**Supplemental Materials**

**Study design and patient eligibility**

In Part A, eligible patients received ralimetinib Q12H at dose levels ranging from 10 to 560 mg either as capsules or tablets on days 1-14 on a 28-day cycle until the criteria for maximum tolerated dose (MTD) were met. In Part B, patients were treated with 420 mg Q12H of ralimetinib on days 1-14 on a 28-day cycle. During the DDI sub-study, patients received 2 mg of midazolam orally 2 days before starting ralimetinib and again with the morning dose of ralimetinib on day 8 in cycle 1 only. Dosing with ralimetinib continued uninterrupted on days 1-14 in Part B. After review of the Part B safety data, the dose was de-escalated and Part C) was initiated to obtain additional data on the recommended phase II dose, safety, PK/PD and efficacy of 300 mg Q12H of ralimetinib on days 1-14 on a 28-day cycle. Part D consisted of an expansion cohort of patients with hormone receptor-positive metastatic breast cancer with prior progression or intolerance to aromatase inhibitors who received 200 or 300 mg of ralimetinib Q12H days 1-14 in combination with tamoxifen 20 mg every 24 hours administered on days 1-28 on a 28-day cycle.

**Pharmacokinetic studies**

Ralimetinib drug concentrations were determined for all patients using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. For ralimetinib, the lower limit of quantification was 1 ng/mL and the upper limit of quantification was 100 ng/mL. The interassay accuracy (% relative error) during validation ranged from -5.00% to 3.36% and the interassay precision (% relative SD) during validation was ≤5.26%.  For midazolam and 1’‑hydroxymidazolam, the lower limit of quantitation was 0.1 ng/mL and the upper limit of quantitation was 100 ng/mL. The interassay accuracy (% relative error) during validation ranged from –11.8% to –1.3% for midazolam and from –15.5% to 2.0% for 1’‑hydroxymidazolam.  The interassay precision (% relative SD) during validation was ≤17.9% for midazolam and ≤18.3% for 1’‑hydroxymidazolam. For tamoxifen and its 2 metabolites, the lower limit of quantification was 0.5 ng/mL and the upper limit of quantification was 500 ng/mL.  For tamoxifen, the interassay accuracy (% relative error) ranged from -1.6% to 0.5% and the interassay precision (% relative SD) ranged from 1.8% to 9.9%.  For 4-hydroxytamoxifen, the interassay accuracy (% relative error) ranged from 1.7% to 6.3% and the interassay precision (% relative SD) ranged from ‑0.4% to 3.3%.  For endoxifen, the interassay accuracy (% relative error) ranged from ‑9.2% to ‑1.5% and the interassay precision (% relative SD) ranged from 3.2% to 10.1%.

**Pharmacodynamic studies**

Intracellular levels of pMAPKAP-K2 were measured in peripheral blood mononuclear cells by flow cytometry following methodology previously published by Esoterix (Esoterix, US Patent No. 7326577). Briefly, CD3-FITC (BD Biosciences #349201) and CD14-PE (BD Pharmingen #555398) were added to plasma from patients. The samples were then fixed (Esoterix, US Patent No. 7326577), and pMAPKAPK-2-Alexa Fluor 647 (Cell Signaling Technology #4320) was added.  The samples were then analyzed on FACSCalibur flow cytometer (BD Biosciences) and pMAPKAP-K2 levels were assessed in the CD14 positive monocyte population.

**Table S1. Summary of baseline pathological diagnosis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristic** | **Part A****n=54** | **Part B****n=18** | **Part C****n=8** | **Part D****n=9** | **Total****n=89** |
| Tumor type [n (%)] |  |  |  |  |  |
| Colorectal | 18 (33.3) | 6 (33.3) | 1 (12.5) | - | 25 (28.1) |
| Breast | 3 (5.6) | 4 (22.2) | 3 (37.5) | 9 (100.0) | 19 (21.3) |
| Sarcoma | 7 (13.0) | 3 (16.7) | 1 (12.5) | - | 11 (12.4) |
| NSCLC | 3 (5.6) | 2 (11.1) | 1 (12.5) | - | 6 (6.7) |
| Pancreas | 2 (3.7) | 1 (5.6) | 2 (25.0) | - | 5 (5.6) |
| Renal | 4 (7.4) | 1 (5.6) | - | - | 5 (5.6) |
| Melanoma | 3 (5.6) | - | - | - | 3 (3.4) |
| Ovary | 2 (3.7) | - | - | - | 2 (2.2) |
| Other | 12 (22.2) | 1 (5.6) | - | - | 13 (14.6) |

