



**Patient derived organoids and xenografts identify neratinib plus HER2 antibody drug conjugate as a synergistic drug combination for HER2 mutated, non-amplified metastatic breast cancer.**

**Shunqiang Li, Maureen Highkin, Tina M. Primeau, Stephanie L. Pratt, Irmina Diala, Jingqin Luo, Richard E. Cutler Jr., Grace Mann, Alshad S. Lalani, Cynthia X. Ma, and Ron Bose**

**AACR Annual Meeting, April 2, 2019**



**Washington University in St. Louis • School of Medicine**

# Disclosure of unlabeled or unapproved uses of drugs

- All of the drugs tested here do not have FDA indications for HER2 mutated breast cancer.
- Vinorelbine is FDA approved for metastatic breast cancer. Trastuzumab and T-DM1 (ado-trastuzumab-emtansine) are FDA approved for HER2 amplified breast cancer.

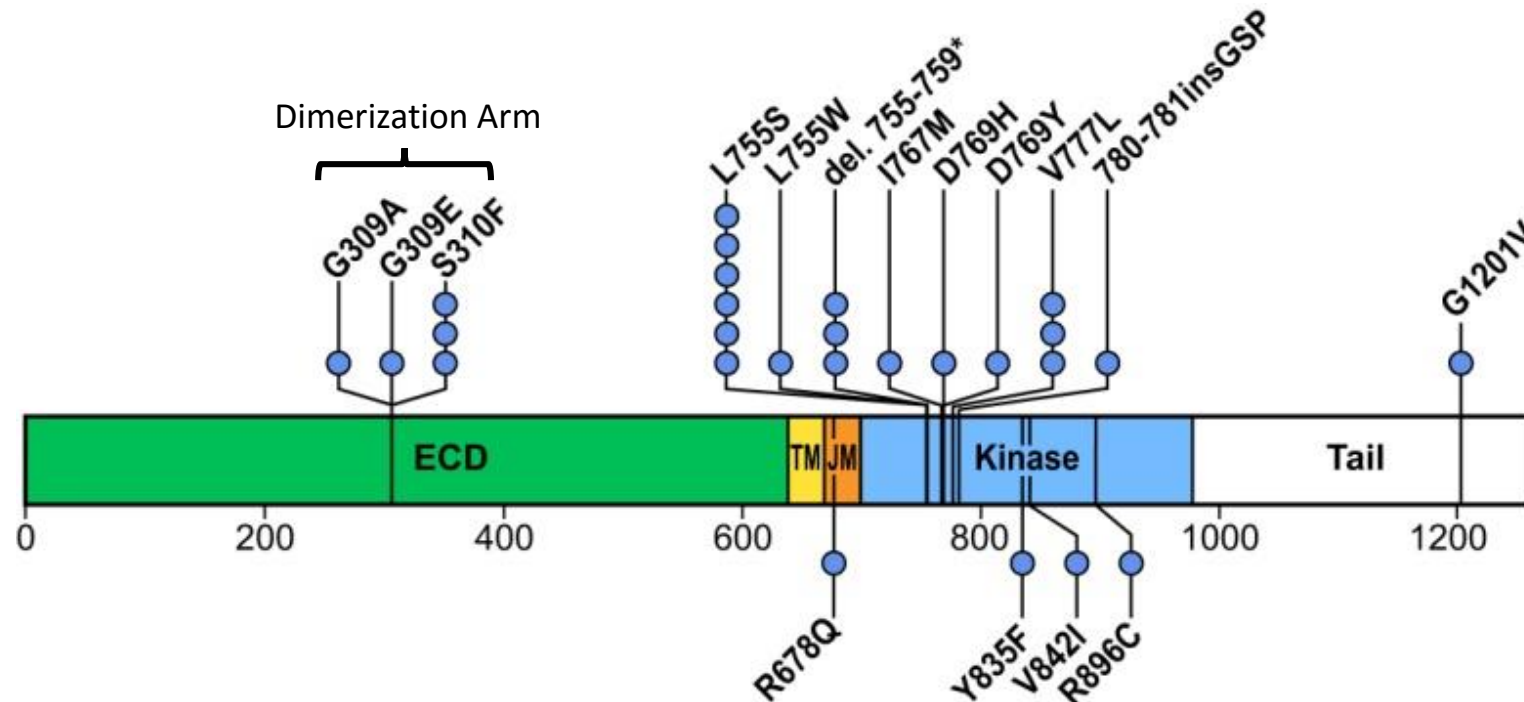
# HER2 Mutated Breast Cancer Cases Identified by The Cancer Genome Atlas (TCGA) Project.

Somatic HER2 mutations	Stage	ER	PR	HER2 Status	HER2 IHC	HER2 FISH Ratio
G309A	IIB	+	+	Negative	1+	
R678Q and L755W	IIB	+	+	Negative	1+	
L755S	IIA	+	+	Negative*	2+	Not available
L755S	I	+	-	Negative*	2+	2.05
D769H	IIB	-	-	Positive	3+	
V777L	IIIA	+	+	Negative	0	
V842I	IIIB	+	+	Negative	1+	

\* Confirmed by SNP chip and/or exome sequencing read number.

# HER2 Gene Sequencing from 1,499 Breast Cancer Patients

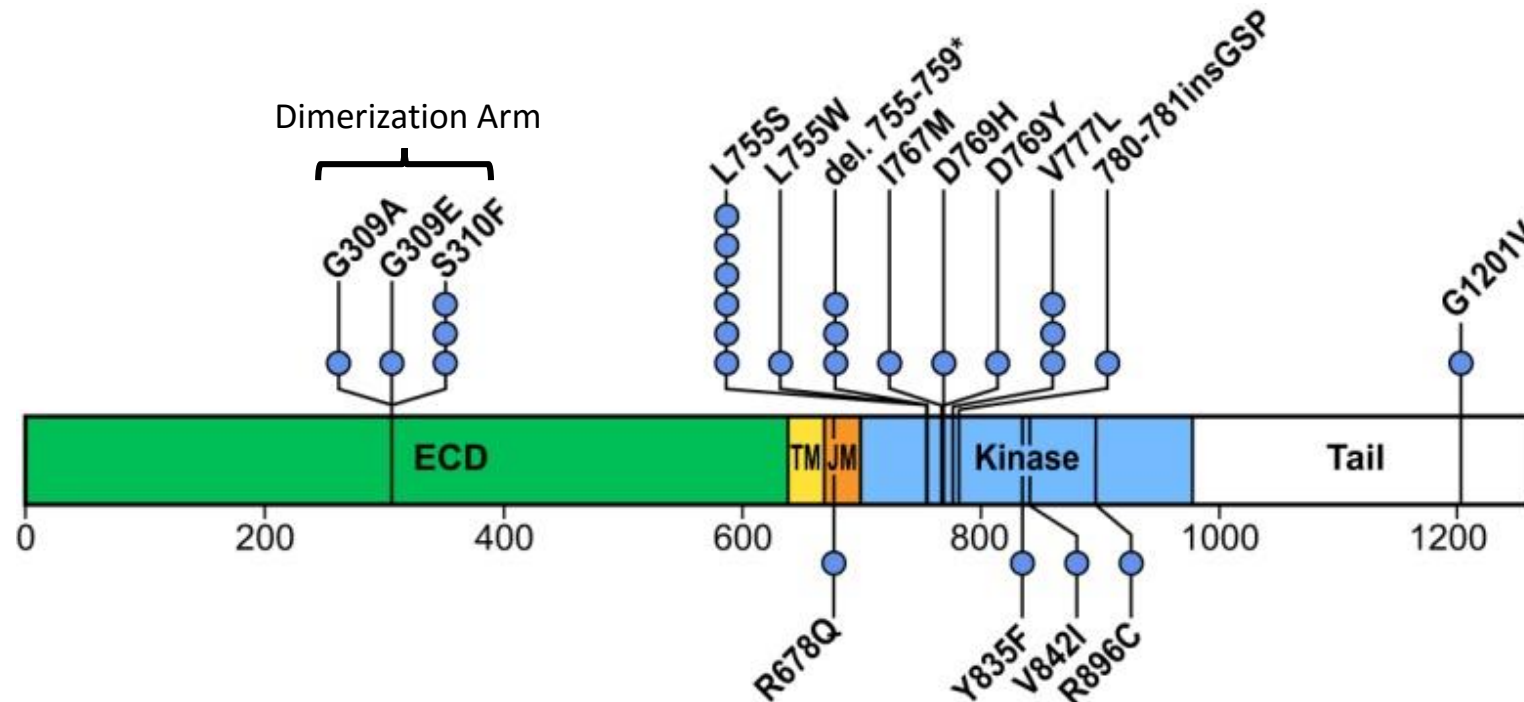
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Each blue circle represents a patient.

This pattern of mutations strongly suggests this is an Activated Oncogene.  
Mirrors the pattern of mutations in RAS or PIK3CA.

