**Supplementary Figure S1. Comparative potency of tucatinib, neratinib, and lapatinib in cellular HER2 and EGFR phosphorylation assays**

Inhibition of HER2 and EGFR phosphorylation by tucatinib, neratinib, or lapatinib in tumor-derived cell lines. BT-474, A431, and NCI-N87 cells were treated with tucatinib, neratinib, or lapatinib using an 11-point titration. Samples were analyzed by ELISA using a protein or phospho-specific protein (anti-HER2, anti–phospho-HER2, anti-EGFR, anti–phospho-EGFR). Results are expressed as total phosphoprotein signal normalized to total protein ± SD.



**Supplementary Figure S2. Effect of tucatinib, trastuzumab, or combination treatment on mouse body weight in HER2+ PDX models**

Percent body weight change in mice treated with vehicle, 50 mg/kg BID tucatinib, 20 mg/kg QW trastuzumab or the combination of tucatinib + trastuzumab. Error bars represent ± SEM.

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**Supplementary Table S1. Tucatinib activity correlates with HER2 surface receptor density**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cell line** | **HER2 status** | **HER2 expression** | **Tucatinib potency (EC50 in nM)** | **T-DM1 potency(EC50 in ng/mL)** |
| HCC-1419 | High | 1,515,661 | 23 | 704 |
| SK-BR-3 | High | 1,384,157 | 27 | 13 |
| HCC-1954 | High | 1,259,398 | 50 | 7 |
| ZR-75-30 | High | 1,240,426 | 156 | 79 |
| AU-565 | High | 1,153,419 | 33 | 31 |
| HCC-1569 | High | 938,858 | 249 | 118 |
| HCC2218 | High | 905,340 | 18 | 52 |
| BT-474 | High | 864,064 | 33 | 191 |
| UACC-893 | High | 775,719 | 431 | > 25000 |
| UACC-812 | High | 722,241 | 64 | 37 |
| HCC-202 | High | 303,584 | 282 | 40 |
| JIMT-1 | Low | 123,626 | 15147 | 1395 |
| BT-483 | Low | 82,187 | > 25000 | > 25000 |
| CAMA-1 | Low | 57,568 | 7309 | 4997 |
| T47D | Low | 25,797 | 4938 | > 25000 |
| MCF7 (ATCC) | Low | 20,855 | 10020 | > 25000 |
| MCF7 (NCI) | Low | 17,859 | > 25000 | > 25000 |
| MDA-MB-231 | Low | 4,350 | 16482 | > 25000 |
| MDA-MB-436 | Negative | 1,702 | 17601 | 23878 |
| Hs578T | Negative | 1,663 | 12394 | 11493 |
| MDA-MB-468 | Negative | 1a | 9617 | 7695 |
| DU4475 | Negative | 1a | 8973 | > 25000 |

T-DM1, ado-trastuzumab emtansine.

a Expression below the quantifiable level of detection.

Tucatinib activity was investigated in 22 breast cancer cell lines with or without HER2 overexpression.

**Supplementary Table S2. HER2 copy number variation in PDX models**

|  |  |  |
| --- | --- | --- |
| **Model** | **Histology** | **HER2 Copy Number (NGS)** |
| CTG-0807CTG-0708CTG-0717 | Breast CancerBreast CancerBreast Cancer | 68\*69\*77\* |
| CTG-0137 | Esophageal Cancer | 85\* |
| CTG-0138 | Esophageal Cancer | 60\* |
| CTG-0121 | Colorectal | 34\* |
| CTG-0383 | Colorectal | 41\* |
| CTG-0784 | Colorectal | 80\* |
|  |  |  |
| **Model** | **Histology** | **HER2 Copy Number (PICNIC score)** |
| GXA-3038 | Gastric | 12# |
| GXA-3039 | Gastric | 14# |
| GXA-3054 | Gastric | 14# |

\*HER2 copy number provided by Champions Oncology and are based on NGS analysis of PDX tumor samples.

#HER2 copy number provided by Charles River Laboratories and are based on Affymetrix® Genome-Wide Human SNP Array 6.0.