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## A low serum level of soluble tumor necrosis factor receptor p55 predicts response to thalidomide in advanced multiple myeloma

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A B S T R A C T

**Background and Objectives.** Thalidomide modulates the production of tumor necrosis factor (TNF- $\alpha$ ). Soluble TNF receptors, TNFR p55 and TNFR p75, modify TNF- $\alpha$  activity. In this study, we explored the relation between soluble TNF receptors and outcome in patients with advanced multiple myeloma treated with thalidomide.

**Design and Methods.** The levels of soluble TNF receptor p55 and p75 were assessed in serum from 34 myeloma patients with relapsed or refractory disease before starting thalidomide treatment. Serial measurements were performed for 16 patients in serum collected during treatment.

**Results.** The pre-treatment serum level of soluble TNFR p55 in thalidomide responders was significantly lower than that in non-responders (median 1.75 ng/mL (range 1.19–2.84) vs. 2.79 ng/mL (1.36–5.51),  $p=0.004$ ). The levels of p55 declined significantly during treatment. The levels of p75 showed the same pattern as p55, but the differences were not significant. The median survival of myeloma patients with pre-treatment levels of p55 < 2.79 ng/mL was 404 days; the median survival of patients with pre-treatment levels  $\geq 2.79$  ng/mL was shorter (65 days, log-rank test  $p=0.02$ ).

**Interpretation and Conclusions.** We conclude that soluble TNFR p55 is an adverse prognostic factor in myeloma patients with relapsed or refractory disease treated with thalidomide. Patients with a low pre-treatment level of this receptor have a better response rate and a longer overall survival.

**Key words:** advanced myeloma, thalidomide, soluble TNFR.

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Thalidomide was introduced as a treatment for multiple myeloma in 1999.<sup>1</sup> Clinical trials have reported an effect in 25–35% of the patients with relapsed or refractory disease. The mechanism of action of this drug is not completely understood.

Thalidomide has immunomodulatory properties, and inhibits the production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in lipopolysaccharide-stimulated monocytes.<sup>2,3</sup> More recent studies show that thalidomide enhances TNF- $\alpha$  production in activated T-cells.<sup>4</sup> Several authors have suggested that TNF- $\alpha$  has an important role in the pathogenesis of multiple myeloma, and TNF- $\alpha$  is reported to be elevated in myeloma patients.<sup>5</sup> TNF- $\alpha$  exerts its effects by binding to two different receptors, tumor necrosis factor receptor (TNFR) p55 (CD120a) and p75 (CD120b).<sup>6</sup> Soluble TNF- $\alpha$  receptors are derived from proteolytic cleavage of cell sur-

face receptors, and modify the activity of TNF- $\alpha$ .<sup>7–10</sup> Infusion of TNF- $\alpha$  leads to an increase in the level of circulating soluble TNF receptors.<sup>11</sup> These observations prompted us to explore the relation between the level of soluble TNF receptors and outcome in patients with advanced multiple myeloma treated with thalidomide.

### Design and Methods

#### Patients and serum samples

Of a total of 65 patients from 30 different hospitals who were included in the Nordic Myeloma Study Group (NMSG) trial #10/99 (Waage *et al.* in press), 34 patients (20 men, 14 women) had serum samples collected by the time of analysis, and these patients constituted our study population. Inclusion criteria were: 1) patients with primary or sec-

ondary refractiveness to treatment with melphalan and prednisone, or who were refractory to other types of chemotherapy, 2) patients who relapsed within 6 months after high-dose therapy with peripheral blood stem cell support. Thalidomide was given as a single agent at a dose of 100 mg × 2 which was escalated to 800 mg × 2 over 7 weeks. The patients' characteristics were registered before starting thalidomide treatment, and laboratory values and disease status were also recorded after 1, 3, 5, 7, 12, 16, 20 and 24 weeks of treatment, and after 1 and 2 years. Patients gave written consent to participate in this study, which was approved by official Medical Authorities and Ethical Committees in each country.

The serum samples were collected at initiation of treatment (30 patients) or after one week (4 patients). In addition, serial samples from 16 of these patients (10 men, 6 women) collected at initiation of treatment or after 1 week (n = 16), after 3 weeks (n = 15), after 12-16 weeks (n = 15) and after 20-24 weeks (n = 11), were analyzed. The patients' characteristics are shown in Table 1. These characteristics were not significantly different from the original population of 65 patients. All patients with available serum samples at the time of analysis were included in the study. Patients were divided into two groups according to response to thalidomide after 12 weeks of treatment. Response was defined as a >25% decrease in monoclonal immunoglobulin concentration (15 patients), whereas no response was defined as a ≤25% decrease or an increase (17 patients). This definition of response differs from the definition used in the original study by Waage *et al.*, which also includes clinical response parameters. For 2 patients information about response was missing. The original study of 65 patients demonstrated that all patients with an immunoglobulin response had responded within 12 weeks of treatment.

