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Pilot study of recombinant human soluble tumor necrosis factor (TNF) receptor (p75) fusion protein (TNFR:Fc; Enbrel) in patients with refractory multiple myeloma: increase in plasma TNFα levels during treatment

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Abstract

Elevated tumor necrosis factor (TNF)-α levels are associated with poor prognosis in patients with multiple myeloma (MM). Enbrel is a TNF antagonist fusion protein consisting of the extracellular, ligand-binding domain of the human p75 TNF receptor linked to the Fc portion of human IgG1. Ten patients with refractory MM were treated with Enbrel 25 mg s.c twice weekly for a minimum of eight median age was 63 years (range, 43–76). The total number of Enbrel doses was 191 (median 16; range, 3–55). TNFα plasma levels increased significantly during treatment with Enbrel. No objective response occurred. Acceleration of disease occurred in four patients. While well-tolerated, Enbrel did not have anti-myeloma activity as administered on this study.

Keywords: Refractory; Multiple myeloma; TNFα; Enbrel

1. Introduction

The management of patients with relapsed or refractory multiple myeloma (MM) remains inadequate and novel treatment modalities are urgently needed. The response rate with standard therapy, including combination chemotherapy with vincristine, adriamycin, and dexamethasone (VAD) is approximately 60% [1–3]. Thalidomide single-agent therapy is associated with overall response rates of approximately 30% and 2 years overall and failure-free survival rates of 48 and 20%, respectively [4,5].

Among the potential MM growth factors, tumor necrosis factor (TNF)-α is a survival factor for MM cell lines, induces MM cells in the cell-cycle and promotes long-term growth of malignant plasma cells [6]. It promotes the growth of MM cell lines, sometimes in a synergistic manner with interleukin-6 (IL-6), but also may clearly act through a pathway independent of IL-6, having a growth-promoting effect at least equal to that of IL-6 [7–11]. TNFα is also a potent bone-resorbing factor and plays an important role in the development of the osteolytic bone lesions observed in MM patients [12–15]. In some models, the role of TNFα in MM is more complex; it stimulates both growth and apoptosis of some plasma cell lines and some ex-vivo plasma cells [8,16]. Fillela et al. found that TNFα serum levels were increased in 44% of patients with newly diagnosed MM and 50% of those with progressive disease [11]. TNFα serum levels were significantly higher in persons with monoclonal gammopathy of undetermined significance (MGUS), or patients with progressive MM compared with healthy subjects; patients with progressive MM also had significantly higher TNF levels than patients with stable MM. Furthermore, concentrations of TNFα are significantly higher in patients with bone disease than in those without overt lesions [17].

Two distinct receptors for TNF of 55 and 75 kDa have been identified [18,19]. A recombinant TNF receptor p75-Fc fusion protein (Enbrel, Immunex, Seattle) was developed targeting to neutralize TNF, reducing its biologic activity [20]. DNA encoding the Fc portion of a human immunoglobulin (Ig) G1 molecule was linked to DNA encoding the soluble portion of human p75 TNF receptor. The combined DNA was expressed in a mammalian cell line, resulting to an Ig-like dimer. This soluble TNFR-Fc fusion construct acts

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as a competitive inhibitor of TNF, preventing its binding to the cell surface TNF receptors; it also renders it biologically unavailable [20].

Studies in healthy normal volunteers and in patients with rheumatoid arthritis [21–24] Wegener's granulomatosis [25] and advanced heart failure have shown that Enbrel is safe [21–28]. However, in patients with established septic shock caused by Gram-positive organisms there was a non-significant trend toward increased rates of mortality in those treated with higher doses of Enbrel in comparison with the placebo group; a similar tendency to increased mortality rates was also noted with the use of an anti-TNF monoclonal antibody on a prior study in a similar patient population [29–31].

An effective anti-TNF agent might be of therapeutic benefit in patients with MM. As an initial investigation of the safety of Enbrel in this immunocompromised population, already prone to sepsis, we conducted a pilot study of Enbrel, as a single agent, in patients with advanced or refractory MM. Measurements of TNFα, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and IL-6 were performed before and during treatment with Enbrel.

