 28) reported their results in a large-scale (375 patients) randomized placebo-controlled multicenter phase III clinical trial, using rHuEpo to treat anemia of various neoplasms. Although the study was not designed and lacked the statistical power to evaluate the effect of Epo on the course of the disease and survival, they observed a tendency towards longer survival among responders compared with non-responders. The Kaplan–Meier estimate of 12-month survival was 60% for the Epo-treated group vs. 49% for the placebo group. The median survival was 17 months for the Epo group compared with 11 months in the controls. These researchers suggest that Epo might result in a survival benefit unrelated to the increased Hct, as blood transfusions may not be as effective (29). Wallvik *et al.* (30) have recently reported longer median survival (49 months) among patients with myelodysplastic syndromes responding to rHuEpo, compared with the non-responding patients (18 months). Pangalis *et al.* (31) treated 25 patients with chronic lymphocytic leukemia and anemia with rHuEpo. Twenty (80%) patients not only responded to Epo with improved Hb and QoL, but also were 'down staged' in their hematologic disease. In most of the responders chemotherapy could be postponed. Although no prolonged survival has been shown, down staging, i.e. earlier and less advanced phase of the disease, is indeed interpreted as consistent with more favorable prognosis and longer survival (32). Interestingly, a single patient with erythroleukemia demonstrated *in vitro* and *in vivo* complete remission following administration of rHuEpo, after failure of conventional chemotherapy (33). It was also evident that Epo induced leukemic-cell differentiation.

The incidence of the antineoplastic effect of Epo is currently unknown, as all data collected and reported so far are retrospective and observational. Most MM patients are on chemotherapy and prolonged survival is often attributed to chemotherapy. Although myeloma patients were treated with rHuEpo, most of them were on chemotherapy, thus hindering the estimation of the true incidence of the antimyeloma effect of Epo. Prospective trials are required to answer such questions.

The mechanism explaining the prolonged survival in the above-mentioned patients has not been fully elucidated. In animal models for MM Epo induces a CD8+ T-cell-mediated anti-MM effect (24).

Many tumor cells suffer from hypoxia, associated with radio resistance, genomic instability, inhibition of apoptosis and angiogenesis, leading to malignant progression with a negative impact on long-term outcome (34). Epo improves hypoxia and is associated with longer survival in patients with oral squamous cell cancer and head and neck cancers (34–37).

An important point is the prevalence and location of the erythropoietin receptors (EpoR). EpoR have been found not only on erythroid progenitors but also on megakaryocytes, other BM progenitors, endothelial cells, brain capillaries, rodent placenta cells, neuronal cells, cardiac tissue and several tumor cell lines (38–48). The presence of EpoR on these cells, suggests an additional biologic function to Epo in certain conditions or during evolution. This is also supported by the complex signal transduction pathways, which share some features, common to other cytokines and molecules (49). Suzuki *et al.* (50) suggested that EpoR might not be necessary, at least not for non-erythroid effects. Recently, Lappin *et al.* (51) have reviewed various multiple actions of Epo.

In fact, the presence of EpoR on various myeloma (44) and other tumor cells (52, 53), in cell lines, have raised some questions. Possible Epo stimulation of proliferation of cancer cells, particularly breast, is of concern (53). So far, a single patient with increased proteinuria following Epo administration has been reported (54). However, a recent trial aiming to show the impact of Epo on outcome of cancer chemotherapy in patients with advanced breast cancer had to be terminated prematurely due to poorer survival in the Epo-treated group (55). While these data should be taken into consideration, explanations for the early termination of the study are not clear enough. Only well-designed future clinical trials will clarify these points. Till then, rHuEpo can be regarded as a safe medication.

