San Antonio Breast Cancer Symposium - December 6-10, 2016

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

S1-08

Prognostic associations of tumor-infiltrating lymphocytes (TILs) in advanced HER2-positive breast cancer treated with pertuzumab and trastuzumab: *a secondary analysis of the CLEOPATRA study*

Stephen J Luen*, Roberto Salgado, Stephen Fox, Peter Savas, Jennifer Eng-Wong, Emma Clark, Astrid Kiermaier, Sandra Swain, Jose Baselga, Stefan Michiels, Sherene Loi

*SABCS clinical scholar award 2016

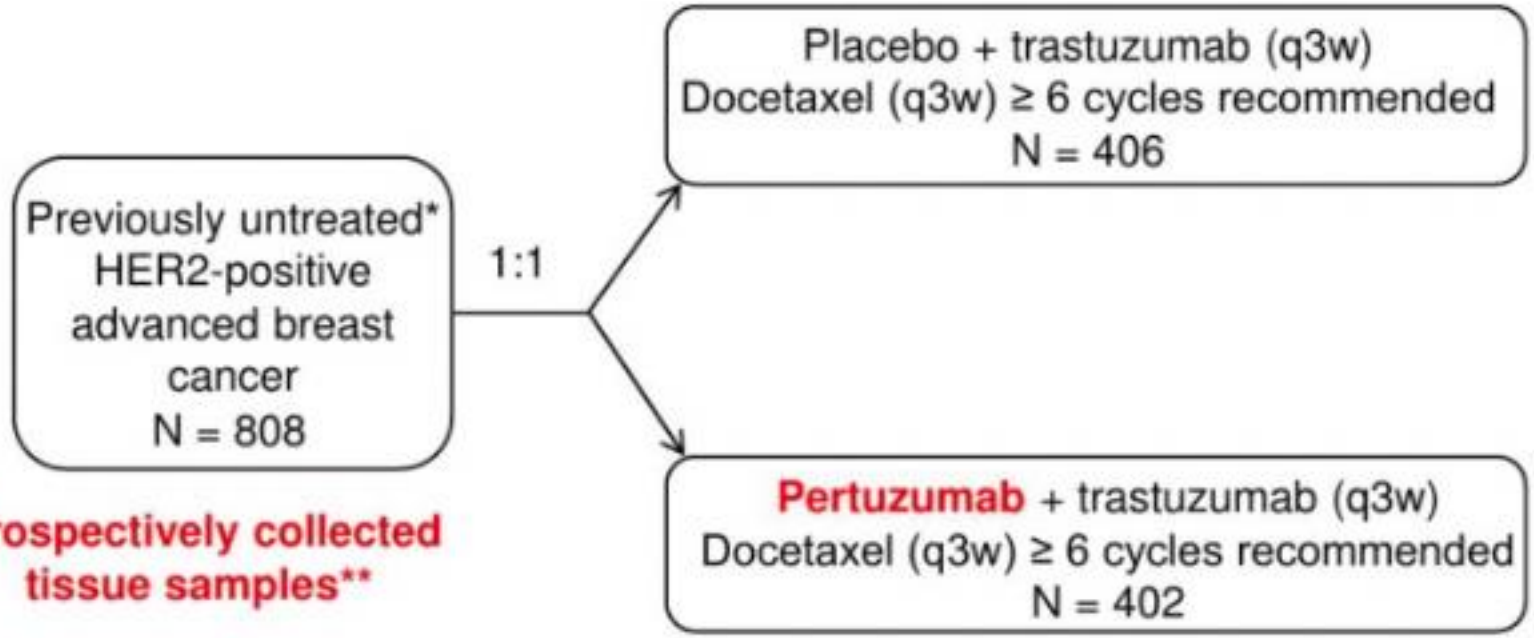
Background

- Retrospective analyses from clinical trials of early HER2-positive breast cancer have demonstrated significant associations of increasing tumor-infiltrating lymphocytes (TILs) with:
  - Improved pathological complete response (pCR) rates
  - Improved event-free survival, disease free and overall survival

- **The prognostic association of TILs in the setting of advanced HER2-positive breast cancer is unknown**

Denkert et al, J Clin Oncol 2015
Salgado et al, JAMA Oncol 2015

CLEOPATRA clinical trial



After a median of follow up of 50 months:

	Improvement in median survival	Hazard ratio (95% confidence interval)	*P* value
PFS	6.3 months	0.68 (0.58 – 0.80)	< 0.001
OS	15.7 months	0.68 (0.56 – 0.84)	< 0.001

* Prior neo(adjuvant) chemotherapy and/or trastuzumab allowed. Prior endocrine therapy allowed.
** Optional metastatic tumor tissue collection included in analyses

Swain et al, NEJM 2015

Objectives

- **Primary:**
  - **To determine the prognostic association between stromal TILs and survival (PFS) in patients with advanced HER2-positive breast cancer treated in the first line setting**

- **Secondary:**
  - **To determine associations between TILs and overall survival (OS)**
  - **To determine association of TILs with clinicopathological factors**
  - **To investigate if the benefit of the addition of pertuzumab significantly differed by TIL level (potential predictive factor)**
  - **To investigate the above associations by ER status**

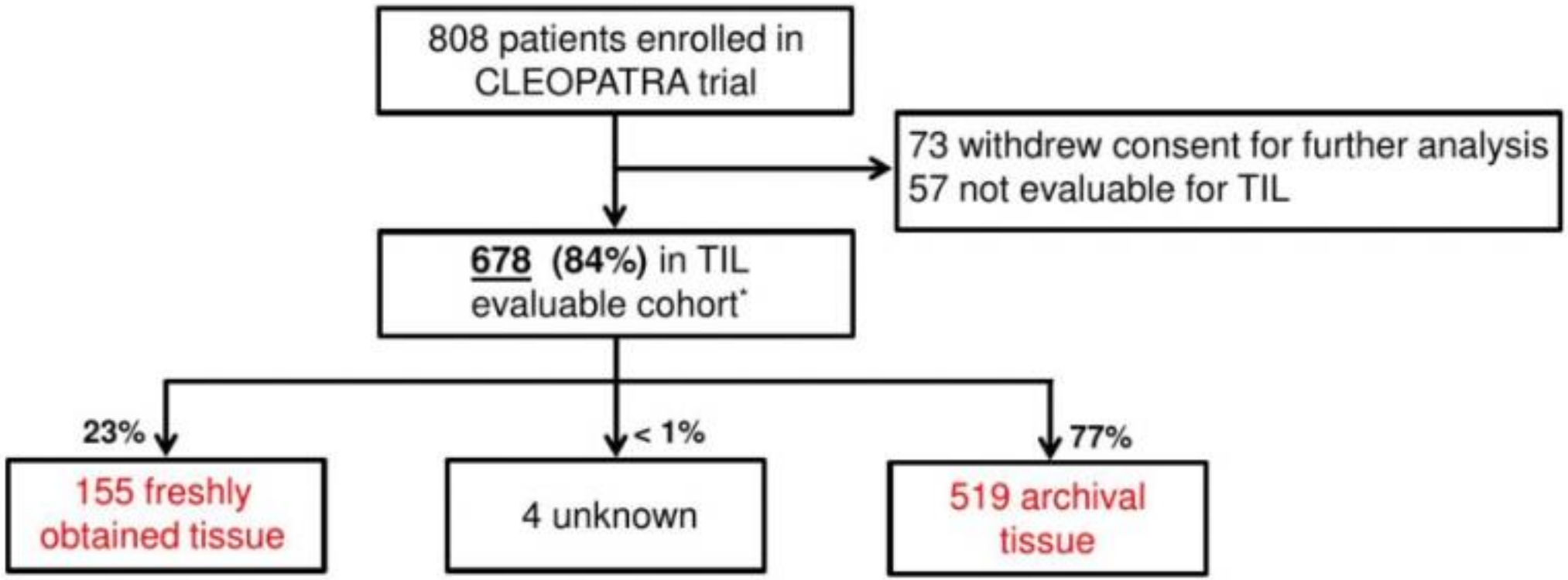
Methods

- **TILs were evaluated in prospectively collected pre-treatment tumor samples using our previously described method by analysis of haematoxylin and eosin tumor sections***

- **Statistical analyses:**
  - **The primary endpoint was PFS**
  - **Secondary endpoints were OS and clinico-pathological associations**
  - **Analyses were pre-specified using stromal TILs as a predefined TIL biomarker, measured as a continuous variable (per 10% increment)**
  - **Cox proportional hazard models were used to assess survival and interactions with pertuzumab treatment**

** Salgado, Denkert et al. Annals of Oncology 2014*

Consort diagram



Fresh obtained tissue – **obtained ≤ 45 days** from the date of randomization and they had not received prior endocrine therapy for advanced disease (all others were defined as archival)

*There are an additional 20 paired primary and metastasis samples

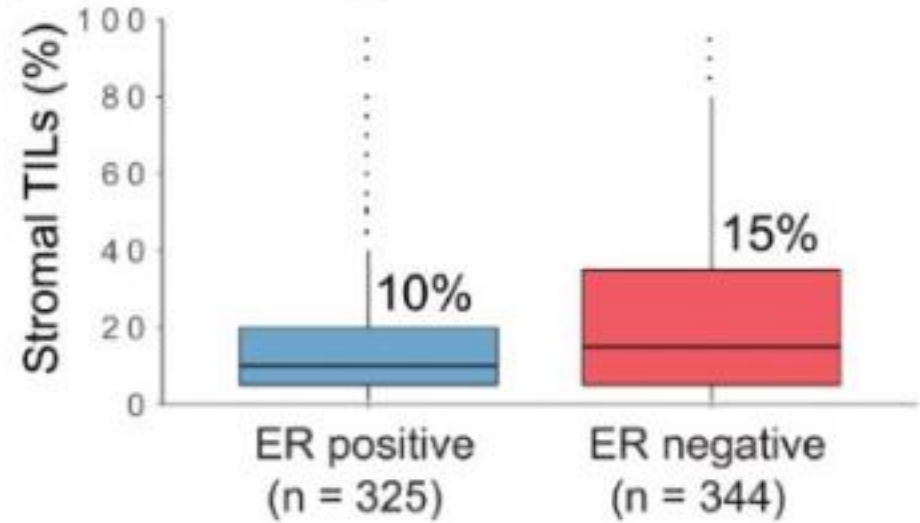
Patient characteristics

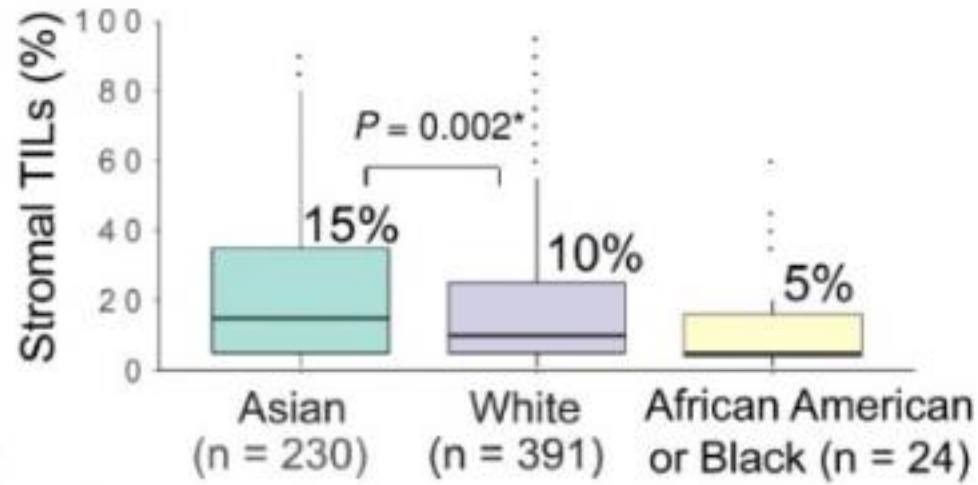
TIL evaluable cohort (n = 678), median follow up 50 months	
Age	Median 54 years (range 22 - 89)
Ethnicity*	
White	391 (58%)
Asian	230 (34%)
African American or Black	24 (4%)
ER status* - Positive / Negative	325 (48%) / 344 (51%)
PIK3CA genotype* - Mutated / Wild type	144 (22%) / 318 (47%)
Prior (neo)adjuvant chemotherapy**	284 (42%)
Prior (neo)adjuvant trastuzumab	75 (11%)
Visceral disease at screening (%)	538 (79%)
Stromal TILs (%)	Median 10%; Mean 21%; Range 1-95%

* Patients with unknown status or other status not listed; ** Anthracycline and/or taxane chemotherapy

TIL association with clinico-pathological factors

- **Age, tumor grade, and presence of visceral disease at screening were not significantly associated with TIL levels**
- ***ER-negative* tumors had significantly *higher* TIL levels ($P < 0.001$)***
- **TIL levels significantly differed by *ethnicity* ($P < 0.001$)****

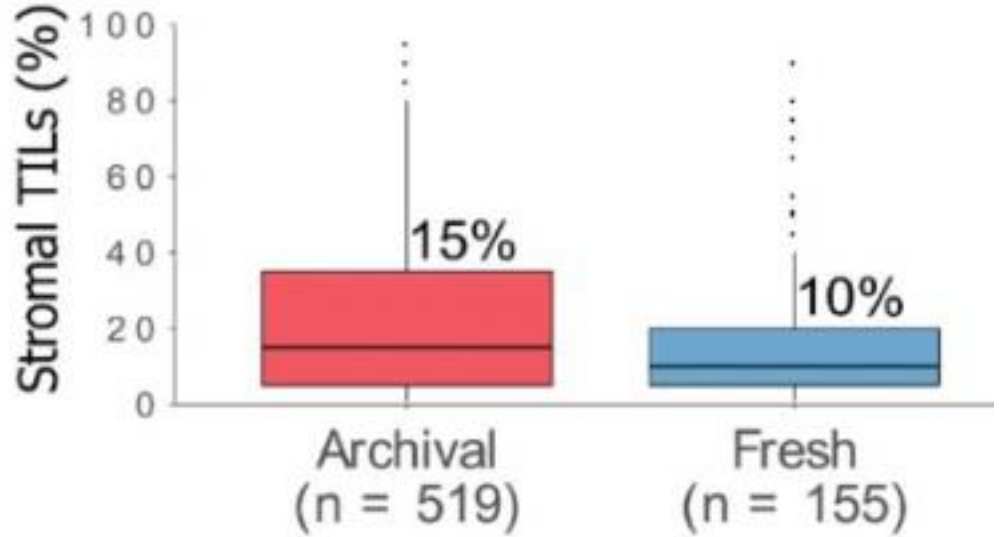


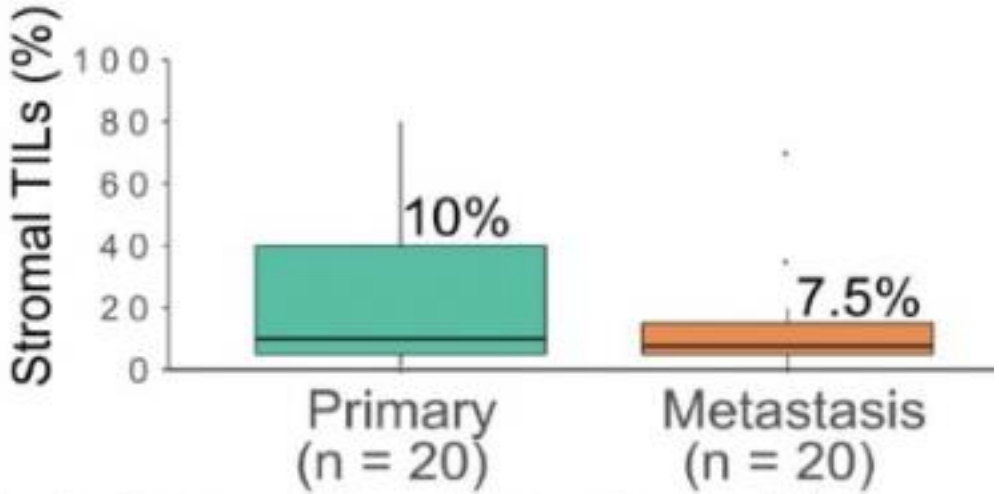


Median values shown. *Wilcoxon-Mann-Whitney test; **Kruskal-Wallis test

TIL associations with metastatic tissue type

- ***Freshly obtained*** **tumor samples had significantly *lower* TIL levels ($P < 0.001$)***
- **In the 20 paired primary and metastasis samples, there was a trend towards *lower* TIL levels in *metastatic samples* ($P = 0.07$)***





Median values shown. *Wilcoxon-Mann-Whitney test

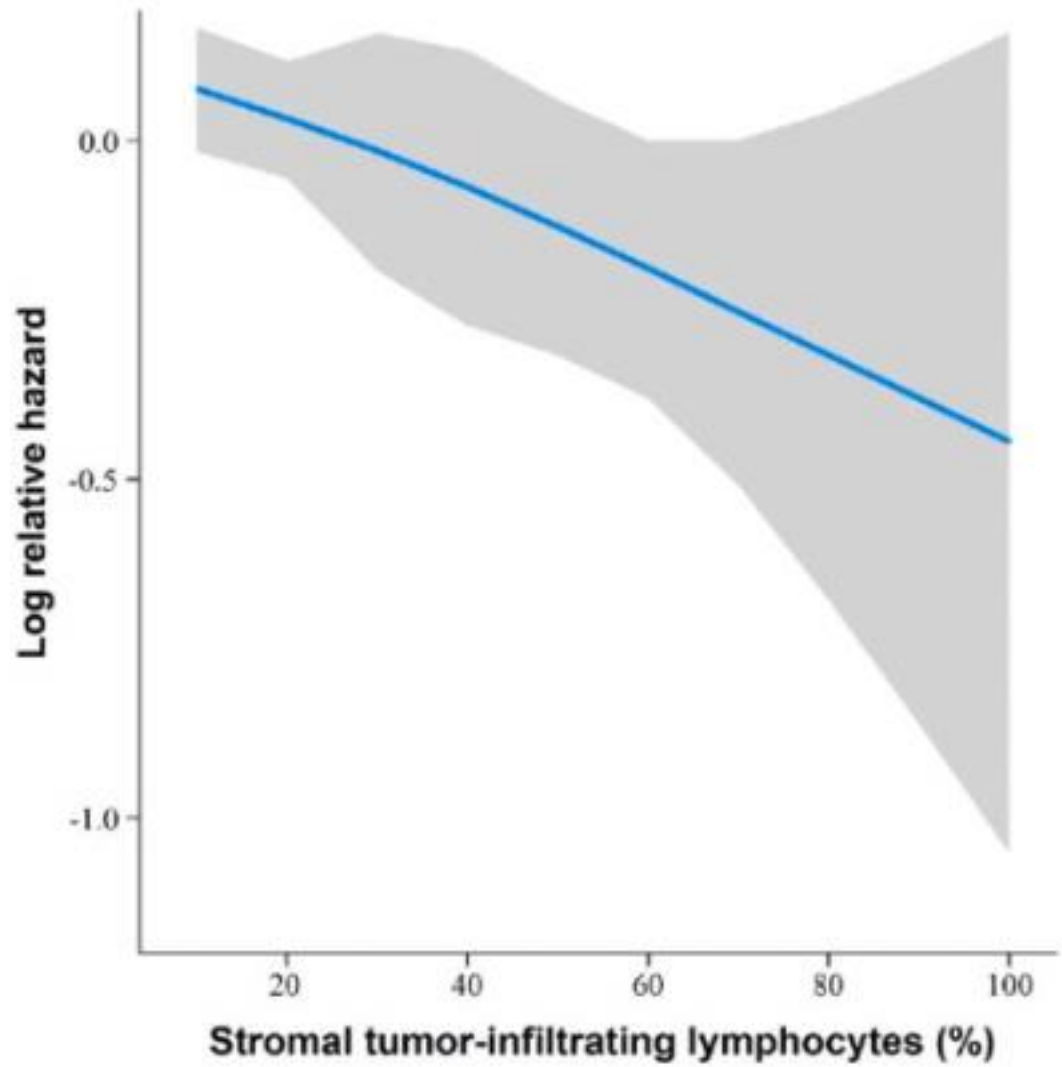
TIL association with survival – multivariate Cox analysis (adjusted)

	PFS			OS		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Stromal TILs (per 10% increment)	0.95	0.90 - 1.00	0.06	0.89	0.83 - 0.96	0.001
Age (< 65 vs ≥ 65 years)	1.01	0.74 - 1.38	0.95	1.03	0.70 - 1.53	0.86
Race - White vs Asian	1.29	1.03 - 1.62	0.028	1.08	0.82 - 1.42	0.6
ER - positive vs negative	1.07	0.86 - 1.33	0.57	0.79	0.60 - 1.04	0.09
PIK3CA - mutated vs wild type	1.81	1.43 - 2.29	< 0.001	1.65	1.24 - 2.19	< 0.001
Treatment naive vs prior (neo)adjuvant therapy	1.04	0.83 - 1.30	0.73	0.87	0.66 - 1.15	0.33
Visceral disease at screening - yes vs no	1.3	1.00 - 1.70	0.06	1.86	1.27 - 2.71	0.001
Treatment arm - Pertuzumab vs placebo	0.69	0.55 - 0.86	0.001	0.66	0.51 - 0.87	0.003

TILs evaluated as a continuous variable; race evaluated as White vs Asian as there were only small numbers of other ethnicities; *P* values calculated using Wald test.

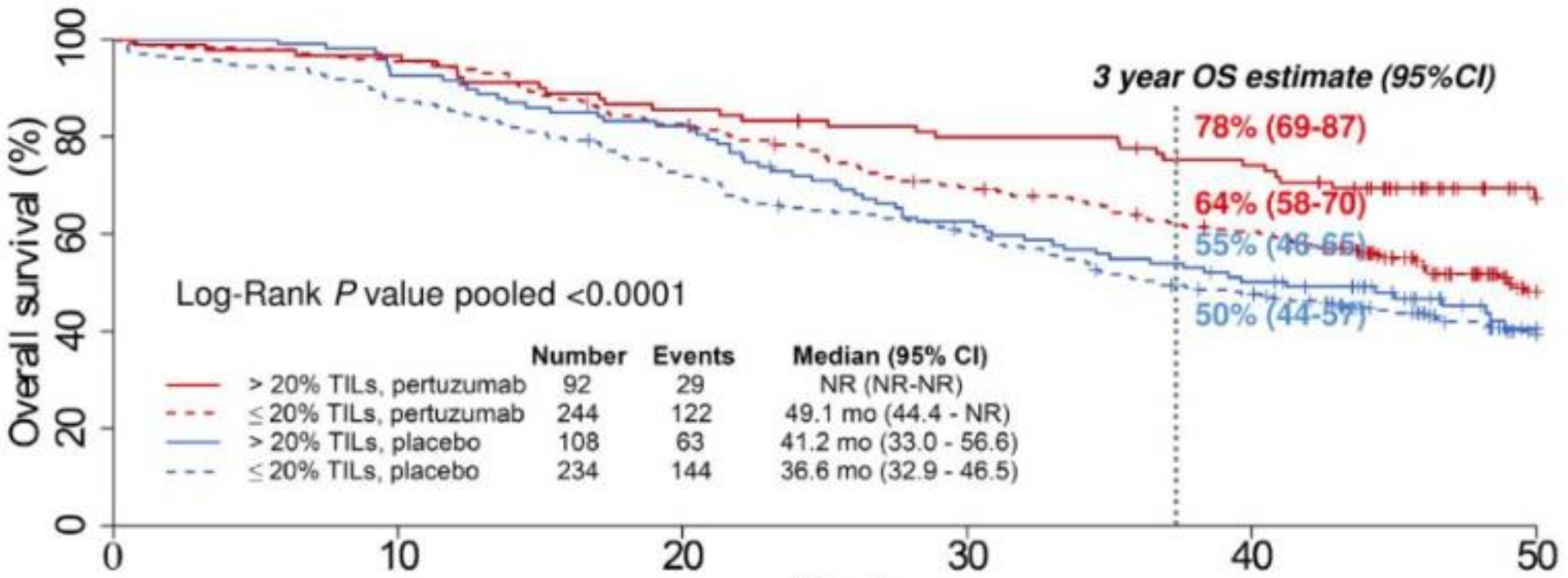
TIL effect is linearly related to survival

- **Plot demonstrating the log-relative HR for death vs stromal TIL per 10% increment**



Cubic smoothing spline for log relative hazard for death.
95% confidence interval shown in grey.

OS by mean TIL level by treatment arm



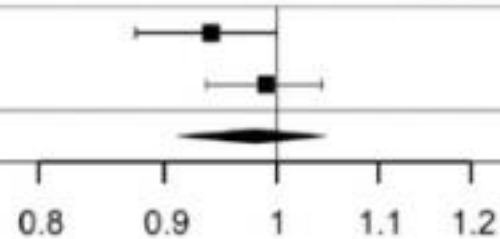
No. at risk:

> 20% TILs, pertuzumab	92 (0)	87 (2)	76 (3)	70 (4)	63 (6)	33 (31)
≤ 20% TILs, pertuzumab	244 (0)	231 (2)	198 (4)	164 (7)	139 (11)	61 (66)
> 20% TILs, placebo	108 (0)	99 (1)	88 (1)	66 (2)	52 (3)	25 (23)
≤ 20% TILs placebo	234 (0)	202 (3)	165 (4)	136 (6)	105 (9)	53 (46)

No significant interaction between TILs effect and pertuzumab treatment

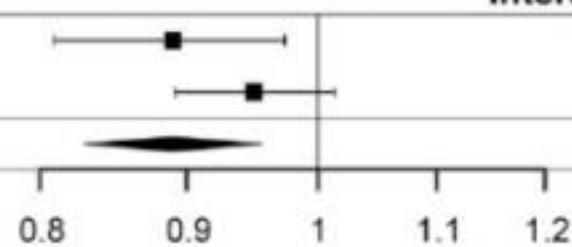
Progression-free survival

	Number	PFS Events	HR (95% CI)	Interaction P value
Pertuzumab	336	241	0.94 (0.88 – 1.00)	0.23
Placebo	342	278	0.99 (0.94 – 1.04)	
Overall	**678**	**519**	**0.98 (0.91 – 1.05)**	



Overall survival

	Number	Deaths	HR (95% CI)	Interaction P value
Pertuzumab	336	151	0.89 (0.81 – 0.97)	0.21
Placebo	342	207	0.95 (0.89 – 1.01)	
Overall	**678**	**358**	**0.89 (0.83 – 0.96)**	



TILs evaluated as a continuous variable per 10% increment; *P* values calculated using likelihood ratio test.

Conclusions

- This is the first study investigating associations between TILs and survival in advanced HER2-positive breast cancer treated with first line pertuzumab.

- There was a non-significant trend between higher TILs & improved PFS
- There was a significant association between higher TILS & improved OS
  - Each 10% increase in stromal TILs was associated with an 11% reduction in the risk of death - the TIL effect is linear
  - 3 year OS in patients who received pertuzumab and had stromal TILs > 20% was 78% (CI: 69-87%)

- Prognostic effect of TILs was not different according to treatment arm
  - No predictive effect was observed with regard to pertuzumab treatment

- The positive influence of pre-existing anti-tumor immunity persists in the advanced setting. Strategies to augment immunity may further improve survival.

THE LANCET Oncology

Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab and trastuzumab: a retrospective analysis of the CLEOPATRA study

Authors

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THE LANCET Oncology

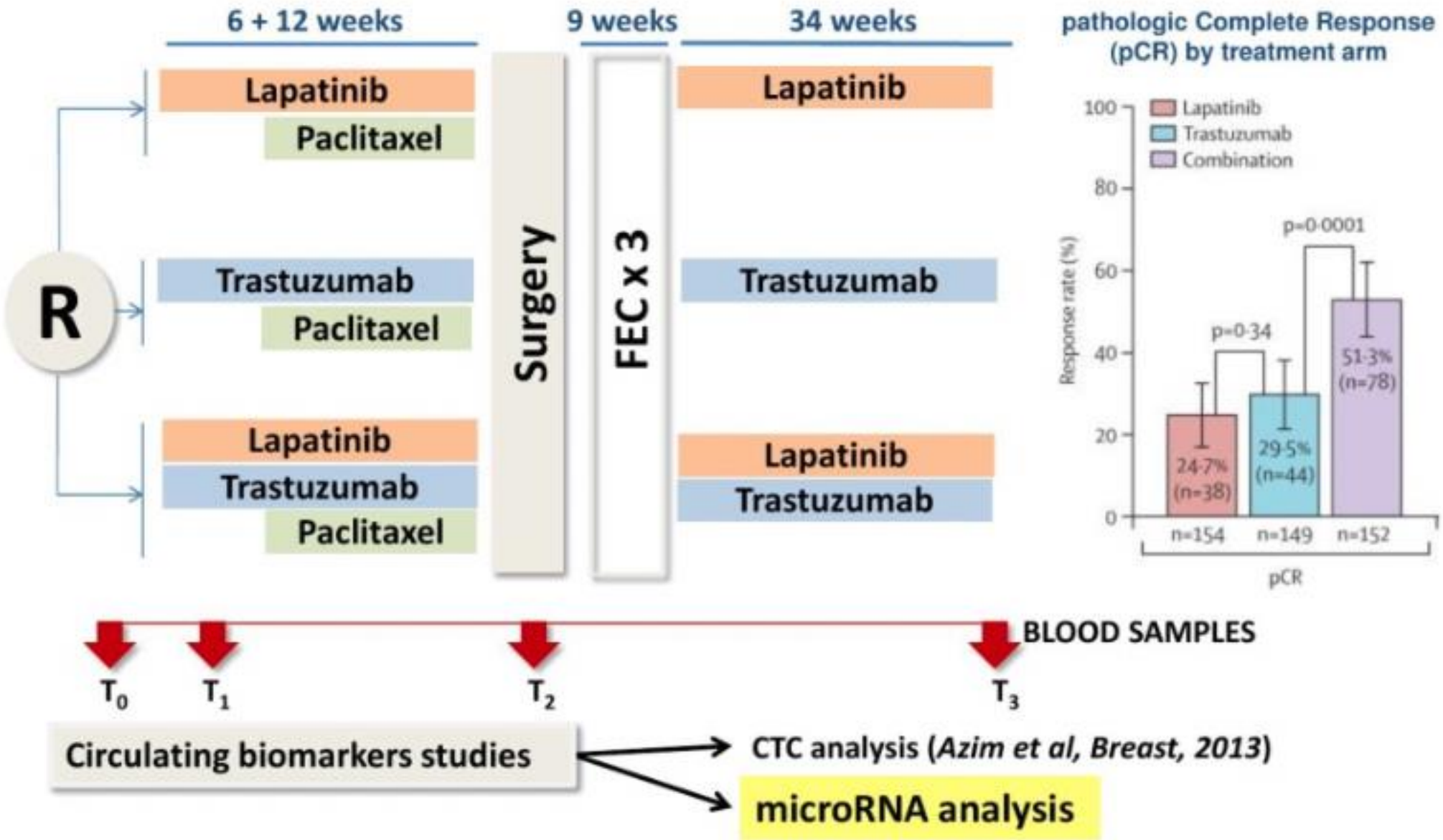
The best science for better lives

S3-02

Plasma microRNA levels for predicting therapeutic response to neoadjuvant treatment in HER2-positive breast cancer Results from NeoALTTO

Serena Di Cosimo, Valentina Appierto, Paola Tiberio, Paolo Verderio, Sara Pizzamiglio, Stefano Bottelli, Marilena Iorio, José Baselga, Martine Piccart, Jens Huober, Jan Brase, Lorena de la Peña, Debora Fumagalli, Filippo de Braud, Maria Grazia Daidone

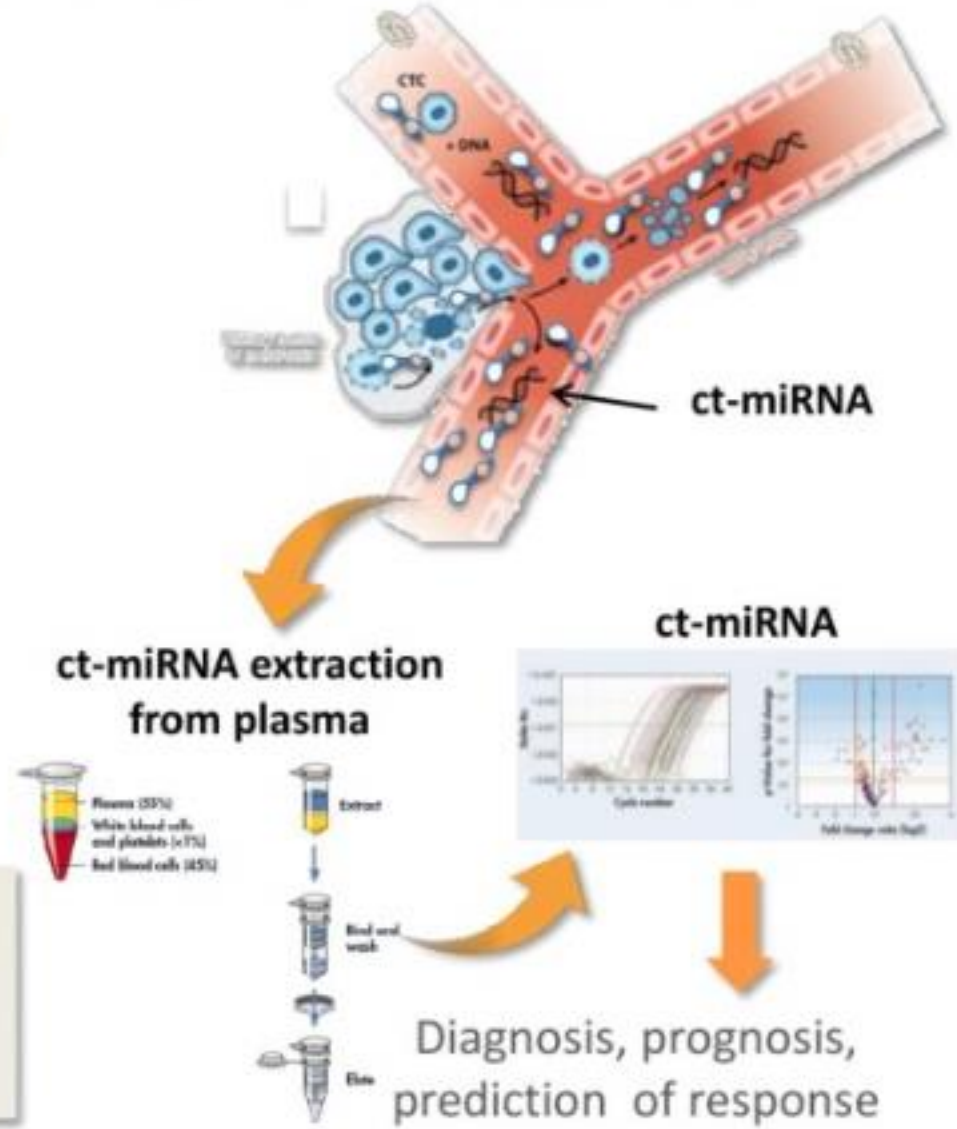
NeoALTTO Study *(J. Baselga, Lancet 2012)*