2. Materials and methods

2.1. Study group

Patients with refractory MM were entered onto the study between August and December 2000, after written informed consent was obtained according to institutional guidelines. Refractory MM was defined as: (a) primary resistant MM, progressive disease during receipt of at least two courses of induction chemotherapy, which includes an alkylating agent and/or a topoisomerase II inhibitor; (b) transient response, defined as response but relapse while still on induction therapy; or (c) relapsed disease, i.e. post-remission or -plateau relapse Eligibility criteria included patients with a quantifiable serum paraprotein or Bence-Jones proteinurea and a bone marrow plasmacytosis >5%, without overt infection, hypotension, concurrent chemotherapy, systemic radiotherapy, pregnancy or overt psychosis.

Pretreatment evaluation included history taking and physical examination; complete blood count, differential, and platelets count; serum chemistries, including liver and renal function studies; bone marrow aspiration with or without biopsy; β_2-M, serum immunoelectrophoresis, serum protein, immunoglobulin assay and M-band quantitation by immunofixation, 24 h urine collection for Bence-Jones protein, total protein, and creatinine; and radiologic assessment as indicated.

2.2. Measurement of cytokine levels

2.2.1. Plasma and serum collection

Plasma and serum samples were collected and stored according to approved protocols from eight patients on study who consented to provide these specimens for cytokine assay prior to first Enbrel therapy, at 2–3 week intervals while on study, and after completion of study therapy.

2.2.2. Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assays (ELISAs) for TNFα, VEGF, bFGF, HGF, and IL-6 were performed using commercially available kits from R&D Systems (Minneapolis, MN). Manufacturer's recommended protocols were followed. Briefly, plasma was collected in tubes with EDTA and stored at −82 °C. Patient samples were added to separate microplates, each containing a specific monoclonal antibody and mixtures were incubated at room temperature for 2 h. The plates were washed three times to remove any unbound substances. Protein-specific enzyme-linked polyclonal antibodies were added to the wells. Subsequently, the mixtures were incubated at room temperature for 2 h followed by another washing to remove any unbound antibody or enzyme reagent. A substrate solution was added to the wells, and a blue color developed. The intensity of the blue was proportionate to the amount of cytokine bound in the initial step. The color development was stopped, and the intensity of the color was measured and compared with a standard curve. Reading was done at 450 nm wavelength for TNFα, VEGF, bFGF, HGF, and IL-6.

2.3. Therapy

Treatment consisted of Enbrel 25 mg twice weekly subcutaneously (s.c.) for a minimum of eight doses (4 weeks; one cycle). If patient developed toxicity of grade 3–4 (NCI toxicity criteria), Enbrel was held until resolution to at least grade 1. Supportive care, including transfusion of blood and blood products, antibiotics, and analgesics were administered as needed.

2.3.1. Course timing

Enbrel was given for one course of treatment (4 weeks); if patients responded or had no signs of progression they received 16 additional doses (two courses) of Enbrel without interruption, at the same dose. Further courses were given if patients continued to respond or not to progress.

2.4. Endpoints and statistical methods

Complete response (CR) was defined as disappearance of serum and urine M-protein on electrophoresis and immunofixation in two determinations at least 4 weeks apart, <5% plasma cells in the bone marrow, normalization of peripheral blood values or biochemical abnormalities assignable to MM, and resolution of all soft tissue plasmatocytomas.

Partial response (PR) was defined as ≥50% reduction of serum M-protein; ≥50% reduction in the urine M-protein if the baseline value was ≥1 g/24 h and <0.1 g/24 h if baseline value was 0.5–1 g/24 h; and ≥50% reduction of sum

of the products of the cross diameters of each measurable lesion. Disease progression was defined as ≥50% increase in the serum or urine M-protein above the lowest previous level, and appearance of new plasmatocytomas or increase by ≥50% of soft tissue plasmatocytomas. Failure to meet criteria for response or progression was categorized as stable disease.

