**Supporting Information**

# Preclinical Pharmacokinetics of the Marine-Derived PDE4 Inhibitor LY104 and Its Active Metabolite M1 in Beagle Dogs Following Intravenous Infusion

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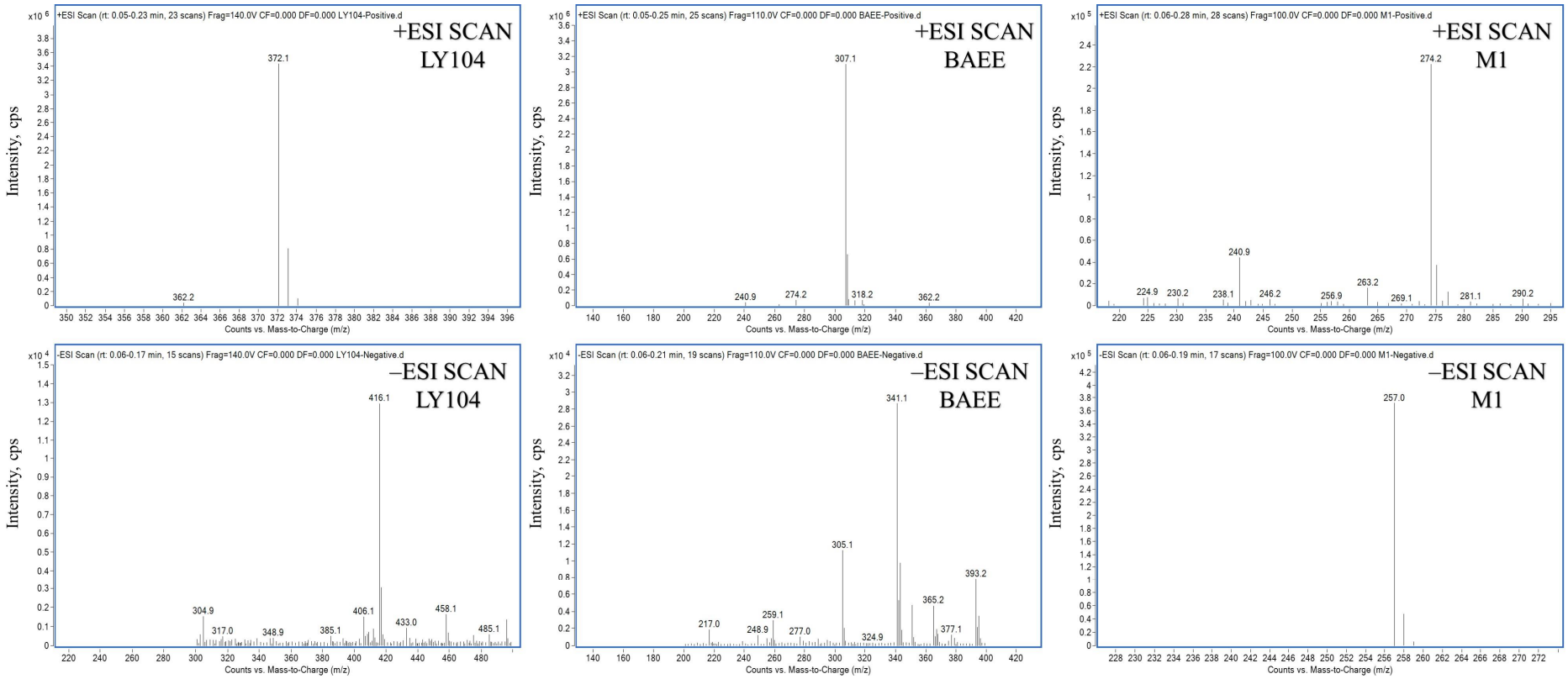



**Figure S1.** Representative full-scan mass spectra of LY104, BAEE, and M1 in positive (+ESI) and negative (−ESI) electrospray ionization modes.

**Table S1.** Inter- and intra-batch precision and accuracy for LY104.

| Detection Time | Run batch number | LLOQ (20 ng/mL) | RE% | LQC (40 ng/mL) | RE% | MQC (800 ng/mL） | RE% | HQC (1800 ng/mL） | RE% |
|---|---|---|---|---|---|---|---|---|---|
| 2024.04.08 | LY104-dog-plasma-20240408-run1 | 20.51 | 2.53 | 42.55 | 6.37 | 789.62 | -1.30 | 1563.69 | -13.13 |
| | | 19.86 | -0.68 | 42.52 | 6.30 | 778.32 | -2.71 | 1595.03 | -11.39 |
| | | 19.42 | -2.90 | 39.47 | -1.33 | 840.51 | 5.06 | 1558.04 | -13.44 |
| | | 22.31 | 11.57 | 42.09 | 5.24 | 790.02 | -1.25 | 1541.61 | -14.35 |
| | | 18.60 | -7.02 | 41.18 | 2.94 | 808.51 | 1.06 | 1582.89 | -12.06 |
| | | 19.83 | -0.83 | 41.45 | 3.63 | 779.56 | -2.56 | 1708.14 | -5.10 |
| Mean | | 20.09 | | 41.54 | | 797.76 | | 1591.57 | |
| SD (intra-batch) | | 1.26 | | 1.16 | | 23.57 | | 60.12 | |
| %CV (intra-batch) | | 6.26 | | 2.79 | | 2.96 | | 3.78 | |
| RE% (intra-batch) | | 0.44 | | 3.86 | | -0.28 | | -11.58 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| 2024.04.08 | LY104-dog-plasma-20240408-run2 | 21.31 | 6.54 | 45.80 | 14.50 | 836.71 | 4.59 | 1707.40 | -5.14 |
| | | 17.07 | -14.65 | 40.80 | 2.00 | 741.59 | -7.30 | 1736.34 | -3.54 |
| | | 18.36 | -8.18 | 41.98 | 4.94 | 787.96 | -1.51 | 1681.04 | -6.61 |
| | | 17.22 | -13.89 | 41.70 | 4.26 | 786.32 | -1.71 | 1620.04 | -10.00 |
| | | 19.77 | -1.16 | 42.31 | 5.77 | 836.27 | 4.53 | 1582.70 | -12.07 |
| | | 20.39 | 1.94 | 42.84 | 7.11 | 829.87 | 3.73 | 1620.41 | -9.98 |
| Mean | | 19.02 | | 42.57 | | 803.12 | | 1657.99 | |
| SD (intra-batch) | | 1.74 | | 1.72 | | 38.05 | | 59.38 | |
| %CV (intra-batch) | | 9.15 | | 4.04 | | 4.74 | | 3.58 | |
| RE% (intra-batch) | | -4.90 | | 6.43 | | 0.39 | | -7.89 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| 2024.04.09 | LY104-dog-plasma-20240409-run1 | 19.76 | -1.21 | 40.32 | 0.79 | 791.58 | -1.05 | 1592.35 | -11.54 |
| | | 18.28 | -8.61 | 39.43 | -1.42 | 773.55 | -3.31 | 1630.32 | -9.43 |
| | | 19.38 | -3.12 | 40.43 | 1.07 | 799.77 | -0.03 | 1609.78 | -10.57 |
| | | 20.07 | 0.36 | 38.28 | -4.29 | 795.85 | -0.52 | 1655.88 | -8.01 |
| | | 19.88 | -0.60 | 39.56 | -1.09 | 784.60 | -1.93 | 1575.41 | -12.48 |
| | | 20.51 | 2.56 | 40.68 | 1.69 | 788.80 | -1.40 | 1604.08 | -10.88 |
| Mean | | 19.65 | | 39.78 | | 789.03 | | 1611.30 | |
| SD (intra-batch) | | 0.77 | | 0.88 | | 9.25 | | 28.47 | |
| %CV (intra-batch) | | 3.90 | | 2.22 | | 1.17 | | 1.77 | |
| RE% (intra-batch) | | -1.77 | | -0.54 | | -1.37 | | -10.48 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| Mean (three batches) | | 19.58 | | 41.30 | | 796.63 | | 1620.29 | |
| SD (inter-batch) | | 1.32 | | 3.46 | | 17.42 | | 83.55 | |
| %CV (inter-batch) | | 6.72 | | 8.37 | | 2.19 | | 5.16 | |
| RE% (inter-batch) | | -2.08 | | 3.25 | | -0.42 | | -9.98 | |
| n | | 18 | | 18 | | 18 | | 18 | |

