Supplementary materials

The absolute bioavailability, absorption, distribution, metabolism and excretion of BI 425809 administered as an oral dose or an oral dose with an intravenous microtracer dose of $[^{14}\text{C}]-\text{BI 425809}$ in healthy males

Ute Burkard¹, Michael Desch¹, Yury Shatillo², Glen Wunderlich³, Salome Rebecca Mack¹, Christina Schlecker², Aaron M. Teitelbaum³, Pingrong Liu³, and Tom Chan³

¹Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; ²Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; ³Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA

Corresponding Author: Ute Burkhard

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Supplementary Table 1. Baseline demographics and clinical characteristics.

|                                | Absolute bioavailability study (NCT03783000) | Mass balance recovery study (NCT03654170) |
|--------------------------------|--------------------------------------------|------------------------------------------|
| Number of subjects (N, %)      | 6 (100.0)                                  | 6 (100.0)                                |
| Sex (N, %)                     |                                            |                                          |
| Male                           | 6 (100.0)                                  | 6 (100.0)                                |
| Race (N, %)                    |                                            |                                          |
| White                          | 5 (83.3)                                   | 5 (83.3)                                 |
| Multiple                       | 1 (16.7)                                   | 0 (0.0)                                  |
| Black or African American      | 0 (0.0)                                    | 1 (16.7)                                 |
| Hispanic/Latino (N, %)         |                                            |                                          |
| No                             | 6 (100)                                    | 5 (83.3)                                 |
| Yes                            | 0 (0)                                      | 1 (16.7)                                 |
| Age (Years)                    |                                            |                                          |
| Mean                           | 35.0                                       | 45.7                                     |
| SD                             | 14.5                                       | 17.7                                     |
| Height (cm)                    |                                            |                                          |
| Mean                           | 182.5                                      | 180.8                                    |
| SD                             | 6.0                                        | 3.4                                      |
| Weight (kg)                    |                                            |                                          |
| Mean                           | 83.3                                       | 84.9                                     |
| SD                             | 12.2                                       | 11.3                                     |
| BMI (kg/m²)                    |                                            |                                          |
| Mean                           | 25.0                                       | 26.1                                     |
| SD                             | 3.6                                        | 4.0                                      |
| Smoking status (N, %)          |                                            |                                          |
| Never                          | 4 (66.7)                                   | 2 (33.3)                                 |
| Current                        | 2 (33.3)                                   | 1 (16.7)                                 |
| Former                         | 0 (0.0)                                    | 3 (50.0)                                 |

BMI, body mass index
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**Supplementary Table 2.** Subjects with adverse events following a single oral dose of 25 mg BI 425809 plus an IV microtracer infusion of [14C]-BI 425809 30 μg (treated set) (absolute bioavailability study).

| System organ class – preferred term | BI 425809 on-treatment period |          |          |
|------------------------------------|-------------------------------|----------|----------|
|                                    | Subjects with AEs N (%)       | Subjects with related AEs<sup>a</sup> N (%) |          |
| Number of subjects                 | 6 (100)                       | 6 (100)  |          |
| Total with AEs                     | 3 (50.0)                      | 2 (33.3) |          |
| Gastrointestinal disorders         |                               |          |          |
| Abdominal pain                     | 2 (33.3)                      | 0        |          |
| Diarrhea                           | 1 (16.7)                      | 0        |          |
| Nervous system disorders           | 1 (16.7)                      | 1 (16.7) |          |
| Somnolence                         | 1 (16.7)                      | 1 (16.7) |          |
| Eye disorders                      | 1 (16.7)                      | 1 (16.7) |          |
| Visual brightness                  | 1 (16.7)                      | 1 (16.7) |          |

<sup>a</sup>As assessed by the investigator

AEs, adverse events; IV, intravenous
**Supplementary Table 3.** Overview of individual total recovery from time point 0 to 677 h [% of dose] in study 2 (mass balance recovery of BI 425809).

| Subject | $f_{e_{\text{total, 0-677}}}$ (%) | $f_{e_{\text{urine, 0-677}}}$ (%) | $f_{e_{\text{feces, 0-677}}}$ (%) |
|---------|----------------------------------|----------------------------------|----------------------------------|
| 1001    | 93.9                             | 46.4                             | 47.5                             |
| 1002    | 98.1                             | 46.8                             | 51.3                             |
| 1003    | 87.6                             | 50.8                             | 36.8                             |
| 1004    | 105.0                            | 56.3                             | 48.6                             |
| 1005    | 97.0                             | 42.5                             | 54.5                             |
| 1006    | 99.9                             | 46.8                             | 53.1                             |
| gMean   | 96.7                             | 48.1                             | 48.3                             |
| gCV (%) | 6.10                             | 9.60                             | 14.3                             |

