Farnesyl transferase (FTase) inhibitors were originally developed to inhibit the farnesylation of mutant, oncogenic Ras proteins. However, recent results have shown in vitro and in vivo antitumor activity independent of Ras status, indicating that Ras farnesylation may not be the most clinically relevant target. FTIs such as tipifarnib (Zarnestra, R115777; Johnson & Johnson, Pharmaceutical Research and Development LLC), an oral, selective, and potent inhibitor of FTase, has been shown to modulate multiple intracellular signaling pathways, including Rheb, Rac, CENP-E, CENP-F, lamin proteins, and others (1, 2).

Regardless of the biomedical mechanism, tipifarnib has potent antiangiogenic, antiproliferative, and proapoptotic effects that have translated into significant clinical activity (3). A phase I study of tipifarnib showed about a 30% response rate in patients with refractory or relapsed acute myeloid leukemia (4). Activity has also been observed in other malignancies, including myelodysplastic syndrome (MDS; refs. 5–7), multiple myeloma (8), and chronic myeloid leukemia (9).

Similar to other targeted therapeutics, one of the major challenges encountered with tipifarnib has been selecting an optimal biological dose and schedule. Earlier studies identified a maximum tolerated dose (MTD) for tipifarnib of 600 mg twice daily on a 3 weeks on/1 week off schedule in patients with relapsed and refractory hematologic malignancies (4, 10). A phase I study conducted by our group in an MDS patient population identified a lower MTD of 400 mg twice daily on a 3 weeks on/1 week off schedule (6). A simultaneously done phase II study found that 600 mg twice daily on a 4 weeks on/2 weeks off schedule was too toxic for the majority of MDS patients (7). Moreover, the latter dosing schedule was associated with a lower response rate (11.1% versus 30.0%) compared with the phase I trial (6, 7). Cumulative, drug-induced myelosuppression observed with higher doses and prolonged use in the phase II study may have concealed challenges encountered with tipifarnib has been selecting an optimal biological dose and schedule. Earlier studies identified a maximum tolerated dose (MTD) for tipifarnib of 600 mg twice daily on a 3 weeks on/1 week off schedule in patients with relapsed and refractory hematologic malignancies (4, 10). A phase I study conducted by our group in an MDS patient population identified a lower MTD of 400 mg twice daily on a 3 weeks on/1 week off schedule (6). A simultaneously done phase II study found that 600 mg twice daily on a 4 weeks on/2 weeks off schedule was too toxic for the majority of MDS patients (7). Moreover, the latter dosing schedule was associated with a lower response rate (11.1% versus 30.0%) compared with the phase I trial (6, 7). Cumulative, drug-induced myelosuppression observed with higher doses and prolonged use in the phase II study may have concealed...
hematologic improvements experienced by some patients. A phase I study conducted by Crul et al. (11) suggested that chronic dosing is associated with severe myelosuppression and neurotoxicity at significantly lower doses compared with intermittent schedules.

Tipifarnib inhibits FTase activity in human peripheral blood cells isolated from patients with relapsed/refractory acute myeloid leukemia after doses as low as 100 mg twice daily (4). Responses including a complete remission (CR) were also observed at these low doses (4). Considering these results and the observation that FTase inhibition may persist for several days during rest periods (6), as well as the fact that tipifarnib is likely to be combined with other therapies in the treatment of acute myeloid leukemia and MDS, we explored a more intermittent schedule (1 week on/1 week off), starting with a dose of 100 mg twice daily in the initial cohort and escalating to the MTD in subsequent patients.

**Patients and Methods**

**Eligibility.** Patients were eligible if they were 18 years or older, had histologically confirmed MDS including all French-American-British (12) types refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts (RAEB), RAEB in transformation, and chronic myelomonocytic leukemia. Patients were classified according to French-American-British classification because at the time the protocol was written, this was the most common classification used. The WHO criteria (13) further subdivides these groups and considers RAEB in transformation as acute myeloid leukemia.] Prognosis was assessed by International Prognostic Scoring System (IPSS) criteria (14) from low risk (0 points) to high risk (2.5 points). Patients with an IPSS score of zero had to have significant cytopenia of at least 4 weeks duration (hematocrit <26%, absolute neutrophil count <1,000/mm³ or platelet count ≤50,000/mm³). Patients had to have an Eastern Cooperative Oncology Group performance status of ≤2, life expectancy >12 weeks, and normal hepatic and renal function (bilirubin ≤1.5 mg/100 mL; creatinine ≤1.5%). Patients were excluded if they had received chemotherapy or radiotherapy within 4 weeks before study entry, were receiving other investigational agents, had a history of hypersensitivity to similar compounds such as imidazoles, had uncontrolled intercurrent illness, or were pregnant. Growth factors other than granulocyte colony stimulating factor were excluded, and patients must not have received growth factors for 2 weeks before study entry. Patients eligible for bone marrow transplant (≤60 years old) with a compatible sibling and no contraindications for transplant were excluded from the study. Patients could be previously treated or untreated. The Institutional Review Board approved the study protocol and all patients signed an informed consent in keeping with the policies of the Surveillance Committee of the M. D. Anderson Cancer Center.

