Table S1 Related to Figure 1: Total number of patients by cancer type across databases

Cancer Type	Total n	Weighted Average frequency of *ERBB2* mutations (Figure 1A)	Weighted Average frequency of *ERBB2* Exon 20 Mutations (Figure 1B)
Bile Duct	829	5.307%	0.724%
Bladder	3146	8.295%	0.858%
Brain	10105	0.350%	0.040%
Breast	29609	3.115%	0.882%
Cervix	1301		0.384%
Colorectal	33302	2.185%	0.287%
Early Gastric Cancer	341	3.812%	0.293%
Endometrial	4962	2.156%	0.181%
Esophageal	4824	2.902%	0.435%
Head and Neck	3428	1.083%	0.146%
Kidney	3600	1.164%	0.167%
Leukemia	2451	0.122%	0.082%
Non-small Cell Lung Cancer	7859	2.150%	1.525%
Melanoma	7409	0.892%	0.165%
Neuroendocrine	60085	0.896%	0.121%
Ovarian	11762	2.380%	0.188%
Pancreatic	7988	0.964%	0.100%
Peritoneal	693	0.937%	0.433%
Prostate	5319	1.154%	0.019%
salivary gland	962	0.303%	0.832%
Sarcoma	3198	0.534%	0.063%
Small Cell	2380		0.336%
Small Intestine	1028	4.730%	1.751%
Stomach	2969	4.515%	0.370%
Thyroid	2175	0.181%	0.046%

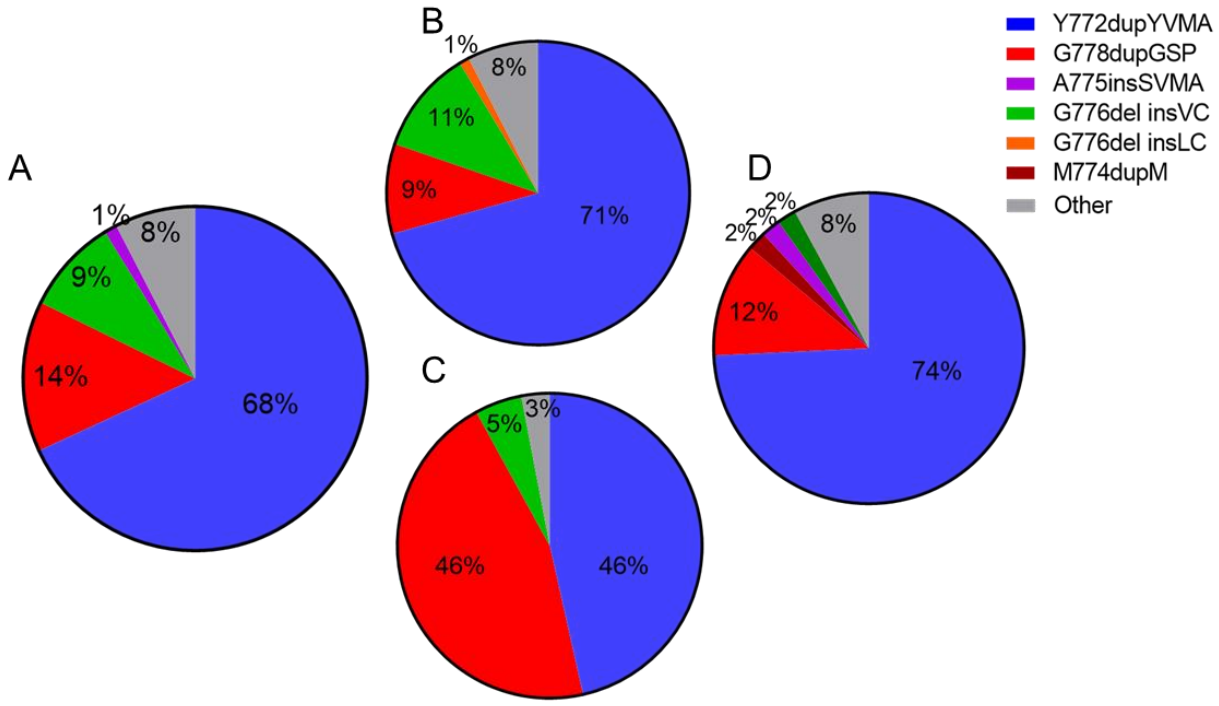


Figure S1 Related to Figure 2: Exon 20 insertion mutations vary in sequence.
(A) *ERBB2* exon 20 insertion mutation frequency in all cancer types.
(B-D) Frequency of *ERBB2* exon 20 insertion mutations in lung cancer **(B),** breast cancer **(C),** and other cancers **(D).**

