High Plasma Basic Fibroblast Growth Factor Concentration Is Associated with Response to Thalidomide in Progressive Multiple Myeloma

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ABSTRACT

The aim of this study was to define prognostic factors that might be predictive for response to thalidomide (Thal) in progressive multiple myeloma (n = 54). We examined the concentration of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), two potent heparin-binding mediators of angiogenesis in peripheral blood (PB; PB-VEGF and PB-bFGF) and bone marrow (BM; BM-VEGF and BM-bFGF), in combination with well-characterized predictors for response and survival to chemotherapy. After a median follow-up time of 15 months (range, 0.3–20), 29 patients (pts.) showed at least a minimal response to Thal therapy, whereas 25 pts. were nonresponsive. As shown by univariate analysis, responsive pts. had statistically significant higher concentrations of PB-bFGF (P = 0.009) and β2-microglobulin (P = 0.03) before therapy, as well as lower hemoglobin (P = 0.008) and albumin (P = 0.02) levels, whereas no statistically significant difference was found for PB-VEGF (P = 0.93). When a multiple logistic regression analysis was performed, PB-bFGF was the only statistically significant predictor for response to therapy (P = 0.01). None of these variables was associated with a prolonged progression-free survival. In conclusion, our findings indicate that high pretreatment plasma bFGF levels in pts. with progressive multiple myeloma are associated with unfavorable parameters of response and survival but nevertheless predict for response to Thal therapy.

INTRODUCTION

Angiogenesis plays a central role in embryonic development, wound healing, and reproduction (1). This highly regulated process is normally activated for short periods only and then completely inhibited through a cascade of different mechanisms (2). Induction of angiogenesis is an important feature of malignancy and plays a role in progression of tumor growth, invasion, and metastasis. Within the last few years, the knowledge about angiogenesis in hematological diseases has expanded. In BM biopsy of pts. with acute lymphoblastic leukemia (3), B-cell NHL (4), acute myeloid leukemia (5), and MM (6), an increase in blood vessel density was found. In MM, BM angiogenesis is increased in active myeloma compared with monoclonal gammopathy of undetermined significance or smouldering myeloma (6). It correlates positively with a high plasma cell-labeling index, suggesting that proliferative capacity of neoplastic plasma cells is related to angiogenesis.

Thal is a drug that was originally marketed as a sedative between 1956 and 1961. Interest in this drug has revived because of its anti-inflammatory (7), immunomodulatory (8–10), and antiangiogenic activity (11). Clinical trials have shown that Thal is effective in the treatment of a variety of diseases including erythema nodosum leprosum (12) and oral aphthous ulcers in HIV-positive pts. (13). In addition, Thal has been used successfully in the treatment of malignancies like AIDS-related Kaposi sarcoma (14), high-grade glioma (15), and MM (16).

Tumor angiogenesis is influenced by positive and negative regulatory molecules. bFGF and VEGF are two potent heparin-binding mediators of angiogenesis with a synergistic effect in vitro and in vivo (17, 18). Both angiogenic factors are involved in an autocrine endothelial cell mitogenic loop (19, 20). Thal has been reported to be capable of inhibiting the formation of new blood vessels from sprouts of preexisting vessels in a rabbit model in which corneal neovascularization was induced with the angiogenic protein bFGF (11). In a mouse model, Thal and related analogues also inhibited angiogenesis in the cornea induced by VEGF as well as bFGF (21).

For a better understanding of the role of bFGF and VEGF in MM, we measured both angiogenic cytokines from PB and BM before Thal and compared these data to a group of healthy volunteers. In addition, VEGF and bFGF levels were measured after 3 and 6 months of treatment to explore whether a clinical response to Thal might be associated with a decline in angiogenic cytokine levels. Furthermore, bFGF and VEGF levels were analyzed in combination with well-characterized predictors of response and survival in MM like β2-microglobulin (22), C-reactive protein (CRP), leucocyte (Hb), progressive disease (PD), and C-reactive protein (CRP).