Circulating microRNAs as cancer biomarkers

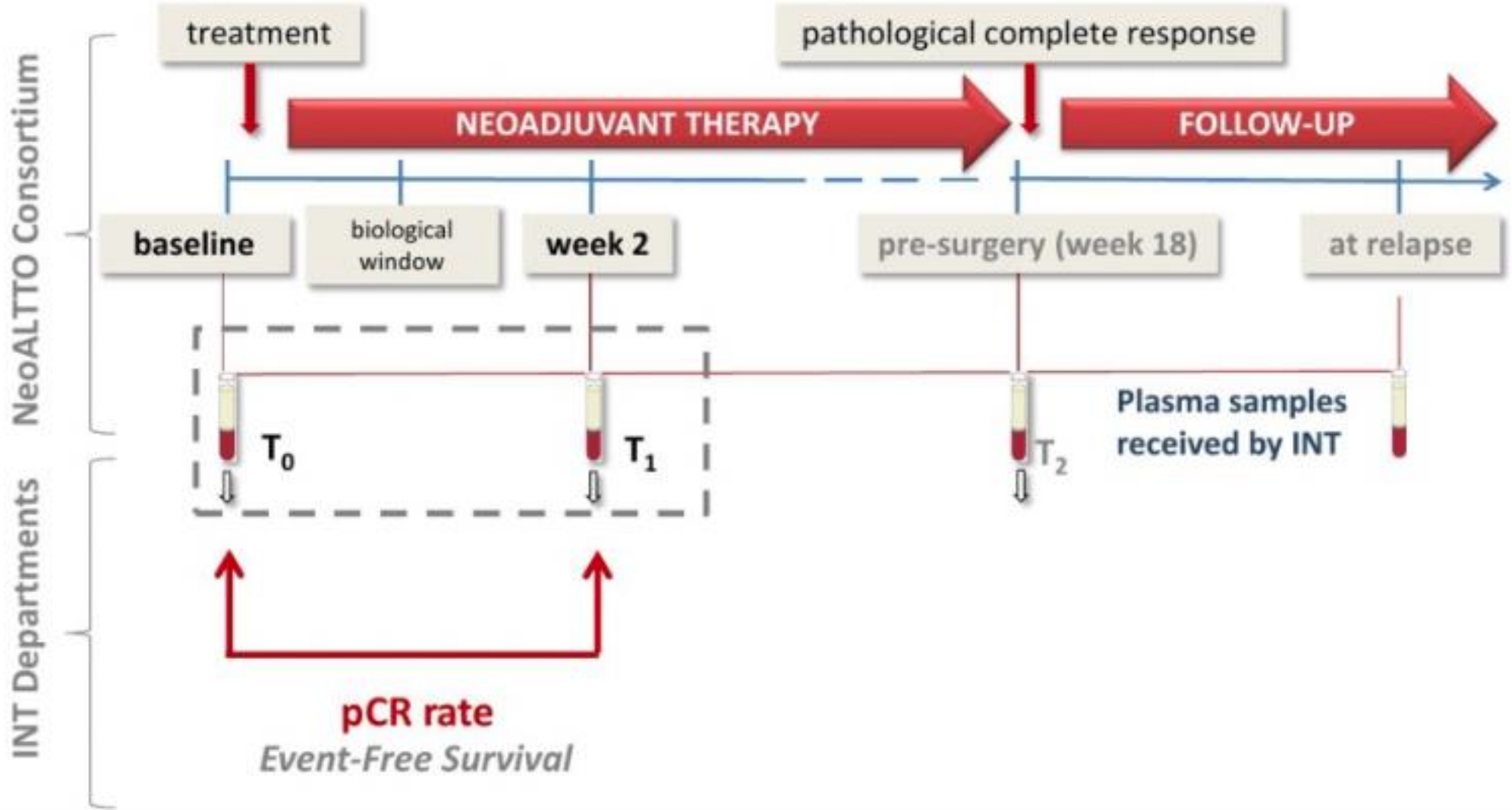
- microRNAs (miRNAs) are small, non-coding RNAs, known to regulate gene expression
- miRNAs can disseminate from tumor cells to peripheral circulation
- Circulating miRNAs (ct-miRNAs) are stable and detectable in many biological fluids
- ct-miRNAs may function as non-invasive liquid biopsies.

Are ct-miRNAs associated with clinical outcome in NeoALTTO breast cancer patients?



modified from K. Pantel and C. Alix-Panabieres, Cancer Res 2013

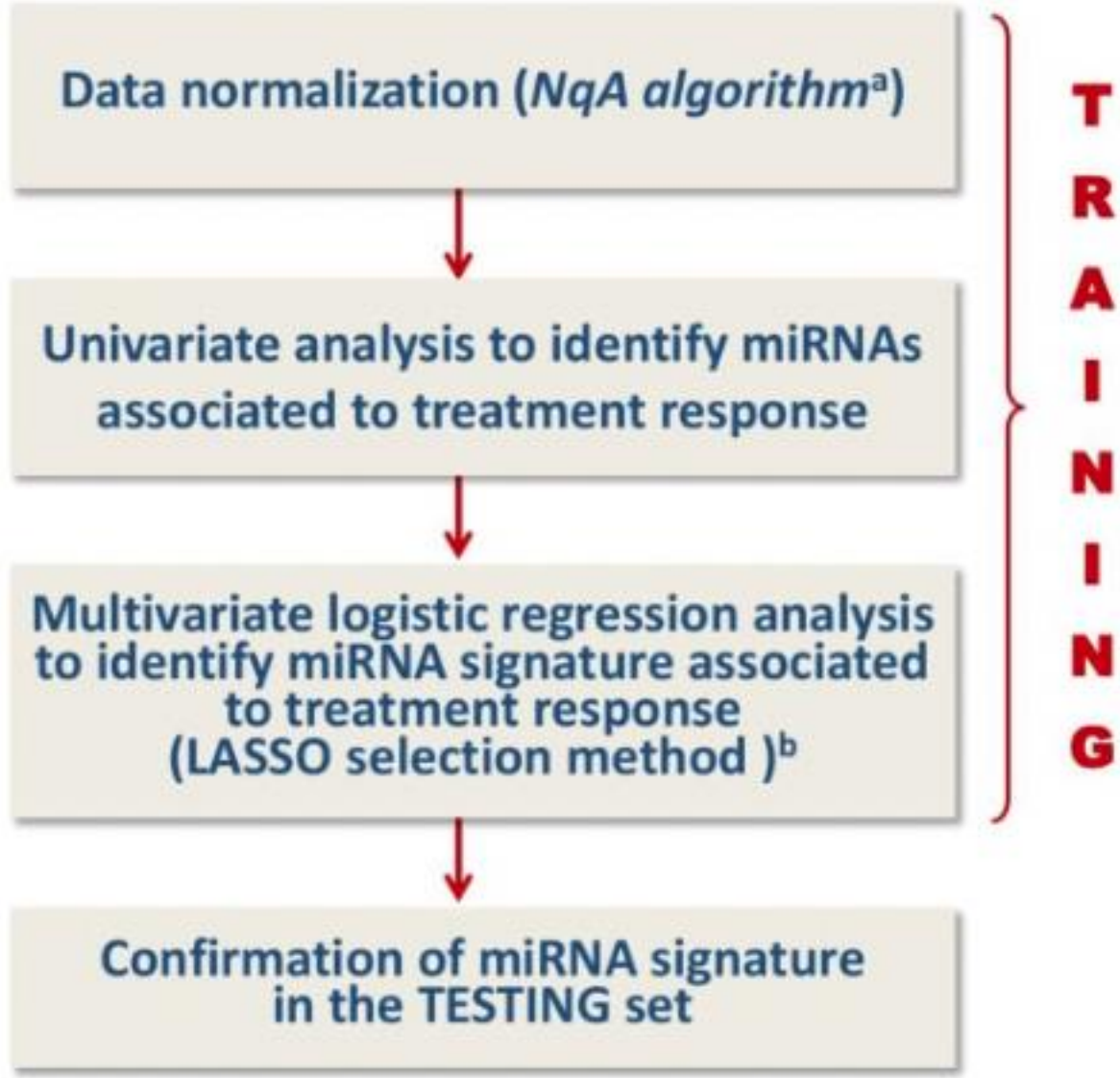
Longitudinal blood sampling for circulating biomarkers in NeoALTTO



Statistical analysis

[a] Verderio et al.
Analytical Biochemistry 2014

[b] Verderio et al. BJC 2016



Patient population

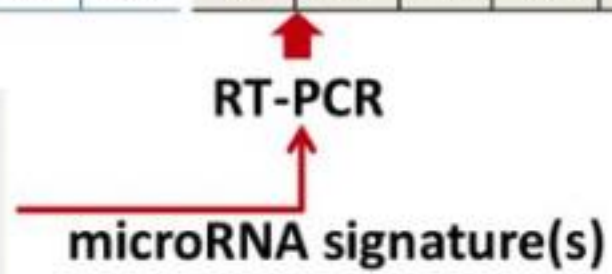
Cases were randomly split according to Tx arm and pCR rate

		TRAINING set (N=185, 43%)						TESTING set (N=250, 57%)					
		L (N=64)		T (N=60)		L+T (N=61)		L (N=87)		T (N=81)		L+T (N=82)	
		#	%	#	%	#	%	#	%	#	%	#	%
ER Status	-ve	34	**53**	29	**48**	31	**51**	48	**55**	47	**58**	42	**51**
	+ve	30	**47**	31	**52**	30	**49**	39	**45**	34	**42**	40	**49**
N Status (clinical)	N 0/1	52	**81**	56	**92**	50	**83**	74	**85**	69	**85**	66	**80**
	N 2+	12	**19**	5	**8**	10	**17**	13	**15**	12	**15**	16	**20**
Tumor Size	≤ 5cm	40	**63**	39	**64**	38	**63**	52	**60**	50	**62**	48	**59**
	> 5cm	24	**37**	22	**36**	22	**37**	35	**40**	31	**38**	34	**41**
pCR	Yes	16	**25**	18	**30**	31	**51**	21	**24**	24	**30**	42	**51**
	No	48	**75**	42	**70**	30	**49**	66	**76**	57	**70**	40	**49**

RT-PCR panel analysis of miRNAs

Exiqon miRCURY LNA™ Universal RT microRNA PCR panel I+II:

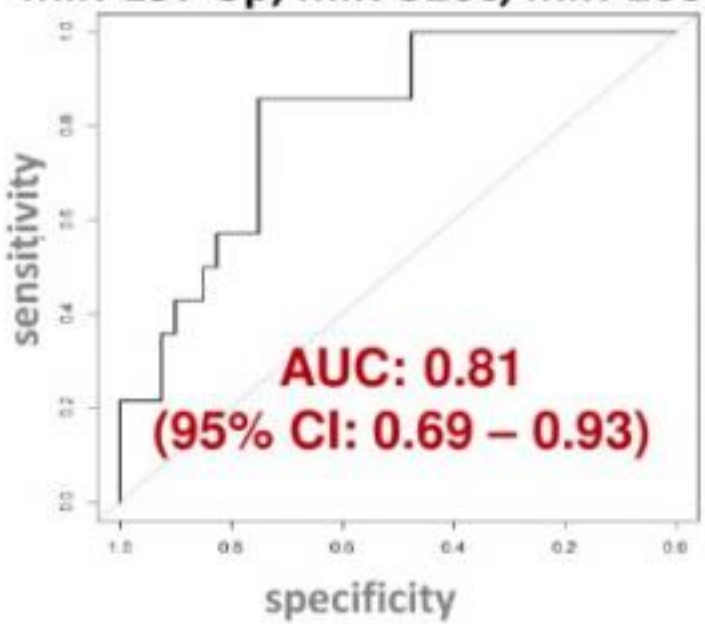
6 technical controls spike-in + 674 assays



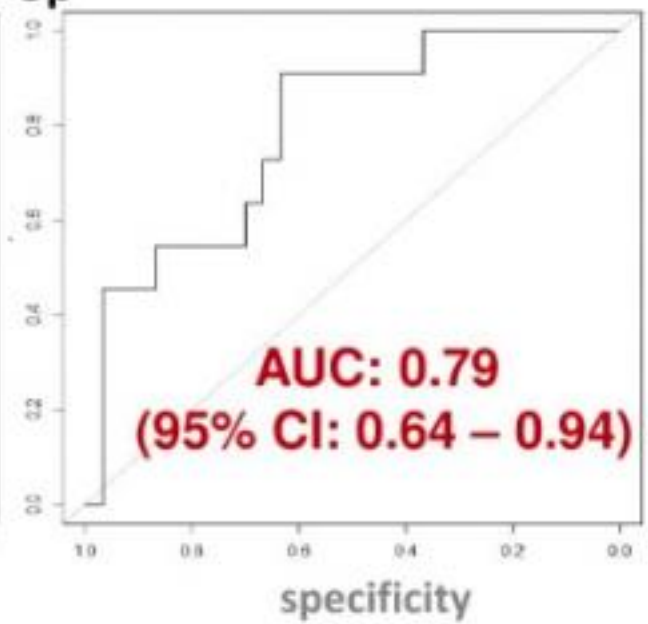
ct-miRNA signatures associated with pCR at baseline

Results from training set by treatment arm

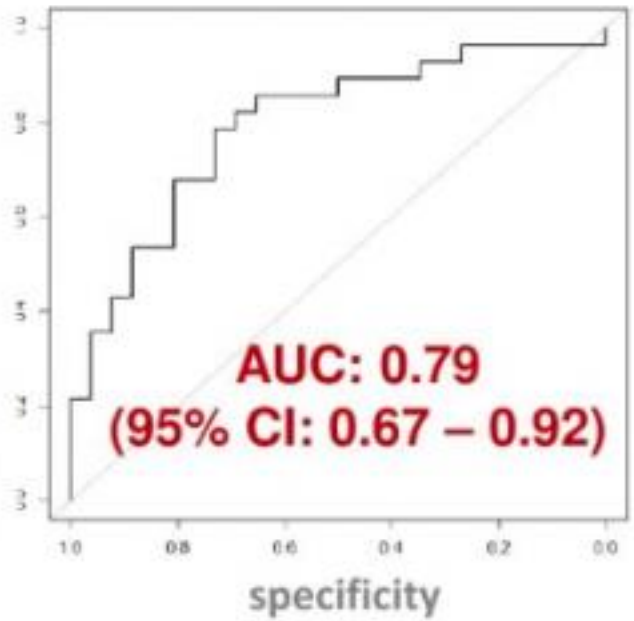
Lapatinib (L)
(pCR Yes=14; No=40)
miR-376c-3p; miR-874-3p; miR-197-3p; miR-320c; miR-100-5p



Trastuzumab (T)
(pCR Yes=11; No=30)
miR-143-3p; miR-96-5p



L+T
(pCR Yes=28; No=26)
miR-126-3p; miR-133b



Discrimination of the final predictive model was assessed using AUC (optimal values in the range 0.7-0.9)

ct-miRNA signatures associated with pCR at baseline

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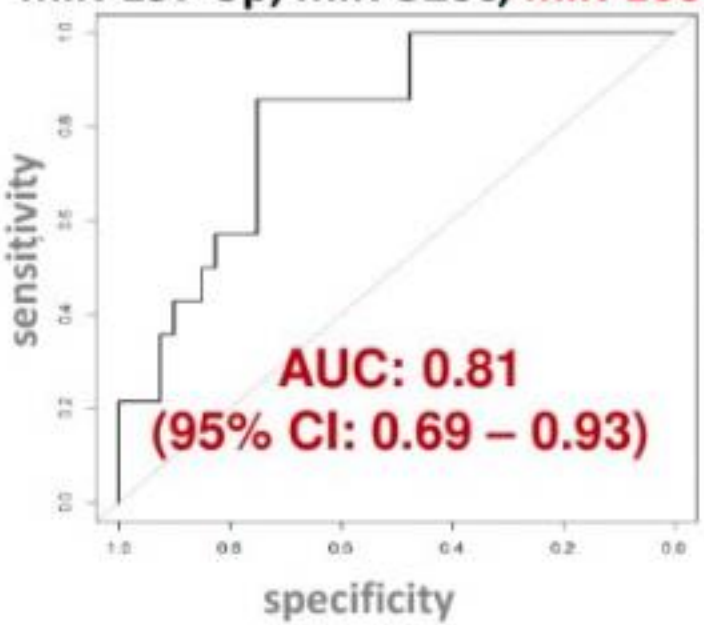

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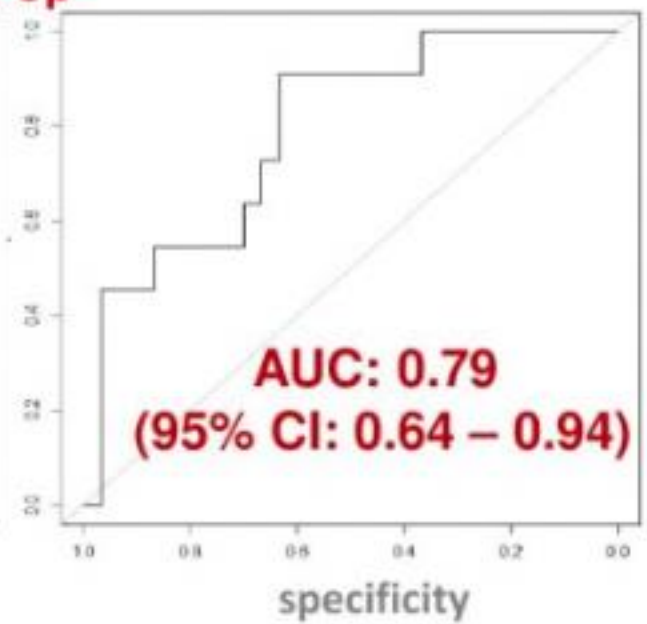
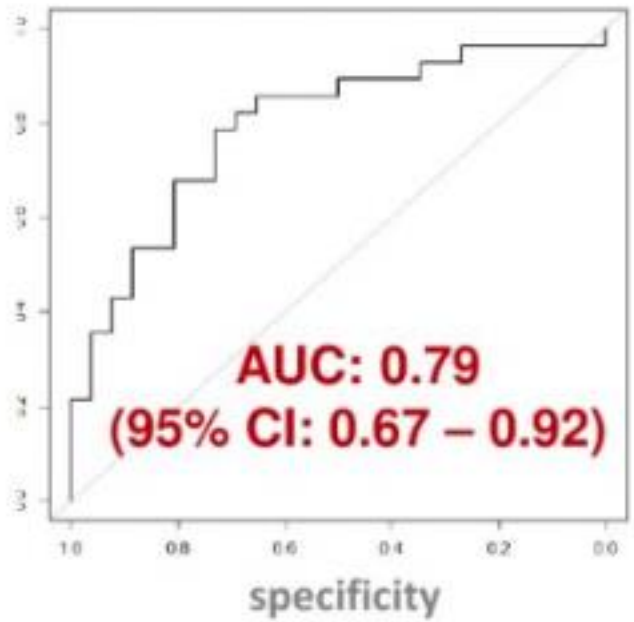


L+T

(pCR Yes=28; No=26)

miR-126-3p; miR-133b



APOPTOSIS

ct-miRNA signatures associated with pCR at baseline

Results from training set by treatment arm

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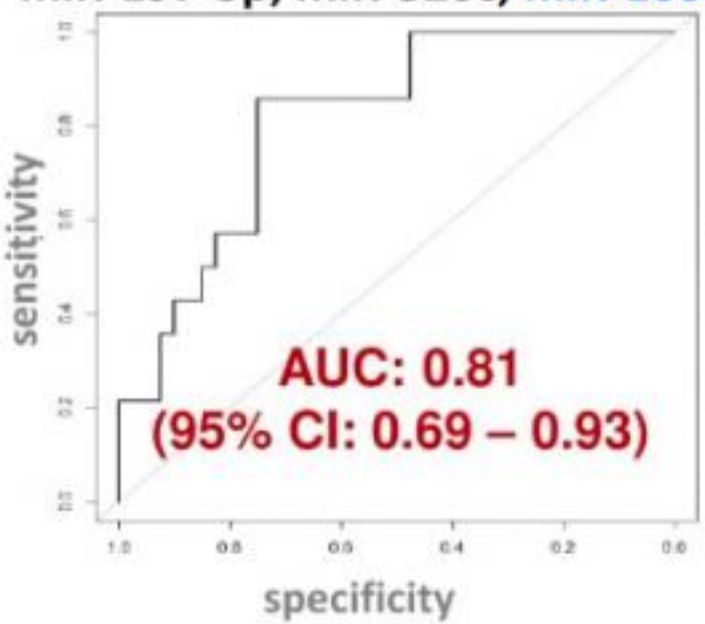

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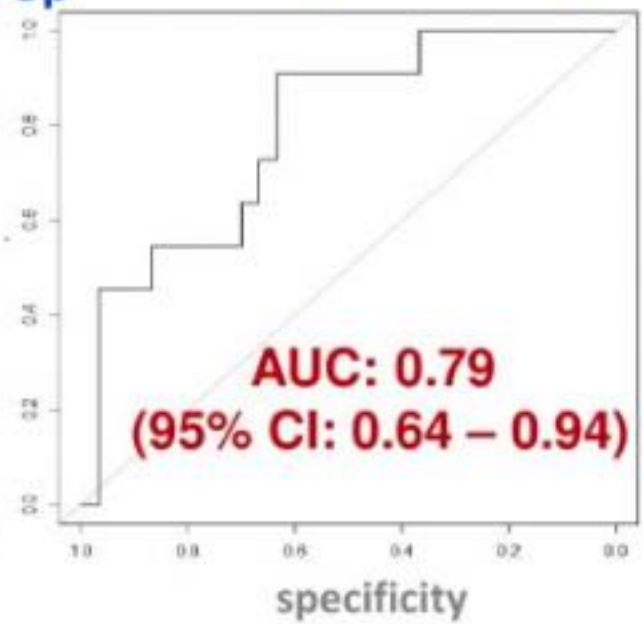
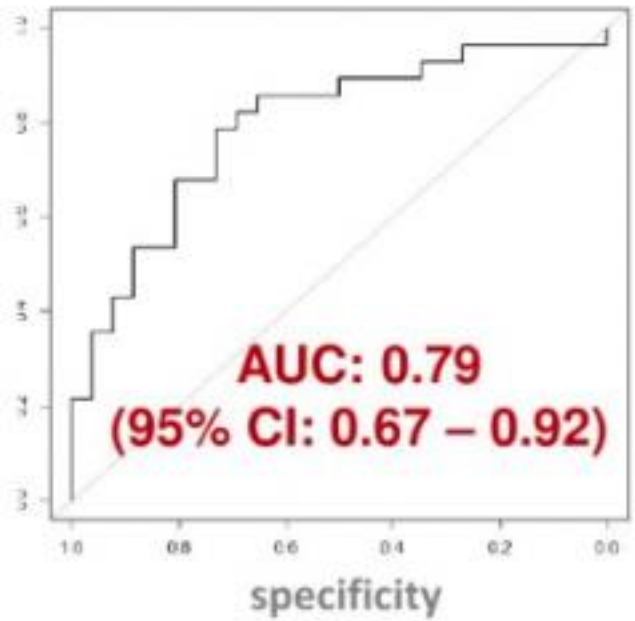
miR-143-3p; miR-96-5p



L+T
(pCR Yes=28; No=26)
miR-126-3p; miR-133b



PROLIFERATION

ct-miRNA signatures associated with pCR at baseline

Results from training set by treatment arm

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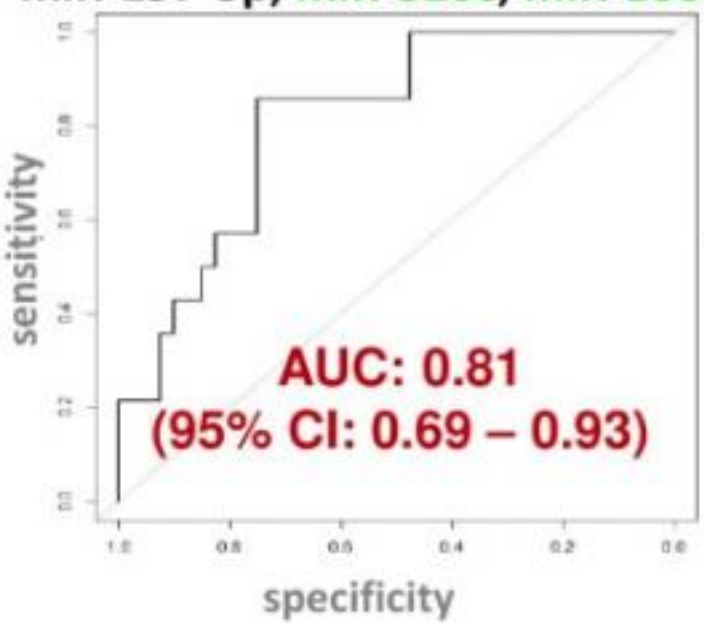

Trastuzumab (T)

(pCR Yes=11; No=30)

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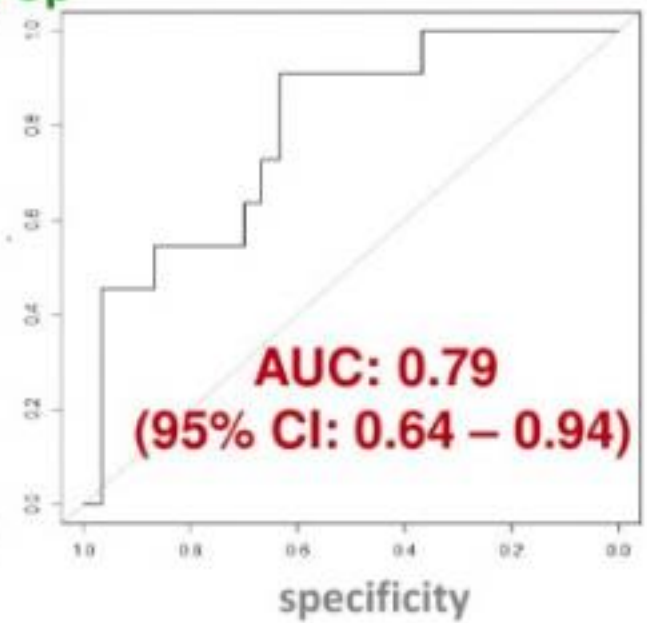
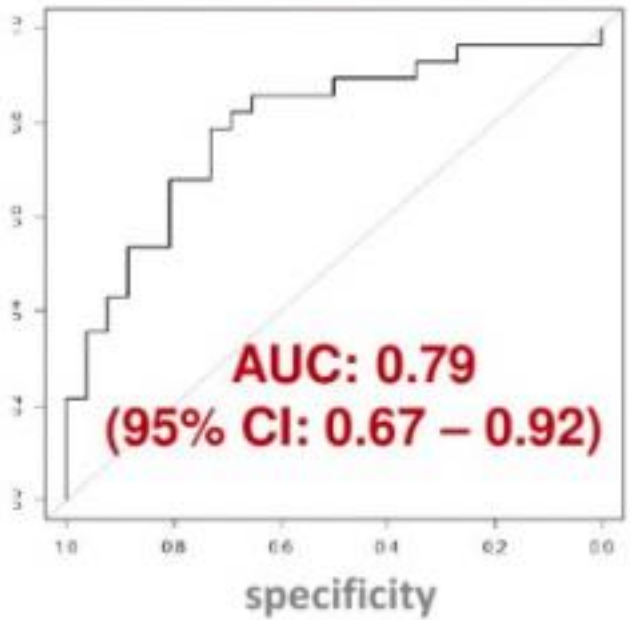


L+T

(pCR Yes=28; No=26)

miR-126-3p; miR-133b



INVASIVENESS

ct-miRNA signatures associated with pCR at baseline

Results from training set by treatment arm

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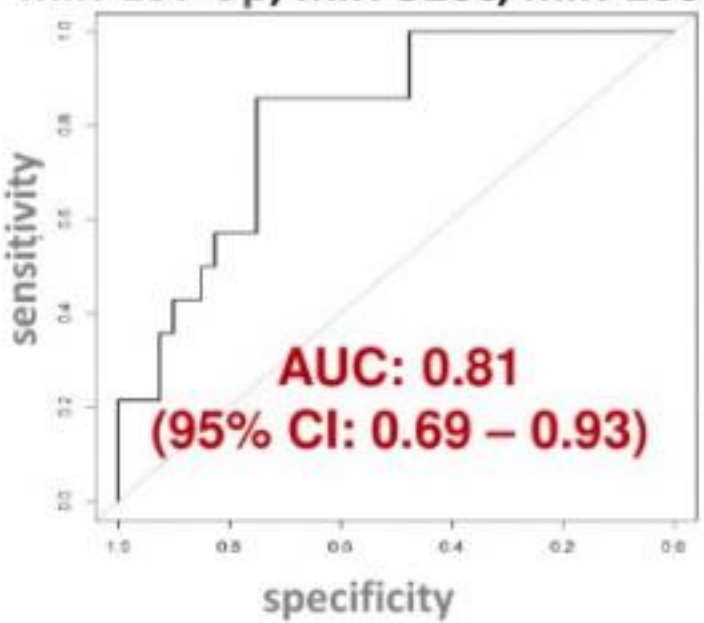
miR-376c-3p; miR-874-3p;
miR-197-3p; **miR-320c; miR-100-5p**



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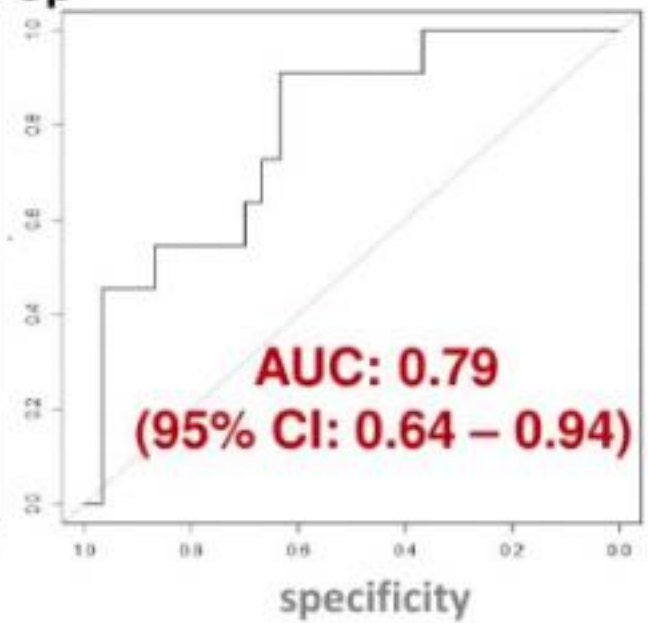
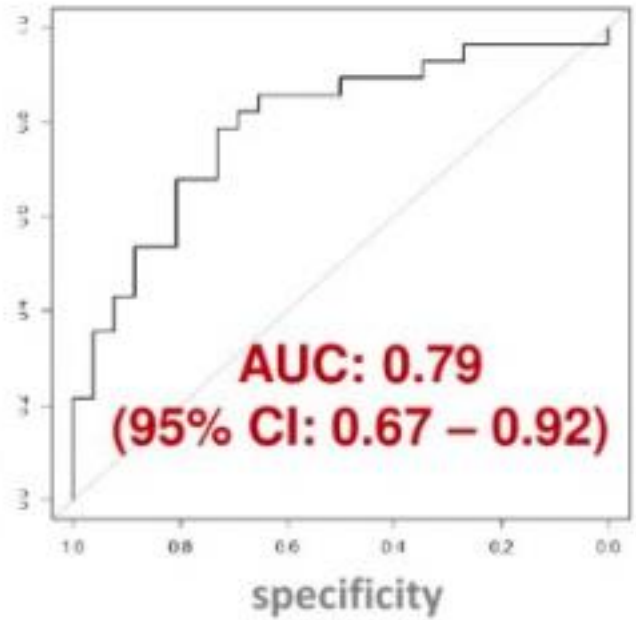
miR-143-3p; **miR-96-5p**