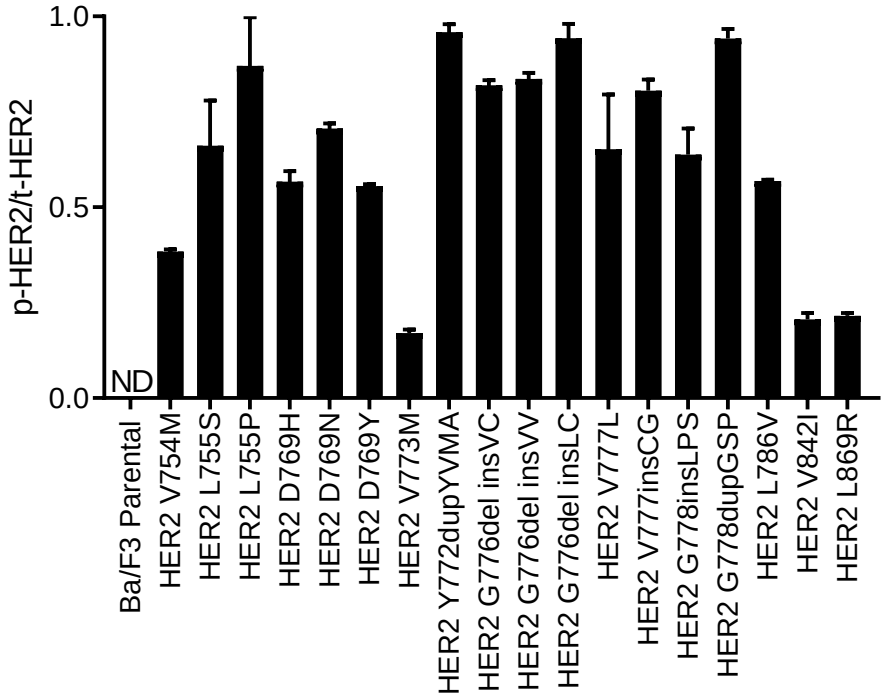


Figure S2 Related to Figure 3: Common HER2 mutants are constitutively, but differentially phosphorylated.

Relative p-HER2 expression was determined by taking the ratio of p-HER2 over total HER2 expression. Bars are representative of the mean ± SEM, and n = 3. ND = below the limit of detection.

A

IC_{50} (nM)	Sapitinib	Laptinib	Afatinib	Dacomitinib	Neratinib	Tarlox-TKI	Poziotinib	Pyrotinib	Ibrutinib	Osimertinib	Nazartinib
EGFR WT (+10 ng/µl)	230.90	176.77	20.20	19.26	38.75	9.85	6.49	639.95	80.87	142.27	349.18
HER2 L755S	376.30	112.46	4.37	12.49	3.28	3.67	0.53	41.60	51.54	12.41	23.48
HER2 L755P	587.25	474.68	20.54	25.16	19.71	5.61	3.65	55.18	190.24	69.58	68.99
HER2 D769H	231.00	31.66	2.71	7.04	2.50	0.57	0.35	5.36	7.38	13.80	35.55
HER2 D769N	83.79	1.76	0.81	2.65	0.51	0.93	0.12	4.53	3.74	8.10	10.10
HER2 D769Y	58.15	8.68	1.48	3.67	1.02	0.59	0.36	0.78	6.68	7.38	23.13
HER2 V773M	115.95	57.57	3.27	15.19	2.99	0.50	1.07	0.57	8.80	29.44	76.71
HER2 V777L	21.19	6.01	1.17	3.42	1.62	0.50	0.13	0.74	4.48	6.96	10.96
HER2 Y772dupYVMA	1274.72	2253.86	42.05	143.43	31.08	27.55	1.87	118.80	343.71	577.77	428.94
HER2 G776del insVC	419.75	2340.50	29.34	84.87	33.59	9.30	4.85	89.82	232.31	412.60	669.49
HER2 G776del insVV	5188.24	972.53	14.90	45.37	20.85	4.83	2.99	1665.65	162.58	188.06	316.36
HER2 G776del insLC	164.00	319.54	7.01	25.11	6.55	2.13	1.13	11.42	40.80	46.96	118.03
HER2 G778insLPS	63.77	6.96	1.67	3.39	1.99	0.87	0.18	0.98	7.02	16.90	44.48
HER2 P780insGSP	299.25	892.97	27.59	95.57	32.27	6.28	4.12	23.10	121.05	440.31	350.86
HER2 L786V	163.28	21.42	5.02	9.78	1.41	1.64	1.22	2.26	8.09	8.30	25.37
HER2 V842I	49.27	8.25	4.05	6.48	1.51	0.82	0.45	0.65	9.13	24.31	49.03
HER2 L869R	195.70	35.57	1.44	3.07	0.96	0.77	0.81	4.96	25.37	27.61	29.06