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2 The abbreviations used are: bFGF, basic fibroblast growth factor; Thal, thalidomide; VEGF, vascular endothelial growth factor; PB, peripheral blood; BM, bone marrow; pts., patients; MM, multiple myeloma; CRP, C-reactive protein; Hb, hemoglobin; PD, progressive disease; PFS, progression-free survival; CI, confidence interval; HDT, high-dose chemotherapy; PBSCT, peripheral blood stem cell transplantation; NHL, non-Hodgkin’s lymphoma.
CRP (22), albumin (23), and Hb (24) to define factors that might predict for response before Thal treatment.

PATIENTS AND METHODS

Treatment Design. From December 1998 to March 2000, 54 pts. with progressive MM were enrolled in a clinical Phase II trial and treated with Thal. The study has been approved by the ethical guidelines of the Joint Committee of Clinical Investigation of the University of Heidelberg. All pts. had to sign an informed consent indicating the potential benefit and toxicities associated with the treatment. Thal was supplied in 100-mg tablets by Gruenenthal GmbH (Aachen, Germany). At the start of Thal treatment, the drug was administered nightly at a dose of 100 mg daily after a weekly dose increase of 100 mg daily for a final dose of 400 mg daily beginning at day 22.

Laboratory and Clinical Evaluation. The pretreatment and monthly follow-up evaluations included: full blood counts; renal and liver function tests; serum levels of immunoglobulins, β2-microglobulin, lactate dehydrogenase, C-reactive protein, and Bence Jones protein in urine; and serum and urine protein electrophoresis. In addition, VEGF and bFGF were measured every 3 months from PB (PB-VEGF and PB-bFGF). BM aspirations were performed in most of the pts. before and 3 and 6 months after the start of Thal to determine the percentage of plasma cells in the BM and to measure VEGF and bFGF levels (BM-VEGF and BM-bFGF). X-rays of the skull, thorax, spine, pelvis, humera, and femura were obtained before and every 6 months during treatment to assess the number and size of bone lesions.

For VEGF and bFGF measurements, plasma from PB and BM was obtained on months 0, 3, and 6 after the start of Thal. Aliquots were frozen at −80°C. The laboratory assays for VEGF and bFGF were performed by quantitative sandwich enzyme immunoassay (ELISA; R 38 D Systems, Minneapolis, MN) according to the manufacturer’s instructions. For quality control, the samples of each patient and volunteer were measured twice.

Assessment of Response. All of the pts. were evaluated for response at least monthly. The criteria for response included the decline in the level of monoclonal protein in serum or urine of ≥25, 50, or 90% on at least two occasions apart. In case pts. had detectable levels of monoclonal protein in urine and serum, the response was evaluated on the component showing the smaller decline. Pts. with a reduction of <25% and those who were withdrawn from the study before a response could be evaluated were considered to have had no response to Thal and accounted as “no change.” New lytic lesions (but not compression fractures), hypercalcemia, an increase in monoclonal protein of >25% from nadir, or other new evidence of disease constituted PD. In addition, the response to Thal was evaluated by changes in the percentage of plasma cells in the BM aspirate measured every third month. Additionally, Hb levels were compared monthly with baseline values before Thal.

The disease status was assessed every 3 months using the criteria of the European Bone Marrow Transplantation Group (25). All pts. who discontinued treatment before a response could be assessed were considered to have no response to Thal. Disease progression and death from any cause were the only events that accounted for PFS.

Assessment of Adverse Effects. All of the pts. who received Thal for ≥1 month were included in the evaluation of side effects. Pts. received diaries, including questions to somnolence, constipation, tremor, fatigue, dizziness, nausea, fever, infections, dryness of mouth, rash, headache, mood changes, tingling or numbness, and incoordination. Adverse effects were evaluated from diaries of the pts. and verified in the presence of each patient by a direct interview. In addition, hematological values and other laboratory data were evaluated on every visit. The system of classification of the WHO was used.