#### **Immunoassay for detection of soluble tumor necrosis factor receptors (sTNFR) in serum**

An enzyme-linked immunosorbent assay was used to detect soluble TNFR p55 and p75 as described elsewhere.<sup>12,13</sup> The immunoassays were based on monoclonal antibodies recognizing a non-TNF-binding site of the TNF receptor for capture, and bound TNF receptors were detected with DIG-labeled TNF-α. These assays detected free as well as reversibly ligand-bound soluble TNF receptors. The detection limit of both assays was 0.3 ng/mL. Intra-assay coefficients of variation were 8.6% for the p55 assays and 24.2% for the p75 assays measuring TNF receptor levels in pre-treatment serum.

#### **Statistical analyses**

All statistical analyses were done with the SPSS computer program, version 11.0.1. (SPSS, Chicago, IL, USA).

**Table 1. Patients' characteristics at time of inclusion.**

<i>Characteristic</i>	<i>Study population (n = 34)</i>
Male/ female (No. of pts.)	20 / 14
Age, years (median, range)	66 (31-78)
Stage according to Durie and Salmon (N. of pts. )	
I	2
II	3
III	29
Monoclonal immunoglobulin (No. of pts. )	
IgG	24
IgA	5
Light chain	5
Previous treatment (No. of pts. )	
PBM <sup>1</sup>	12
Other <sup>2</sup>	22
Periods of treatment	
1-2	9
3 or more	25
Dosage of thalidomide (% of expected dose, median, range)	61 (0-100)
s-Creatinine, μmol/L (median, range)	89.5 (59-231)
Creatinine clearance mL/min (median, range) <sup>3</sup>	62.7 (27.7-108.2)
C-reactive protein, mg/L (median, range)	7.5 (0-187)
β2 microglobulin, mg/L (median, range)	4.45 (1.2-30)
Median survival, days	369

<sup>1</sup>Patients receiving high dose therapy with peripheral blood stem cell support.

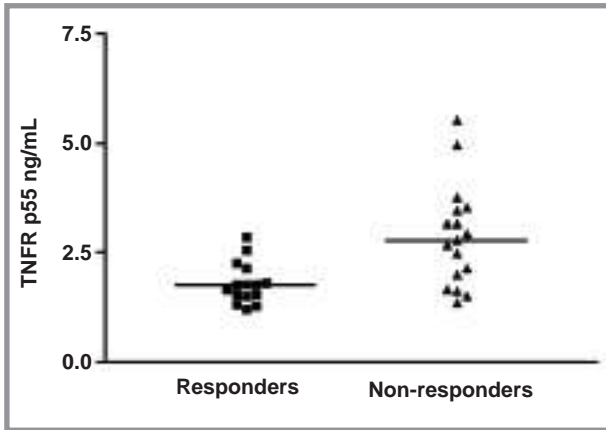
<sup>2</sup>Patients receiving melphalan and prednisone or other types of chemotherapy.

<sup>3</sup>Creatinine clearance was calculated by the formula:

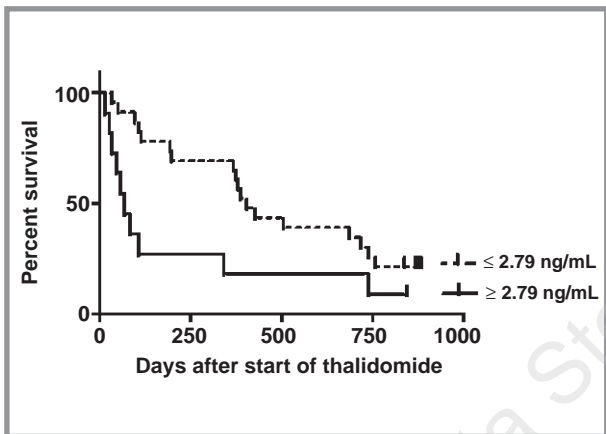
Creatinine clearance:  $(k \times 1.23 \times (140 - \text{age in years}) \times \text{lean bodyweight in kg}) / \text{s-creatinine}$  (the value of k is 1 for men and 0.85 for women).

