UNICANCER

Centre Jean Perrin

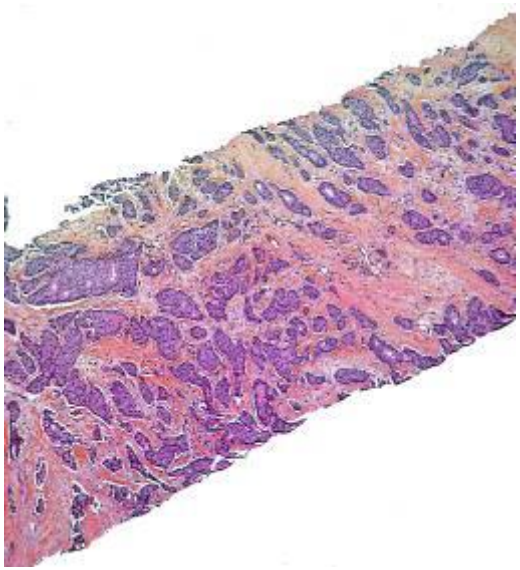
Centre de Lutte contre le Cancer d'Auvergne

Clermont-Ferrand - France -

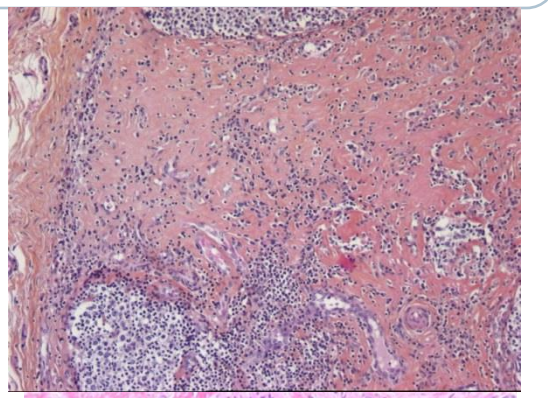
Biological profiling of residual disease (Incl biomarkers)

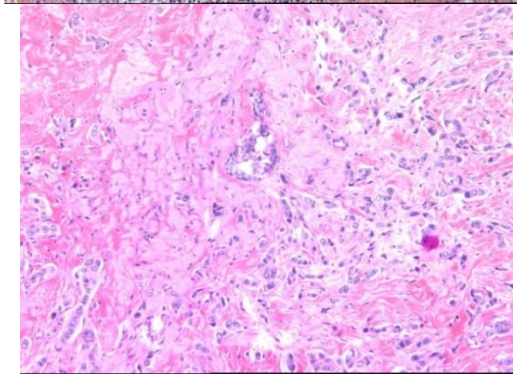
Frédérique Penault-Llorca and Nina Robin

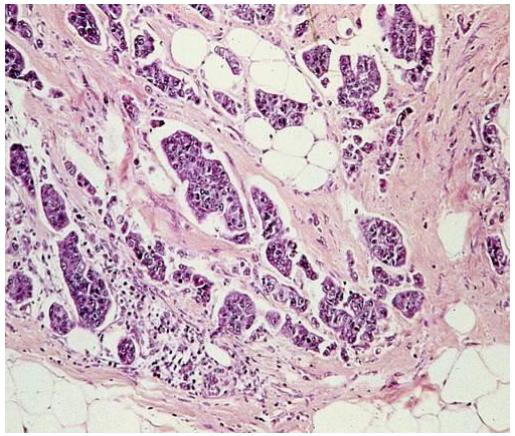
When we don't have a ypT0/is ypN0



Before







After

RATIONALE AND OUTLINES

- Significant biological differences between breast cancer before and after neoadjuvant therapy.
- Differences for classical parameters
- Evaluation of specific parameters within the different BC categories
- => IMPORTANCE OF THE RESIDUAL DISEASE for **prognosis** and in search of specific and targetable **markers of resistance or sensitivity**.