**Treatment plan.** The initial cohort of patients was treated at a dose level of 100 mg orally twice per day. In subsequent cohorts of patients, the plan was to escalate by 100 mg twice daily until grade 2 toxicity was observed and then by 100 mg/d thereafter until the MTD was determined. At least three patients were treated at each dose level and evaluated for 4 weeks before doses were escalated in subsequent cohorts. Therapy was administered on a 1 week on/1 week off basis for an 8-week induction cycle. Patients with stable disease or better were maintained on therapy at the same dose and schedule until loss of response provided that there was no unacceptable toxicity.

Dose modification was allowed as follows. Those with persistent grade 2 toxicity, other than alopecia, received a 50% dose reduction (rounded down to the nearest 100 mg). The drug was stopped in patients with grade 3 nonhematologic toxicity or grade 2 neurologic toxicity until resolution of side effects to less than grade 1. The drug could then be resumed with a 50% dose reduction. If grade 3 toxicity recurred, the drug was discontinued. Patients with a deterioration in counts persisting for up to 4 weeks (platelet count drop by >50% and to <5,000/mm³ if baseline between 20,000 and 50,000/mm³ or absolute neutrophil count drop by >50% and to <500/mm³ if baseline absolute neutrophil count >1,500/mm³) had the drug held but could restart after resolution of toxicity.

**Definitions of response.** Hematologic CR, partial remission, stable disease, and hematologic improvement were defined as per the International Working Group criteria (15).

**Determination of minimum tolerated dose.** A major objective of the study was to determine a tolerable dose of tipifarnib administered on a 1 week on/1 week off schedule for further evaluation in a phase II setting. A minimum of three patients were entered at each dose level beginning with the lowest dose of 100 mg twice daily. Dose-limiting toxicity was determined during the first 8 weeks of therapy. If one patient experienced dose-limiting toxicity, defined as a grade 3 nonhematologic toxicity (except for alopecia or nausea adequately treated with a well-tolerated anti-emetic) or grade 4 hematologic toxicity (lasting >28 days after the last day of therapy or with serious infection or hemorrhage and accompanying hematologic deterioration), the cohort was expanded to six patients. If no additional patients in the expanded cohort experienced a dose-limiting toxicity, dose escalation was resumed at 100 mg/d for the next cohort. If a second patient enrolled at the same dose level in an expanded cohort experienced a dose-limiting toxicity, the MTD was exceeded and dose escalations ceased. The next lower dose level was considered the MTD. Therefore, the MTD was the dose level below that at which one third or greater patients experienced dose-limiting toxicity. Toxicity was evaluated and graded according to the Cancer Therapy Evaluation Program Common Toxicity Criteria (15).

**FTase I and GGTTase I enzyme activity assays.** Patient peripheral blood mononuclear cells (PBMC) were sonicated in 150 μL sonication buffer [50 mmol/L Tris (pH 7.5), 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L DTT, 2 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, and 25 μg/mL leupeptin]. Each sample was then prepared for FTase and GGTTase I enzymatic assays by spinning at 12,000 × g for 30 min. The protein content of the resultant supernatants was determined, and each sample was then assayed for its FTase and GGTTase I enzyme activity by its ability to transfer [3H]farnesyl and [3H]geranylgeranyl from [3H]farnesyl and [3H]geranylgeranyl Ppi to recombinant H-Ras-CVLS and H-Ras-CVLL protein substrates, respectively, as described previously (6). Briefly, 40 μL of 12,000 × g supernatants was incubated for 30 min at 37°C in 50 mmol/L Tris (pH 7.5), 50 μmol/L ZnCl₂, 20 mmol/L KCl, 1 mmol/L DTT, and 3 mmol/L MgCl₂ in the presence of either 1 μCi (0.037 MBq) [3H]farnesyl Ppi and 50 μg H-Ras-CVLS (FTase) or 1.0 μCi (0.037 MBq) [3H]geranylgeranyl Ppi and 25 μg H-Ras-CVLL (GGTase I). The reaction was then stopped by the addition of 4% SDS, and the proteins were precipitated with 30% trichloroacetic acid and collected on glass microfiber filters; final results were determined by scintillation counting.