Statistical Analysis. Comparisons between cytokine levels in the PB and BM of volunteers and myeloma pts. were performed by the Mann-Whitney U test. Pairwise correlations between parameters before treatment were estimated using Spearman’s rank correlation coefficient. Binary logistic regression was used to identify possibly relevant prognostic factors for response to therapy. Changes in clinical parameters and cytokine levels within the first 6 months of treatment were evaluated by using Friedman’s rank sum test. Individual slope estimates were used as summary measures to correlate the changes of cytokine levels over time with response to Thal therapy (26). The median follow-up duration was estimated according to the method of Korn (27). Survival probabilities were estimated by the method described by Kaplan and Meier. Predictors used were bFGF and VEGF from PB together with β2-microglobulin, albumin, CRP, and Hb. The proportional hazards regression model as proposed by Cox was used for the analysis of the survival time data. Primary end points are response to Thal therapy and PFS of the pts. An effect was considered as statistically significant if the P of its corresponding test statistic was ≤5% (P ≤ 0.05). To provide quantitative information of the relevance of statistically significant results, 95% CIs for correlation coefficients, odds ratios, and hazard ratios were also computed. The statistical analyses were performed using the software packages StatXact (Cytel Software Corp, Cambridge, MA) and S-Plus (MathSoft, Inc., Seattle, WA) together with the Design software library.

RESULTS

Patient Characteristics. According to the classification of Salmon and Durie, 1 patient had stage I, 6 pts. had stage II, and 47 pts. had stage III MM. There were 37 males and 17 females with a median age of 57 years (range, 34–79). The median time from diagnosis to entry into the study was 34 months (range, 1–163). Of all 54 of the pts., 49 received chemotherapy before Thal, and there was a median number of 6 (range, 3–30) chemotherapy cycles, including at least 1 cycle of HDT and PBSCT in 39 pts. In addition, 5 pts. with stage I or II disease received Thal as first-line treatment. Before Thal, all of the pts. had PD according to the European Bone Marrow Transplantation Group criteria. The median interval from the last chemotherapy to the start of Thal was 11 months (range, 3–65). In particular the last treatment before Thal was as follows: 5 pts. had progressive MM without previous treatment, 16 pts. were treated with conventional chemotherapy, 15 pts. received the first cycle, 17 pts. received the second cycle, and 1 patient...
showing a decline in monoclonal protein-type PBSCT achieved a complete response for a period of 10 months, who had a relapse 8 months after the first cycle of HDT and Thal, respectively (Fig. 1). One patient with a Bence Jones MM minimal response after 3, 6, 9, and 12 months after the start of 18 of 23 (78%), and 12 of 15 (80%) pts. showed at least a 6-month PFS for the whole group of 54 pts. after initiating treatment with Thal was 73% (95% CI; range, 62–86%). Thus far, 4 pts. died, 2 were nonresponsive to Thal, and 2 died in PD after initial response.

To define prognostic factors that predict for response to Thal, we compared patient characteristics between pts. showing at least a minimal response to Thal (n = 29; criteria of the European Bone Marrow Transplantation Group) and pts. who were nonresponsive (n = 25). As shown by univariate analysis in Table 1, responsive pts. had statistically significant higher concentrations of PB-bFGF (P = 0.009) and β2-microglobulin.

received the third cycle of HDT and PBSCT. Cytogenetical data of the pts., such as chromosome 13 abnormalities, were not available. The patient characteristics are summarized in Table 1.

