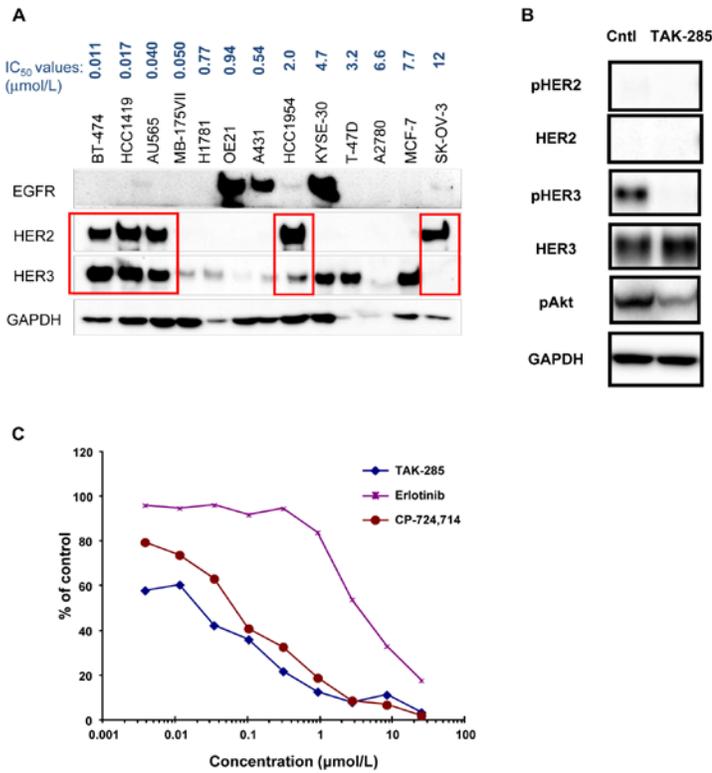
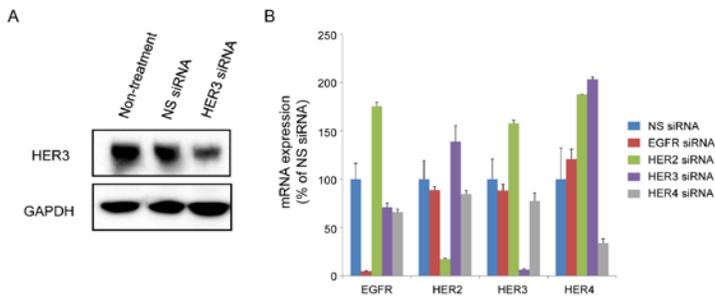


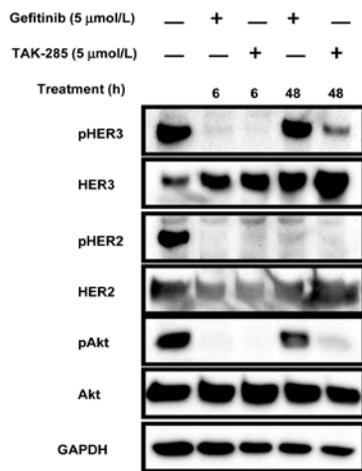
HER2 and HER3 cooperatively regulate cancer cell growth and determine sensitivity to the novel investigational EGFR/HER2 kinase inhibitor TAK-285 – Takagi et al



Supplementary Figure 1: HER2 and HER3 expression in cell lines. A. Coexpression of HER2 and HER3 proteins in TAK-285-sensitive cells. IC₅₀ values of TAK-285 are presented in blue above the names of cell lines. B. A431 cells were treated with 1 μmol/L TAK-285 for 2 h, the cells were lysed, and immunoblot analyses were performed. C. Cell growth inhibition assays were performed using HCC1419 cells, which express both HER2 and HER3.



Supplementary Figure 2: Knockdown of the HER family using siRNAs. A. SK-BR-3 cells were transfected with siRNAs and lysed after 48 h incubation. Immunoblot analyses were performed using anti-HER3 antibody. GAPDH was used as a loading control. B. SK-BR-3 cells were transfected with siRNAs and total RNA was extracted 48 h after transfection. Quantitative PCR was performed.



Supplementary Figure 3: HER3 reactivation in HER tyrosine kinase inhibitor-treated cells. SK-BR-3 cells were treated with 5 $\mu\text{mol/L}$ gefitinib or TAK-285 for 6 or 48 h. Although gefitinib is best known as an EGFR kinase inhibitor, at this concentration it inhibited not only EGFR but also HER2 in cultured cells [37].

Supplementary Table 1: IC₅₀ values and HER family mRNA expression levels. IC₅₀ values of TAK-285 for cell growth inhibition and HER family, PTEN, and IGF1-R mRNA expression levels are presented.

