Background and Objectives. Patients with high-risk essential thrombocythemia require cytoreductive therapy in order to normalize the elevated platelet counts. We evaluated the efficacy and toxicity of pegylated interferon in high-risk essential thrombocythemia in a phase II trial.

Design and Methods. Thirty-six patients with high-risk essential thrombocythemia (median age 54 years; range, 24-72 years) were studied. The dose of pegylated interferon was initially 50 µg per week and could be escalated up to 150 µg per week.

Results. During the first three months platelet counts decreased significantly from a median baseline count of 895×10^9/L (range: 383-1779) to a median count of 485×10^9/L (range: 211-1283; p≤0.001). A complete response was defined as platelet counts < 450×10^9/L. The complete response rate was 39%, 47%, 58% and 67% at 3, 6, 9 and 12 months of treatment, respectively. There were 25%, 11%, 8% and 0% poor responders, defined as patients with platelet counts > 600×10^9/L, at 3, 6, 9 and 12 months of treatment, respectively. After a median time of 23 months (range 3-39 months) 23 of 36 patients (64%) are still receiving pegylated interferon. In ten patients (28%) treatment was stopped due to grade 1 to 2 toxicity, classified according to the WHO standard toxicity scale. One patient, who responded partially to pegylated interferon (platelet count 542×10^9/L), had a cerebral stroke after 23 months of treatment.

Interpretations and Conclusions. In high-risk essential thrombocythemia sustained treatment with pegylated interferon is effective and safe in reducing platelet counts with a toxicity comparable to that of conventional interferon.

Key words: pegylated interferon, treatment, essential thrombocythemia, ET.

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Essential thrombocythemia is a Philadelphia-negative chronic myeloproliferative disorder with an incidence of 1.55 per 10^5 persons/year. Its prognosis is mainly determined by the occurrence of thromboembolic complications. However, in about one third of all patients the disease is benign and there are no complications for many years. Given this variable clinical course the major problem is to identify patients who will benefit from cytoreductive therapy. Therefore, the identification of patients at risk is important. According to retrospective studies, high-risk patients are characterized by an age > 60 years, a very high platelet count or a previous thromboembolic event. The need for cytoreductive therapy in these high-risk patients is widely accepted. Treatment options include cytoreductive drugs, such as hydroxyurea, anagrelide, a specific platelet lowering drug, or interferon-α. Hydroxyurea is the best studied drug and has been proven to reduce complications related to essential thrombocythemia in prospective trials; however, this drug has a potential to induce secondary malignancies. Many physicians are still reluctant to treat young thrombocythemic patients with hydroxyurea. However, there is no clear proof that hydroxyurea alone is increasing the risk of secondary malignancy and new data suggest that the mutagenic potential of hydroxyurea is very low. A non-mutagenic drug, conventional interferon-α, has been used in the treatment of essential thrombocythemia for more than 20 years. The major disadvantage of interferon is its frequent side effects. Modification of the pharmacokinetic profile of interferon-α through addition of a polyethyleneglycol (pegylation) has resulted in slower absorption and a lower elimination rate, thus enabling weekly administration, potentially improving compli-
The aim of this phase II study was to evaluate the safety, toxicity and efficacy of pegylated interferon in a group of 36 patients with high-risk essential thrombocythemia.

**Design and Methods**

**Patients’ characteristics and study design**

Between December 2001 and September 2003 a total of 36 patients with high-risk essential thrombocythemia were enrolled into this study by 16 German and Austrian centers. A total of 48 patients had been eligible for the trial, but 12 patients refused to give their consent and were thus excluded from the protocol. Patients were eligible if they were older than 18 years, had a diagnosis of essential thrombocythemia meeting the WHO criteria for this disease and fulfilled at least one of the following criteria for high-risk essential thrombocythemia: a platelet count > 1500\times10^9/L, age > 60 years, or previous complications related to essential thrombocythemia (thromboembolic or major hemorrhagic events). Microvascular disturbances were not regarded as an inclusion criterion for high-risk essential thrombocythemia. With the exception of conventional interferon-α cytoreductive pretreatment was permitted. A previous history of psychiatric disorders (in particular previous depression) or severe cardiac, liver or renal dysfunction were regarded as exclusion criteria. The study was approved by the local ethic committees of the participating centers and written informed consent was obtained from each patient enrolled into this trial.