B

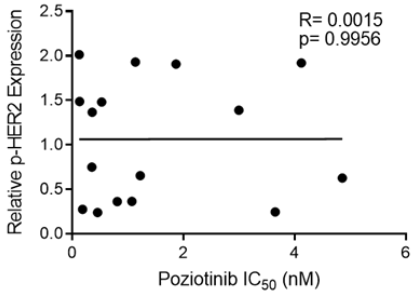


Figure S3 related to Figure 4: Ba/F3 cell drug sensitivity does not correlate with HER2 expression.

(A) Chart of average IC_{50} (nM) values of Ba/F3 cell lines treated with indicated inhibitors for 72 hr (n = 3 biologically independent experiments).

(B) Correlation of the relative p-HER2 was plotted against poziotinib IC_{50} values for Ba/F3 HER2 mutant cell lines. Pearson correlations and p values were determined by GraphPad Prism (n = 3).

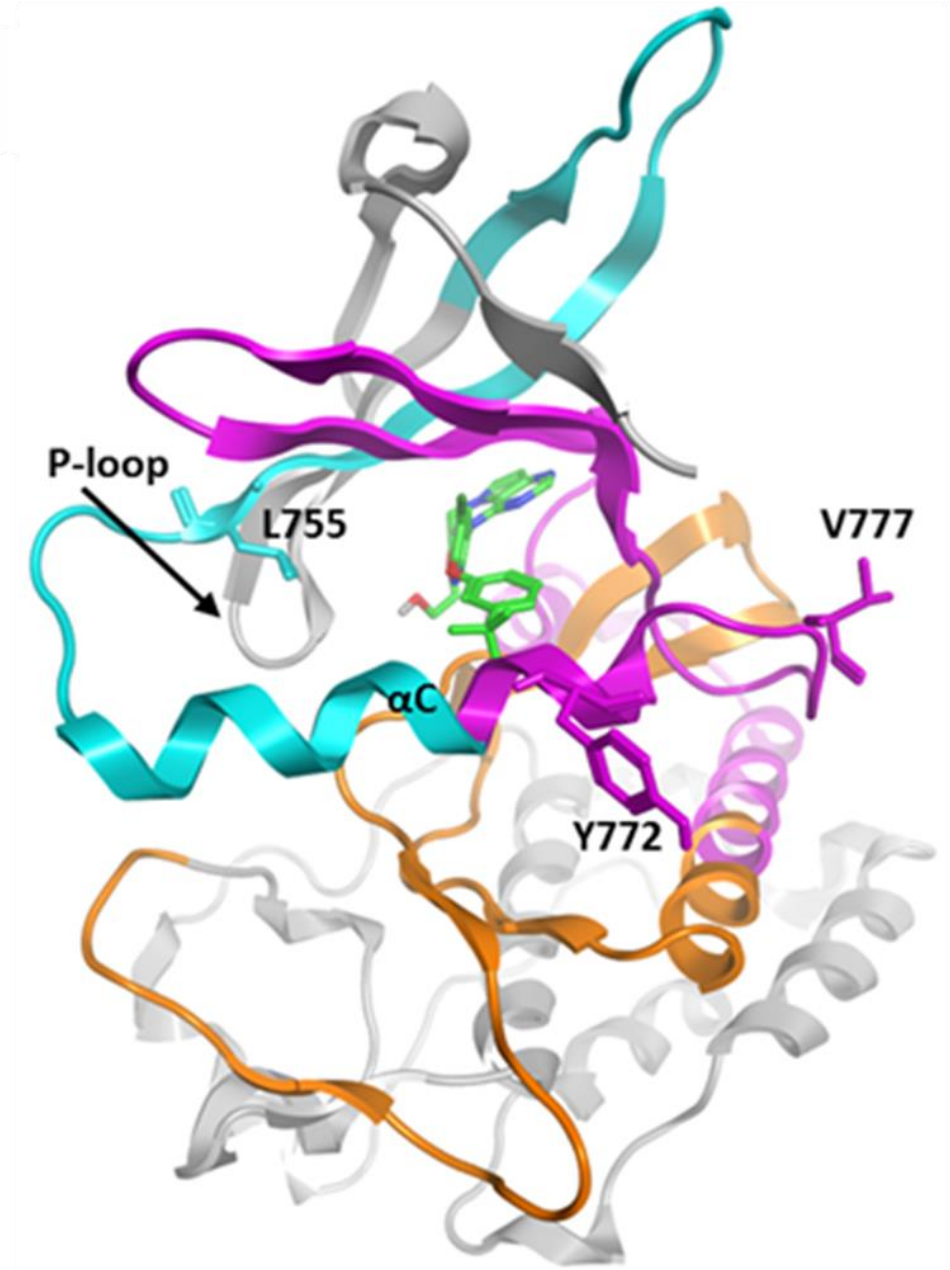
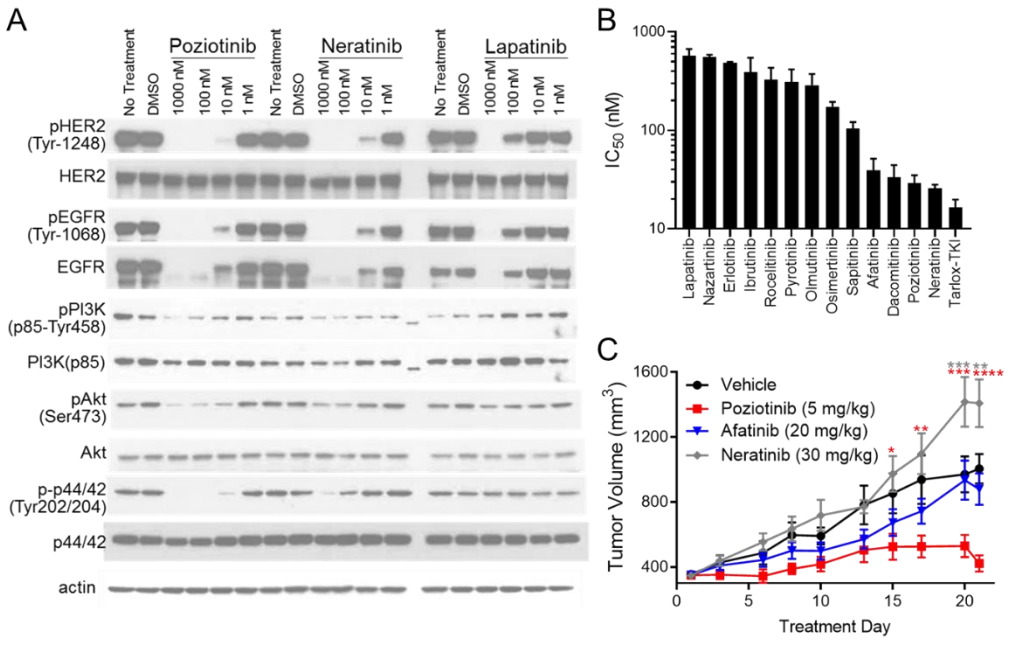


Figure S4 Related to Figure 5: Molecular modeling reveals HER2 mutants differ in binding pocket size.
The protein backbone of HER2 kinase domain encoded by exons 19, 20 and 21 are colored in blue, pink, and orange, respectively. The ligand from the template X-ray structure (PDB 3PP0) is rendered in green sticks and labels are provided for mutated residues/insertion locations.



