The small molecule tyrosine kinase inhibitor NVP-BHG712 antagonizes ABCC10-mediated paclitaxel resistance: a preclinical and pharmacokinetic study

Supplementary Material

Supplemental Figure 1: The effect of NVP-BHG712 administration on HEK293/pcDNA3.1 tumor growth in vivo. (A) Changes in tumor volume over time are shown. Bar graphs represent

1
mean tumor volume for each experimental group (n = 8) during treatment. Each point on the graph represents the mean tumor volume (mm$^3$) at a particular time after treatment. Error bars represent SEM. *, $p < 0.05$ versus the vehicle group. (B) The bar graph represents the mean tumor weight (n = 8) of the excised HEK293/pCdNA3.1 tumors from different mice. The treatments were as follows: vehicle, paclitaxel, NVP-BHG712 and NVP-BHG712 plus paclitaxel. Each column represents the mean tumor weight determinations. Error bars represent SEM. *, $p < 0.05$ versus the control group.

Supplemental Figure 2: The changes in mean body weight of mice (n = 8) after treatment.
The mean body weight (n = 8) of the the mice treated with with vehicle, paclitaxel, NVP-BHG712 or combination of NVP-BHG712 plus paclitaxel, at the end of the 18-day treatment period. Error bars represent SEM. *: $p < 0.05$ versus vehicle group.
Supplemental Figure 3: Concentration-response curve for sensitization to paclitaxel with or without TKIs. The sensitization effect of TKIs in combination with paclitaxel is shown in ABCC10-expressing HEK293/ABCC10 cells. Error bars represent SD.
Supplemental Table 1: NVP-BHG712 sensitizes HEK293/ABCC10 cells to vinblastine and vincristine with no or minimal effect on HEK293/pcDNA3.1 cells

| Compounds               | HEK293/pcDNA3.1 | HEK293/ABCC10 |
|-------------------------|-----------------|---------------|
|                         | IC₅₀ ± SDᵃ (nM) | FRᵇ | IC₅₀ ± SD (nM) | FR |   |
| Docetaxel               | 11.01 ± 1.2     | [1.0] | 102.22 ± 5.0   | [9.3] |   |
| +NVP-BHG712 0.25 μM    | 10.59 ± 0.9     | [1.0] | 29.60 ± 9.4**  | [2.7] |   |
| +NVP-BHG712 0.5 μM     | 10.12 ± 1.0     | [0.9] | 10.63 ± 1.0**  | [1.0] |   |
| +Cepharanthine 2.5 μM  | 10.08 ± 0.9     | [0.9] | 10.86 ± 1.0**  | [1.0] |   |
| Vinblastine             | 10.21 ± 0.9     | [1.0] | 93.39 ± 6.4    | [9.1] |   |
| +NVP-BHG712 0.25 μM    | 10.03 ± 0.8     | [1.0] | 33.78 ± 5.6**  | [3.3] |   |
| +NVP-BHG712 0.5 μM     | 9.92 ± 0.8      | [1.0] | 10.66 ± 0.8**  | [1.0] |   |
| +Cepharanthine 2.5 μM  | 10.15 ± 0.9     | [1.0] | 11.86 ± 3.3**  | [1.1] |   |

ᵃIC₅₀: The drug concentration that inhibited cell survival by 50% (mean ± SD). ᵇFR: fold-resistance was determined by dividing the IC₅₀ values of substrate in HEK293/ABCC10 cells by the IC₅₀ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712; or the IC₅₀ of substrate in HEK293/pcDNA3.1 cells in the presence of NVP-BHG712 divided by the IC₅₀ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712. Values in the table are representative of at least 3 independent experiments performed in triplicate. ** indicate significant statistical difference from the IC₅₀ values of HEK293/ABCC10 without the reversal drug. **: p <0.01.
Supplemental Table 2: The effect of NVP-BHG712 on the cytotoxicity of paclitaxel, vinblastine and colchicine to HEK293/pcDNA3.1 and HEK293/ABCB1 cells

| Compounds      | HEK293/pcDNA3.1 |         | HEK293/ABCB1 |         |
|----------------|-----------------|---------|--------------|---------|
|                | IC₅₀ ± SDᵃ (nM) | FRᵇ    | IC₅₀ ± SD (nM) | FR     |
| Paclitaxel     | 10.60 ± 1.3     | [1.0]   | 2507.49 ± 424.7 | [236.5] |
| +NVP-BHG712 0.25 μM | 10.20 ± 0.9   | [1.0]   | 2013.14 ± 443.5 | [189.9] |
| +NVP-BHG712 0.5 μM | 9.60 ± 0.5    | [0.9]   | 1089.10 ± 399.9* | [102.7] |
| +Verapamil 2.5 μM  | 9.72 ± 0.7     | [0.9]   | 52.05 ± 19.2**  | [5.0]   |
| Vinblastine    | 10.21 ± 0.9     | [1.0]   | 937.53 ± 62.6   | [91.8]  |
| +NVP-BHG712 0.25 μM | 10.03 ± 0.8   | [1.0]   | 781.68 ± 84.4   | [76.5]  |
| +NVP-BHG712 0.5 μM | 9.92 ± 0.8    | [1.0]   | 250.27 ± 67.3** | [24.5]  |
| +Verapamil 2.5 μM  | 10.15 ± 0.9    | [1.0]   | 39.87 ± 8.4**   | [3.9]   |
| Colchicine     | 10.72 ± 1.6     | [1.0]   | 787.24 ± 148.3  | [73.4]  |
| +NVP-BHG712 0.25 μM | 10.24 ± 1.3   | [1.0]   | 551.50 ± 187.6  | [51.4]  |
| +NVP-BHG712 0.5 μM | 9.55 ± 1.5    | [0.9]   | 245.95 ± 58.2** | [22.9]  |
| +Verapamil 2.5 μM  | 9.61 ± 1.2     | [0.9]   | 35.69 ± 10.3**  | [3.3]   |

