We carried out a multicenter dose-escalation phase I study of oral OPB-51602, a signal transducer and activator of transcription 3 phosphorylation inhibitor, in patients with relapsed or refractory hematological malignancies to evaluate the safety, maximum tolerated dose (MTD), pharmacokinetics, and preliminary antitumor activity. Twenty patients were treated with OPB-51602 at doses of 1, 2, 3, 4, and 6 mg in the “3 + 3” dose escalation design. The most common treatment-related adverse events included nausea (55%), peripheral sensory neuropathy (45%), and diarrhea (40%). The most frequently observed grade 3 or 4 drug-related adverse events were neutropenia (20%), leukopenia (15%), lymphpenia (10%), and thrombocytopenia (10%). The MTD was 6 mg, with dose-limiting toxicities of grade 3 lactic acidosis and increased blood lactic acid levels observed in one of three patients and grade 1–2 peripheral neuropathy in three of three patients. The recommended dose was determined to be 4 mg. OPB-51602 was rapidly absorbed, and exposure tended to increase in a dose-dependent manner. Accumulation of OPB-51602 was seen with 4 weeks of multiple treatments. No clear therapeutic response was observed. Durable stable disease was observed in two patients with acute myeloid leukemia and one with myeloma. In conclusion, the MTD of OPB-51602 was 6 mg. OPB-51602 was safe and well tolerated in a dose range of 1–4 mg. However, long-term administration at higher doses was difficult with the daily dosing schedule, and no response was seen. Therefore, further clinical development of OPB-51602 for hematological malignancies with a daily dosing schedule was terminated.
treatment; (iii) Eastern Cooperative Oncology Group performance status of 0–1; and (iv) aged 20–75 years. Adequate bone marrow, hepatic, and renal functions were mandatory and were defined as: hemoglobin, ≥8.0 g/dL; absolute neutrophil count, ≥1.5 × 10^9/L; platelet count, ≥75 × 10^9/L (not applicable for leukemia); bilirubin, ≤1.5 × upper limit of normal (ULN); aspartate aminotransferase, ≤2.5 × ULN; alanine aminotransferase, ≤2.5 × ULN; and creatinine, ≤1.5 × ULN. All patients signed written informed consent. The study was approved by the institutional review board at each participating institute.

**Study design.** The primary objective of this study was to determine the tolerability, safety profile, and MTD of OPB-51602 in patients with relapsed or refractory hematological malignancies. Secondary objectives included determination of pharmacokinetics and the preliminary antitumor activity of OPB-51602 in this patient population.

OPB-51602 was given orally once daily, continuously for 4 weeks per cycle, until disease progression or unacceptable toxicity was observed. The starting dose was 1 mg, and the dose was escalated to 2, 3, 4, and 6 mg. Dose escalation was based on the “3 + 3” design. Maximum tolerated dose was defined as the dose in which dose-limiting toxicities (DLTs) in the first treatment cycle were observed in two or more out of six patients. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. A DLT was defined as any of the following that were related to OPB-51602 during the first treatment cycle: grade 3 nausea, vomiting, or diarrhea despite the use of antiemetic or anti-diarrheal drugs; any grade 3 non-hematologic toxicity, excluding alopecia; grade 4 neutropenia lasting ≥8 days (not applicable for leukemia); grade 3 febrile neutropenia or infection due to neutropenia (not applicable for leukemia); and grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring platelet transfusion (not applicable for leukemia).

Assessment of the treatment response was evaluated according to internationally recognized response criteria for MM, non-Hodgkin’s lymphoma, AML, or chronic myeloid leukemia (19–25).

**Pharmacokinetics.** Blood samples were collected for pharmacokinetic analysis in the first treatment cycle on days 1–4 and on days 28–31. The time course of the plasma concentration of OPB-51602 in this patient population.

**Results**

**Patients.** From May 2011 to August 2013, a total of 20 patients were enrolled in this study and treated with oral OPB-51602 (1 mg, n = 4; 2 mg, n = 3; 3 mg, n = 4; 4 mg, n = 6; 6 mg, n = 3).

