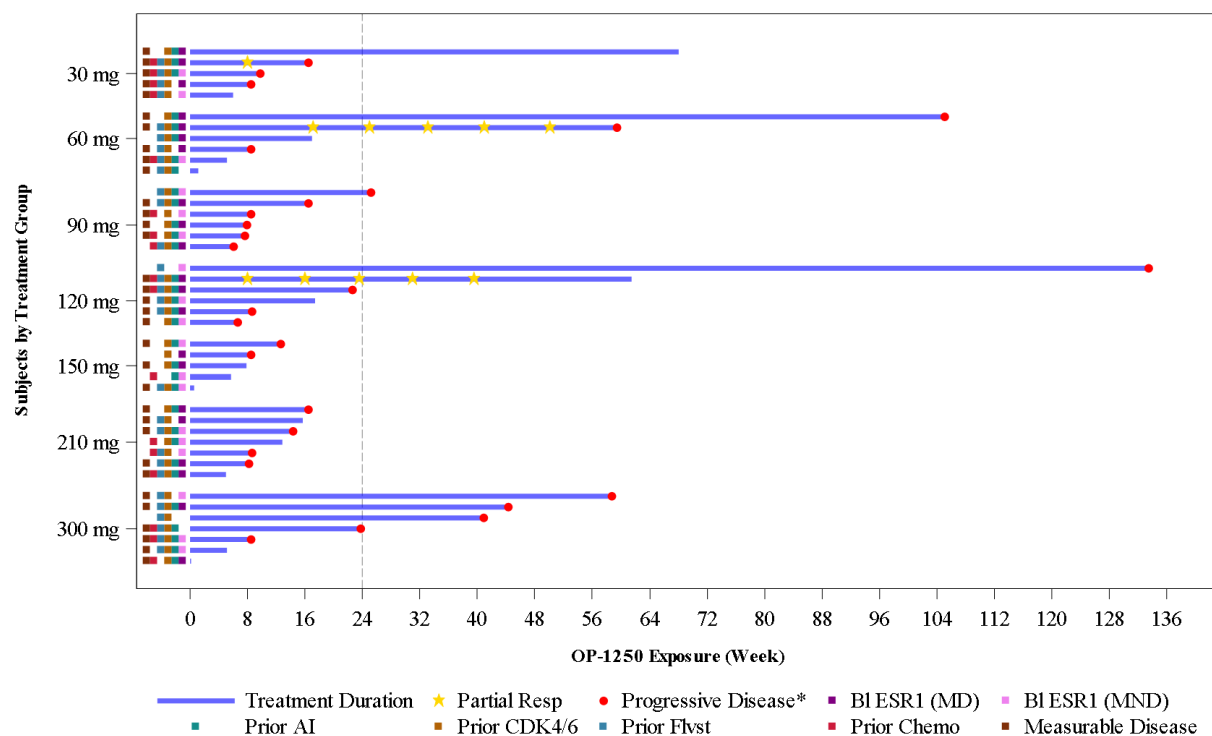


**Supplemental Figure 1. Duration of treatment and best overall responses**

Duration of treatment and best overall responses in patients in the dose-escalation cohorts (phase 1a). Each lane represents one patient. Solid purple boxes represent *ESR1* mutation, solid pink boxes represent *ESR1* wild-type, and the absence of a box indicates missing data. \*Progression by radiographic assessment only.



AI, aromatase inhibitor; BI, baseline; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; Chemo, chemotherapy; *ESR1*, estrogen receptor 1; Fulv, fulvestrant, MD, mutation detected, MND, mutation not detected.

