The therapeutic superiority of neratinib in combination with trastuzumab compared to pertuzumab plus trastuzumab in HER2-positive *in vivo* breast cancer models

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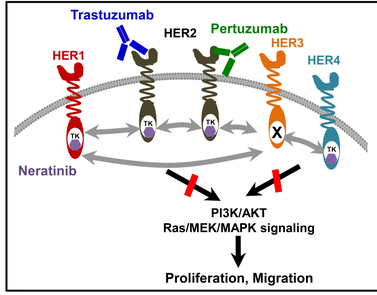
Introduction

Lapatinib (L) plus trastuzumab (T) alone or with endocrine therapy for HER2+/ER+ tumors but without chemotherapy, yielded complete tumor eradication in xenograft models. In neoadjuvant trials (NCT00548184, 00999804, 01973660), a substantial number of patients achieved pathologic complete response with this same strategy. The irreversible pan-HER inhibitor neratinib (N) has been recently approved by the FDA for early stage HER2+ breast cancer and has shown greater potency compared to L in the preclinical setting. However, the therapeutic efficacy of N in combination with T (N+T) and how it compares to pertuzumab (P) +T (without chemotherapy) has not been well studied. Here, we evaluate the therapeutic efficacy of N, P, and T, either alone or in combination, with a primary focus on comparing N+T vs. P+T in established cell line- and patient-derived xenograft (PDX) models.

Hypothesis

Dual HER2 inhibition using N+T will be highly efficacious and equally potent or more effective than P+T due to more complete blockade of the HER pathway.

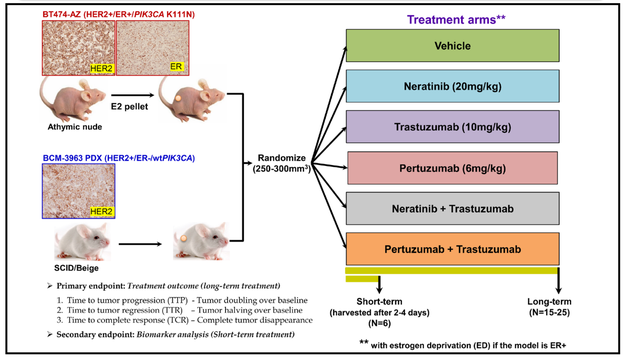
HER signaling pathway and targeted therapy



Trastuzumab and pertuzumab - bind to the extracellular domain of HER2

Neratinib - Irreversible tyrosine kinase inhibitor that targets HER1, HER2, and HER4

Experimental Plan



➢ **Primary endpoint:** *Treatment outcome (long-term treatment)*

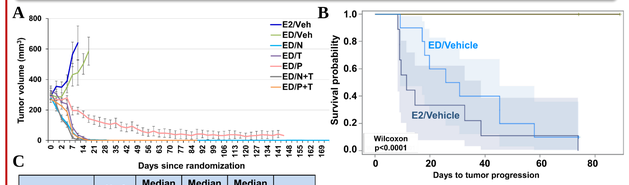
1. Time to tumor progression (TTP) - Tumor doubling over baseline
2. Time to tumor regression (TTR) - Tumor halving over baseline
3. Time to complete response (TCR) - Complete tumor disappearance

➢ **Secondary endpoint:** *Biomarker analysis (Short-term treatment)*

** with estrogen deprivation (ED) if the model is ER+

Results

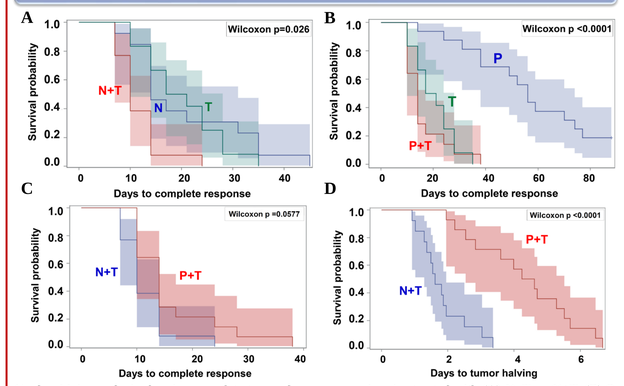
BT474-AZ :Tumor growth in response to HER2-targeted treatments



Treatment	N of mice	Median TTP (Days)	Median TTR (Days)	Median TCR (Days)	CR (%)
E2/Vehicle	9	8	-	-	0
ED/Vehicle	10	25	-	-	0
ED/N	13	-	2	14	100
ED/T	12	-	5	19	100
ED/P	12	-	18	54	92
ED/N+T	13	-	2	10	100
ED/P+T	14	-	4	14	100

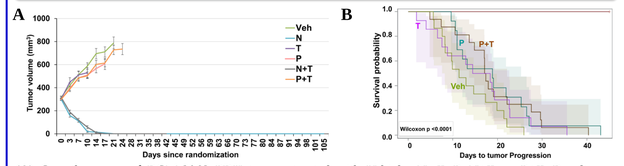
(**A**) Growth curves of BT474-AZ xenografts treated with Vehicle, N, T, P, N+T, or P+T. Results are presented as mean tumor volume±SEM. (**B**) Kaplan-Meier analysis of time to *tumor progression* in mice treated with anti-HER2 therapies. (**C**) Table summarizing the different primary study endpoints following anti-HER2 therapies.