# HER2 Gene Sequencing from 1,499 Breast Cancer Patients



## Mutation frequency:

Newly diagnosed patients = 1.6%

Metastatic breast cancer = 2 - 5%

Invasive lobular breast cancer = 5-7%

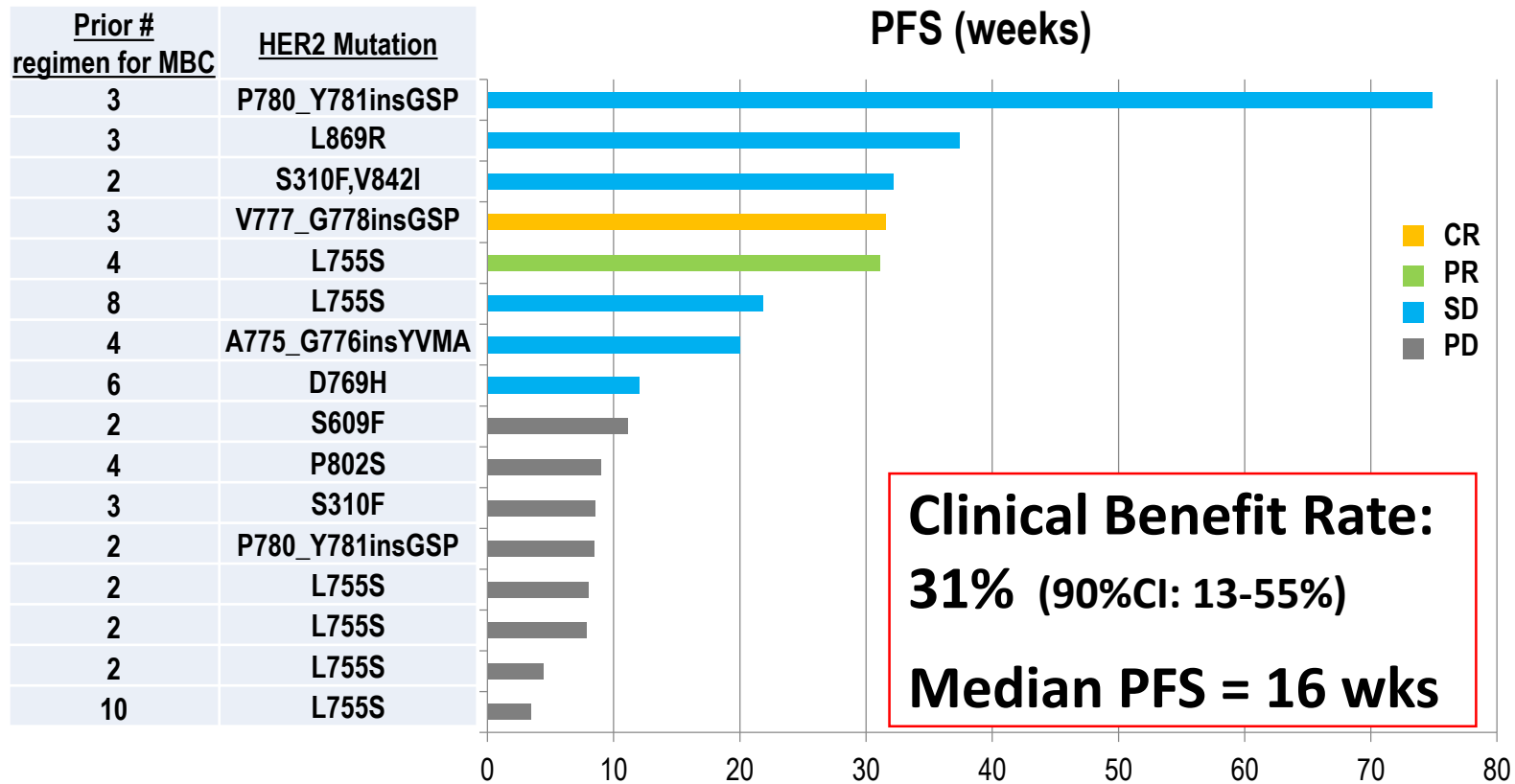
## Incidence/prevalence in US

→ 4,000 patients annually.

→ 3,000 – 7,500 MBC patients

# Phase II Trials treating HER2 Mutated Breast Cancer with the TKI, Neratinib

## MutHER Trial

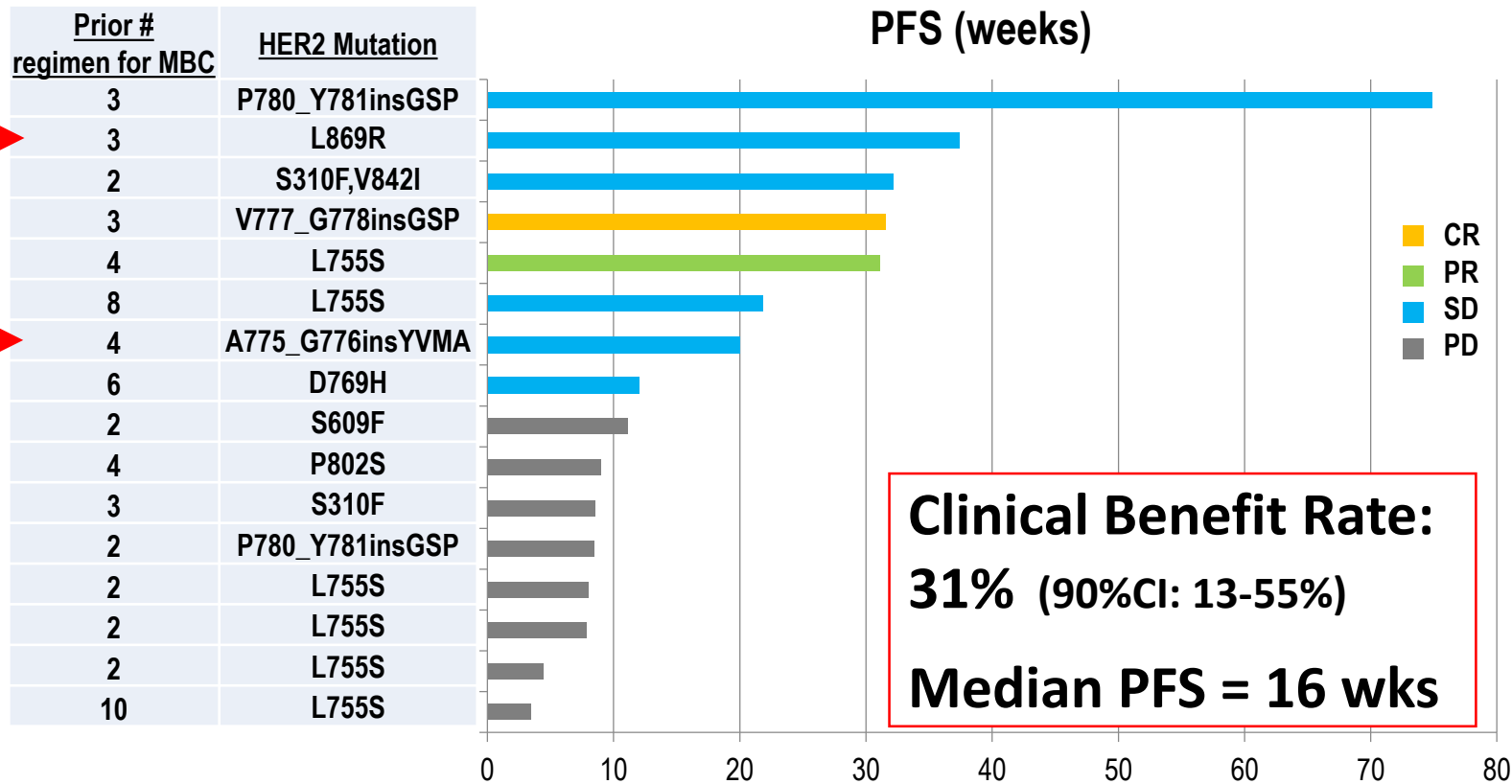


**CBR = CR + PR + SD  $\geq$ 24 wks**

Ma et al., Clin Cancer Res. Oct 2017

# Phase II Trials treating HER2 Mutated Breast Cancer with the TKI, Neratinib

## MutHER Trial



CBR = CR + PR + SD  $\geq$ 24 wks

Ma et al., Clin Cancer Res. Oct 2017

## SUMMIT Trial

Neratinib Basket Trial for HER2 Mutated Cancers.

Breast Cancer Enrollment = 25

### 1° Endpoint

**ORR at week 8 = 32%**  
(95% CI = 15–54%)

### 2° Endpoints

**Clinical Benefit Rate = 40%**  
(95% CI = 21–61%)

**Median PFS = 3.5 months**

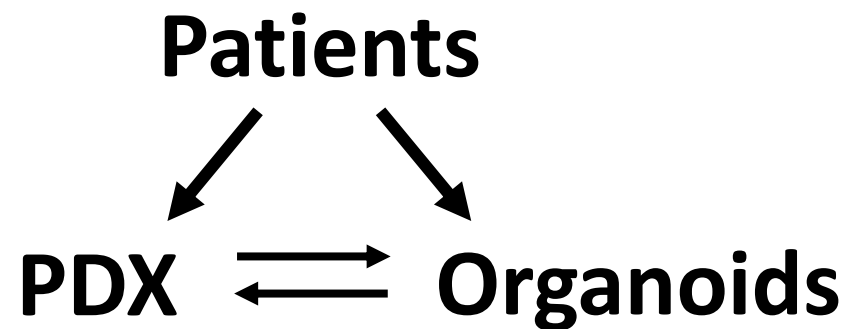
Hyman et al., Nature. Feb 2018

# How can we rapidly test Neratinib combination regimens?

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Preclinical testing of Neratinib combinations  
using Patient Derived Organoids and Xenografts.

- **Fundamental Change in Tissue Culture**
- **High Culture Establishment Rate**
- **Fast! Time Scale = Weeks.**



Nomenclature used  
in the literature:  
PDxO vs PDO

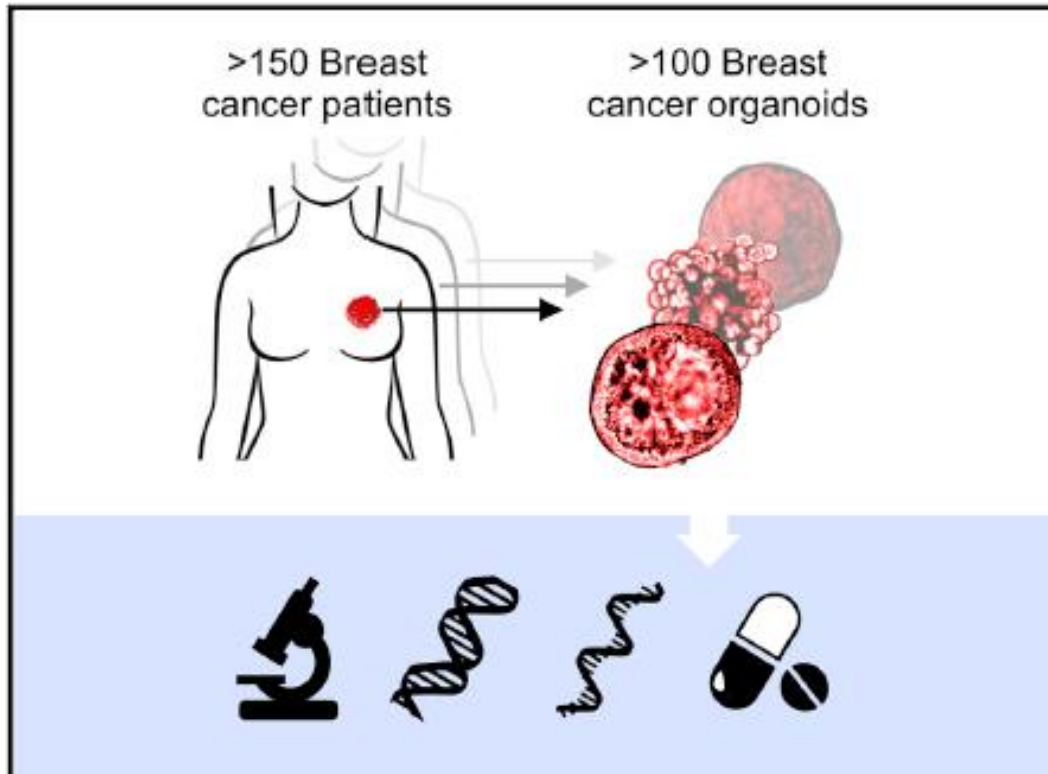


# Breakthrough Paper describing how to generate Organoids from Breast Cancer Patient Samples

Cell

## A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity

### Graphical Abstract



### Authors

Norman Sachs, Joep de Ligt,  
Oded Kopper, ...,  
Robert Gerhardus Jacob Vries,  
Edwin Cuppen, Hans Clevers

### Correspondence

[h.clevers@hubrecht.eu](mailto:h.clevers@hubrecht.eu)

### In Brief

The heterogeneity of breast cancer subtypes can be captured using organoid cultures that can facilitate drug screens that corroborate with patient responses.