### Table 1 Patient characteristics

Responsive pts. showed at least a minimal response to Thal1, while all of the other pts. were considered to be nonresponsive (criteria of the European Bone Marrow Transplantation Group).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All pts. (n = 54)</th>
<th>Responders (n = 29)</th>
<th>Nonresponders (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of pts. (%)</td>
<td>No. of pts.</td>
<td>No. of pts.</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>37 (69%)</td>
<td>20</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Stage III according to Salmon and Durie</td>
<td>47 (87%)</td>
<td>26</td>
<td>21</td>
<td>0.69</td>
</tr>
<tr>
<td>IgG monoclonal protein</td>
<td>24 (44%)</td>
<td>15</td>
<td>8</td>
<td>0.11</td>
</tr>
<tr>
<td>IgA monoclonal protein</td>
<td>19 (35%)</td>
<td>7</td>
<td>12</td>
<td>0.09</td>
</tr>
<tr>
<td>k light chain</td>
<td>38 (70%)</td>
<td>24</td>
<td>14</td>
<td>0.04</td>
</tr>
<tr>
<td>Prior high-dose chemotherapy</td>
<td>39 (72%)</td>
<td>21</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Prior total-body irradiation</td>
<td>9 (17%)</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>57 (34–79)b</td>
<td>57c</td>
<td>56c</td>
<td>0.70</td>
</tr>
<tr>
<td>No. of pretreatment chemotherapy cycles</td>
<td>6 (0–30)b</td>
<td>7c</td>
<td>6c</td>
<td>0.05</td>
</tr>
<tr>
<td>Hb (12–17 g/dl)c</td>
<td>11.5 (8–15)b</td>
<td>10.9c</td>
<td>11.9c</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum albumin (30–50 g/liter)c</td>
<td>40 (30–59)b</td>
<td>38c</td>
<td>41c</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum calcium (2.1–2.65 mm)c</td>
<td>2.39 (2.18–2.63)b</td>
<td>2.39e</td>
<td>2.42e</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum β2-microglobulin (≤2.5 mg/l)c</td>
<td>2.8 (1.3–10.4)b</td>
<td>3.5c</td>
<td>2.5c</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum CRP (≤5 mg/l)c</td>
<td>2 (&lt;2–101)b</td>
<td>3.7c</td>
<td>≤2c</td>
<td>0.86</td>
</tr>
<tr>
<td>PB-bFGF (pg/ml)c</td>
<td>55.2 (1–226.2)b</td>
<td>67.7c</td>
<td>50.2c</td>
<td>0.009</td>
</tr>
<tr>
<td>PB-VEGF (pg/ml)c</td>
<td>196.6 (12.5–750.6)b</td>
<td>202.8c</td>
<td>176.2c</td>
<td>0.93</td>
</tr>
<tr>
<td>BM plasmocytosis (%)c</td>
<td>25 (&lt;5–95) [n = 43]</td>
<td>30 [n = 21]c</td>
<td>15–20 [n = 22]c</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Characteristic (normal range).
* Median (range).
* Median.

As shown in Fig. 2, the estimated 6-month PFS for the whole group of 54 pts. after initiating treatment with Thal was 73% (95% CI; range, 62–86%). Thus far, 4 pts. died, 2 were nonresponsive to Thal, and 2 died in PD after initial response.

### Clinical Response

The median follow-up time was 15 months (range, 0.3–20). Using the criteria of the European Bone Marrow Transplantation Group, 20 of 47 (43%), 24 of 37 (65%), 18 of 23 (78%), and 12 of 15 (80%) pts. showed at least a minimal response after 3, 6, 9, and 12 months after the start of Thal, respectively (Fig. 1). One patient with a Bence Jones MM who had a relapse 8 months after the first cycle of HDT and PBSCT achieved a complete response for a period of 10 months, showing a decline in monoclonal protein-type k from 3048 mg/24 h to a negative immunofixation within 4 weeks after the start of Thal.

The maximum decline of monoclonal protein is illustrated in Table 2. The serum or urine levels were reduced by ≥90% in 5 pts. (including 1 complete remission), ≥50% in 14 pts., and ≥25% in 31 pts., for a total rate of response of 57%. Within the first 6 months of treatment, the content of plasma cells in the BM decreased from a median value of 25% down to 15% (n = 18; P = 0.003), whereas the median Hb level increased from 11.5 g/dl up to 13.1 g/dl (n = 25; P < 0.001). There were 2 pts. who had a transfusion-dependent anemia before Thal and became transfusion-independent after 2 and 3 months of treatment, respectively.

### Survival Analysis

As shown in Fig. 2, the estimated 6-month PFS for the whole group of 54 pts. after initiating treatment with Thal was 73% (95% CI; range, 62–86%). Thus far, 4 pts. died, 2 were nonresponsive to Thal, and 2 died in PD after initial response.

To compare cytokine levels from pts. with controls, plasma samples from PB and BM were obtained from 22 healthy volunteers. All of the volunteers had neither a known malignant disorder nor an acute infection. In particular there were 13 males and 9 females with a median age of 56 years (range, 21–69).