Silverberg *et al.* (56) suggested that using rHuEpo may improve the cardiac function and the

clinical picture in patients with CHF, besides correcting the anemia. Although they assumed that correction of anemia improved cardiac function, a direct effect of Epo on the myocardium cannot be excluded. This is supported by experiments suggesting that Epo stimulated arteriolar proliferation in hearts of rats (57) and also by the presence of EpoR in the cardiac tissue (48). Of note, our patient J.S. responded to rHu-Epo with higher Hb and also with a significantly improved CHF.

Administration of rHuEpo 24 h before or up to 6 h after focal ischemic stroke significantly reduced the extent of infarction (46, 47). rHuEpo also attenuated concussive brain injury, kainate-induced seizure activity, and autoimmune encephalomyelitis. These preclinical findings suggest that rHuEpo may have a therapeutic potential for stroke, head trauma, and epilepsy. Digicaylioglu and Lipton (58) suggest that this Epo-mediated neuroprotection involves cross-talk between Jak2 and NF-kappa B signaling cascades.

Grimm *et al.* (59) reported that Epo expression in the photoreceptor cells in mouse retina increases following exposure to low concentration of oxygen. This increased Epo production, induced by increased amounts of hypoxia-inducible factor, prevents apoptosis of these retinal cells, and protects against retinal degeneration. Exogenous administration of Epo to the abdomen in these mice similarly protected the retina.

Shurtz-Swirski *et al.* (60) described *in vitro* immunologic effect to Epo on T cells in dialysis patients. Interestingly, data from patients with HIV infection show an adverse impact of anemia on survival and suggest that treatment with rHuEpo can prolong survival compared with patients receiving no treatment or blood transfusions (61). Whether rHuEpo stimulates a T-cell response in these patients is unknown.

Finally, Sigounas *et al.* (62) reported that Epo improves the pulmonary fibrosis related to bleomycin-induced lung fibrosis. They assume that better oxygenation of the pulmonary tissue protects against fibrosis.

The MM patients described here do not provide results of a clinical study. Rather, they represent a clinical observation of physicians treating patients with a particular problem (anemia) and obtaining an unexpected result (anti-MM effect). When one observes a single patient responding in an unexpected fashion, it can be considered as an anecdote. However, when the number of these patients increases, a 'phenomenon' is suspected. The mice experiments together with other cited reports suggest that Epo induces an additional non-erythroid antineoplastic, apparently immune, effect. In fact, the mice experiments allowed us to prove that we are dealing with two separate effects of Epo, i.e. an erythroid effect and the immunologic effect.

In four of the six reported patients no reduction in the M-protein was noted. However, in patients 1 and 6, there was a transient protein level reduction and then stabilization, while the other patients demonstrated a stable disease for a relatively long period of time. This stabilization was absolutely unexpected and could not be related to the mild chemotherapy they had been on. The number of the observed patients is too small to draw conclusions on that issue and these patients had very advanced disease. In addition, a regular nephlometry and electrophoresis was used to analyze the proteins. Applying a more sensitive Western blot analysis with anti λ-mouse antibodies, we observed a decreased amount of the pathologic protein in the mouse model (24). Future application this more sensitive method in testing patient samples is warranted.

The optimal dose and regimen of Epo required to achieve antineoplastic effect has not been established. The first observed MM patients (see table), were treated with a dose of 150 U/kg three times per week and a few required a double dose, as they were all treated for anemia and indeed received the usual 'anti-anemic' regimens. This may not be the case in treating neoplastic disease. The mice experiments suggest higher doses (24). Future clinical trials should focus on anticancer regimens. These may be different from the antianemic regimens, characterized by a loading dose, followed by a lower maintenance dose, and dose modification according to the Hb level. In addition, it may be the case that patients with MM will be treated regardless of having anemia. Of course, precautions will have to be taken to avoid dangerous Hb rise.

Of course, this new biologic effect of Epo which has been observed in several patients and has been well studied and characterized in mouse models will have to be further tested in a randomized controlled trial. If this is confirmed, this may initiate a significant progress in the treatment of MM, in addition to other new agents that are expected to enter the market.

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