D

Tukey's multiple comparisons test	Summary	Adjusted p value
Day 10		
Vehicle vs. Poziotinib (5 mg/kg)	ns	0.3888
Vehicle vs. Afatinib (20 mg/kg)	ns	0.8492
Vehicle vs. Neratinib (30 mg/kg)	ns	0.6826
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	ns	0.8809
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	*	0.0333
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	ns	0.2222
Day 13		
Vehicle vs. Poziotinib (5 mg/kg)	ns	0.057
Vehicle vs. Afatinib (20 mg/kg)	ns	0.2563
Vehicle vs. Neratinib (30 mg/kg)	ns	0.9996
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	ns	0.9266
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	ns	0.0737
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	ns	0.3041
Day 15		
Vehicle vs. Poziotinib (5 mg/kg)	*	0.0152
Vehicle vs. Afatinib (20 mg/kg)	ns	0.391
Vehicle vs. Neratinib (30 mg/kg)	ns	0.7201
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	ns	0.5209
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	***	0.0003
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	*	0.0452

Tukey's multiple comparisons test	Summary	Adjusted p value
Day 17		
Vehicle vs. Poziotinib (5 mg/kg)	**	0.0012
Vehicle vs. Afatinib (20 mg/kg)	ns	0.3219
Vehicle vs. Neratinib (30 mg/kg)	ns	0.5028
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	ns	0.193
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	****	<0.0001
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	*	0.0118
Day 20		
Vehicle vs. Poziotinib (5 mg/kg)	***	0.0005
Vehicle vs. Afatinib (20 mg/kg)	ns	0.9896
Vehicle vs. Neratinib (30 mg/kg)	***	0.0007
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	**	0.0015
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	****	<0.0001
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	***	0.0002
Day 21		
Vehicle vs. Poziotinib (5 mg/kg)	****	<0.0001
Vehicle vs. Afatinib (20 mg/kg)	ns	0.6886
Vehicle vs. Neratinib (30 mg/kg)	**	0.0029
Poziotinib (5 mg/kg) vs. Afatinib (20 mg/kg)	***	0.0003
Poziotinib (5 mg/kg) vs. Neratinib (30 mg/kg)	****	<0.0001
Afatinib (20 mg/kg) vs. Neratinib (30 mg/kg)	****	<0.0001

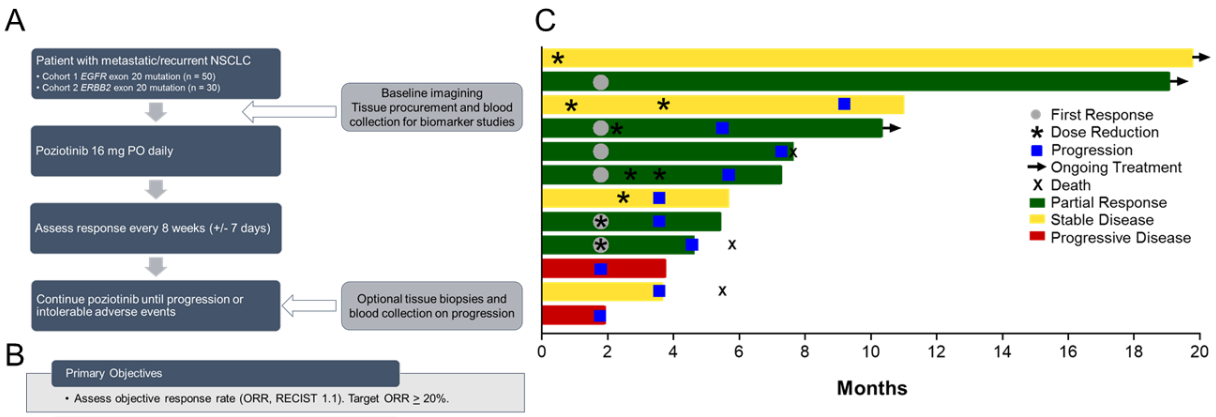
Figure S5 Related to Figure 6: Poziotinib inhibits p-HER2 in HER2 mutant cell lines and inhibits tumor growth in a xenograft of exon 19 mutant colorectal cancer.

(A) Western blot of MCF10A cells expressing G776delinsVC after 2 hr treatment of the indicated drugs and doses.

(B) Bar plot of average IC_{50} values of MCF10A cells expressing WT HER2 treated with indicated inhibitors for 72 hr. Bars are representative of mean ± SEM (n = 3).