$Fe_{\text{feces, 0-677}}$, fraction excreted in feces after 677 hours; $F_{\text{e_{total, 0-677}}}$, fraction excreted after 677 hours; $F_{\text{e_{urine, 0-677}}}$, fraction excreted in urine after 677 hours; gCV, geometric coefficient of variation; gMean, geometric mean.
Supplementary Table 4. Radioactive contributions of BI 425809 and metabolites in male human plasma

| Compounds | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 | Average |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
|           | % [14C]   | nM*h     | % [14C]   | nM*h     | % [14C]   | nM*h     | % [14C] | nM*h |
| BI 425809 | 44.3      | 16,229   | 48.9      | 14,540   | 43.2      | 14,795   | 43.5    | 19,513 |
| BI 758790 | 30.6      | 11,213   | 34.1      | 10,134   | 41.2      | 14,113   | 31.5    | 14,124 |
| BI 761036 | 11.5      | 4212     | 11.4      | 3386     | 6.5       | 2229     | 7.1     | 3166  |
| BI 764352 | 6.5       | 2386     | 6.1       | 1819     | 3.8       | 1313     | 7.8     | 3512  |
| M510(3)   | 0.4       | 155.0    | 0.3       | 100.7    | 0.4       | 139.3    | 0.3     | 130.5 |
| M528(1)   | 0.7       | 255.0    | 0.6       | 184.7    | 0.8       | 265.3    | 0.9     | 394.9 |
| M544(1)   | 0.7       | 253.0    | 0.5       | 145.7    | 0.7       | 246.9    | 1.2     | 521.0 |
| M544(2)   | ND        | ND       | 0.4       | 113.0    | 0.6       | 192.0    | ND      | 0.9   |
| M580(1)   | 0.7       | 267.6    | 0.5       | 148.6    | ND        | ND       | ND      | 0.8   |
| M665(1)   | ND        | ND       | ND        | ND       | 0.4       | 137.2    | ND      | 0.6   |
| M706(1)   | 1.1       | 414.2    | 1.0       | 282.4    | 2.0       | 689.2    | ND      | 2.1   |
| Others     | 3.5       | 1272     | ND        | ND       | 0.5       | 168.0    | 7.9     | 3548  |
| Total      | 100       | 36,658   | 100       | 29,727   | 100       | 34,288   | 100     | 44,910|
|           | 100       | 27,249   | 100       | 33,223   | 100       | 34,342   |         |       |

ND: Radioactive peak not detected or below the limit of quantitation (<20 cpm in peak height)

Peak co-eluted, the % [14C] was calculated based on MS response. Sum of peaks were <20 cpm on the radiochromatogram
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**Supplementary Table 5.** Radioactive contributions of BI 425809 and metabolites in male human urine based on metabolite radioprofiling

| Compounds   | % of Dose Administered | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 | Average |
|-------------|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| BI 425809   |                         | 7.9       | 8.7       | 15.5      | 15.1      | 2.4       | 6.8       | 9.4     |
| BI 758790   |                         | 6.0       | 5.3       | 7.6       | 6.2       | 2.8       | 4.7       | 5.4     |
| BI 761036   |                         | 2.5       | 1.8       | 1.4       | 3.3       | 1.8       | 3.3       | 2.3     |
| BI 764352   |                         | 0.6       | 0.4       | 0.9       | 0.8       | 0.3       | 0.6       | 0.6     |
| M234(1)     |                         | 1.4       | 2.7       | 2.0       | 1.4       | 2.0       | 2.4       | 2.0     |
| M357(1)     |                         | 0.6       | 0.4       | ND        | 0.6       | 0.5       | 0.4       | 0.5     |
| M378(1)     |                         | 1.6       | 1.3       | 0.4       | 1.2       | 1.5       | 1.6       | 1.3     |
| M510[3]     |                         | 0.7       | 0.6       | 0.3       | 0.7       | 0.5       | 0.7       | 0.6     |
| M520(1)     |                         | 0.5       | 0.4       | 0.4       | 0.6       | 0.5       | 0.5       | 0.5     |
| M528(1)     |                         | 1.1       | 1.5       | 1.1       | 1.5       | 1.1       | 1.4       | 1.3     |
| M532[3]     |                         | 1.2       | 0.9       | 1.5       | 0.7       | 1.3       | 0.9       | 1.1     |
| M532(1)     |                         | 0.7       | 1.2       | 1.2       | 0.6       | 1.0       | 0.7       | 0.9     |
| M544(1)     |                         | 5.4       | 3.9       | 4.6       | 7.8       | 7.3       | 3.9       | 5.5     |
| M544(2)     |                         | 0.3       | 0.5       | 0.2       | 0.3       | 1.9       | 0.3       | 0.6     |
| M706(1)     |                         | 4.0       | 2.9       | 5.6       | 2.6       | 4.5       | 2.9       | 3.7     |
| M733(1)     |                         | 0.4       | 0.1       | 0.6       | 0.1       | 0.2       | 0.2       | 0.3     |
| Others      |                         | 2.8       | 3.5       | 1.2       | 1.6       | 3.6       | 3.0       | 2.5     |
| Total (0-288 h) |                     | 37.7      | 36.2      | 44.3      | 45.1      | 33.3      | 34.2      | 38.5    |
| Total (0-677 h) |                     | 40.5      | 38.9      | 46.7      | 47.7      | 36.1      | 37.8      | 41.3    |