**Table S2 Noncompartmental Pharmacokinetic Summary Following Oral Administration of ralimetinib on Day 1 and on Day 14 (Cycle 1) – Capsule Formulation**

|  |  |  |
| --- | --- | --- |
|  |  | **Geometric Mean (CV%)** |
|  |  | **Cohort 1 (10 mg Q12H)** | **Cohort 2 (20 mg Q12H)** | **Cohort 3 (40 mg Q12H)** | **Cohort 4 (65 mg Q12H)** | **Cohort 5 (90 mg Q12H)** | **Cohort 6 (120 mg Q12H)** | **Cohort 7 (160 mg Q12H)** | **Cohort 8 (200 mg Q12H)** |
| **Day 1** | **n** | **4** | **3** | **3** | **3** | **5** | **3** | **3** | **7** |
|  | Cmax(ng/mL) | 14.7(75) | 90.1(75) | 144(83) | 135(87) | 202(62) | 405(68) | 606(53) | 673(55) |
| tmaxa(hr) | 2.51(1.00–6.00) | 1.00(0.52–7.57) | 1.02(1.00–1.02) | 2.02(2.00–6.03) | 2.00(1.00–3.00) | 1.05(1.00–2.95) | 2.03(1.00–3.00) | 2.00(1.00–3.02) |
| AUC(0-8)(ng·hr/mL) | 55.6(36) | 257(26) | 375(79) | 445(83) | 707(45) | 1310(33) | 2120(39) | 2550(55) |
| **Day 14** | **n** | **3** | **3** | **3** | **3** | **3** | **3** | **3** | **6** |
|  | Cmax(ng/mL) | 28.4(63) | 81.4(63) | 252(144) | 415(14) | 208(74) | 530(38) | 1930(64) | 1330(56) |
| tmaxa(h) | 1.03(1.00–2.00) | 1.00(1.00–4.05) | 1.00(0.98–1.98) | 1.00(0.98–1.00) | 2.00(1.97–2.00) | 1.05(0.98–2.00) | 1.02(0.50–2.03) | 1.50(1.00–3.00) |
| AUC(0-8)(ng·hr/mL) | 151(83) | 372(28) | 1070(130) | 1300(13) | 827(61) | 1960(50) | 6000(29) | 6220(42) |
| AUC(0-24,ss)b(ng·hr/mL) | 407(87) | 1020(22) | 2840(133) | 3220(18) | 2230(69) | 5150(48) | 14900(27) | 17000(42) |
| CLss/F(L/hr) | 49.1(87) | 39.4(22) | 28.1(133) | 40.4(18) | 80.9(69) | 46.6(48) | 21.5(27) | 23.5(42) |
| Vdss/F(L) | 3850(72) | 4500(122) | 3080(180) | 1510(45) | 5600(74) | 1640(79) | 1010(11) | 1860(61) |
| t1/2(hr) | 66.3(99) | 284(140) | 123(13) | 71.5(120) | 93.3(18) | 44.0(172) | 61.3(11) | 113(71) |
| RAc | 2.52(65) | 1.45(51) | 2.84(35) | 2.93(65) | 1.30(6) | 1.49(44) | 2.82(58) | 2.45(38) |

Abbreviations: AUC(0-8) = area under the baseline-corrected serum concentration versus time curve from time zero to 8 hours; AUC(0‑12,ss) = area under the baseline-corrected serum concentration versus time curve from time zero to 12 hours at steady state; AUC(0-24,ss) = area under the baseline-corrected serum concentration versus time curve from time zero to 24 hours at steady state; CLss/F = apparent total body clearance at steady state after extra-vascular administration; Cmax = maximum plasma concentration; CV% = coefficient of variation; Q12H = every 12 hours; RA = accumulation ratio; t1/2 = terminal half-life; tmax = time to reach Cmax; Vdss/F = apparent volume of distribution at steady state after extra-vascular administration.

a Median (range).

b AUC(0-24,ss) represents the sum of AUC(0-12,ss) + AUC(0-12,ss).

c RA: accumulation ratio between Day 1 and Day 14 of Cycle 1 (Ratio of AUC[0-8] [Day 14]/AUC[0-8] [Day 1]).