(C) Poziotinib inhibits tumor growth in a xenograft of exon 19 mutant colorectal cancer**.** CW-2 cells harboring an *ERBB2* L755S mutation were injected into the flanks of 6-week-old female nu/nu nude mice. When tumors reached 350 mm^3 mice were randomized into four groups: 20 mg/kg afatinib, 5 mg/kg poziotinib, 30 mg/kg neratinib, or vehicle control. Tumor volumes were measured three times per week, and mice received drug Monday- Friday (5 days per week). Symbols are representative of the mean ± SEM for each time point.

(D) Two-Way ANOVA with Tukey's multiple comparisons test was used to determine statistical significance. Asterisk indicate significance between vehicle and poziotinib (red) or neratinib (grey). p values for each comparison are listed below beginning at 10 day when significant differences were first detected.



B

Primary Objectives

- Assess objective response rate (ORR, RECIST 1.1). Target ORR ≥ 20%.

Secondary Objectives

- Progression free survival (PFS)
- Overall Survival (OS)
- Disease control rate
- Duration of response
- Safety and toxicity

Eligibility

- *EGFR* or *ERBB2* exon 20 insertion or point mutation excluding acquired T790M
- One or more systemic therapy (amended to include treatment-naive patients)
- Previous EGFR/HER2 targeted therapies allowed
- Brain metastases permitted if asymptomatic and stable, and without escalating steroids or anticonvulsants.

D

Age	Sex	# of prior lines	Final dose of poziotinib	*ERBB2* Mutation	Co-Occurring Mutations
57	F	1	16 mg	Y772_A775dupYVMA	*TP53* p.R306*
64	F	6	12 mg	Y772_A775dupYVMA	
54	F	1	12 mg	Y772_A775dupYVMA	*KRAS* Y64N
59	F	0	12 mg	Y772_A775dupYVMA	
58	F	3	16 mg	Y772_A775dupYVMA	*TP53* I162fs*8 *CCNE1* Amp
60	F	1	16 mg	G778_P780dupGSP	
61	F	3	8 mg	G778_P780dupGSP	*CDKN2A/B* loss *TP53* S149fs*11 *TERT* promoter -124C>T
62	F	0	12 mg	Y772_A775dupYVMA	
55	F	2	12 mg	G778_P780dupGSP	
61	M	4	8 mg	Y772_A775dupYVMA	*MYC* E251A *APC* R499Q *ARID1A* A578V *NOTCH1* V2476G *CCND2* L118R
63	M	1	8 mg	Y772_A775dupYVMA	
60	F	3	16 mg	Y772_A775dupYVMA	*TP53* P151H

E

Treatment related AEs	n (%)
Grade 3-4	8 (67%)
Grade 5	-
AE leading to treatment dose reduction	8 (67%)
AE leading to treatment discontinuation	-

F

Treatment Related AEs >n=1	Grade 1-2 n (%)	Grade 3-4 n (%)
Alkaline phosphatase increased	2 (17%)	
Alopecia	5 (42%)	
Anorexia	6 (50%)	
Diarrhea	9 (69%)	2 (17%)
Dry Skin	10 (77%)	
Dysgeusia	3 (25%)	
Hypomagnesemia	2 (17%)	
Oral Mucositis	10 (77%)	
Nausea	4 (31%)	1 (8%)
Paronychia	10 (77%)	1 (8%)
Rash	5 (42%)	7 (58%)
Weight loss	2 (17%)	1 (8%)

Figure S6 Related to Figure 7: Study schema, objectives, eligibility, and patient characteristics and co-mutations for clinical trial NCT03066206.

(A) Flow chart of study schema including timeline of patient assessments.
(B) Chart of primary objectives, secondary objectives, and patient eligibility.
(C) Swimmers' plots of individual patients' treatment and response to treatment over time.
(D) Patient characteristics, dose of poziotinib, *ERBB2* mutations, and co-occurring mutations.
(E) Safety summary for n = 12 patients.
(F) Treatment related adverse events (AEs) occurring in more than one patient for n = 12 patients.