**Fig. 1** Response to Thal treatment using the criteria of the European Bone Marrow Transplantation Group.
Thalidomide and bFGF in Multiple Myeloma

Toxicity Evaluation. The following mild or moderate adverse effects (grade 1 and 2 toxicity) were observed most frequently: somnolence (48%), constipation (44%), dryness of mouth (36%), tingling or numbness (34%), fatigue or weakness (28%), tremor (26%), infection (26%), dizziness (22%), rash (18%), and mood changes (16%). In addition, there were eight grade 3 toxicities including cardiovascular complications (n = 3), polyneuropathy (n = 3), and infections (n = 2). In one case, a grade 4 toxicity was observed; a 74-year-old woman had an acute myocardial infarction leading to a Thal break for 6 weeks. Grade 3 polyneuropathy lead to a withdrawal from study in 2 cases. Symptoms like fever, diarrhea, and sensory loss within the first 2 weeks of treatment in 1 patient were interpreted as intolerance to Thal.

In 35 of all 54 pts. (65%), the daily Thal dosage had to be reduced as shown in Fig. 3. At the start of Thal we aimed at a maximum dosage of 400 mg daily that was reached by 49 of 54 pts. (91%). This rate declined; after 3 months 23 of 47 pts. (91%) had an intolerance to Thal.

Cytokine Measurements. To determine whether pts. with progressive MM had increased levels of VEGF or bFGF in PB or BM, we measured cytokine levels by ELISA from plasma and compared the data with a group of healthy volunteers. As shown in Fig. 4, myeloma pts. had statistically significant higher PB-VEGF (P = 0.002) and BM-bFGF (P = 0.01) levels than healthy volunteers. In addition, there was a trend toward higher BM-bFGF levels than PB-bFGF (P = 0.18). A statistically significant difference between PB and BM for the group of healthy volunteers (bFGF, P = 0.001; VEGF, P = 0.02) was found.

Before Thal therapy, we analyzed the correlation of the plasma cell content in the BM to cytokine levels in both compartments of PB and BM. The estimated correlation coefficient was statistically significant for the plasma cell content in the

**Table 2** Decline in monoclonal protein levels (n = 54) evaluated on the basis of the percentage changes from baseline to the time of maximal response

<table>
<thead>
<tr>
<th>Maximum decline in monoclonal protein levels</th>
<th>No. of pts. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>&gt;90% of monoclonal protein</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>&gt;50% of monoclonal protein</td>
<td>9 (16.7)</td>
</tr>
<tr>
<td>&gt;25% of monoclonal protein</td>
<td>17 (31.5)</td>
</tr>
<tr>
<td>No change/PD</td>
<td>23 (42.6)</td>
</tr>
</tbody>
</table>

**Table 3** Logistic regression analysis of bFGF, VEGF, β2-microglobulin, albumin, Hb, and CRP before Thal1 treatment

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimated odds ratio</th>
<th>Estimated 95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-FGF (pg/ml) 50–100</td>
<td>3.33</td>
<td>(1.33, 8.33)</td>
<td>0.01</td>
</tr>
<tr>
<td>PB-VEGF (pg/ml) 100–300</td>
<td>0.56</td>
<td>(0.22, 1.41)</td>
<td>0.22</td>
</tr>
<tr>
<td>β2-microglobulin 2.5–5</td>
<td>2.16</td>
<td>(0.67, 6.94)</td>
<td>0.20</td>
</tr>
<tr>
<td>Albumin (g/liter) 35–45</td>
<td>0.24</td>
<td>(0.04, 1.30)</td>
<td>0.10</td>
</tr>
<tr>
<td>Hb (g/dl) 9–12</td>
<td>0.44</td>
<td>(0.10, 1.93)</td>
<td>0.27</td>
</tr>
<tr>
<td>CRP (mg/liter) &gt;2:&lt;2</td>
<td>0.66</td>
<td>(0.16, 2.80)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Table 4** Estimated effects of bFGF, VEGF, β2-microglobulin, albumin, Hb, and CRP before Thal treatment on PFS (Cox proportional hazards regression)