Tissue/Disease	Cell lines	IC ₅₀ (μmol/L)	EGFR	HER2	HER3	HER4	PTEN	IGF-1R
Breast	AU565	0.040	0.001253346	0.318640157	0.012516717	8.94802E-06	0.000109256	0.000956465
Breast	BT-474	0.011	0.000618045	0.655196702	0.031467361	0.001202289	0.004549741	0.007340021
Breast	MCF-7	7.7	0.000104805	0.007976657	0.019640834	0.000709948	0.000425074	0.014179987
Breast	MDA-MB-175VII	0.050	0.000167913	0.015092755	0.0119239	3.36275E-05	0.000372622	0.001185737
Breast	MDA-MB-231	15	0.00837323	0.005640348	0.000104081	5.75E-07	0.001145347	0.002649618
Breast	MDA-MB-361	1.1	0.000440064	0.077481731	0.016515907	0.000911165	0.000557014	0.01067219
Breast	MDA-MB-453	0.54	1.53649E-05	0.079109787	0.021492841	0.000342882	0.002064488	0.001270842
Breast	HCC1419	0.017	6.42411E-07	0.489710149	0.018971795	0.000766195	0.000509016	0.004775939
Breast	HCC1954	2.0	0.00232267	0.420448208	0.003239529	3.74119E-07	0.000280444	0.001809742
Breast	SK-BR-3	0.15	0.001835005	0.297301779	0.011841536	0.000190226	0.000357443	0.000596992
Breast	T-47D	3.2	0.001532391	0.018325546	0.046070913	0.006434305	0.001076079	0.023195681
Breast	UACC-812	0.027	4.14003E-05	0.795536484	0.023035457	0.000491678	0.000618045	0.022561394
Breast	UACC-893	0.25	0.002107868	0.655196702	0.01286861	0.000832651	0.000407758	0.000105534
Breast	ZR-75-1	15	0.000232578	0.016064279	0.009752582	0.00182233	0.000352522	0.001511294
CML	K562	12	1.61504E-06	0.002152158	0.003773189	1.87858E-05	0.000526967	0.001371528
Colon	HCT116	10	0.002371474	0.003594483	0.002182201	5.00544E-07	0.00041345	0.000734984
Colon	HT-29	11	0.003043612	0.008789519	0.012259127	8.18792E-07	0.00085606	0.004157696
Epidermis	A-431	0.54	0.036397925	0.009485898	0.004613253	6.07758E-07	0.000709948	0.006003419
Esophagus	KYSE-30	4.7	0.034196678	0.002781348	0.002724109	6.02754E-06	0.000201072	0.002686605
Esophagus	OE19	0.35	0.001994164	0.673616788	0.015409886	0.000523327	0.000290334	0.00579892
Esophagus	OE21	0.94	0.105112052	0.001046654	0.000911165	2.73871E-07	0.000296434	0.007089993
Esophagus	OE33	9.4	0.005640348	0.052192995	0.006258358	4.06568E-07	0.00054555	0.002800694
Lung	Calu-3	0.14	0.004613253	0.578344092	0.022405551	0.000201072	0.000838443	0.006479059
Lung	H1781	0.77	0.0078125	0.110337875	0.140632311	1.77724E-05	0.001129578	0.015625
Lung	HCC4006	3.2	0.011841536	0.006992383	0.007188966	5.29E-07	0.001297545	0.002919627
Lung fibroblast	MRC-5	17	0.00390625	0.004128977	2.4788E-05	3.90006E-07	0.000512557	0.004978752
Ovary	A2780	6.6	0.000709948	0.010237448	0.000516122	0.001410087	0.000880127	0.001784827
Ovary	OV90	9.5	0.000549345	0.004978752	0.001153313	3.27079E-05	0.000596992	0.007391075
Ovary	SKOV3	12	0.0078125	0.768437591	0.000324386	0.002762136	0.000471649	0.005336095
Prostate	PC-3	12	0.007391075	0.001784827	0.000526967	1.1564E-05	6.77227E-05	0.003172861
Skin	A375	11	0.000151332	0.001532391	0.020333466	2.99695E-07	0.000232578	0.006258358
Skin	Cell	7.7	0.003172861	0.0036194	0.0004192	4.15111E-	0.0009175	0.0048091

	Systems-Fb			85	21	07	03	58
Stomach	NCI-N87	0.11	0.009226505	0.8827029 96	0.0188407 47	9.94173E- 07	0.0015537 82	0.0030017 09
Uterine	MES-SA	15	1.23084E-05	0.0045497 41	8.39564E- 05	5.59251E- 07	0.0001317 42	0.0010180 33
Uterine	MES-SA/Dx- 5	16	8.93607E-05	0.0044871 03	0.0006953 37	2.41424E- 06	0.0001212 27	0.0008269