L+T
(pCR Yes=28; No=26)
miR-126-3p; miR-133b

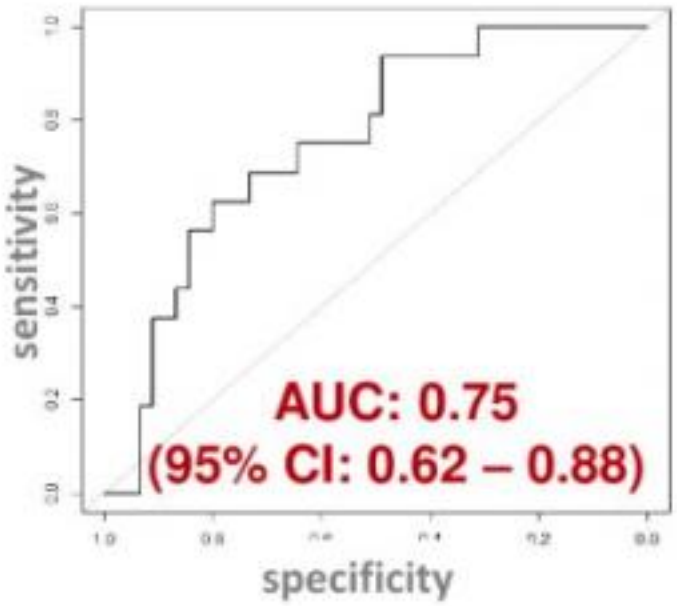


RESPONSE TO TREATMENT

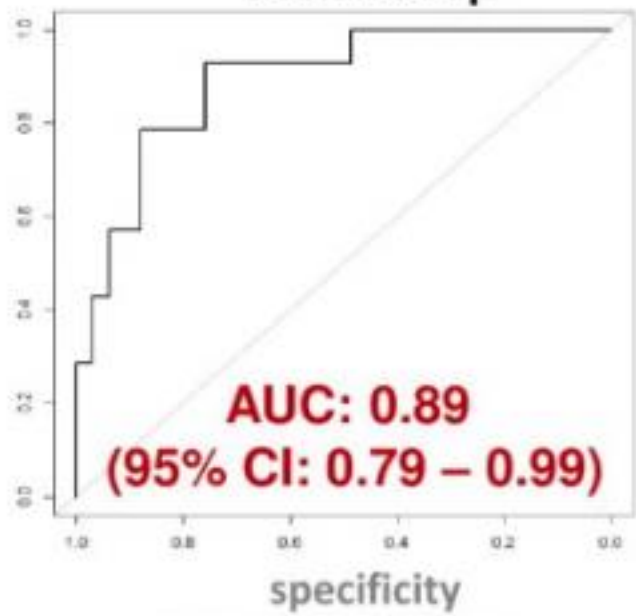
ct-miRNA signatures associated with pCR at week 2

Results from training set by treatment arm

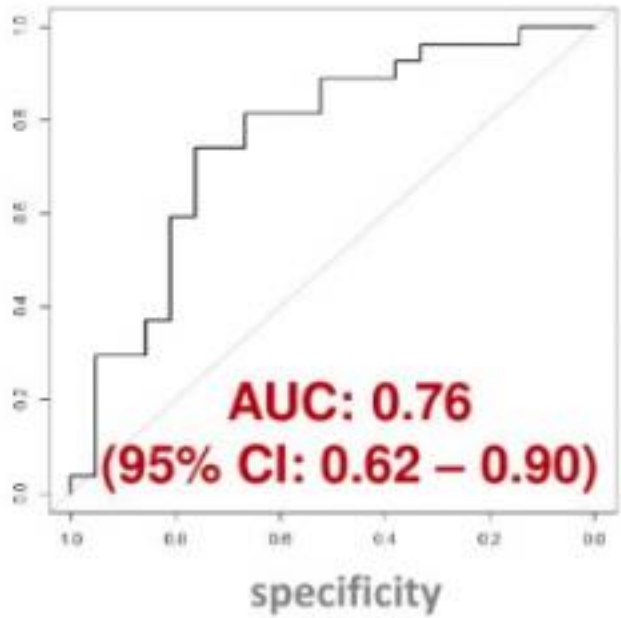
Lapatinib (L)
(pCR Yes=16; No=45)
miR-144-3p;
miR-362-3p; miR-100-5p



Trastuzumab (T)
(pCR Yes=14; No=33)
miR-374a-5p; miR-574-3p;
miR-140-5p; miR-328-3p;
miR-145-5p



L+T
(pCR Yes=27; No=21)
miR-34a-5p;
miR-98-5p; miR-100-5p

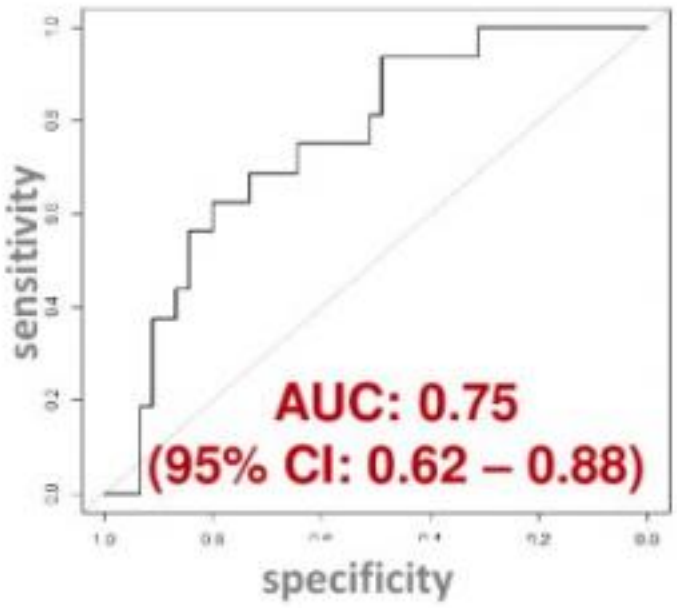


PROLIFERATION

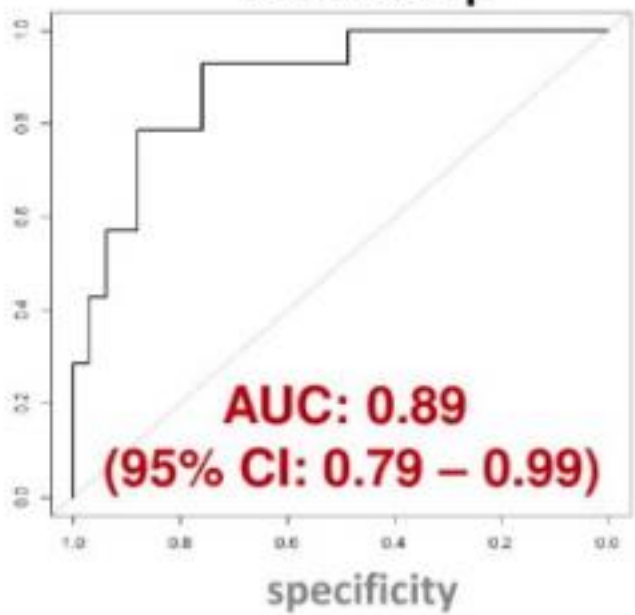
ct-miRNA signatures associated with pCR at week 2

Results from training set by treatment arm

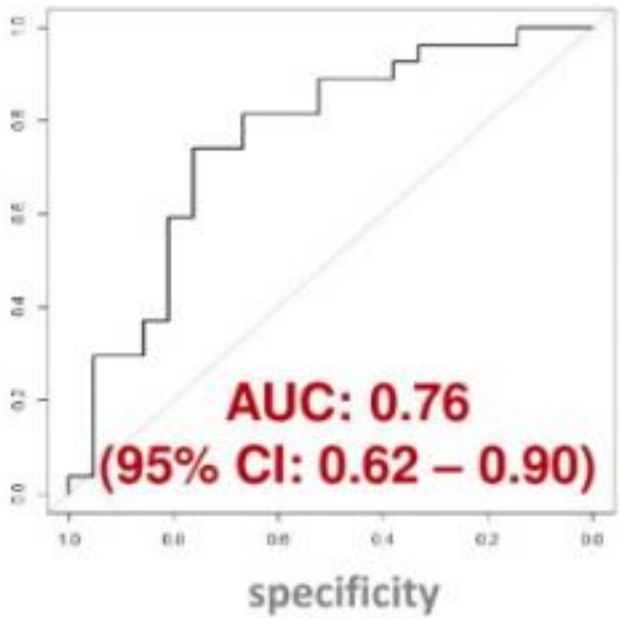
Lapatinib (L)
(pCR Yes=16; No=45)
miR-144-3p; miR-362-3p; miR-100-5p



Trastuzumab (T)
(pCR Yes=14; No=33)
miR-374a-5p; miR-574-3p; miR-140-5p; miR-328-3p; miR-145-5p



L+T
(pCR Yes=27; No=21)
miR-34a-5p; miR-98-5p; miR-100-5p



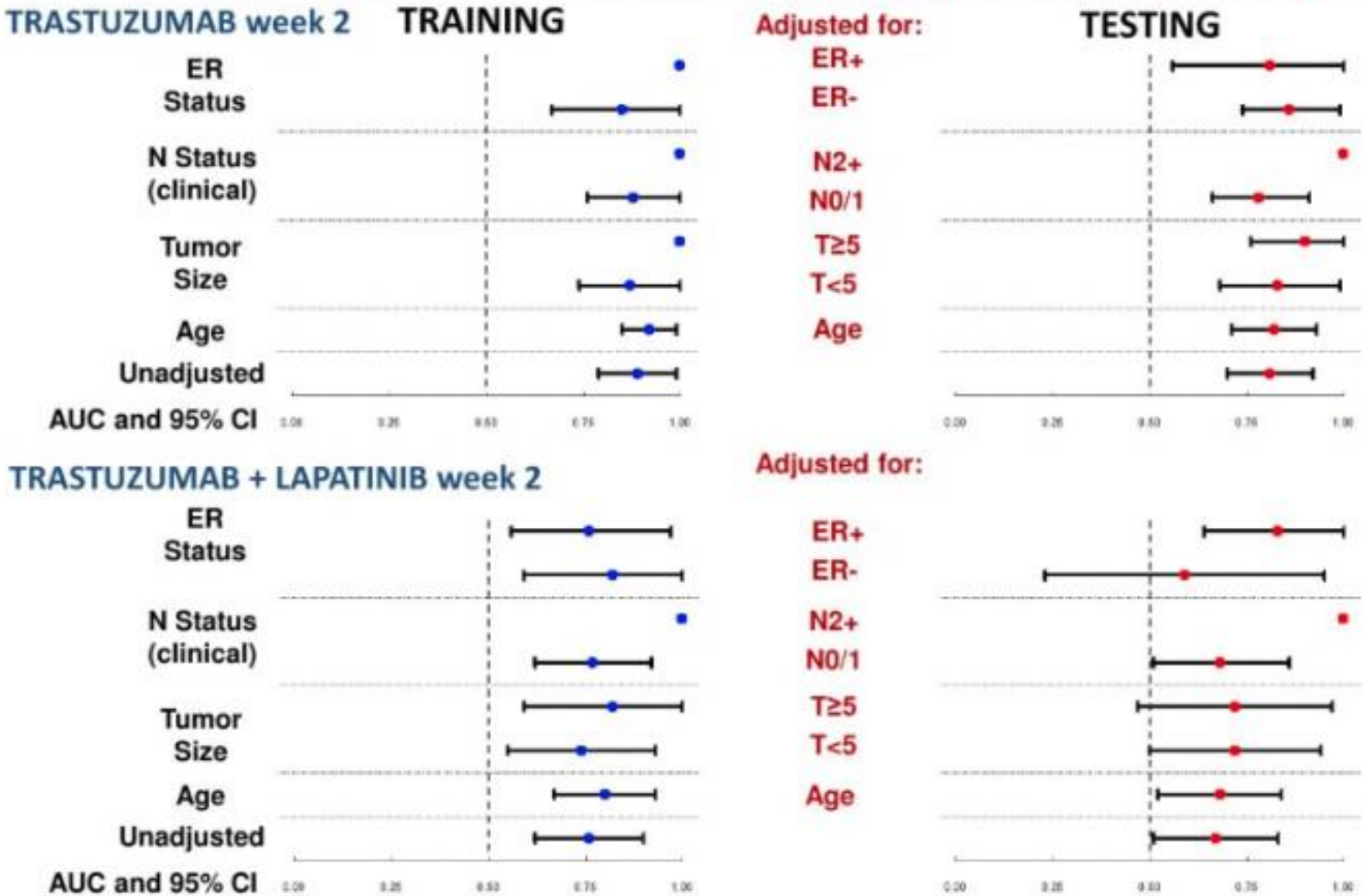
RESPONSE TO TREATMENT

ct-miRNA signatures associated with pCR in training set and confirmed in testing

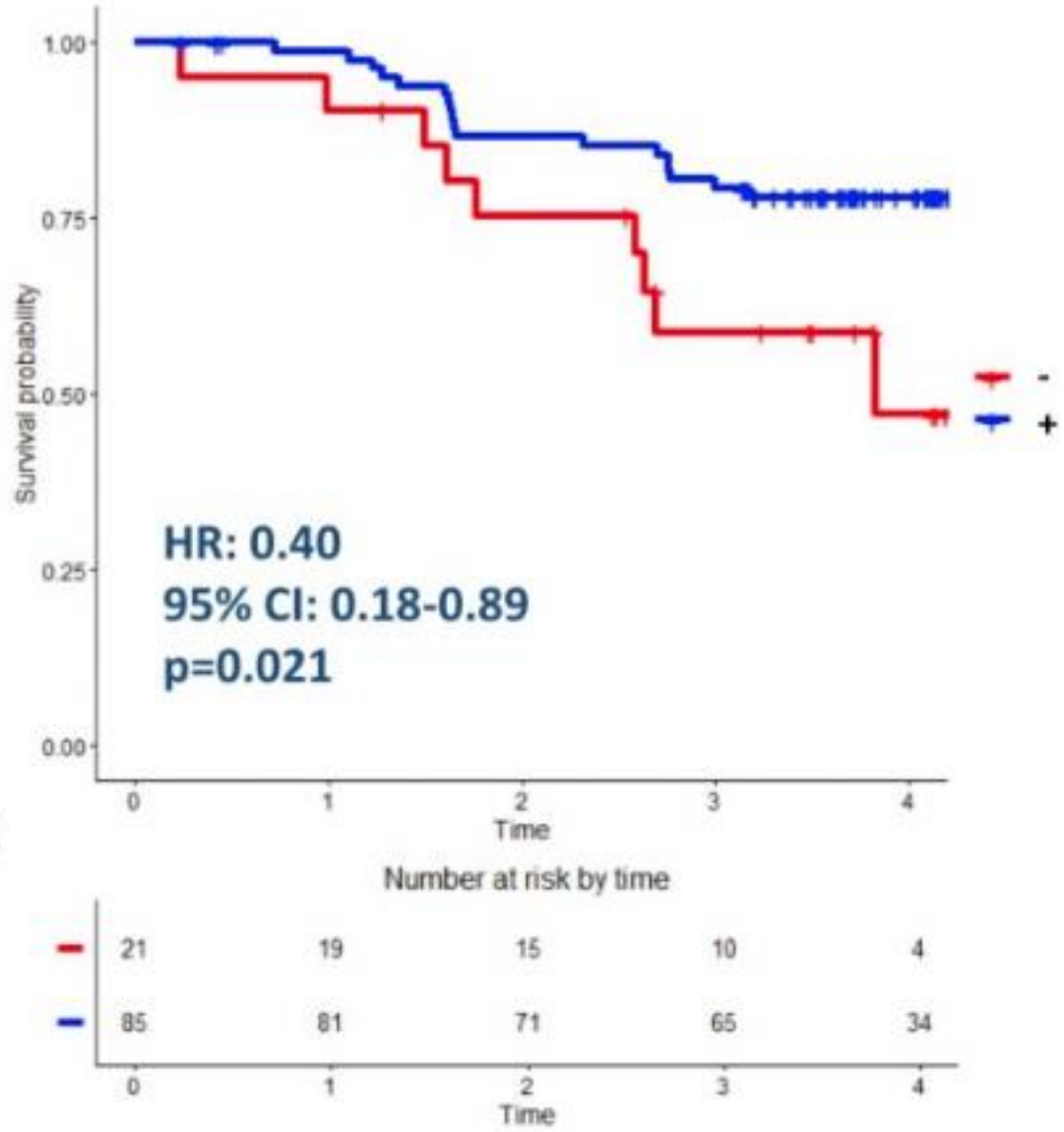
Set of analysis	# of pts.	Final multivariate model	AUC (95% CI) TRAINING	AUC (95% CI) TESTING
Lapatinib at baseline	Train=54 Test=42	**5 miRNAs** **miR-376c-3p; miR-874-3p; miR-197-3p; miR-320c; miR-100-5p**	0.81 (0.69 – 0.93)	**0.86 (0.73 - 0.98)**
Lapatinib at week 2	Train=61 Test=66	**3 miRNAs** **miR-144-3p; miR-362-3p; miR-100-5p**	0.75 (0.62 – 0.88)	**0.71 (0.55 - 0.86)**
Trastuzumab at baseline	Train=41 Test=57	2 miRNAs miR-143-3p; miR-96-5p	0.79 (0.64 – 0.94)	*0.47 (0.30 - 0.65)*
Trastuzumab at week 2	Train=47 Test=59	**5 miRNAs** **miR-374a-5p; miR-574-3p; miR-140-5p; miR-328-3p; miR-145-5p**	0.89 (0.79 – 0.99)	**0.81 (0.70 - 0.92)**
Lapatinib + Trasuzumab at baseline	Train=54 Test=59	2 miRNAs miR-126-3p; miR-133b	0.79 (0.67 – 0.92)	*0.55 (0.40 – 0.70)*
Lapatinib + Trastuzumab at week 2	Train=48 Test=47	**3 miRNAs** **miR-34a-5p; miR-98-5p; miR-100-5p**	0.76 (0.62 – 0.90)	**0.67 (0.51 - 0.83)**

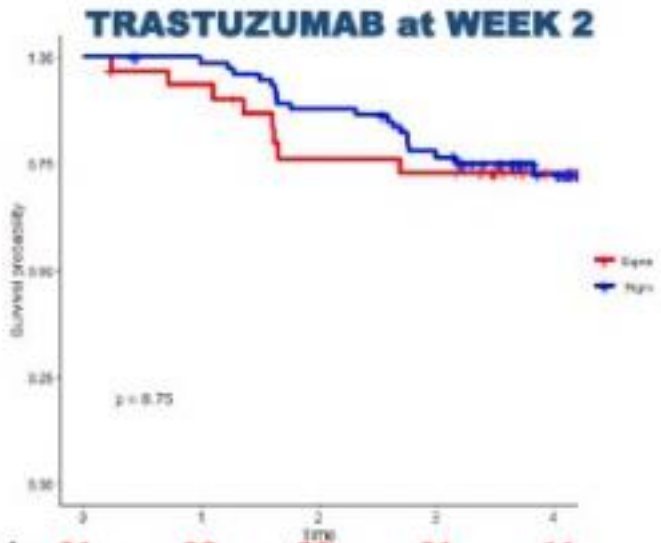
ct-miRNAs discriminating capability in classifying responsive and unresponsive cases held across different subgroups

ct-miRNAs discriminating capability in classifying responsive and unresponsive cases held across different subgroups



ct-miRNA 140-5p is associated with EFS





Conclusions

- This is the first evidence of the potential of circulating miRNAs to discriminate between responsive and unresponsive HER2 positive BC patients
- Four ct-miRNA signatures were found to identify patients with and without pCR in a time and treatment specific manner
- Results obtained early post-treatment are of special value: women with unfavorable miRNA signature can be expected to have poor response after just 2 weeks of treatment
- At present, none of the ct-miRNA signatures are associated with EFS
- Functional studies are ongoing to investigate the biological role of miRNAs identified in the signatures
- Independent validation studies are planned.

San Antonio Breast Cancer Symposium, December 6-10, 2016

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Pamela

S3-03

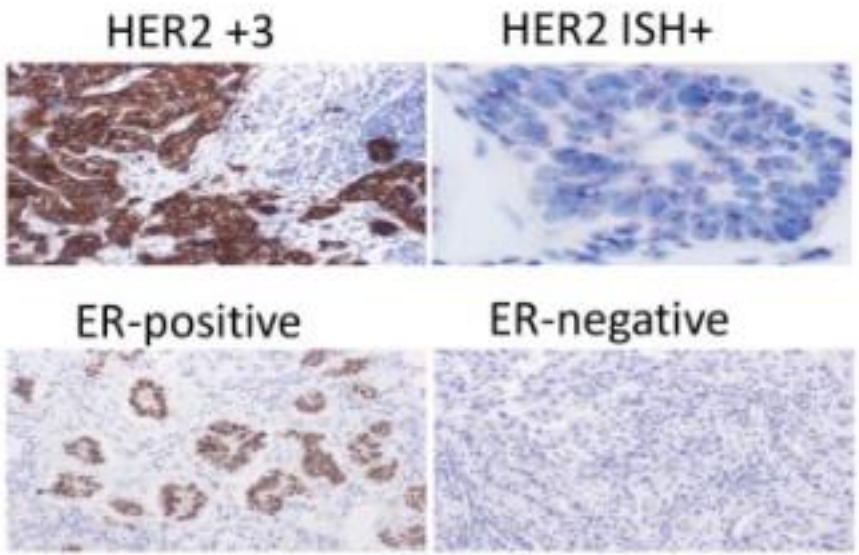
PAM50 intrinsic subtype as a predictor of pathological complete response following neoadjuvant dual HER2 blockade without chemotherapy in HER2-positive breast cancer: first results of the PAMELA clinical trial

Aleix Prat, Javier Cortés, Laia Paré, Patricia Galván, Mafalda Oliveira, Begoña Bermejo, Noelia Martínez, Maria Vidal, Sonia Pernas, Rafael López, Montserrat Muñoz, Paolo Nuciforo, Roberta Fasani, Serafin Morales, Lorena de la Peña, Alexandra Peláez and Antonio Llombart-Cussac, on behalf of SOLTI

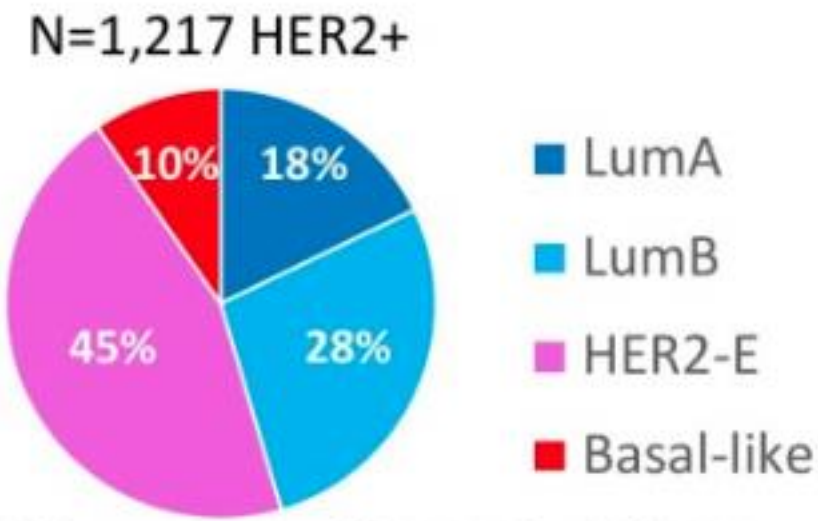
SOLTI

Background

- HER2-positive (HER2+) breast cancer is clinically and biologically heterogenous.
- Based on gene expression, HER2+ breast cancer is composed of 4 intrinsic molecular subtypes (**Luminal A**, **Luminal B**, **HER2-enriched [HER2-E]** and **Basal-like**) and a **Normal-like group**.
- These intrinsic subtypes are not fully recapitulated by hormone receptor status.



Courtesy of Dr. Pedro Fernández



TCGA Nature 2012; Prat CCR 2014; Prat JNCI 2014;
Ferrari Nat Com 2015; Carey JCO 2016; Fumagalli JAMA Oncol 2016

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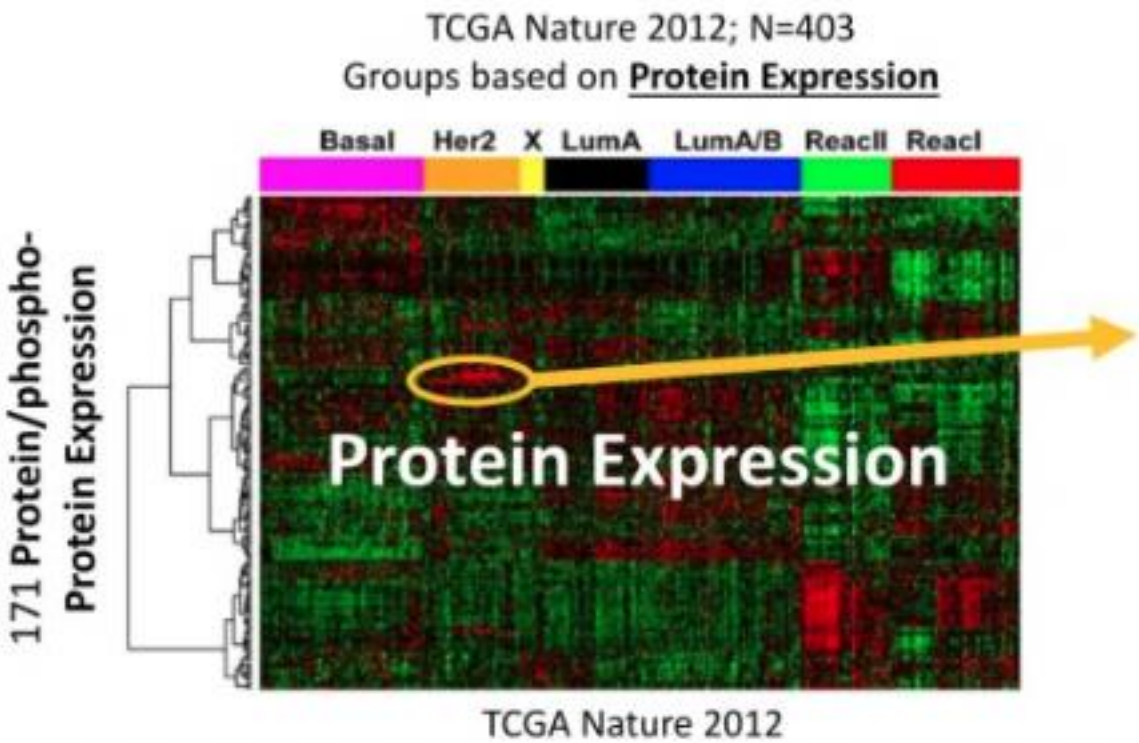
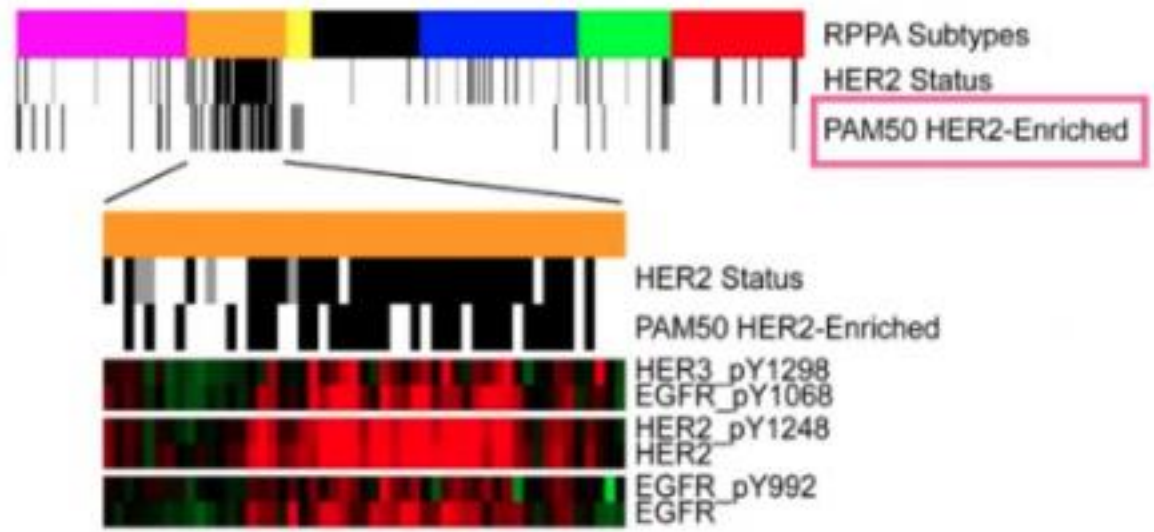
Background

- Among the different subtypes, the HER2-E is characterized by the highest expression of HER2/EGFR proteins and phospho(p)-HER2/p-EGFR.
- Thus, HER2+/HER2-E disease is likely to have the highest activation of the HER2/EGFR pathway.



TCGA Nature 2012



TCGA Nature 2012

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Hypothesis

- We hypothesized that the HER2+/HER2-E intrinsic subtype:
  - Benefits the most from dual HER2 blockade in the absence of chemotherapy.
  - Provides independent predictive information beyond hormone receptor (HR) status.

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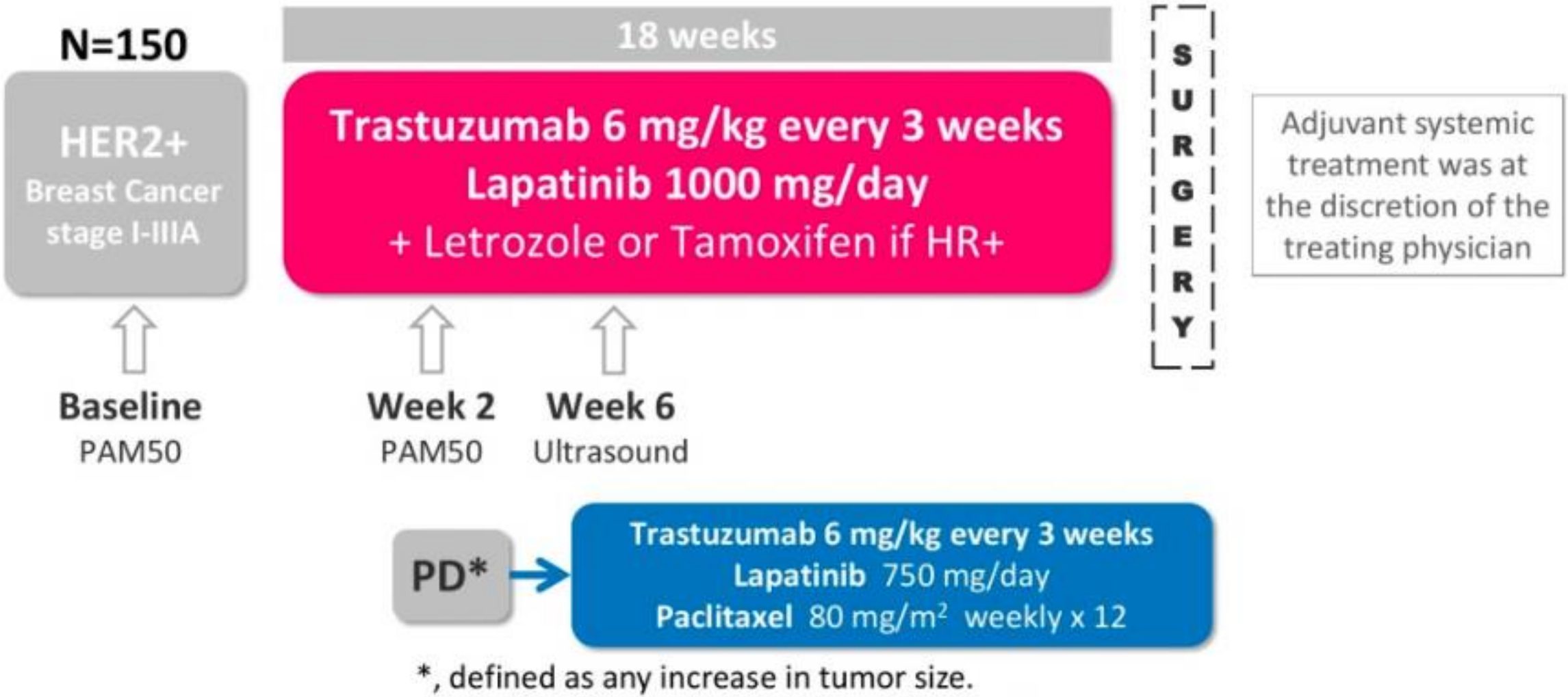
PAMELA trial schema



*, defined as any increase in tumor size.

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Primary and Secondary Objectives

- Primary objective:
  - To evaluate the ability of the **HER2-E** subtype to predict pathological complete response (pCR) in the breast (ypT_{0-is}) in all patients (ITT population) at the time of surgery.

- Secondary objectives included:
  - pCR in the breast and axilla ($ypT_{0-is}N0$).
  - Association of subtype at baseline with pCR beyond HR status.
  - Changes in subtype calling at baseline vs. week 2.
  - Association of subtypes identified at week 2 with pCR.
  - Safety.

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Statistical Design

- 150 patients were needed to provide 95% power to detect an absolute difference in pCR in the breast of 27% between the HER2-E and the **non-HER2-E subtypes** (including normal-like).
- Intrinsic subtype was identified in FFPE tumor samples using a research-based version of the PAM50 intrinsic subtype predictor on the nCounter platform (Prat et al. JAMA Oncol 2016).
- Identification of intrinsic subtype was performed blinded from clinical data.

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Main Eligibility Criteria

- Pre or post-menopausal patients.
- Stage I-IIIA breast cancer with primary tumors >1 cm in diameter.
- Adequate organ function.
- Performance status (WHO/ECOG scale) 0-2
- Baseline left ventricular ejection fraction of ≥50%.
- Centrally confirmed HER2 status (under ISO15189 accreditation).
- Centrally performed estrogen and progesterone receptors by immunohistochemistry (under ISO15189 accreditation).

