Proteomics: Clinical Applications at the Bedside

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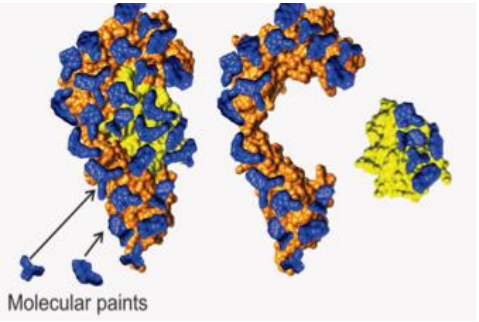
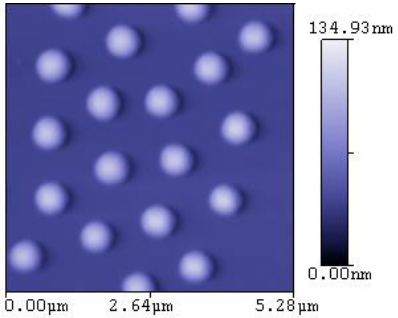
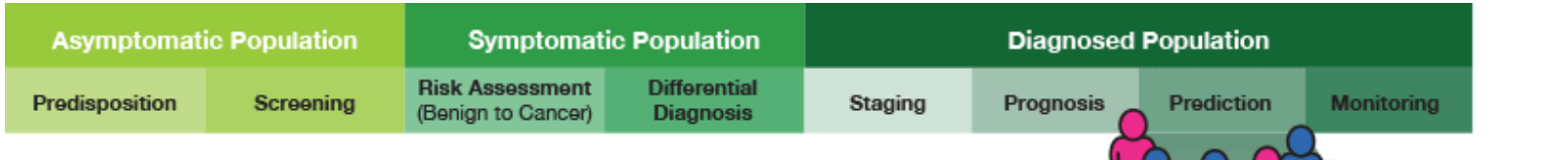
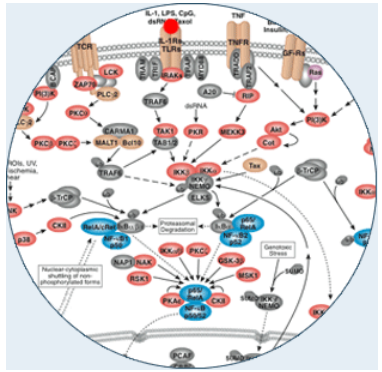
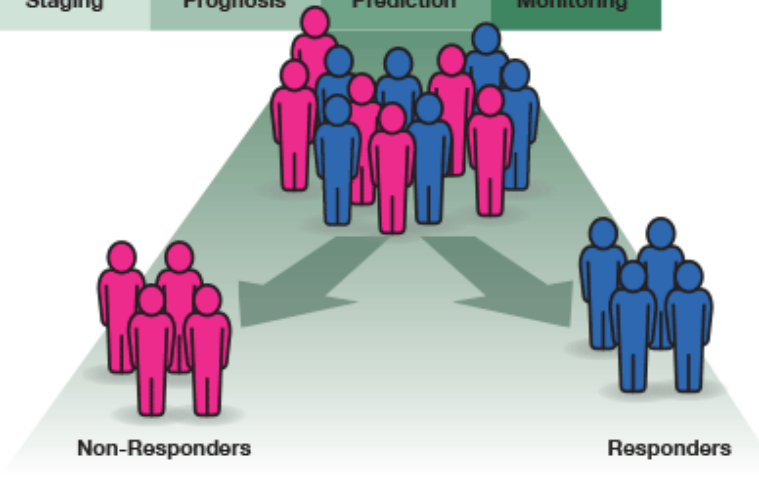


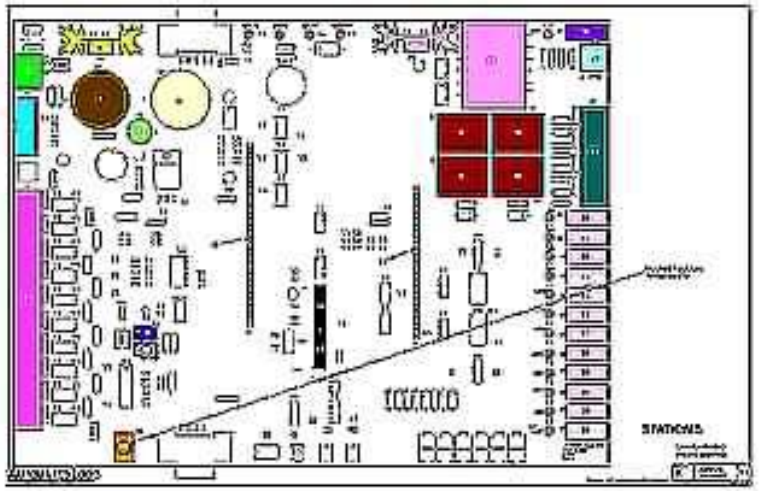








DNA: The “blueprint”/the ”wiring diagram”



PROTEINS: The working machinery of the cell

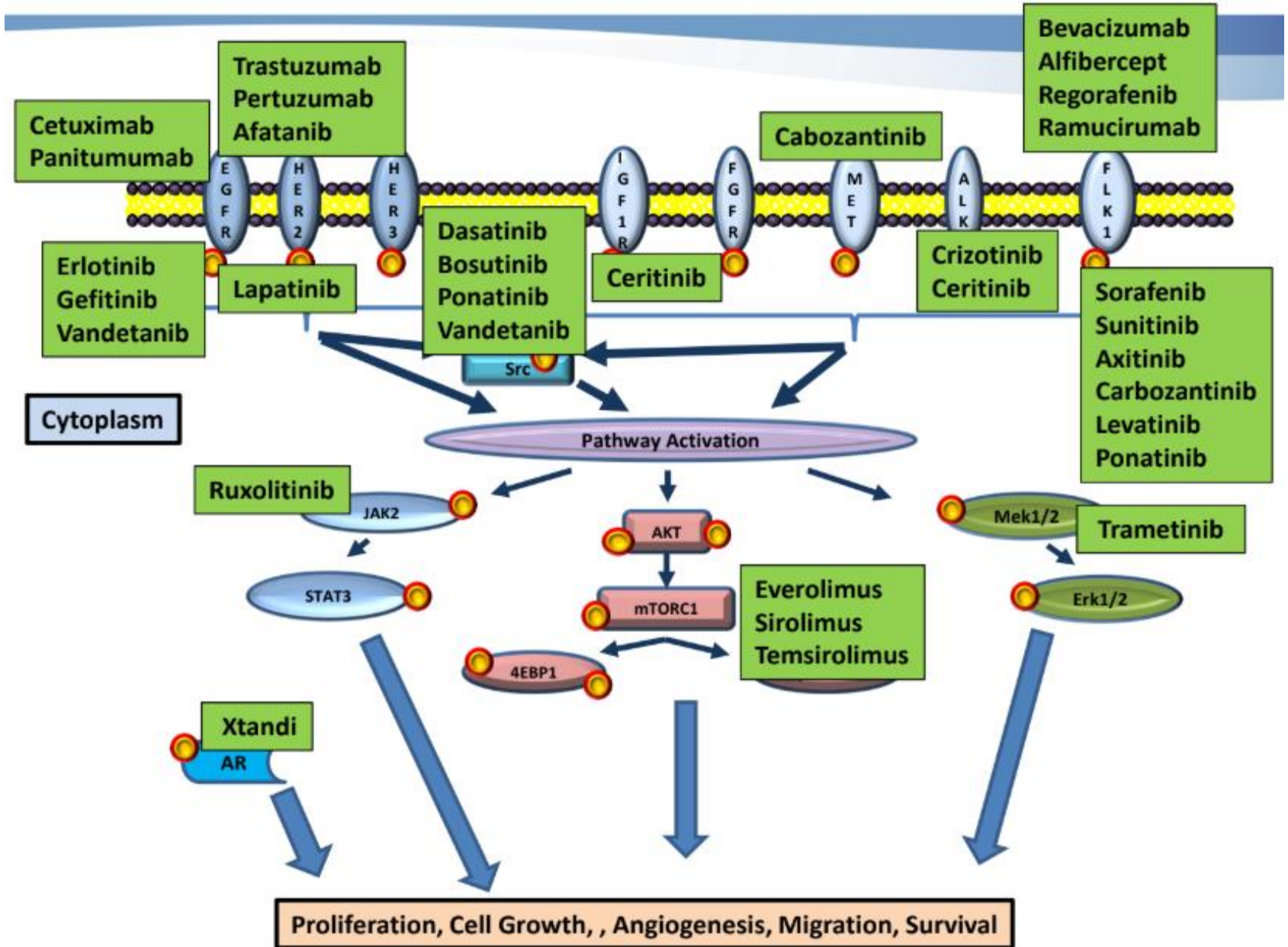
- **Most often the drug target itself** (Capivasertib targets AKT protein kinase activity)
- **Sometimes the therapy** (Enhertu is a protein-drug conjugate, Cetuximab is a protein (antibody).

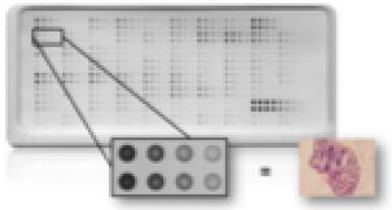
12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

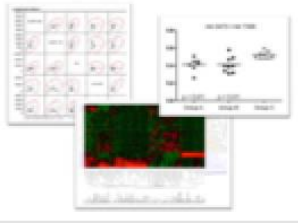
Capivasertib is an inhibitor of all 3 isoforms of serine/threonine kinase AKT (AKT1, AKT2 and AKT3) and inhibits phosphorylation of downstream AKT substrates. AKT activation in tumors is a result of activation of upstream signaling pathways, mutations in *AKT1*, loss of phosphatase and tensin homolog (PTEN) function and mutations in the catalytic subunit alpha of phosphatidylinositol 3-kinase (*PIK3CA*).