Results were considered statistically significant when  $p \leq 0.05$ . Comparisons between groups were made using the Mann-Whitney U test. Serial measurements were evaluated using the method of summary measures.<sup>14</sup> Individual slopes were estimated by linear regression coefficients.

To evaluate whether the mean slope was different from zero, we used a one-sample t test. Correlations were estimated by Spearman's rank correlation analysis. The median follow-up duration was estimated according to Korn's method.<sup>15</sup> The Kaplan Meier method was used to compute the survival curves and to estimate median survival. A log-rank test was used to compare the survival curves. We used binary logistic regres-



**Figure 1.** Pre-treatment serum levels of soluble TNFR p55. Median level (range) of p55 was 1.75 ng/mL (1.19-2.84) in thalidomide responders and 2.79 ng/mL (1.36-5.51) in non-responders ( $p = 0.004$ ).



**Figure 2.** Kaplan-Meier survival plots for patients with advanced MM. Median overall survival for patients with serum levels of soluble TNFR p55 < 2.79 ng/mL ( $n = 23$ ) was 404 days, whereas for patients with serum levels  $\geq 2.79$  ng/mL ( $n = 11$ ) it was 65 days (log-rank test,  $p = 0.02$ ).

sion analysis to evaluate the value of TNF receptors as predictors of response.

The receptor concentrations were categorized into tertiles for univariate analysis. For multivariate analysis, factors were analyzed as continuous variables. Because of the limited number of patients in our study, only two variables were entered simultaneously in the multivariate model.

## Results

### Serum levels of soluble TNF receptors

Responders to thalidomide had a significantly lower pre-treatment level of soluble p55 (median 1.75 ng/mL

**Table 2.** Univariate binary logistic regression analysis of the pre-treatment level of TNFR p55: TNFR p55 is a predictor of response to thalidomide.

Tertiles of TNFR p55 (ng/mL)	No.	N. of responders	Estimated odds ratio	95% CI	p value
1.19-1.66	11	7	1.0	Ref.	
1.75-2.67	11	7	1.0	0.18-5.7	1.0
2.79-5.51	10	1	0.06	0.006-0.70	0.03

Treating TNFR p55 as a continuous variable, the estimated odds ratio of response was 0.17 ( $p = 0.01$ ).

(range 1.19-2.84) than did non-responders (2.79 ng/mL (1.36-5.51),  $p = 0.004$ , Figure 1). The same tendency was observed for the level of soluble p75 as the median level in responders was 3.30 ng/mL (2.39-7.09) and in non-responders 4.25 ng/mL (2.02-9.70), although this difference was not statistically significant ( $p = 0.28$ , data not shown).

There was a strong positive correlation between the pre-treatment levels of the two receptors in serum ( $r = 0.71$ ,  $p < 0.001$ ).

In 16 patients, serial samples collected at initiation of treatment or after 1 week, and after 3,12-16 and 20-24 weeks, were analyzed. We observed a significant decline in the level of soluble TNFR p55 during this period (mean slope of  $-0.014$  ng/mL/week,  $p = 0.004$ ). The decline in the level of soluble p75 during the same period was not significant (mean slope of  $-0.023$  ng/mL/week,  $p = 0.13$ , data not shown).

### Soluble TNF receptors as predictors of response

To estimate the value of soluble TNF receptors as predictors of response to thalidomide, the receptor levels were categorized into tertiles. In a univariate binary logistic regression model, the estimated odds ratio of response was 0.06 for patients with serum levels of soluble p55  $\geq 2.79$  ng/mL (upper tertile) relative to patients with serum levels  $\leq 1.66$  ng/mL (lower tertile,  $p = 0.03$ , Table 2). The estimated odds ratio of response for patients with serum levels of soluble p75  $\geq 4.61$  ng/mL (upper tertile) was 0.48 relative to patients with serum levels  $\leq 3.24$  ng/mL (lower tertile,  $p = 0.4$ ).

Soluble TNF receptor levels were further analyzed as continuous variables. Inclusion of age, C-reactive protein or  $\beta 2$  microglobulin, recorded before the start of treatment, in a multivariate model did not change the estimate of p55 as a predictor of response.

In the univariate model, serum (s)-creatinine concentration ( $\mu\text{mol/L}$ ) was a significant predictor of response with an estimated odds ratio of 0.95 ( $p = 0.03$ ). To further investigate the effect of renal function on response, creatinine clearance (mL/min) was calculated and analyzed in the univariate model. Creatinine

clearance had no effect on response in this model (estimated odds ratio 1.03,  $p = 0.17$ , *data not shown*).