**Supplemental Figure 2. Biomarker analyses following treatment with palazestrant.**

Quantitative analyses were performed on patient tissue and blood samples collected at baseline and

cycle 3 day 1 of palazestrant treatment. A) The percentage of Ki67-positive nuclei was significantly

decreased after treatment with palazestrant compared ( $P=.041$ ). B) ER Allred scores were decreased

after treatment with palazestrant ( $P=.13$ ). C) Sankey diagram showing the number of mutations detected

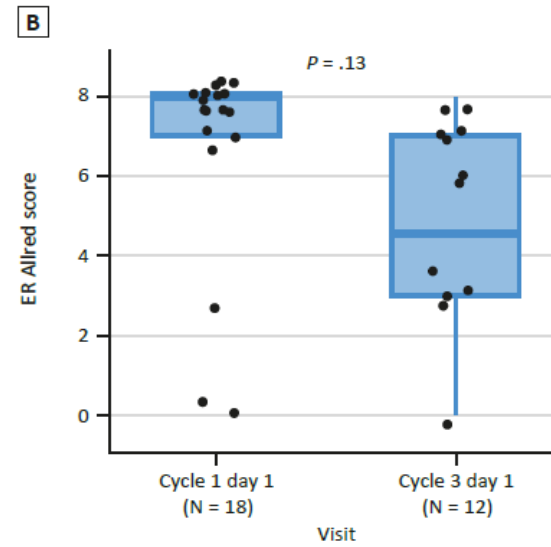
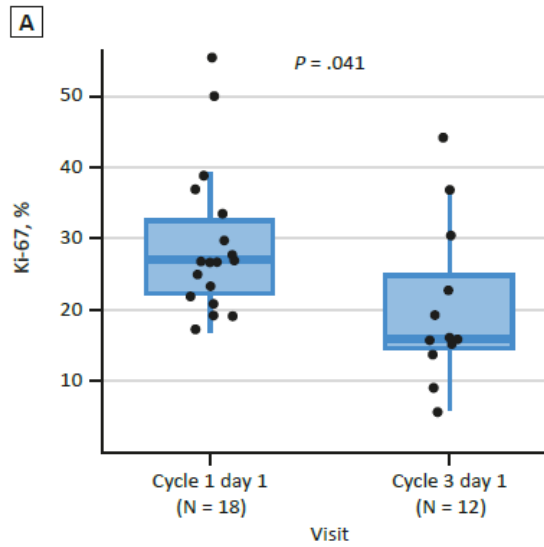
in cfDNA for each *ESR1* variant before and after treatment with palazestrant. N = number of patients in

the analysis; n= number of mutations. Out of 64 patients who had an *ESR1* mutation detected at C1D1,