Precision cancer therapeutics target *proteins*

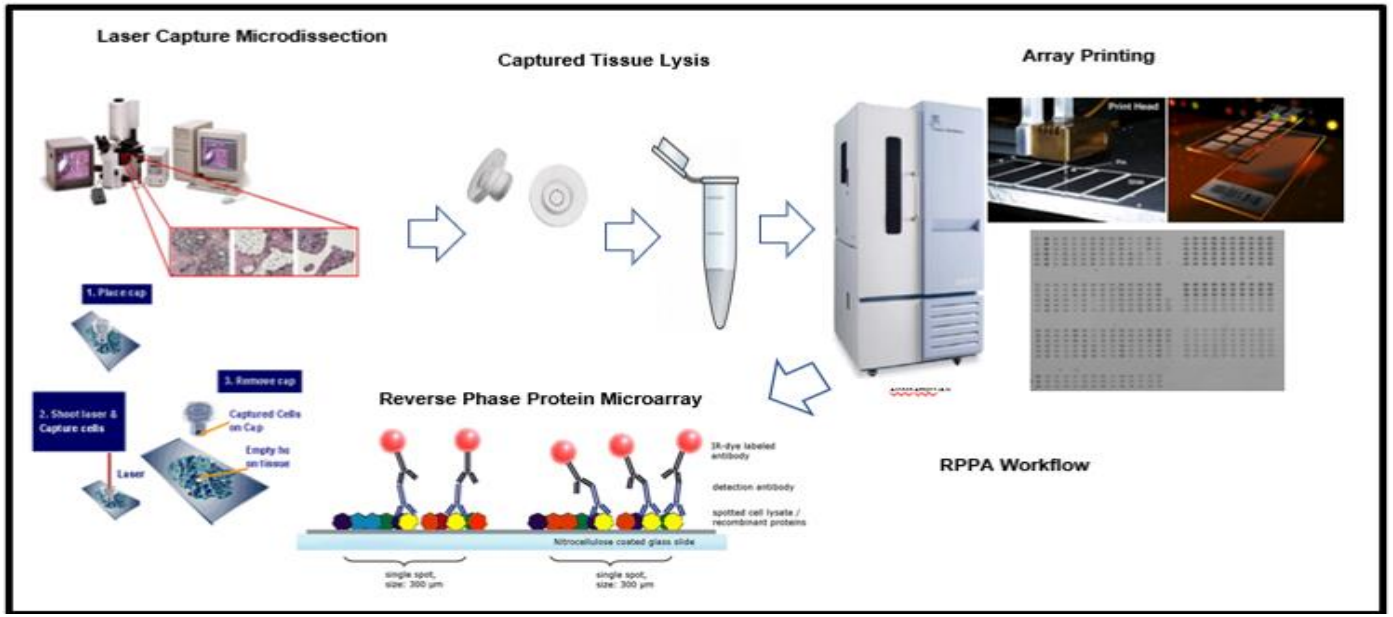




RPPA: Multiplexed Mapping of the Drug Target Activation Landscape



- **Multiplex quantitative measurement of phosphoprotein epitopes or protein analytes in small numbers of cultured cells or tissue (biopsy or LCM):**
  - **Low abundance signal pathway proteins and transcription factors not accessible by mass spectrometry**
  - **Requires only one antibody (no antibody sandwich) per analyte: often an anti-peptide antibody derived from the gene sequence.**
  - **Quantitation, sensitivity, and multiplex capacity vastly exceeds western blotting or IHC.**
  - **Can utilize FFPE CNB/small surgical samples tissue processed under normal pathology SOP**



4E-BP1 (S65)
4E-BP1 (T37/46)
4E-BP1 (T70)
4G10 (anti Phosphotyrosine)
c-Abl (T735)
c-Abl (Y245)
Acetyl-CoA Carboxylase (S79)
Adducin (S662)
AFX (S193)
Akt (S473)
Akt (S473)
Akt (S473) (587F11)
Akt1/PKB alpha (S473) (SK703)
Akt (S473) (736E11)
Akt (T308)
Akt (Y326)
ALK (Y1604)
AMPKalpha (T172)
AMPKalpha1 (S485)
AMPKbeta1 (S108)
AMPKbeta1 (S182)
Arrestin (beta) 1 (S412) (6-24)
ASK1 (S83)
ASK1 (T845)
ATF-2 (T71)
ATF-2 (T69/71)
Aurora A (T288)/B (T232)/C (T198)
Bad (S112)
Bad (S112) (7E11)
Bad (S136)
Bad (S155)
Bcl-2 (S70) (5H2)
Bcl-2 (T56)
Bcr (Y177)
BLNK (Y96)
Btk (S180) (7A12)
Caspase-3, cleaved (D175)
Caspase-3, cleaved (D175) (5A1)
Caspase-6, cleaved (D162)
Caspase-7, cleaved (D198)
Caspase-8, cleaved (D374)
Caspase-9, cleaved (D315)
Caspase-9, cleaved (D330)
Catenin (beta) (S45)
Catenin (beta) (S33/37/T41)

Catenin (beta) (T41/S45)
Caveolin-1 (Y14)
c-Cbl (Y731)
c-Cbl (Y774)
CD19 (Y513)
Chk1 (S345)
Chk2 (S33/35)
Chk2 (T68) (80F5)
Cofilin (S3)
Cofilin (S3) (77G2)
Connexin 43 (S368)
CREB (S133)
CREB (S133) (1B6)
CrkII (Y221)
Cyclin B1 (S147)
DFF45, cleaved (D224)
eEF2 (T56)
EGFR (S1046/1047)
EGFR (S1047) (1H9)
EGFR (T654) (3F2)
EGFR (Y845)
EGFR (Y992)
EGFR (Y1045)
EGFR (Y1068)
EGFR (Y1068) (1H12)
EGFR (Y1148)
EGFR (Y1148)
EGFR (Y1173)
pEGFR (Y1173) (9H2)
EGFR (Y1173) (53A3)
eIF2alpha (S51)
eIF2alpha (S51) (119A11)
eIF4E (S209)
eIF4G (S1108)
Elk-1 (S383)
eNOS (S113)
eNOS (S1177)
eNOS (S1177)
eNOS (T495)
eNOS/NOS III (S116)
Ephrin B (Y324/329)
ErbB2/HER2 (Y877)
ErbB2/HER2 (Y1221/1222)
ErbB2/HER2 (Y1248)
ErbB2/HER2 (Y1248)
ErbB2/HER2 (Y1248)/EGFR (Y1173)
ErbB3/HER3 (Y1222) (50C2)
ErbB3/HER3 (Y1289) (21D3)
ERK 1/2 (T202/Y204)
ERK 1/2 (T202/Y204) (E10)
Estrogen Receptor alpha (S118)
Estrogen Receptor alpha (S118) (16JR)

Etk (Y40)
Ezrin (Y353)
Ezrin (T567)/Radixin (T564)/Moesin (T558)
FADD (S194)
FAK (Y397) (18)
FAK (Y576/577)
FAK (Y925)
FCgamma Rec IIb (Y292)
FGF Receptor (Y653/654)
FHIT (Y114)
FKHR (S256)
FKHRL1 (S253)
FKHR (T24)/FKHRL1 (T32)
FLT3 (Y591) (54H1)
alpha-Fodrin, cleaved (D1185)
FRS2-alpha (Y436)
Gab1 (Y627)
Gab2 (S159)
Gab2 (Y452)
GCN2 (T898)
Glucocorticoid Receptor (S211)
GSK-3alpha (S21) (46H12)
GSK-3alpha/beta (S21/9)
GSK-3alpha (Y279)/beta (Y216)
GSK-3beta (S9)
Histone H3 (S10) Mitosis Marker
Histone H3 (S28)
Histone H3 (T11)
HSP27 (S15)
IGF-1 Rec (Y1131)/Insulin Rec (Y1146)
IGF-1R (Y1135/36)/IR (Y1150/51) (19H7)
IkappaB-alpha (S32)
IkappaB-alpha (S32) (14D4)
IkappaB-alpha (S32/36) (5A5)
IkappaB-alpha (S32/36) (39A1431)
IKKalpha (S176)/IKKbeta (S180)
IKKalpha (S180)/IKKbeta (S181)
IL-1beta, cleaved (D116)
IRAK1 (S376)
IRS-1 (S302)
IRS-1 (S307)
IRS-1 (S612)
IRS-1 (S636/639)
IRS-1 (S789)
IRS-1 (S1101)
IRS-1 (Y612)
Jak1 (Y1022/1023)
Jak2 (Y221)
Jak2 (Y1007/1008)
c-Jun (S63) II
c-Kit (Y703)
c-Kit (Y719)
c-Kit (Y721)
Lamin A, cleaved (D230)
LAT (Y171)
LAT (Y191)
Lck (Y192)
Lck (Y505)
Lck (Y505)
LIMK1 (T508)/LIMK2 (T505)
LKB1 (S334)
LKB1 (S428)
LKB1 (T189)
Lyn (Y507)
MAPK (pTEpY)
MAPKAPK-2 (T334)
MARCKS (S152/156)
M-CSF Receptor (Y723)
MDM2 (S166)
MEK1 (S298)
MEK1/2 (S217/221)
Met (Y1234/1235)
MKK3/MKK6 (S189/207)

Mnk1 (T197/202)
MSK1 (S360)
Mst1 (T183)/Mst2 (T180)
mTOR (S2448)
mTOR (S2481)
c-Myc (T58/S62)
Myosin Light Chain 2 (T18/S19)
NF-kappaB p65 (S536)
NPM (T199)
p27 (T187)
p27 (T187) (2B10B7)
p38 MAP Kinase (T180/Y182)
p40 phox (T154)
p56Dok-2 (Y351)
p70 S6 Kinase (S371)
p70 S6 Kinase (T389)
p70 S6 Kinase (T412)
p70 S6 Kinase (T421/S424)
p90RSK (S380)
p130 Cas (Y165)
PAK1 (S144)/PAK2 (S141)
PAK1 (S199/204)/PAK2 (S192/197)
PAK1 (T423)/PAK2 (T402)
PAK2 (S20)
PAK4 (S474)/PAK5 (S602)/PAK6 (S560)
PARP, cleaved (D214)
PARP, cleaved (D214) (19F4)
Paxillin (Y118)
PDGF Receptor alpha (Y754) (23B2)
PDGF Receptor beta (Y716)
PDGF Receptor beta (Y751)
PDK1 (S241)
PI3-Kinase p85(Y458)/p55(Y199)
PKA C (T197)
PKC alpha (S657)
PKC alpha/beta II (T638/641)
PKC (pan) (betaII S660)
PKC delta (T505)
PKC theta (T538)
PKC zeta/lambda (T410/403)
PKR (T446)
cPLA2 (S505)
PLCgamma1 (Y783)
PLCgamma2 (Y759)
PLD1 (S561)
PLK1 (T210)
PRAS40 (T246)
PRK1 (T774)/PRK2 (T816)
Progesterone Receptor (S190)
PTEN (S380)
Pyk2 (Y402)
Rac1/cdc42 (S71)
Raf (S259)
A-Raf (S299)