1. This paper describes the conditions to establish Breast Cancer, Patient-Derived Organoids (PDO)
2. They established about 100 PDO's.
3. These Organoids recapitulate histological and genetic features of the original tumors
4. Organoids allow high-throughput drug screening and potentially aid personalized therapy

# Organoids from HER2 mutated, ER+ PDX's:

## PDX-51

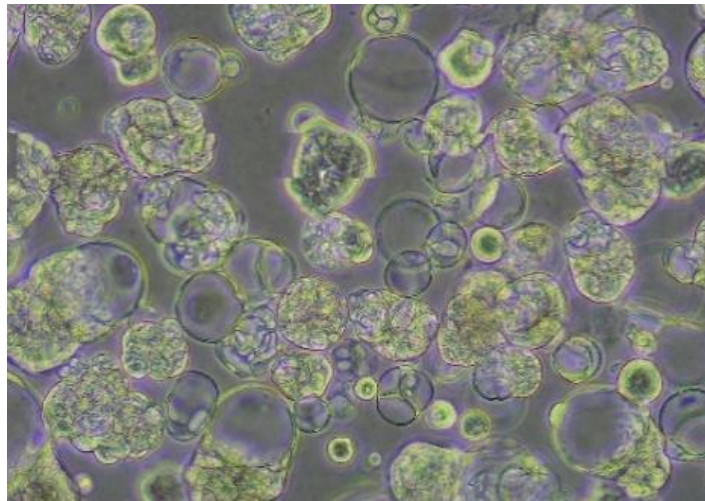
***ERBB2* G776insYVMA (HER2 exon20)**  
*PIK3CA* H1047R

### Prior Hormonal Therapy

Tamoxifen  
 Letrozole  
 Exemestane  
 Fulvestrant  
 Exemestane + Everolimus

### Prior Chemotherapy

CMF  
 Paclitaxel  
 Capecitabine  
 Carboplatin  
 Gemcitabine



### Pt. IHC

ER +  
 PR +  
 HER2 IHC 2+

### Histology:

Ductal carcinoma

## PDX-64

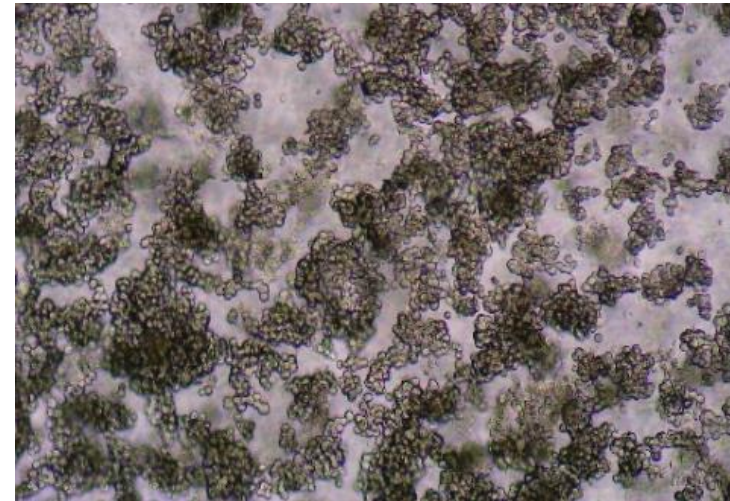
***ERBB2* L869R (HER2 Kinase Domain)**  
*PIK3CA* WT

### Prior Hormonal Therapy

Tamoxifen  
 Anastrozole  
 Exemestane + Everolimus

### Prior Chemotherapy

Docetaxel  
 Doxorubicin  
 Cyclophosphamide  
 Capecitabine



### Pt. IHC

ER +  
 PR negative  
 HER2 IHC score 0

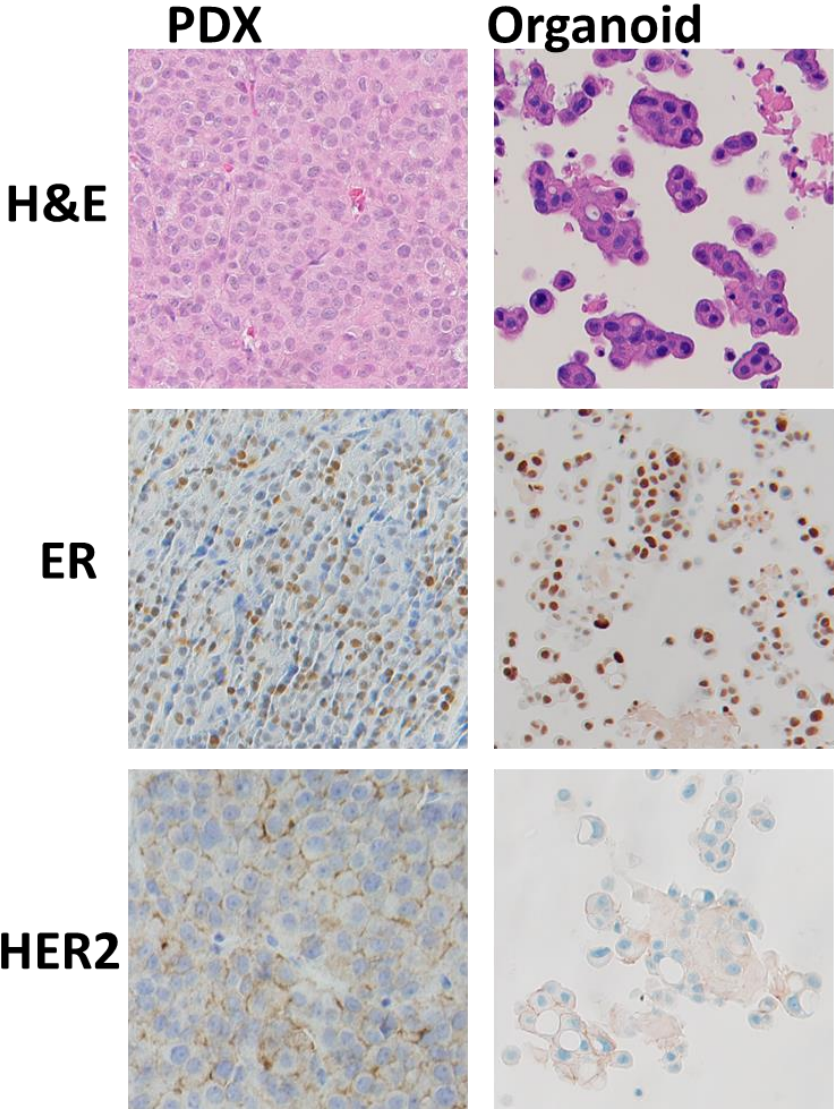
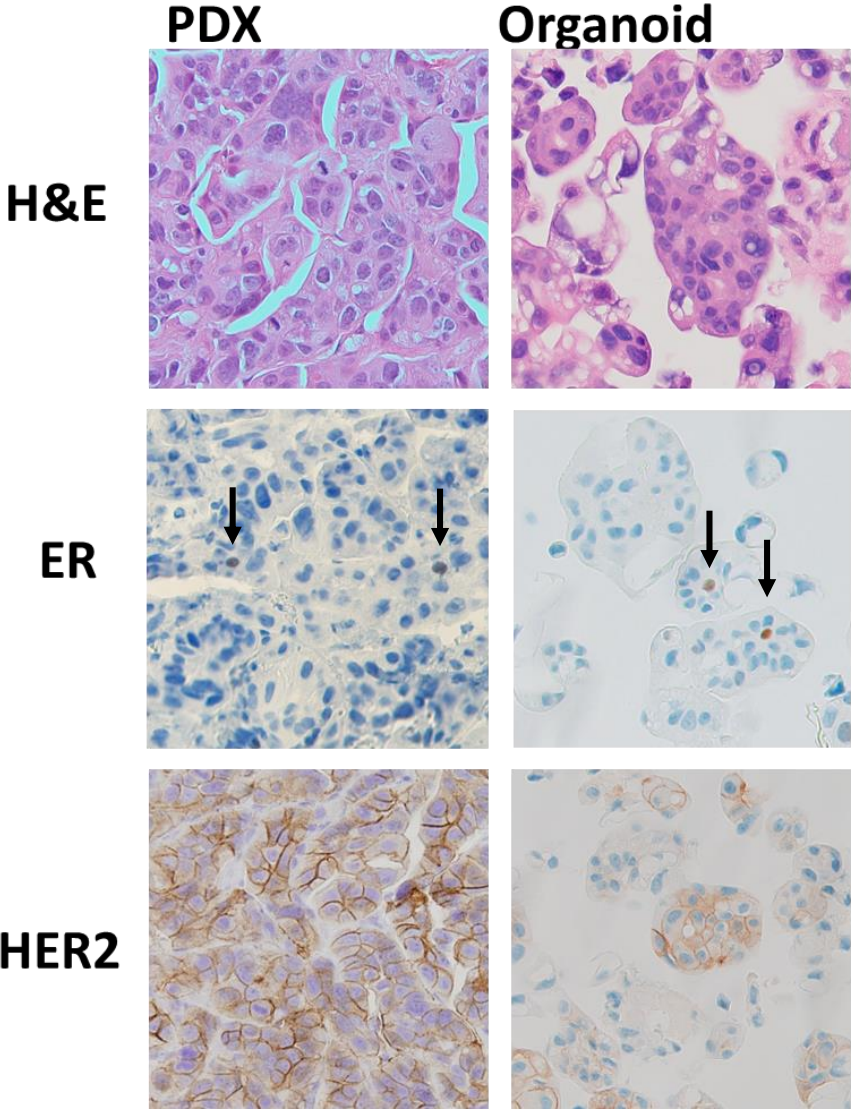
### Histology:

Lobular carcinoma



# PDX-51 (HER2 G776insYVMA)

# PDX-64 (HER2 L869R)



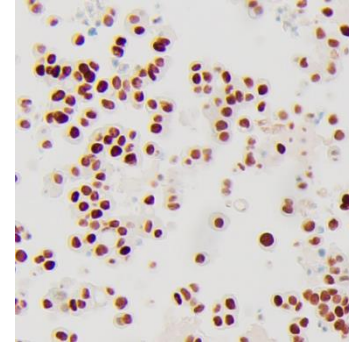
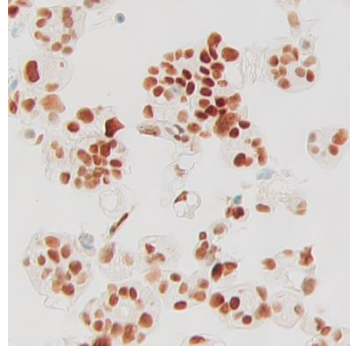
# IHC on Organoids demonstrates Breast Carcinoma cells

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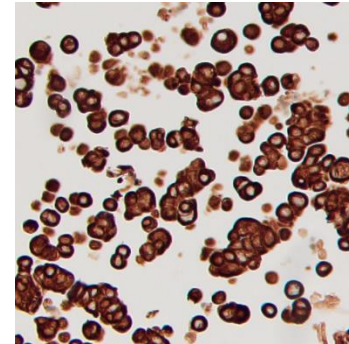
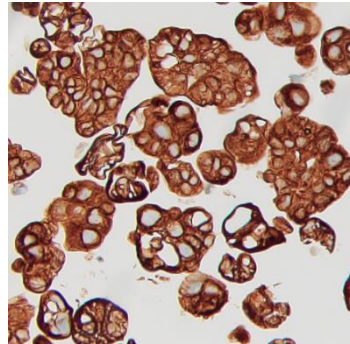
**PDX-51**

**PDX-64**

**GATA3**

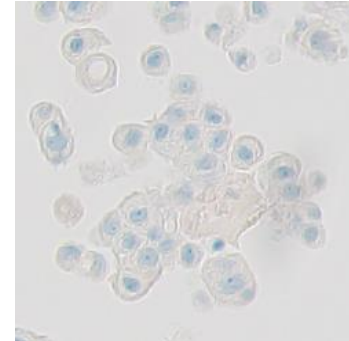
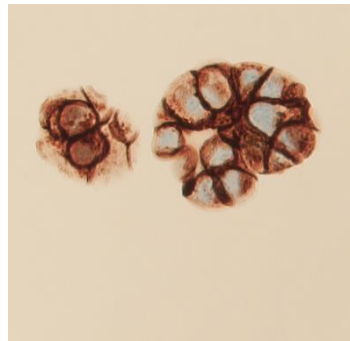


**Pan  
Cytokeratin**



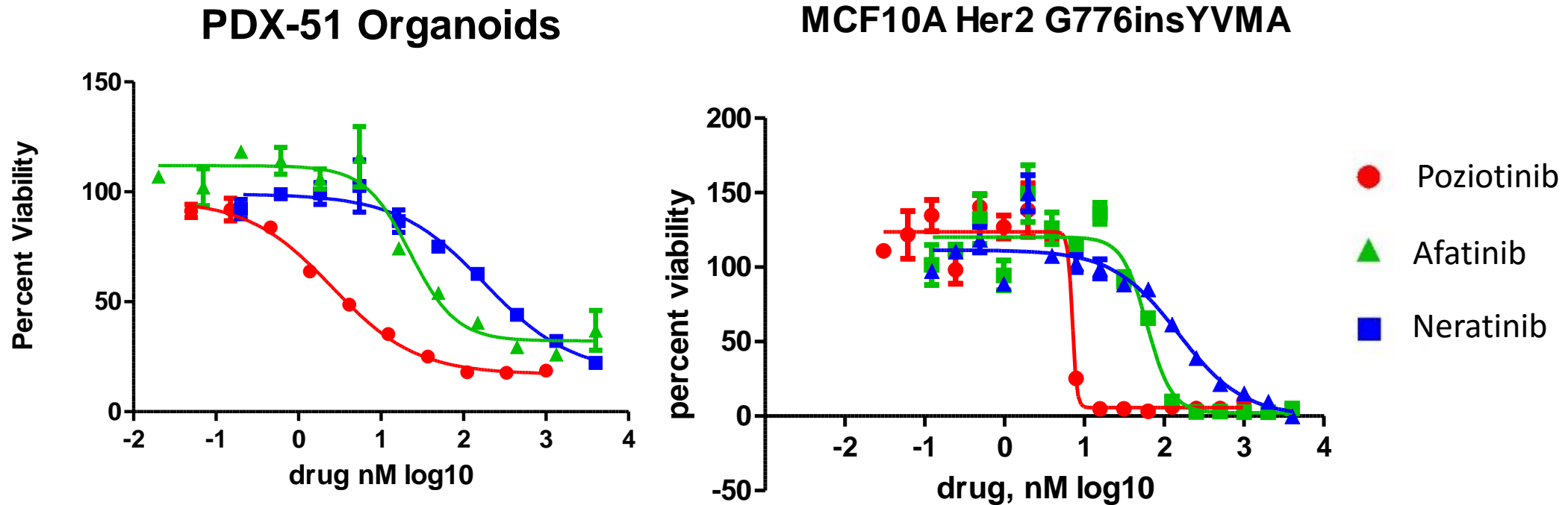
**E-Cadherin**

**Invasive Ductal Carcinoma  
E-cadherin positive**



**Invasive Lobular Carcinoma  
with known loss of E-cadherin**

# Drug Testing on Organoids recapitulates Cell Line Data



Drug	Organoid IC50 nM	MCF10A IC50 nM
Poziotinib	3	7
Afatinib	23	61
Neratinib	180	149

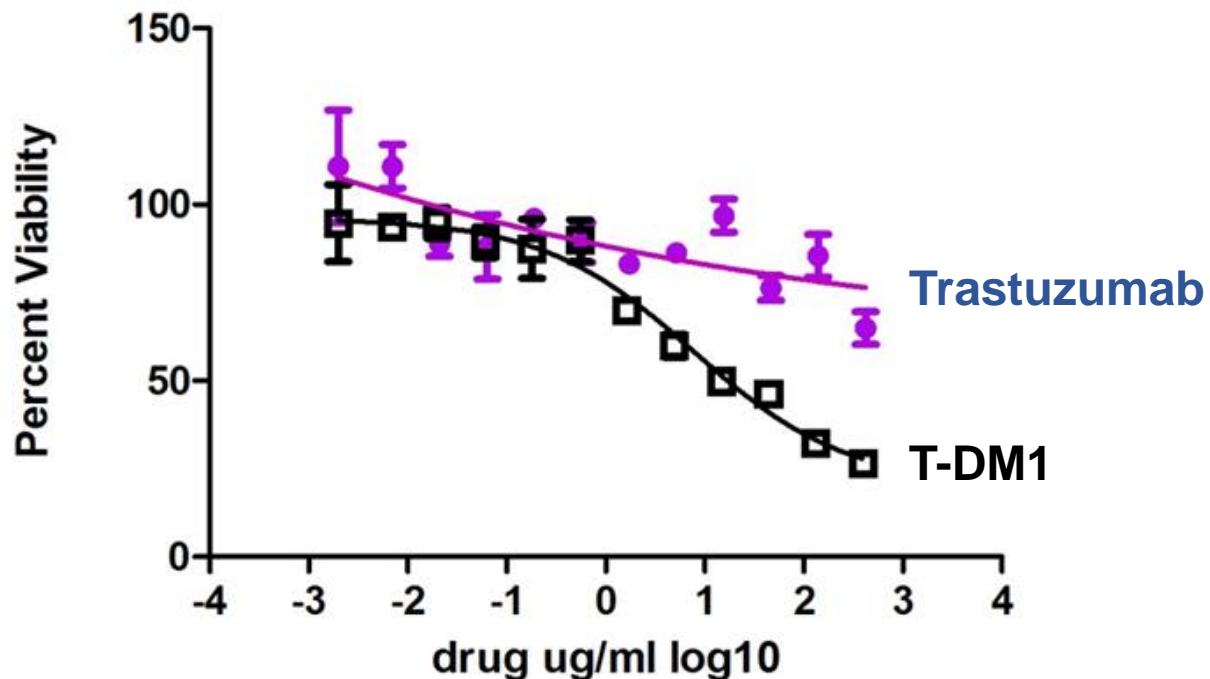
# Single Drug Sensitivity on PDX-51 (HER2 G776insYVMA) in 96 or 384 well plates

**Drug Dose**

Neratinib		Afatinib		Lapatinib		Poziotinib		Vinorelbine	
31	45	35	38	101	105	24	26	33	46
58	67	38	34	102	107	26	23	53	58
73	72	43	41	116	102	32	27	57	52
83	98	43	54	102	90	44	42	53	49
97	93	76	65	103	94	52	53	49	57
76	81	92	102	98	106	84	77	46	48
84	87	80	81	106	111	66	70	51	52
89	96	87	104	107	108	73	79	54	61
109	117	114	94	118	132	96	88	65	56
123	102	95	98	105	102	84	79	68	56
107	110	101	104	116	117	104	89	60	62
90	100	111	96	109	100	102	106	71	74
123	124	104	101	106	110	106	98	78	80
107	120	104	104	93	109	100	98	70	73
103	115	114	105	98	92	93	108	102	97
0	0	0	0	0	0	0	0	0	0