ANALYSIS OF RESIDUAL DISEASE AFTER NEOADJUVANT THERAPY (NAT) FOR BC

TUMOR CELLS

TUMOR MICROENVIRONMENT
- **CELLULAR COMPONENTS**
- **ACELLULAR COMPONENTS**

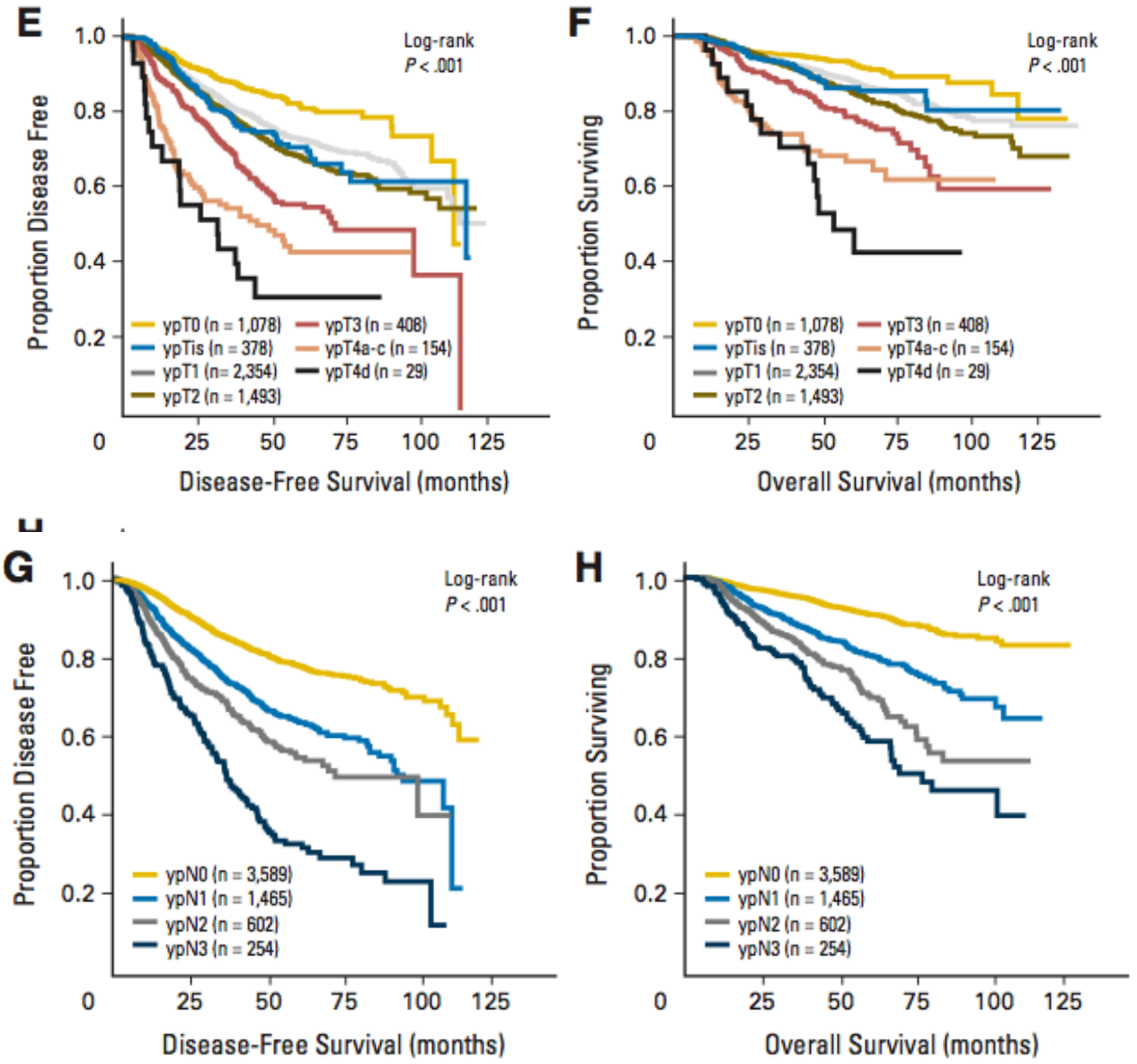
most of the published studies have analyzed post-NAT changes without separating tumor cells and their microenvironment

METHODS OF POST-NAT BIOMARKER ANALYSIS

- **PROTEOMIC (protein expression incl IHC)**
- **GENOMIC (gene or gene regulators expression)**
- **CELLULAR (analysis of cellular populations)**

CLASSICAL PARAMETERS

Size matters….