**Study drug.** Tipifarnib (Zarnestra, R115777) was supplied by the Division of Cancer Treatment and Diagnosis of the National Cancer Institute in an oral 100 mg tablet formulation.

**Results**

**Patient characteristics.** Sixty-three patients with MDS entered the study. All were evaluable for toxicity and 61 were evaluable for response. (One patient was noncompliant and one patient was found to be ineligible because diagnosis of MDS could not

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verified.) Patient demographics and disease characteristics are listed in Table 1. The median age was 68 years and 73% (46 of 63) of the patients were men. All subtypes of MDS were included, the majority being RAEBs. Most patients had IPSS INT-1 and INT-2 disease. Three patients had an N-Ras mutation and one had a K-Ras mutation, all tests being done on bone marrow. The median number of prior therapies was 2 (range, 0-3).

### Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>No. patients (%)</th>
<th>Total 63 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 46 (73) Female 17 (27)</td>
</tr>
<tr>
<td>Age, y</td>
<td>Median 68 Range 35-84</td>
</tr>
<tr>
<td>MDS diagnosis by FAB*</td>
<td>RA 15 (24) RARS 6 (10) RAEB 26 (41) RAEB-t 8 (13) CMML 8 (13)</td>
</tr>
<tr>
<td>MDS type by IPSS score</td>
<td>Low 5 (8) INT-1 27 (43) INT-2 23 (36) High 8 (13)</td>
</tr>
<tr>
<td>RAS mutations</td>
<td>N-Ras 3 K-Ras 1</td>
</tr>
<tr>
<td>Assessable for toxicity</td>
<td>63 (100)</td>
</tr>
<tr>
<td>Assessable for response</td>
<td>61 (97)</td>
</tr>
</tbody>
</table>

Abbreviations: FAB, French-American-British classification; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB-t, refractory anemia with excess blasts in transformation; CMML, chronic myelomonocytic leukemia; INT, intermediate.

*At the time protocol was written, this was the most common classification used, and it was therefore the one used in the trial.

Dose levels and toxicity. The initial cohort of patients was treated with tipifarnib 100 mg orally twice daily on days 1 to 7 every 2 weeks. At least 3 patients were treated at each dose level and dose-limiting toxicity was assessed during the first 8 weeks. All 63 patients were evaluable for toxicity. Nonhematologic toxicities are shown in Table 2. The most common toxicity was myelosuppression, experienced by 60% of patients (all grades). Notably, no side effects were reported by 20% of patients. Nonhematologic toxicities (all grades) included fatigue (20%), diarrhea (16%), increase in transaminases (14%), increase in bilirubin (11%), nausea (11%), and skin rash (9%). Dose-limiting toxicities of ataxia (n = 1), fatigue (n = 1), nausea (n = 1), and neutropenic fever (n = 2) occurred at tipifarnib doses above 1,200 mg/d. The MTD of tipifarnib in this patient population was 600 mg orally twice daily using an alternate-week schedule. For patients on maintenance therapy, no previously undescribed long-term toxicities were noted. All patients treated at doses above those later determined to be the MTD (600 mg twice daily) required dose reductions during therapy, mostly due to myelosuppression and fatigue and skin rash.

Efficacy. Sixteen of 61 (26%) evaluable patients achieved a response (Table 3). Three patients attained a CR and 13 showed hematologic improvement: 2 with a trilineage response, 1 with a bilineage response, and 10 with a single-lineage response. Major platelet responses were the most common and occurred in 11 of the 16 responders (69%). Figure 1 depicts the gradual time course of one such platelet response.

### Table 2. Nonhematologic toxicity

<table>
<thead>
<tr>
<th>No. patients (%)</th>
<th>Total evaluable 63 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLT, n (%)</td>
<td>Fatigue 13 (20) Diarrhea 10 (16) Increase in transaminases 9 (14) Increase in bilirubin 7 (11) Nausea 7 (11) Skin rash 6 (9) Ataxia 1 (2)</td>
</tr>
</tbody>
</table>

Abbreviation: DLT, dose-limiting toxicity.