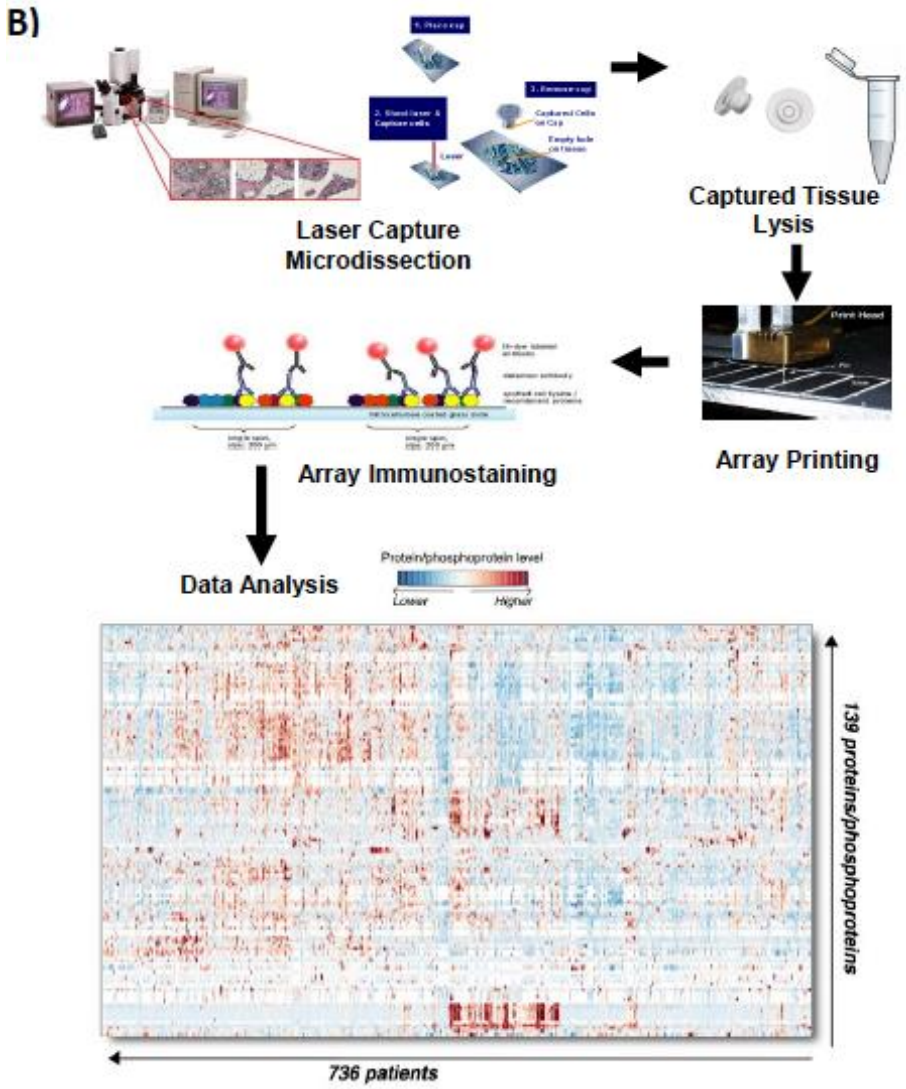
B-Raf (S445)
c-Raf (S338) (56A6)
Ras-GRF1 (S916)
Ret (Y905)
RSK3 (T356/S360)
S6 Ribosomal Protein (S235/236) (2F9)
S6 Ribosomal Protein (S240/244)
SAPK/JNK (T183/Y185)
SEK1/MKK4 (S80)
SGK (S78)
Shc (Y317)
Shc (Y317)
SHIP1 (Y1020)
SHP2 (Y542)
SHP2 (Y580)
Smad1 (S/S)/Smad5 (S/S)/Smad8 (S/S)
Smad2 (S465/467)
Smad2 (S245/250/255)
Smad3 (S433/435)
Src Family (Y416)
Src (Y527)
SRF (S103)
Stat1 (S727)
Stat1 (S727)
Stat1 (Y701)
Stat1 (Y701)
Stat2 (Y689)
Stat3 (S727)
Stat3 (S727)
Stat3 (Y705) (9E12)
Stat3 (Y705) (58E12)
Stat5 (Y694)
Stat6 (Y641)
Syk (Y323)
Syk (Y525/526)
TAK1 (T184)
TAK1 (T184/187)
Tie2 (S1119)
Tie2 (Y992)
Tpl2 (S400)
Troponin I (Cardiac) (S23/24)
Tuberin/TSC2 (Y1571)
Tyk2 (Y1054/1055)
VASP (S157)
VASP (S239)
VEGFR 2 (Y951)
VEGFR 2 (Y996)
VEGFR 2 (Y1175) (19A10)
WNK1 (T60)
Zap-70 (Y315/319)
Zap-70 (Y493)
Zap-70 (Y319)/Syk (Y352)

MEASURE ALL OF THESE IN

Reverse phase protein (RPPA) analysis in I-SPY2

- Protein/phosphoprotein (RPPA) data from pre-treatment laser micro-dissected (LMD) tumor epithelium

- 139 key cancer signaling proteins/phosphoproteins from pathways:
  - hormone receptor (n=4),
  - HER family (n=14),
  - cell cycle/proliferation (n=20),
  - immune (n=18),
  - DNA repair deficiency (DDR; n=15)
  - AKT/mTOR/PI3K (n=7),
  - apoptosis/autophagy (n=10)
  - IGF1R (n=6)
  - TIE/ANG (n=4)
  - growth/survival/metabolism (n=22)
  - RTK (n=19) pathways.



139 proteins/phosphoproteins profiled

ER/AR Hormone

Hormone Rec	Androgen Rec S650
Hormone Rec	Androgen Rec total
Hormone Rec	Estrogen Rec alpha S118
Hormone Rec	Estrogen Rec alpha total

HER

HER Family	EGFR total
HER Family	EGFR Y1068
HER Family	EGFR Y1148
HER Family	EGFR Y1173
HER Family	EGFR Y992
HER Family	ERBB2 total
HER Family	ERBB2 Y1248
HER Family	ERBB2 Y877
HER Family	ERBB3 total
HER Family	ERBB3 Y1289
HER Family	ERBB4 total
HER Family	ERBB4 Y1284
HER Family	Heregulin total
HER Family	SHC Y317

AKT/mTOR/PI3K

AKT/mTOR/PI3K	AKT S473
AKT/mTOR/PI3K	AKT T308
AKT/mTOR/PI3K	mTOR S2448
AKT/mTOR/PI3K	mTOR total
AKT/mTOR/PI3K	PI3K p85 Y458 p55 Y199
AKT/mTOR/PI3K	PTEN S380
AKT/mTOR/PI3K	PTEN total

IGF1R

IGF1R	IGF1R total
IGF1R	IGF1R Y1131 IR Y1146
IGF1R	IGF1R Y1135 Y1136 IR Y1150 Y
IGF1R	IGFBP5 total
IGF1R	Insulin Rec beta total
IGF1R	IRS1 S612

Immune

Immune	CD3 epsilon
Immune	CD3 zeta
Immune	HLA DR DP DQ DX total
Immune	HLA DR total
Immune	JAK1 Y1022 Y1023
Immune	JAK2 Y1007
Immune	PD1 Nivolumab
Immune	PD1 Pembrolizumab
Immune	PDL1 E1L3N
Immune	PDL1 SP142
Immune	PDL1 22C3
Immune	PDL1 28 8
Immune	PDL1 Atezolizumab
Immune	STAT1 Y701
Immune	STAT3 S727
Immune	STAT3 Y705
Immune	STAT5 Y694
Immune	TYK2 Y1054 Y1055

TIE2/ANG

TIE/ANG	TIE2 S1119
TIE/ANG	TIE2 total
TIE/ANG	TIE2 Y992
TIE/ANG	VEGFR2 Y996

Apoptosis/autophagy

Apoptosis/Autophagy	BAD S136
Apoptosis/Autophagy	Caspase 3 cleaved D 175
Apoptosis/Autophagy	Caspase 7 cleaved D198
Apoptosis/Autophagy	Caspase 9 cleaved D330
Apoptosis/Autophagy	FADD S194
Apoptosis/Autophagy	IkBa S32 S36
Apoptosis/Autophagy	LC3B total
Apoptosis/Autophagy	MDM2 S166
Apoptosis/Autophagy	NFkB p65 S536
Apoptosis/Autophagy	Survivin total

DNA Damage Repair (DDR)

DDR	ATM S1981
DDR	ATR S428
DDR	BRCA1 S1524
DDR	CHK1 S345
DDR	CHK2 S33 S35
DDR	DNAPK T2609
DDR	H2A X S139
DDR	MLH1 total
DDR	MSH2 total
DDR	MSH6 total
DDR	p53 S15
DDR	p53 total
DDR	PARP cleaved D214
DDR	PARP total
DDR	PLK1 T210

Cell cycle/proliferation

Cell Cycle/Proliferation	A RAF S299
Cell Cycle/Proliferation	Aurora A T288 B T232 C T198
Cell Cycle/Proliferation	B RAF S445
Cell Cycle/Proliferation	C RAF S338
Cell Cycle/Proliferation	Cofilin S3
Cell Cycle/Proliferation	CREB S133
Cell Cycle/Proliferation	Cyclin A2 total
Cell Cycle/Proliferation	Cyclin B1 total
Cell Cycle/Proliferation	Cyclin D1 total
Cell Cycle/Proliferation	E cadherin total
Cell Cycle/Proliferation	Ki67 total
Cell Cycle/Proliferation	MEK1 2 S217 S221
Cell Cycle/Proliferation	MSK1 S360
Cell Cycle/Proliferation	p38 MAPK T180 Y182
Cell Cycle/Proliferation	P90RSK S380
Cell Cycle/Proliferation	RB S780
Cell Cycle/Proliferation	RSK3 T356 S380
Cell Cycle/Proliferation	S6RP S240 S244
Cell Cycle/Proliferation	SRC Y527
Cell Cycle/Proliferation	YAP S127

Growth/metabolism

Growth/Survival/Metabolism	4EBP1 S65
Growth/Survival/Metabolism	4EBP1 T70
Growth/Survival/Metabolism	Acetyl CoA Carboxylase S79
Growth/Survival/Metabolism	AMPKa1 S485
Growth/Survival/Metabolism	AMPKb1 S108
Growth/Survival/Metabolism	B catenin S33 S37 T41
Growth/Survival/Metabolism	eIF4E S209
Growth/Survival/Metabolism	eIF4G S1108
Growth/Survival/Metabolism	eNOS NOSIII S116
Growth/Survival/Metabolism	eNOS S113
Growth/Survival/Metabolism	ERK1 2 T202 Y204
Growth/Survival/Metabolism	FOXO1 S256
Growth/Survival/Metabolism	FOXO1 T24 FOXO3a T32
Growth/Survival/Metabolism	FOXO3a S253
Growth/Survival/Metabolism	GSK3aB S21 S9
Growth/Survival/Metabolism	Histone H3 S10
Growth/Survival/Metabolism	p27 T187
Growth/Survival/Metabolism	p70S6K S371
Growth/Survival/Metabolism	p70S6K T389
Growth/Survival/Metabolism	p70S6K T412
Growth/Survival/Metabolism	SGK S78
Growth/Survival/Metabolism	Tuberin TSC2 Y1571