BT474-AZ: N+T regressed tumors faster than P+T



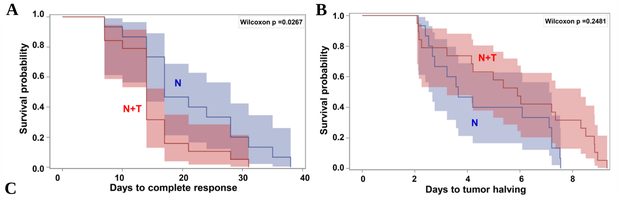
Kaplan-Meier analysis showing *complete tumor disappearance* in mice treated with (**A**) N, T, or N+T, (**B**) P, T, or P+T, (**C**) N+T or P+T, and (**D**) *tumor regression* following treatment with N+T or P+T.

BCM-3963 PDX: Tumor growth in response to HER2-targeted treatments



(**A**) Growth curves of BCM-3963 PDX tumors treated with Vehicle, N, T, P, N+T, or P+T. Results are presented as mean tumor volume±SEM. (**B**) Kaplan-Meier analysis of time to *tumor progression* in mice treated with anti-HER2 therapies.

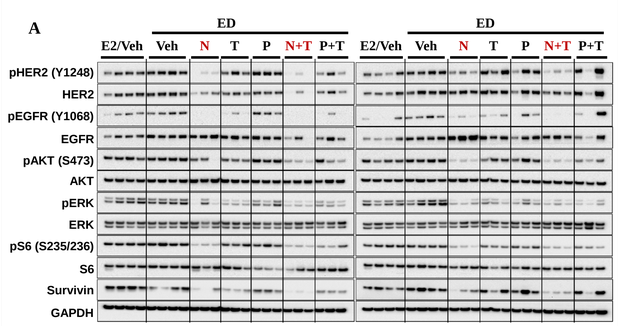
BCM-3963 PDX: N+T accelerated tumor regression compared to N alone



Treatment	N of mice	Median TTP (Days)	Median TTR (Days)	Median TCR (Days)	CR (%)
Vehicle	15	11	-	-	0
N	15	-	4	17	100
T	14	16	-	-	0
P	13	19	-	-	0
N+T	19	-	6	14	100
P+T	16	17	-	-	0

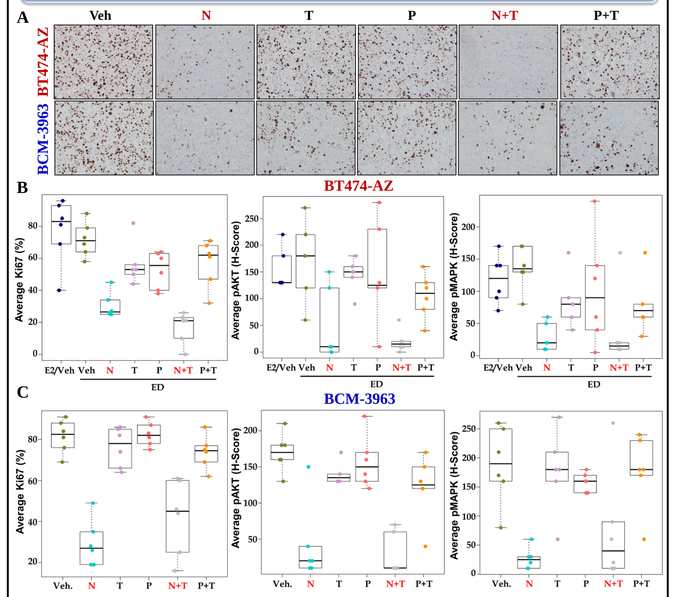
Kaplan-Meier analysis showing (**A**) time to *complete tumor disappearance* in mice treated with N and N+T, and (**B**) time to *tumor regression* in mice treated with N and N+T. (**C**) Table summarizing the different primary study endpoints following anti-HER2 therapies.

N containing regimens yield a more complete blockade of the HER pathway



Western blots showing alterations in the levels and activation of proteins along the HER signaling axis following short-term treatment with different anti-HER2 therapies in (**A**) BT474-AZ cell, and (**B**) BCM-3963 PDX tumors.

N containing regimens significantly inhibit tumor cell proliferation (Ki67) and AKT and MAPK activation



(**A**) Representative Ki67 IHC staining in BT474-AZ (*top panel*) and BCM-3963 PDX (*bottom panel*) tumors. Box plots showing the average Ki67 (%), pAKT (H-Score), and pMAPK (H-Score) protein levels by immunohistochemistry in short-term treated (**B**) BT474-AZ cell, and (**C**) BCM-3963 PDX tumors.

Conclusions and clinical significance

Conclusions

- N achieved 100% complete tumor disappearance in both xenograft models, and accelerated both tumor regression and tumor disappearance compared to T and P, either alone or in combination.
- N+T was more effective than T and P, either alone or in combination in achieving faster tumor regression and a more complete blockade of the HER signaling pathway.
- N, both alone and in combination with T significantly suppressed tumor cell proliferation (Ki67) after short-term treatment.
- pAKT and pMAPK levels were significantly inhibited by N and N+T, but not by T, P, or P+T

Clinical significance

- N is a potent, irreversible pan-HER inhibitor and yields a more comprehensive blockade of the HER layer and its downstream signaling.
- Our findings strongly support future clinical investigations of the combination of N with T in a chemotherapy-sparing setting for patients with HER2+ breast cancer.

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