Figure S4

A

H1781 cells

![Graph showing the effect of different drugs on H1781 cell viability.](image)

- erlotinib
- afatinib
- AZD9291
- AZ5104

B

100 nM, 8h

![Western blot analysis showing protein expression levels under different conditions.](image)

- pHER2
- HER2
- pAKT
- AKT
- pERK
- ERK
- Actin

C

<table>
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<tr>
<th>Condition</th>
<th>DMSO</th>
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<th>AZD9291</th>
<th>AZ5104</th>
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![Additional western blot analysis showing protein expression under different conditions.](image)

- pHER2
- HER2
- Actin
Figure S4. Efficacy of AZD9291 and other EGFR TKIs against lung-cancer associated HER2 mutants. 

A, H1781 cells, which contain a VC insertion at G776 in exon 20 of HER2, were treated with each drug for 5 days. Cell growth relative to controls was assessed using Cell Titer Blue according to the manufacturer's instructions. All experimental points were plated in hextuplicate; bars, SD. 

B, H1781 cells were treated with each drug for 8 hours, after which cell lysates were subjected to immunoblot analysis with antibodies against the indicated proteins. 

C, 293 cells were transiently transfected with either empty vector (mock) or a HER2 mutant (exon20 YVMA 776-779ins) expression vector. Transfectants were treated with various drugs for 4 h. Corresponding cell lysates were subjected to immunoblotting with the indicated antibodies.