RTK

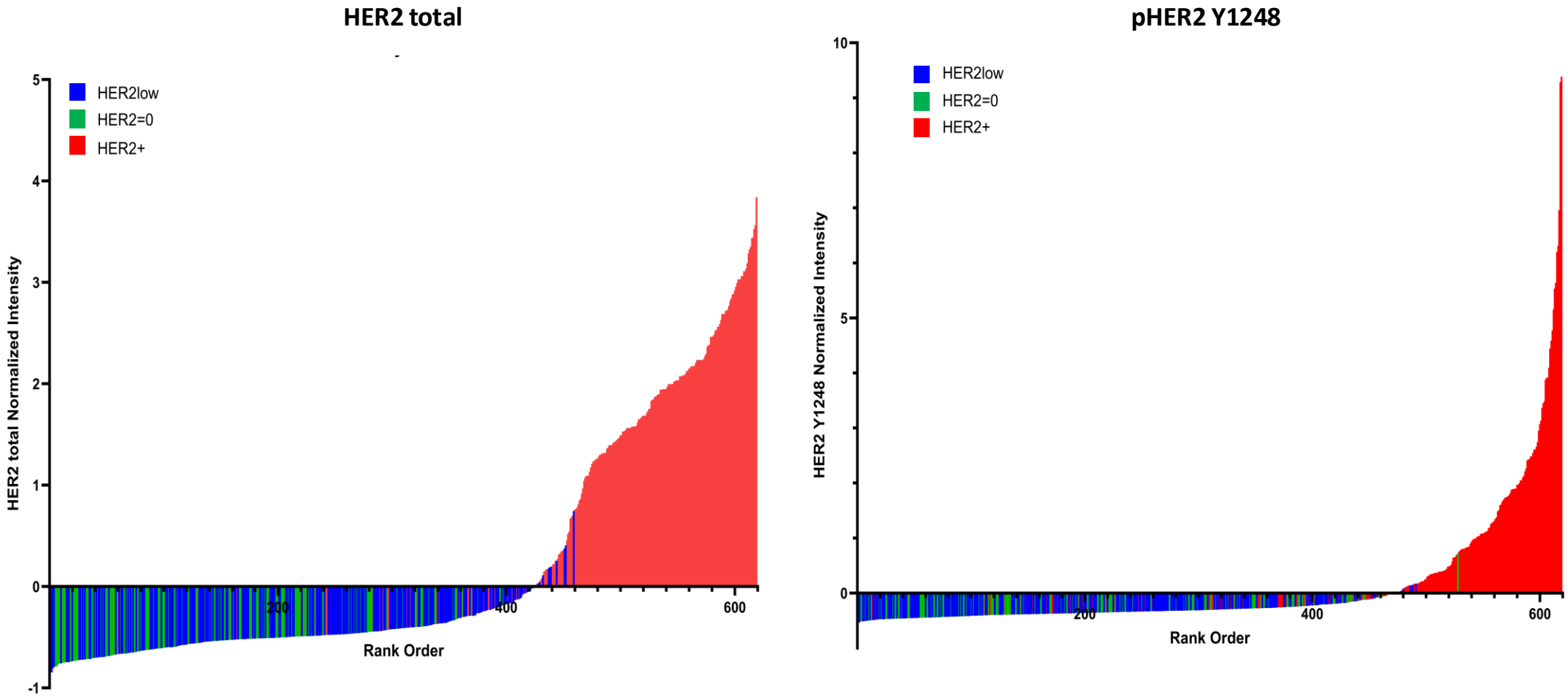
RTK	ALK total
RTK	ALK Y1586
RTK	ALK Y1604
RTK	cABL T735
RTK	cABL Y245
RTK	cKIT Y703
RTK	Ephrin A3 Y799 A4 Y799 A5 Y8
RTK	FAK Y397
RTK	FAK Y576 Y577
RTK	MCSF Rec Y723
RTK	MET total
RTK	MET Y1234 Y1235
RTK	PDGFRa Y754
RTK	PDGFRb Y751
RTK	RET Y905
RTK	RON Y1353
RTK	ROS Y2274
RTK	RTK ROR1 total
RTK	ZAP70 Y319 SYK Y352

An alternative to HER2 IHC 0/1+/2+ status to predict which clinically HER2-negative patients will respond to anti-HER2 therapies: A rationale for the likely superiority of quantitative HER2 pathway RPPA measurements

Julia Wulfkuhle[1], Denise M Wolf[2], Angela DeMichele[3], Christina Yau[2], Laura van 't Veer[2], Hope Rugo[2], Lajos Pusztai[4], I-SPY2 Investigators[5], Gillian Hirst[2], Rosa I Gallagher[1], Amy Delson[5], Alexander Borowsky[6], Laura J Esserman[2], Paula Pohlmann[7], Emanuel F. Petricoin[1]

[1]George Mason University; [2]University of California, San Francisco; [3]University of Pennsylvania; [4]Yale University; [5]Quantum Leap Healthcare Collaborative; [6]University of California, Davis; [7]MD Anderson Cancer Center

RPPA-BASED MEASUREMENTS OF TOTAL HER2 AND PHOSPHO-HER2 SHOW EXCELLENT CONCORDANCE WITH CLINICAL IHC-DETERMINED HER2



Waterfall plots of total HER2 **(A)** and pHER2 Y1248 **(B)** for dataset including all patients/subtypes across 8 arms of I-SPY2 TRIAL colorized by HER2+ (red); HER2 0 (green); HER2low (blue).

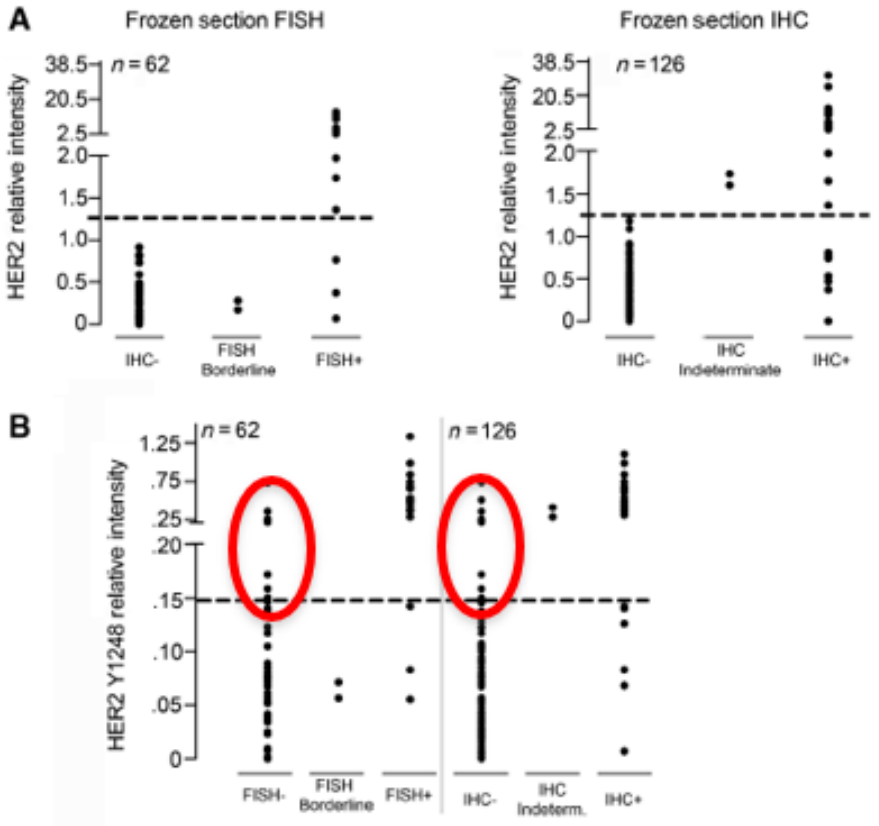
HER2 is activated even in HER2- (0-1+) patients!

uman Cancer Biology

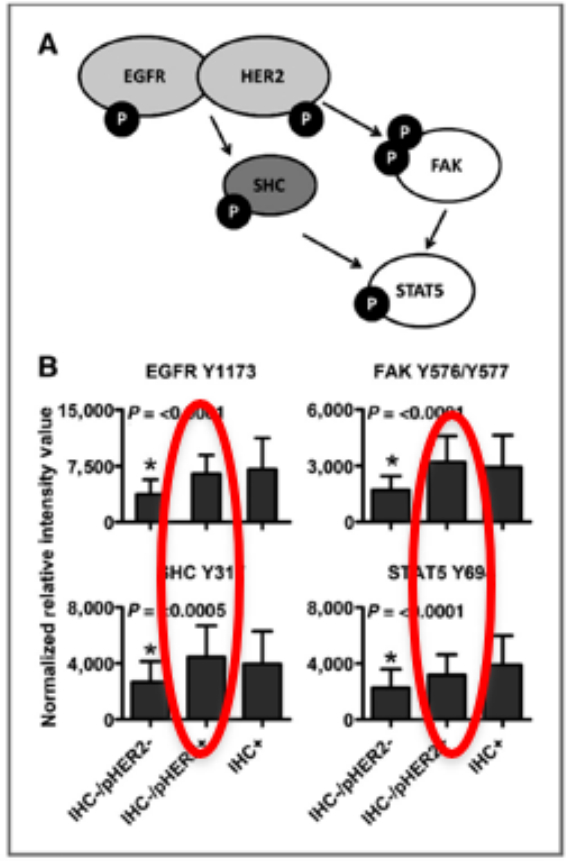
Clinical Cancer Research

lolecular Analysis of HER2 Signaling in Human Breast ancer by Functional Protein Pathway Activation Mapping

lia D. Wulfkuhle[1], Daniela Berg[2], Claudia Wolff[2], Rupert Langer[2], Kai Tran[2], Julie III[4], Virginia Espina[1], ariaelena Pierobon[1], Jianghong Deng[1], Angela DeMichele[5], Axel Walch[6], Holger Bronger[3], grid Becker[7], Christine Waldhör[8], Heinz Höfler[2], Laura Esserman[4], on behalf of the I-SPY 1 TRIAL vestigators, Lance A. Liotta[1], Karl-Friedrich Becker[2], and Emanuel F. Petricoin III[1]



~20% of FISH/IHC- are pHER2+ and are pathway activated



Dual activation/phosphorylation of HER2 and EGFR predict response in HER2 -/LOW setting Signature present in > 40% of TNBC (HER2 0/1+) with pCR rate of ~80%

original report

Evaluation of the HER/PI3K/AKT Family Signaling Network as a Predictive Biomarker of Pathologic Complete Response for Patients With Breast Cancer Treated With Neratinib in the I-SPY 2 TRIAL

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abstract

Purpose In the I-SPY 2 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis 2), the pan–erythroblastic oncogene B inhibitor neratinib was available to all hormone receptor (HR)/human epidermal growth factor receptor 2 (HER2) subtypes and graduated in the HR-negative/HER2-positive signature. We hypothesized that neratinib response may be predicted by baseline HER2 epidermal growth factor receptor (EGFR) signaling activation/phosphorylation levels independent of total levels of HER2 or EGFR proteins.