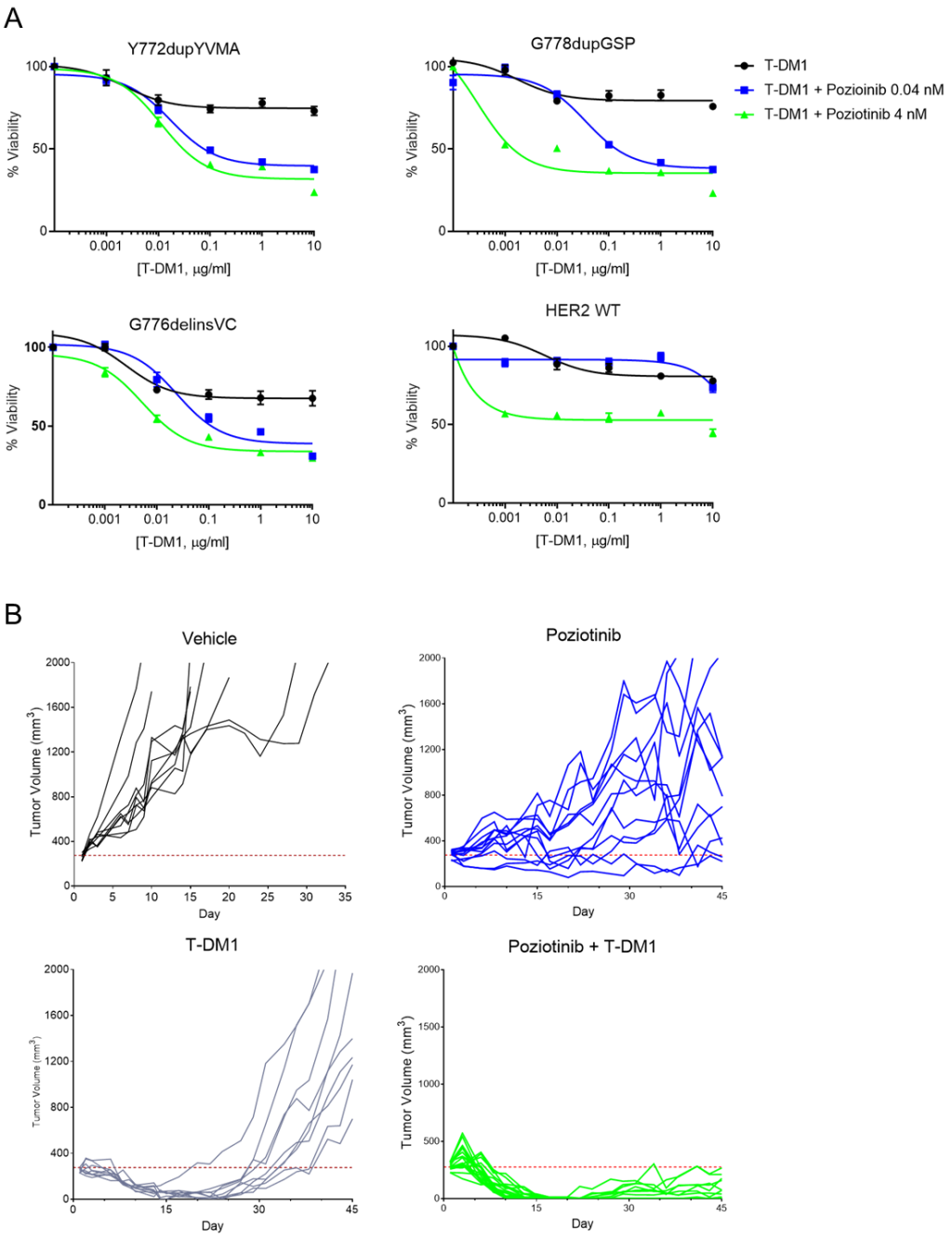


Figure S7 Related to Figure 8: Combination of poziotinib and T-DM1 treatment potentiates anti-tumor activity *in vitro* and *in vivo.*

(A) Dose response curves of MCF10A cell lines expressing HER2 Y772dupYVMA, HER2 G778dupGSP, HER2 G776delinsVC or WT HER2 with T-DM1 alone (black) or T-DM1 in combination with poziotinib (0.04 nM blue and 4 nM green). Cells were treated for 72 hr, and cell viability was determined by Cell Titer Glo Assay. Symbols are representative a mean ±SEM. Graphs are representative of n = 2 independent experiments.

(B) Spider plots of tumor volume of HER2 Y772dupYVMA PDX mice treated with indicated inhibitors. The red dotted line indicates the point of randomization (275 mm^3).