Vehicle  
No Cells

**384 well plate result vs. 96 well plate result**



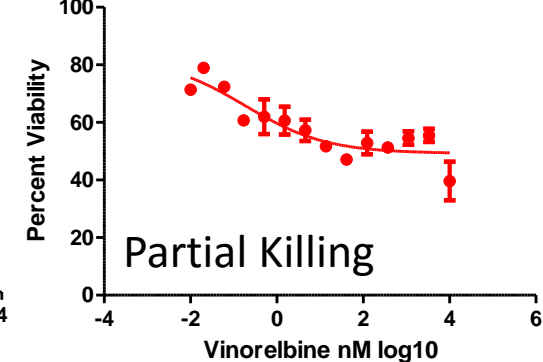
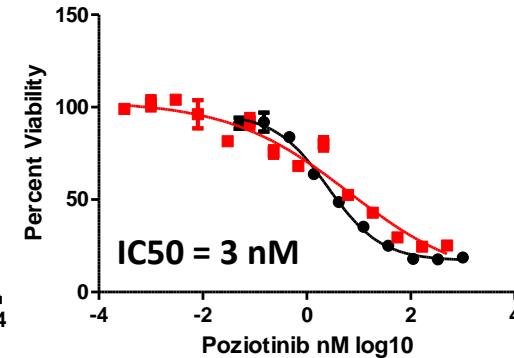
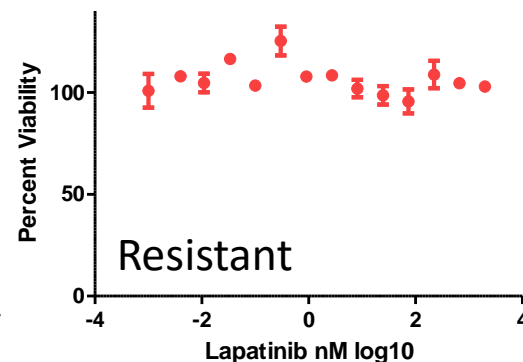
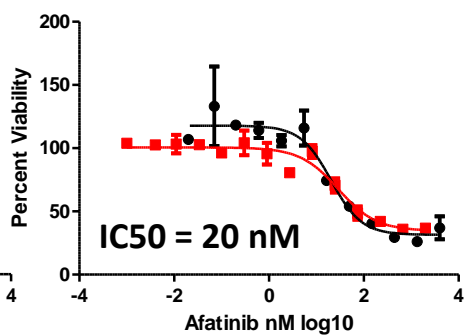
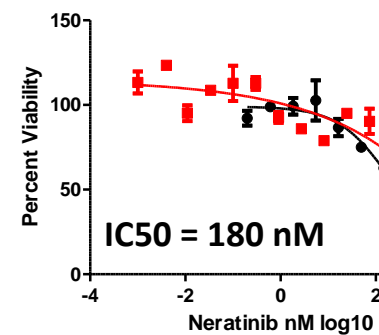
**Neratinib**

**Afatinib**

**Lapatinib**

**Poziotinib**

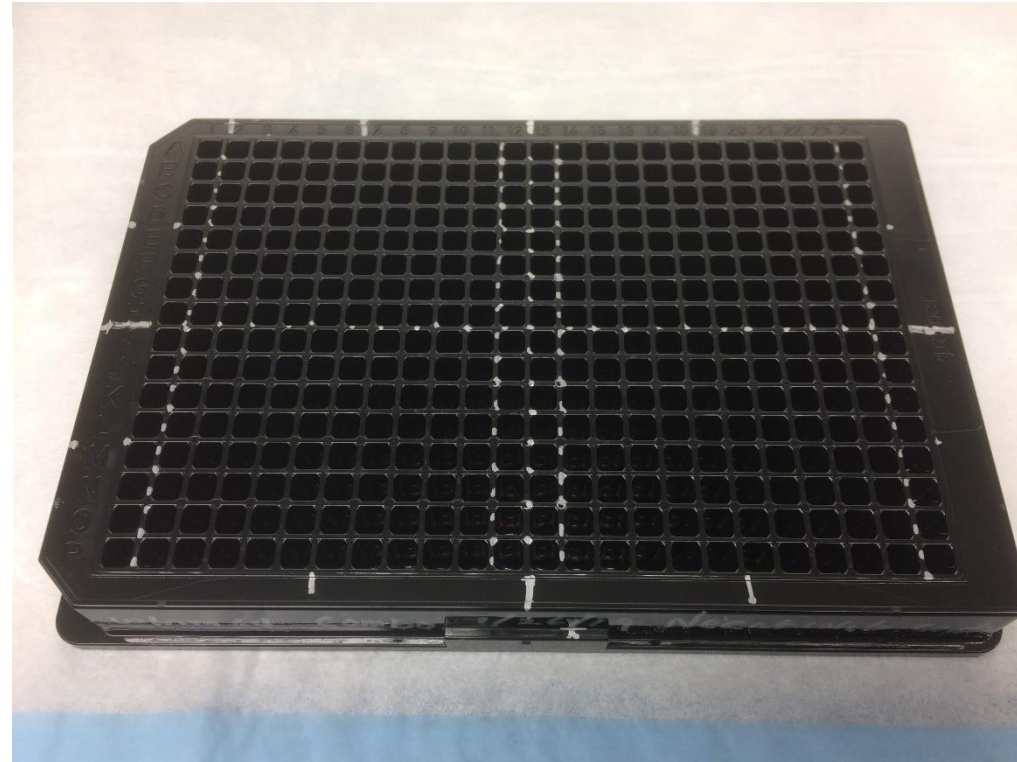
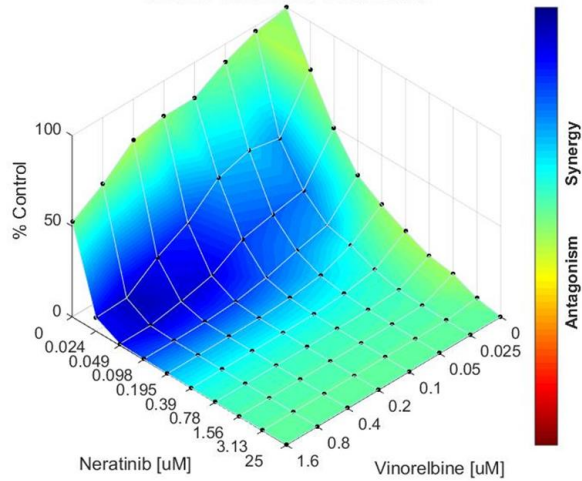
**Vinorelbine**



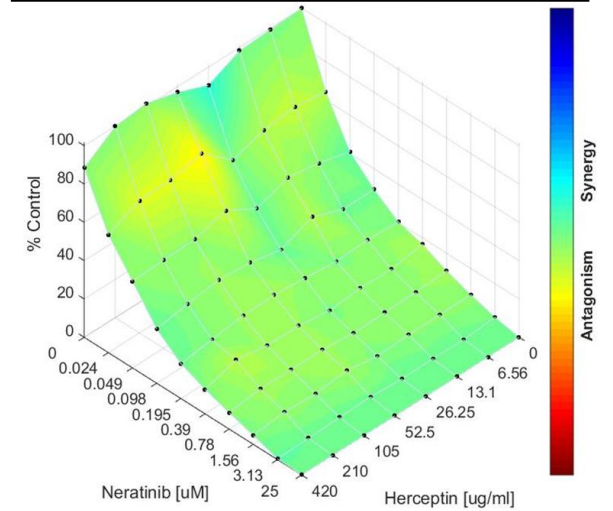


# Four Drug Combinations Tested on PDX-64 in a single 384-well Plate

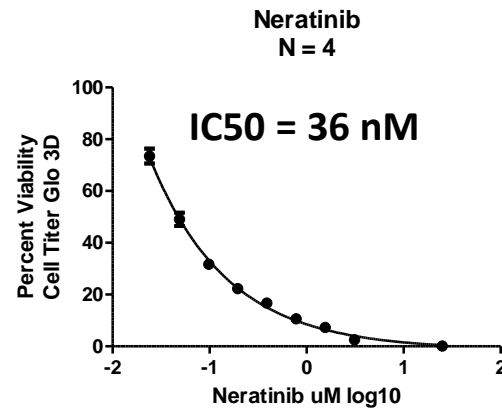
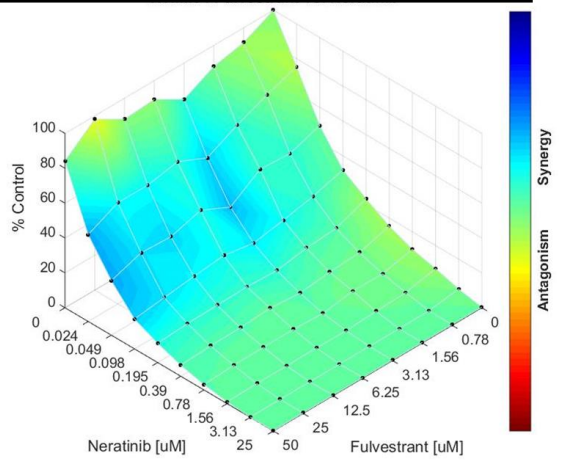
**Neratinib+Vinorelbine**