**Table 1. Patients’ demographics and characteristics**

| Number of enrolled patients | n = 20 |
|-----------------------------|-------|
| Age, years, median (range)  | 64 (49–74) |
| Gender, n (%) (male/female) | 13 (65)/7 (35) |
| ECOG performance status, n (%) (0/1) | 20 (100)/0 |

**Prior chemotherapy**

| Median number of prior treatment regimens (range) | 3.5 (1–9) |

**Table 2. Treatment-related adverse events that occurred in ≥20% of patients with relapsed or refractory hematological malignancies treated with oral OPB-51602**

| Treatment-related adverse events | 1 mg (n = 4) | 2 mg (n = 3) | 3 mg (n = 4) | 4 mg (n = 6) | 6 mg (n = 3) | Total (n = 20) |
|---------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Nausea                          | 4 (100)     | 2 (33)      | 1 (33)      | 0 (0)       | 0 (0)       | 11 (55)     |
| Peripheral sensory neuropathy   | 2 (50)      | 3 (50)      | 3 (100)     | 3 (33)      | 0 (0)       | 9 (45)      |
| Diarrhea                        | 2 (50)      | 3 (50)      | 3 (100)     | 1 (33)      | 0 (0)       | 8 (40)      |
| Decreased appetite              | 3 (75)      | 3 (50)      | 1 (3)       | 0 (0)       | 0 (0)       | 1 (5)       |
| Anemia                          | 2 (50)      | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 2 (10)      |
| Malaise                         | 1 (25)      | 1 (17)      | 0 (0)       | 0 (0)       | 0 (0)       | 1 (5)       |
| Vomiting                        | 3 (75)      | 1 (33)      | 1 (33)      | 0 (0)       | 0 (0)       | 1 (5)       |
| Neutropenia                     | 2 (50)      | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 2 (10)      |
| Leukopenia                      | 2 (50)      | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 2 (10)      |
| Thrombocytopenia                | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       |
| Fatigue                         | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       | 0 (0)       |

**Gr, grade;**
Safety. The DLT analysis set consisted of 15 patients (three patients per dose) among the 20 patients enrolled in this study. The remaining five patients were excluded from the DLT analysis set because of early progression of disease or request from the patient to discontinue administration of OPB-51602 before completion of the first treatment cycle. No DLT was found in the 1- to 4-mg cohorts, but DLT did emerge in one of three patients in the 6-mg cohort. The DLT appeared as grade 3 lactic acidosis and an increased blood lactic acid level, which were observed on day 13 after the start of administration and quickly resolved with suitable medical intervention after discontinuation of OPB-51602.

During the first treatment cycle, grade 1–2 peripheral sensory neuropathy was also found in three patients in the 6-mg cohort. The frequency of peripheral sensory neuropathy tended to exacerbate in a dose-dependent manner, and because it was thought to influence long-term administration of OPB-51602, dose escalation was discontinued without supplementing the 6-mg cohort with an additional three patients. The MTD of OPB-51602 for hematological malignancies was estimated to be 6 mg, and the recommended dose was determined to be 4 mg.

Treatment-related AEs were reported in 18 patients (90%). Table 2 lists the treatment-related AEs that occurred in ≥20% of patients. The common treatment-related AEs were nausea (n = 11, 55%), peripheral sensory neuropathy (n = 9, 45%), diarrhea (n = 8, 40%), decreased appetite (n = 7, 35%), anemia (n = 6, 30%), malaise (n = 5, 25%), vomiting (n = 5, 25%), neutropenia (n = 5, 25%), leukopenia (n = 5, 25%), thrombocytopenia (n = 5, 25%), and fatigue (n = 4, 20%). Grade 3 or 4 drug-related AEs were neutropenia (n = 4, 20%), leukopenia (n = 3, 15%), lymphopenia (n = 2, 10%), thrombocytopenia (n = 2, 10%), anemia (n = 1, 5%), diarrhea (n = 1, 5%), increased blood lactic acid levels (n = 1, 5%), acidosis (n = 1, 5%), and hypophosphatemia (n = 1, 5%). Treatment-related AEs leading to discontinuation of treatment occurred in two patients (palpitations in one patient and acidosis, peripheral sensory neuropathy, and blood lactic acid increases in another patient) in the 6-mg cohort.

Responses. No objective response, including complete response or partial response, was obtained. Durable stable disease (>6 months) was observed in three patients: two patients with AML in the 1- and 2-mg cohorts and one patient with MM in the 4-mg cohort. The patient with acute megakaryoblastic leukemia (AML-M7) in the 1-mg cohort continued OPB-51602 administration for 8 months (eight cycles). Another patient with acute myelomonocytic leukemia (AML-M4) in the 2-mg cohort continued OPB-51602 administration for 15 months (15 cycles). The patient with MM in the 4-mg cohort continued OPB-51602 administration for 8 months (eight cycles).