Toxicity was graded on a scale of 0–5 using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2.0 criteria [32].

3. Results

3.1. Study group

The clinical characteristics of the 10 patients are summarized in Table 1. Median age was 63 years (range, 43–76; 70%) were older than 60 years. Eight patients (80%) were male; one had a performance status (PS) of 2 (10%). Five patients had progressive and five stable/refractory MM.

Table 1
Patients' characteristics

Characteristic	$N = 10$	%
Age		
Median	63	
Range	43–76	
>60	7	70
Male	8	80
PS >1	1	10
High β_2M (>3 mg/l)	8	80
Immunoglobulin type		
IgG	7	70
IgA	3	30
IgG Kappa chain deposition disease	1	10
Marrow involvement	6	60
Prior regimens		
0–2	1	10
3–7	9	90
Hb <10 g/dl	4	40
WBC $<1 \times 10^9\,l^{-1}$	3	30
PLT $<100 \times 10^9\,l^{-1}$	2	20
M-protein >3 g/dl	5	50
Bone lesions		
0–2	3	30
>3	7	70
Karyotype		
Diploid	4/5	80
48–49, XY, −1, +add(3)(p26), −11, del(13)(q12q14), +14, −17 × 2, +19, −22, +4mar	1/5	20
Prior thalidomide	10	100
Prior hyper-CVAD	6	60
Prior allogeneic transplant	2	20

Median number of prior treatments was five (range, 2–7). All patients had received prior therapy with thalidomide, six had received fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD) regimen, five had received high-dose melphalan, two had received stem cell transplant; two had received Biaxin, and two patients had received IFN-α. The maximum response to prior treatment was CR in one patient, PR in six patients, and SD in three patients. One patient had IgG Kappa chain deposition disease and Guillen-Barre like syndrome, six had IgG and three patients had IgA MM. Five patients had Stage I, two Stage II, and three Stage III disease as per the Durie and Salmon classification [33]. All patients with Stage I disease had received prior therapy for their disease based on symptoms attributable to their disease, usually fatigue and/or bone pain. Prior medical history was significant for recurrent severe infections in three patients: one had sinusitis, one urinary track infection due to β-hemolytic streptococcus, and one patient had bronchitis. The median Hgb value was 11 g/dl (range, 8.9–13.8); the median WBC $4.7 \times 10^9\,l^{-1}$ (range, 2.9–5.6) and the median platelet count was $199 \times 10^9\,l^{-1}$ (range, 57–356). The median M-protein was 2.7 g/dl (range, 0–4.7; M-protein was zero in a patient with Bence-Jones proteinuria, bone marrow infiltration, and >3 bone lytic lesions). Six patients had bone marrow infiltration. Two patients had 0–1 bone lesions, one patient 2, and seven patients had >3 bone lesions. The median creatinine was 1.0 mg/dl (range, 0.7–2.6), the median β_2-M 6.9 mg/l (range, 1.2–17.5) and the median serum calcium was 8.7 mg/dl (range, 7.7–10.5). Cytogenetic studies were successful in five patients; four had diploid karyotype and one patient had multiple chromosome abnormalities (48–49, XY, −1, +add (3)(p26), −11, del (13)(q12q14), +14, −17 × 2, +19, −2, +4mar).

3.2. Treatment results

Ten patients received a total of 25 cycles of Enbrel therapy. The median number of cycles administered was 2 (range, 1–7). The median number of doses was 16 (range, 3–55) and the total number of doses was 191.

3.2.1. Response

No patient had a complete or partial response to therapy. Four patients had progressive disease on study, including two patients who were withdrawn early (after 2 and 4 weeks; Table 2). Among the four patients who progressed, three patients had stable MM on study entry.