G Von Minckwitz J Clin Oncol 2012; 30:1796-1804

Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Ki67 Measured after Neoadjuvant Chemotherapy for Primary Breast Cancer

Gunter von Minckwitz[1,2], Wolfgang D. Schmitt[5], Sibylle Loibl[1,4], Berit M. Müller[5], Jens U. Blohmer[6], Bruno V. Sinn[5], Holger Eidtmann[7], Wolfgang Eiermann[8], Bernd Gerber[9], Hans Tesch[3], Jörn Hilfrich[10], Jens Huober[11], Tanja Fehm[12], Jana Barinoff[13], Thomas Rüdiger[14], Erhard Erbstoesser[15], Peter A. Fasching[16], Thomas Karn[2], Volkmar Müller[17], Christian Jackisch[4], and Carsten Denkert[5]

« CLASSICS » (1)

Ki67 INDEX (post-NAT)

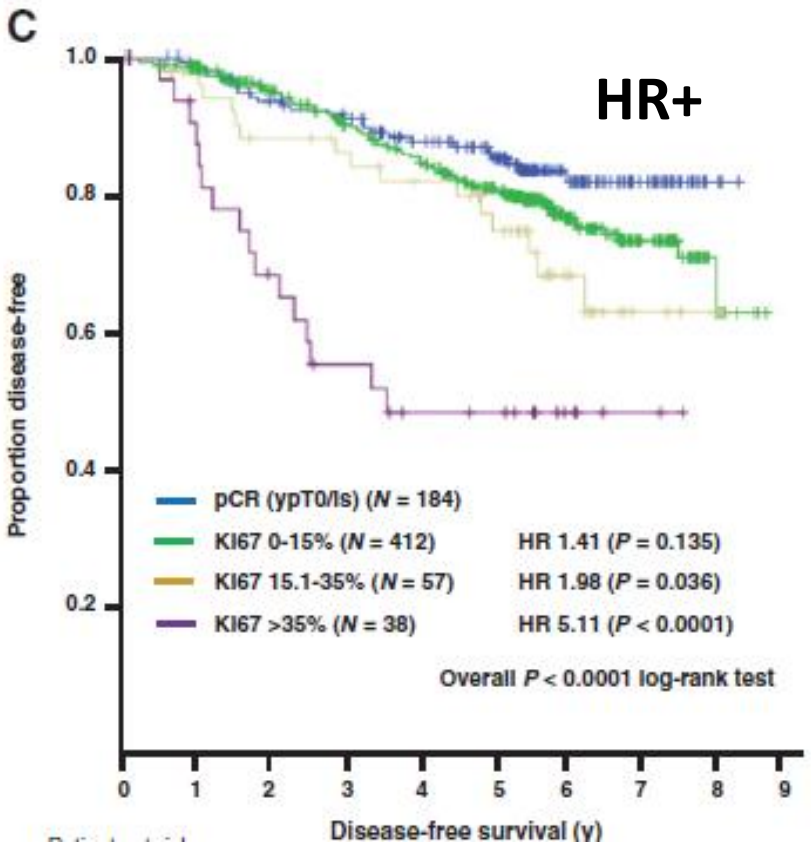
Von Minckwit G et al, Clin Canc Res 2013 (PMID 23812670)
GeparTrio : 667 pts with post-NAT RT, all BC subtypes