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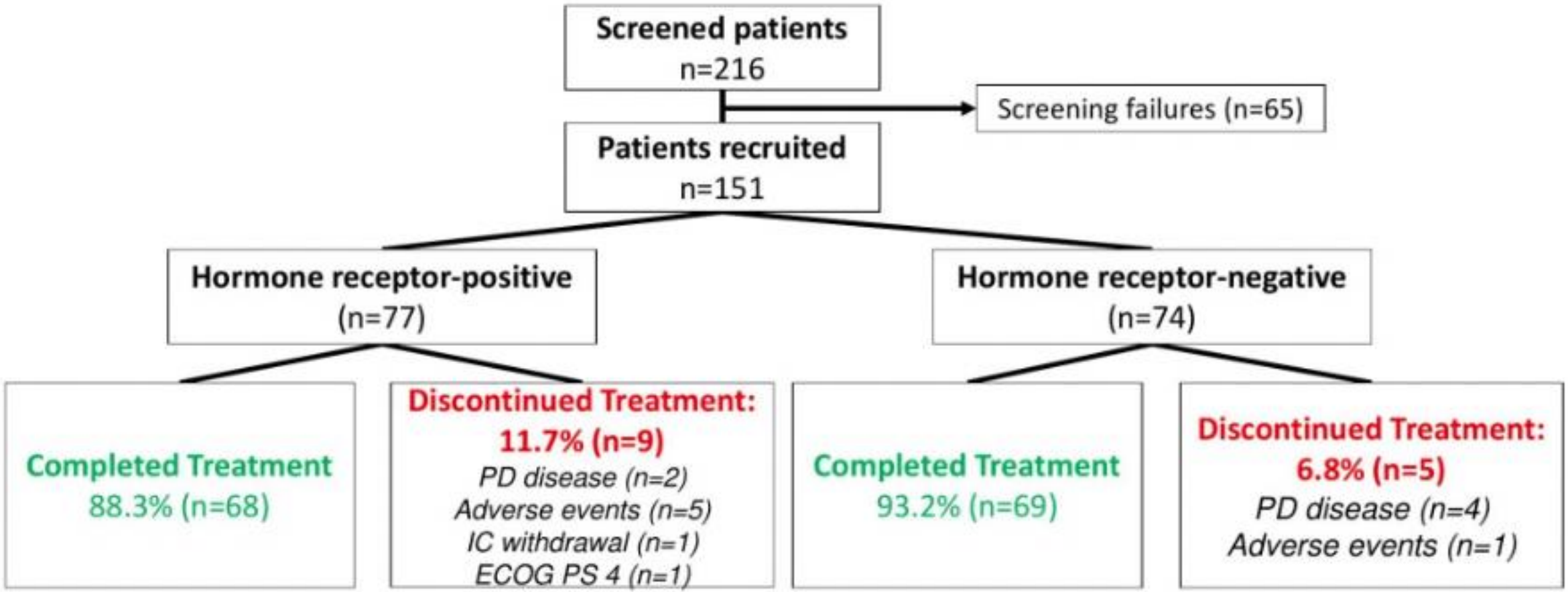
Study Flow Diagram

- **From October 2013 to October 2015:** 151 patients were recruited across 19 sites.



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Patient Demographics at Baseline

	N	%
N	151	-
Age, mean (range)	55 (29-86)	
Menopausal status		
Pre-menopausal	61	40.4%
Post-menopausal	90	59.6%
Tumor size (mm), median (range)	24 (10-110)	
Tumor stage		
T1	60	39.7%
T2	79	52.3%
T3	12	8%
Clinical nodal status		
N0	98	64.9%
N1	50	33.1%
N2	3	2%
Hormone receptor status		
Negative	74	49%
Positive	77	51%
Letrozole	*37*	*48%*
Tamoxifen	*40*	*52%*

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Safety

Characteristic	All grades	Grade 3	Grade 4
Diarrhea	244 (36.6%)	12 (1.8%)	-
Rash	173 (25.9%)	5 (0.7%)	-
Asthenia	53 (7.9%)	1 (0.2%)	-
Pain	52 (7.8%)		-
ALT/AST increased	38 (5.8%)	13 (1.9%)	1 (0.2%)

- No other Grade 4 toxicity was observed.
- Six patients (4%) discontinued study treatment due to side effects.

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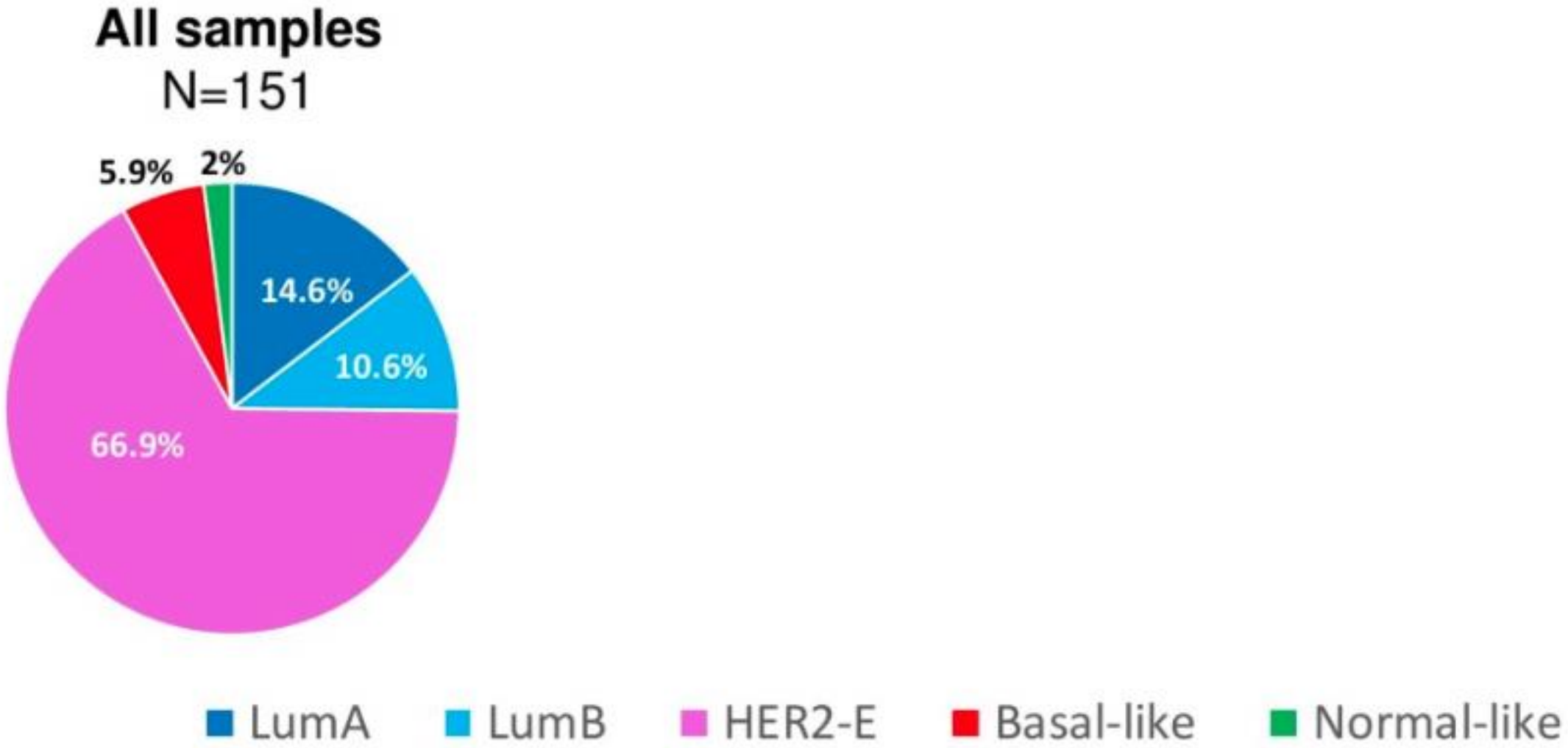
Intrinsic subtype distribution at baseline



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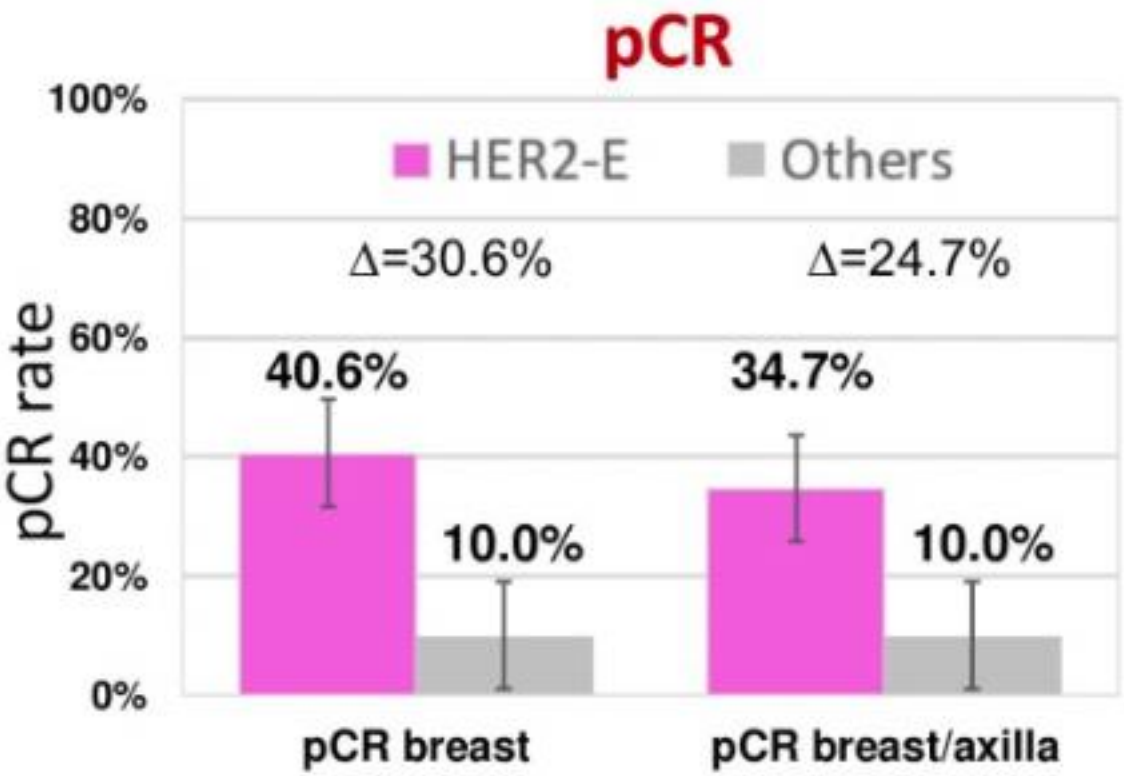
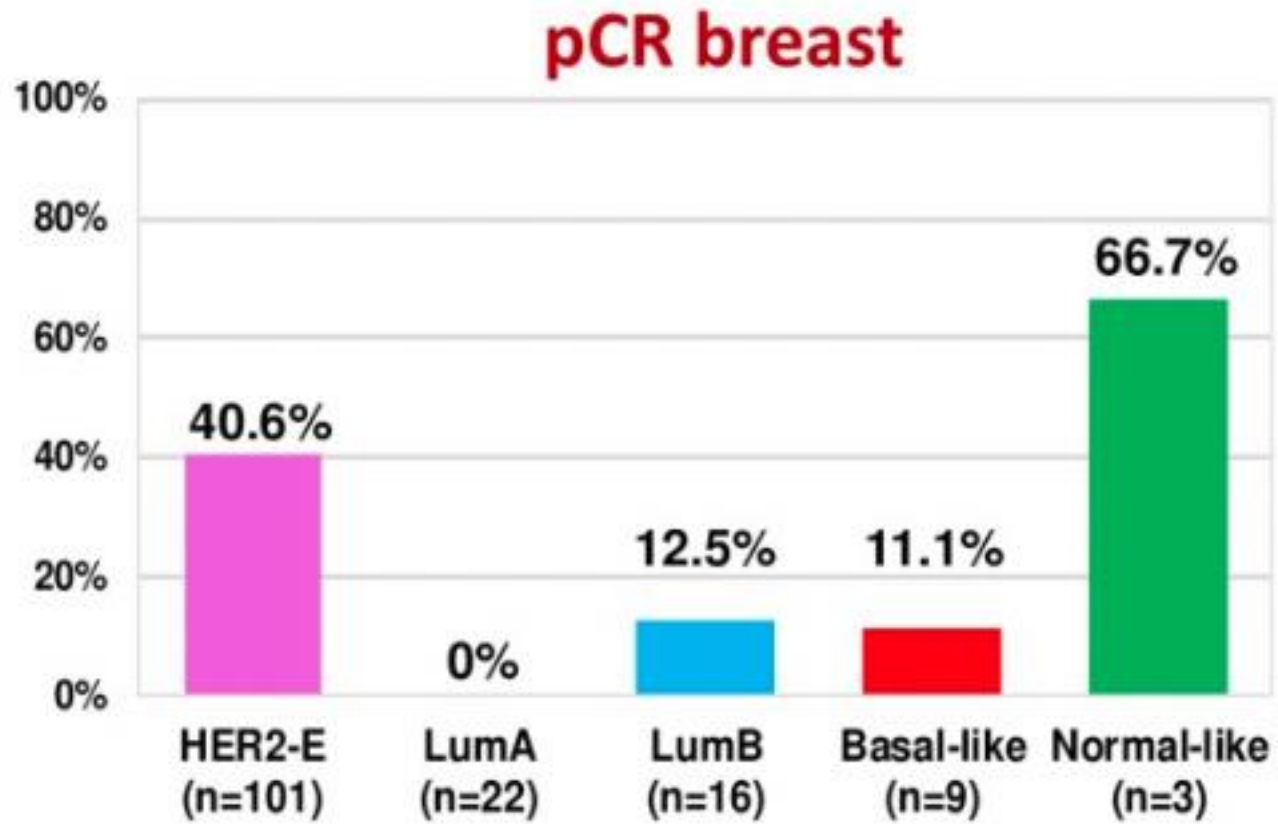
Intrinsic subtype at baseline vs. pCR

Baseline samples (N=151)





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Intrinsic subtype at baseline vs. pCR in the breast

Signatures	N	Breast pCR rate
HR status		
HR+	77	18.2%
HR-negative	74	43.2%
Intrinsic subtype		
nonHER2-E	50	10.0%
HER2-E	101	40.6%

- No other clinical-pathological variable was found associated with pCR.

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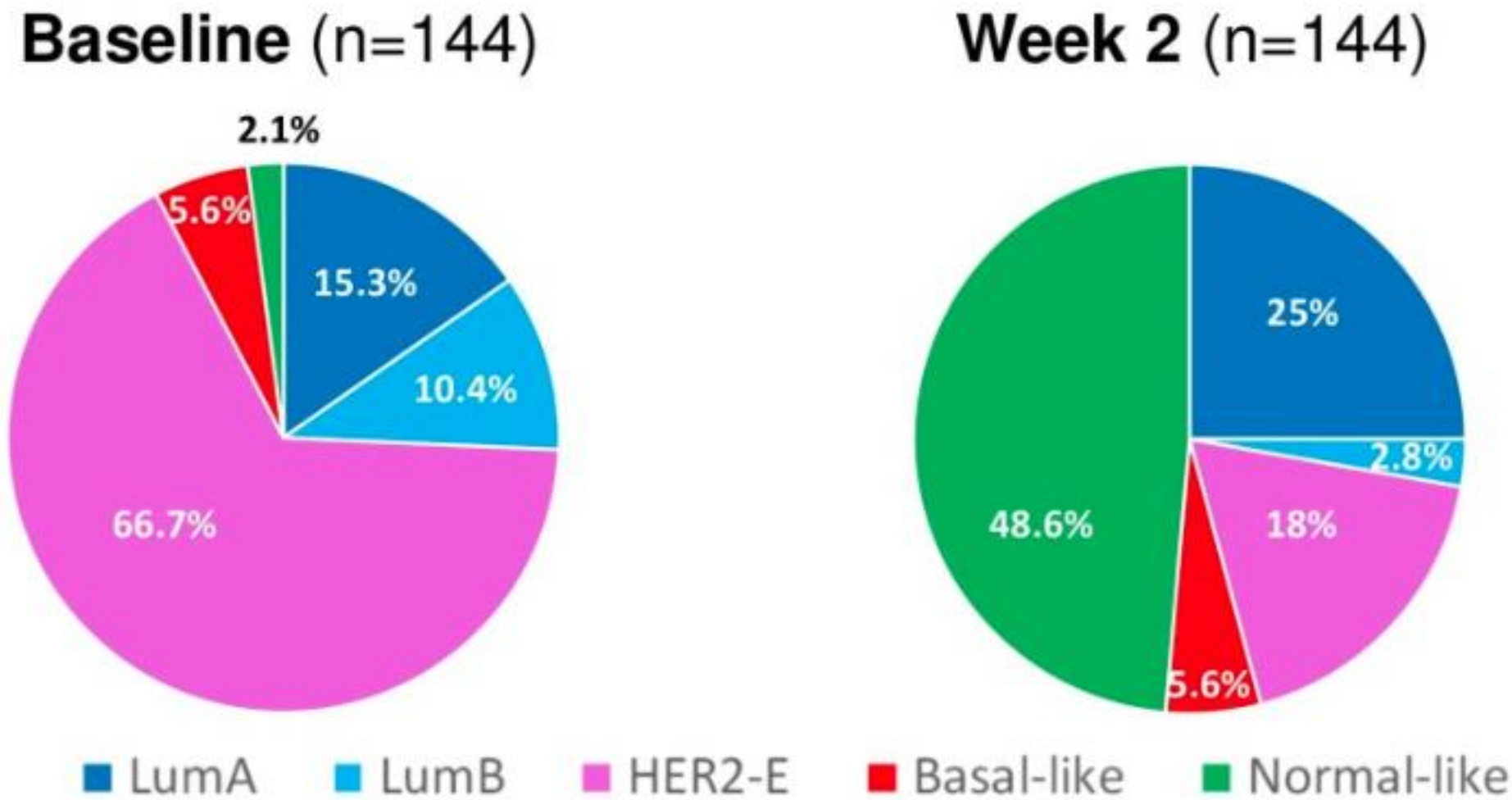
Intrinsic subtype distribution at baseline vs. week 2



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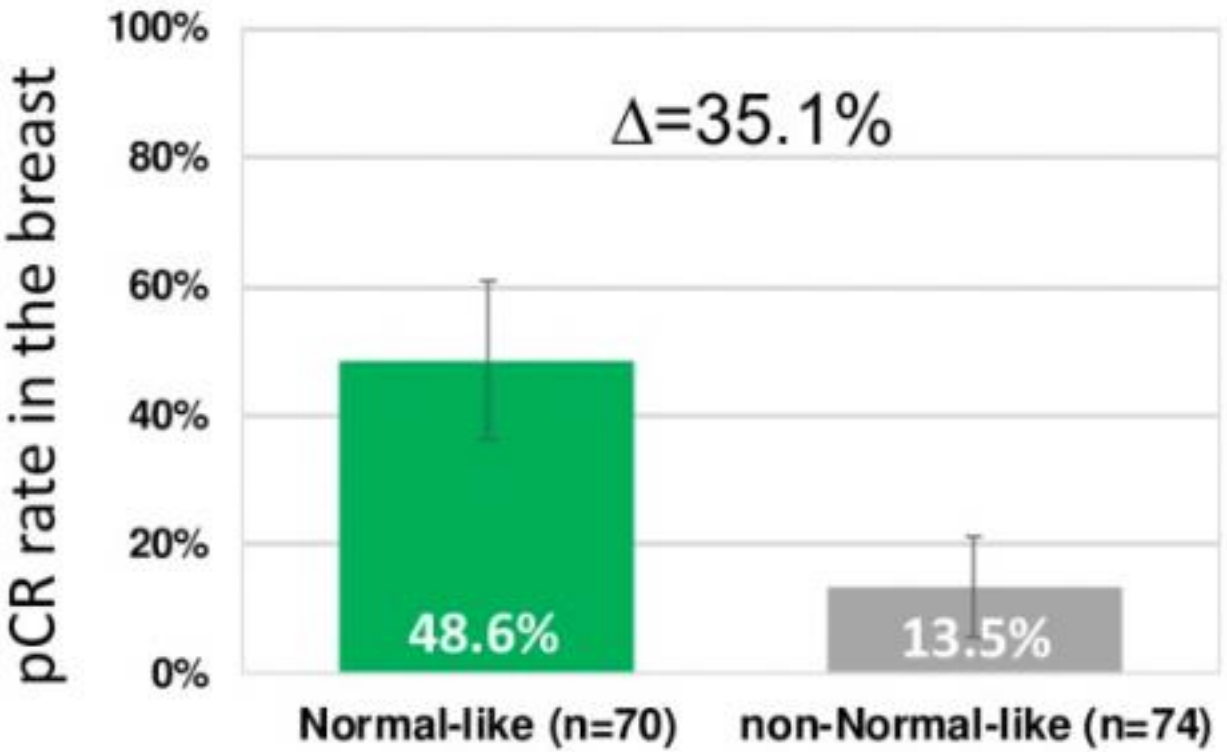
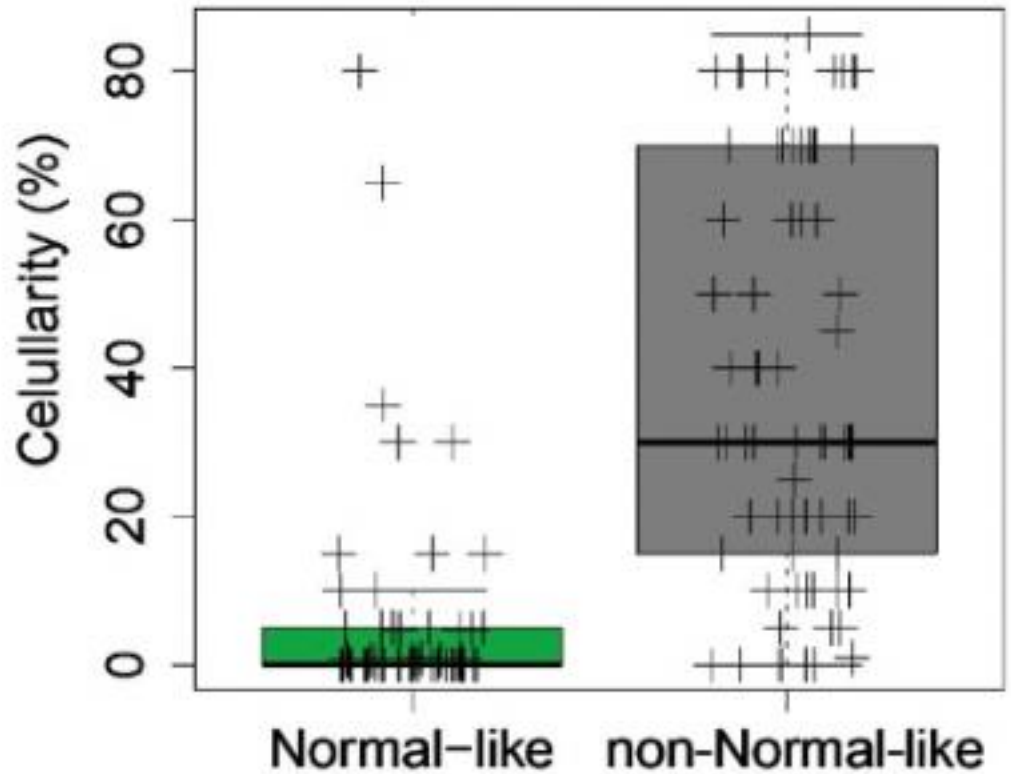
Intrinsic subtype at week 2 vs. pCR in the breast

Week 2 samples (N=144)





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Conclusions

- We prospectively confirmed that the **HER2-E subtype** is a strong predictor of sensitivity to dual HER2 blockade within HER2+ breast cancer in the absence of chemotherapy.
- PAM50 at baseline, and at week 2, provides independent information compared to HR status, which is the only molecular predictor to date consistently found associated with pCR in HER2+ disease.
- Studies evaluating the long-term survival outcomes of chemotherapy-free dual HER2 blockade are justified after selecting patients based on variables such as intrinsic subtyping.
- Further validation of PAM50, PIK3CA mutations, and PTEN-loss, is ongoing in collaboration with The Translational Breast Cancer Research Consortium (TBCRC) group.

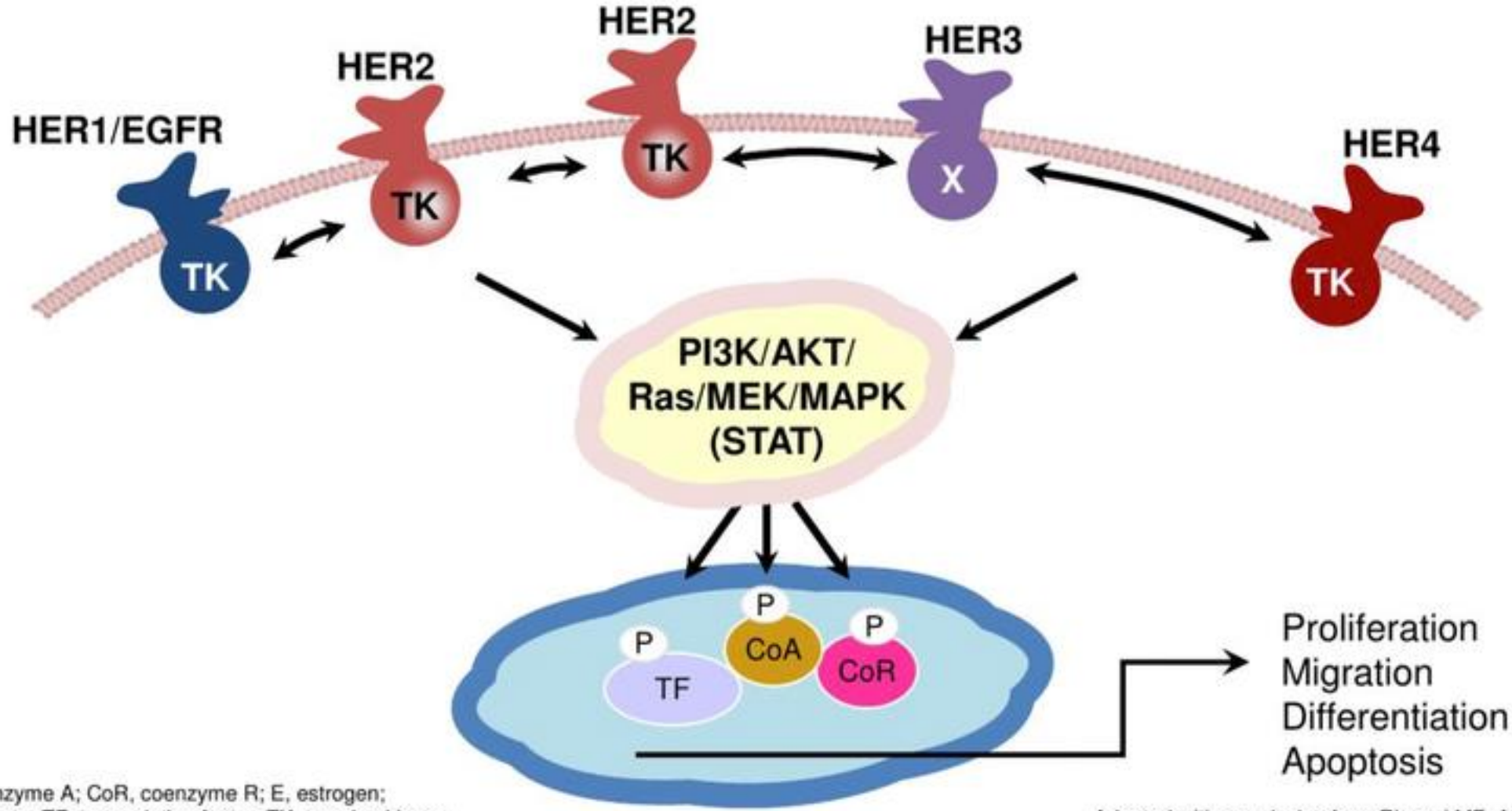
S3-04

Primary analysis of PERTAIN: A randomized, two-arm, open-label, multicenter phase II trial assessing the efficacy and safety of pertuzumab given in combination with trastuzumab plus an aromatase inhibitor in first-line patients with HER2-positive and hormone receptor-positive metastatic or locally advanced breast cancer

Mothaffar Rimawi,[1] Jean-Marc Ferrero,[2] Juan de la Haba-Rodriguez,[3] Valerie Easton,[4] Christine Schuhmacher,[4] Eleonora Restuccia,[4] and Grazia Arpino[5]

[1]Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX;
[2]Département d'Oncologie Médicale, Centre Antoine Lacassagne, Nice, France;
[3]Oncology Department, Maimonides Institute of Biomedical Research, Reina Sofía Hospital, University of Córdoba, Córdoba, Spain;
[4]F. Hoffmann-La Roche Ltd, Basel, Switzerland;
[5]Department of Clinical Medicine and Surgery, Università degli Studi di Napoli Federico II, Naples, Italy

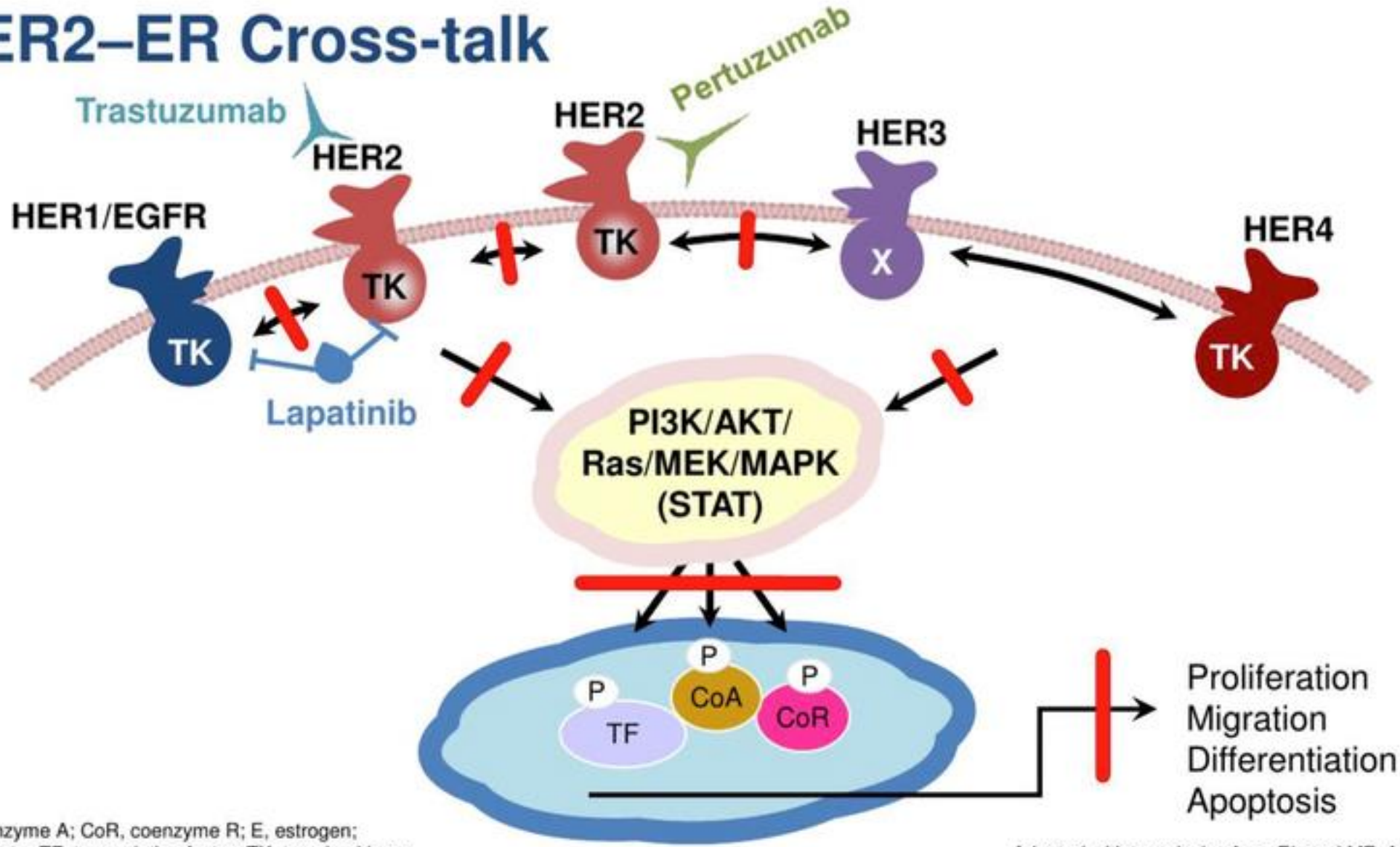
HER2–ER Cross-talk



CoA, coenzyme A; CoR, coenzyme R; E, estrogen; P, phosphate; TF, transcription factor; TK, tyrosine kinase.

Adapted with permission from Rimawi MF. ASCO 2015.