**Table S2.** Inter- and intra-batch precision and accuracy for M1.

| Detection Time | Run batch number | LLOQ (20 ng/mL) | RE% | LQC (40 ng/mL) | RE% | MQC (800 ng/mL） | RE% | HQC (1800 ng/mL） | RE% |
|---|---|---|---|---|---|---|---|---|---|
| 2024.04.08 | LY104-dog-plasma-20240408-run1 | 26.27 | 31.36 | 40.19 | 0.49 | 841.68 | 5.21 | 1760.40 | -2.20 |
| | | 20.76 | 3.81 | 43.24 | 8.11 | 810.85 | 1.36 | 1728.08 | -4.00 |
| | | 21.56 | 7.82 | 41.65 | 4.13 | 803.18 | 0.40 | 1731.88 | -3.78 |
| | | 22.61 | 13.07 | 44.31 | 10.78 | 813.23 | 1.65 | 1680.06 | -6.66 |
| | | 22.04 | 10.18 | 44.35 | 10.87 | 832.75 | 4.09 | 1740.72 | -3.29 |
| | | 20.92 | 4.59 | 40.98 | 2.44 | 823.69 | 2.96 | 1698.23 | -5.65 |
| Mean | | 22.36 | | 42.45 | | 820.90 | | 1723.23 | |
| SD (intra-batch) | | 2.04 | | 1.77 | | 14.52 | | 29.24 | |
| %CV (intra-batch) | | 9.11 | | 4.16 | | 1.77 | | 1.70 | |
| RE% (intra-batch) | | 11.80 | | 6.14 | | 2.61 | | -4.27 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| 2024.04.08 | LY104-dog-plasma-20240408-run2 | 20.56 | 2.81 | 43.35 | 8.36 | 800.31 | 0.04 | 1729.31 | -3.93 |
| | | 21.34 | 6.69 | 39.83 | -0.43 | 741.18 | -7.35 | 1802.70 | 0.15 |
| | | 18.91 | -5.47 | 45.23 | 13.08 | 796.51 | -0.44 | 1721.34 | -4.37 |
| | | 22.09 | 10.46 | 38.29 | -4.28 | 760.24 | -4.97 | 1701.55 | -5.47 |
| | | 21.61 | 8.05 | 40.89 | 2.23 | 775.89 | -3.01 | 1599.58 | -11.13 |
| | | 19.94 | -0.32 | 41.00 | 2.51 | 815.06 | 1.88 | 1658.07 | -7.88 |
| Mean | | 20.74 | | 41.43 | | 781.53 | | 1702.09 | |
| SD (intra-batch) | | 1.18 | | 2.49 | | 27.62 | | 68.82 | |
| %CV (intra-batch) | | 5.70 | | 6.01 | | 3.53 | | 4.04 | |
| RE% (intra-batch) | | 3.70 | | 3.58 | | -2.31 | | -5.44 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| 2024.04.09 | LY104-dog-plasma-20240409-run1 | 18.38 | -8.08 | 39.33 | -1.67 | 772.08 | -3.49 | 1611.34 | -10.48 |
| | | 18.78 | -6.11 | 36.19 | -9.53 | 738.56 | -7.68 | 1663.21 | -7.60 |
| | | 18.67 | -6.65 | 41.23 | 3.07 | 829.12 | 3.64 | 1609.02 | -10.61 |
| | | 21.99 | 9.93 | 40.83 | 2.08 | 769.69 | -3.79 | 1650.41 | -8.31 |
| | | 21.15 | 5.77 | 36.41 | -8.97 | 769.31 | -3.84 | 1589.27 | -11.71 |
| | | 21.07 | 5.36 | 39.01 | -2.47 | 780.75 | -2.41 | 1611.04 | -10.50 |
| Mean | | 20.01 | | 38.83 | | 776.58 | | 1622.38 | |
| SD (intra-batch) | | 1.57 | | 2.14 | | 29.48 | | 28.21 | |
| %CV (intra-batch) | | 7.84 | | 5.51 | | 3.80 | | 1.74 | |
| RE% (intra-batch) | | 0.03 | | -2.92 | | -2.93 | | -9.87 | |
| n | | 6 | | 6 | | 6 | | 6 | |
| Mean (three batches) | | 21.04 | | 40.91 | | 793.00 | | 1682.57 | |
| SD (inter-batch) | | 2.95 | | 4.57 | | 59.48 | | 130.27 | |
| %CV (inter-batch) | | 14.02 | | 11.18 | | 7.50 | | 7.74 | |
| RE% (inter-batch) | | 5.18 | | 2.27 | | -0.87 | | -6.52 | |
| n | | 18 | | 18 | | 18 | | 18 | |

**Table S3.** Stability of LY104, M1, and IS stock and working solutions under various storage conditions (n = 3).

| Stability Conditions | | Analyte | Concentration | Stability (%) |
|---|---|---|---|---|
| Stock solutions | Room temperature for 12 h | LY104 | 5 mg/mL | –1.98 |
| | | M1 | 5 mg/mL | –0.80 |
| | | IS | 10 mg/mL | 12.56 |
| | Long-term for 7 days(–20°C) | LY104 | 5 mg/mL | –0.10 |
| | | M1 | 5 mg/mL | –0.43 |
| | | IS | 10 mg/mL | 11.49 |
| Working solutions | Room temperature for 12 h | LY104 | 20 ng/mL | –2.92 |
| | | | 20,000 ng/mL | 3.54 |
| | | M1 | 20 ng/mL | –1.23 |
| | | | 20,000 ng/mL | 3.71 |
| | | IS | 50 ng/mL | 7.05 |
| | Long-term for 7 days(2–8°C) | LY104 | 20 ng/mL | 3.51 |
| | | | 20,000 ng/mL | 2.49 |
| | | M1 | 20 ng/mL | 4.19 |
| | | | 20,000 ng/mL | 1.16 |
| | | IS | 50 ng/mL | 10.98 |