Materials and Methods Complete experimental and response data were available for between 130 and 193 patients. In qualifying analyses, which used logistic regression and treatment interaction analysis, 18 protein/phosphoprotein, 10 mRNA, and 12 DNA biomarkers that related to *HER* family signaling were evaluated. Exploratory analyses used Wilcoxon rank sum and *t* tests without multiple comparison correction.

Results *HER* pathway DNA biomarkers were either low prevalence or nonpredictive. In expression biomarker analysis, only one gene (*STMN1*) was specifically associated with response to neratinib in the HER2-negative subset. In qualifying protein/phosphoprotein analyses that used reverse phase protein microarrays, six HER family markers were associated with neratinib response. After analysis was adjusted for HR/HER2 status, EGFR Y1173 (pEGFR) showed a significant biomarker-by-treatment interaction ($P = .049$). Exploratory analysis of HER family signaling in patients with triple-negative (TN) disease found that activation of EGFR Y1173 ($P = .005$) and HER2 Y1248 (pHER2) ($P = .019$) were positively associated with pathologic complete response. Exploratory analysis in this pEGFR/pHER2–activated TN subgroup identified elevated levels of estrogen receptor α ($P < .006$) in these patients.

Conclusion Activation of HER family phosphoproteins associates with response to neratinib, but only EGFR Y1173 and *STMN1* appear to add value to the graduating signature. Activation of HER2 and EGFR in TN tumors may identify patients whose diseases respond to neratinib and implies that there is a subset of patients with TN disease who paradoxically exhibit HER family signaling activation and may achieve clinical benefit with neratinib; this concept must be validated in future studies.

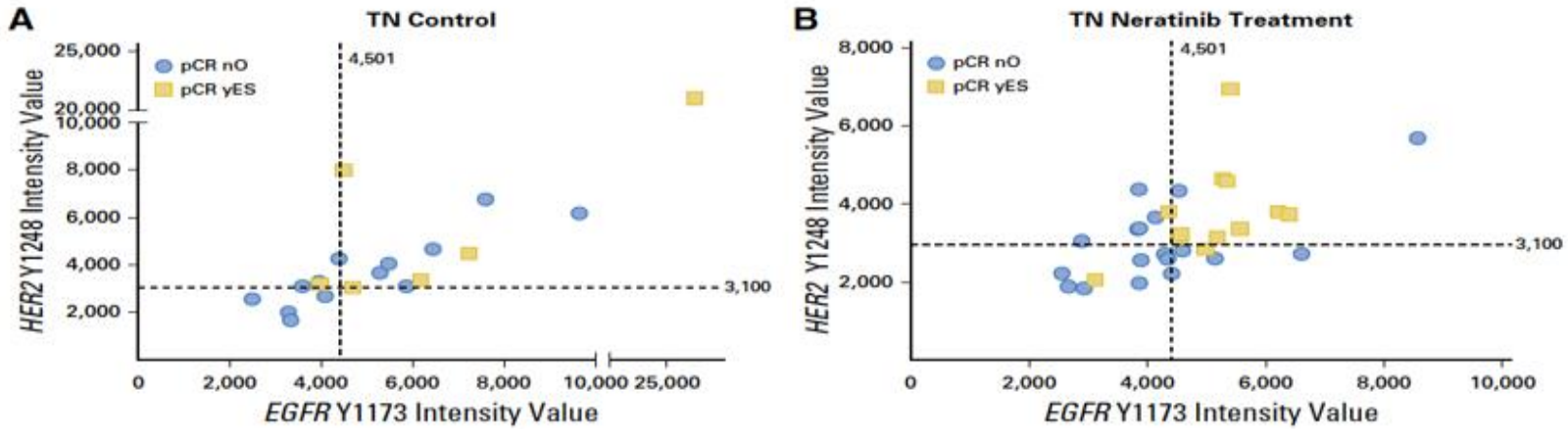


Table A5. Bayesian Probabilities and Biomarker Prevalence for TN Population

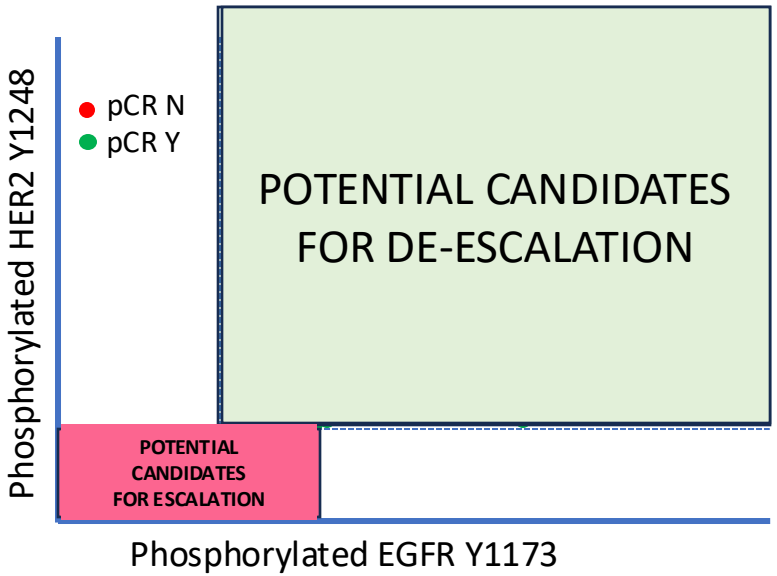
Patient Subset	Probability, Neratinib > Control	Predictive Probability of Phase III success (N = 300)	TN Prevalence (%)
Unselected TN (n = 49)	0.76	0.42	100
TN/EGFR Y1173-high (n = 27)	0.88	0.72	55
TN/HER2 Y1248-high (n = 30)	0.95	0.82	61
TN/EGFR Y1173-high and HER2 Y1248-high (n = 21)	0.99	0.95	43

Abbreviations: EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; TN, triple negative.

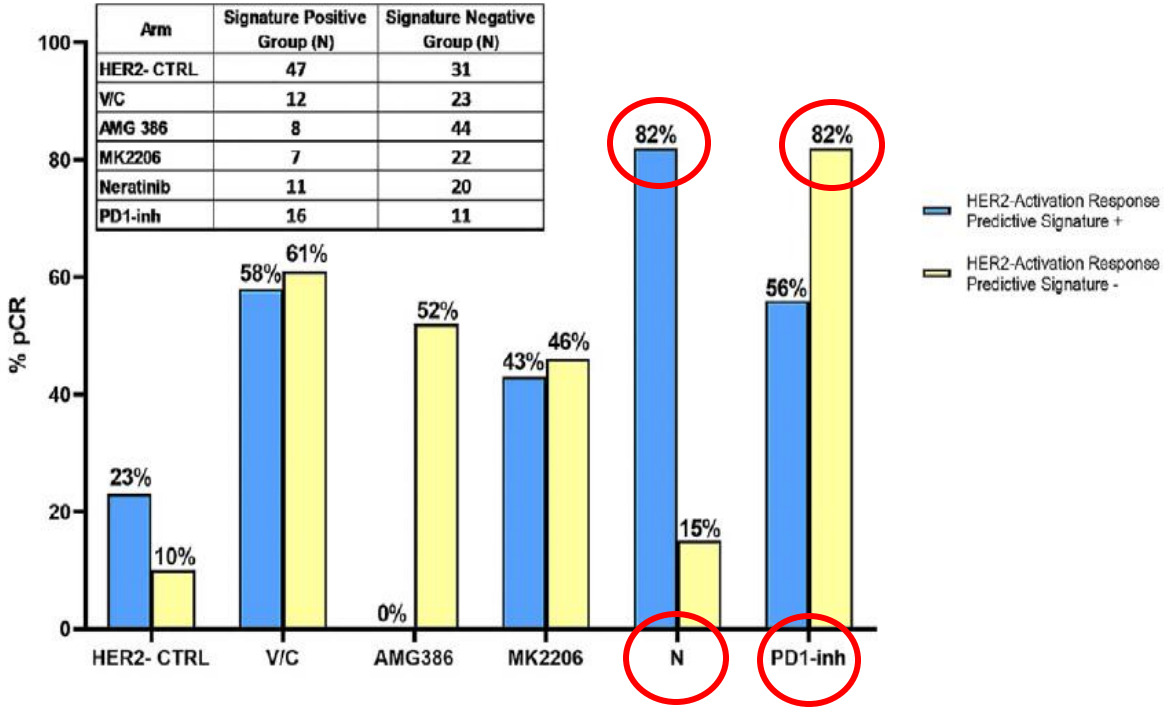
HER2 ACTIVATION RESPONSE PREDICTIVE SIGNATURE (HARPS)