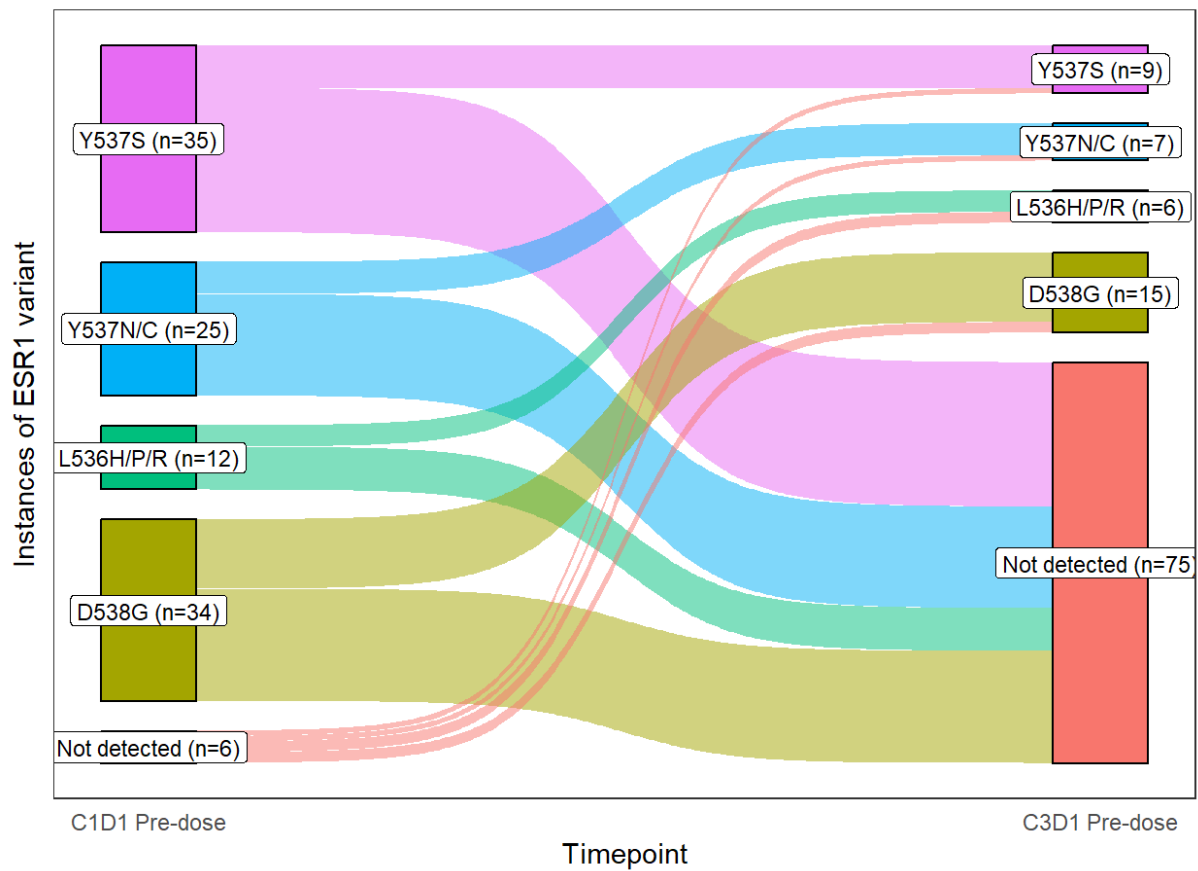
49 (77%) had at least one mutation become “Not detected” at C3D1. Out of 64 patients who had an

*ESR1* mutation detected at C1D1, 43 (67%) had all their detected mutation(s) become “Not detected” at

C3D1.



**C**



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26 cfDNA, cell free DNA; ER, estrogen receptor; ESR1, estrogen receptor 1 gene.

27 **Supplementary Table 1. Patient baseline characteristics and demographics (dose escalation cohort; phase 1a)**