**Table S4.** Reinjection reproducibility of samples stored in the autosampler for 24 h (n = 3).

| Analyte | QC (ng/mL) | Mean ± SD (ng/mL) | RSD (%) | RE (%) |
|---|---|---|---|---|
| LY104 | 40 | 41.29 ± 1.39 | 3.36 | 3.22 |
| | 1800 | 1734.13 ± 81.00 | 4.67 | –3.66 |
| M1 | 40 | 38.36 ± 2.93 | 7.64 | –4.11 |
| | 1800 | 1689.38 ± 90.77 | 5.37 | –6.15 |

**Table S5.** Extraction recovery of the IS in beagle dog plasma (n = 9).

| Number | Post-extraction IS peak area | Unextracted peak area of IS | Extraction recovery rate of the IS |
|---|---|---|---|
| 1 | 3331.63 | 3600.13 | 108.06 |
| 2 | 3297.39 | 3658.78 | 110.96 |
| 3 | 3416.21 | 3762.34 | 110.13 |
| 4 | 3311.15 | 3652.30 | 110.30 |
| 5 | 3379.68 | 3615.19 | 106.97 |
| 6 | 3401.72 | 3660.64 | 107.61 |
| 7 | 3325.30 | 3718.88 | 111.84 |
| 8 | 3363.32 | 3661.92 | 108.88 |
| 9 | 3432.33 | 3627.30 | 105.68 |
| Mean ± SD | 3362.08 ± 48.48 | 3661.94 ± 50.79 | 108.94 ± 2.02 |
| RSD (%) | 1.44 | 1.39 | 1.6 |

**Table S6.** Individual whole blood, blood cell and plasma concentrations of LY104 in beagle dogs.

| Concentration (ng/mL) | No. | $C_b$ (ng/mL) | $C_e$ (ng/mL) | $C_p$ (ng/mL) |
|---|---|---|---|---|
| 200 | 1 | 132.62 | 24.10 | 288.48 |
| | 2 | 130.77 | 22.54 | 290.25 |
| | 3 | 177.88 | 37.84 | 370.06 |
| 1000 | 1 | 621.52 | 126.77 | 1513.95 |
| | 2 | 620.16 | 153.14 | 1540.60 |
| | 3 | 857.66 | 195.55 | 1890.51 |
| 5000 | 1 | 3363.08 | 507.93 | 7248.10 |
| | 2 | 4042.54 | 630.50 | 8316.95 |
| | 3 | 4293.49 | 685.46 | 9084.17 |

$C_b$: the whole-blood concentration; $C_e$: the red blood cells concentration; $C_p$: plasma concentration.

**Table S7.** Pharmacokinetic parameters of LY104 and M1 in beagle dogs after single intravenous injections of LY104 (0.2 mg/kg, n = 6).

| Analytes | Parameters | Units | 1101 | 1102 | 1103 | 1201 | 1202 | 1203 | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| LY104 | $AUC_{0-t}$ | h·μg/L | 10.01 | 18.56 | 11.10 | 18.85 | 20.48 | 16.74 | 15.96 | 4.36 |
| | $AUC_{0-\infty}$ | h·μg/L | / | / | / | / | / | / | / | / |
| | $t_{1/2}$ | h | / | / | / | / | / | / | / | / |
| | Vz | L/kg | / | / | / | / | / | / | / | / |
| | CLz | L/h/kg | / | / | / | / | / | / | / | / |
| | $MRT_{0-t}$ | h | / | / | / | / | / | / | / | / |
| | $T_{max}$ | h | 0.29 | 0.32 | 0.32 | 0.29 | 0.32 | 0.33 | 0.31 | 0.01 |
| | $C_{max}$ | ug/L | 83.29 | 143.94 | 147.96 | 174.10 | 151.99 | 122.73 | 137.34 | 31.17 |
| M1 | $AUC_{0-t}$ | h·μg/L | 1699.33 | 905.65 | 1578.65 | 530.04 | 919.25 | 445.58 | 1013.08 | 522.79 |
| | $AUC_{0-\infty}$ | h·μg/L | 2522.87 | 1386.36 | 1642.16 | 924.02 | 1325.68 | 1375.90 | 1529.50 | 538.83 |
| | $t_{1/2}$ | h | 15.73 | 16.38 | 4.83 | 6.49 | 13.72 | 17.07 | 12.37 | 5.34 |
| | Vz | L/kg | 1.80 | 3.41 | 0.85 | 2.03 | 2.99 | 3.58 | 2.44 | 1.06 |
| | CLz | L/h/kg | 0.08 | 0.14 | 0.12 | 0.22 | 0.15 | 0.15 | 0.14 | 0.04 |
| | $MRT_{0-t}$ | h | 10.72 | 9.07 | 9.49 | 3.77 | 9.83 | 3.56 | 7.74 | 3.20 |
| | $T_{max}$ | h | 12.28 | 0.80 | 6.30 | 0.78 | 1.30 | 0.81 | 3.71 | 4.72 |
| | $C_{max}$ | ug/L | 91.54 | 85.12 | 121.87 | 91.05 | 70.95 | 93.87 | 92.40 | 16.65 |

**Table S8.** Pharmacokinetic parameters of LY104 and M1 in beagle dogs after the first and seventh intravenous injections of LY104 (1 mg/kg, n = 6).

