Doc S1. Additional methods and pharmacokinetic results

A phase I study of tirabrutinib (ONO-4059/GS-4059) in patients with relapsed or refractory B-cell malignancies in Japan

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METHODS

Definition of dose-limiting toxicity

Dose-limiting toxicity (DLT) criteria were defined as the adverse events (AEs), stipulated below, that occurred in Cycle 1 and for which a drug relationship cannot be ruled out (AEs for which the relationship to the study drug has been determined to be “definitely related,” “probably related,” or “possibly related”). If the study was prematurely terminated or suspended for a patient for a reason other than DLT during DLT evaluation, a new patient was enrolled in the relevant cohort for evaluation:

- Thrombocytopenia requiring platelet transfusion or Grade 4 thrombocytopenia accompanied by spontaneous hemorrhage (<25,000/mm^3)
- Febrile neutropenia that occurred when not using supportive care with granulocyte colony-stimulating factor (G-CSF) (neutrophil count <1000/mm^3 and fever >38.3°C)
- Grade 4 neutropenia persisting for at least 8 days when not using supportive care with G-CSF (neutrophil count <500/mm^3)
- Other Grade 4 or greater hematologic toxicities and Grade 3 or greater non-hematologic toxicities.

Criteria for discontinuation or interruption, resumption, and dose modifications

If a patient experienced any AE during Cycle 1 that met the DLT criteria and required follow-up for determination of causality, the study treatment for the patient was interrupted, and the patient was followed up. If the potential benefits of resumed or continued treatment with tirabrutinib outweighed the possible risks, the study treatment was resumed after consultation with the sponsor while carefully monitoring the patient's condition, even if at least 14 days were required for recovery (disappearance of the AE or relief to equal or lower grade compared with the pre-onset state).

If the AE leading to interruption of the study treatment was adequately controlled from a medical point of view, and the potential benefits of resuming the study treatment outweighed the risks of the AE, the study treatment was resumed at the same dose used before interruption. If the investigator and sub-investigator decided to resume the study treatment at a lower dose than that used before the interruption, the study treatment was resumed from a lower dose, followed by dose escalation to the dose level that was used before interruption. In this trial, a dose reduction to 160 mg/day was allowed, as necessary, for use as a maintenance dose in individual patients.

If a patient fulfilled the following criteria after completion of the DLT-evaluation period, the patient was allowed to proceed to the extended treatment period by maintaining the same dose after providing informed consent again:

1. Patients who experience no DLT (in consideration of ethical aspects, continuation of the study treatment was considered even in patients who experience a DLT).
2. Other patients whom the investigator deems eligible to proceed to the extended treatment period.

After determination of a clinically recommended dose of tirabrutinib based on the results of late-phase clinical development, the dose was increased (or decreased) to the clinically recommended dose in the subsequent cycle and thereafter, at the discretion of the investigator. If a dose reduction was required after a dose increase due to the onset of an

adverse drug reaction, the dose was returned to the level used during participation in the trial.

When a patient met any of the following criteria, the investigator immediately discontinued the patient's study treatment:

1. When the patient wishes to discontinue participation in the study.
2. When the patient is found to be pregnant.
3. Progressive disease.
4. When the patient does not meet all of the inclusion criteria.
5. When the patient meets any of the exclusion criteria.
6. When any serious non-compliance with the study procedure is detected.
7. Onset of a DLT (only during the DLT-evaluation period).

   However, the study treatment can be resumed after the patient fulfils the criteria presented in Section 8.2 of the study protocol, Criteria for Interruption, Resumption, and Dose Modifications of the Study Drug. At the time of resumption of the study treatment after onset of a DLT, the patient's informed consent should be obtained again.
8. When the investigator or sub-investigator determines that continued study treatment for the patient is difficult because of the onset of an AE, regardless of the AE's causal relationship to the study drug.
9. When the patient fails to return for visits, and continued study treatment becomes impossible.
10. Other patients whom the investigator or sub-investigator deem inappropriate to continue the trial.

Pharmacodynamics assay

The blood sample at pre-dose on Day 1 of Cycle 1 was obtained at screening. A blood sample was taken pre-dose on Days 2, 8, and 28 of Cycle 1 and pre-dose on Day 1 of Cycle 3. For patients who discontinued or dropped out of the trial before completing Cycle 6, a

blood sample was taken at the time of discontinuation. Samples from patients were heated at 95°C for 5 minutes, and then separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (10%) and transferred onto a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). Anti-pBTK antibody (Abcam, Cambridge, MA, USA), anti-BTK antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-β-actin antibody (Sigma-Aldrich) were used as primary antibodies. Secondary antibodies used were HRP-labeled anti-rabbit IgG (H+L) antibody (Life Technologies, Carlsbad, CA, USA) and HRP-labeled anti-mouse IgG (γ) antibody (KPL, Milford, MA, USA). After washing, Chemi-Lumi One L (Nacalai Tesque, Inc., Kyoto, Japan) or ECL prime (GE Healthcare Biosciences, Piscataway, NJ, USA) was added onto the membrane, and the bands were detected by LAS-3000 imaging analyzer. Cells were incubated with a cell extraction buffer (Thermo Scientific, Rockford, IL, USA) solution containing 1 mM phenylmethanesulfonyl fluoride (Sigma-Aldrich, St. Louis, MO, USA) and Halt Protease Inhibitor Single-Use Cocktail [100×] (Thermo Scientific) following the manufacturer's instructions.

RESULTS

Pharmacokinetics

The tirabrutinib C_{max} on Day 1 was 611, 1220, 1280, and 886 ng/mL for Cohorts 1–4, respectively, and these were reached 2.0 to 3.0 h after treatment. The C_{max} on Day 28 after the first dosing was 484, 971, 1940, and 961 ng/mL for Cohorts 1–4, respectively. Systemic tirabrutinib exposure, as measured by the area under the curve (AUC_{12h}) on Day 1, was 3030, 5090, 7050, and 5000 ng•h/mL for Cohorts 1–4, respectively. The AUC_{12h} on Day 28 was 2720, 5530, 13,000, and 5880 ng•h/mL, respectively. The increases in C_{max} and AUC were approximately dose-proportional from 160 to 480 mg QD. The mean $T_{1/2}$ of tirabrutinib was comparable across dose levels, with a $T_{1/2}$ of approximately 4.7, 4.8, 6.2, and 4.9 h on Day 1 for Cohorts 1–4, respectively. On Day 28, the $T_{1/2}$ was approximately 5.4, 4.7, 3.9, and 6.5 h for Cohorts 1–4, respectively.