|                                                                            |                                                | Treatment         |                   |                   |                    |                    |                    |                    |                 |
|----------------------------------------------------------------------------|------------------------------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|-----------------|
|                                                                            |                                                | 30 mg QD<br>(N=5) | 60 mg QD<br>(N=6) | 90 mg QD<br>(N=6) | 120 mg QD<br>(N=6) | 150 mg QD<br>(N=5) | 210 mg QD<br>(N=7) | 300 mg QD<br>(N=7) | Total<br>(N=42) |
| Age* (year)                                                                | n                                              | 5                 | 6                 | 6                 | 6                  | 5                  | 7                  | 7                  | 42              |
|                                                                            | Mean (SD)                                      | 62.0 (7.58)       | 62.2 (15.57)      | 54.2 (7.03)       | 57.8 (10.61)       | 70.8 (17.91)       | 67.7 (8.24)        | 63.9 (14.58)       | 62.6 (12.41)    |
|                                                                            | Median                                         | 63.0              | 65.0              | 57.0              | 62.5               | 76.0               | 65.0               | 64.0               | 63.5            |
|                                                                            | Min, Max                                       | 54, 72            | 34, 79            | 40, 58            | 40, 67             | 40, 85             | 57, 77             | 37, 82             | 34, 85          |
| Age Group*                                                                 | < 65                                           | 3 (60.0)          | 3 (50.0)          | 6 (100)           | 4 (66.7)           | 1 (20.0)           | 3 (42.9)           | 4 (57.1)           | 24 (57.1)       |
|                                                                            | 65 - <75                                       | 2 (40.0)          | 2 (33.3)          | 0 (0.0)           | 2 (33.3)           | 1 (20.0)           | 2 (28.6)           | 1 (14.3)           | 10 (23.8)       |
|                                                                            | ≥ 75                                           | 0 (0.0)           | 1 (16.7)          | 0 (0.0)           | 0 (0.0)            | 3 (60.0)           | 2 (28.6)           | 2 (28.6)           | 8 (19.0)        |
| Sex                                                                        | Male                                           | 0 (0.0)           | 0 (0.0)           | 0 (0.0)           | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 0 (0.0)            | 1 (2.4)         |
|                                                                            | Female                                         | 5 (100)           | 6 (100)           | 6 (100)           | 6 (100)            | 5 (100)            | 6 (85.7)           | 7 (100)            | 41 (97.6)       |
| Race                                                                       | Black or African American                      | 0 (0.0)           | 0 (0.0)           | 0 (0.0)           | 1 (16.7)           | 0 (0.0)            | 0 (0.0)            | 0 (0.0)            | 1 (2.4)         |
|                                                                            | White                                          | 5 (100)           | 5 (83.3)          | 4 (66.7)          | 5 (83.3)           | 5 (100)            | 7 (100)            | 6 (85.7)           | 37 (88.1)       |
|                                                                            | Not Reported                                   | 0 (0.0)           | 1 (16.7)          | 2 (33.3)          | 0 (0.0)            | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 4 (9.5)         |
| Menopausal Status (Female)                                                 | Post-menopausal                                | 5 (100)           | 6 (100)           | 6 (100)           | 6 (100)            | 4 (80.0)           | 6 (100)            | 7 (100)            | 40 (97.6)       |
|                                                                            | Peri/pre-menopausal and taking an LHRH agonist | 0 (0.0)           | 0 (0.0)           | 0 (0.0)           | 0 (0.0)            | 1 (20.0)           | 0 (0.0)            | 0 (0.0)            | 1 (2.4)         |
| Baseline ECOG Score                                                        | 0                                              | 2 (40.0)          | 5 (83.3)          | 1 (16.7)          | 1 (16.7)           | 1 (20.0)           | 2 (28.6)           | 6 (85.7)           | 18 (42.9)       |
|                                                                            | 1                                              | 3 (60.0)          | 1 (16.7)          | 5 (83.3)          | 5 (83.3)           | 4 (80.0)           | 5 (71.4)           | 1 (14.3)           | 24 (57.1)       |
| Presence of Visceral Disease (Liver, Lung, Pleura, Ascites, or Peritoneum) | Yes                                            | 3 (60.0)          | 4 (66.7)          | 2 (33.3)          | 5 (83.3)           | 2 (40.0)           | 4 (57.1)           | 5 (71.4)           | 25 (59.5)       |
|                                                                            | No                                             | 2 (40.0)          | 2 (33.3)          | 4 (66.7)          | 1 (16.7)           | 3 (60.0)           | 3 (42.9)           | 2 (28.6)           | 17 (40.5)       |
| Prior Lines of Therapy in Advanced Setting                                 |                                                | 5 (100)           | 6 (100)           | 6 (100)           | 6 (100)            | 5 (100)            | 7 (100)            | 7 (100)            | 42 (100)        |
|                                                                            | 1                                              | 1 (20.0)          | 2 (33.3)          | 1 (16.7)          | 1 (16.7)           | 2 (40.0)           | 0 (0.0)            | 2 (28.6)           | 9 (21.4)        |
|                                                                            | 2                                              | 1 (20.0)          | 2 (33.3)          | 2 (33.3)          | 2 (33.3)           | 2 (40.0)           | 3 (42.9)           | 0 (0.0)            | 12 (28.6)       |
|                                                                            | 3                                              | 0 (0.0)           | 0 (0.0)           | 2 (33.3)          | 1 (16.7)           | 1 (20.0)           | 2 (28.6)           | 2 (28.6)           | 8 (19.0)        |