| Analytes | Parameters | Units | 2101 | 2102 | 2103 | 2201 | 2202 | 2203 | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| LY104 | | | | | **first day** | | | | | |
| | $AUC_{0-t}$ | h·μg/L | 202.39 | 197.58 | 235.02 | 178.13 | 181.57 | 200.70 | 199.23 | 20.26 |
| | $AUC_{0-\infty}$ | h·μg/L | / | 201.99 | 242.60 | 183.51 | 186.60 | 205.46 | 204.03 | 23.55 |
| | $t_{1/2}$ | h | / | 0.13 | 0.15 | 0.15 | 0.14 | 0.13 | 0.14 | 0.01 |
| | Vz | L/kg | / | 0.96 | 0.86 | 1.21 | 1.07 | 0.94 | 1.01 | 0.14 |
| | CLz | L/h/kg | / | 4.95 | 4.12 | 5.45 | 5.36 | 4.87 | 4.95 | 0.53 |
| | $MRT_{0-t}$ | h | / | 0.17 | 0.22 | 0.16 | 0.21 | 0.22 | 0.19 | 0.03 |
| | $T_{max}$ | h | 0.17 | 0.17 | 0.33 | 0.17 | 0.17 | 0.33 | 0.22 | 0.08 |
| | $C_{max}$ | ng/mL | 498.84 | 489.81 | 570.66 | 409.32 | 442.23 | 507.06 | 486.32 | 55.83 |
| | | | | | **seventh day** | | | | | |
| | $AUC_{0-t}$ | ug/L*h | 192.42 | 187.82 | 195.63 | 201.83 | 170.77 | 175.53 | 187.33 | 11.99 |
| | $AUC_{0-\infty}$ | ug/L*h | 198.41 | 193.60 | 202.23 | 207.89 | 175.77 | / | 195.58 | 12.25 |
| | $t_{1/2}$ | h | 0.14 | 0.15 | 0.15 | 0.15 | 0.14 | / | 0.14 | 0.00 |
| | Vz | L/kg | 1.00 | 1.09 | 1.04 | 1.01 | 1.16 | / | 1.06 | 0.07 |
| | CLz | L/h/kg | 5.04 | 5.17 | 4.95 | 4.81 | 5.69 | / | 5.13 | 0.34 |
| | $MRT_{0-t}$ | h | 0.21 | 0.19 | 0.24 | 0.19 | 0.23 | / | 0.21 | 0.02 |
| | $T_{max}$ | h | 0.29 | 0.17 | 0.33 | 0.17 | 0.33 | 0.33 | 0.27 | 0.08 |
| | $C_{max}$ | ng/mL | 506.79 | 450.64 | 541.53 | 490.06 | 448.50 | 463.35 | 483.48 | 36.51 |
| M1 | | | | | **first day** | | | | | |
| | $AUC_{0-t}$ | h·μg/L | 5087.26 | 6520.52 | 4256.26 | 3905.81 | 5062.88 | 3857.81 | 4781.76 | 1009.23 |
| | $AUC_{0-\infty}$ | h·μg/L | 10,074.23 | 8254.56 | 7807.70 | 4018.72 | 8803.25 | 3965.41 | 7153.98 | 2564.51 |
| | $t_{1/2}$ | h | 26.61 | 10.05 | 21.21 | 4.75 | 18.83 | 4.88 | 14.39 | 9.14 |
| | Vz | L/kg | 3.81 | 1.76 | 3.92 | 1.71 | 3.09 | 1.78 | 2.68 | 1.06 |
| | CLz | L/h/kg | 0.10 | 0.12 | 0.13 | 0.25 | 0.11 | 0.25 | 0.16 | 0.07 |
| | $MRT_{0-t}$ | h | 9.87 | 9.72 | 9.01 | 8.62 | 9.13 | 8.95 | 9.21 | 0.48 |
| | $T_{max}$ | h | 1.28 | 0.79 | 1.31 | 1.30 | 0.80 | 0.81 | 1.05 | 0.27 |
| | $C_{max}$ | ng/mL | 427.52 | 507.97 | 454.61 | 395.50 | 561.47 | 468.80 | 469.31 | 58.99 |
| | | | | | **seventh day** | | | | | |
| | $AUC_{0-t}$ | h·μg/L | 5467.39 | 12,968.63 | 5239.67 | 6784.49 | 10,301.97 | 6868.49 | 7938.44 | 3057.94 |
| | $AUC_{0-\infty}$ | h·μg/L | 5602.65 | 21,351.77 | 5300.26 | 7928.39 | 1,1173.94 | 6928.69 | 9714.28 | 6081.11 |
| | $t_{1/2}$ | h | 4.67 | 18.29 | 3.71 | 8.19 | 6.44 | 3.36 | 7.44 | 5.61 |
| | Vz | L/kg | 1.20 | 1.24 | 1.01 | 1.49 | 0.83 | 0.70 | 1.08 | 0.29 |
| | CLz | L/h/kg | 0.18 | 0.05 | 0.19 | 0.13 | 0.09 | 0.14 | 0.13 | 0.05 |
| | $MRT_{0-t}$ | h | 7.91 | 10.30 | 7.69 | 7.76 | 9.34 | 7.86 | 8.48 | 1.09 |
| | $T_{max}$ | h | 0.78 | 8.29 | 0.81 | 1.30 | 1.31 | 0.82 | 2.22 | 2.99 |
| | $C_{max}$ | ng/mL | 886.33 | 836.75 | 714.81 | 646.81 | 857.79 | 758.19 | 783.44 | 92.70 |

/: no data.

**Table S9.** Pharmacokinetic parameters of LY104 and M1 in beagle dogs after single intravenous injections of LY104 (5 mg/kg, n = 6).

| Analytes | Parameters | Units | 3101 | 3102 | 3103 | 3201 | 3202 | 3203 | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| LY104 | $AUC_{0-t}$ | h·μg/L | 765.65 | 820.76 | 931.44 | 658.55 | 977.81 | 544.89 | 783.18 | 163.64 |
| | $AUC_{0-\infty}$ | h·μg/L | 816.13 | 902.73 | 989.81 | 842.55 | 1281.55 | 614.28 | 907.84 | 221.48 |
| | $t_{1/2}$ | h | 1.32 | 1.64 | 1.77 | 5.45 | 3.53 | 1.34 | 2.51 | 1.66 |
| | Vz | L/kg | 11.67 | 13.08 | 12.90 | 46.68 | 19.87 | 15.71 | 19.98 | 13.40 |
| | CLz | L/h/kg | 6.13 | 5.54 | 5.05 | 5.93 | 3.90 | 8.14 | 5.78 | 1.40 |
| | $MRT_{0-t}$ | h | 0.48 | 0.50 | 0.71 | 1.51 | 0.52 | 0.52 | 0.71 | 0.40 |
| | $T_{max}$ | h | 0.45 | 0.46 | 0.46 | 0.30 | 0.46 | 0.33 | 0.41 | 0.07 |
| | $C_{max}$ | ug/L | 1883.03 | 2056.56 | 2355.15 | 2184.92 | 2263.49 | 2174.77 | 2152.99 | 165.38 |
| M1 | $AUC_{0-t}$ | h·μg/L | 30,971.32 | 24,096.03 | 33,788.65 | 19,019.97 | 46,815.36 | 20,559.74 | 29,208.51 | 10,381.05 |
| | $AUC_{0-\infty}$ | h·μg/L | 98,259.12 | 27,633.62 | 43,659.30 | 188,114.71 | 58,081.01 | 23,747.41 | 73,249.20 | 62,380.17 |
| | $t_{1/2}$ | h | 47.27 | 8.94 | 10.10 | 185.62 | 9.77 | 7.99 | 44.95 | 70.58 |
| | Vz | L/kg | 3.47 | 2.33 | 1.67 | 7.12 | 1.21 | 2.43 | 3.04 | 2.14 |
| | CLz | L/h/kg | 0.05 | 0.18 | 0.12 | 0.03 | 0.09 | 0.21 | 0.11 | 0.07 |
| | $MRT_{0-t}$ | h | 10.09 | 8.57 | 9.03 | 10.38 | 9.06 | 7.59 | 9.12 | 1.02 |
| | $T_{max}$ | h | 0.17 | 0.79 | 0.79 | 0.17 | 1.29 | 0.17 | 0.56 | 0.47 |
| | $C_{max}$ | ug/L | 2745.73 | 2939.89 | 2796.62 | 2075.20 | 3688.07 | 2630.69 | 2812.70 | 522.35 |