The baseline characteristics at enrollment are shown in Table 1. Treatment was indicated for at least one of the following reasons: age > 60 years in 14 patients (39%), a platelet count > 1500\times10^9/L in 13 patients (36%) or previous complications related to essential thrombocythemia in 18 patients (50%). Ten patients (28%) had already received some treatment: eight had been treated with hydroxyurea and two with anagrelide. The median time between diagnosis and entry into the study was 6.6 months (range: 0.3–95 months).

**Pretreatment evaluation and follow-up**

Pretreatment evaluation included a complete medical history and physical examination, abdominal ultrasound, assessment of cardiovascular risk factors (cigarette smoking, hypertension, diabetes mellitus, and hyperlipidemia), full blood count, electrolytes, liver and renal functions, baseline coagulation tests, bone marrow cytology and histology and qualitative polymerase chain reaction analysis to rule out the bcr-abl rearrangement. All 36 patients were bcr-abl negative. At study entry, PRV-1 positivity or negativity was determined in a quantitative RT-PCR analysis in 26 patients as previously described. Data is presented as a ratio between PRV-1 and the housekeeping gene GAPDH (PRV-1/GAPDH ratio). PRV-1 positivity was defined as a PRV-1/GAPDH ratio of < 1.15 and PRV-1 negativity defined as a PRV-1/GAPDH ratio of > 1.19. Out of the 26 patients, 18 were negative and 8 were positive for PRV-1. Follow-up data are available for six patients: one patient became PRV-1 negative after 11 months and is still negative after 24 months of therapy with pegylated interferon, the other five patients with follow-up data were already negative at entry into the study.

Follow-up evaluations were performed on a weekly basis during the first three months and subsequently every third month. The follow-up evaluations consisted of medical history, physical examination, full blood count, serum biochemical assays including liver and renal function tests.

**Treatment plan and study endpoints**

Pegylated interferon α-2b (Peg-IFNα2b, PegIntron) was supplied by Essex Pharma, Germany in vials containing 50 μg, 100 μg and 150 μg lyophilized powder, along with sterile water for injection. Pegylated interferon was administered subcutaneously once weekly at a starting dose of 50 μg. In those patients who did not achieve a platelet count below 500\times10^9/L after 8–12 weeks, the dosages were increased by 25 μg per week up to 150 μg per week. Dose reduction by 25 μg per week was recommended in the case of adverse effects related to pegylated interferon and in patients with a stable platelet count below <450\times10^9/L for at least four weeks. Concomitant therapy with acetaminophen

### Table 1. Baseline characteristics of the 36 high-risk patients with essential thrombocythemia at enrollment into the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n=36 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
</tr>
<tr>
<td>Range</td>
<td>24–72</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>20/16</td>
</tr>
<tr>
<td>Leukocytes (\times10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>9.2</td>
</tr>
<tr>
<td>Range</td>
<td>4.9–19.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>13.9</td>
</tr>
<tr>
<td>Range</td>
<td>10.4–16.2</td>
</tr>
<tr>
<td>Platelets (\times10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>895</td>
</tr>
<tr>
<td>Range</td>
<td>383–1779</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td>25 (69%)</td>
</tr>
<tr>
<td>Aspirin therapy at enrollment</td>
<td>9 (25%)</td>
</tr>
</tbody>
</table>
(500-1000 mg) was recommended approximately 30 minutes before administration of the pegylated interferon to reduce flu-like symptoms. The use of low dose aspirin (100 mg/day) was optional and at the discretion of the attending physician. No other cytoreductive therapy was permitted while the patient was in the study.

