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Pilot study of recombinant human soluble tumor necrosis factor receptor (TNFR:Fc) in patients with low risk myelodysplastic syndrome

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Abstract

In low risk myelodysplastic syndrome (MDS), increased apoptosis of marrow cells is a reproducible finding. Cytokines may drive this apoptosis. Several studies have demonstrated elevated levels of tumor necrosis factor-alpha (TNF-α) in MDS. Soluble tumor necrosis factor receptor (TNFR:Fc) can eliminate biologically active TNF in vivo. This data provided the rationale for a clinical trial of TNFR:Fc in low risk MDS. Eligibility was limited to cytopenic MDS patients with < 10% marrow blasts. Secondary MDS was an exclusion. The study design was to administer 25 mg TNFR:Fc twice a week for 10 weeks. Toxicity did not exceed grade 1. No responses were observed in the 10 treated patients and one had disease progression. At this dosing schedule, TNFR:Fc is unlikely to ameliorate cytopenias in low risk MDS.

Keywords: Myelodysplastic syndrome; Tumor necrosis factor; Cytogenetics; Acute non-lymphocytic leukemia

1. Introduction

Despite more that two decades of myelodysplastic syndrome (MDS) clinical trials, there is no FDA approved therapy. Recent advances in the understanding of the pathophysiology of MDS may provide a platform for rational drug development [1]. One important concept is that MDS can be divided into low (<10% marrow blasts) and high risk disease (>10% blasts) [2]. Some characteristics of low risk disease include lack of DNA hypermethylation, increased apoptosis and worse prognosis compared to patients with > 10% marrow blasts [2–4]. Clinical trials may be more productive if focused on these risk groups compared to French–American–British (FAB) cytologic groups. Other prognostic factors, such as cytogenetics, are also important.

Elevated serum levels of tumor necrosis factor receptor (TNF-α) in MDS have been reported by several investigators [5–8]. Increased TNF-α production by blood mononuclear cells was observed in several patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) but not RAEB or RAEB-t [9]. Furthermore, overexpression of TNF-α mRNA from marrow was detected in most cases of MDS but not in normal controls or AML patients [10]. One probable source of TNF-α is marrow macrophages which are increased in MDS patients [11]. The physiological significance of TNF-α in MDS may be appreciated by (1) enhanced formation of CFU-GM in vitro when anti-TNF-α was added to the MDS in vitro cultures (2) inverse correlation between serum TNF-α concentration and hemoglobin (3) inverse correlation between clinical response to erythropoietin and TNF-α levels and (4) positive correlation between TNF-α positive cells in the marrow (by immunohistochemistry) and apoptosis (by in situ end labeling of fragmented DNA) [6–8,12]. The therapeutic implication is that inhibition of TNF-α activity should improve blood counts. Pentoxifylline can inhibit TNF-α mRNA transcription. Combination therapy with pentoxifylline + ciprofloxacin was not effective in one study but a triple drug regimen of pentoxifylline + ciprofloxacin + dexamethasone produced hemopoietic responses in 35% (18/51) and 28% (5/18) of responders demonstrated a cytogenetic response [13,14].

These observations indicate that TNF-α may have an important role in the cytopenias of low risk MDS. The implication is that therapies which ameliorate the action of TNF may be effective in low risk MDS. As noted earlier, transcriptional inhibition of TNF-α secretion was effective in one study [14]. Another approach may be to directly

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remove TNF-α by administration of soluble TNFR:Fc. In vitro, incubation of MDS marrow with TNFR:Fc enhanced CFU-GM formation [8]. These two observations (TNF-α may be related to cytopenias and enhancement of CFU-GM by TNFR:Fc) formed the preclinical basis for this clinical trial of TNFR:Fc in low risk MDS.

2. Patients, materials and methods

2.1. Patients

Eligibility was limited to MDS patients with < 10% marrow blasts. In addition, patients were required to be red cell transfusion dependent as defined by a requirement for at least two red cell transfusions in the month prior to study initiation, absolute neutrophil count (ANC) < 1000/μl, or a platelet count < 50,000/μl. Exclusions included ECOG performance status > 2, myelosclerosis, therapy related MDS, any prior transplant, or administration of MDS disease modifying therapy in the 4 weeks prior to starting TNFR:Fc. All marrows were reviewed independently by a consulting pathologist and the principal investigator. Patients in this study provided written informed consent that had been approved by the Institutional Review Board of PRN Research, Inc.