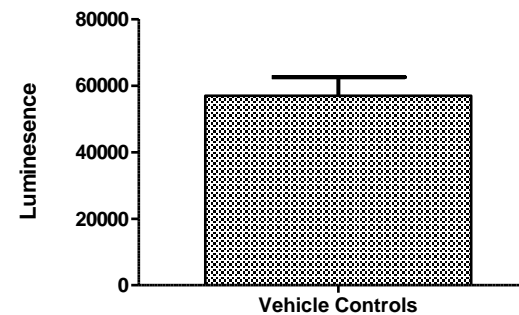
**Neratinib+Trastuzumab**



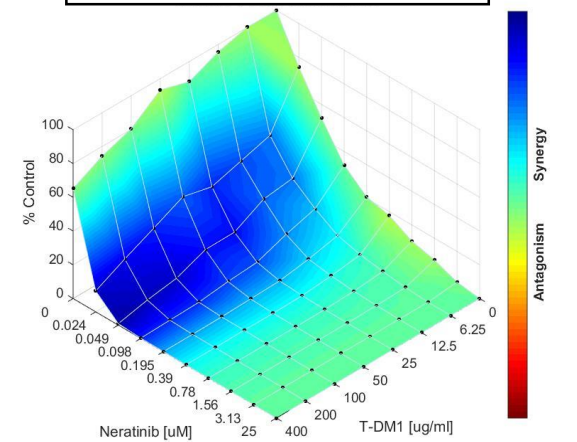
**Neratinib+Fulvestrant**



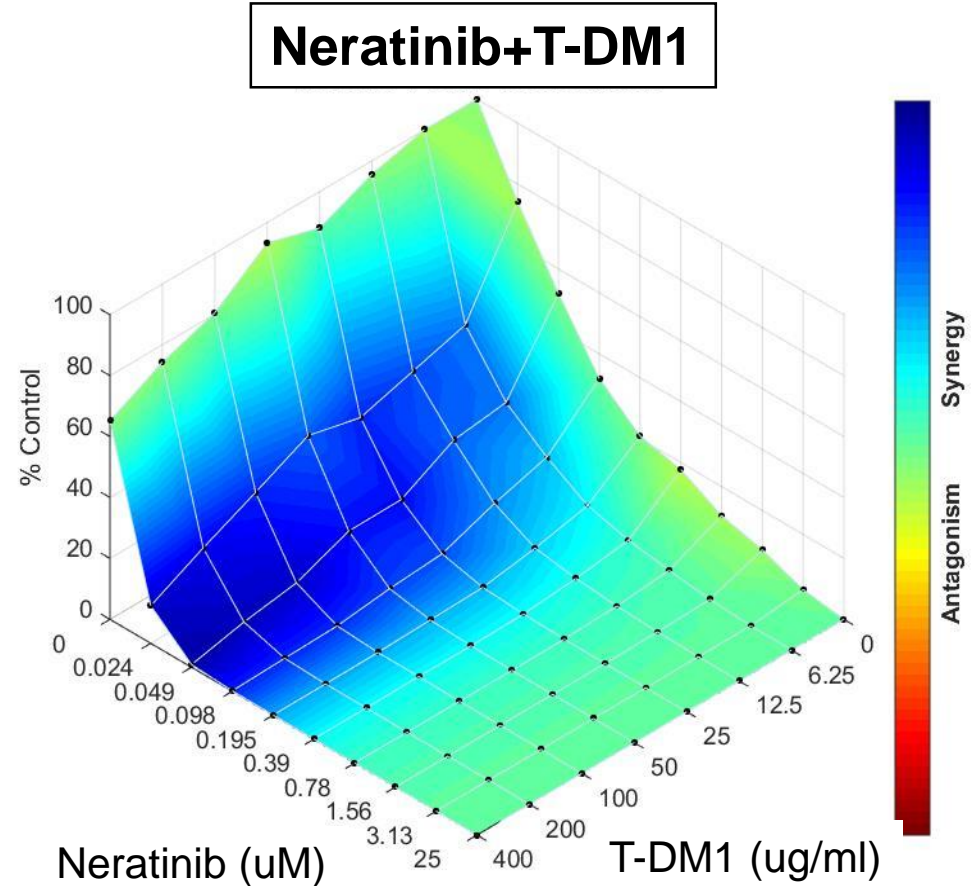
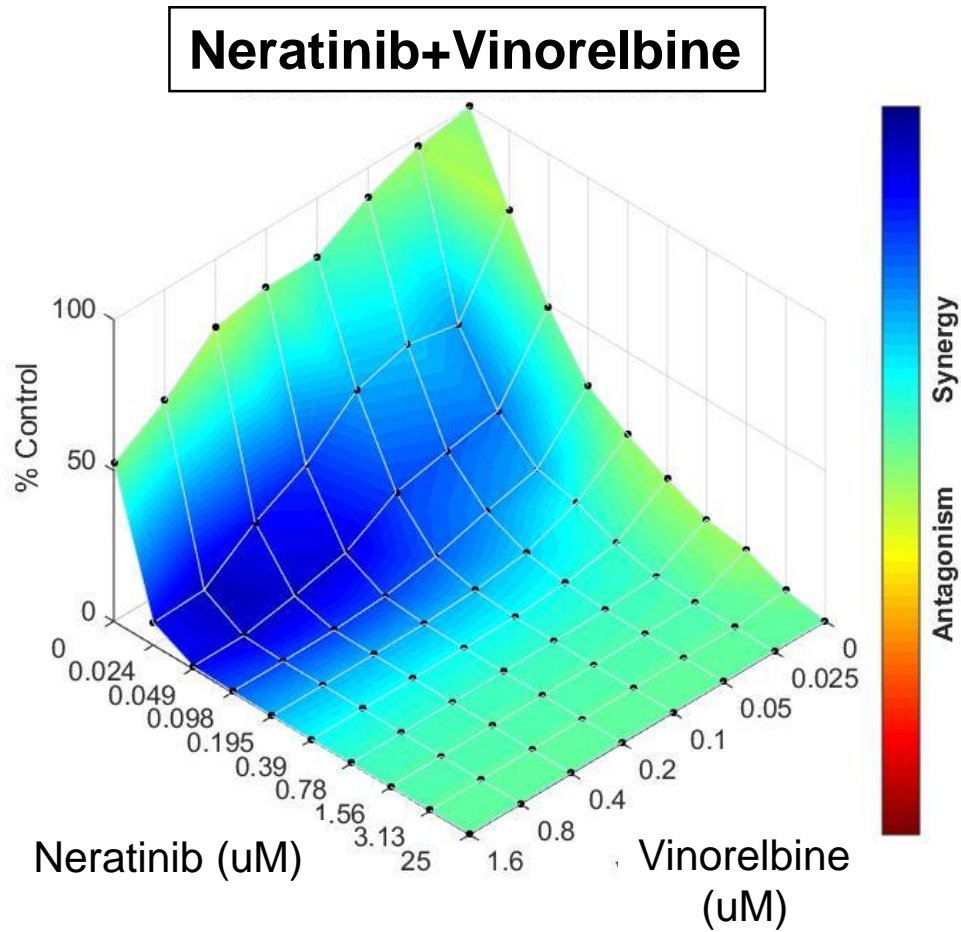
vehicle controls, with SD  
N = 52



**Neratinib+T-DM1**



# Four Drug Combinations Tested on PDX-64 in a single 384-well Plate

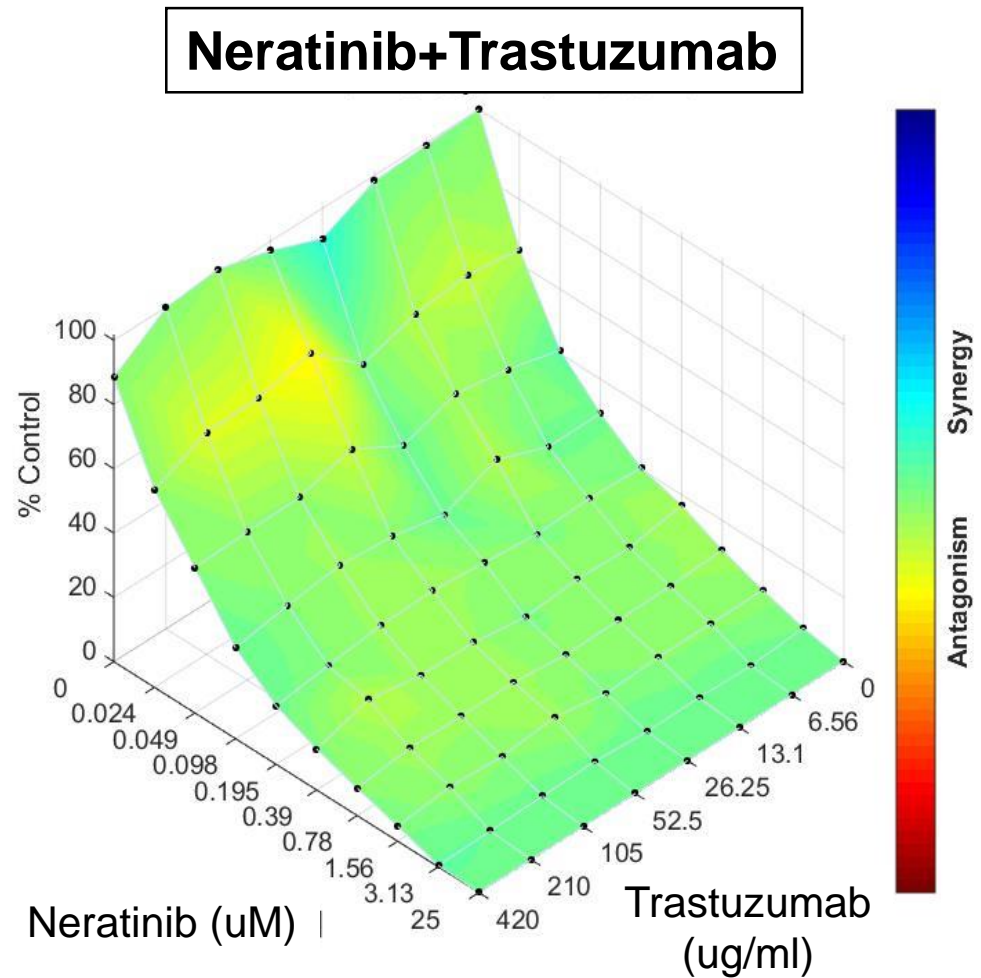
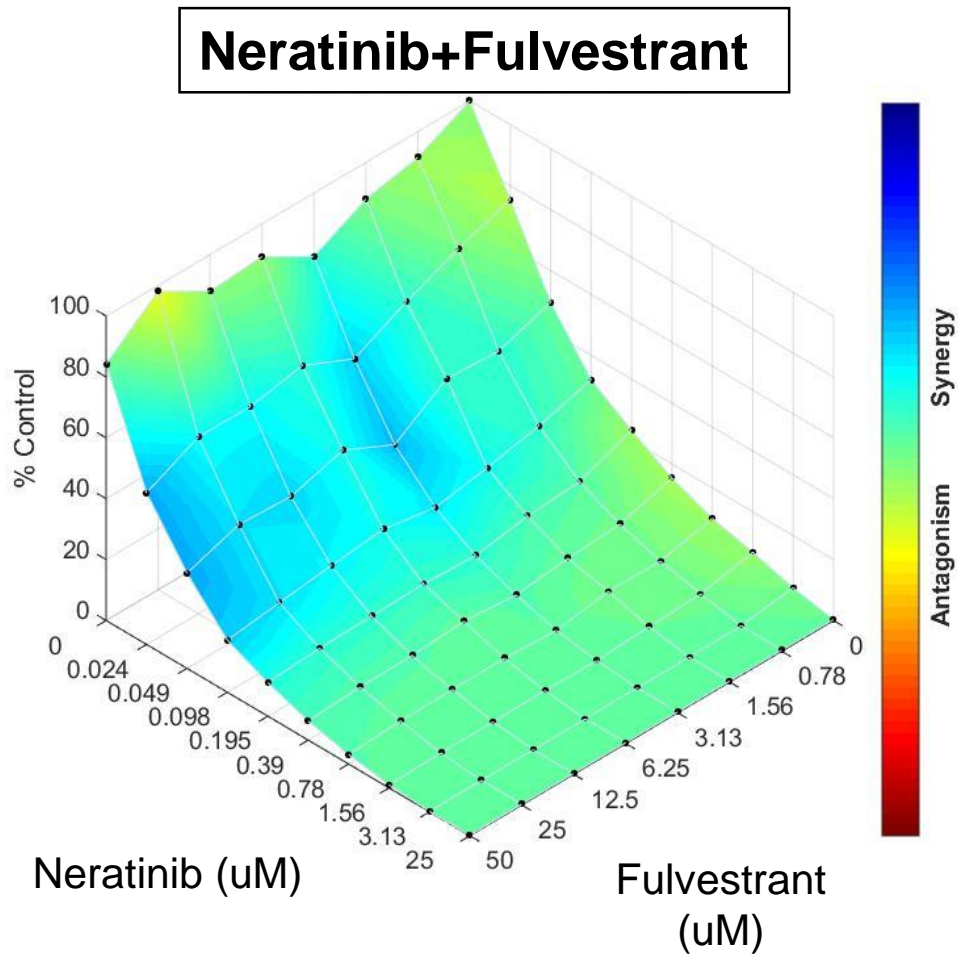


Methods: Drug synergy calculated as per the Loewe model.