### Table 3. Responses

| Result | Total evaluable, n (%) 61 (100) | Objective response, n (%) 16 (26) | CR 3 (5) | HI 13 (21) | Median time to response, wk (range) 8 (4-32) | Median duration of response, wk (range) 20 (8-101) | Death within 4 mo 6 (10) |

Abbreviation: HI, hematologic improvement.

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**Fig. 1.** Gradual increase in platelet count from a baseline of $10^5/L$ to a peak level $10^9/L$ in a 75-year-old man with refractory anemia treated with a total daily dose of 800 mg/d.
patient had a CR on this low dose level, and the dose level was expanded to 15 patients to gain experience with it.) The other two patients who achieved CR had RAEB in transformation, with a deletion on chromosome 5 and trisomy 8 (IPSS high-risk disease; dose, 400 mg twice daily reduced to 200 mg twice daily) and RAEB with normal karyotype (IPSS INT-1; dose, 1,400 mg/d, reduced to 700 mg/d). Time to CR was 22 weeks for the patient at the lowest dose level, and 7 and 4 weeks for the other two patients, respectively. The duration of CR was 17, 13, and 91+ weeks, respectively.

The median time to response was 8 weeks (range 4-32 weeks) and the median duration of response was 20 weeks (range 8-101 weeks). The median time to response for patients at the lowest dose level (100 mg twice daily) was 17 weeks (range, 7-32 weeks); for patients at higher dose levels, it was 6.5 weeks (range, 1-16 weeks).

To determine the most effective dose of tipifarnib that inhibits FTase and/or GGTase I, we prepared PBMCs from patient blood and processed these for FTase and GGTase I

### Table 4. Correlation of dose and response to alternate-week tipifarnib

<table>
<thead>
<tr>
<th>Total dose per day (mg)</th>
<th>No. responders/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>4/15 (27)</td>
</tr>
<tr>
<td>400</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>600-700</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>800-900</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>1,000-1,100</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>≥1,200</td>
<td>3/21 (14)</td>
</tr>
</tbody>
</table>

### Table 5. Percentage inhibition of PBMC FTase and GGTase is shown during study

<table>
<thead>
<tr>
<th>% Inhibition FTase</th>
<th>Daily dose</th>
<th>Response*</th>
<th>% Inhibition GGTase 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1 on</td>
<td>Week 2 off</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26-50%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76-100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Inhibition FTase</td>
<td>Daily dose</td>
<td>Response*</td>
<td>% Inhibition GGTase 1</td>
</tr>
<tr>
<td>Week 1 on</td>
<td>Week 2 off</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Percent inhibition of PBMC FTase and GGTase is shown during week 1 (on drug) and week 2 (off drug) of study. The table shows that during the week on drug, FTase inhibition was present in all but one patient tested, regardless of dose, and was mostly more than 76%. In addition, in over half the patients, FTase inhibition persisted during the week off study. In contrast, effect on GGTase was variable, with activation, inhibition, and no effect seen.

*Only patients with responses are shown.
enzymatic activities as described above. The blood was collected from patients before the first dose was taken, as well as at various times after initiation of treatment. Table 5 shows that during the week on drug, FTase inhibition was present in all but one patient tested, regardless of dose, and was mostly more than 76%. [Indeed, at the lowest daily dose given (200 mg; 5 patients analyzed) as well as at the next dose level (400 mg; 1 patient analyzed), FTase was inhibited by over 76% during the first week of treatment (days 2 to 7) in all patients analyzed.] In addition, in more than half the patients tested, FTase inhibition persisted during the week off study, regardless of dose level, albeit not to the same degree as while on drug.

Table 5 also shows the effects of tipifarnib treatment on GGTase I activity in PBMCs from the same patients analyzed for FTase. In contrast to FTase, tipifarnib treatment during the first week either had no effect on GGTase I, inhibited it, or actually activated it. For example, of 32 patients analyzed, the GGTase I activity in only 4 patients was inhibited by over 76%, in 3 patients by 51% to 75%, in 4 patients by 26% to 50%, and in 7 patients between 0% and 25%. In 15 patients, GGTase I was actually activated by tipifarnib, in some cases by as much as 20-fold (data not shown). This observation is consistent with other studies reported previously (6, 8). During the week off drug, many of these changes persisted, at least partially. As with FTase, the ability of tipifarnib to either inhibit or stimulate GGTase I activity was not dependent on the doses used.