Predicts response to anti-HER2 therapy in both HER2+ and HER2- BC

HER2 POSITIVE BREAST CANCER



TRIPLE NEGATIVE BREAST CANCER

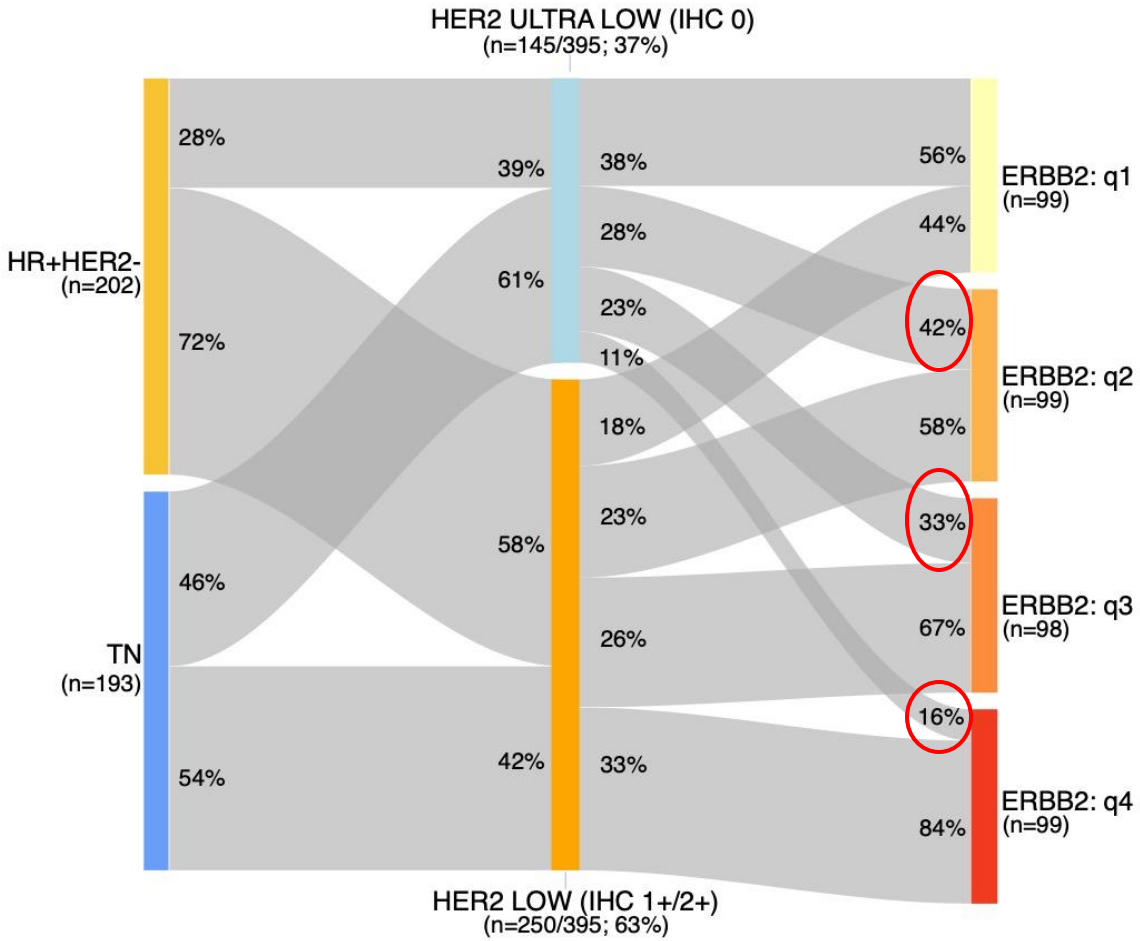


Anti-HER2 Therapy: TDM-1 (ADC)

Pertuzumab (MoAb)

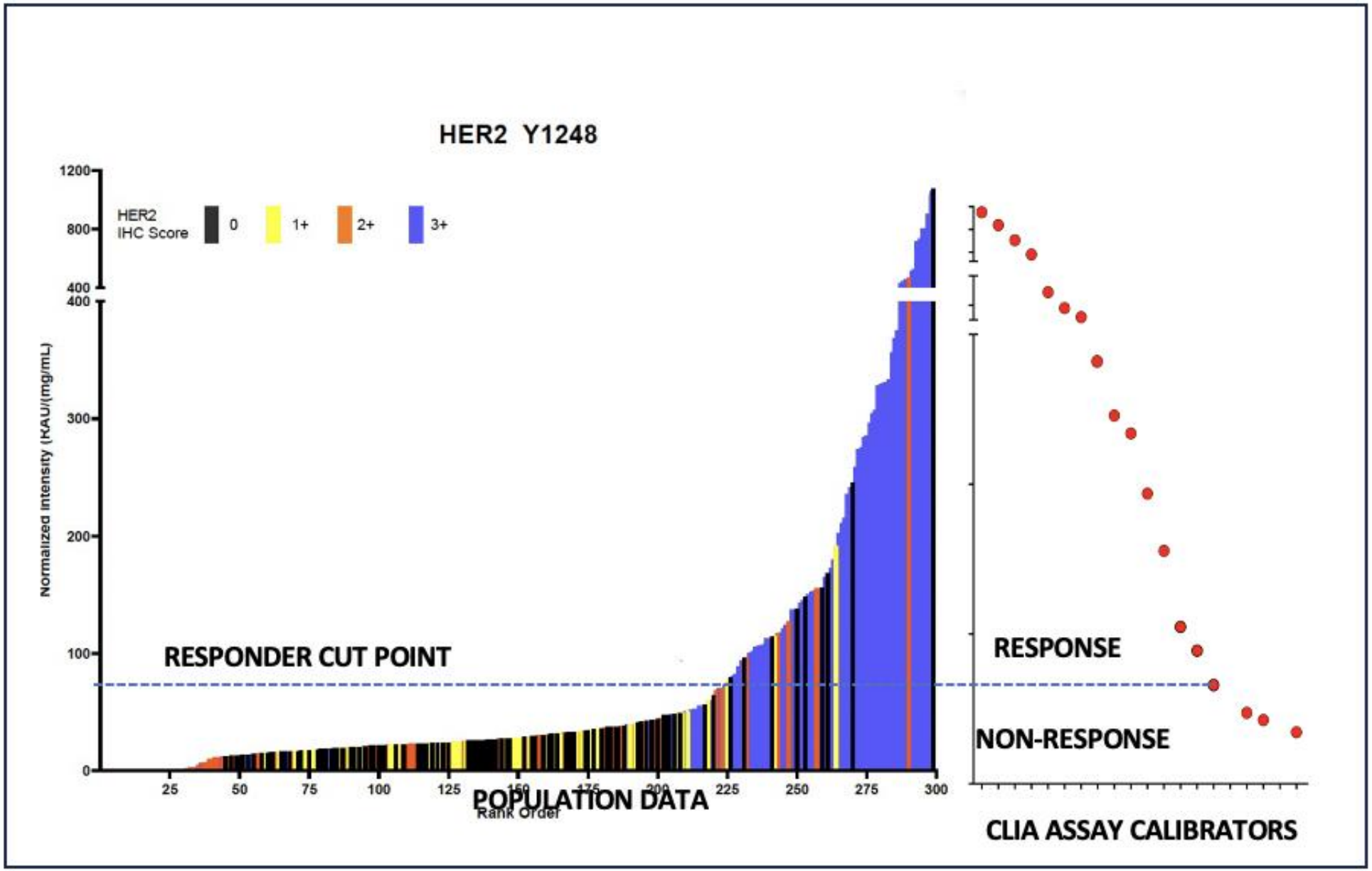
Trastuzumab (MoAb)

RPPA TOTAL HER2 MEASUREMENT IDENTIFIES ADDITIONAL POTENTIAL CANDIDATES FOR T-DXd THERAPY



RPPA quantitative total HER2 measurements found that 62% of HER2 IHC 0/ULTRA LOW ("NULL": e.g. ostensibly 0% staining) tumors express HER2 at levels within the expression range of HER2 LOW tumors from patients that are currently candidates for T-DXd and increases the putative T-DXd response candidate group by 26% (red circles)

QUANTITATIVE TOTAL AND PHOSPHO-HER2 PREDICT RESPONSE TO TDX-d IN HER2 LOW MBC



Overall Survival

<= 25%
>25%-50%
>50%-75%
>75%

months
1 months
6 months
nonths

48

0 (2)
0 (0)
1 (1)
1 (4)

Ignite CLI
response (
HER2 level
Review Annals

predict
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npj | precision oncology

Case report

Published in partnership with The Hormel Institute, University of Minnesota

SEPTEMBER 2024

https://doi.org/10.1038/s41698-024-00696-6

Proteomics based selection achieves complete response to HER2 therapy in HER2 IHC 0 breast cancer

Laura E. Johnston[1], Jamie Randall[1], Safae Chouraichi[1], Mary Luu[1], Allison L. Hunt[2], Lauren Mauro[1], Claudius Mueller[3], Justin B. Davis[3], Emanuel F. Petricoin[4], Thomas P. Conrads[2], Timothy L. Cannon[1] & Jasmine Huynh[1]

patient's insurance company. The patient had an excellent response with near-resolution of her hepatic lesions after four cycles of T-DXd. No measurable disease was observed after nine cycles, including no new brain metastases, consistent with a complete response to therapy

eligible.

Published OnlineFirst December 14, 2021; DOI: 10.1158/1078-0432.CCR-21-2498

Functional Mapping of AKT Signaling and Biomarkers of Response from the FAIRLANE Trial of Neoadjuvant Ipatasertib plus Paclitaxel for Triple-Negative Breast Cancer

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ABSTRACT

Purpose: Despite extensive genomic and transcriptomic profiling, it remains unknown how signaling pathways are differentially activated and how tumors are differentially sensitized to certain perturbations. Here, we aim to characterize AKT signaling activity and its association with other genomic or IHC-based PI3K/AKT pathway biomarkers as well as the clinical activity of ipatasertib (AKT inhibitor) in the FAIRLANE trial.

Experimental Design: In FAIRLANE, 151 patients with early triple-negative breast cancer (TNBC) were randomized 1:1 to receive paclitaxel with ipatasertib or placebo for 12 weeks prior to surgery. Adding ipatasertib did not increase pathologic complete response rate and numerically improved overall response rate by MRI. We used reverse-phase protein microarrays (RPPA) to examine the total level and/or phosphorylation states of over 100 proteins in various signaling or cell processes including PI3K/AKT and mTOR signaling. One hundred and twenty-five baseline and 127 on-treatment samples were evaluable by RPPA, with 110 paired samples at both time points.

Results: Tumors with genomic/protein alterations in PIK3CA/AKT1/PTEN were associated with higher levels of AKT phosphorylation. In addition, phosphorylated AKT (pAKT) levels exhibited a significant association with enriched clinical benefit of ipatasertib, and identified patients who received benefit in the absence of PIK3CA/AKT1/PTEN alterations. Ipatasertib treatment led to a downregulation of AKT/mTORC1 signaling, which was more pronounced among the tumors with PIK3CA/AKT1/PTEN alterations or among the responders to the treatment.

Conclusions: We showed that the high baseline pAKT levels are associated with the alterations of PI3K/AKT pathway components and enriched benefit of ipatasertib in TNBC.