Di Veroli et al. Bioinformatics 32(18):2866, 2016.



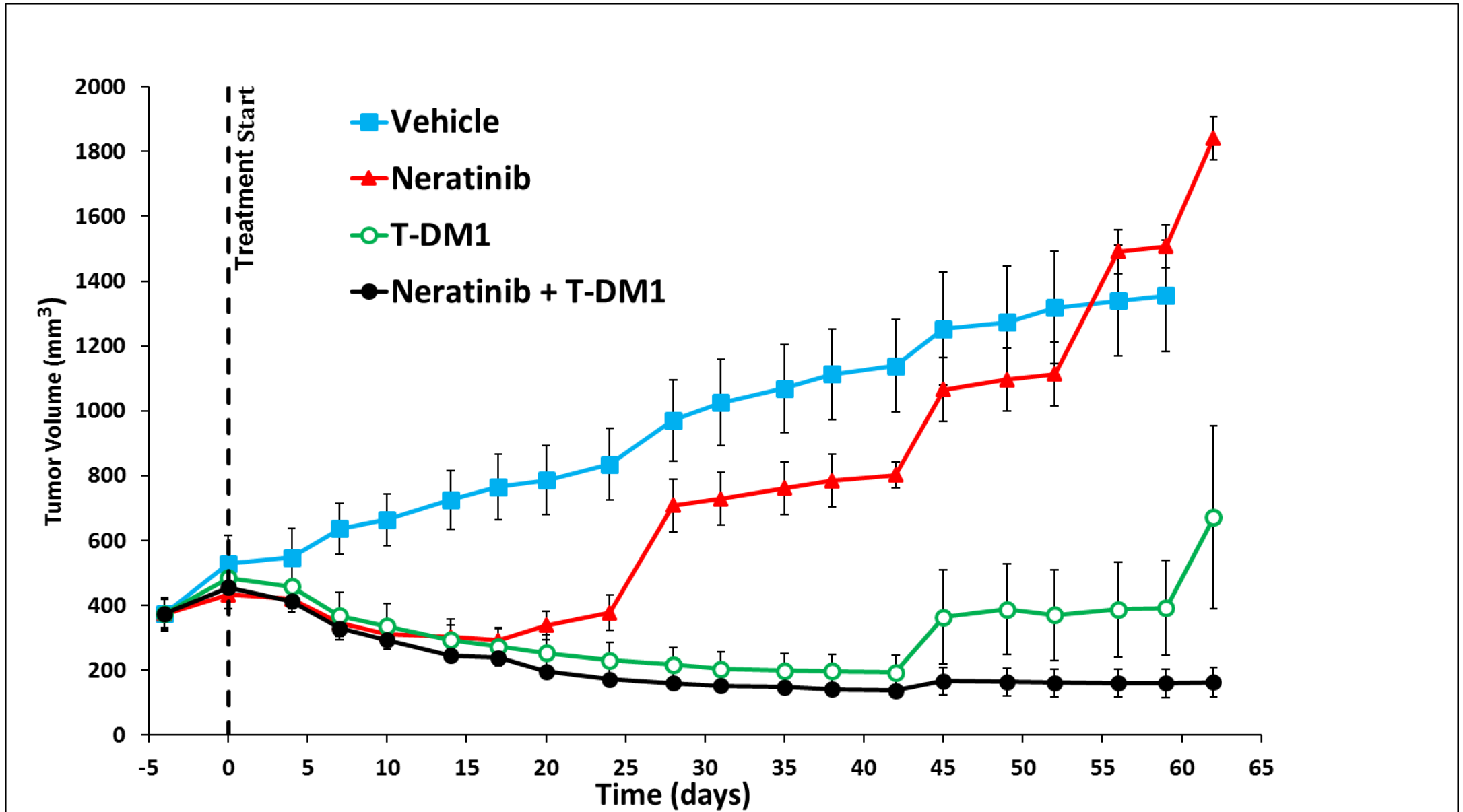
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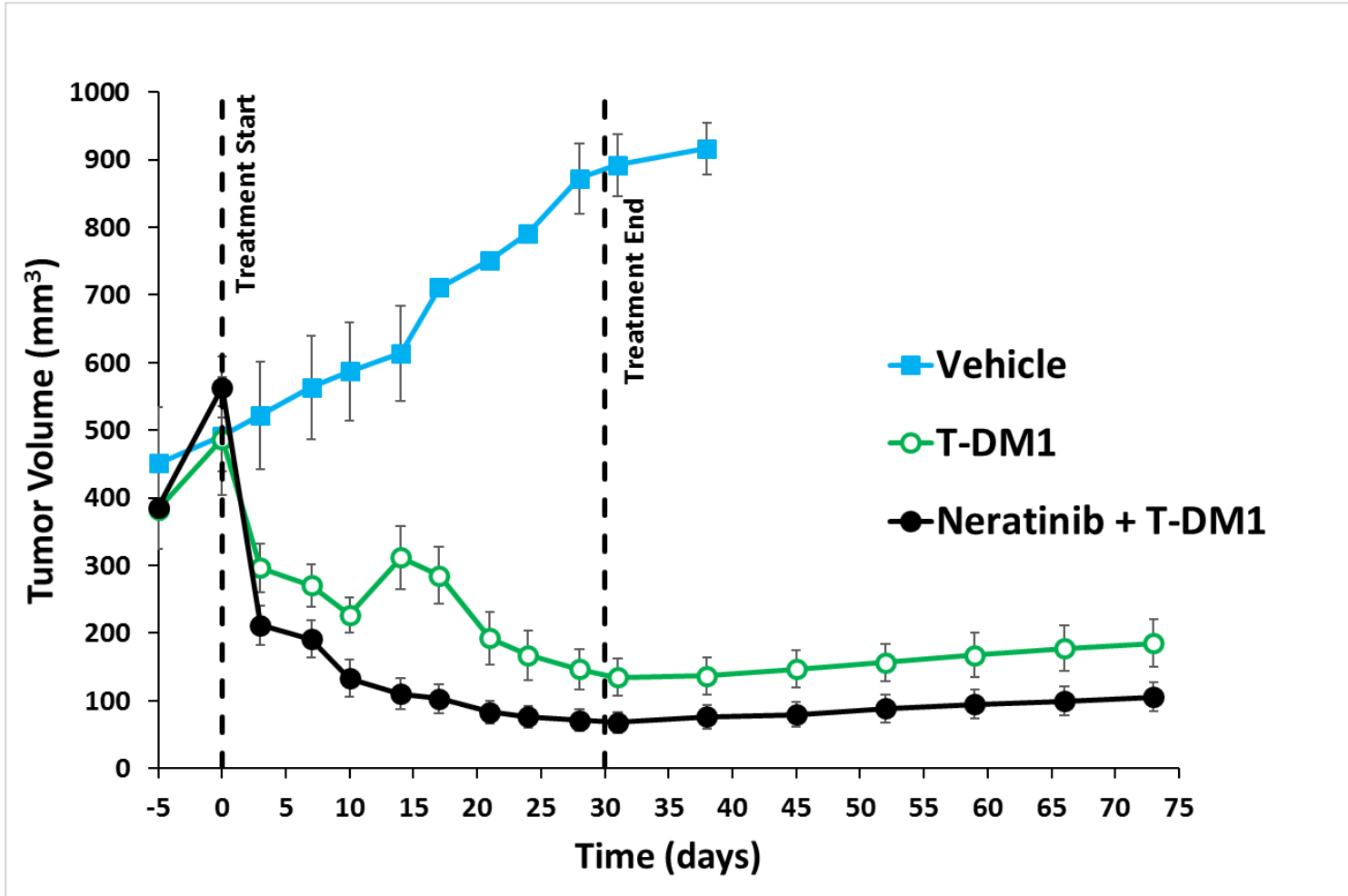
Di Veroli et al. Bioinformatics 32(18):2866, 2016.