ND: Radioactive peak not detected or below the limit of quantitation (<20 cpm in peak height)

*Peak co-eluted, the % dose was calculated based on MS response. *Sum of metabolites <1.0% of dose in feces. *Subject 2, 4, and 6: 0-509 h. Other subjects: 0-677 h
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**Supplementary Table 6.** Radioactive contributions of BI 425809 and metabolites in male human feces

| Compounds     | % of Dose Administered | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 | Average |
|---------------|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| BI 425809     |                         | 0.4       | ND        | ND        | 0.2       | ND        | ND        | 0.3     |
| BI 758790     |                         | 6.8       | 3.6       | 2.2       | 5.1       | 3.2       | 3.8       | 4.1     |
| BI 761036     |                         | 7.0       | 7.4       | 3.8       | 4.0       | 8.0       | 6.0       | 6.0     |
| M450(1)¹      |                         | 0.8       | 1.7       | 2.0       | 0.6       | 1.2       | 1.0       | 1.2     |
| M528(1)       |                         | 0.4       | 0.2       | 0.3       | 0.3       | 0.5       | 0.2       | 0.3     |
| M544(1)       |                         | 13.5      | 1.6       | 2.5       | 13.6      | 7.3       | 13.1      | 8.6     |
| M546(1)       |                         | 9.1       | 22.3      | 14.5      | 9.7       | 22.5      | 14.2      | 15.4    |
| M612(1)²      |                         | 0.9       | 0.7       | 0.3       | 0.7       | 1.8       | 1.0       | 0.9     |
| Others²       |                         | 4.5       | 6.6       | 7.5       | 5.0       | 5.2       | 6.6       | 5.7     |
| Total (0-240 h)³ |                       | 43.5      | 44.1      | 33.1      | 39.1      | 49.7      | 46.0      | 42.6    |
| Total (0-677 h)⁴ |                       | 46.1      | 49.2      | 35.5      | 42.6      | 52.3      | 49.9      | 45.9    |

ND: Radioactive peak not detected or below the limit of quantitation (<20 cpm in peak height)

¹Peak co-eluted, the % dose was calculated based on MS response. ²Sum of metabolites <1.0% of dose in feces. ³Subject 2: 0-264 h. Other subjects: 0-240 h. ⁴Subject 2, 4, and 6: 0-509 h. Other subjects: 0-677 h
Supplementary Table 7. Frequency of subjects with on-treatment AEs (mass balance recovery of BI 425809)

| System organ class                       | Preferred term                  | BI 425809 |
|------------------------------------------|---------------------------------|-----------|
|                                           |                                 | N         | %          |
| Number of subjects                       |                                 | 6         | 100.00     |
| Total with on-treatment adverse events   |                                 | 5         | 83.3       |
| Nervous system disorders                 |                                 |           |            |
|                                           | Headache                        | 3         | 50.0       |
|                                           | Dizziness                       | 1         | 16.7       |
| Gastrointestinal disorders               |                                 | 3         | 50.0       |
|                                           | Abdominal discomfort            | 1         | 16.7       |
|                                           | Diarrhea                        | 1         | 16.7       |
|                                           | Feces discolored                | 1         | 16.7       |
|                                           | Flatulence                      | 1         | 16.7       |
|                                           | Frequent bowel movements        | 1         | 16.7       |
| Infections and infestations              |                                 | 1         | 16.7       |
|                                           | Conjunctivitis                  | 1         | 16.7       |
|                                           | Rhinitis                        | 1         | 16.7       |
| Musculoskeletal and connective tissue disorders |           | 1         | 16.7       |
|                                           | Myalgia                         | 1         | 16.7       |
| Psychiatric disorders                    |                                 | 1         | 16.7       |
|                                           | Insomnia                        | 1         | 16.7       |
| Renal and urinary disorders              |                                 | 1         | 16.7       |
|                                           | Pollakiuria                     | 1         | 16.7       |

AEs, adverse events