HER2–ER Cross-talk



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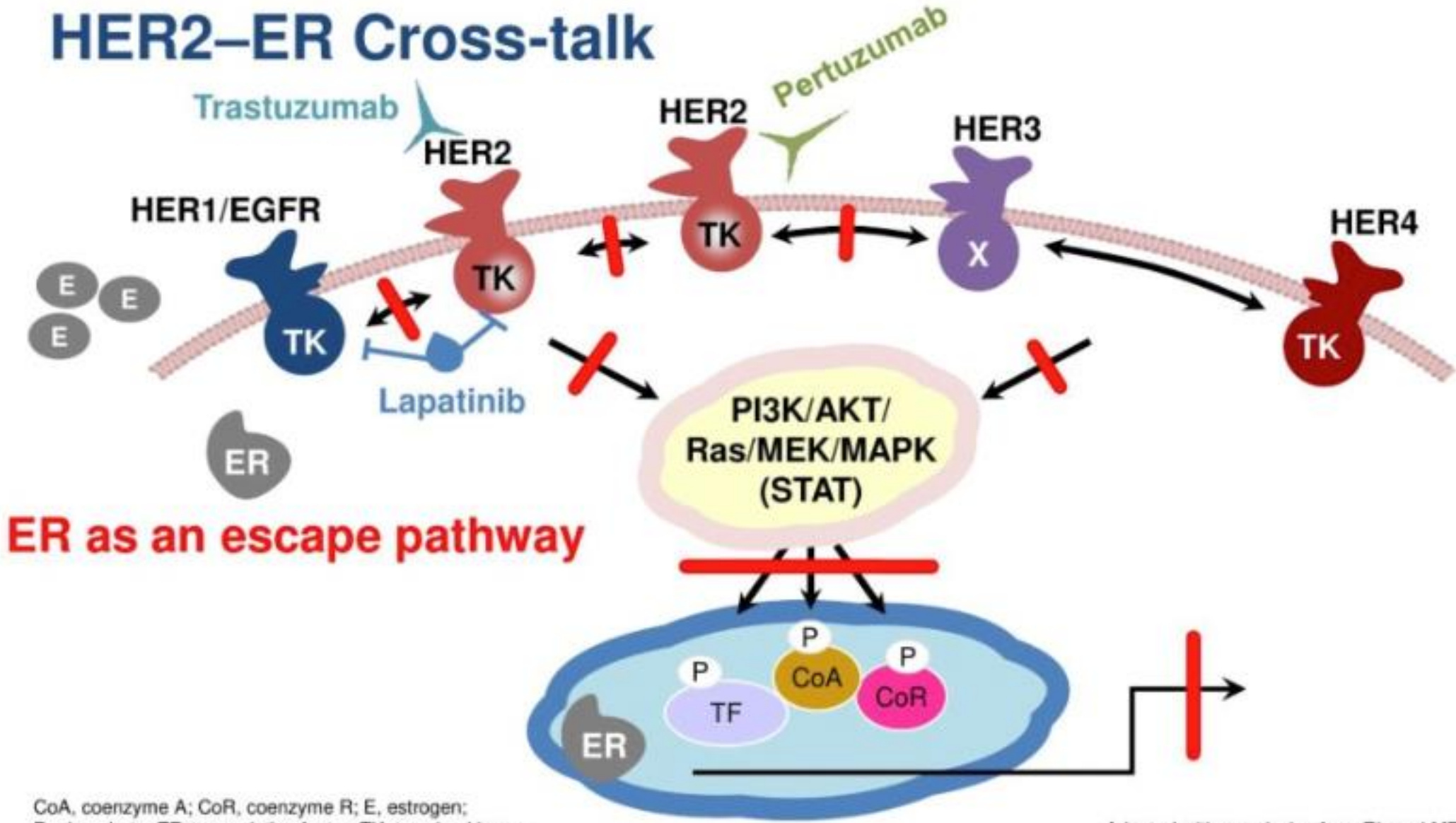
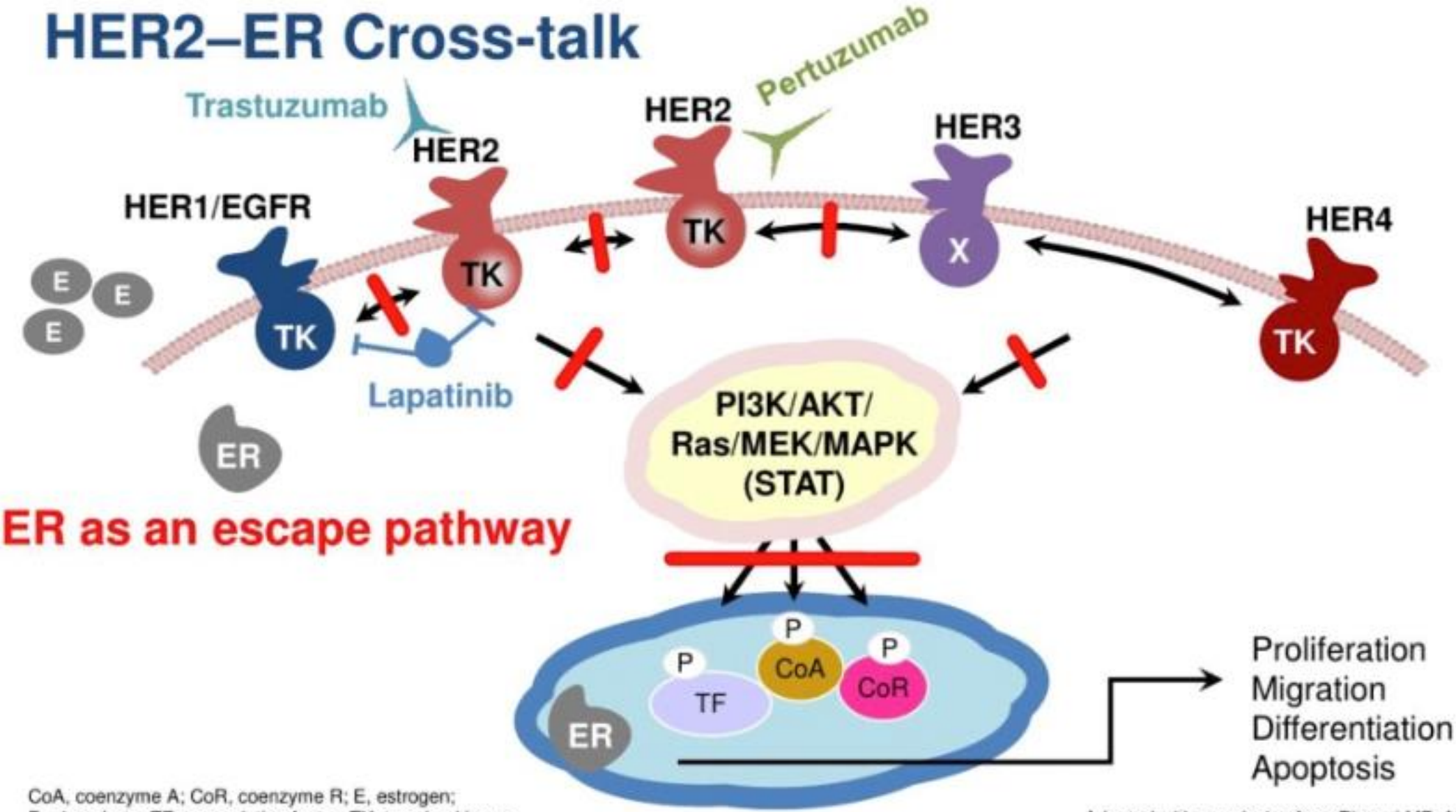


ER as an escape pathway

CoA, coenzyme A; CoR, coenzyme R; E, estrogen; P, phosphate; TF, transcription factor; TK, tyrosine kinase.

Adapted with permission from Rimawi MF. ASCO 2015.

HER2–ER Cross-talk



ER as an escape pathway

CoA, coenzyme A; CoR, coenzyme R; E, estrogen; P, phosphate; TF, transcription factor; TK, tyrosine kinase.

Adapted with permission from Rimawi MF. ASCO 2015.

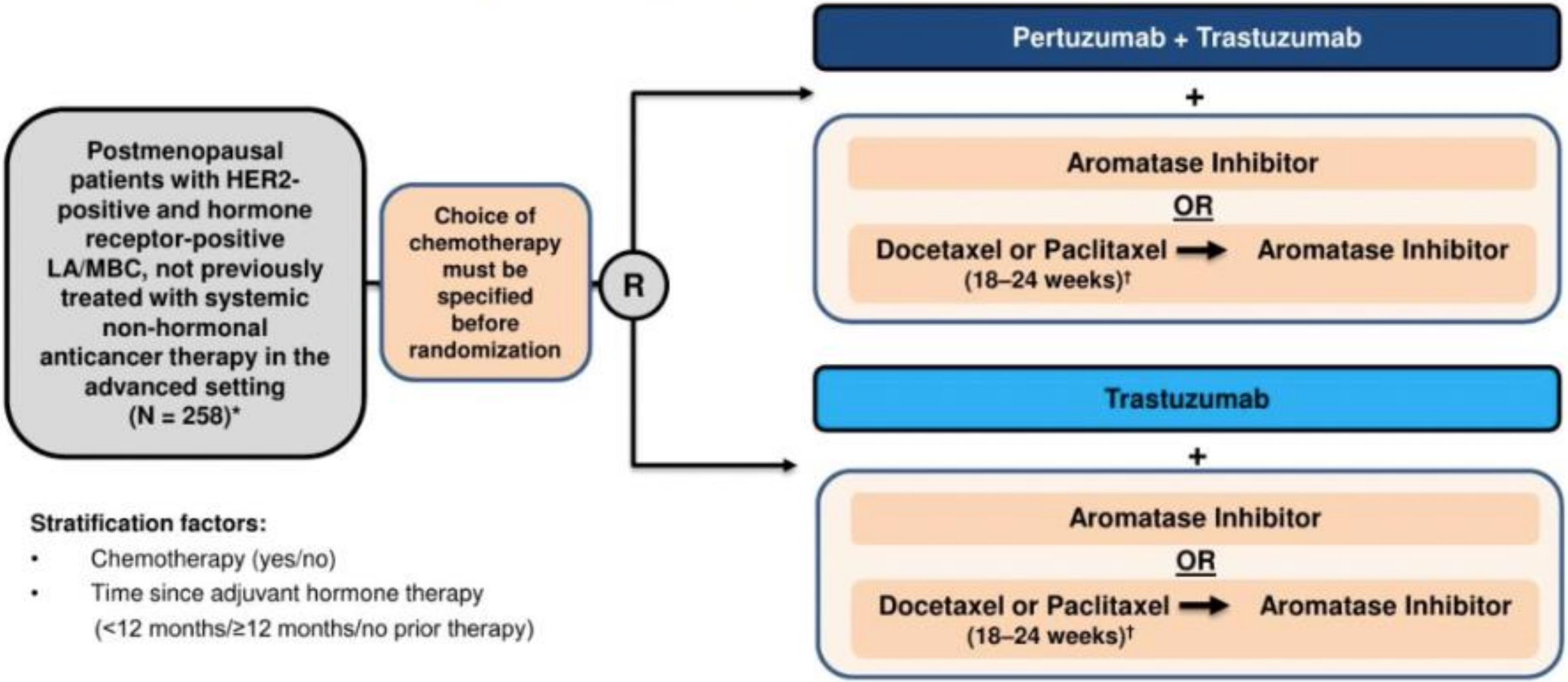
Background

- HER2–ER bidirectional cross-talk may contribute to resistance to hormonal and anti-HER2 therapies[1–6]
- TAnDEM: The addition of trastuzumab to anastrozole significantly improved PFS vs. anastrozole alone in HER2-positive/hormone receptor-positive MBC[7]
- CLEOPATRA: The addition of pertuzumab to trastuzumab + docetaxel significantly improved PFS and OS vs. trastuzumab + docetaxel in first-line, HER2-positive MBC[8,9]
- Pertuzumab + trastuzumab + AIs could therefore offer additional benefits

AI, aromatase inhibitor; MBC, metastatic breast cancer; OS, overall survival; PFS, progression-free survival.

1. Pietras RJ, *et al. Oncogene* 1995; **10**:2435–2446; 2. Arpino G, *et al. J Natl Cancer Inst* 2007; **99**:694–705; 3. Johnston S, *et al. J Clin Oncol* 2009; **27**:5538–5546; 4. Koeberle D, *et al. Endocr Relat Cancer* 2011; **18**:257–264; 5. Wang YC, *et al. Breast Cancer* Res 2011; **13**:R121; 6. Rimawi MF, *et al. Clin Cancer Res* 2011; **17**:1351–1361; 7. Kaufman B, *et al. J Clin Oncol* 2009; **27**:5529–5537; 8. Baselga J, *et al. N Engl J Med* 2012; **366**:109–119; 9. Swain SM, *et al. N Engl J Med* 2015; **372**:724–734.

PERTAIN Study Design (Phase II Trial)



Stratification factors:

- Chemotherapy (yes/no)
- Time since adjuvant hormone therapy (<12 months/≥12 months/no prior therapy)

* 165 events to detect significant improvement in PFS from 7 months to 10.8 months (i.e. HR 0.645) with 80% power and a 2-sided log-rank test at an alpha level of 0.05.
† Choice of chemotherapy must be specified before randomization; administered per product labelling. LA, locally advanced; R, randomization.

Study Endpoints

Primary endpoint

- **PFS**
  - Event-driven analysis
  - 165 events needed; 166 events observed; median follow-up 31 months

Secondary endpoints

- OS
  - Final analysis after a minimum follow-up of 60 months for all patients
- **ORR**
- CBR
- **DoR**
- Time to response
- **Safety and tolerability**
- QoL

CBR, clinical benefit rate; DoR, duration of response; ORR, overall response rate; QoL, quality of life.

Eligibility Criteria

Inclusion Criteria

- Postmenopausal (fulfilling ≥1 NCCN criteria[1])
- First-line patients with hormone receptor-positive and HER2-positive LA/MBC as per local laboratory assessment
- ≥1 measurable lesion and/or non-measurable disease (per RECIST Version 1.1[2])
- ECOG PS 0 or 1
- LVEF ≥50%
- Life expectancy ≥12 weeks

Exclusion Criteria

- Prior systemic non-hormonal anticancer therapy for MBC
- DFI <6 months from completion of (neo)adjuvant systemic non-hormonal treatment
- Anti-HER2 agents for BC, except trastuzumab and/or lapatinib in the (neo)adjuvant setting
- PD during trastuzumab and/or lapatinib in the adjuvant setting
- Patients with uncontrolled CNS metastases

CNS, central nervous system; DFI, disease-free interval; ECOG PS, Eastern Cooperative Oncology Group performance status; LVEF, left ventricular ejection fraction; NCCN, National Comprehensive Cancer Network; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors.

1. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Breast Cancer. Version 2.2011;
2. Eisenhauer EA, *et al. Eur J Cancer* 2009; **45**:228–247.

Demographics and Baseline Characteristics (ITT Population)

	Pertuzumab + Trastuzumab + AI (n = 129)	Trastuzumab + AI (n = 129)
Median age, years (min, max)	59.0 (35, 87)	61.0 (31, 89)
Age by category, n (%)		
<65 years	86 (66.7)	86 (66.7)
≥65 years	43 (33.3)	43 (33.3)
Region, n (%)		
Asia	10 (7.8)	16 (12.4)
Europe	82 (63.6)	70 (54.3)
North America	18 (14.0)	22 (17.1)
South America	19 (14.7)	21 (16.3)
ECOG PS, n (%)*		
0	85 (65.9)	89 (69.0)
1	43 (33.3)	39 (30.2)

* Missing: n = 1 in each arm; both patients were randomized but not treated.

ITT, intention-to-treat.

Previous Systemic Therapy for Breast Cancer (ITT Population)

	Pertuzumab + Trastuzumab + AI (n = 129)	Trastuzumab + AI (n = 129)
Previous systemic therapy for BC, n (%)*	67 (51.9)	67 (51.9)
Chemotherapy, n (%)		
Neoadjuvant	20 (15.5)	18 (14.0)
Adjuvant	51 (39.5)	41 (31.8)
Anthracyclines	53 (41.1)	36 (27.9)
Taxanes	33 (25.6)	36 (27.9)
Trastuzumab, n (%)		
Neoadjuvant	10 (7.8)	8 (6.2)
Adjuvant	30 (23.3)	24 (18.6)
Hormonal therapy, n (%)		
Neoadjuvant	1 (0.8)	1 (0.8)
Adjuvant	54 (41.9)	51 (39.5)
Other†	2 (1.6)	4 (3.1)

Patients could be counted under >1 treatment setting, e.g. neoadjuvant/adjuvant if they received >1 treatment with a different purpose. * Includes previous lapatinib (n = 1 in each arm) and bevacizumab (n = 1 in Arm A).† Metastatic disease (n = 3), bone metastasis (n = 1), first-line metastatic (n = 1), cancer treatment (n = 1).

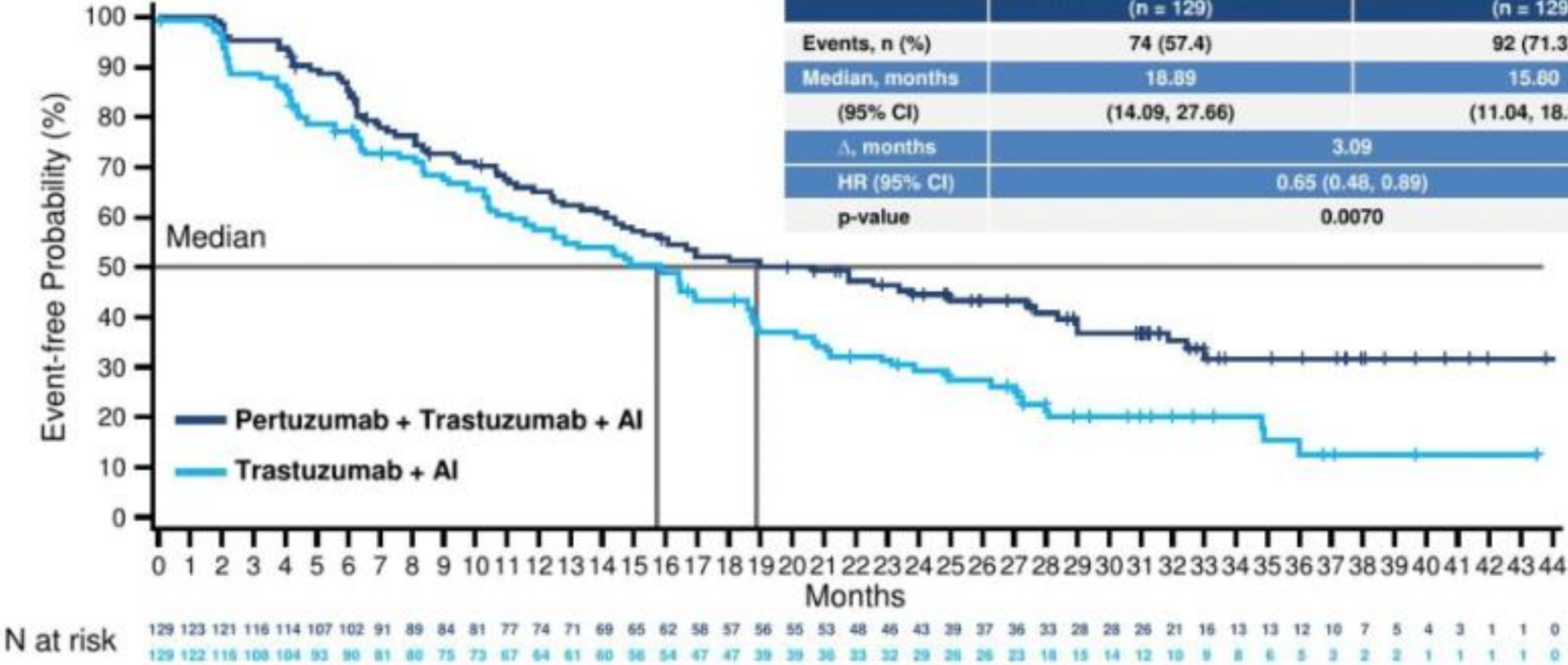
Baseline Disease Status and Induction Chemotherapy (ITT Population)

	Pertuzumab + Trastuzumab + AI (n = 129)	Trastuzumab + AI (n = 129)
LA/MBC at study entry, n (%)		
LABC	8 (6.2)	7 (5.4)
MBC	121 (93.8)	122 (94.6)
Disease type at screening, n (%)*		
Visceral	94 (72.9)	88 (68.2)
Non-visceral	35 (27.1)	41 (31.8)
Number of organs involved, n (%)*		
≥3	42 (32.6)	44 (34.1)
<3	87 (67.4)	85 (65.9)
Induction chemotherapy, n (%)		
Yes	75 (58.1)	71 (55.0)
No	54 (41.9)	58 (45.0)

* Based on baseline tumor assessment (target and non-target lesions).

Primary Progression-Free Survival Analysis (Stratified, ITT Population)

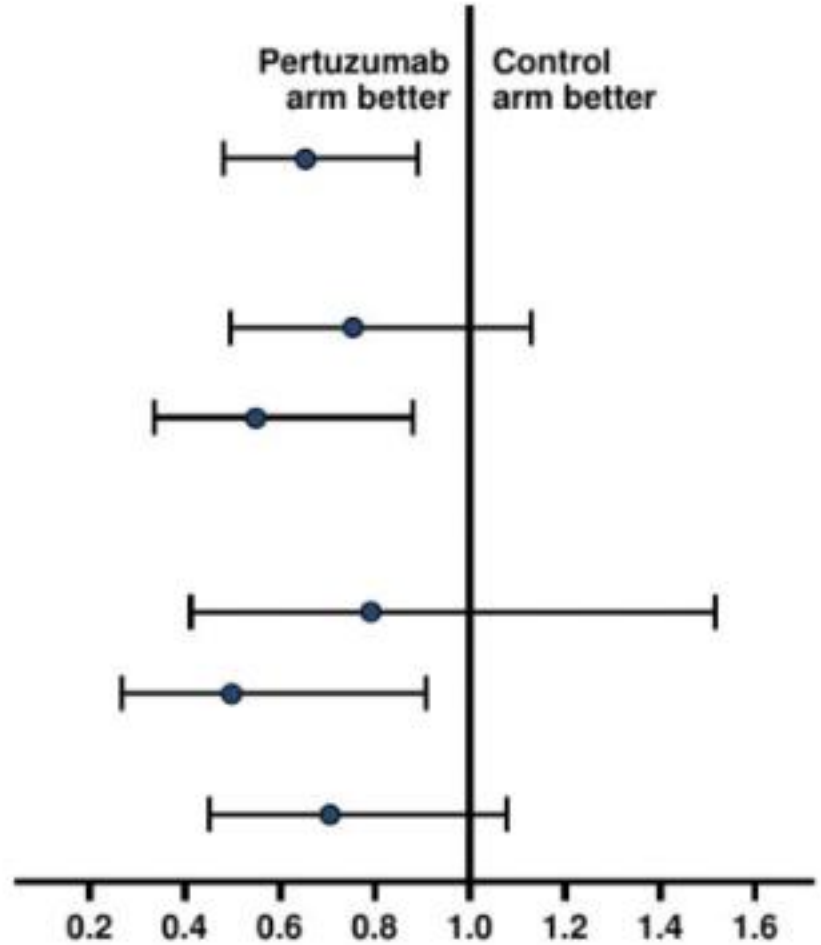
	Pertuzumab + Trastuzumab + AI (n = 129)	Trastuzumab + AI (n = 129)
Events, n (%)	74 (57.4)	92 (71.3)
Median, months	18.89	15.80
(95% CI)	(14.09, 27.66)	(11.04, 18.56)
Δ, months	3.09	
HR (95% CI)	0.65 (0.48, 0.89)	
p-value	0.0070	



Analysis based upon Kaplan–Meier approach including stratification factors from IXRS. HR from a stratified Cox proportional hazards model including stratification factors from IXRS. Median time of follow-up: 31 months. CI, confidence interval; HR, hazard ratio.

Progression-Free Survival by Stratification Subgroups

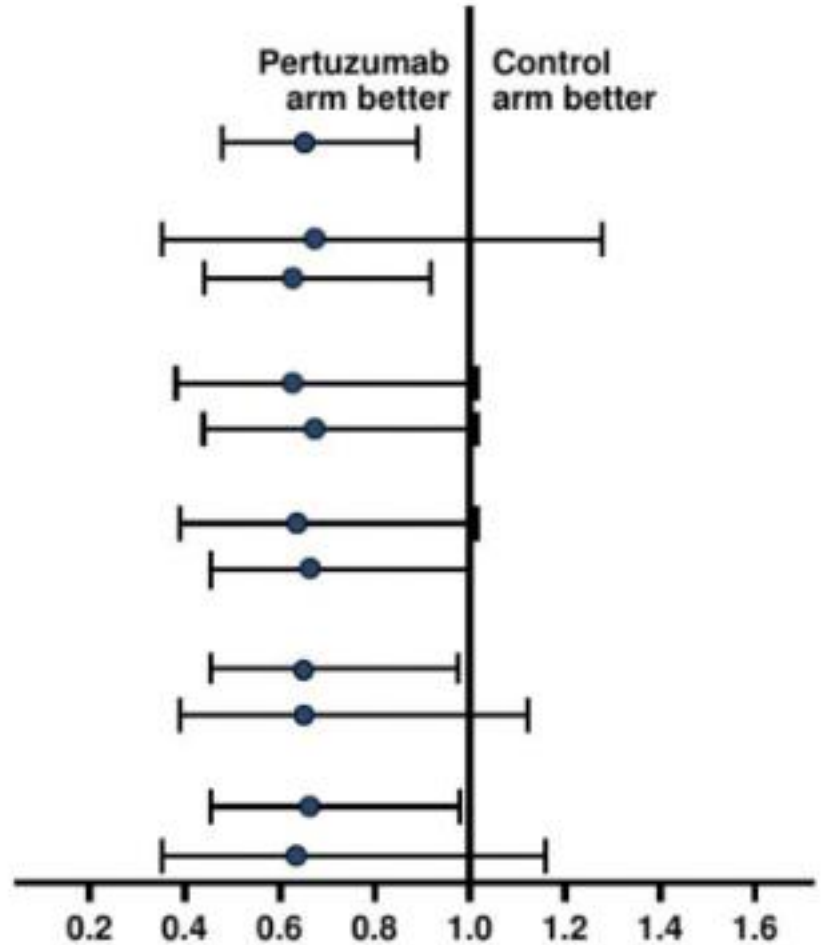
Subgroup	n	Events	HR*	(95% CI)
ITT population	258	166	**0.66**	(0.48, 0.89)
Chosen to receive induction chemotherapy				
Yes	148	94	**0.75**	(0.50, 1.13)
No	110	72	**0.55**	(0.34, 0.88)
Time since adjuvant hormone therapy				
<12 months	48	37	**0.79**	(0.42, 1.52)
≥12 months	84	45	**0.50**	(0.27, 0.91)
No prior hormone therapy	126	84	**0.71**	(0.46, 1.09)



* HR for pertuzumab arm vs. control arm (control arm, reference category) from an unstratified Cox model.

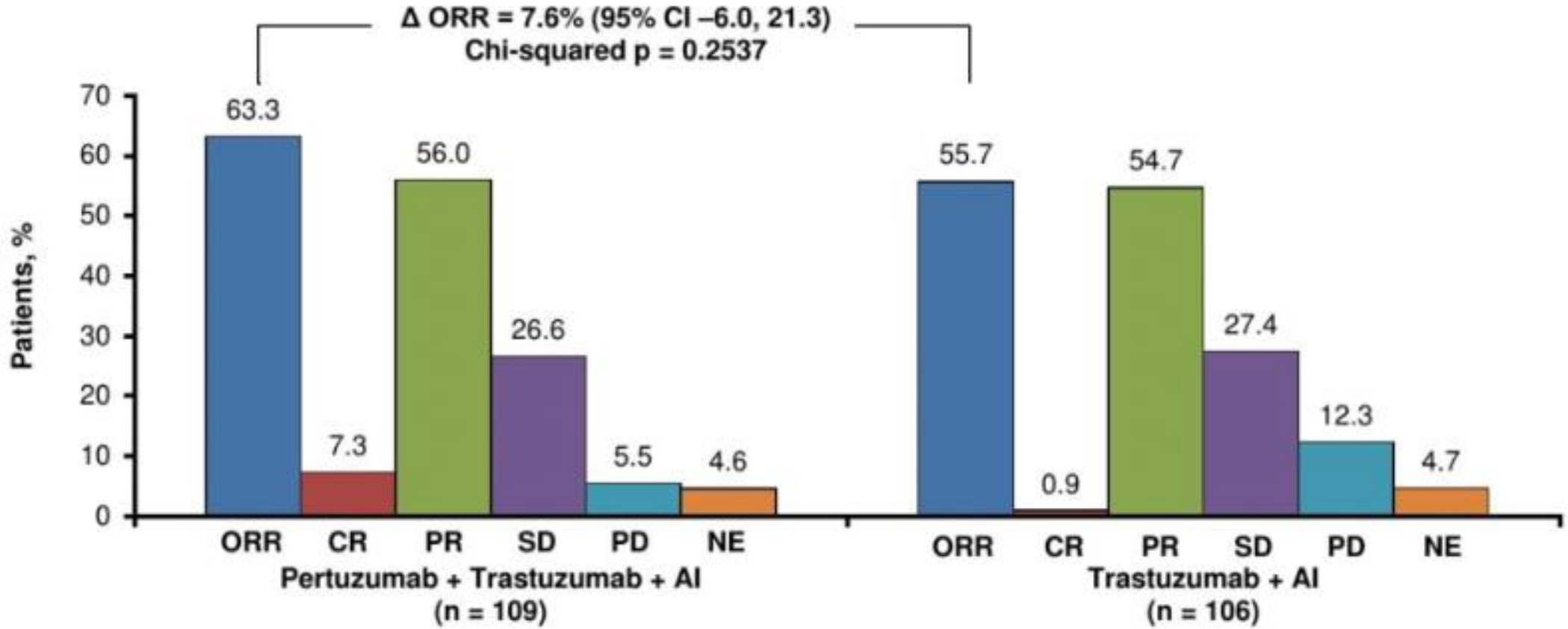
Progression-Free Survival by Baseline Subgroups

Subgroup	n	Events	HR*	(95% CI)
ITT population	258	166	**0.66**	(0.48, 0.89)
Prior (neo)adjuvant treatment with trastuzumab				
Yes	59	40	**0.68**	(0.37, 1.27)
No	199	126	**0.64**	(0.45, 0.92)
Prior (neo) adjuvant chemotherapy				
Yes	115	71	**0.64**	(0.40, 1.02)
No	143	95	**0.67**	(0.44, 1.02)
Prior hormone therapy†				
Yes	110	70	**0.64**	(0.40, 1.02)
No	148	96	**0.67**	(0.44, 1.00)
Age category				
<65 years	172	108	**0.66**	(0.45, 0.97)
≥65 years	86	58	**0.66**	(0.39, 1.12)
Disease type at screening				
Visceral	182	118	**0.67**	(0.47, 0.97)
Non-visceral	76	48	**0.65**	(0.36, 1.16)



* HR for pertuzumab arm vs. control arm (control arm, reference category) from an unstratified Cox model.
† Includes treatment in the neoadjuvant, adjuvant, and other settings.

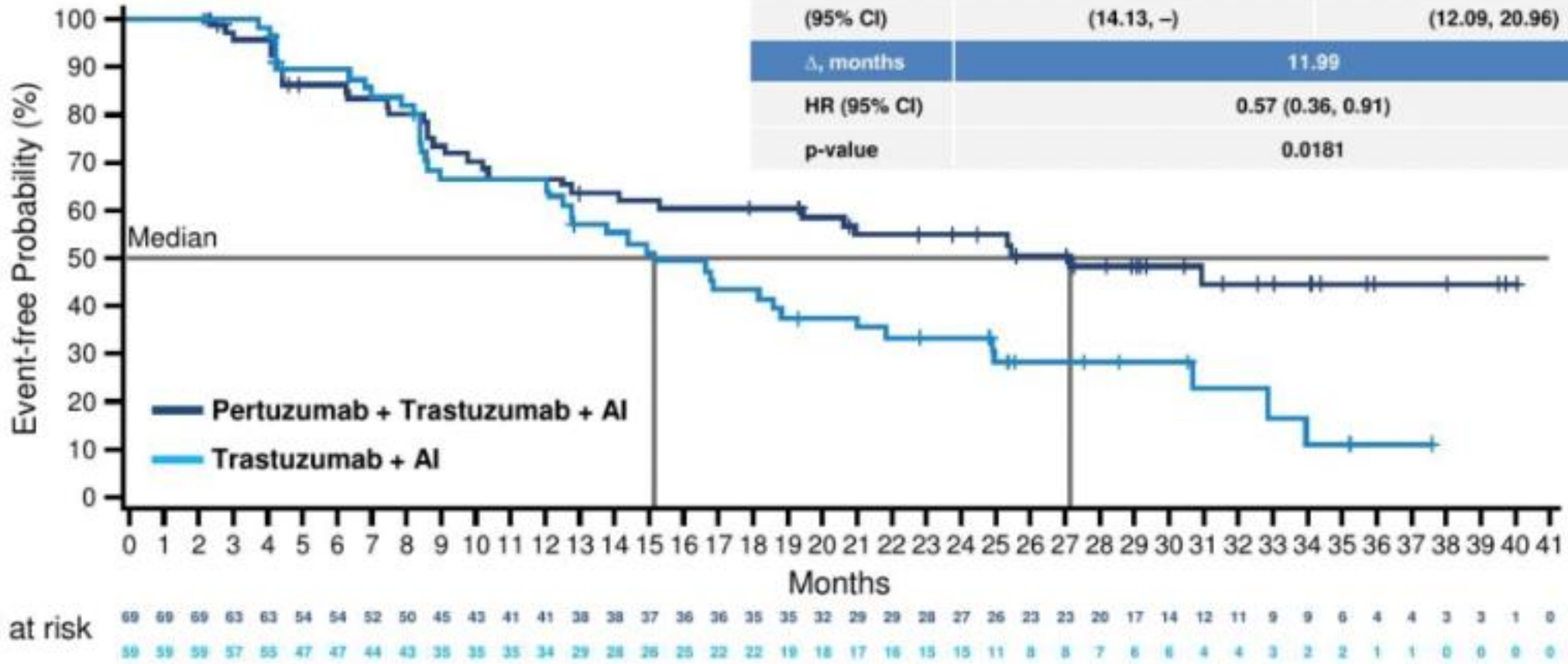
Overall Response Rate (ITT Population with Measurable Disease at Baseline)



Based on best overall response according to RECIST Version 1.1. NE: Patients who did not have any evaluable post-baseline assessments.
95% CIs were computed using the Clopper–Pearson approach. 95% difference in ORR between treatment arms with associated 95% CIs calculated using the Hauck–Anderson approach.
CR, complete response; NE, not evaluable; PR, partial response; SD, stable disease.