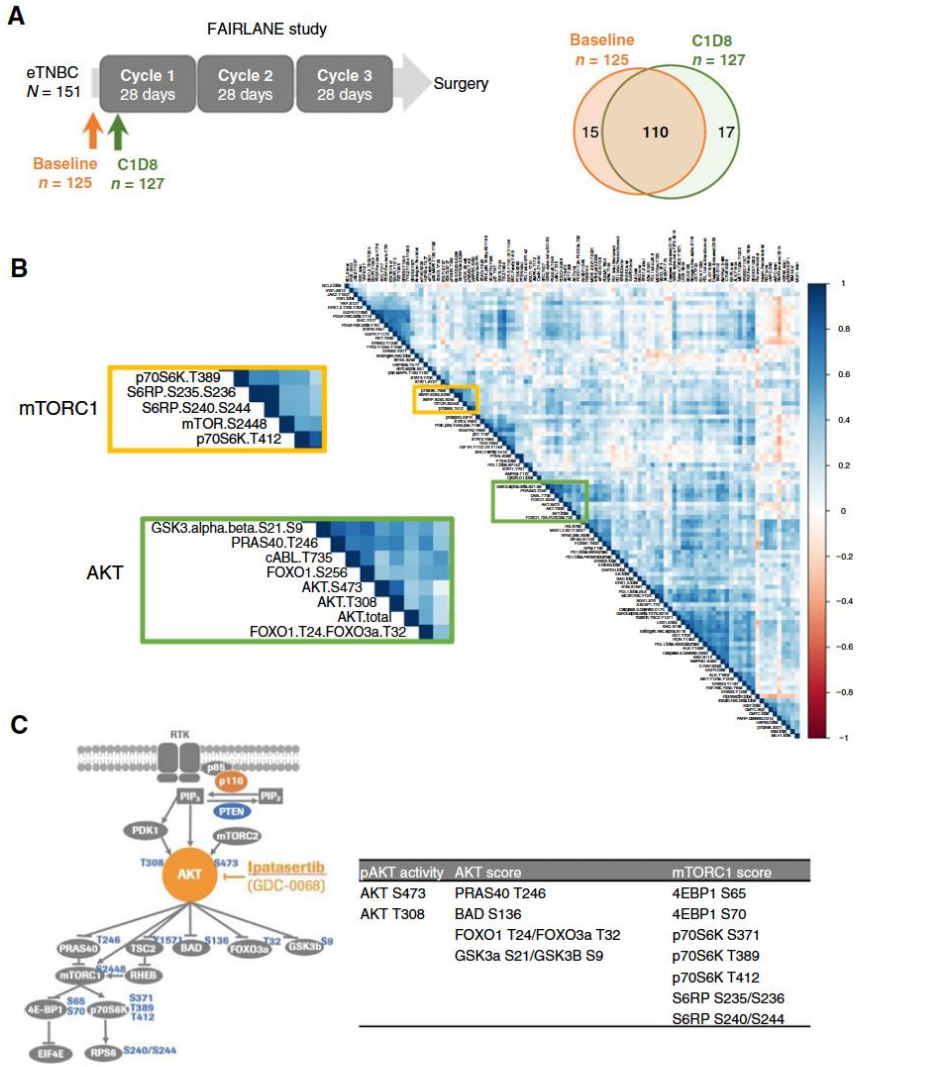
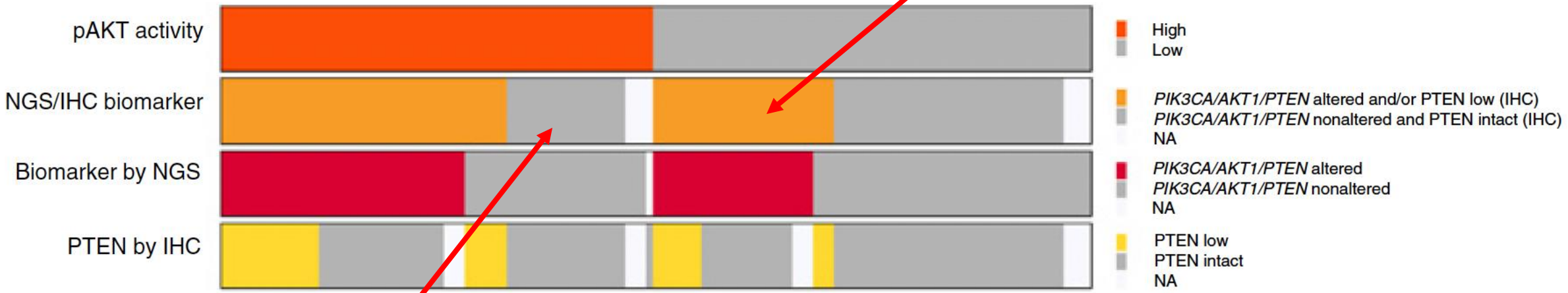


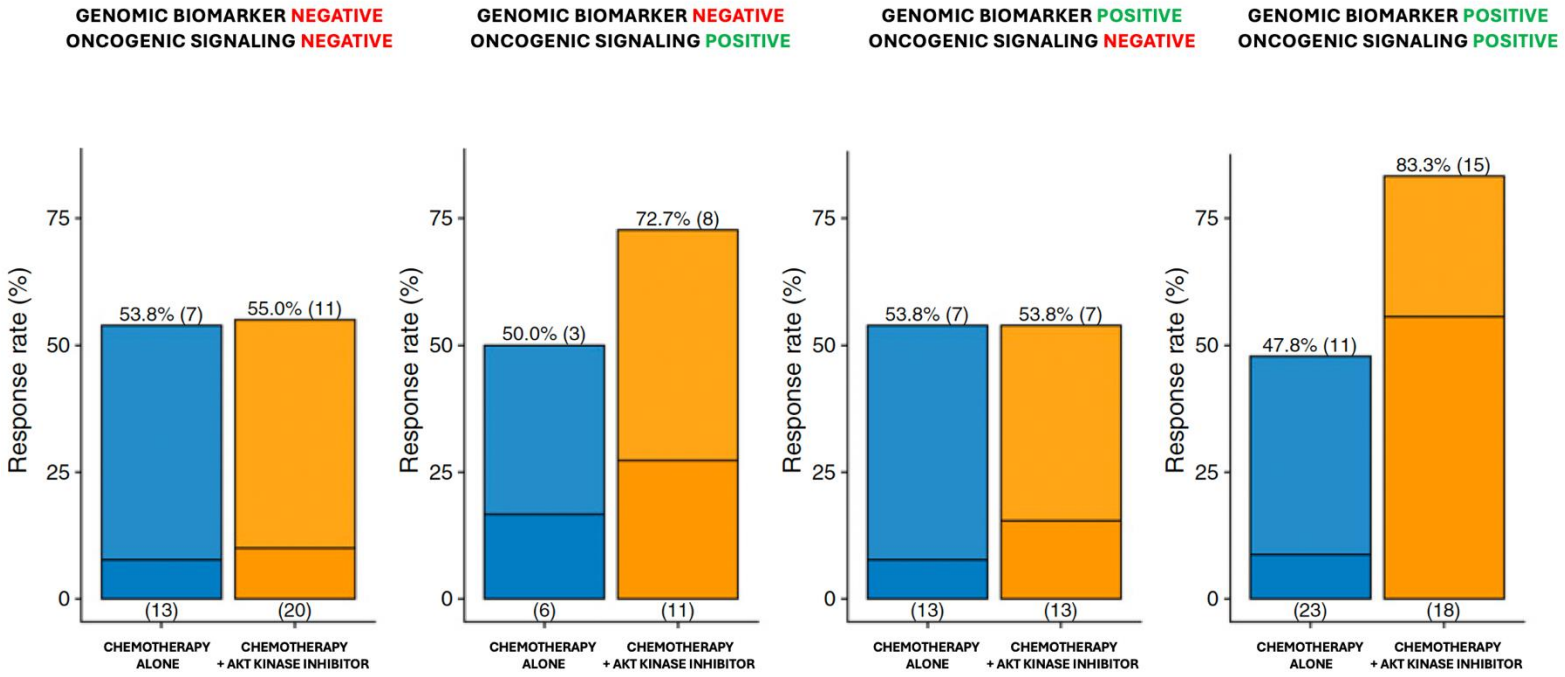
Figure 1.

RPPA analysis of cell signaling proteins from frozen tumor samples in FAIRLANE study. **A,** Schematic showing the collection of baseline and cycle 1 day 8 (C1D8) tumor samples for RPPA analysis. Venn diagram shows the number of the baseline and C1D8 RPPA samples. **B,** Correlation plot showing the pair-wise correlation between all endpoints measured by RPPA at baseline. The AKT and mTORC1 downstream components cluster tightly together shown in the zoomed plot. **C,** Diagram of the AKT/mTORC1 signaling pathway highlighting the phosphorylation sites measured by RPPA in this study. pAKT activity, AKT score, and mTORC1 score were calculated by the phosphorylation levels of AKT itself, AKT, and mTORC1 direct substrates, respectively.

NON-RESPONSIVE PATIENTS GIVEN WRONG THERAPY BECAUSE OF GENOMICS ASSAYS



RESPONSIVE PATIENTS MISSED BY GENOMICS ASSAYS

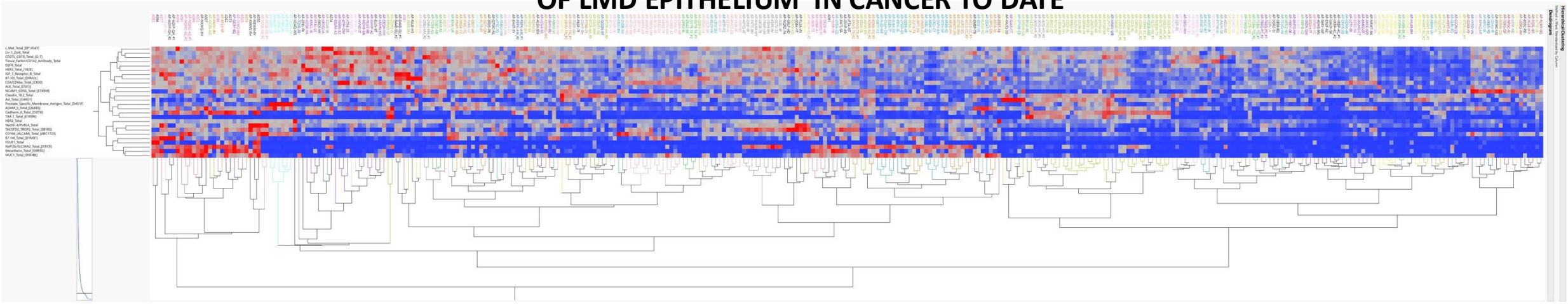


Translational Relevance

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer with poor prognosis and high recurrence and metastasis rate, highlighting the need for more effective therapeutic approaches with appropriate diagnostic biomarkers. Due to the molecular heterogeneity of TNBC, a key aspect for targeted therapy is identifying tumors that are most likely to be sensitive to the specific oncogenic signaling perturbation to maximize the clinical benefit. Here, we showed that PIK3CA/AKT1/PTEN alterations, together with multiple cell signaling activities, modulate the level of phosphorylated AKT (pAKT) on Serine473 and Threonine308. Importantly, tumors with high pAKT levels exhibited the strongest association with enriched ipatasertib activity, suggesting that the pAKT-high tumors are most addicted to AKT signaling. This study provides proof-of-concept that the baseline phosphorylation levels of AKT, the direct target of ipatasertib, could have predictive value and may possess an improved means of biomarker-based patient selection for AKT inhibitors and diagnostic utility for precision medicine.