3.3. Cytokine levels

Cytokine plasma levels were measured in eight patients who agreed to provide samples before and during treatment with Enbrel (Table 3). TNFα plasma levels were significantly higher during Enbrel treatment compared with the levels before treatment. In contrast, there was no significant

Table 2
Response

Patients	MM status on entry	Duration of Rx (weeks)	M-protein baseline	M-protein min during Rx	M-protein (end of Rx)	M-protein increase (%)	Response
1	SD/refractory	12	0.10	0.20	0.20	50	PD
2	PD	8	3.70	4.30	4.80	30	SD[a]
3	PD	26	3.20	3.50	3.50	9	SD[b]
4	SD/refractory	4	1.30	2.20	2.20	69	PD
5	PD	2	0.13	0.42	0.56	460	PD[c]
6	SD/refractory	9	4.70	4.50	5.30	13	SD
7	SD/refractory	12	4.00	4.00	6.80	70	PD
8	PD	3	2.20	2.20	3.10	41	SD
9	PD	8	0.30	0.30	0.30	0	SD
10	SD/refractory	27	3.40	3.20	3.50	3	SD

[a] Improvement in Hgb from 9.7 to 10.4 g/dl, WBC (4.4–5.8) $\times$ 10^6 l^{-1}, bone marrow plasma cells reduced from 32 to 18%.
[b] Progressive growth of myeloma slowed.
[c] Early removal from the study.

Table 3
Cytokine levels in plasma of patients treated with Enbrel

Cytokine levels	Median (range)	Mean (±2S.D.)	*P*-value
TNFα			
Pretreatment	8.0 (6.7–9.1)	7.9 (±1.4)	0.006
During treatment	243.6 (72.7–413.9)	250.8 (±243.93)	
VEGF			
Pretreatment	77.7 (38.5–294.1)	118.3 (±196.7)	0.20
During treatment	68.3 (43.4–91.4)	64.6 (±39.4)	
Bfgf			
Pretreatment	20.4 (8.6–57.8)	25.4 (±38.0)	0.22
During treatment	17.2 (7.3–25.2)	16.0 (±16.3)	
HGF			
Pretreatment	786.3 (540.3–1527.5)	855.1 (±743.6)	0.63
During treatment	743.2 (371.8–2009.3)	898.3 (±1148.4)	
IL-6			
Pretreatment	2.7 (2.4–8.9)	3.89 (±7.7)	0.44
During treatment	3.4 (1.9–8.4)	4.08 (±5.6)	

difference before and during treatment with Enbrel in the plasma levels of VEGF, bFGF, HGF, and IL-6.

3.4. Toxicity

Enbrel was associated with grade 2 fever in two patients; grade 1 fatigue in one; grade 2 flu-like syndrome in two; grade 2 chest pain in one; grade 2 abdominal discomfort in one; and grade 1 hyperbilirubinemia in one. There were no overt allergic reactions to Enbrel. No patient was withdrawn from the study because of toxicity. There was no increased mortality rate in patients treated with Enbrel. No patients developed sepsis while on study.

4. Discussion and conclusion

The administration of Enbrel at the dose of 25 mg s.c. twice weekly was not associated with overt serious adverse events in these patients with heavily pretreated refractory MM. There was no evidence of cumulative toxicity, and the more common adverse events were fever and flu-like syndrome. More importantly, there was no increased mortality rate among patients treated with Enbrel. However, no responses were observed. Four patients progressed and six patients had stable disease.

The safety profile of Enbrel in patients with MM in our study is in line with other reports in patients with rheumatoid arthritis [21–24], Wegener's granulomatosis [25], and advanced heart failure, [27,28] showing that Enbrel is well-tolerated. The most common side effects, such as injection site reactions and upper respiratory tract infections, seen in other disorders, were not noted in our study population [34,35]. The stimulus to perform the currently reported pilot safety study was the observation of a trend toward increased mortality rates with higher doses of Enbrel compared with a placebo group in patients with documented sepsis from Gram-positive bacteria [29]. The same trend has been observed in two trials of an anti-TNFα monoclonal antibody for the treatment of sepsis: in non-shock patients receiving a 15 mg/kg dose in one study [30], and in shock patients treated with the same dose in a second study [31].