Duration of Response (Unstratified, ITT Responders)

	Pertuzumab + Trastuzumab + AI (n = 69)	Trastuzumab + AI (n = 59)
Median, months	27.10	15.11
(95% CI)	(14.13, –)	(12.09, 20.96)
Δ, months	11.99	
HR (95% CI)	0.57 (0.36, 0.91)	
p-value	0.0181	



Unstratified analysis based upon Kaplan–Meier approach. HR from a stratified Cox proportional hazards model including stratification factors from IXRS.

Adverse Events (Safety Population)

	Pertuzumab + Trastuzumab + AI (n = 127)	Trastuzumab + AI (n = 124)
Any AE	122 (96.1)	122 (98.4)
NCI-CTCAE grade ≥3 AE	64 (50.4)	48 (38.7)
Serious AE	42 (33.1)	24 (19.4)
AE leading to discontinuation of pertuzumab	13 (10.2)	NA
AE leading to interruption of pertuzumab	34 (26.8)	NA

Data are number of patients, n (%).
There were no deaths due to AEs.
AE, adverse event; NA, not applicable; NCI-CTCAE, National Cancer Institute – Common Terminology Criteria for Adverse Events.

Most Common Adverse Events (Incidence ≥20%; Safety Population)

	Pertuzumab + Trastuzumab + AI (n = 127)	Trastuzumab + AI (n = 124)
Diarrhea	70 (55.1)	45 (36.3)
Alopecia	36 (28.3)	40 (32.3)
Nausea	41 (32.3)	32 (25.8)
Asthenia	39 (30.7)	31 (25.0)
Arthralgia	37 (29.1)	29 (23.4)
Edema peripheral	31 (24.4)	22 (17.7)
Vomiting	29 (22.8)	22 (17.7)
Anemia	26 (20.5)	18 (14.5)

Data are number of patients, n (%).

Worst LVEF While on Treatment (Safety Population)

LVEF	Pertuzumab + Trastuzumab + AI (n = 127)	Trastuzumab + AI (n = 124)
>45%	110 (86.6)	112 (90.3)
40–45% and ≥10% fall from baseline*	6 (4.7)	4 (3.2)
<40%	5 (3.9)	3 (2.4)
No LVEF measurement on treatment†	6 (4.7)	5 (4.0)

Data are number of patients, n (%).

Local assessment by ECHO or MUGA; change from baseline was only calculated where the type of scan was the same as at baseline.

* Seven patients had an LVEF of exactly 45%.

† Eight patients discontinued before post-baseline LVEF assessment was due, two patients discontinued and left the study before LVEF was completed, one patient discontinued and a post-baseline assessment was not done (site error).

Conclusions

- **PERTAIN met its primary PFS objective:**

  Pertuzumab + Trastuzumab + AI was superior to Trastuzumab + AI in postmenopausal women with HER2-positive/hormone receptor-positive LA/MBC

- Secondary efficacy endpoints (ORR and DoR) supported the primary PFS analysis
- Subgroup analyses were generally consistent with the primary analysis
- Pertuzumab + Trastuzumab + AI was well tolerated and no new safety signals were identified

S3-05

Integrated analysis of multidimensional genomic data on CALGB 40601 (Alliance), a randomized neoadjuvant phase III trial of weekly paclitaxel (T) and trastuzumab (H) with or without lapatinib (L) for HER2-positive breast cancer

Maki Tanioka, Cheng Fan, Lisa A. Carey, Terry Hyslop, Brandelyn Pitcher, Joel S. Parker, Katherine Hoadley, N. Lynn Henry, Sara Tolaney, Chau Dang, Ian E. Krop, Lyndsay Harris, Donald A. Berry, Elaine Mardis, Charles M. Perou, Eric P Winer, Clifford A Hudis

Lineberger Comprehensive Cancer Center

The University of North Carolina at Chapel Hill

UNC LINEBERGER

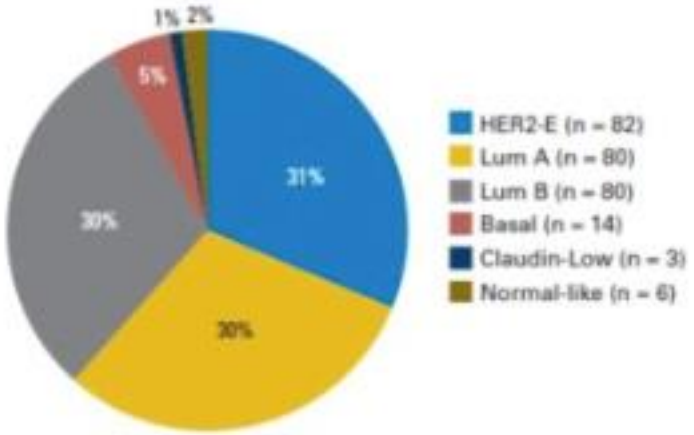
ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

CALGB 40601

Clinical Stage II - III
HER2+
N=305

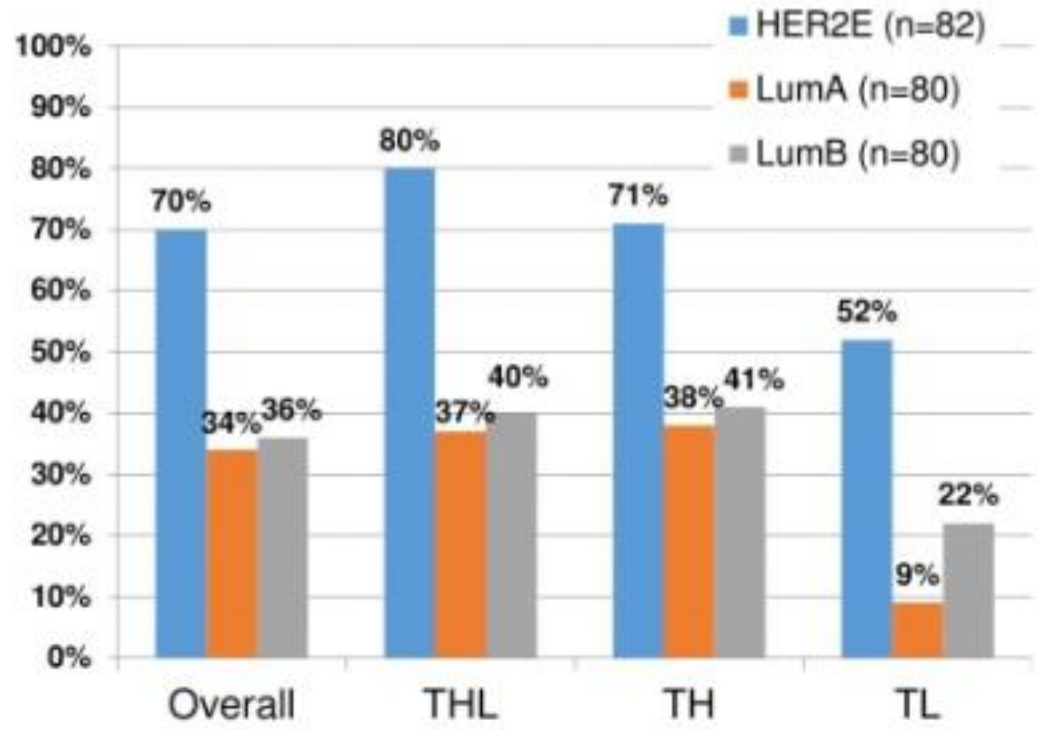
- Paclitaxel + Trastuzumab x 16wk
- Paclitaxel + Lapatinib x 16wk
- Paclitaxel + Trastuzumab + Lapatinib x 16wk

→ SURGERY



Pretreatment intrinsic subtype frequency

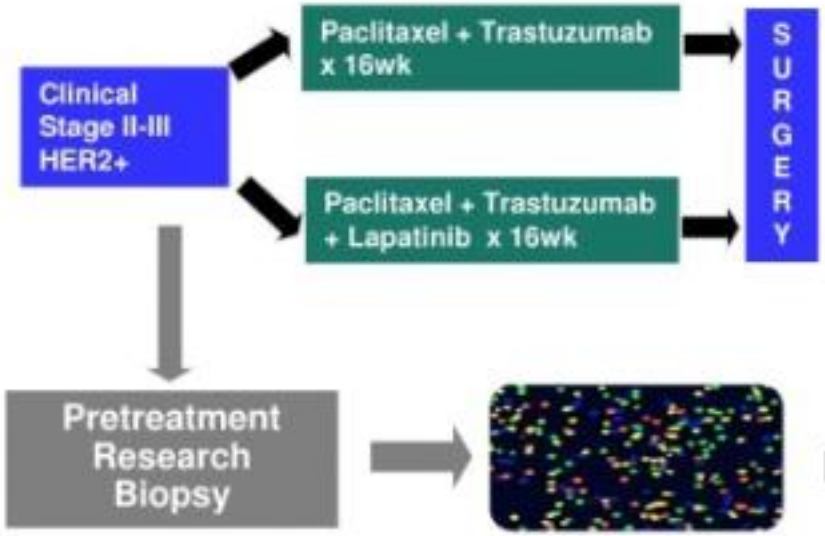
pCR rates according to Intrinsic Subtypes



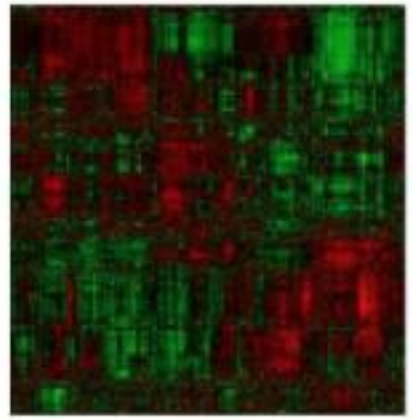
pCR was defined as no invasive tumor in the breast

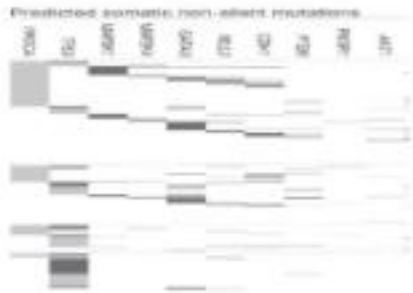
Carey et al., JCO 2015 (PMID:26527775)

Genomic Methods for predicting pCR



Illumina HiSeq sequencing from RNAseq + DNA Exomes





The unique feature of our analyses is the integration of RNA and DNA data to predict pCR.

Our analyses include Elastic Net and DawnRank.

mRNA Gene Expression:
518 gene signatures (GS) representing multiple biological pathways and cell types#

mRNA-seq data quantitated using RSEM

#Fan C, et al. BMC Med Genomics, 2011

DNA Copy Number (CN) from Exomes:
515 genomic segment-level features from 473 cancer-specific segments and 42 chromosome arm features.

CN segment values were determined using Synthex*

*https://github.com/ChenMengjie/SynthEx

Somatic Mutations:
12 genes with mutations in more than 10 patients (>6%), or only TP53 and PIK3CA.

UNCeqR: an integrated DNA & RNA mutation caller**

**Wilkerson MD, et al. Nucleic Acids Res, 2014

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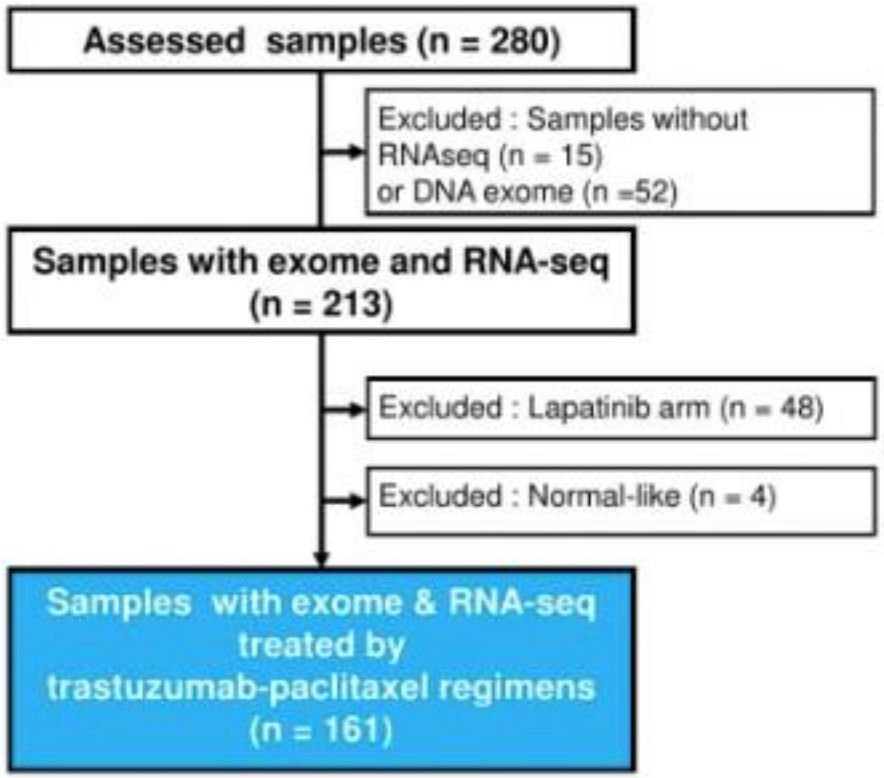
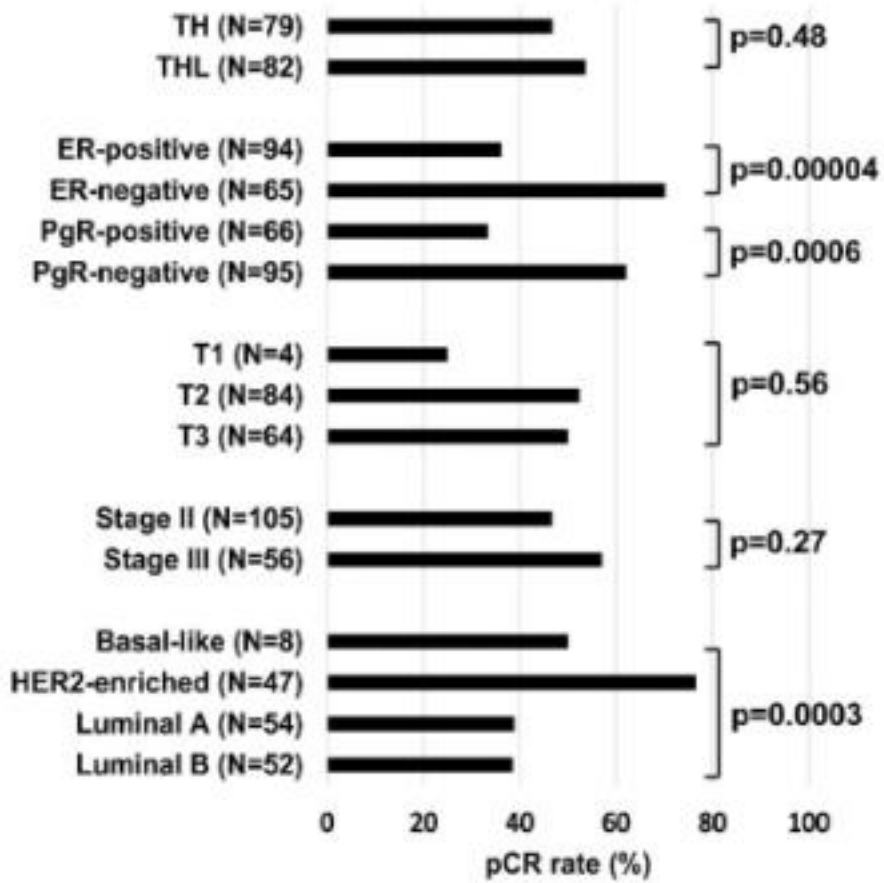
ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

Patient Subset Tested

Consort diagram



Clinical Characteristics versus pCR (n=161)



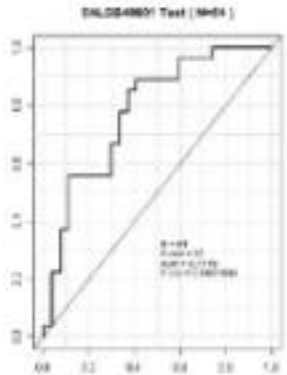
Logistic Regression Model building for pCR using Elastic Net

161 samples

Train/Test sets balanced for: Treatment arm, Intrinsic Subtype, ER / PgR status, And Stage

Train N= 107

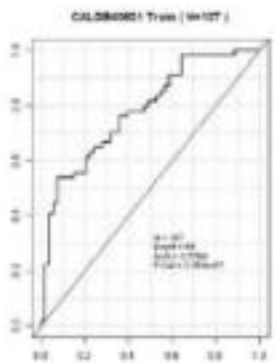
Test N= 54



10-fold cross validation for estimating elastic net α & λ tuning parameters

Repeat 10 times using different train/test splits

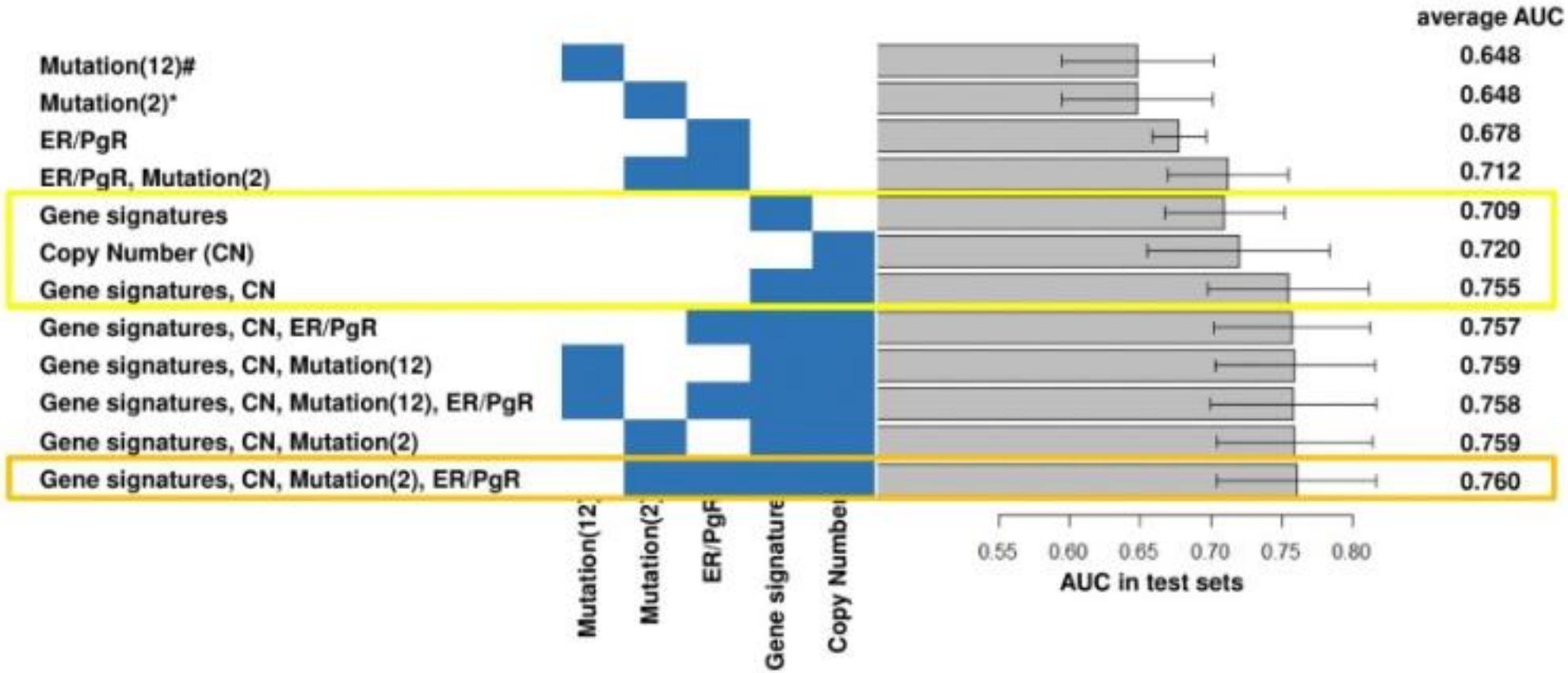
Use α & λ values and the entire training set to **select the features, determine weights, and calculate AUC** (Area under ROC)



Apply the model to the test set and calculate the AUC

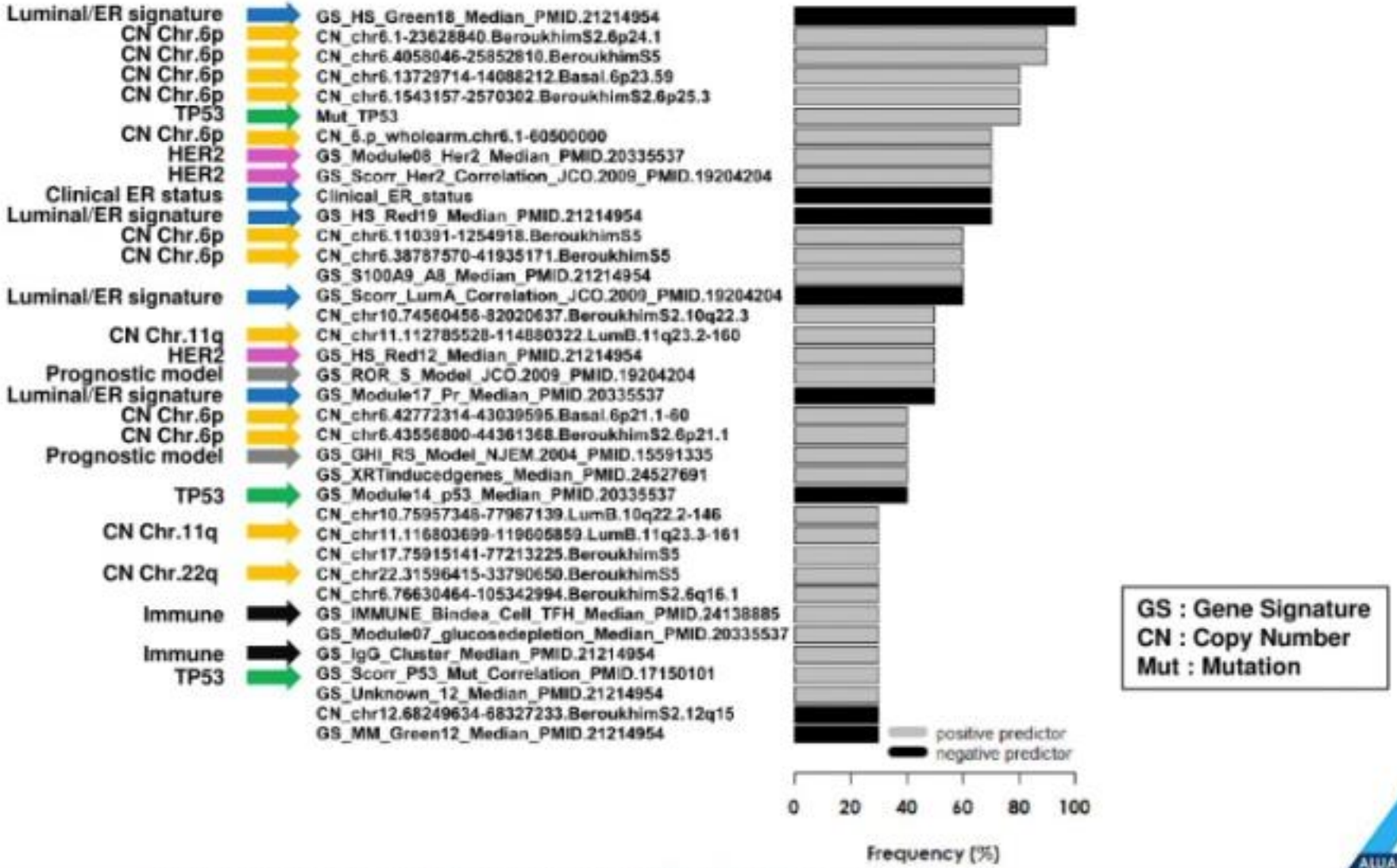
The goal is biological discovery to understand trastuzumab-based regimen responsiveness

AUC Scores in Test sets through 10 repeated Elastic Net analysis

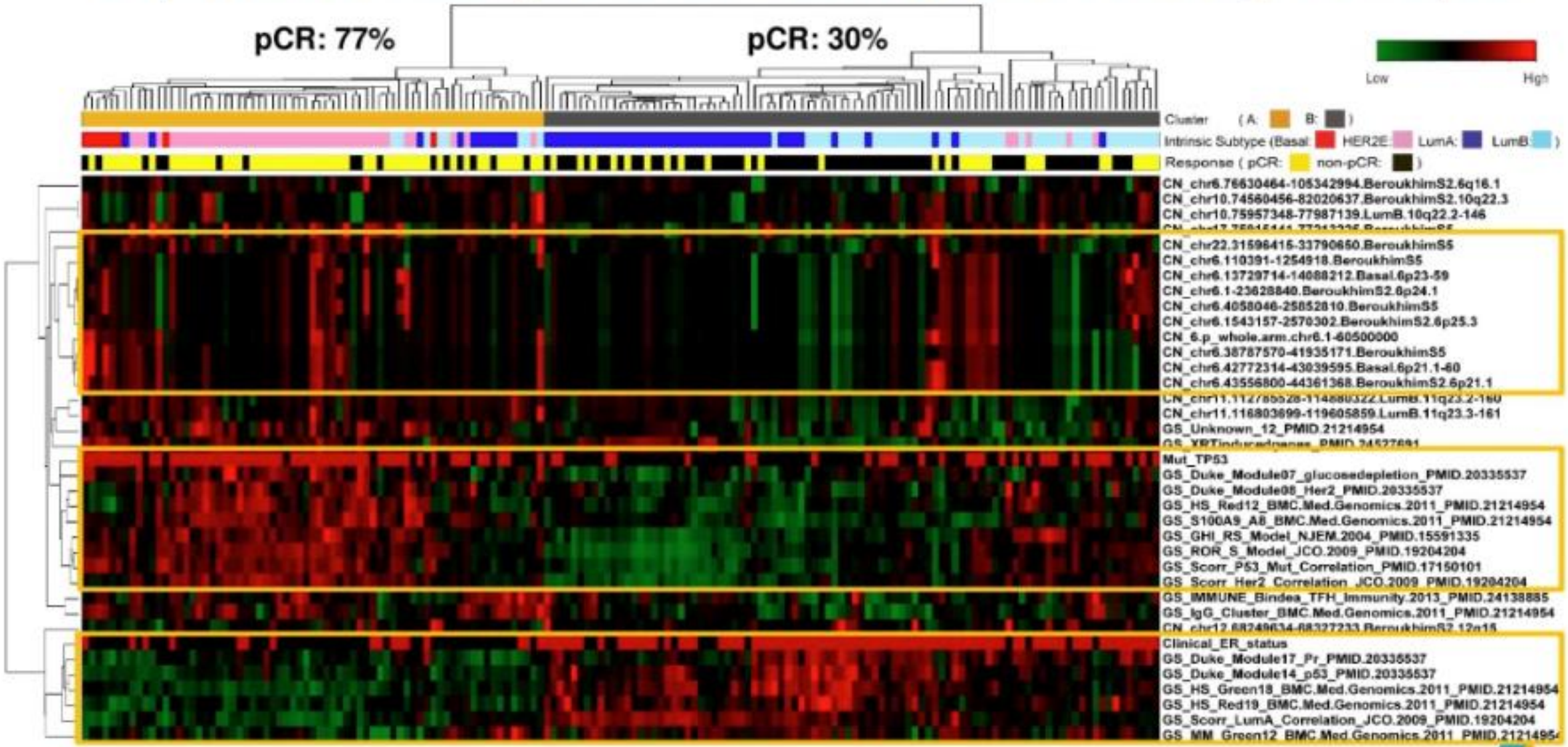


#Mutation(12): 12 genes with somatic mutations in more than 10 patients (>6%)
*Mutation(2): PIK3CA and TP53 mutation only

Features selected ≥3/10 times from the 10 rounds of testing using Gene Signatures + DNA Copy Number + Mutation + clinical ER/PgR status



Supervised Clustering of selected Elastic Net Features using 161 samples



DawnRank: Identifying Functional Genetic Drivers using DNA & RNA expression data together

1. Start with the knowledge of known Protein-Protein interaction networks (KEGG, MEMo, Reactome)
2. Populate network with RNA gene expression data for a single patient
3. Calculate a score for each gene based upon expression of the connected genes in the network
4. Using somatically altered genes, individual patient scores are aggregated for subjects with pCR or non-pCR, separately
5. Output rank-ordered gene list:
The top ranked genes = the most altered networks in that group (pCR or non-pCR subjects)

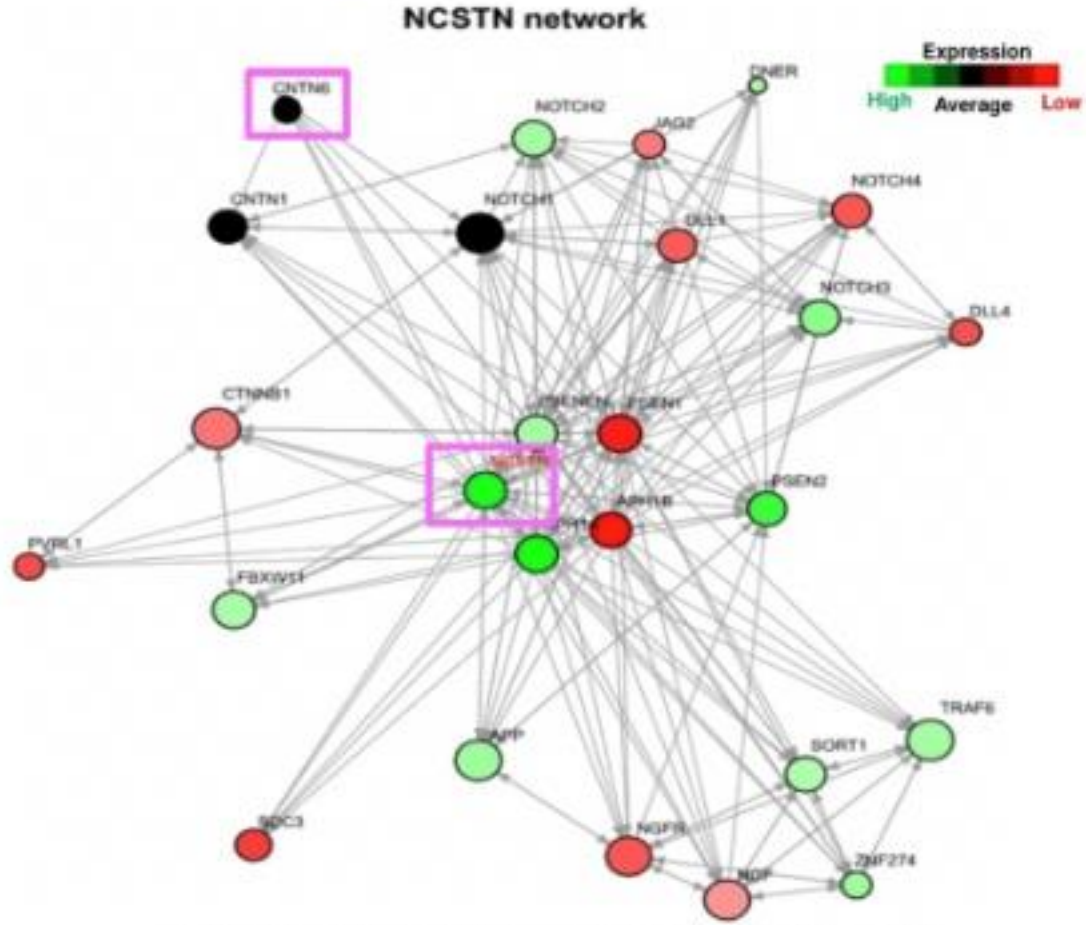


Figure adopted from Silva G, et al., BCRT, 2015, PMID:26109346

Hou J, Ma J. 2014. DawnRank: discovering personalized driver genes in cancer. *Genome Medicine*. PMID: 25177370