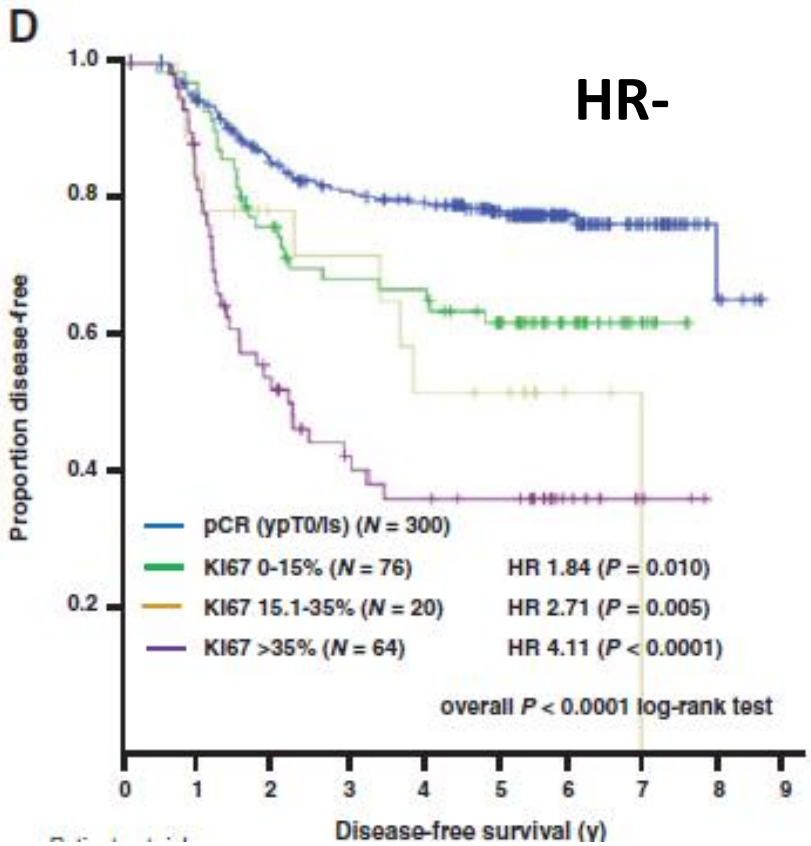
post-NAT Ki67

low (0 – 15 %) : 488 pts

intermediate (15.1 – 35 %) : 77 pts

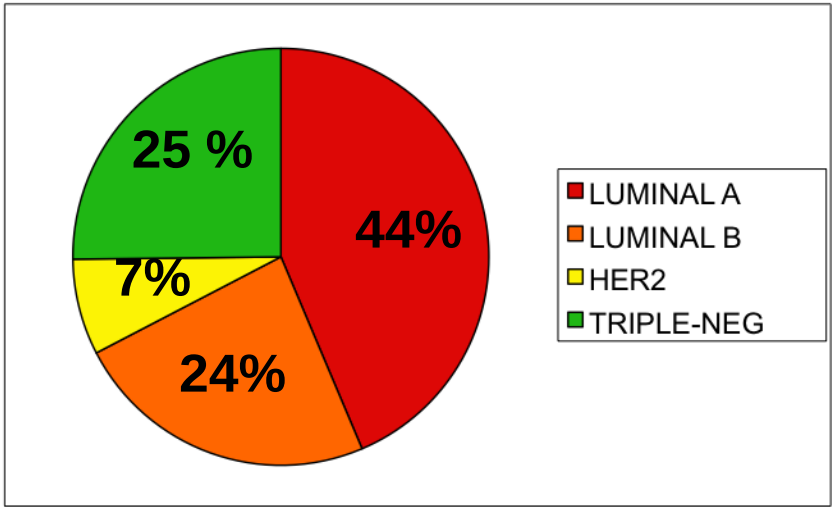
high (> 35%) : 102 pts





HIGH POST-NAT Ki67 : higher risk of relapse and death (HR+ & HR-)

Intrinsic subtypes before and after NACT

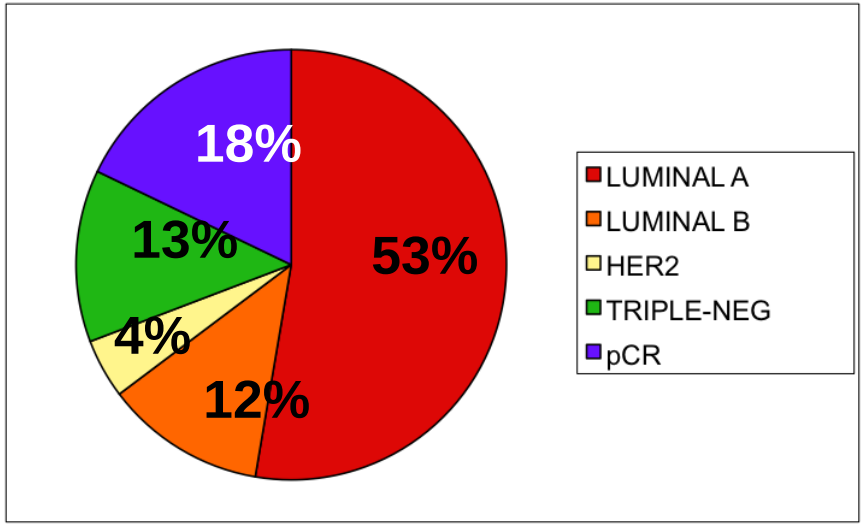


Intrinsic subtypes before NACT

(n = 282 patients)

Intrinsic subtypes after NACT

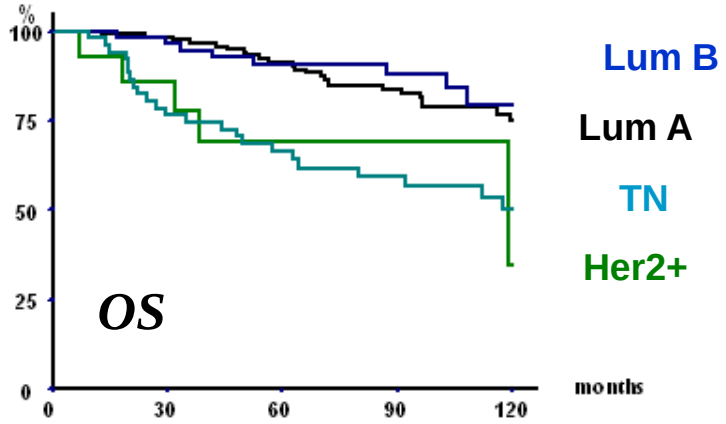
(n = 224 patients, 58 missing data)