Enbrel is a dimer of the p80 TNF receptor linked by the Fc portion of IgG1, which binds TNFα and lymphotoxin, neutralizing their effects. This dimeric construct of Enbrel has a higher affinity for TNF than the monomeric forms of the receptor. Additionally, the Fc peptide gives a longer half-life to the molecule [20]. The primary mechanism of its action is by binding to the TNFα, rendering it biologically unavailable. Preclinical and clinical studies have shown that Enbrel does not cause rapid removal of TNF from the biologic fluids, but does prolong TNF's half-life [20,36]. Enbrel has been reported to act as a TNF "carrier" [21,36]. This "carrier" activity of Enbrel may explain the finding of significantly higher TNFα values in patients during treatment on this study compared with their respective pretreatment values. This observation is in agreement with the study of Eason et al., who showed similar effects in patients with OKT3-acute

clinical syndrome [36]. These investigators demonstrated that the high TNFα antigenic levels were associated with concomitant low or undetectable TNFα bioactivity; high levels of TNF receptors were also noted >13 days after the administration of Enbrel, indicating its long half-life [36].

Despite this data suggesting that the elevated TNFα levels associated with Enbrel use are not bioactive, some caution must be applied in accepting that this is always so. A noteworthy event on the current study was the acceleration of MM in four patients soon after commencing Enbrel therapy, three of whom had entered on study with an immediate prior history of stable disease. In this study, we focused on safety in terms of lack of overt adverse events—Enbrel was clearly "safe" from this perspective. However, its safety in terms of modulation of disease activity in patients with MM will require much more attention in other studies. The source of the elevated circulating TNFα in patients with MM receiving Enbrel is of interest. Serial quantitative RT-PCR analyses of mRNA expression for relevant cytokines in both myeloma and stromal cells would be of benefit in future studies.

Neben et al. have investigated the genetic polymorphism in the TNFα in patients with relapsed and refractory MM treated with thalidomide [37]. Eight patients with MM carrying the −238A allele had higher TNFα levels in peripheral blood, prolonged 12 months progression free survival and a trend towards longer overall survival compared with patients with the −238G allele [37]. Among patients with the −238G allele, only one patient had achieved a CR. These investigators suggest that regulatory polymorphisms of the TNFα gene can affect TNFα production and the response to thalidomide. Of particular interest is the fact that all patients in our study had previously failed thalidomide; although no studies for genetic polymorphism were performed, it is possible that patients who progressed may have been carriers of the −238G allele.

Enbrel is also being investigated in patients with other hematologic malignancies. In a cohort of seven patients with acute myelogenous leukemia (AML), a single s.c. 25 mg dose resulted to a reduction of apoptosis in three out of five evaluable patients and increase of proliferation in three out of five patients [38]. The drug was well-tolerated without any side effects. In six patients, the WBC count stabilized or decreased, but no patient achieved an objective response. In patients with myelodysplastic syndromes, the combination of Enbrel with thalidomide was well-tolerated, and produced significant hematologic improvement in 4 out of 18 patients who completed 16 weeks of therapy [39]. Five patients had stable disease and three had a major erythroid response. In a pilot study in patients with myelofibrosis with myeloid metaplasia, Enbrel relieved constitutional symptoms and was well-tolerated but no objective responses were documented [40]. In a Phase 2 study of Enbrel, in 26 patients with refractory myeloproliferative malignancies, the agent was very well-tolerated, but no patients had a clinically meaningful response to therapy [41].

In conclusion, Enbrel had an acceptable safety profile in patients with refractory MM. As a single agent it did not induce any remissions. This pilot study involved a small patient cohort and thus, its findings are not definitive. Longer-term follow-up of a larger patient cohort would be required to properly assess any relationship between the increased levels of plasma TNFα associated with Enbrel therapy and disease behavior in patients with MM.

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