We defined the following events as end-points regarding the treatment with pegylated interferon: (i) major thromboembolic events. (ii) major bleeding, defined as a decrease in hemoglobin of more than 2 g/dL, life-threatening bleeding or cerebral bleeding; (iii) microvascular disturbances; (iv) transformation into myelofibrosis or blast crisis; (v) intolerable side effects or patient’s refusal to continue the treatment.

**Response and toxicity criteria**

A complete hematologic response was defined as a sustained reduction of platelet counts below 450×10^9/L for at least one month. A partial hematologic response was defined as the reduction of platelets counts to between 450 and 600×10^9/L. A poor hematologic response or no response was defined as a sustained platelet count > 600×10^9/L in spite of therapy. Treatment-related toxicity was graded according to the WHO standard toxicity scale on a scale of 0 to 4.

**Statistical analyses**

The SAS system was used for the statistical analyses. All response data of patients who received at least one dose of pegylated interferon were calculated according to an intention-to-treat analysis. Values were compared using the two-sided Wilcoxon test for parallel groups. All significance levels are two-sided. A box plot analysis was used for the course of the platelet counts at baseline and after 3, 6, 9, 12, 18 and 21 months of therapy. The box itself represents the middle 50% of the data. The upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The line in the box indicates the median value of the data. The ends of the vertical lines indicate the minimum and maximum data values.

**Result**

**Patients and dosage level of pegylated interferon**

In this phase II study 36 patients with high-risk essential thrombocythemia were treated with pegylated interferon (PegIntron). The initial dosage level of pegylated interferon used was 50 µg per week. During follow up the median dose was 50 µg per week (range 12.5-150 µg). After 12 months 81% of all treated patients were on a dose ≤50 µg per week and after 21 months 88% of all treated patients were on a dose ≤50 µg per week.

**Efficacy of treatment with pegylated interferon**

During the first three months of treatment platelet counts decreased significantly from a median baseline count of 895×10^9/L (range: 383-1779) to a median count of 485×10^9/L (range: 211-1283; p<0.001). Figure 1 represents the course of the platelet counts as box plots at baseline and after 3, 6, 9, 12, 18 and 21 months of treatment with pegylated interferon. The complete response rate (platelet counts <450×10^9/L) was 39% (14 of 36 patients), 47% (17 of 36 patients), 58% (21 of 36 patients) and 67% (24 of 36 patients) at 3, 6, 9 and 12 months of treatment, respectively. The overall response rate (complete and partial response, i.e. platelet counts <600×10^9/L) was 72% (26 of 36 patients), 72% (26 of 36 patients), 69% (25 of 36 patients) and 75% (27 of 36 patients) at 3, 6, 9 and 12 months of therapy, respectively. The rate of poor responders (platelet counts > 600×10^9/L) was 25% (9 of 36 patients), 11% (4 of 36 patients), 8% (3 of 36 patients) and 0% (0 of 36 patients) at 3, 6, 9 and 12 months of treatment, respectively. Figure 2 shows the efficacy of pegylated interferon treatment for all 36 patients with essential thrombocythemia at 3, 6, 9, 12, 18 and 21 months.

**Discontinuation and side effects of pegylated interferon therapy during follow-up**

After a median time of 23 months (range 3–39 months) with a total observation time of 763 months, 25 of 36 patients (64%) are still receiving pegylated interferon. During the first six months of therapy with pegylated interferon 28 of 36 patients (78%) reported flu-like symptoms including fever, fatigue, headache and myalgia. After 12 months of treatment
the frequency of flu-like symptoms among patients still on therapy decreased to 36%. Other reported side effects included mild depression in up to 15% of treated patients as well as mild hair loss in up to 18% of treated patients. Furthermore, skin changes, mainly consisting of increased dryness, were reported by 37% of the patients treated over time. All reported side effects decreased in intensity over time. None of the reported adverse events exceeded a grade 2 toxicity according to the WHO standard toxicity scale. Table 2 shows the reasons for discontinuation of pegylated interferon in the 13 patients who ceased to take the drug. In ten patients (28%) therapy was stopped at the patients’ request due to grade 1 or 2 drug related adverse effects: flu-like symptoms with sustained fatigue (n=6), depression (n=1), prolonged diarrhea (n=1) and alopecia in female patients (n=2). One patient was lost to follow-up after 2.6 months. In another patient the platelet count could not be lowered sufficiently with pegylated interferon and an alternative cytoreductive therapy was initiated. The median duration of treatment until its cessation was 8.5 months (range 2.6-23.2 months).