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimated hazard ratio</th>
<th>Estimated 95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-FGF (pg/ml) 50–100</td>
<td>0.87</td>
<td>(0.59, 1.27)</td>
<td>0.47</td>
</tr>
<tr>
<td>PB-VEGF (pg/ml) 100–300</td>
<td>0.83</td>
<td>(0.47, 1.46)</td>
<td>0.52</td>
</tr>
<tr>
<td>β2-microglobulin 2.5–5</td>
<td>1.33</td>
<td>(0.82, 2.11)</td>
<td>0.24</td>
</tr>
<tr>
<td>Albumin (g/liter) 35–45</td>
<td>1.21</td>
<td>(0.58, 2.53)</td>
<td>0.62</td>
</tr>
<tr>
<td>Hb (g/dl) 9–12</td>
<td>1.19</td>
<td>(0.53, 2.69)</td>
<td>0.67</td>
</tr>
<tr>
<td>CRP (mg/liter) &gt;2:&lt;2</td>
<td>1.26</td>
<td>(0.56, 2.87)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Fig. 2 PFS for 54 pts. with progressive MM during Thal therapy. At the start of Thal1 treatment, the drug was administered nightly at a dose of 100 mg daily, following a weekly dose increase of 100 mg daily for a final dose of 400 mg daily beginning at day 22. PFS was calculated from the start of Thal to disease progression or death from any cause. The estimated 6-month PFS was 73% (95% CI; range, 62% to 86%).

(P = 0.03) before therapy, as well as lower Hb (P = 0.008) and albumin (P = 0.02) levels, whereas no statistically significant difference was found for PB-VEGF (P = 0.93). In addition, a difference in the content of plasma cells in the BM (P = 0.01) and frequency of light chain (P = 0.04) was found.

Because of the limited size of the data set (54 pts.), we concentrated on both angiogenic cytokine levels (bFGF and VEGF) and well-characterized predictors for response and survival in MM (β2-microglobulin, Hb, albumin, and CRP) for multivariate analysis. As shown in Table 3 using a logistic regression analysis, bFGF was the only statistically significant predictor for response to therapy (P = 0.01). To evaluate the effect of these five variables on PFS, a Cox proportional hazards regression analysis was performed. There was a tendency that pts. with a low pretreatment β2-microglobulin level had a better PFS (P = 0.24), but no statistically significant effect was observed (Table 4).
BM compared with PB-bFGF (r = –0.32; P = 0.036) and BM-bFGF (r = –0.37; P = 0.045), whereas no statistically significant results were obtained for PB-VEGF and BM-VEGF.

To examine whether Thal treatment might lead to a decline in angiogenic cytokine levels, VEGF and bFGF levels were measured after 3 and 6 months of treatment. By focusing on pts. who had a complete follow-up for 6 months (three examinations), we found no statistically significant changes for PB-bFGF (n = 24; P = 0.44), BM-bFGF (n = 5; P = 0.37), PB-VEGF (n = 24; P = 0.37), and BM-VEGF (n = 7; P = 0.42).

Summary measures were used to correlate the changes of cytokine levels over time with response. Decreasing values of PB-bFGF (P = 0.10) and PB-VEGF (P = 0.04) were found to be associated with response, whereas no effect was observed for BM-bFGF and BM-VEGF.

DISCUSSION

To examine the impact of angiogenic cytokines on the response to Thal therapy MM, we measured bFGF and VEGF levels in PB and BM. Compared with a group of healthy volunteers, the cytokine levels in myeloma pts. were elevated from 1.3-fold for bFGF up to 2.4-fold for VEGF. Consistent with our observations, abnormally elevated levels of bFGF have been reported in serum and urine of pts. with various types of cancer (28). In particular, a high pretreatment serum bFGF concentration was found to be correlated with a poor prognosis in NHL (29). Pts. with diffuse large-cell and immunoblastic lymphomas had a significantly poorer survival of 28% versus 56% at 5 years if the pretreatment bFGF level was within the highest quartile of 160 pts. Also, pts. with low grade NHLs (n = 38) and a bFGF concentration within the highest quartile had an inferior survival rate in comparison with those with lower bFGF concentrations (5-year survival rates of 56% and 72%, respectively). In MM, Vacca et al. (6) found that plasma cells isolated from the BM of pts. with active myeloma produce higher levels of bFGF compared with smouldering MM and monoclonal gammopathy of undetermined significance pts., suggesting that this angiogenic factor is produced by myeloma cells and plays a role in BM neovascularization during disease progression. Recently, Sezer et al. (30) showed that serum bFGF levels were statistically significantly higher in stage II and III than in stage I myeloma pts. Consistent with this observation we found a positive correlation of the plasma cell content in the BM and bFGF levels. This association was observed in samples from PB as well as from BM, emphasizing the relevance of bFGF in disease progression.