**Table S10.** The concentration of LY104 in beagle dogs after a single intravenous injection of LY104 (0.2 mg/kg) (ng/mL) (n=6).

| Time (h) in Figure | 1101 | | 1102 | | 1103 | | 1201 | | 1202 | | 1203 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 |
| 0.167 | 0.167 | 32.93 | 0.167 | 49.00 | 0.167 | 0.00 | 0.167 | 54.57 | 0.167 | 57.28 | 0.167 | 43.32 |
| 0.292 | 0.292 | 83.29 | 0.317 | 143.94 | 0.317 | 147.96 | 0.292 | 174.10 | 0.317 | 151.99 | 0.325 | 122.73 |
| 0.442 | 0.442 | ND | 0.467 | ND | 0.467 | BLOQ | 0.442 | ND | 0.467 | ND | 0.475 | ND |
| 0.775 | 0.775 | ND | 0.800 | ND | 0.800 | ND | 0.775 | ND | 0.800 | ND | 0.808 | ND |
| 1.275 | 1.275 | ND | 1.300 | ND | 1.300 | ND | 1.275 | ND | 1.300 | ND | 1.308 | ND |
| 2.275 | 2.275 | ND | 2.300 | ND | 2.300 | ND | 2.275 | ND | 2.300 | ND | 2.308 | ND |
| 4.275 | 4.275 | ND | 4.300 | ND | 4.300 | ND | 4.275 | ND | 4.300 | ND | 4.308 | ND |
| 6.275 | 6.275 | ND | 6.300 | ND | 6.300 | ND | 6.275 | ND | 6.300 | ND | 6.308 | ND |
| 8.275 | 8.275 | ND | 8.300 | ND | 8.300 | ND | 8.275 | ND | 8.300 | ND | 8.308 | ND |
| 12.275 | 12.275 | ND | 12.300 | ND | 12.300 | ND | 12.275 | ND | 12.300 | ND | 12.308 | ND |
| 24.275 | 24.275 | ND | 24.300 | ND | 24.300 | ND | 24.275 | ND | 24.300 | ND | 24.308 | ND |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S11.** The concentration of M1 in beagle dogs after a single intravenous injection of LY104 (0.2 mg/kg) (ng/mL) (n=6).

| Time (h) in Figure | 1101 | | 1102 | | 1103 | | 1201 | | 1202 | | 1203 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 |
| 0.167 | 0.167 | 0.00 | 0.167 | 0.00 | 0.167 | 0.00 | 0.167 | 0.00 | 0.167 | 0.00 | 0.167 | 0.00 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.292 | 0.292 | 35.49 | 0.317 | 46.21 | 0.317 | 0.00 | 0.292 | 47.33 | 0.317 | 49.98 | 0.325 | 64.99 |
| 0.442 | 0.442 | 41.45 | 0.467 | 55.02 | 0.467 | 25.41 | 0.442 | 63.91 | 0.467 | 55.21 | 0.475 | 73.66 |
| 0.775 | 0.775 | 50.22 | 0.800 | 85.12 | 0.800 | 42.79 | 0.775 | 91.05 | 0.800 | 66.80 | 0.808 | 93.87 |
| 1.275 | 1.275 | 50.80 | 1.300 | 76.25 | 1.300 | 61.57 | 1.275 | 89.63 | 1.300 | 70.95 | 1.308 | 80.94 |
| 2.275 | 2.275 | 79.71 | 2.300 | 54.35 | 2.300 | 104.91 | 2.275 | 81.38 | 2.300 | 60.42 | 2.308 | 73.07 |
| 4.275 | 4.275 | 84.32 | 4.300 | 52.22 | 4.300 | 117.10 | 4.275 | 62.53 | 4.300 | 36.71 | 4.308 | 44.59 |
| 6.275 | 6.275 | 73.82 | 6.300 | 44.09 | 6.300 | 121.87 | 6.275 | 56.68 | 6.300 | 45.47 | 6.308 | 40.65 |
| 8.275 | 8.275 | 80.61 | 8.300 | 39.52 | 8.300 | 89.60 | 8.275 | 42.47 | 8.300 | 46.10 | 8.308 | 37.91 |
| 12.275 | 12.275 | 91.54 | 12.300 | BLOQ | 12.300 | 51.28 | 12.275 | BLOQ | 12.300 | 37.61 | 12.308 | BLOQ |
| 24.275 | 24.275 | 35.91 | 24.300 | 20.37 | 24.300 | 32.36 | 24.275 | BLOQ | 24.300 | 20.53 | 24.308 | BLOQ |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S12.** The concentration of LY104 in beagle dogs after a single intravenous injection of LY104 (1 mg/kg) (ng/mL) (n=6).

| **Time (h) in Figure** | **2101** | | **2102** | | **2103** | | **2201** | | **2202** | | **2203** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0.000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.167 | 0.167 | 498.84 | 0.17 | 489.81 | 0.17 | 545.32 | 0.17 | 409.32 | 0.17 | 442.23 | 0.17 | 470.32 |
| 0.292 | 0.292 | 281.14 | 0.31 | 336.01 | 0.33 | 570.66 | 0.31 | 224.39 | 0.31 | 425.97 | 0.33 | 507.06 |
| 0.442 | 0.442 | 17.25 | 0.46 | 23.64 | 0.48 | 40.35 | 0.47 | 30.89 | 0.47 | 29.73 | 0.48 | 26.05 |
| 0.775 | 0.775 | BLOQ | 0.79 | 21.74 | 0.81 | 32.32 | 0.80 | 24.09 | 0.80 | 23.13 | 0.81 | 21.78 |
| 1.275 | 1.275 | BLOQ | 1.29 | BLOQ | 1.31 | BLOQ | 1.30 | BLOQ | 1.30 | BLOQ | 1.31 | BLOQ |
| 2.275 | 2.275 | ND | 2.29 | BLOQ | 2.31 | BLOQ | 2.30 | BLOQ | 2.30 | BLOQ | 2.31 | BLOQ |
| 4.275 | 4.275 | ND | 4.29 | ND | 4.31 | BLOQ | 4.30 | BLOQ | 4.30 | ND | 4.31 | ND |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.275 | 6.275 | ND | 6.29 | BLOQ | 6.31 | BLOQ | 6.30 | ND | 6.30 | ND | 6.31 | ND |
| 8.275 | 8.275 | ND | 8.29 | ND | 8.31 | ND | 8.30 | ND | 8.30 | ND | 8.31 | ND |
| 12.275 | 12.275 | BLOQ | 12.29 | ND | 12.31 | ND | 12.30 | ND | 12.30 | ND | 12.31 | ND |
| 24.275 | 24.275 | ND | 24.29 | ND | 24.31 | ND | 24.30 | ND | 24.30 | ND | 24.31 | BLOQ |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S13.** The concentration of M1 in beagle dogs after a single intravenous injection of LY104 (1 mg/kg) (ng/mL) (n=6).