2.2. Study drug

Recombinant human TNFR:Fc is a dimer of two molecules of the extracellular portion of the p75 TNF receptor each consisting of 235 amino acids. The two receptors are fused to the Fc portion of human IgG1 consisting of 232 amino acids. The gene fragments encoding the truncated TNFR and the Fc portion of human IgG1 are fused using an nucleotide expressed in a Chinese hamster ovary (CHO) expression vector. TNFR:Fc was supplied as a sterile lyophilized powder containing 25 mg TNFR:Fc; 40 mg mannitol, USP; 10 mg sucrose, NF; and 1.2 mg tromethamine (TRIS), USP per vial. TNFR:Fc was reconstituted with 1.0 ml bacteriostatic water for injection, USP (0.9% benzyl alcohol).

2.3. Treatment plan

The dose of TNFR:Fc was fixed at 25 mg twice weekly. The justification for this dose was that there was little experience with higher doses at the time of study initiation and 25 mg twice weekly was known to have superior biological effectiveness in blocking TNF activity compared to lower doses. In the absence of safety data at higher doses, 25 mg twice weekly was chosen for this study. TNFR:Fc was administered subcutaneously. The planned treatment duration of 10 weeks was based on the delay to detect a response with antithymocyte globulin was 65 days (median) and 3–9 months for cyclosporine [15,16]. Since, TNFR:Fc therapy could be considered a more specific type of immunosuppression compared to ATG or cyclosporine, it seemed reasonable that the duration of TNFR:Fc should be similar to the time required for a response with ATG or cyclosporine. TNFR:Fc would be discontinued for grade 3 or 4 toxicity or disease progression.

2.4. Definitions

2.4.1. For response

Disease progression was defined as the appearance ≥ 5% blasts on at least two occasions or a marrow with > 10% blasts. Toxicity was evaluated by NCI common toxicity criteria. IPSS scores were calculated retrospectively [2] (Table 1).

2.4.2. Study design

This was a phase II study that was limited to 10 evaluable patients.

Table 1
Response criteria

Response category	Patient category	Major response	Minor response
Red cell	Red cell transfusion dependent	Transfusion-independent throughout the study period or ≥ 2.0 g/dl rise in hemoglobin without transfusion	< 2.0 g/dl incremental rise in hemoglobin with a decrease in transfusion requirements of at least 50% compared to the mean 5-week pre-study period
	Non red cell transfusion dependent	> 2.0 g/dl elevation in hemoglobin sustained throughout the study period	≥ 1.0 g/dl elevation in hemoglobin sustained throughout the study period
Platelet	Platelet transfusion dependent	A sustained platelet count at or above the baseline value with a decrease in platelet transfusion requirements of at least 50%	
	Non platelet transfusion dependent	≥ 50% increase in platelet count and incremental net increase ≥ 20000/μl	≥ 50% increase in platelet count with net increase < 20000/μl
ANC		ANC < 1500/μl at screening: ANC increase exceeding 2X baseline, and absolute increase ≥ 500/μl	ANC increase > 2X baseline, but absolute increase < 500/μl

Table 2
Characteristics of the patients under study[a]