**Thrombotic complications during follow-up**

After 23.0 months on therapy with pegylated interferon a 45-year old patient suffered from a cerebral stroke and was subsequently taken out of the study. A cerebral stroke had been the initial presenting symptom of his essential thrombocythemia and the reason why he had entered the study. At the time of the second event the dose of pegylated interferon being administered was 100 μg per week and his platelet count was 542x10⁹/L. This complication accounted for the total complication rate of 1.7% per patient-year. No further thrombocythemia-related complications were observed in other patients during the total observation period of our study.

<table>
<thead>
<tr>
<th>Reasons for discontinuation of pegylated interferon</th>
<th>Number of patients</th>
<th>WHO standard toxicity scale</th>
<th>Months until discontinuation of pegylated interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu-like symptoms and fatigue</td>
<td>n=6</td>
<td>grade 1 (n=2)</td>
<td>5.9; 23.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>grade 2 (n=4)</td>
<td>3.6; 9.5; 15.1; 19.1</td>
</tr>
<tr>
<td>Alopecia</td>
<td>n=2</td>
<td>grade 2 (n=2)</td>
<td>4.4; 8.5</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>n=1</td>
<td>grade 2 (n=1)</td>
<td>5.9</td>
</tr>
<tr>
<td>Depression</td>
<td>n=1</td>
<td>grade 2 (n=1)</td>
<td>4.4</td>
</tr>
<tr>
<td>Insufficient platelet count reduction</td>
<td>n=1</td>
<td>—</td>
<td>8.8</td>
</tr>
<tr>
<td>Cerebral stroke*</td>
<td>n=1</td>
<td>—</td>
<td>23.0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>n=1</td>
<td>—</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*not related to pegylated interferon.

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**Table 2. Reasons for therapy discontinuation in 13 out of 36 patients treated with pegylated interferon. Side effects are classified according to the WHO standard toxicity scale.**

**Figure 2. Efficacy of pegylated interferon treatment for all 36 patients with essential thrombocythemia at 3, 6, 9, 12, 18 and 21 months. CR: complete remission; PR: partial remission; NC: no change or poor response. The black bars indicate patients who discontinued therapy with pegylated interferon at the respective times.**
**PRV-1 expression at study entry and during follow up**

At study entry, PRV-1 positivity or negativity was determined in quantitative reverse transcription polymerase chain reaction analysis in 26 patients. Of these 26 patients, 18 were negative and 8 were positive for PRV-1. Follow-up data are available for six patients: one patient became PRV-1 negative after 11 months and is still negative after 24 months of therapy with pegylated interferon (Figure 3). The other five patients with follow-up data were already negative at entry into the study.

**Discussion**

In this phase II trial 36 patients with high-risk essential thrombocythemia were treated with pegylated interferon (PegIntron) to evaluate the safety, toxicity and efficacy of this drug. Conventional, recombinant interferon-α is an effective treatment in essential thrombocythemia and a complete hematologic remission is obtained in about 54% of patients. However, about 15% of all essential thrombocythemia patients are primarily resistant to interferon-α and frequent side effects limit therapy with this drug. In a meta-analysis of 273 patients with essential thrombocythemia, interferon-α was terminated in 25% of all patients and in up to 66% of patients in individual trials. Utilization of new interferon-α formulations such as pegylated interferon may help to overcome some of these problems.