# In vivo testing of Neratinib + T-DM1 on PDX-51

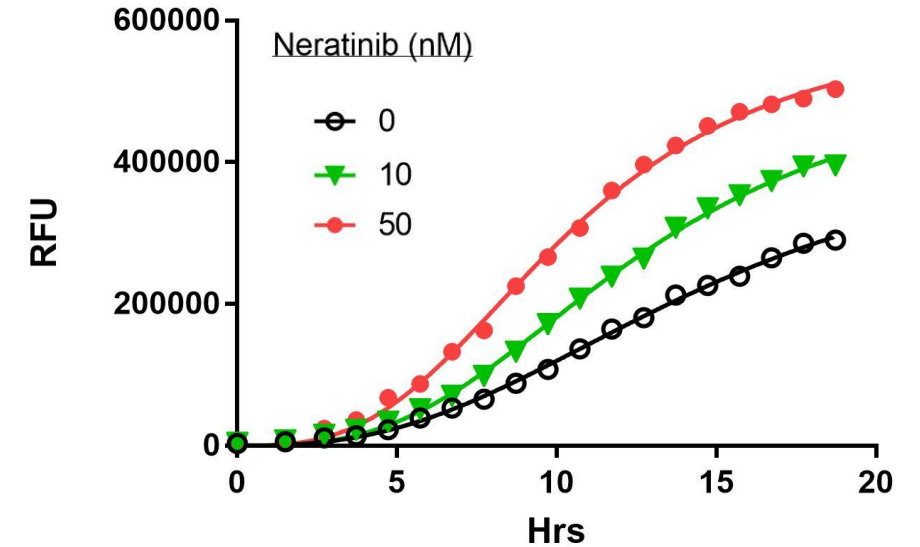


# Neratinib + T-DM1: PDX-64 in vivo results and Mechanism of Synergy

PDX-64 – HER2 L869R



Effect of Neratinib on Antibody Uptake by BT-474 Cells



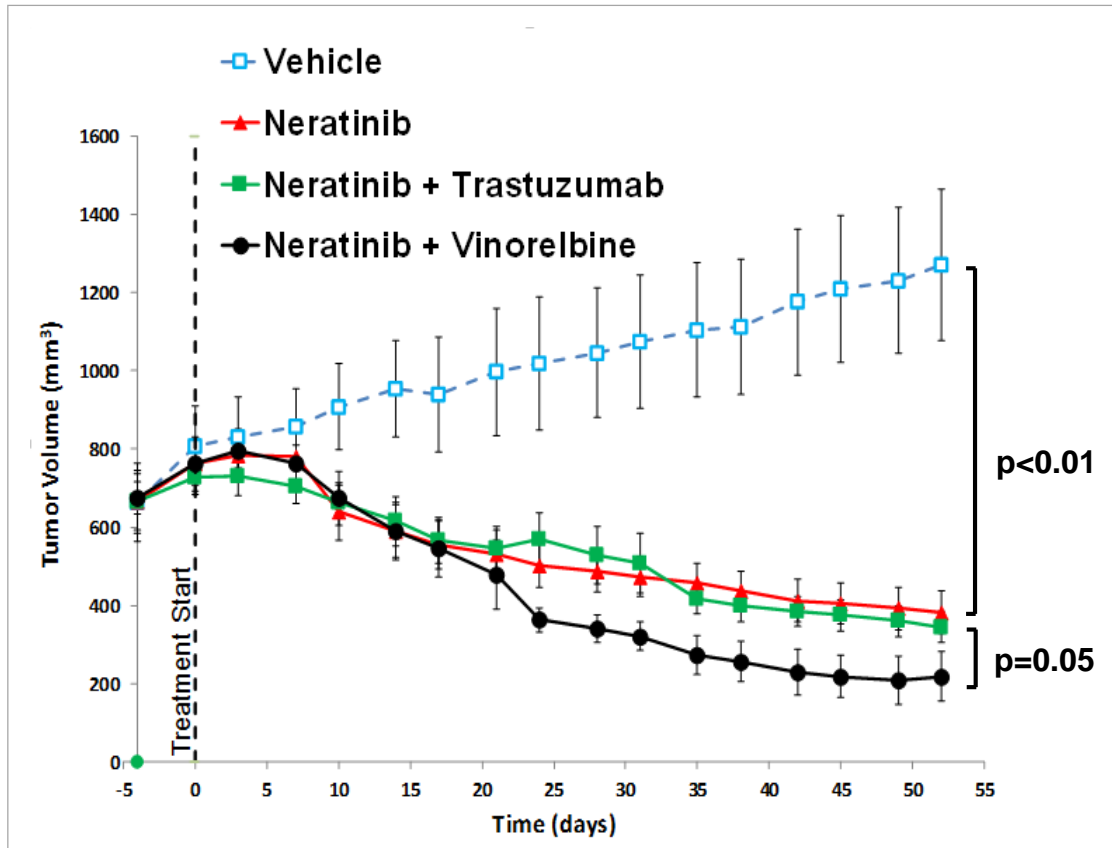
**Prior evidence that Neratinib or CI-1033 enhances HER2 internalization**

Citri et al, EMBO Journal 2002

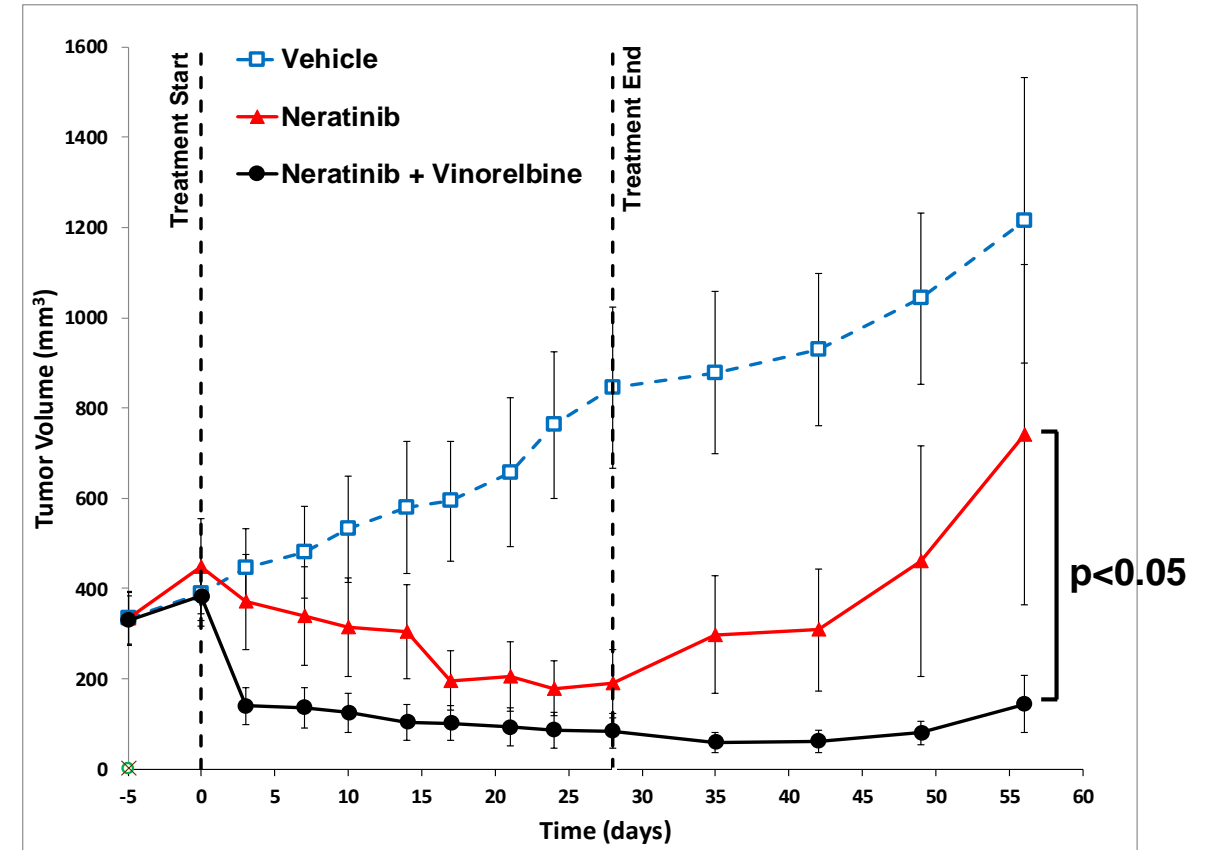
Zhang et al, Cancer Lett. 2016

# In vivo testing of Neratinib + Vinorelbine on both PDX's

## PDX-51 – HER2 G776insYVMA



## PDX-64 HER2 L869R



# Safety Data on Neratinib + T-DM1 and Neratinib + Vinorelbine

BREAST CANCER—METASTATIC

ASCO Annual  
Meeting 2018

NSABP FB-10: Phase Ib dose-escalation trial evaluating trastuzumab emtansine (T-DM1) with neratinib (N) in women with metastatic HER2+ breast cancer (MBC).

[Jame Abraham](#), [Shannon Puhalla](#), [William M. Sikov](#), [Alberto J. Montero](#), [Mohamad Adham Salkeni](#), [Wajeeha Razaq](#), [Jan Hendrik Beumer](#), [Brian Kiesel](#), [Marc E. Buyse](#), [Laura M Adamson](#), [Ashok Srinivasan](#), [Katherine L. Pogue-Geile](#), [Carmen Joseph Allegra](#), [Samuel A. Jacobs](#)

Annals of Oncology

original articles

*Annals of Oncology* 24: 109–116, 2013  
doi:10.1093/annonc/mds284  
Published online 11 September 2012

Annals of  
Oncology 2013

**Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy**

A. Awada<sup>1\*</sup>, L. Dirix<sup>2</sup>, L. Manso Sanchez<sup>3</sup>, B. Xu<sup>4</sup>, T. Luu<sup>5</sup>, V. Diéras<sup>6</sup>, D. L. Hershman<sup>7</sup>, V. Agrapart<sup>8</sup>, R. Ananthakrishnan<sup>9</sup> & E. Staroslawska<sup>10</sup>

<sup>1</sup>Medical Oncology Clinic, Jules Bordet Institute, Brussels University, Brussels; <sup>2</sup>Medical Oncology Department, Sint-Augustinus, Antwerp, Belgium; <sup>3</sup>Medical Oncology

# Conclusions

- **HER2 activating mutations are a druggable target in HER2 gene amplification negative breast cancer.**
- **Two independent, phase II clinic trials demonstrate that neratinib has clinical benefit rate of 31-40% for HER2 mutated, Stage IV breast cancer.**
- **Patient-derived organoids (PDO's) accelerated drug testing for these rare mutations.**
- **Using PDO's, we identified two synergistic drug combinations for HER2 mutated, metastatic breast cancer.**
- **These drug combinations were validated *in vivo* and phase I clinical trial data shows that these combinations are safe for patients.**

# Acknowledgements

---

## Bose Lab

Maureen Highkin

McKenna Wilhelm

John Monsey

Wei Shen

## Siteman Cancer Center at

## Washington University

Cynthia Ma

Shunqiang Li

Tina Primeau

Stephanie Pratt

## Puma Biotechnology, Inc.

Alshad S. Lalani

Richard E. Cutler Jr.

Irmina Diala

Grace Mann

## Funding

DoD CDMRP Breast Cancer

Research Program

Puma Biotechnology, Inc.

We thank Bryan Welm and Katrin Guillen (Univ. of Utah, Huntsman Cancer Institute) and Jennifer Rosenbluth and Nikhil Wagle (Dana-Farber Cancer Institute) for helpful discussions on breast cancer PDO's.