ᵃIC₅₀: The drug concentration that inhibited cell survival by 50% (means ± SD). ᵇFR: fold-resistance was determined by dividing the IC₅₀ values of substrate in HEK293/ABCB1 cells by the IC₅₀ value of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712; or the IC₅₀ of substrate in HEK293/pcDNA3.1 cells in the presence of NVP-BHG712 divided by the IC₅₀ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712. Values in table are
representative of at least three independent experiments performed in triplicate. * and ** indicate significant statistical difference from the IC$_{50}$ values of HEK293/ABCB1 without the reversal drug. *: $p < 0.05$; **: $p < 0.01$. 
Supplemental Table 3: The effect of NVP-BHG712 on the cytotoxicity of vincristine to HEK293/pcDNA3.1 and HEK293/ABCC1 cells

| Compounds                  | HEK293/pcDNA3.1 | HEK293/ABCC1 |
|----------------------------|----------------|-------------|
|                            | IC$_{50}$ ± SD$^a$ (nM) | FR$^b$ | IC$_{50}$ ± SD (nM) | FR |
| Vincristine                | 9.92 ± 0.6      | [1.0]       | 139.30 ± 17.0       | [14.0] |
| +NVP-BHG712 0.5 μM        | 8.68 ± 1.4      | [0.9]       | 43.03 ± 6.0*        | [4.3] |
| +PAK-104P 5 μM            | 8.58 ± 0.2      | [0.9]       | 8.61 ± 1.4**        | [0.9] |

$^a$IC$_{50}$: The drug concentration that inhibited cell survival by 50% (means ± SD). $^b$FR: fold-resistance was determined by dividing the IC$_{50}$ values of substrate in HEK293/ABCC1 cells by the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712; or the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the presence of NVP-BHG712 divided by the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712. Values in table are representative of at least three independent experiments performed in triplicate. * and ** indicate significant statistical difference from the IC$_{50}$ values of HEK293/ABCC1 without the reversal drug. *: $p < 0.05$; **: $p < 0.01$. 

Supplemental Table 4: The effect of NVP-BHG712 on the cytotoxicity of vincristine to HEK293/pcDNA3.1, ABCG2-482-R2, ABCG2-482-T7 and ABCG2-482-G2 cells

| Compounds | HEK293/pcDNA3.1 | ABCG2-482-R2 | ABCG2-482-T7 | ABCG2-482-G2 |
|-----------|----------------|--------------|--------------|--------------|
|           | IC$_{50}$ ± SD$^a$ (nM) | FR$^b$ | IC$_{50}$ ± SD (nM) | FR | IC$_{50}$ ± SD (nM) | FR | IC$_{50}$ ± SD (nM) | FR |
| Mitoxantrone | 23.29 ± 4.5 | [1.0] | 132.11 ± 14.1 | [5.7] | 482.18 ± 73.2 | [20.8] | 645.80 ± 47.2 | [27.2] |
| +NVP-BHG712 0.25 µM | 21.45 ± 5.0 | [0.9] | 94.58 ± 20.1 | [4.0] | 440.58 ± 97.9 | [19.0] | 386.91 ± 57.6* | [16.7] |
| +NVP-BHG712 0.5 µM | 20.48 ± 1.6 | [0.9] | 83.99 ± 9.0* | [3.6] | 403.48 ± 91.5 | [17.4] | 326.06 ± 52.6* | [14.3] |
| +Nilotinib 2.5 µM | 26.04 ± 6.7 | [1.1] | 33.64 ± 14.1** | [1.5] | 13.35 ± 6.7** | [0.6] | 18.36 ± 5.9** | [0.8] |

$^a$IC$_{50}$: The drug concentration that inhibited cell survival by 50% (means ± SD). $^b$FR: fold-resistance was determined by dividing the IC$_{50}$ values of substrate in ABCG2-482-R2, ABCG2-482-T7 or ABCG2-482-G2 cells by the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712; or the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the presence of NVP-BHG712 divided by the IC$_{50}$ of substrate in HEK293/pcDNA3.1 cells in the absence of NVP-BHG712. Values in table are representative of at least three independent experiments performed in triplicate. * and ** indicate significant statistical difference from the IC$_{50}$ values of ABCG2-482-R2, ABCG2-482-T7 or ABCG2-482-G2 without the reversal drug. *: $p < 0.05$; **: $p < 0.01$. 