Dawnrank results

The most differently ranked genes between pCR vs. non-pCR samples

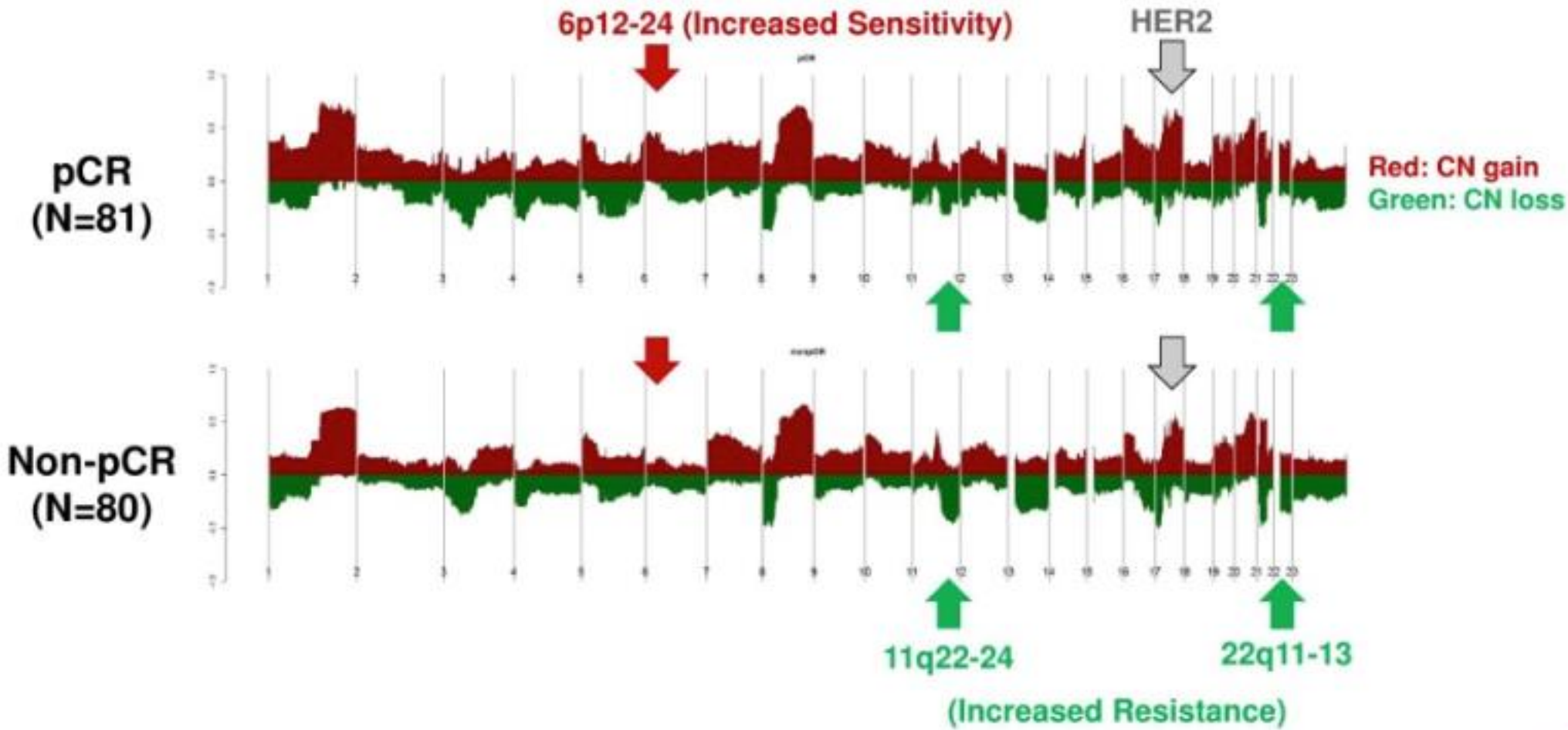
pCR / sensitivity (N=81)

Gene		Chr.	Rank pCR	Rank Non-pCR	Difference
HLA-A	mutation	6p21.3	30	-	-
MAPK14	mutation	6p21.2-3	159	-	-
ADCY2	mutation	5p15.3	183	-	-
EDN1	amplification	6p24.1	161	1067	906
HLA-DRA	amplification	6p21.3	174	973	799
C2	amplification	6p21.3	141	939	798
HLA-DRB1	amplification	6p21.3	157	941	784
VEGFA	amplification	6p12	147	924	777
TNF	amplification	6p21.3	134	852	718
DAXX	amplification	6p21.3	102	781	679
CDKN1A	amplification	6p21.2	66	699	633
MAPK14	amplification	6p21.2-3	55	654	599
HLA-A	amplification	6p21.3	89	676	587
CBLB	deletion	3q13.11	96	599	503
CDKN1B	deletion	12p12-13.1	178	594	416
CRK	amplification	17p13.3	167	523	356
ARRB2	amplification	17p13	138	492	354
E2F4	amplification	16q21-22	172	526	354
GNB1	amplification	1p36.33	126	461	335
CDH1	amplification	16q22.1	129	457	328
CDC42	amplification	1p36.1	160	475	315
TP53	amplification	17p13.1	182	476	294
HDAC1	amplification	1p34	150	438	288
EP300	amplification	22q13.2	59	342	283
CAMK4	deletion	5q21.3	127	401	274
CREB1	deletion	2q34	133	397	264
HDAC2	deletion	6q21	163	391	228
MAPK1	amplification	22q11.21	184	407	223
FYN	deletion	6q21	146	368	222
PRKACA	amplification	19p13.1	93	313	220
IL12RB2	deletion	1p31.2-3	110	310	200
HSP90AA1	amplification	14q32.33	56	250	194
JAK1	deletion	1p31-32	98	277	179
APC	deletion	5q21-q22	31	203	172
TAF9	deletion	5q11-13	122	291	169
CCL5	amplification	17q11-12	106	261	155
LEF1	deletion	4q23-25	152	306	154

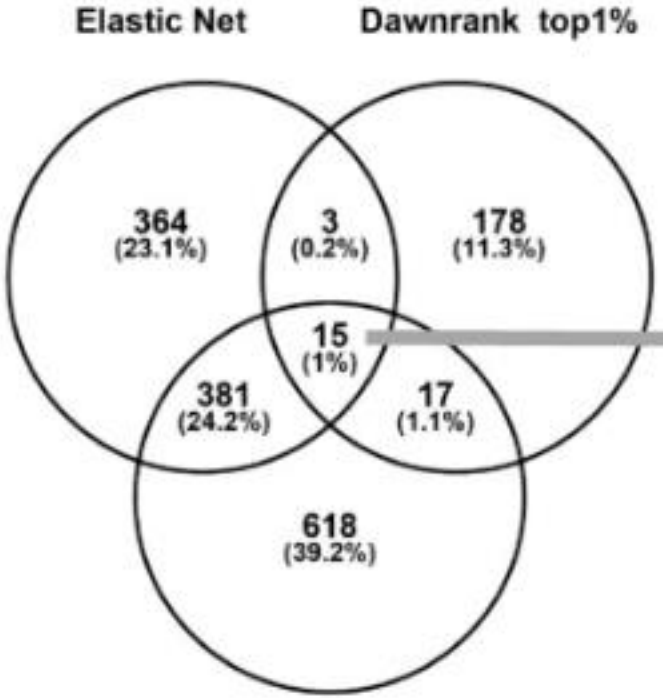
Non-pCR / resistance (N=80)

Gene		Chr.	Rank Non-pCR	Rank pCR	Difference
CABIN1	mutation	22q11.23	118	-	-
GATA3	mutation	10p15	109	1630	1521
AP1B1	deletion	22q12.2	114	916	802
GRAP2	deletion	22q13.2	140	925	785
IL10RA	deletion	11q23	136	783	647
CDH1	mutation	16q22.1	159	749	590
CSNK1E	deletion	22q13.1	115	640	525
MAPK12	deletion	22q13.33	121	639	518
BCR	deletion	22q11.23	117	625	508
FLI1	deletion	11q24.1-3	151	596	445
CRKL	deletion	22q11.21	60	406	346
IL2RB	deletion	22q13.1	81	422	341
GLI3	amplification	7p13	150	485	335
EP300	deletion	22q13.2	42	346	304
FOXA1	mutation	14q12-q13	110	402	292
MAPK1	deletion	22q11.21	56	337	281
APOA1	deletion	11q23-24	34	309	275
CHEK1	deletion	11q24.2	77	330	253
NCOA3	amplification	20q12	148	377	229
FASLG	amplification	1q23	79	299	220
EPHB2	deletion	1p35-36	152	372	220
EGFR	amplification	7p12	67	279	212
RBBP4	deletion	1p35.1	153	365	212
IL2RA	amplification	10p14-15	164	364	200
RBL1	amplification	20q11.2	120	312	192
CD3G	deletion	11q23	31	217	186
E2F4	deletion	16q21-22	104	288	184
MTOR	deletion	1p36.2	112	295	183
ARRB1	amplification	11q13	144	322	178
CD3E	deletion	11q23	29	205	176
CD3D	deletion	11q23	28	200	172
CALML3	amplification	10pter-p13	161	328	167
BIRC2	deletion	11q22	107	270	163
FLT1	deletion	13q12	132	291	159
CCNE2	amplification	8q22.1	156	306	150
CDC25A	deletion	3p21	131	280	149
CBL	deletion	11q23.3	21	169	148

Dawnrank Drivers on Copy Number Landscape: pCR vs. non-pCR



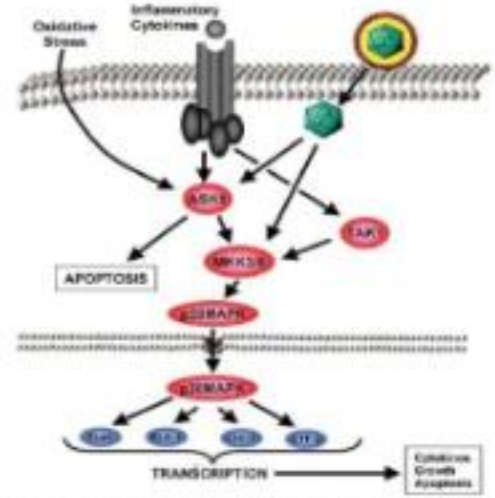
Computational analysis to find common drivers between Elastic Net, Dawnrank, and supervised analysis of Copy Number data



Supervised analysis (q<1%) of DNA Copy Number: pCR vs non-pCR

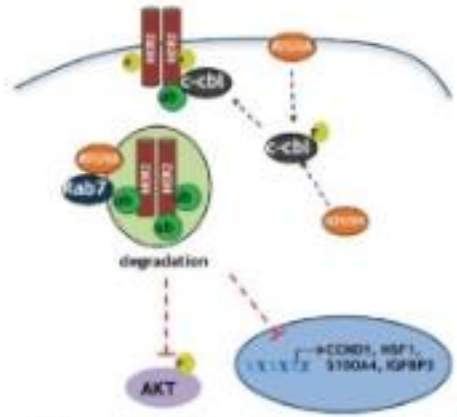
EDN1	6p24.1
CDKN1A	6p21.2
MAPK14	6p21.2-3
C2	6p21.3
DAXX	6p21.3
TNF	6p21.3
HLA-DRA	6p21.3
HLA-DRB1	6p21.3
VEGFA	6p12
CD3D	11q23
CD3E	11q23
CD3G	11q23
CBL	11q23
IL10RA	11q23
ACTG1	17q25

p38 (amplification & high mRNA expression)



Aurelian, et al. Frontiers in Bioscience, 2005

E3 ubiquitin ligase (deletion & low mRNA expression)



Nunes, et al. Oncotarget, 2016

Summary

- Gene Expression Signatures and DNA Copy Number changes were the most predictive of pathological complete response in CALGB 40601. Using the Elastic Net selected features, our hypothesis is that tumor subtype (HER2E vs Luminal), tumor genetics (mutation, amplification, deletion), and the microenvironment (immune cells) were each independent predictors of response.

- Integrated Elastic Net models could be used to develop valuable biomarkers. To accomplish this goal we would use all 161 samples as a training set, and then apply this model onto the test data set(s). The test sets will require both DNA Exome and RNAseq data, and we are actively looking for such test sets.

- Multiple bioinformatics methods identified Chromosome 6p gain (MAPK14) as a predictor of sensitivity, and 11q (CBL) and 22q loss as predictors of resistance to trastuzumab-paclitaxel regimens. Experimental validation is needed and is ongoing.

San Antonio Breast Cancer Symposium, December 6-10, 2016

S3-06

NSABP B-52 (NRG Oncology)

Evaluating Pathologic Complete Response Rates in Patients with Hormone Receptor-Positive, HER2-Positive Breast Cancer treated with Neoadjuvant Therapy of Docetaxel, Carboplatin, Trastuzumab, and Pertuzumab (TCHP) with or without Concurrent Estrogen Deprivation Therapy

Mothaffar F. Rimawi, Reena S. Cecchini, Priya Rastogi, Charles E. Geyer, Jr, Louis Fehrenbacher, Philip J. Stella, Zoneddy Dayao, Rachel Rabinovitch, Stephen H. Dyar, Patrick J. Flynn, Luis Baez-Diaz, Soonmyung Paik, Sandra M. Swain, Eleftherios P. Mamounas, C. Kent Osborne, Norman Wolmark

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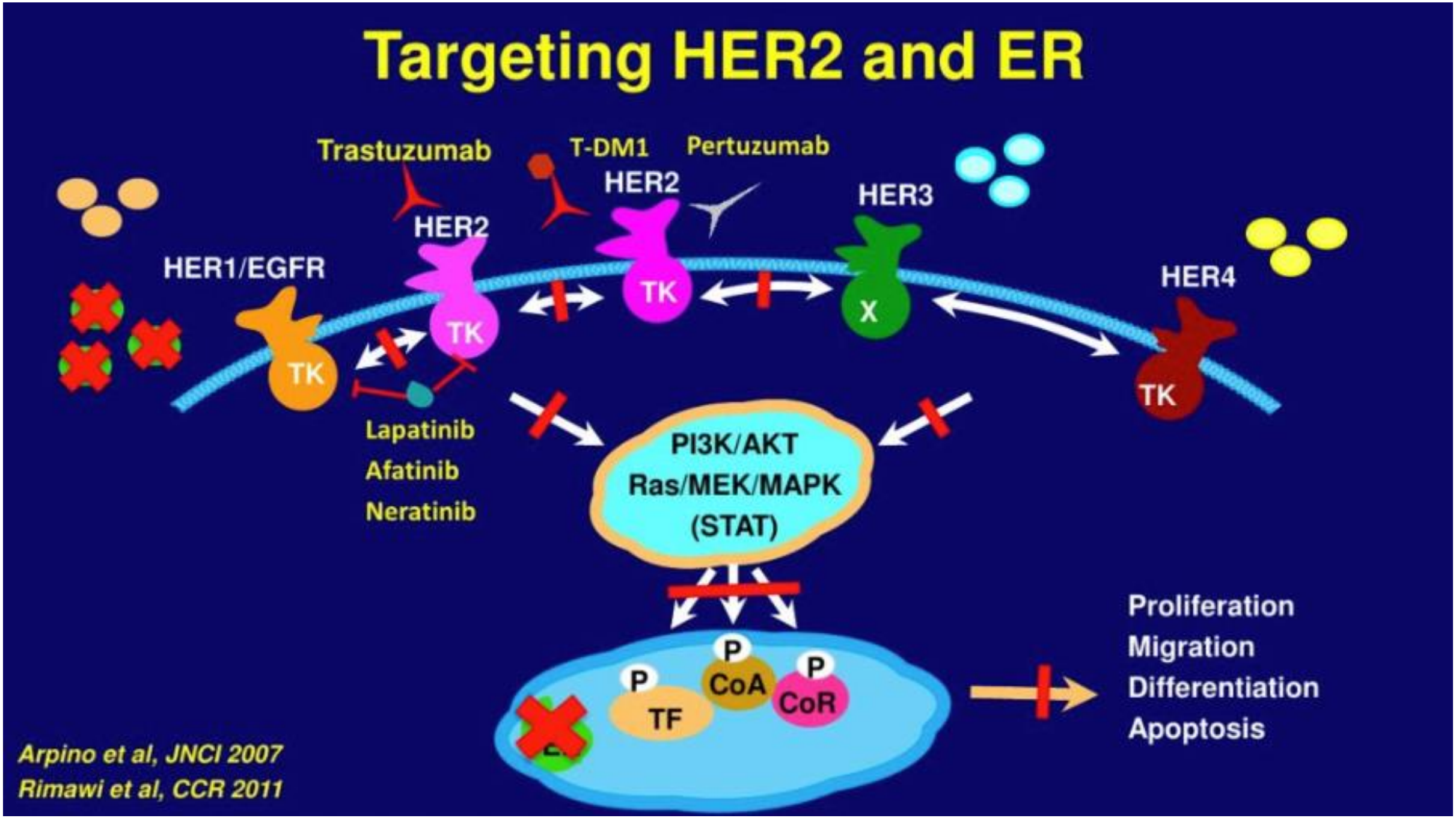
Advancing Research. Improving Lives.™

Targeting HER2 and ER



Arpino et al, JNCI 2007
Rimawi et al, CCR 2011

Dual HER2 inhibition by ER status

Trial	HER2 Inhibition	pCR in ER-positive	pCR in ER-negative
NeoSphere	Per/Tras	26%	63%
NeoALTTO	Lap/Tras	42%	61%
CALGB 40601	Lap/Tras	42%	77%
NSABP B-41	Lap/Tras	56%	73%
TRYPHAENA	Per/Tras	46-50%	65-84%

Rationale

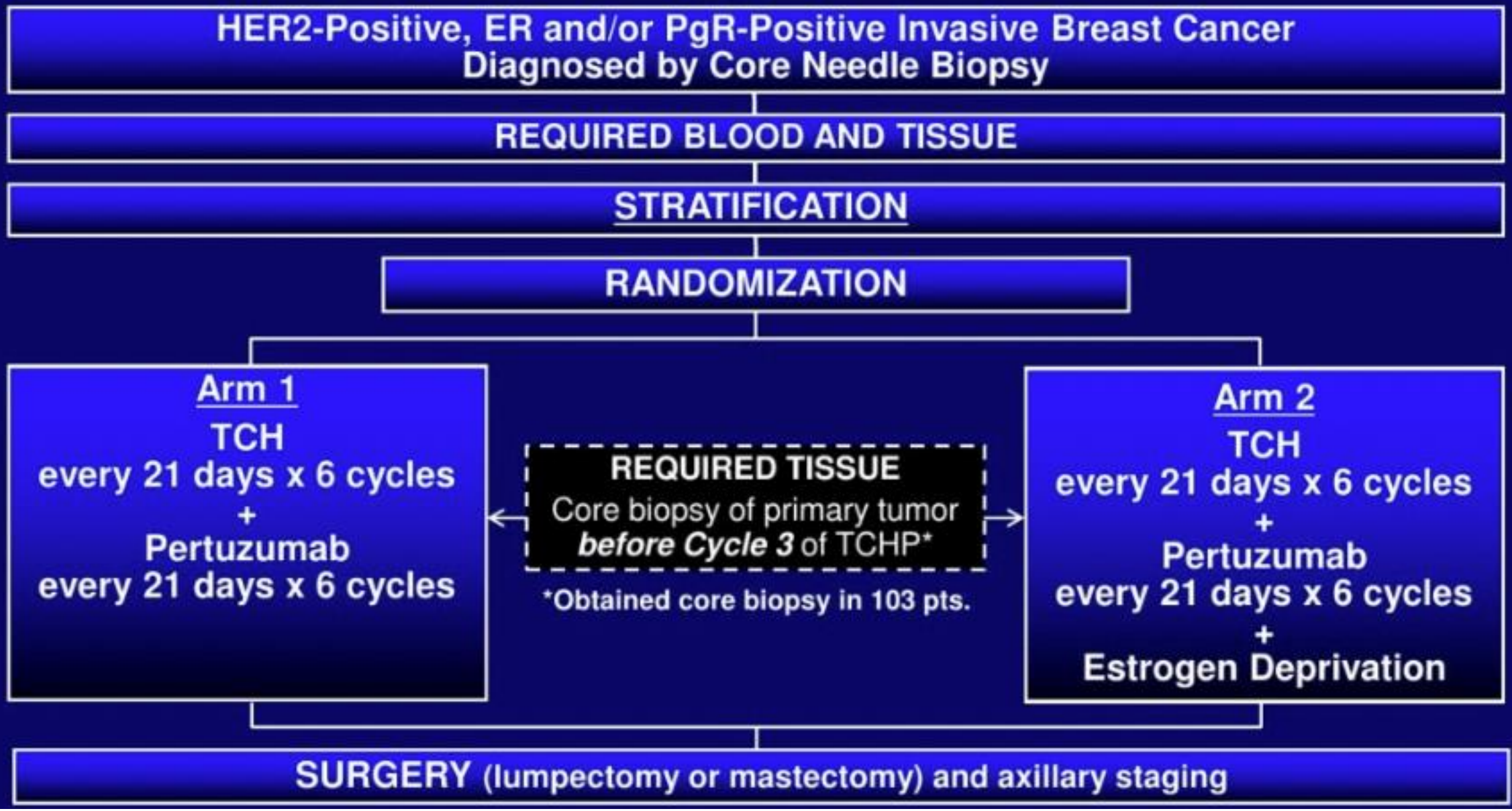
- ER+/HER2+ tumors are less likely than ER-/HER2+ tumors to respond to dual anti-HER2 therapy.
- ER may act as a pathway of resistance to anti-HER2 treatment.
- Older trials suggested antagonistic effects of chemotherapy and endocrine therapy.

Hypothesis

- We hypothesized that concurrent inhibition of ER and HER2, plus chemotherapy, **will not be antagonistic, and will overcome resistance** to treatment thus improving pCR rates in pts with ER+/HER2+ breast cancer.

SABCS, December 6-10, 2016

NRG Oncology/NSABP B-52



Eligibility Criteria

- Invasive adenocarcinoma of the breast *diagnosed by core needle biopsy*
- Clinical tumor ≥2.0 cm if clinically node negative. Any size if node positive.
- Tumors must be hormone receptor positive and HER2+ by ASCO/CAP
- The LVEF must be ≥50% regardless of the testing facility's lower limit of normal.
- Adequate organ function

Dose Regimen

- **TCH: Docetaxel 75 mg/m2 IV + carboplatin AUC of 6 IV + trastuzumab IV (administer a loading dose of 8mg/kg; then 6 mg/kg every 3 wks for the remaining doses).**
- **Pertuzumab: Administer a loading dose of 840 mg; then 420 mg every 3 wks for the remaining doses.**
- **Estrogen deprivation therapy determined by menopausal status:**

  Postmenopausal: **Aromatase inhibitor**

  Premenopausal: **Aromatase inhibitor plus ovarian suppression**

Endpoints

Primary

- pCR rate in the breast and nodes ($ypT_{0\text{-}is}$ ypN_0)

Secondary

- pCR rate in the breast
- Clinical complete response
- Toxicity
- Recurrence-free interval
- OS

} ~ 8 yrs after start of trial

Statistical Considerations

- The expected rate of pCR in the group not treated with estrogen deprivation is 45%.
- Between January 2014 and February 2016, 315 patients were enrolled to provide 80% power to detect a 33% improvement, increasing the path CR rate from 45% to 60%.

NSABP B-52
Patient Characteristics*

- **Age**
  - ≤ 49 — 46%
  - 50 – 59 — 32%
  - ≥ 60 — 22%
- **Race**
  - White — 79%
  - Black — 12%
  - Other/Unk — 9%

- **Tumor staging**
  - cT0-cT2 — 74%
  - cT3-cT4c — 24%
  - cT4d — 2%
- **Clinical Nodal Status**
  - Pos. — 57%
  - Neg. — 43%

* Patient characteristics were balanced between treatment regimens

NSABP B-52
Toxicity

Toxicity	TCHP (n=154)				TCHP +Est Dep (n=157)			
	Gr 0-1	Gr 2	Gr 3	Gr 4	Gr 0-1	Gr 2	Gr 3	Gr 4
Diarrhea	42%	34%	23%	<1%	43%	35%	22%	0%
Nausea	60%	31%	9%	0%	65%	29%	6%	0%
Vomiting	82%	10%	8%	<1%	82%	13%	5%	0%
Dehydration	71%	20%	8%	<1%	78%	17%	5%	0%

NSABP B-52
Toxicity

Toxicity	TCHP (n=154)				TCHP +Est Dep (n=157)			
	Gr 0-1	Gr 2	Gr 3	Gr 4	Gr 0-1	Gr 2	Gr 3	Gr 4
Anemia	53%	35%	12%	0%	56%	26%	18%	0%
Hypokalemia	83%	5%	10%	2%	80%	8%	10%	1%
Febrile Neutropenia	-	-	5%	<1%	-	-	7%	1%
Overall	**3%**	**29%**	**59%**	**10%**	**5%**	**37%**	**52%**	**6%**

NSABP B-52
Completion of Neoadjuvant Therapy

	TCHP (n=158)	TCHP + Est Dep (n=157)
TCHP*	89.9%	90.4%

* Completed at least 5 cycles of all 4 drugs comprising TCHP

NSABP B-52
Completion of Estrogen Deprivation among the TCHP+Est Dep Group

Aromatase Inhibitor

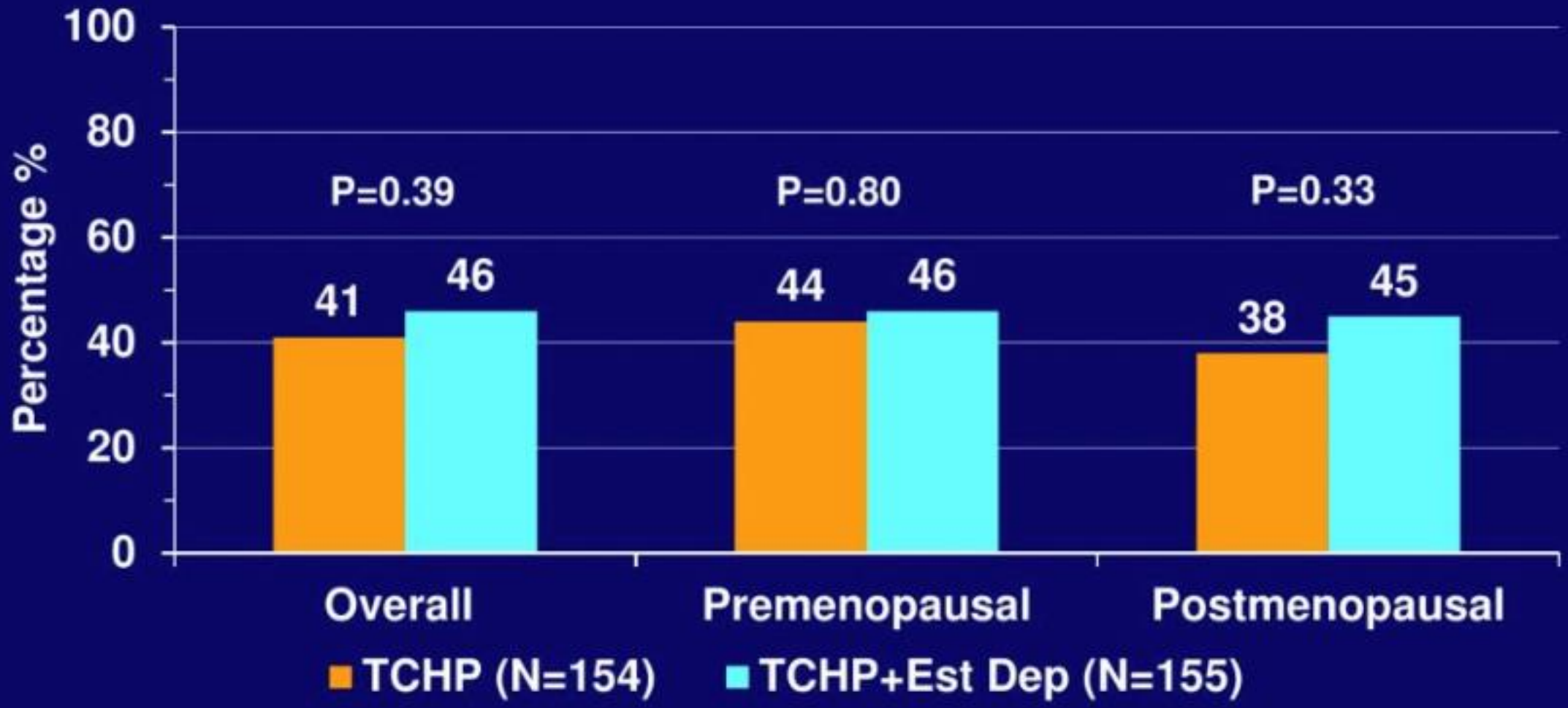
% completed of total exp daily doses

≥ 90%	79.6%
80–89%	10.2%
< 80%	10.2%

Goserelin/LHRH agonist
(Among premenopausal women only) 89.9%

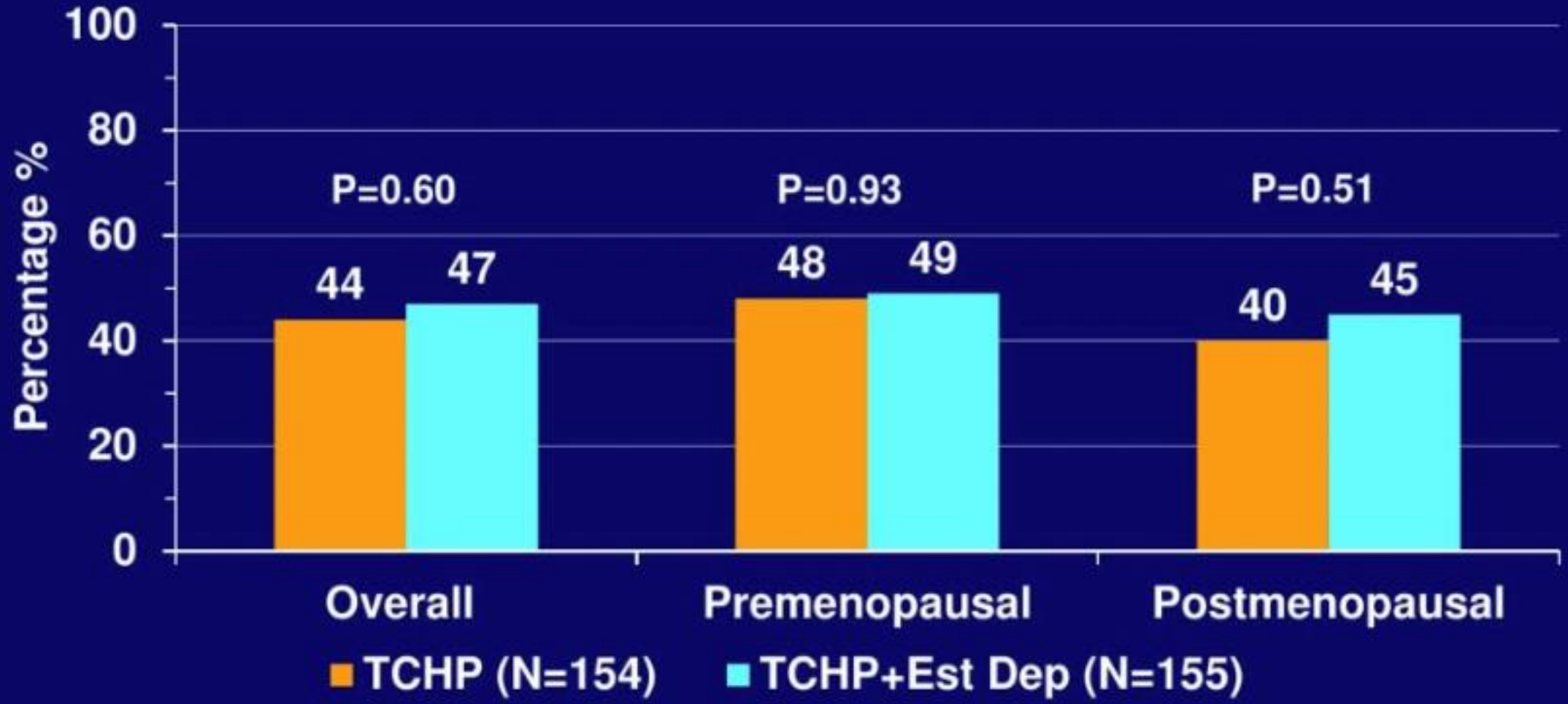
NSABP B-52

pCR Breast and Nodes



NSABP B-52

pCR Breast



NSABP B-52
Clinical Complete Response

cCR	TCHP (n=138)	TCHP + Est Dep (n=142)	p
Overall	68.1%	73.9%	0.28

NSABP B-52
Surgery

Type of Surgery	TCHP (n=158)	TCHP +Est Dep (n=157)
Lumpectomy	33.5%	42.7%
Mastectomy	63.9%	56.1%
No Surgery	2.5%	1.3%

Conclusion

- The addition of estrogen deprivation to neoadjuvant chemotherapy was not antagonistic and did not increase toxicity.
- The combination increased pCR rates numerically, but the improvement was not statistically significant.
- Correlative science studies, evaluation of residual cancer burden (RCB), and long-term outcomes may help define the role of estrogen deprivation in the treatment of HER2+ early breast cancer.

Conclusion

- Given the toxicity of standard chemotherapy observed on this trial, findings from NSABP B52 argue for a tailored de-escalation approach where toxic treatments are omitted or replaced with less toxic ones without compromising outcomes.