| **Time (h) in Figure** | **2101** | | **2102** | | **2103** | | **2201** | | **2202** | | **2203** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0.000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.167 | 0.167 | 77.41 | 0.17 | 80.09 | 0.17 | 97.93 | 0.17 | 59.18 | 0.17 | 99.84 | 0.17 | 122.13 |
| 0.292 | 0.292 | 233.69 | 0.31 | 327.68 | 0.33 | 302.89 | 0.31 | 228.67 | 0.31 | 313.74 | 0.33 | 317.09 |
| 0.442 | 0.442 | 359.26 | 0.46 | 472.16 | 0.48 | 424.64 | 0.47 | 325.76 | 0.47 | 504.73 | 0.48 | 444.46 |
| 0.775 | 0.775 | 392.89 | 0.79 | 507.97 | 0.81 | 444.30 | 0.80 | 379.52 | 0.80 | 561.47 | 0.81 | 468.80 |
| 1.275 | 1.275 | 427.52 | 1.29 | 434.42 | 1.31 | 454.61 | 1.30 | 395.50 | 1.30 | 509.50 | 1.31 | 437.24 |
| 2.275 | 2.275 | 346.94 | 2.29 | 361.67 | 2.31 | 368.27 | 2.30 | 353.92 | 2.30 | 397.76 | 2.31 | 328.56 |
| 4.275 | 4.275 | 288.40 | 4.29 | 304.30 | 4.31 | 260.76 | 4.30 | 265.47 | 4.30 | 242.37 | 4.31 | 242.22 |
| 6.275 | 6.275 | 213.51 | 6.29 | 234.14 | 6.31 | 215.62 | 6.30 | 231.11 | 6.30 | 193.02 | 6.31 | 200.95 |
| 8.275 | 8.275 | 190.92 | 8.29 | 324.67 | 8.31 | 188.87 | 8.30 | 165.55 | 8.30 | 357.10 | 8.31 | 144.20 |
| 12.275 | 12.275 | 199.02 | 12.29 | 315.12 | 12.31 | 99.00 | 12.30 | 95.54 | 12.30 | 115.78 | 12.31 | 84.87 |
| 24.275 | 24.275 | 130.35 | 24.29 | 115.50 | 24.31 | 116.49 | 24.30 | 90.24 | 24.30 | 132.17 | 24.31 | 110.68 |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S14.** The concentration of LY104 in beagle dogs after a single intravenous injection of LY104 (5 mg/kg) (ng/mL) (n=6).

| Time (h) in Figure | 3101 | | 3102 | | 3103 | | 3201 | | 3202 | | 3203 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 |
| 0.167 | 0.167 | 115.74 | 0.167 | 114.74 | 0.167 | 169.98 | 0.167 | 128.78 | 0.167 | 175.45 | 0.167 | 101.27 |
| 0.292 | 0.3 | 1578.13 | 0.308 | 1502.07 | 0.308 | 1035.25 | 0.300 | 2184.92 | 0.308 | 1962.88 | 0.325 | 2174.77 |
| 0.442 | 0.45 | 1883.03 | 0.458 | 2056.56 | 0.458 | 2355.15 | 0.450 | 429.18 | 0.458 | 2263.49 | 0.475 | 400.05 |
| 0.775 | 0.783 | 63.36 | 0.792 | 61.00 | 0.792 | 90.90 | 0.783 | 57.21 | 0.792 | 82.98 | 0.808 | 76.07 |
| 1.275 | 1.283 | 39.54 | 1.292 | 58.95 | 1.292 | 73.21 | 1.283 | 60.67 | 1.292 | 68.82 | 1.308 | 62.89 |
| 2.275 | 2.283 | 27.64 | 2.292 | 33.49 | 2.292 | 44.87 | 2.283 | 24.83 | 2.292 | 60.72 | 2.308 | 35.46 |
| 4.275 | 4.283 | BLOQ | 4.292 | BLOQ | 4.292 | 22.90 | 4.283 | BLOQ | 4.292 | BLOQ | 4.308 | BLOQ |
| 6.275 | 6.283 | BLOQ | 6.292 | BLOQ | 6.292 | BLOQ | 6.283 | BLOQ | 6.292 | BLOQ | 6.308 | BLOQ |
| 8.275 | 8.283 | BLOQ | 8.292 | BLOQ | 8.292 | BLOQ | 8.283 | 23.29 | 8.292 | BLOQ | 8.308 | BLOQ |
| 12.275 | 12.283 | BLOQ | 12.292 | BLOQ | 12.292 | BLOQ | 12.283 | BLOQ | 12.292 | BLOQ | 12.308 | BLOQ |
| 24.275 | 24.283 | ND | 24.292 | ND | 24.292 | ND | 24.283 | ND | 24.292 | ND | 24.308 | ND |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S15.** The concentration of M1 in beagle dogs after a single intravenous injection of LY104 (5 mg/kg) (ng/mL) (n=6).

| Time (h) in Figure | 3101 | | 3102 | | 3103 | | 3201 | | 3202 | | 3203 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 |
| 0.167 | 0.167 | 2745.73 | 0.167 | 2792.84 | 0.167 | 2794.86 | 0.167 | 2075.20 | 0.167 | 2858.40 | 0.167 | 2630.69 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.292 | 0.3 | 1754.32 | 0.308 | 2010.17 | 0.308 | 2048.91 | 0.300 | 1970.91 | 0.308 | 2138.54 | 0.325 | 1943.96 |
| 0.442 | 0.45 | 1418.72 | 0.458 | 1550.24 | 0.458 | 1405.22 | 0.450 | 1012.97 | 0.458 | 1582.09 | 0.475 | 1075.18 |
| 0.775 | 0.783 | 2408.58 | 0.792 | 2939.89 | 0.792 | 2796.62 | 0.783 | 1990.60 | 0.792 | 3321.96 | 0.808 | 2442.64 |
| 1.275 | 1.283 | 2018.90 | 1.292 | 2731.69 | 1.292 | 2642.78 | 1.283 | 1909.19 | 1.292 | 3688.07 | 1.308 | 2012.89 |
| 2.275 | 2.283 | 1592.20 | 2.292 | 2023.54 | 2.292 | 1979.44 | 2.283 | 1286.62 | 2.292 | 2687.01 | 2.308 | 1518.57 |
| 4.275 | 4.283 | 2347.54 | 4.292 | 1228.26 | 4.292 | 1520.43 | 4.283 | 539.26 | 4.292 | 2725.77 | 4.308 | 1318.89 |
| 6.275 | 6.283 | 1107.91 | 6.292 | 1130.73 | 6.292 | 2157.33 | 6.283 | 584.84 | 6.292 | 2941.07 | 6.308 | 1351.81 |
| 8.275 | 8.283 | 1472.51 | 8.292 | 917.56 | 8.292 | 2216.13 | 8.283 | 494.64 | 8.292 | 2188.88 | 8.308 | 1077.24 |
| 12.275 | 12.283 | 940.26 | 12.292 | 702.59 | 12.292 | 1029.46 | 12.283 | 818.99 | 12.292 | 1800.57 | 12.308 | 528.53 |
| 24.275 | 24.283 | 968.34 | 24.292 | 542.10 | 24.292 | 670.99 | 24.283 | 587.55 | 24.292 | 809.65 | 24.308 | 277.42 |

BLOQ: data below the limit of quantification; ND: not detected.