**Table S3 Noncompartmental Pharmacokinetic Summary Following Oral Administration of ralimetinib on Day 1 and on Day 14 (Cycle 1) – Tablet Formulation**

|  |  |  |
| --- | --- | --- |
|  |  | **Geometric Mean (CV%)** |
|  |  | **Cohort 10 (160 mg Q12H)** | **Cohort 11 (200 mg Q12H)** | **Cohort 12 + Part C (300 mg Q12H)** | **Cohort 13 + Part B (420 mg Q12H)** | **Cohort 14 (560 mg Q12H)** |
| **Day 1** | **n** | **3** | **3** | **11** | **21** | **5** |
|  | Cmax(ng/mL) | 454(18) | 636(78) | 1020(58) | 1700(71) | 2360(28) |
| tmaxa(hr) | 2.00(1.00–2.00) | 1.00(0.50–1.00) | 1.00(0.98–3.00) | 1.00(0.50–8.00) | 1.98(1.00–2.07) |
| AUC(0-8) (ng·hr/mL) | 1750(20) | 1720(70) | 3460(51) | 5780(57) | 8880(37) |
| **Day 14** | **n** | **3** | **3d** | **9e** | **14f** | **1** |
|  | Cmax(ng/mL) | 574(11) | 963(82) | 1400(81) | 2230(53) | 3060 |
| tmaxa(hr) | 1.00(0.98–1.00) | 1.00(0.55–1.03) | 2.02(1.00–11.72) | 1.53(0.50–4.00) | 3.00 |
| AUC(0-8) (ng·hr/mL) | 2810(22) | 2140 - 7090 | 6620(85) | 10200(55) | 15200 |
| AUC(0-24,ss)b (ng·hr/mL) | 7590(30) | 4920 – 17600 | 17900(85) | 26200(57) | 40500 |
| CLss/F(L/hr) | 42.2(30) | 22.7 – 81.3 | 33.6(85) | 32.1(57) | 27.7 |
| Vdss/F(L) | 2580(44) | 1280 – 2250 | 2130(100) | 1620(165) | 3930 |
| t1/2(hr) | 86.8(27) | 111 – 193 | 92.6(46) | 77.4(150) | 193 |
| RAc | 1.60(17) | 0.948 – 2.63 | 1.89(33) | 1.79(28) | 1.52 |

Abbreviations: AUC(0-8) = area under the baseline-corrected serum concentration versus time curve from time zero to 8 hours; AUC(0‑12,ss) = area under the baseline-corrected serum concentration versus time curve from time zero to 12 hours at steady state; AUC(0-24,ss) = area under the baseline-corrected serum concentration versus time curve from time zero to 24 hours at steady state; CLss/F = apparent total body clearance at steady state after extra-vascular administration; Cmax = maximum plasma concentration; CV = coefficient of variation; PK = pharmacokinetic; Q12H = every 12 hours; RA = accumulation ratio; t1/2 = terminal half-life; tmax = time to reach Cmax; Vdss/F = apparent volume of distribution at steady state after extra-vascular administration.

a Median (range).

b AUC(0-24,ss) represents the sum of AUC(0-12,ss) + AUC(0-12,ss).

c RA: accumulation ratio between Day 1 and Day 14 of Cycle 1 (ratio of AUC[0-8] [Day 14]/AUC[0-8] [Day 1]).

d N=2 for all parameters except Cmax and tmax. Individual PK parameters reported as a range after excluding one patient due to missing results in terminal phase.

e N=8 for all parameters except t1/2. Excluded PK parameters other than t1/2 for one patient due to missing PK results at early sampling time points.