Our results on response and toxicity are in line with the findings of Singhal et al. (16). We found that Thal therapy induced a marked response in MM, resulting in an overall response rate of 57% as measured by a decline in monoclonal protein of ≥25%. The decline in monoclonal protein was accompanied by a reduction of plasma cells in the BM as well as an increase of Hb levels, implying that the tumor burden was reduced. In comparison to Singhal et al. (16), we found a higher response rate of 57% versus 32%. This is probably related to a different patient selection. All of our pts. had PD, including 72% who relapsed after high-dose therapy. Once a relapse was diagnosed, we aimed at an early start of treatment, when the tumor burden was low and pts. were in a good physical condition. Compared with previous studies with daily Thal dosages up to 800 mg/day (14–16), we used a maximum dosage of 400 mg that was reached by 49 of 54 pts. (91%). Although most of the adverse effects were moderate, we observed 9 pts. (17%) with grade 3 or 4 toxicity. However, the toxicity did not seem to be Thal-related in all cases. Two of our pts. had pneumonia, a common complication in MM pts. (31). In addition, major cardiovascular complications were observed in 4 pts. Consistently, a French group reported on thrombotic events during Thal therapy in 5 pts. with nonmalignant disorders, including 4 pts. with lupus erythematosus (32). This observation suggests that Thal might act as a precipitating factor for cardiovascular complications. Additional studies should evaluate the need of an anticoagulant treatment during Thal therapy.

Before conventional chemotherapy, β2-microglobulin (22), CRP (22), albumin (23), and Hb (24) are known to be predictive for response and survival. We found that responsive pts. had (at least in univariate analysis) statistically significant higher concentrations of PB-bFGF and β2-microglobulin before therapy, as well as lower Hb and albumin levels, suggesting that a high bFGF concentration is associated with unfavorable clinical characteristics. In our study none of these parameters was predictive for PFS before Thal therapy. In line with these findings, Hideshima et al. (33) showed that Thal is active in MM cell lines that are resistant to melphalan, doxorubicin, and dexamethasone, and able to overcome classical drug resistance by inducing apoptosis or G1 growth arrest.

As proposed by Raje and Anderson (34), Thal might act by a variety of different mechanisms in MM, including direct effects on survival and growth of myeloma cells, modulation of the cytokine milieu in the BM, alteration of the profile of adhesion molecules, or inhibition of angiogenesis. To study the effect of Thal therapy on angiogenesis, a repeated BM biopsy was performed in the Arkansas study, finding a decrease of microvessel density in some responsive pts. but without a statistically significant effect (16).

In our study, the bFGF concentration was the only statistically significant predictor for response to Thal therapy when a...
logistic regression analysis was performed. Vacca et al. (6) showed that antibodies to bFGF cause a significant inhibition (>50%) of angiogenesis induced by myeloma cells, suggesting a role for bFGF in initiating or sustaining the angiogenesis seen in myeloma.

As shown by serial measurements, we found no decline in angiogenic cytokine levels over a period of 6 months for the whole group of pts. This persistence of angiogenic cytokine secretion makes it unlikely that this drug acts by a specific inhibition of bFGF or VEGF secretion in MM, although decreasing values of PB-bFGF and PB-VEGF were found to be associated with response to Thal therapy. However, decreasing bFGF serum levels are also found in responsive pts. after chemotherapy (30), suggesting that this effect on angiogenic cytokine secretion might be attributable to a reduction of plasma cells in the BM. More likely, an effect of Thal on cell surface receptors or intracellular signaling events could explain its efficacy in pts. with high pretreatment bFGF levels. For example, the low affinity receptor for bFGF, syndecan-1 (CD 138), was recognized on the surface of myeloma cells (35).

In conclusion, Thal is a novel agent with a variety of different effects in MM that might improve, either alone or in combination with conventional chemotherapy, the prognosis in this presently incurable disease.

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Kai Neben, Thomas Moehler, Gerlinde Egerer, et al.

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