Pharmacokinetics. OPB-51602 was rapidly absorbed after both single and multiple administrations. The median time to reach the maximum plasma concentration (t_{max}) was between 2.0 and 4.5 h (Table 3, Fig. 1). For the lower dose groups (the 1- and 2-mg cohorts), terminal-phase elimination half-life (t_{1/2,z}) was evaluable in a few patients due to low OPB-51602 plasma levels after single administration. The mean t_{1/2,z} was 50.1–240.0 h after multiple administrations. Exposure (area under the plasma concentration–time curve from 0 to 24 h [AUC_{24}]) and maximum plasma drug concentration [C_{max}] tended to increase in a dose-dependent manner following mul-

---

Table 3. Pharmacokinetic parameters (mean) of OPB-51602 after single and repeated dosing in patients with relapsed or refractory hematological malignancies

| Dose | 1 mg | 2 mg | 3 mg | 4 mg | 6 mg |
|------|------|------|------|------|------|
| n    | 3    | 2    | 4    | 4    | 2    |
| t_{max} (h) | 0.3 | 0.2 | 0.7 | 3.8 | 2.2 |
| AUC_{24} h | 0.8 | 1.0 | 3.0 | 18.0 | 10.6 |
| t_{1/2,z} (h) | 6.6 | 4.6 | 4.6 |

†Median; 1; n = 2; n = 3. Not calculated, plasma concentrations below the lower limit of quantification were set to 0 ng/mL; AUC_{24}, area under the plasma concentration–time curve from 0 to 24 h; C_{max}, maximum plasma drug concentration; n, number of patients; t_{1/2,z}, terminal-phase elimination half-life; t_{max}, time to maximum plasma concentration.

---

Fig. 1. Time-course of the mean plasma concentration of OPB-51602 after single administration (a) and multiple administrations on day 28 (b). Plasma concentrations below the lower limit of quantification were considered to be 0 ng/mL. Values are the mean ± SD.
multiple administrations across the dose range from 1 to 4 mg (Table 3). We observed accumulation of OPB-51602 with a 2.6–10.2-fold increase in AUC$_{24\text{h}}$ and a 1.4–4.6-fold increase in C$_{\text{max}}$ with doses of 1, 2, 3, and 4 mg.

**Phosphorylated STAT levels.** Pharmacodynamic effects of OPB-51602 on the expression of phosphorylated STAT3 at tyrosine 705 (pY705-STAT3) were measured using immunostaining. pY705-STAT3 was assessed in 16 patients (Table 4). Four patients (follicular lymphoma, patient number 001S003; AML, patient numbers 003S003 and 003S009; and diffuse large B-cell lymphoma, patient number 004S003) were positive for pY705-STAT3 before treatment with OPB-51602. Among these four positive patients, the pY705-STAT3 status changed from positive to negative after OPB-51602 treatment in one patient with AML-M4 (patient number 003S003) in the 2-mg cohort. This patient experienced long-term stable disease. Immunostaining for pY705-STAT3 after treatment with OPB-51602 was not carried out in two patients (patient numbers 003S009 and 004S003) because these patients discontinued OPB-51602 administration within cycle 1 due to progressive disease or DLT. The relationship between pY705-STAT3 and efficacy (antitumor effect) was not investigated due to the lack of response.

**Discussion**

The Jak–STAT pathway is activated in most hematopoietic tumors and is considered a good target for anticancer ther-