**Table S16.** The concentration of LY104 in beagle dogs after the seventh intravenous injection of LY104 (1 mg/kg) (ng/mL) (n=6).

| **Time (h) in Figure** | **2101** | | **2102** | | **2103** | | **2201** | | **2202** | | **2203** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0.000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.167 | 0.167 | 480.23 | 0.17 | 450.64 | 0.17 | 432.84 | 0.17 | 490.06 | 0.17 | 385.60 | 0.17 | 416.99 |
| 0.292 | 0.292 | 506.79 | 0.31 | 329.94 | 0.33 | 541.53 | 0.31 | 396.96 | 0.33 | 448.50 | 0.33 | 463.35 |
| 0.442 | 0.442 | 44.10 | 0.46 | 47.98 | 0.48 | 36.54 | 0.47 | 40.14 | 0.48 | 33.16 | 0.48 | 21.00 |
| 0.775 | 0.775 | 27.95 | 0.79 | 26.14 | 0.81 | 27.34 | 0.80 | 27.37 | 0.81 | 21.57 | 0.82 | BLOQ |
| 1.275 | 1.275 | BLOQ | 1.29 | BLOQ | 1.31 | BLOQ | 1.30 | BLOQ | 1.31 | BLOQ | 1.32 | BLOQ |
| 2.275 | 2.275 | ND | 2.29 | ND | 2.31 | BLOQ | 2.30 | BLOQ | 2.31 | ND | 2.32 | ND |
| 4.275 | 4.275 | ND | 4.29 | ND | 4.31 | ND | 4.30 | ND | 4.31 | ND | 4.32 | ND |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.275 | 6.275 | ND | 6.29 | ND | 6.31 | ND | 6.30 | ND | 6.31 | ND | 6.32 | ND |
| 8.275 | 8.275 | ND | 8.29 | ND | 8.31 | ND | 8.30 | ND | 8.31 | ND | 8.32 | ND |
| 12.275 | 12.275 | ND | 12.29 | ND | 12.31 | ND | 12.30 | ND | 12.31 | ND | 12.32 | ND |
| 24.275 | 24.275 | ND | 24.29 | ND | 24.31 | ND | 24.30 | ND | 24.31 | ND | 24.32 | ND |

**Table S17.** The concentration of M1 in beagle dogs after the seventh intravenous injection of LY104 (1 mg/kg) (ng/mL) (n=6).

| **Time (h) in Figure** | **2101** | | **2102** | | **2103** | | **2201** | | **2202** | | **2203** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) | Time (h) | Concentration (ng/mL) |
| 0 | 0.000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.167 | 0.167 | 201.97 | 0.17 | 280.38 | 0.17 | 191.30 | 0.17 | 217.44 | 0.17 | 271.68 | 0.17 | 195.37 |
| 0.292 | 0.292 | 379.83 | 0.31 | 523.30 | 0.33 | 380.78 | 0.31 | 390.21 | 0.33 | 562.99 | 0.33 | 497.04 |
| 0.442 | 0.442 | 525.10 | 0.46 | 716.37 | 0.48 | 558.80 | 0.47 | 582.05 | 0.48 | 743.75 | 0.48 | 690.18 |
| 0.775 | 0.775 | 886.33 | 0.79 | 635.35 | 0.81 | 714.81 | 0.80 | 641.13 | 0.81 | 752.48 | 0.82 | 758.19 |
| 1.275 | 1.275 | 694.43 | 1.29 | 656.93 | 1.31 | 623.30 | 1.30 | 646.81 | 1.31 | 857.79 | 1.32 | 704.98 |
| 2.275 | 2.275 | 484.25 | 2.29 | 558.57 | 2.31 | 509.78 | 2.30 | 538.85 | 2.31 | 657.28 | 2.32 | 550.33 |
| 4.275 | 4.275 | 375.16 | 4.29 | 661.71 | 4.31 | 349.05 | 4.30 | 534.90 | 4.31 | 532.57 | 4.32 | 498.80 |
| 6.275 | 6.275 | 329.55 | 6.29 | 617.30 | 6.31 | 345.92 | 6.30 | 452.90 | 6.31 | 665.98 | 6.32 | 488.16 |
| 8.275 | 8.275 | 179.34 | 8.29 | 836.75 | 8.31 | 207.84 | 8.30 | 366.35 | 8.31 | 505.59 | 8.32 | 355.76 |
| 12.275 | 12.275 | 127.03 | 12.29 | 513.98 | 12.31 | 109.67 | 12.30 | 165.35 | 12.31 | 345.10 | 12.32 | 144.46 |
| 24.275 | 24.275 | 111.94 | 24.29 | 314.86 | 24.31 | 97.76 | 24.30 | 97.03 | 24.31 | 227.28 | 24.32 | 119.45 |

**Table S18.** Individual-animal PK values and ln-transformed values used in the power-model dose-proportionality analysis for LY104 and M1.