San Antonio Breast Cancer Symposium, December 6-10, 2016
Oral Presentation S4-06

S4-06

Biological and clinical effects of abemaciclib in a phase 2 neoadjuvant study for postmenopausal patients with HR+, HER2- breast cancer

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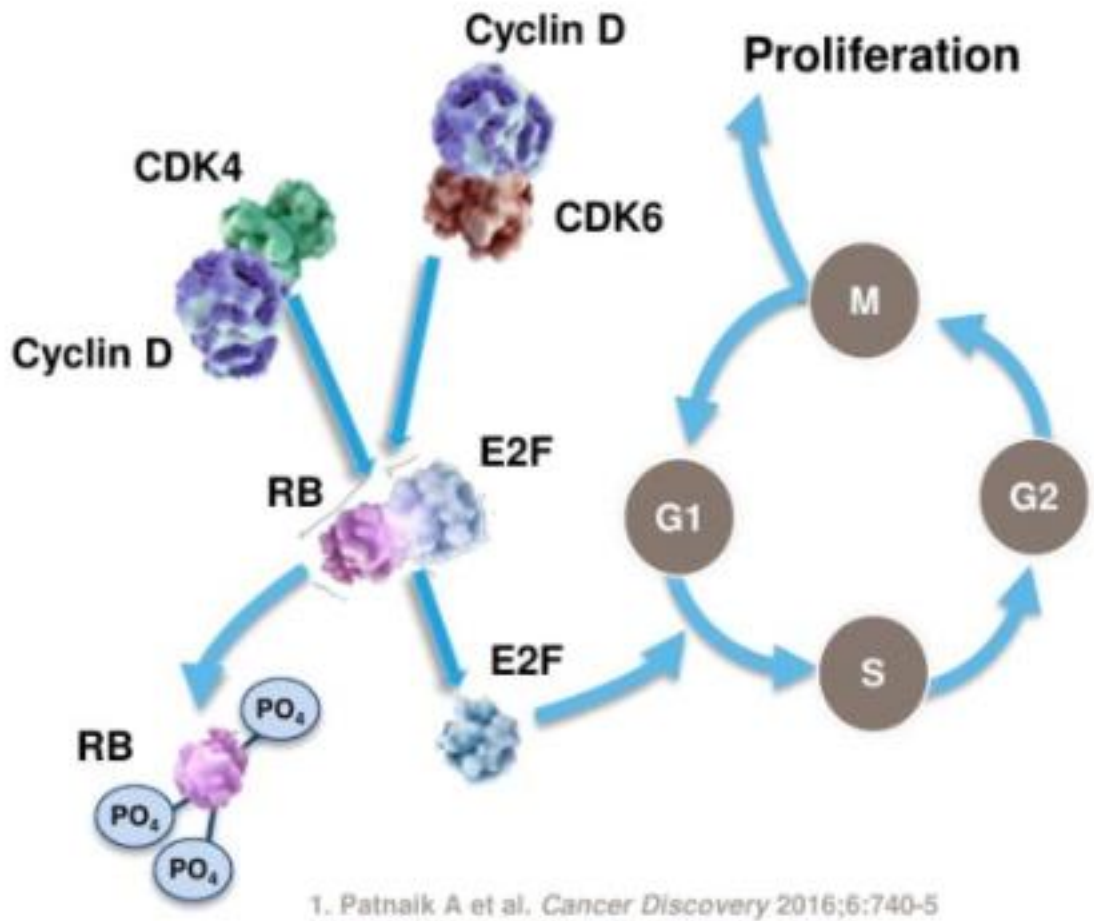
Dysregulation of the CDK4 & CDK6 Pathway

- Activation of cyclin dependent kinases (CDKs) by cyclins leads to the dissociation of the tumor suppressor protein, retinoblastoma (RB), from the transcription factor E2F, resulting in G1 to S cell cycle progression.[1]
- In HR+ breast cancer, estrogen stimulates D-type cyclins resulting in increased activity of CDK4 & CDK6 and cell cycle progression by transcription of E2F-related genes.[2-3]
- Increased Ki67 expression, a proliferation marker, is observed in HR+ breast cancer tissue samples.
- Cell cycle arrest induces senescence, which may implement a senescence-associated secretory phenotype characterized by an immune cell infiltration.[4-5]



1. Patnaik A et al. *Cancer Discovery* 2016;6:740-5
2. Altucci L et al. *Oncogene* 1996;12:15-24
3. Miller TW et al. *Cancer Discovery* 2011;1:338-51
4. Muñoz-Espin and Serrano. *Nat Rev Mol Cell Biol* 2014; 15: 482-496
5. Nardella et al. *Nat Rev Cancer* 2011; 11: 503-511

Phase 2 neoMONARCH Study Design

Rationale:

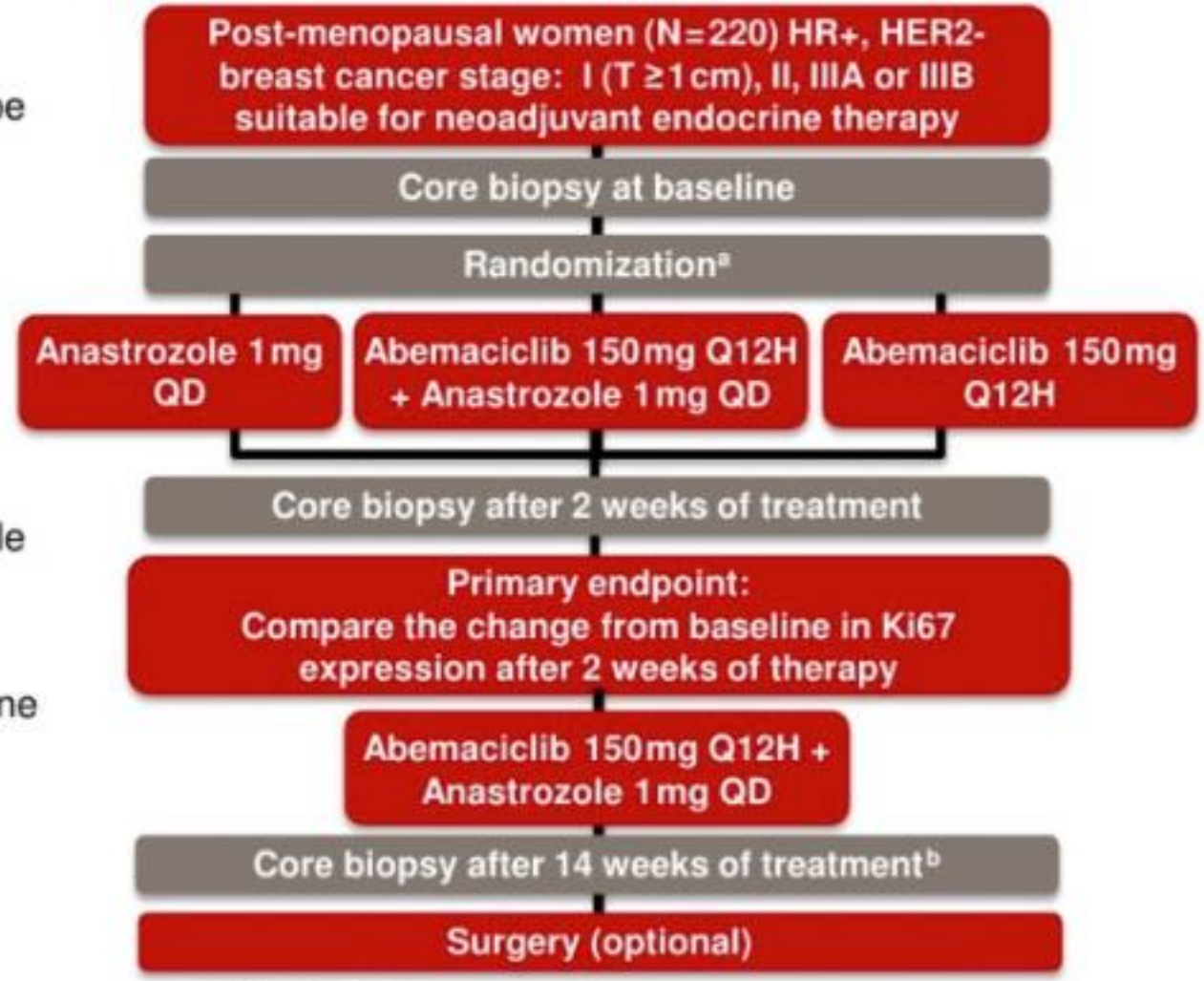
- Change in Ki67 at 2 weeks in neoadjuvant studies may be predictive of improved disease-free survival in adjuvant studies.[1,2]

Secondary and exploratory objectives:

- Safety, clinical, radiologic and pathological response, cell cycle associated gene expression.

Statistical design:

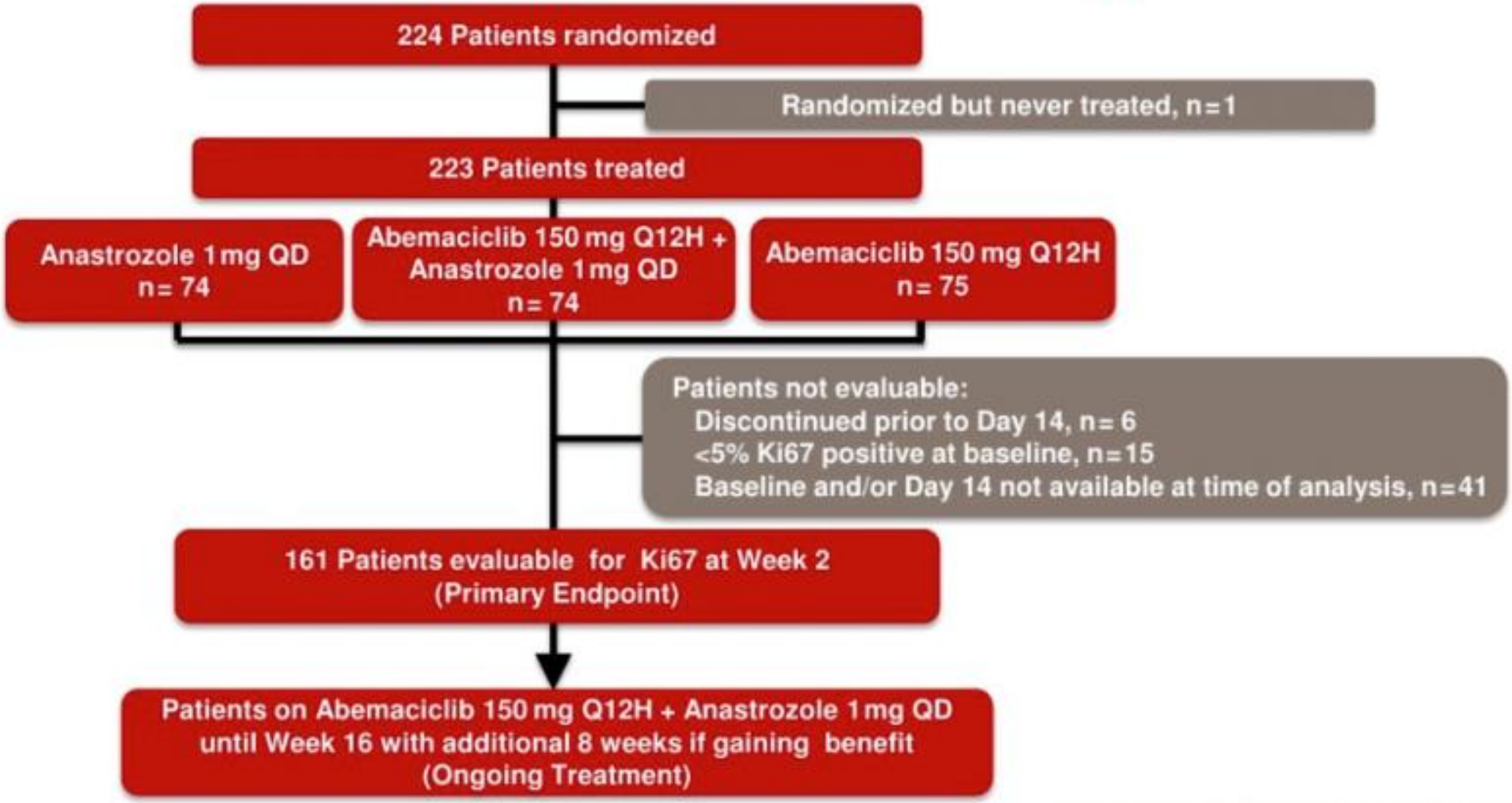
- 220 randomized patients required to achieve 50 evaluable patients in each arm.
- 80% power at one-sided alpha of 0.1, assuming:
  - Assumed mean reduction of 82% for anastrozole alone and 91% for combination.
- 2 mg loperamide was administered prophylactically with each abemaciclib dose for the first 28 days then at discretion of investigator.



[a]Stratified for PR status, tumor size
[b]Participants who experience benefit following 14weeks may remain on neoadjuvant therapy for up to 8additional weeks

1. Dowsett M et al. *Clin Cancer Res* 2005; 11:951s-958s.
2. Dowsett M et al. *J Natl Cancer Inst.* 2011a;103(22):1656-1664.

neoMONARCH Consort Diagram



Abbreviations: Q12H=every 12 hours; QD=once daily

Patient Baseline Characteristics

Characteristic	Anastrozole 1 mg (n=74)	Abemaciclib 150 mg + Anastrozole 1 mg (n=74)	Abemaciclib 150 mg (n=76)	Total (N=224)
Age, years, median (range)	65 (42-83)	63 (52-92)	62 (51-86)	64 (42-92)
Race, Caucasian, n (%)[a]	55 (74.3)	55 (75.3)	57 (75.0)	167 (74.9)
ECOG PS, of 1, n (%)[a]	10 (13.7)	5 (6.8)	5 (6.7)	20 (9.0)
Disease Stage, n (%)[a]				
I/II	48 (81.4)	47 (82.5)	54 (90.0)	149 (84.7)
III	11 (18.6)	10 (17.5)	6 (10.0)	27 (15.3)
Tumor Grade, n (%)[a]				
1	9 (13.2)	8 (12.1)	7 (10.4)	24 (11.9)
2	39 (57.4)	29 (43.9)	40 (59.7)	108 (53.7)
3	12 (17.6)	18 (27.3)	8 (11.9)	38 (18.9)
Tumor Size, median mm (range)[a]	32.0 (10.0-100.0)	30.0 (5.0-100.0)	30.0 (10.0-100.0)	30.0 (5.0-100.0)
<2cm, n (%)	17 (23.0)	16 (21.9)	13 (18.1)	46 (21.0)
≥2and <5cm, n (%)	35 (47.3)	44 (60.3)	42 (58.3)	121 (55.3)
≥5cm, n (%)	22 (29.7)	13 (17.8)	17 (23.6)	52 (23.7)
Baseline Ki67, median % (25^{th}-75^{th} quartile)[b]	25.4 (17.8-34.4)	25.8 (16.0-40.3)	25.0 (19.6-34.4)	25.4 (17.8-36.0)
Hormone Receptor Status, n (%)				
ER+; PR+	64 (86.5)	62 (83.8)	60 (78.9)	186 (83.0)
ER+; PR-	10 (13.5)	11 (14.9)	15 (20.0)	36 (16.1)

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status, ER+ = estrogen receptor positive, PR+ = progesterone receptor positive.
[a]Data not reported for some patients. [b]KE evaluable patients at baseline (n=161)

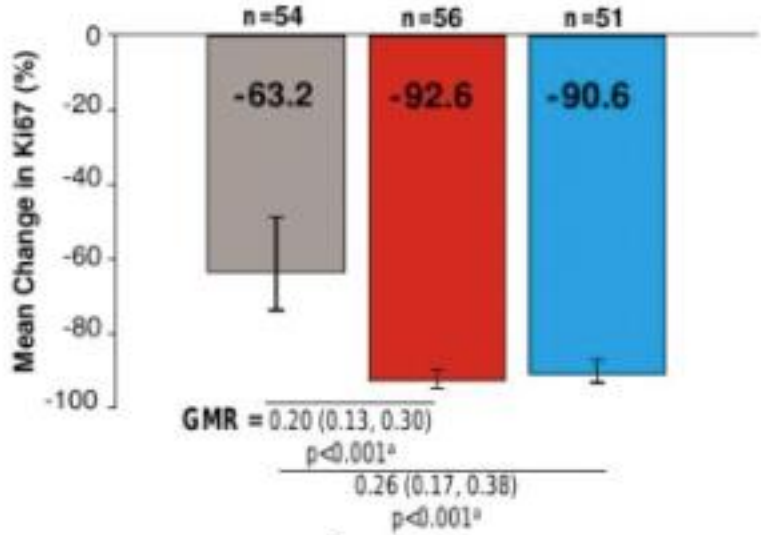
Ki67 Expression and Response at Week 2

Anastrozole 1 mg

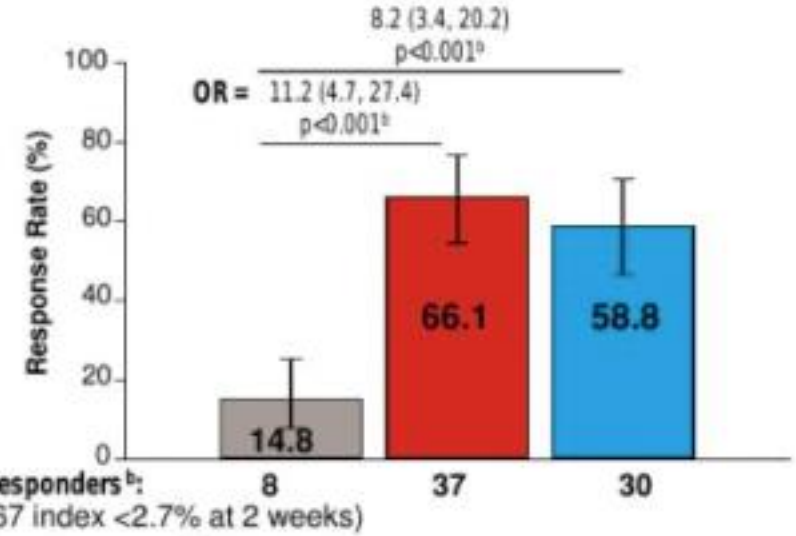
Abemaciclib 150 mg + Anastrozole 1 mg

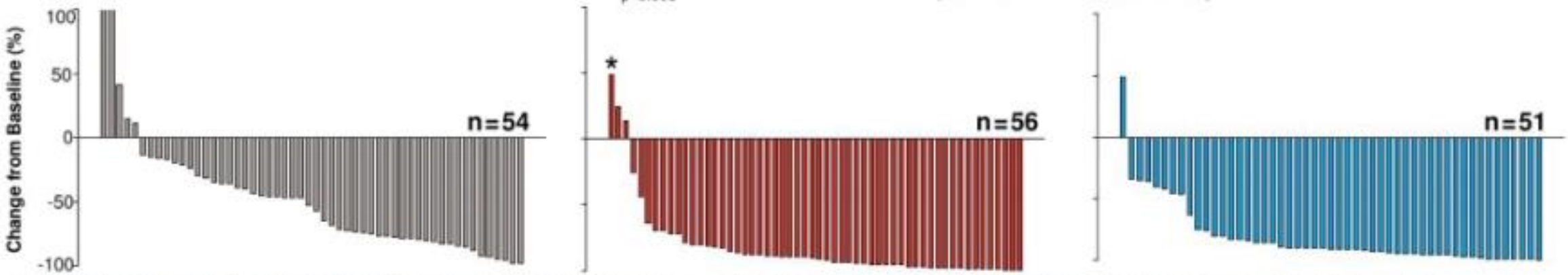
Abemaciclib 150 mg

Geometric Mean Change (Primary Endpoint of Study)



Complete Cell Cycle Arrest





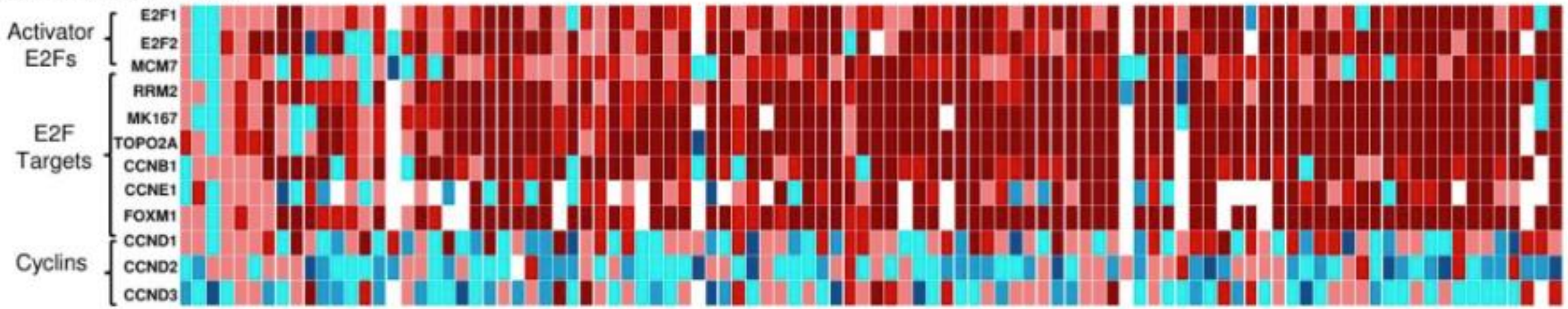
[a]Geometric Mean Ratio (GMR), 2-sided 90% confidence interval (CI), p-value. p-values are based on a one-sided hypothesis test from a linear model with treatment

[b]A responder is identified as a patient with a ln(Ki67) value of less than 1. Odds ratio (OR), 2-sided 90% CI, p value. p value is calculated by Fisher's Exact test of a one-sided hypothesis. * Patient had received dose intensity of 19% for abemaciclib prior to Week 2 biopsy.

Change in Ki67 and mRNA Expression Regardless of Treatment Arm at Week 2

Change in Ki67 Expression:

Tumor tissue mRNA:



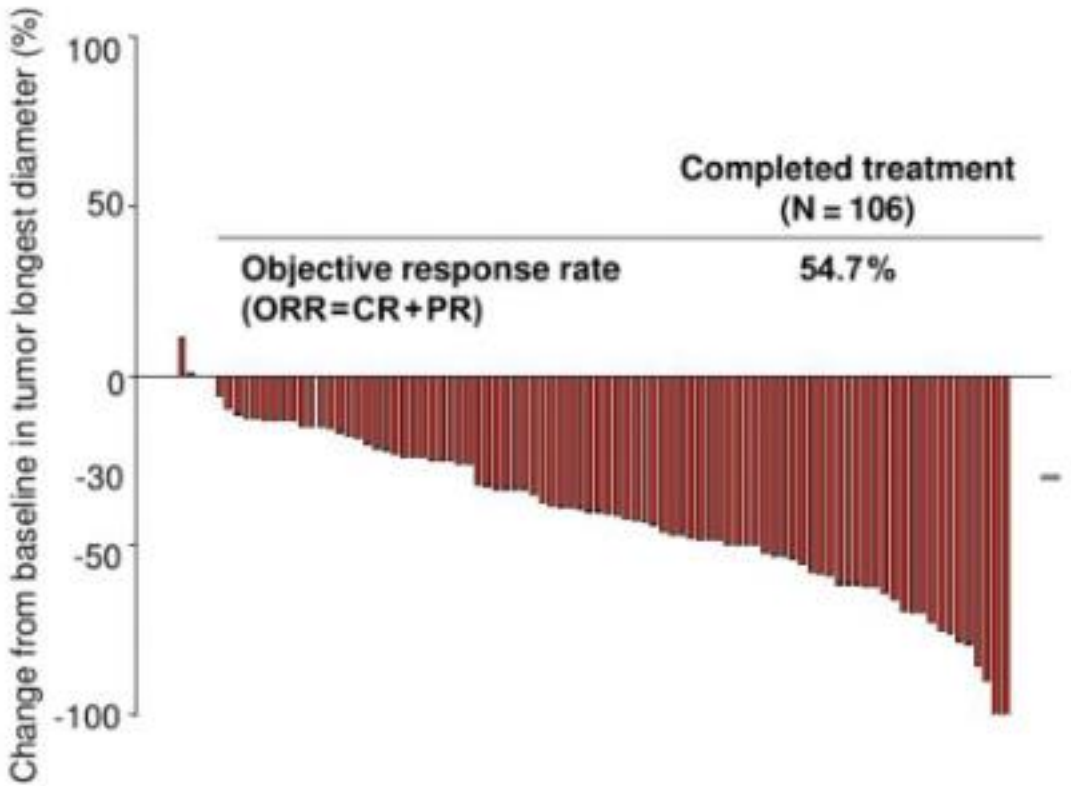
For the heatmap, change from baseline to C1D15 in biomarker expression is defined as log2(marker_C1D15) − log2(marker_baseline).
Color scheme: **very dark red: decrease >2 in expression; bright red: decrease >1 and ≤2 in expression;** light red: decrease ≤ 1 in expression; **light blue: increase ≤1 in expression; dark blue: increase >1 and ≤2 in expression; very dark blue: increase >2 in expression.**

Ki67 Expression on Combination Therapy at Week 16

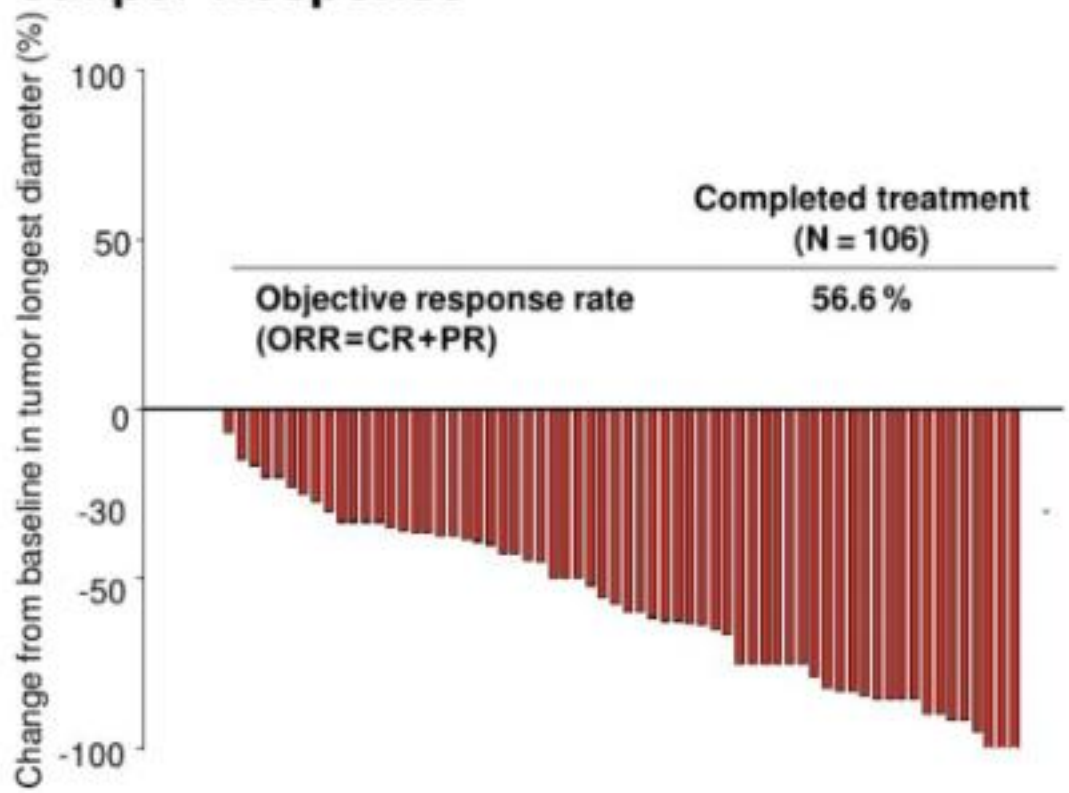
- Patients from all treatment groups received a combination of Abemaciclib 150 mg Q12H and Anastrozole 1 mg QD for a subsequent 14 weeks of therapy.
- Core biopsy was taken after 16 weeks of therapy:
  - At the time of analysis Ki67 expression data was available from 59 patients.

neoMONARCH RECIST Response Data Over Time

Radiologic Response

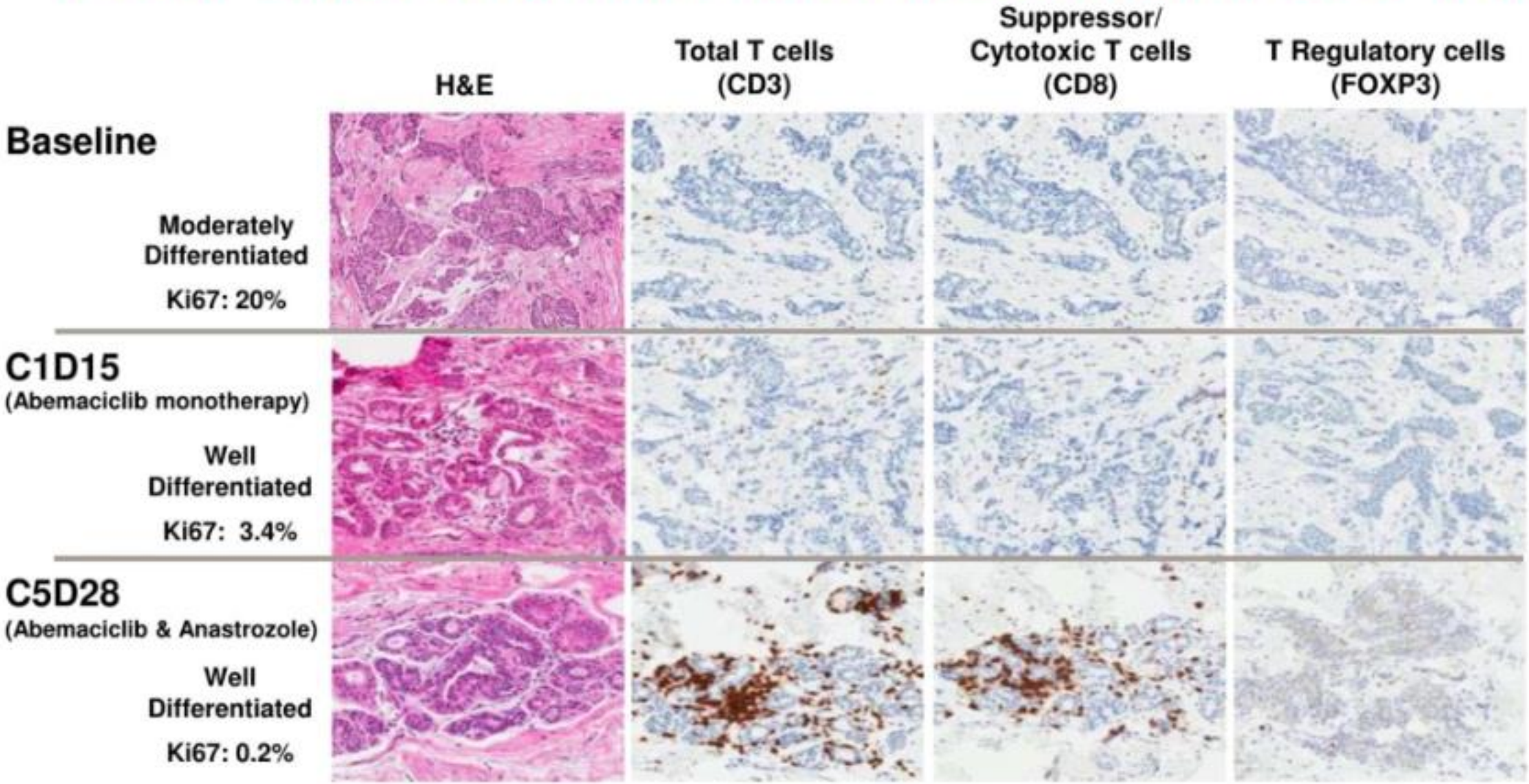


Caliper Response



- At time of analysis:
  - Complete pathologic response in three (3.2%) of 95 patients that underwent surgery.
  - One patient discontinued therapy for progressive disease (20.7% change from baseline in tumor size at week 12).

Tumor Differentiation & Immune Infiltrates Over Time



Most Common Adverse Events

Investigator Assessed TEAEs >10% (N=223)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	All Grades n (%)
Diarrhea	82 (36.8)	32 (14.3)	9 (4.0)	0	123 (55.2)
Constipation[a]	63 (28.3)	18 (8.1)	3 (1.3)	0	87 (39.0)
Nausea	58 (26.0)	19 (8.5)	5 (2.2)	0	82 (36.8)
Fatigue[a]	52 (23.3)	22 (9.9)	3 (1.3)	0	78 (35.0)
Abdominal pain	30 (13.5)	7 (3.1)	7 (3.1)	0	44 (19.7)
Decreased appetite	27 (12.1)	10 (4.5)	4 (1.8)	0	41 (18.4)
Vomiting	19 (8.5)	6 (2.7)	2 (0.9)	0	27 (12.1)
Hot flush	22 (9.9)	4 (1.8)	0	0	26 (11.7)
Laboratory Abnormalities[b]					
Creatinine increased[c]	146 (66.7)	61 (27.9)	3 (1.4)	0	210 (95.9)
Neutrophil count decreased	61 (27.9)	67 (30.6)	16 (7.3)	2 (0.9)	146 (66.7)
WBC decreased	62 (28.3)	66 (30.1)	6 (2.7)	1 (0.5)	135 (61.6)
ALT increased	70 (32.0)	12 (5.5)	10 (4.6)	0	92 (42.0)
AST increased	52 (23.7)	5 (2.3)	5 (2.3)	0	62 (28.3)
Anemia	0	37 (17.7)	0	0	37 (17.7)
Platelet count decreased	32 (14.6)	1 (0.5)	0	0	33 (15.1)

Abbreviations: ALT=alanine aminotransferase, AST=aspartate aminotransferase, TEAE=treatment-emergent adverse event, WBC=white blood cell

[a] Missing patient data; [b] N=219 for lab abnormalities listed, except anemia (N=209); [c] Abemaciclib is a competitive inhibitor of OCT2, MATE1, and MATE2-K, efflux transporters of creatinine

Conclusions

- Abemaciclib, alone or in combination with anastrozole, significantly reduced Ki67 expression compared to anastrozole alone after 2 weeks of treatment based on geometric mean change and complete cell cycle arrest (Ki67< 2.7%). The study met its primary endpoint.
- Abemaciclib induced profound cell cycle arrest, defined by decreased Ki67 and E2F targeted proliferation mRNAs, and reduction of expression of genes associated with senescence [RRM2 and FOXM1].[1,2]
- Exploratory analysis of tissue histology suggests that cell cycle suppression appears to be associated with morphological changes resulting in tumor differentiation.
- Treatment with abemaciclib in combination with anastrozole may induce immune cell infiltration, characterized by an increase in total T cells and cytotoxic/suppressor T cells.
- The majority of patients who received abemaciclib and anastrozole experienced an objective response.
- No new safety signals for abemaciclib dosed at 150 mg BID continuous schedule when administered in combination with anastrozole.
- These data support continued evaluation of abemaciclib in patients with early-stage breast cancer.

1. Anders L et al. *Cancer Cell* 2011; 20:620-634.
2. Aird KM et al. *Cell Reports* 2013; 3:1252-1265.