Patient number	Age	Sex	FAB[b]	Karyotype	IPSS[c]	Blood and marrow counts				Transfusion dependence	
						Hgb (g/dl)	ANC ($\times 10^3/\mu l$)	Platelets ($\times 10^6/\mu l$)	Marrow blasts (%)	RBC	Platelet
1	79	M	RA	46, XY, del(5) (q1?5q3?3), r(7) (p?22q?27) [19]/47, XY, idem, +8[2]	Int-2	7.3	3354	45	0	✓	
2	76	F	RARS	46, XX, t(2;3) (p23;q21) [25/25]	Int-1	11.1	4535	335	6.0	✓	
3	36	F	RA	46, XX	Int-1	6.4	1116	290	0	✓	
4	76	M	RA	46, XY, del(20) (q11.2q13.3) [4]/46, XY [3]	Int-1	8.9	836	64	4.0	✓	
5	82	F	RARS	46, XX	Low	9.2	1798	289	2.0	✓	
6	73	M	RA	46, XY, del(20) (q11.2q13.3) [5]/46, XY [2]	Int-1	10.1	936	31	0.5		
7	62	F	RA	46, XX, add(17) (q25) [15]/46, XX, del(12) (p12)[2]/46, XX [3]	Int-1	10.6	286	258	2.0	✓	
8	61	M	RAEB	46, XY	Int-1	13.9	1836	21	8.5		
9	58	M	RA	46, XY	Low	8.4	2144	173	2.5	✓	
10	84	M	RA	46,XY	Int-1	11.0	1058	38	1.0	✓	

[a] Hgb: hemoglobin; Retic: reticulocyte count; ANC: absolute neutrophil count; RBC: red blood cells; M: male; F: female. Low: low risk; Int: intermediate.
[b] FAB: French–American–British classification [17]; RA: refractory anemia; RARS: refractory anemia with ringed sideroblasts.
[c] Classified according to international prognostic score as described by Greenberg et al. [2].

3. Results

3.1. Patient characteristics

The median age of the 10 patients was 74.5 years (range 36–84) (Table 2). There were six males and four females. According to the IPSS, two patients were classified as low risk, six intermediate-1, and two intermediate-2. Five patients had cytogenetic abnormalities.

3.2. Compliance

Nine patients received all planned 20 doses of TNFR:Fc. TNFR:Fc was discontinued after nine doses in patient 8 due to leukemic progression.

3.3. Toxicity

There were no injection site reactions. Three patients had grade 1 fatigue and two of these also had grade 1 arthralgia/myalgia. There were no grade 2–4 toxicities.

3.4. Response

There were no red cell, platelet or ANC responses. Patient 8 progressed to AML as noted by 23% blood blasts.

4. Discussion

The lack of hemopoietic response to TNFR:Fc suggests that TNF alone is not responsible for cytopenias in MDS. Other apoptotic cytokines, including Fas ligand, IL-1β, interferon-γ and TGF-β, are increased in MDS and may contribute to persistent cytopenias during TNFR:Fc therapy. Our clinical results are consistent with in vitro studies which demonstrated that inhibition of the increased caspase-3 activity in low risk MDS did not increase colony formation.

A potential consideration for treatment failure is that the dose of TNFR:Fc in this study did not eliminate TNF bioactivity. TNF bioactivity was not assayed in this trial. However, at TNFR:Fc doses approximately half of the dose administered in this protocol, TNF bioactivity was eliminated in humans given OKT3 or endotoxin [18,19]. It is also possible that the small sample size of this study precluded detection of a low response rate. Responses to TNFR:Fc have been reported in two other MDS pilot trials. Partial responses in hemoglobin, platelet counts or ANC were observed in 10/18 evaluable patients [20]. Responses correlated with increased marrow cellularity and normal cytogenetics. In another trial, 6/14 MDS patients had “moderate” improvements in hemoglobin, platelet counts, or neutrophil counts [21]. The heterogeneity of MDS patients may explain the responses observed in these other series. For example, the median age of patients in these two other series was about a decade younger than in this trial.

There was a low incidence of adverse events. The most common toxicities were grade 1 arthralgia and myalgia. Interestingly, rheumatoid arthritis patients treated with TNFR:Fc often have resolution of arthalgia [22]. The frequency of disease progression (1/10) is comparable to other MDS trials. Thus, TNFR:Fc does not appear to accelerate MDS progression.

The lack of response to TNFR:Fc provides insights to the activity of effective agents for MDS. For example, transfusion dependence is eliminated in some MDS patients treated with thalidomide [23]. One of the proposed therapeutic activities of thalidomide is TNF-α inhibition. Absence of response to TNFR:Fc in MDS suggests that thalidomide functions through non TNF-α mechanisms.

In summary, TNFR:Fc was well tolerated in MDS patients. Single agent TNFR:Fc has a low likelihood of reversing cytopenias in low risk MDS patients.

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