### Overall survival

To investigate the effect of soluble receptors on overall survival, the patients were divided into groups with high (serum levels  $\geq 2.79$  ng/mL [upper tertile]) and low ( $< 2.79$  ng/mL) pre-treatment levels. The median survival was 404 days for patients with low levels of soluble p55 ( $n = 23$ ) whereas it was only 65 days for patients with high levels ( $n = 11$ ), as shown in Figure 2 (log-rank test  $p = 0.02$ ). We observed the same tendency of longer survival among patients with low pre-treatment levels of soluble p75; patients with low levels ( $< 4.61$  ng/mL) had a median survival of 380 days ( $n=23$ ), and patients with high levels had a median survival of 108 days ( $n=11$ , log-rank test  $p=0.45$ , *data not shown*). Median follow-up was 2 years and 4 months.

## Discussion

In this study we observed that pre-treatment levels of soluble TNFR p55 were significantly lower in patients responding to thalidomide than they were in non-responding patients. Furthermore, the level of soluble p55 declined significantly during the first 5–6 months of treatment. This observation indicates that a reduction of tumor burden also leads to a reduction in the level of this receptor. Soluble TNFR p75 showed the same tendency as p55, although the differences were not statistically significant. This is the first study describing soluble TNF receptors in myeloma patients treated with thalidomide, and our findings suggest a possible role of these receptors on the effect of this drug.

TNF- $\alpha$  activates NF $\kappa$ B and promotes proliferation of myeloma cells.<sup>16</sup> Thalidomide has been shown to inhibit NF $\kappa$ B activation.<sup>17</sup> As TNF- $\alpha$  is captured by its soluble receptor and prevented from stimulating NF $\kappa$ B, an important target for thalidomide may be inactive in patients with high levels of p55.

Soluble TNFR p55 is reported to be a more potent inhibitor of TNF- $\alpha$  than p75.<sup>18</sup> This fact may explain why we observed a significant difference in response and survival between patients with high and low pre-treatment levels of p55, and not p75. However, the number of patients in our study was rather small ( $n=34$ ), and the intra-assay variation coefficient for the p75 assay was as high as 24.2 %. It is, therefore, possible that real differences were not revealed, and that the biological significance of soluble p75 is similar to that of p55.

We also observed that pre-treatment levels of s-creatinine were predictive of response by univariate analysis in our study. Hence it could be speculated that elevated levels of soluble TNF receptors were merely a consequence of impaired renal function.<sup>19</sup> When s-creatinine and soluble TNFR p55 were analyzed as continuous variables in a multivariate model, only soluble TNFR p55 remained as a significant predictor of response. Furthermore, creatinine clearance, which is a more accurate estimate of renal function than s-creatinine, was not predictive of response. We, therefore, conclude that impaired renal function alone cannot explain our observations. This is in accordance with the observations of Iglesias *et al.*, who recently reported that elevated levels of soluble TNF receptors in patients with septic shock were not explained only by decreased renal clearance.<sup>20</sup>

Our study population consisted of patients with advanced, refractory multiple myeloma. We do not know whether soluble TNFR p55 is also predictive of response to thalidomide in newly diagnosed patients, or whether an elevated level of these receptors reflects a general drug resistance. Previous studies have reported an association between high levels of soluble TNFR p55 in sera and an adverse outcome of patients with other hematologic malignancies.<sup>21–23</sup>

Neben *et al.* described that a high pre-treatment level of TNF- $\alpha$  was associated with better outcome after thalidomide therapy, whereas Thompson *et al.* observed that high levels of TNF- $\alpha$  prior to thalidomide treatment adversely affected progression-free survival.<sup>24,25</sup> These results are difficult to interpret not knowing the levels of soluble TNF receptors, since they have an impact on the function of TNF- $\alpha$ .

In conclusion, we found that soluble TNFR p55 concentration is a new adverse prognostic factor in patients with advanced multiple myeloma treated with thalidomide. Our observations may contribute to a better understanding of the mechanisms by which thalidomide acts.

*AW, PG, GJ and IT were responsible for the conception and design of Nordic Myeloma Study Group (NMSG) trial #10/99. AW, ATB, LHR, PR, MB and AS contributed to the design of this study and to the analysis of serum and data interpretation. All authors contributed to the final version of the text. ATB was primarily responsible for data analysis, interpretation and manuscript writing, LHR performed the serum analyses, PR provided statistical assistance (multivariate- and survival analyses), and AW had a major role in supervising and final approval of the manuscript. Primary responsibility for Figures and Tables 1–2: A–TB.*

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