**Discussion**

In this study, we showed that tipifarnib was well tolerated in this MDS population when administered as an alternate-week regimen. The maximum tolerated dose was 1,200 mg/d given orally on a twice daily schedule (600 mg twice daily). This dose provides the monthly equivalent of the MTD dose (400 mg orally twice daily) in the 3 weeks on/1 week off schedule, per our previous report (6). In total, 16 of 61 (26%) evaluable patients responded—3 CRs and 13 hematologic improvements. Major platelet responses were most common; they were observed in 11 of 16 responders. These response rates are consistent with those reported in both the phase I and phase II setting in MDS, as well as the ~15% CR rate described in newly diagnosed poor-prognosis elderly patients with acute myeloid leukemia (6, 16, 17). Only one patient among the responders (hematologic improvement) in this study had a Ras (N-Ras) mutation, an observation consistent with previously published results demonstrating a lack of correlation between Ras mutational status and response to tipifarnib (4, 6, 16). Previous studies also showed variability in the effect of tipifarnib on downstream pathways such as Erk or Stat (6). Which one or more of the >100 farnesylated proteins are therefore responsible for response remains unanswered.

It is important to note that there was no obvious dose-response relationship because responses were observed at all dose levels. In fact, 4 of 15 patients (27%) at the lowest dose level (100 mg twice daily) responded, including one patient who achieved a CR. These results are also consistent with prior observations of responses, including CR in myeloid leukemias at this low dose level (4). Responses were achieved within 8 weeks in nine responders, but took longer in the other seven patients. In particular, achievement of response required more prolonged therapy in the patients at the lowest dose level, with their median time to response being 17 weeks, and the range of times being from 7 weeks up to 32 weeks. Nevertheless, the fact that most patients experienced few to no side effects at this low dose level makes the observation of response of interest.

Maximum response often required multiple cycles of therapy, regardless of the dose. In the three patients who achieved CR, time to CR was 4, 7, and 22 weeks, highlighting the ongoing nature of response and suggesting the advisability of continuing therapy in patients who have stable disease or better. Tipifarnib treatment of the MDS patients was very effective at inhibiting PBMC FTase activity, with 22 of the 31 patients analyzed having their FTase inhibited by more than 76%. There was no correlation between dose and degree of inhibition of FTase, with the lowest daily dose used (200 mg) being as potent as the highest dose (1,500 mg). Interestingly, during week 2 when patients were not taking tipifarnib, FTase was still suppressed in the majority of the patients, with six subjects’ FTase activity inhibited by more than 76%. Although tipifarnib was more selective for FTase over GGTase I, in 4 of 31 patients analyzed, it was able to inhibit GGTase I by more than 76%. However, in 13 patients, tipifarnib treatment resulted in stimulation of GGTase I, a result that is consistent with previous results in patients with MDS and multiple myeloma (6, 8). There was no correlation between clinical activity and the degree of inhibition of either FTase or GGTase I either during week 1 or 2.

In conclusion, an alternate-week regimen of tipifarnib was well tolerated and very effective at blocking FTase. A response rate of 26% was achieved in this study. It seems plausible that there was no dose/response due to the fact that FTase was inhibited at all dose levels. Presumably, there is a lower dose level below which this effect no longer occurs. It also seems that FTase inhibition may be necessary but not sufficient for response to occur after administration of an FTase inhibitor. It seems reasonable to assume that malignant cells may have other relevant pathways driving growth in some patients. The intermittent schedule described herein may be particularly conducive to maintenance therapy or to a combination regimen, especially with drugs such as azacitidine or decitabine (hypomethylating agents) or other molecules that have been approved for therapy of MDS (18–20). Alternate-week treatment may also be attractive because the lowest daily dose (200 mg) of tipifarnib used was very effective at blocking FTase and was also capable of inducing responses, but had little in the way of side effects. However, responses took longer to achieve at lower doses than at higher doses, further suggesting that higher doses may be better suited to treating active diseases, whereas lower doses (which are virtually free of toxicity) may be best applied to maintenance or combination regimens.

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Phase I Study of Alternate-Week Administration of Tipifarnib in Patients with Myelodysplastic Syndrome

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