Table 4. Results of immunostaining for phosphorylated signal transducer and activator of transcription 3 (STAT3) in patients with relapsed or refractory hematological malignancies treated with oral OPB-51602

| Patient no. | Dose (mg) | Tumor type | Sampling point | Type of sample | Result of pSTAT3 † |
|-------------|-----------|------------|----------------|----------------|-------------------|
| 002S001     | 1         | MM         | Baseline       | BMCLOT         | Negative          |
| 002S003     | 2         | AITL       | Baseline       | LN             | Negative          |
| 003S003     | 2         | AML-M4     | Baseline       | BMCLOT         | Positive          |
|             |           |            | End of cycle 1 | BMCLOT         | Negative          |
|             |           |            | End of cycle 2 | BMCLOT         | Negative          |
|             |           |            | End of cycle 3 | BMCLOT         | Negative          |
|             |           |            | End of cycle 4 | BMCLOT         | Positive          |
|             |           |            | End of cycle 5 | BMCLOT         | ND                |
|             |           |            | End of cycle 6 | BMCLOT         | Positive          |
|             |           |            | End of cycle 7 | BMCLOT         | Positive          |
|             |           |            | End of cycle 8 | BMCLOT         | Negative          |
|             |           |            | End of cycle 10| BMCLOT         | Positive          |
|             |           |            | End of cycle 11| BMCLOT         | Negative          |
|             |           |            | End of cycle 12| BMCLOT         | Negative          |
|             |           |            | End of cycle 13| BMCLOT         | Positive          |
|             |           |            | End of cycle 14| BMCLOT         | Negative          |
|             |           |            | End of cycle 15| BMCLOT         | Positive          |
|             |           |            | End of cycle 16| BMCLOT         | Positive          |
| 002S004     | 3         | DLBCL      | Baseline       | LN             | Negative          |
| 002S005     | 3         | DLBCL      | Baseline       | BMCLOT         | Negative          |
| 003S005     | 3         | AML-M2     | Baseline       | BMCLOT         | Negative          |
| 004S001     | 3         | FL         | Baseline       | LN             | Negative          |
| 002S006     | 4         | MM         | Baseline       | BMCLOT         | Negative          |
| 002S007     | 4         | FL         | Baseline       | LN             | Negative          |
| 003S006     | 4         | DLBCL      | Baseline       | BMCLOT         | N.D.              |
| 003S007     | 4         | MM         | Baseline       | BMCLOT         | Negative          |
| 003S008     | 4         | AML-M2     | Baseline       | BMCLOT         | Negative          |
| 003S009     | 4         | AML-M6     | Baseline       | BMCLOT         | Positive          |
| 001S003     | 6         | FL         | Baseline       | LN             | Negative          |
| 004S003     | 6         | DLBCL      | Baseline       | BMCLOT         | Positive          |
| 006S001     | 6         | FL         | Baseline       | BMCLOT         | Negative          |

†Positive pSTAT3 at baseline is shown in italics. AITL, angioimmunoblastic T-cell lymphoma; AML, acute myeloid leukemia; BMCLOT, bone marrow clotted sample; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; LN, lymph node biopsy sample; MM, multiple myeloma; ND, not determined; NHL, non-Hodgkin’s lymphoma; pSTAT3, pY705-STAT3.
apy.\(^{(23,24)}\) The Jak2-specific inhibitor ruxolitinib has been approved for myelofibrosis as an anticancer drug that targets the Jak-STAT pathway.\(^{(25,26)}\) In cancer, however, STAT3 is phosphorylated not only by Jak, but also by other growth factor signals and oncogenic non-receptor-type tyrosine kinases such as Src.\(^{(4,5)}\) STAT3 has both tyrosine and serine phosphorylation sites, and a tyrosine kinase inhibitor such as ruxolitinib can suppress tyrosine phosphorylation by Jak1/2, but not phosphorylation on serine.\(^{(27)}\) OPB-51602 is an inhibitor that targets STAT3 and cannot inhibit phosphorylation on both tyrosine and serine. Thus, OPB-51602 was expected to act as a STAT inhibitor with a different profile from the Jak inhibitors.

In the present phase I, multicenter, open-label, dose-escalation study of oral OPB-51602 as a single agent, OPB-51602 was reasonably well-tolerated with an oral dose of 1–4 mg in patients with relapsed or refractory hematologic malignancies. However, when patients were treated with OPB-51602 at 6 mg, none of the patients remained in the study for 28 days due to grade 3 or 4 treatment-related AEs including neutropenia, diarrhea, acidosis, and hypophosphatemia. These results indicate that long-term administration with OPB-51602 at higher than the recommended dose (4 mg) may be difficult. Goh et al. reported a phase I study of OPB-51602 against solid tumors using a regimen of 2 weeks of administration followed by a 1-week washout period. The main signs of toxicity were nausea/vomiting, diarrhea, peripheral neuropathy, and fatigue.\(^{(28)}\) Although the treatment schedule differed, the findings of this study are consistent with that report, and a similar safety profile was observed in patients with hematological malignancies.