|   |                                                                                       | Treatment         |                   |                   |                    |                    |                    |                    |                 |
|---|---------------------------------------------------------------------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|-----------------|
|   |                                                                                       | 30 mg QD<br>(N=5) | 60 mg QD<br>(N=6) | 90 mg QD<br>(N=6) | 120 mg QD<br>(N=6) | 150 mg QD<br>(N=5) | 210 mg QD<br>(N=7) | 300 mg QD<br>(N=7) | Total<br>(N=42) |
|   | 4                                                                                     | 1 (20.0)          | 1 (16.7)          | 1 (16.7)          | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 1 (14.3)           | 5 (11.9)        |
|   | 5 or More                                                                             | 2 (40.0)          | 1 (16.7)          | 0 (0.0)           | 2 (33.3)           | 0 (0.0)            | 1 (14.3)           | 2 (28.6)           | 8 (19.0)        |
|   | n                                                                                     | 5                 | 6                 | 6                 | 6                  | 5                  | 7                  | 7                  | 42              |
|   | Mean (SD)                                                                             | 3.6 (2.07)        | 3.0 (2.68)        | 2.5 (1.05)        | 3.2 (1.94)         | 1.8 (0.84)         | 3.0 (1.15)         | 3.3 (1.89)         | 2.9 (1.72)      |
|   | Median                                                                                | 4.0               | 2.0               | 2.5               | 2.5                | 2.0                | 3.0                | 3.0                | 2.5             |
|   | Min, Max                                                                              | 1, 6              | 1, 8              | 1, 4              | 1, 6               | 1, 3               | 2, 5               | 1, 6               | 1, 8            |
|   | Prior Lines of Endocrine<br>Therapy in Advanced<br>Setting<br>[AI/Fulv/Inv/LHRH/SERM] | 5 (100)           | 6 (100)           | 6 (100)           | 6 (100)            | 5 (100)            | 7 (100)            | 7 (100)            | 42 (100)        |
|   | 1                                                                                     | 2 (40.0)          | 2 (33.3)          | 3 (50.0)          | 1 (16.7)           | 3 (60.0)           | 1 (14.3)           | 2 (28.6)           | 14 (33.3)       |
|   | 2                                                                                     | 1 (20.0)          | 2 (33.3)          | 2 (33.3)          | 2 (33.3)           | 1 (20.0)           | 3 (42.9)           | 2 (28.6)           | 13 (31.0)       |
|   | 3                                                                                     | 1 (20.0)          | 0 (0.0)           | 0 (0.0)           | 2 (33.3)           | 1 (20.0)           | 2 (28.6)           | 1 (14.3)           | 7 (16.7)        |
|   | 4                                                                                     | 0 (0.0)           | 1 (16.7)          | 1 (16.7)          | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 2 (28.6)           | 5 (11.9)        |
|   | 5 or More                                                                             | 1 (20.0)          | 1 (16.7)          | 0 (0.0)           | 1 (16.7)           | 0 (0.0)            | 0 (0.0)            | 0 (0.0)            | 3 (7.1)         |
|   | n                                                                                     | 5                 | 6                 | 6                 | 6                  | 5                  | 7                  | 7                  | 42              |
|   | Mean (SD)                                                                             | 2.4 (1.67)        | 2.5 (1.64)        | 1.8 (1.17)        | 2.7 (1.37)         | 1.6 (0.89)         | 2.4 (0.98)         | 2.4 (1.27)         | 2.3 (1.25)      |
|   | Median                                                                                | 2.0               | 2.0               | 1.5               | 2.5                | 1.0                | 2.0                | 2.0                | 2.0             |
|   | Min, Max                                                                              | 1, 5              | 1, 5              | 1, 4              | 1, 5               | 1, 3               | 1, 4               | 1, 4               | 1, 5            |
|   | Prior Types of Anti-<br>Cancer Treatment in<br>Advanced Setting                       | 5 (100)           | 6 (100)           | 6 (100)           | 6 (100)            | 5 (100)            | 7 (100)            | 7 (100)            | 42 (100)        |
|   | [- Chemo]                                                                             |                   |                   |                   |                    |                    |                    |                    |                 |
|   | [- Endocrine]                                                                         |                   |                   |                   |                    |                    |                    |                    |                 |
|   | Chemotherapy                                                                          | 4 (80.0)          | 1 (16.7)          | 3 (50.0)          | 2 (33.3)           | 1 (20.0)           | 3 (42.9)           | 3 (42.9)           | 17 (40.5)       |
|   | Aromatase Inhibitor<br>(AI)                                                           | 3 (60.0)          | 5 (83.3)          | 5 (83.3)          | 5 (83.3)           | 4 (80.0)           | 5 (71.4)           | 5 (71.4)           | 32 (76.2)       |
|   | Fulvestrant                                                                           | 4 (80.0)          | 5 (83.3)          | 3 (50.0)          | 5 (83.3)           | 1 (20.0)           | 5 (71.4)           | 6 (85.7)           | 29 (69.0)       |
|   | Investigational<br>Endocrine                                                          | 0 (0.0)           | 1 (16.7)          | 0 (0.0)           | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 0 (0.0)            | 2 (4.8)         |
|   | LHRH Agonist                                                                          | 0 (0.0)           | 0 (0.0)           | 2 (33.3)          | 2 (33.3)           | 0 (0.0)            | 1 (14.3)           | 0 (0.0)            | 5 (11.9)        |
|   | SERM                                                                                  | 2 (40.0)          | 0 (0.0)           | 2 (33.3)          | 1 (16.7)           | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 6 (14.3)        |
|   | CDK4/6 Inhibitor                                                                      | 5 (100)           | 6 (100)           | 6 (100)           | 5 (83.3)           | 4 (80.0)           | 7 (100)            | 7 (100)            | 40 (95.2)       |
|   | Investigational Agent                                                                 | 0 (0.0)           | 0 (0.0)           | 0 (0.0)           | 2 (33.3)           | 0 (0.0)            | 1 (14.3)           | 0 (0.0)            | 3 (7.1)         |
|   | Other Targeted                                                                        | 1 (20.0)          | 1 (16.7)          | 0 (0.0)           | 0 (0.0)            | 0 (0.0)            | 1 (14.3)           | 2 (28.6)           | 5 (11.9)        |
|   | PI3K Inhibitor                                                                        | 1 (20.0)          | 1 (16.7)          | 2 (33.3)          | 1 (16.7)           | 0 (0.0)            | 0 (0.0)            | 0 (0.0)            | 5 (11.9)        |
|   | mTOR Inhibitor                                                                        | 1 (20.0)          | 2 (33.3)          | 2 (33.3)          | 1 (16.7)           | 1 (20.0)           | 2 (28.6)           | 3 (42.9)           | 12 (28.6)       |
| n |                                                                                       | 5                 | 6                 | 6                 | 6                  | 5                  | 7                  | 7                  | 42              |