The administration of pegylated interferon was safe. With a median dosage of 50 μg pegylated interferon per week (range 12.5–150 μg) we observed no case in which a dose reduction was necessary due to impaired hematopoiesis. In the majority of patients 50 μg pegylated interferon per week or less was sufficient to control the platelet count. During the first six months most of our patients suffered from mild to moderate flu-like symptoms. After one year these side effects disappeared in most patients. After a median observation period of 23 months (range 3–39 months) ten of 36 patients (28%) stopped therapy with pegylated interferon due to grade 1 or 2 drug-related side effects. The observed side effects and the drop-out rate due to adverse events are comparable to those reported for conventional interferon-α.

This trial demonstrates that pegylated interferon is an effective platelet-lowering drug in patients with high-risk essential thrombocythemia. Pegylated interferon exerted its platelet-lowering efficacy not only in newly diagnosed patients with a high initial platelet count but also in patients who had been pretreated with hydroxyurea or anagrelide. After three months of therapy with pegylated interferon, the proportion of patients with a reduction of platelet count <600×10^9/L was 72% (26 of 36 patients). However, after three months nine of 36 patients (25%) still had a platelet count >600×10^9/L in spite of therapy with pegylated interferon. The hematologic response improved over time and after 12 months 75% of our patients (27 of 36 patients) achieved a platelet count <600×10^9/L (24 patients with a platelet count <450×10^9/L). At this time there was no patient on treatment with pegylated interferon who had a platelet count >600×10^9/L. The complete hematologic response rate for pegylated interferon was at least as good as that reported for conventional interferon-α, ranging from 54% to 70%. One patient suffered from a cerebral stroke while on pegylated interferon. This event rate of 1.7% per patient-year is comparable to the complication rate in hydroxyurea-treated high-risk essential thrombocythemia patients (1.6% per patient-year) observed in a prospective study after a median observation time of 27 months.

There is one small published pilot study concerning 11 patients with essential thrombocythemia who were treated with pegylated interferon. All patients were in complete remission after 4 months. After a median observation time of 9 months (range 4-17 months) two of 11 patients (18%) discontinued therapy because of side effects. One patient discontinued therapy at 4

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**Figure 3.** PRV-1 expression in a single patient under therapy with pegylated interferon. This patient was PRV-1 positive at entry into the study and became negative after 11 months of therapy with pegylated interferon. PRV-1 mRNA was calculated as previously described. Data are presented as a ratio between PRV-1 and the housekeeping gene GAPDH (PRV-1/GAPDH ratio). PRV-1 positivity is defined as a PRV-1/GAPDH ratio of < 1.15 and PRV-1 negativity is defined as a PRV-1/GAPDH ratio of > 1.19. The other five patients with PRV-1 follow-up data were already negative at entry into the study and remained negative during follow-up (data not shown in this figure).
months because of persistent grade III fatigue and a second patient at 5 months because of anxiety and depression. No events related to essential thrombocytemia were observed during the observation period. The initial dose of pegylated interferon was higher than that used in our study (1.5 – 4.5 μg/kg per week). Further trials with pegylated interferon in essential thrombocytemia are currently under way. Preliminary data from these trials report similar response rates with pegylated interferon but a higher discontinuation rate due to side effects. Our data suggest that pegylated interferon is at least equally effective as unmodified interferon in reducing interferon platelet counts. Due to an initial slow response to pegylated interferon, a large proportion of patients require sustained treatment for several months in order to achieve a hematologic remission. For those patients who respond slowly in the first months of treatment with pegylated interferon, concomitant cytoreductive therapy with hydroxyurea might help to achieve a more effective platelet count reduction.

All authors contributed to the concept of the study, analysis and interpretation of the data, and drafting and revising the article. All approved the final version. In detail, CL was the secretary and coordinator of the trial; EL was in the steering committee of the trial and was one of its coordinators. HLP performed the PRV-1 assays; HB performed the statistical analyses; SS was the data manager of the trial; HG was in the steering committee of the trial and its coordinator in Austria; MG was the principal investigator of this trial. Responsible for all Tables and Figures: MG. The authors also declare that they have no potential conflicts of interest.

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