In the above study, Goh et al.\(^{(28)}\) also reported that after administration of OPB-51602, the expression of pY705-STAT3 was significantly decreased in human peripheral monocytes of solid tumor patients. The expression of pY705-STAT3 was measured in tumor samples in this study, but almost no pY705-STAT3 expression was detected, and no clear decrease after administration was found in the measurable samples. A change in pY705-STAT3 expression after OPB-51602 administration was observed in one patient in the 2-mg cohort. Expression of pY705-STAT3 returned to positive during OPB-51602 administration in this patient. One reason may be that OPB-51602 did not completely inhibit pY705-STAT3 expression completely because the dose of OPB-51602 was low. As described in Goh et al.’s phase I trial, decreased pY705-STAT3 expression was observed in the 4-mg cohort. The details of the reason for this are not clear because no change in pY705-STAT3 expression was observed in the 4-mg or higher cohorts. No clear difference was observed in pharmacokinetic profiles between this study and Goh et al.’s phase I study. We have no data regarding the concentration of OPB-51602 in PBMCs or tumor cells from patients. However, in tumor-bearing mice, the concentrations of OPB-51602 in tumor tissue were higher than in plasma (Otsuka Pharmaceuticals Co., Ltd, unpublished data). From those results and the range of concentrations in which OPB-51602 showed an inhibitory effect against pY705-STAT3 expression in vitro, the concentration of OPB-51602 in tumor cells of patients reached a concentration that is expected to decrease pY705-STAT3 expression. The possibility also remains that inhibition of STAT activation may not lead to antitumor efficacy. However, in order to clarify the effectiveness of OPB-51602, the study population may be required to be restricted to patients with an activated Jak-STAT pathway.

In conclusion, the MTD of OPB-51602 was considered to be ≥6 mg, and OPB-51602 was shown to be safe and well tolerated in a dose range of 1–4 mg. However, long-term administration at higher doses was difficult with a daily dosing schedule, and no therapeutic response was seen. Therefore, further clinical development of OPB-51602 for hematological malignancies with a daily dosing schedule was terminated. Questions still remain unanswered, such as the relationship between STAT inhibition and antitumor efficacy, the optimal patient population, and the optimal dose and schedule, but other STAT inhibitors are currently under development and are expected to clarify these details.

Acknowledgments

The authors would like to thank the patients, their family members, doctors, nurses, and staff members who participated in this trial. We are also grateful to Dr. Kiyoshi Kitamura, Dr. Noriko Usui, and Dr. Norio Komatsu for their helpful advice as members of the Independent Data and Safety Monitoring Board. This study was sponsored by Otsuka Pharmaceutical Co., Ltd.

Disclosure Statement

The authors have no conflict of interest.
18 Germain D, Frank DA. Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy. Clin Cancer Res 2007; 13: 5665–9.
19 Durie BG, Harousseau JL, Miguel JS et al. International uniform response criteria for multiple myeloma. Leukemia 2006; 20: 1467–73.
20 Cheson BD, Bennett JM, Kopecky KJ et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 2003; 21: 4642–9.
21 Cheson BD, Horning SJ, Coiffier B et al. Report of an international workshop to standardize response criteria for non-Hodgkin’s lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999; 17: 1244–53.
22 Baccarani M, Saglio G, Goldman J et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood 2006; 108: 1809–20.
23 Yu H, Lee H, Herrmann A, Buettner R, Iove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer 2014; 14: 736–46.
24 Al Zaid Siddiquee K, Turkson J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. Cell Res 2008; 18: 254–67.
25 Harrison C, Kiladjian J-J, Al-Ali HK et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med 2012; 366: 787–98.
26 Verstovsek S, Mesa RA, Gotlib J et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med 2012; 366: 799–807.
27 Rozovski U, Wu JY, Harris DM et al. Stimulation of the B-cell receptor activates the JAK2/STAT3 signaling pathway in chronic lymphocytic leukemia cells. Blood 2014; 123: 3797–802.
28 Goh BC, Wong ALA, Soo RA et al. Phase I study of OPB51602, a small molecule inhibitor of STAT3 phosphorylation, in patients with refractory solid malignancies. J Clin Oncol. 2012; 30: 173s, (abstr 3002).