|                    |                                      | Treatment         |                   |                   |                    |                    |                    |                    |                 |
|--------------------|--------------------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|-----------------|
|                    |                                      | 30 mg QD<br>(N=5) | 60 mg QD<br>(N=6) | 90 mg QD<br>(N=6) | 120 mg QD<br>(N=6) | 150 mg QD<br>(N=5) | 210 mg QD<br>(N=7) | 300 mg QD<br>(N=7) | Total<br>(N=42) |
|                    | Mean (SD)                            | 4.4 (1.82)        | 3.7 (2.25)        | 4.3 (1.86)        | 4.3 (1.63)         | 2.6 (0.89)         | 4.1 (1.77)         | 3.9 (1.46)         | 3.9 (1.69)      |
|                    | Median                               | 4.0               | 3.0               | 4.0               | 4.5                | 2.0                | 4.0                | 4.0                | 4.0             |
|                    | Min, Max                             | 2, 7              | 2, 8              | 2, 7              | 2, 6               | 2, 4               | 3, 8               | 2, 6               | 2, 8            |
|                    | ESR1 Mutations at<br>Baseline(ctDNA) |                   |                   |                   |                    |                    |                    |                    |                 |
|                    | Patients with C1D1<br>Evaluation     | 5                 | 5                 | 6                 | 6                  | 5                  | 7                  | 5                  | 39              |
|                    | Mutation Detected                    | 3 (60.0)          | 4 (80.0)          | 3 (50.0)          | 3 (50.0)           | 2 (40.0)           | 4 (57.1)           | 2 (40.0)           | 21 (53.8)       |
|                    | Mutation not<br>Detected             | 2 (40.0)          | 1 (20.0)          | 3 (50.0)          | 3 (50.0)           | 3 (60.0)           | 3 (42.9)           | 3 (60.0)           | 18 (46.2)       |
| Measurable Disease |                                      | 5 (100)           | 5 (83.3)          | 4 (66.7)          | 5 (83.3)           | 3 (60.0)           | 5 (71.4)           | 6 (85.7)           | 33 (78.6)       |