APOLLO-5 ADC DRUG TARGET MAPPING

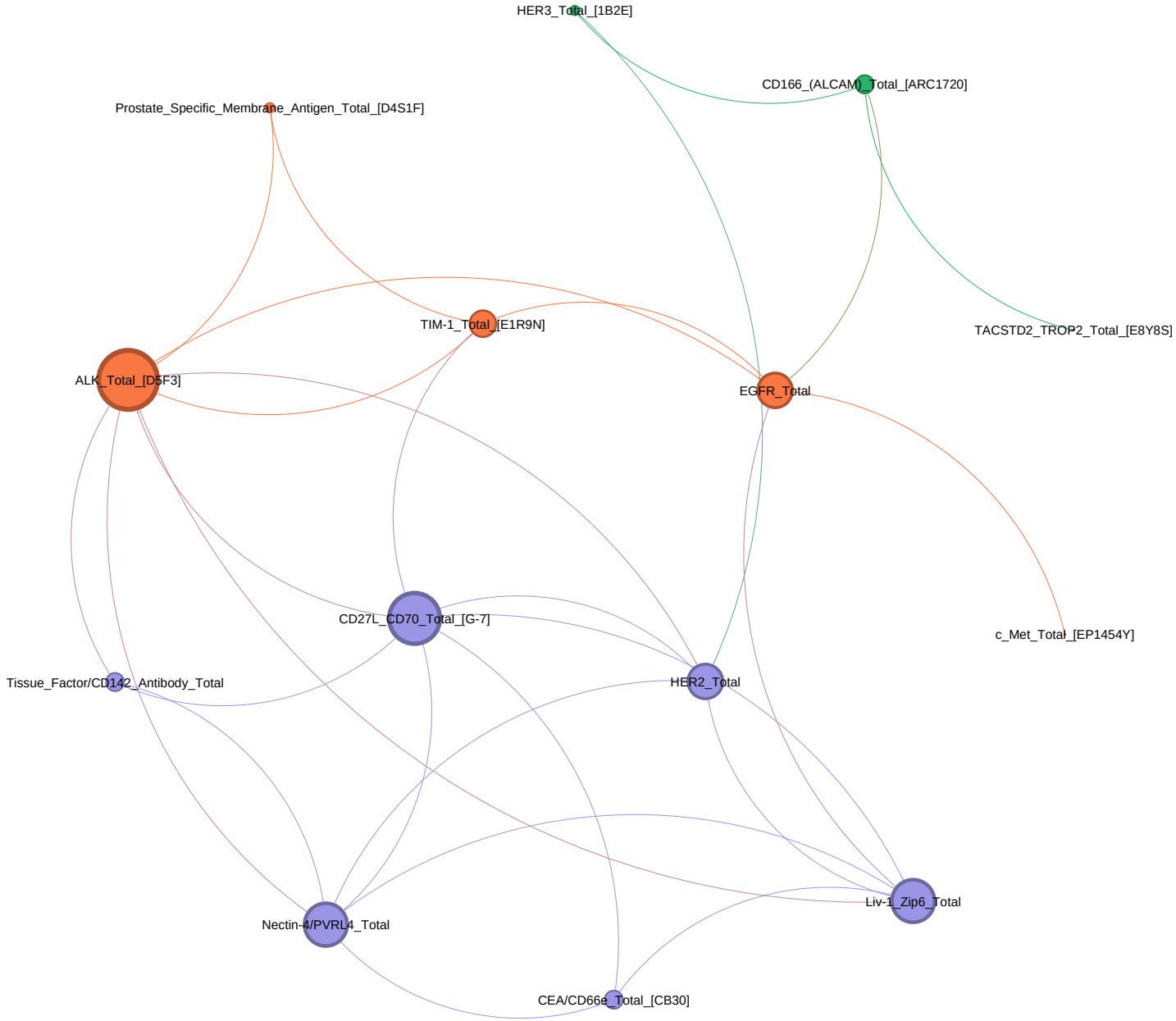
LARGEST PAN-TUMOR ADC DRUG TARGET MAPPING OF LMD EPITHELIUM IN CANCER TO DATE



Tissue Site	Color code on the heatmap
Bile duct	
Brain	
Breast	
Bronchus	
Cervix	
Cul de sac	
Endometrium	
Fallopian Tube	
Gallbladder	
Kidney	
Large instestine	
Larynx	
Liver	
LN, extra-axillary	
Lung	
Mesenteric Lymph Node	
Non-SLN	
Nose, nasal cavity	
Omentum	
Oral Cavity	
Ovary	
Pancreas	
Parotid	
Penis	
Perltoneum	
Prostate	
Rectum	
Right adnexal mass	
Right pelvic mass	
Small Instestine	
Soft tissue	
Spinal Cord	
Spleen	
Stomach	
Testis	
Thyroid	
Tongue	
Tonsil	
Urinary Bladder	
Uterus	
Vulva	

- ALK
- AXL
- B7H3
- B7H4
- MET
- CLAUDIN 18.2
- EGFR
- FOLATE RECEPTOR ALPHA
- HER2
- IGF1R
- HER3
- LIV1
- MESOTHELIN
- MUC-1
- NAPI2B
- NECTIN 4
- PSMA
- ROR1
- TISSUE FACTOR
- TROP2
- CEACAM5
- CD56
- ADAM9
- CL166
- CADHERIN 6
- TIM-1
- ENPP3
- CD70
- DELTA-LIKE PROTEIN 3

29 ADC TARGETS


HER3_Total_[1B2E]
CD166_(ALCAM)_Total_[ARC1720]
Prostate_Specific_Membrane_Antigen_Total_[D4S1F]
TIM-1_Total_[E1R9N]
TACSTD2_TROP2_Total_[E8Y8S]
ALK_Total_[D5F3]
EGFR_Total
CD27L_CD70_Total_[G-7]
c_Met_Total_[EP1454Y]
Tissue_Factor/CD142_Antibody_Total
HER2_Total
Liv-1_Zip6_Total
Nectin-4/PVRL4_Total
CEA/CD66e_Total_[CB30]

CDx Report of the Future: Individualized Protein Pathway Activation Maps

Patient Name: Jane Doe | Report Date: 10/08/2024

Patient

Date of Birth: **01/01/1901**
Sex: **Female**
MRN: **1234567**
Theralink ID: **TT23-00101**

Specimen

Specimen ID: **AB00-12345-67**
Specimen Type: **Biopsy**
Collection Site: **Liver**

Timeline

Specimen Collected: **01/01/1901**
Test Ordered: **11/02/2023**
Specimen Received: **11/20/2023**

Provider

John Doe - Test Cancer Center
1234 Test Center Road, Suite 1111, Warrenton, VA 20187, Phone: 123-456-7890

Diagnosis and Treatment History

Diagnosis: **Metastatic breast cancer**
Stage: **Stage III**
Histology: **Metastastic carcinoma, with morphologic and immunophenotypic features consistent with mammary origin**

Line of Therapy: Faslodex-Everolimus, Tamoxifen+Goserelin (2018), Letrozole, Palbociclib,Goserelin (2021)

Hormonal Status

ER: **Positive 94%, 2+** | PR: **Positive 83%, 1+** | HER2: **Negative (IHC 1+)**

On-Label Options

AKT kinase inhibitor, such as capivasertib

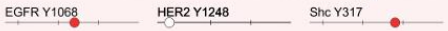
PI3K inhibitor, such as alpelisib

Off-Label Options

anti-PD1 agent, such as pembrolizumab

antiandrogens

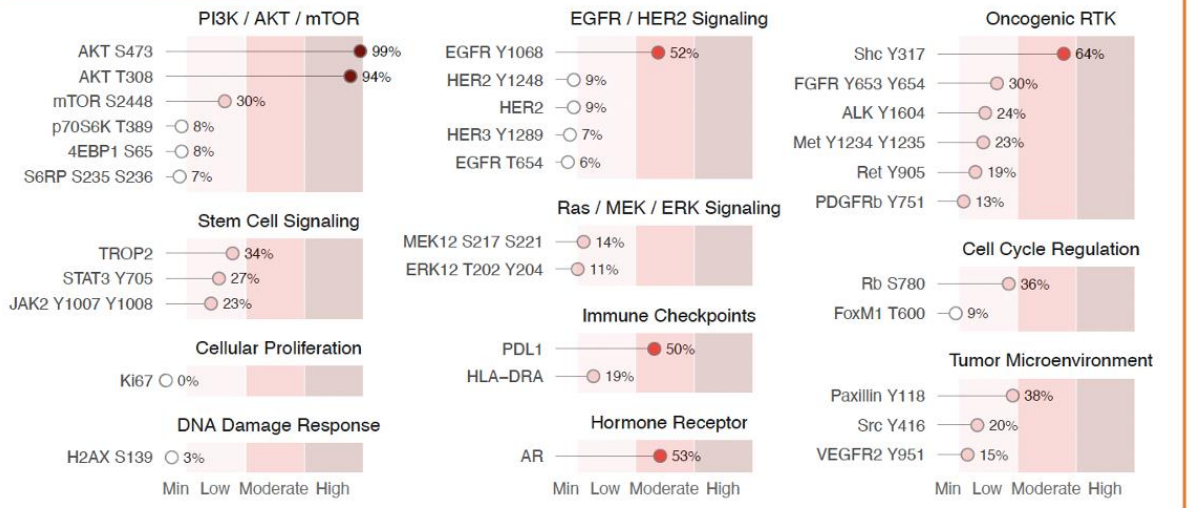
EGFR kinase inhibitor, such as erlotinib, gefitinib



high EGFR activation together with low HER2 activation indicates sensitivity to EGFR-specific kinase inhibitors over EGFR/HER2 kinase inhibitors

Patient Name: Jane Doe | Report Date: 10/08/2024

Complete Assay Results



Acknowledgements

Virginia Legislature