| Analyte | Dose (mg/kg) | Animal ID | $AUC_{0-t}$ (h·μg/L) | $C_{max}$ (μg/L) | Ln (Dose) | Ln (AUC) | Ln ($C_{max}$) |
|---|---|---|---|---|---|---|---|
| LY104 | 0.2 | 1101 | 10.01 | 83.29 | -1.61 | 2.30 | 4.42 |
| | | 1102 | 18.56 | 143.94 | -1.61 | 2.92 | 4.97 |
| | | 1103 | 11.10 | 147.96 | -1.61 | 2.41 | 5.00 |
| | | 1201 | 18.85 | 174.10 | -1.61 | 2.94 | 5.16 |
| | | 1202 | 20.48 | 151.99 | -1.61 | 3.02 | 5.02 |
| | | 1203 | 16.74 | 122.73 | -1.61 | 2.82 | 4.81 |
| | 1 | 2101 | 202.39 | 498.84 | 0.00 | 5.31 | 6.21 |
| | | 2102 | 197.58 | 489.81 | 0.00 | 5.29 | 6.19 |
| | | 2103 | 235.02 | 570.66 | 0.00 | 5.46 | 6.35 |
| | | 2201 | 178.13 | 409.32 | 0.00 | 5.18 | 6.01 |
| | | 2202 | 181.57 | 442.23 | 0.00 | 5.20 | 6.09 |
| | | 2203 | 200.70 | 507.06 | 0.00 | 5.30 | 6.23 |
| | 5 | 3101 | 765.65 | 1883.03 | 1.61 | 6.64 | 7.54 |
| | | 3102 | 820.76 | 2056.56 | 1.61 | 6.71 | 7.63 |
| | | 3103 | 931.44 | 2355.15 | 1.61 | 6.84 | 7.76 |
| | | 3201 | 658.55 | 2184.92 | 1.61 | 6.49 | 7.69 |
| | | 3202 | 977.81 | 2263.49 | 1.61 | 6.89 | 7.72 |
| | | 3203 | 544.89 | 2174.77 | 1.61 | 6.30 | 7.68 |
| M1 | 0.2 | 1101 | 1699.33 | 91.54 | -1.61 | 7.44 | 4.52 |
| | | 1102 | 905.65 | 85.12 | -1.61 | 6.81 | 4.44 |
| | | 1103 | 1578.65 | 121.87 | -1.61 | 7.36 | 4.80 |
| | | 1201 | 530.04 | 91.05 | -1.61 | 6.27 | 4.51 |
| | | 1202 | 919.25 | 70.95 | -1.61 | 6.82 | 4.26 |
| | | 1203 | 445.58 | 93.87 | -1.61 | 6.10 | 4.54 |
| | 1 | 2101 | 5087.26 | 427.52 | 0.00 | 8.53 | 6.06 |
| | | 2102 | 6520.52 | 507.97 | 0.00 | 8.78 | 6.23 |
| | | 2103 | 4256.26 | 454.61 | 0.00 | 8.36 | 6.12 |
| | | 2201 | 3905.81 | 395.50 | 0.00 | 8.27 | 5.98 |
| | | 2202 | 5062.88 | 561.47 | 0.00 | 8.53 | 6.33 |
| | | 2203 | 3857.81 | 468.80 | 0.00 | 8.26 | 6.15 |
| | 5 | 3101 | 30,971.32 | 2745.73 | 1.61 | 10.34 | 7.92 |
| | | 3102 | 24,096.03 | 2939.89 | 1.61 | 10.09 | 7.99 |
| | | 3103 | 33,788.65 | 2796.62 | 1.61 | 10.43 | 7.94 |
| | | 3201 | 19,019.97 | 2075.20 | 1.61 | 9.85 | 7.64 |
| | | 3202 | 46,815.36 | 3688.07 | 1.61 | 10.75 | 8.21 |
| | | 3203 | 20,559.74 | 2630.69 | 1.61 | 9.93 | 7.87 |

**Table S19.** Steady-state pharmacokinetic parameters of LY104 and M1 in beagle dogs after seven consecutive intravenous injections of LY104 (1 mg/kg) (n = 6).

| Analytes | Parameters | Units | 2101 | 2102 | 2103 | 2201 | 2202 | 2203 | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| LY104 | $C_{ss\ min}$ | ng/mL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | $C_{ss\ max}$ | ng/mL | 506.79 | 450.64 | 541.53 | 490.06 | 448.50 | 463.35 | 483.48 | 36.51 |
| | $AUC_{0\text{-}tau}$ | h·μg/L | 192.42 | 187.82 | 195.63 | 201.83 | 170.77 | 175.53 | 187.33 | 11.99 |
| | $C_{ss,av}$ | ng/mL | 8.02 | 7.83 | 8.15 | 8.41 | 7.12 | 7.31 | 7.81 | 0.50 |
| | DF | % | 63.21 | 57.58 | 66.44 | 58.27 | 63.03 | 63.35 | 61.98 | 3.39 |
| | R | / | 0.95 | 0.95 | 0.83 | 1.13 | 0.94 | 0.87 | 0.95 | 0.10 |
| M1 | $C_{ss\ min}$ | ng/mL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | $C_{ss\ max}$ | ng/mL | 886.33 | 836.75 | 714.81 | 646.81 | 857.79 | 758.19 | 783.44 | 92.70 |
| | $AUC_{0\text{-}tau}$ | h·μg/L | 5467.39 | 12,968.63 | 5239.67 | 6784.49 | 10,301.97 | 6868.49 | 7938.44 | 3057.94 |
| | $C_{ss,av}$ | ng/mL | 227.81 | 540.36 | 218.32 | 282.69 | 429.25 | 286.19 | 330.77 | 127.41 |
| | DF | % | 3.89 | 1.55 | 3.27 | 2.29 | 2.00 | 2.65 | 2.61 | 0.86 |
| | R | / | 1.07 | 1.99 | 1.23 | 1.74 | 2.03 | 1.78 | 1.64 | 0.40 |

$C_{ss,min}$: steady-state trough concentration; $C_{ss,max}$: the steady-state peak concentration; $C_{ss,av}$: steady-state average plasma drug concentration; $AUC_{0\text{-}tau}$: steady-state area under the concentration-time curve; DF: The ratio of the difference between the steady-state maximum and minimum plasma concentration to the average steady-state plasma concentration; R: steady state accumulation ratio expressed by AUC, $R = AUC_{0\text{-}t\ (last\ dose)}/AUC_{0\text{-}t\ (first\ dose)}$. $C_{ss}$ was calculated using standard formulas from the Day 7 concentration-time data: $C_{ss,av} = AUC_{0\text{-}\tau} / \tau\ (\tau = 24\ h)$, $C_{ss,max}$ = peak concentration on Day 7, $C_{ss,min}$ = trough concentration on Day 7.

**Table S20.** ICH M10 acceptance criteria applied in the validation of LY104 and M1 in beagle dog plasma.

| Validation parameter | ICH M10 acceptance criterion | Section in this study |
|---|---|---|
| Selectivity | Endogenous interference at analyte retention time ≤ 20% of LLOQ peak area; IS ≤ 5% of LLOQ peak area | Section 2.2 |
| Linearity and Sensitivity | At least 6 calibration levels including LLOQ; back-calculated concentrations within ± 15% of nominal (± 20% at LLOQ); $R^2 > 0.99$ in this study | Section 2.3 |
| Accuracy and precision | LLOQ: RE within ± 20%, RSD ≤ 20%; LQC/MQC/HQC: RE within ± 15%, RSD ≤ 15%; at least 4 concentration levels and 6 replicates per level per batch across ≥3 batches | Section 2.4 |
| Stability | Stored samples within ± 15% of nominal concentration at each QC level; at least triplicate per condition | Section 2.5 |
| Recovery | Recovery should be consistent and concentration-independent, RSD ≤ 15% was applied | Section 2.7 |
| Matrix effect | RE within ± 15% of nominal; RSD ≤ 15% across six individual plasma lots | Section 2.7 |
| Hemolytic matrix effect | Same as matrix effect: RE within ± 15%; RSD ≤ 15% | Section 2.7 |
| Dilution integrity | Diluted samples within ± 15% of nominal concentration | Section 2.8 |
| Carry-over | Residual analyte peak ≤ 20% of LLOQ; IS ≤ 5% of LLOQ | Section 2.8 |
| Reinjection reproducibility | RE within ± 15%; RSD ≤ 15% | Section 2.6 |
| Incurred sample reanalysis (ISR) | At least 67% of reanalyzed samples within ± 20% of the mean of initial and repeat values | Section 2.11 |