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30 **Supplemental Table 2. TEAEs Occurring in ≥15% of Patients Overall (Dose-Escalation Cohort; Phase 1a)**

| Preferred Term           | Toxicity Grade | 30 mg (N=5) | 60 mg (N=6) | 90 mg (N=6) | 120 mg (N=6) | 150 mg (N=5) | 210 mg (N=7) | 300 mg (N=7) | Total (N=42) |
|--------------------------|----------------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|
| Nausea                   | All            | 2 (40.0)    | 2 (33.3)    | 2 (33.3)    | 5 (83.3)     | 4 (80.0)     | 4 (57.1)     | 6 (85.7)     | 25 (59.5)    |
|                          | G3             | 0           | 0           | 0           | 0            | 1 (20.0)     | 0            | 1 (14.3)     | 2 (4.8)      |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Fatigue                  | All            | 2 (40.0)    | 3 (50.0)    | 0           | 2 (33.3)     | 1 (20.0)     | 2 (28.6)     | 4 (57.1)     | 14 (33.3)    |
|                          | G3             | 0           | 0           | 0           | 0            | 1 (20.0)     | 0            | 0            | 1 (2.4)      |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Vomiting                 | All            | 1 (20.0)    | 1 (16.7)    | 1 (16.7)    | 2 (33.3)     | 2 (40.0)     | 1 (14.3)     | 5 (71.4)     | 13 (31.0)    |
|                          | G3             | 0           | 0           | 0           | 1 (16.7)     | 0            | 0            | 0            | 1 (2.4)      |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Constipation             | All            | 1 (20.0)    | 1 (16.7)    | 1 (16.7)    | 0            | 0            | 4 (57.1)     | 3 (42.9)     | 10 (23.8)    |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Decreased appetite       | All            | 1 (20.0)    | 0           | 1 (16.7)    | 1 (16.7)     | 1 (20.0)     | 3 (42.9)     | 3 (42.9)     | 10 (23.8)    |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Headache                 | All            | 1 (20.0)    | 1 (16.7)    | 1 (16.7)    | 1 (16.7)     | 1 (20.0)     | 0            | 4 (57.1)     | 9 (21.4)     |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Neutropenia <sup>a</sup> | All            | 1 (20.0)    | 1 (16.7)    | 0           | 2 (33.3)     | 2 (40.0)     | 1 (14.3)     | 3 (42.9)     | 10 (23.8)    |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 1 (20.0)    | 0           | 0           | 1 (16.7)     | 1 (20.0)     | 0            | 1 (14.3)     | 4 (9.5)      |
| Diarrhea                 | All            | 2 (40.0)    | 2 (33.3)    | 0           | 1 (16.7)     | 1 (20.0)     | 0            | 1 (14.3)     | 7 (16.7)     |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
| Maculopapular rash       | All            | 1 (20.0)    | 1 (16.7)    | 0           | 1 (16.7)     | 0            | 2 (28.6)     | 2 (28.6)     | 7 (16.7)     |
|                          | G3             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |
|                          | G4             | 0           | 0           | 0           | 0            | 0            | 0            | 0            | 0            |

31 <sup>a</sup>Includes preferred terms “neutropenia” and “neutrophil count decreased.” TEAE, treatment-emergent adverse event.