Abrial C, unpublished data

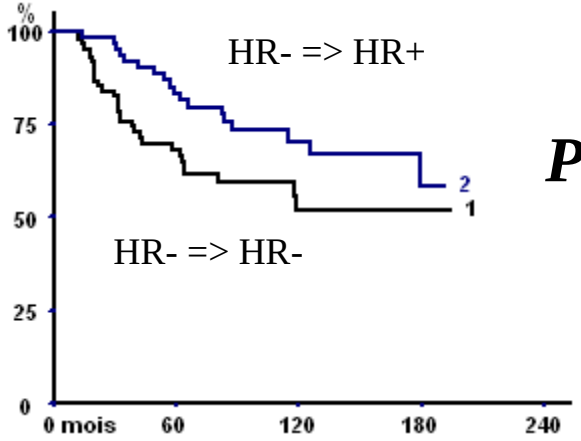
Prognostic factors

Prognostic value of the intrinsic subtypes evaluated on the residual tumor



p = 0.00026

Abrial C, unpublished data

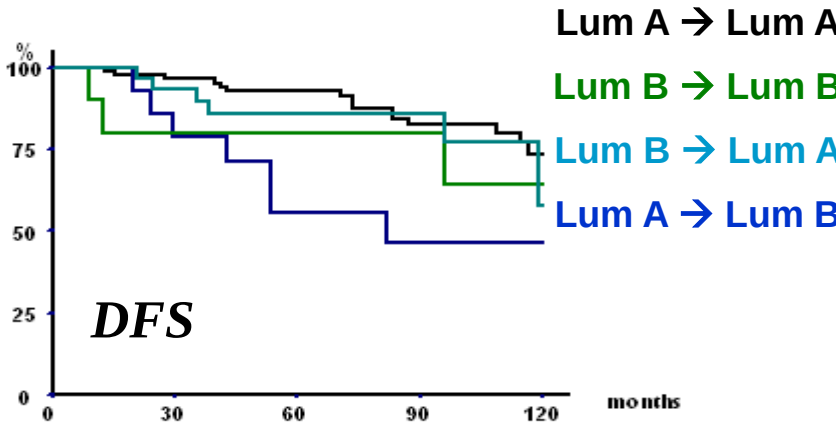


p= 0.045

OS in HR-negative patients with a switch to HR+

(Tacca et al, 2007, The Oncologist)

Prognostic value of switch



Switch in the luminal group

p = 0.05

Abrial C, unpublished data

Cancer Therapy: Clinical

Clinical Cancer Research

Gene Expression, Molecular Class Changes, and Pathway Analysis after Neoadjuvant Systemic Therapy for Breast Cancer

Ana M. Gonzalez-Angulo[1,2], Takayuki Iwamoto[1], Shuying Liu[1], Huiqin Chen[1], Kim-Anh Do[3], Gabriel N. Hortobagyi[1], Gordon B. Mills[2], Funda Meric-Bernstam[4], W. Fraser Symmans[5], and Lajos Pusztai[1]

PROFILING STUDIES

Focus on tumor cells

Gonzalez-Angulo AM et al, Clin Cancer Res 2012 (PMID 22235097)

The MDACC team

1st gene expression analysis conducted in paired pre-NAT + post-NAT TNBC samples

21 pairs, analysis of tumor cells only (no stroma)

Basal samples ($n = 9$)				
1	CXCR4 signaling	220	0.005	Post
2	Thrombin signaling	268	0.010	Post
3	Cardiac hypertrophy signaling	302	0.014	Post
4	Fatty acid biosynthesis	17	0.024	Post
5	Ascorbate and aldarate metabolism	20	0.027	Post
6	Propanoate metabolism	82	0.031	Post
7	Neurotrophin_TRK signaling	101	0.039	Post
8	TR_RXR activation	119	0.039	Post
9	IGF-1 signaling	138	0.045	Post
10	Alanine metabolism	55	0.046	Post
1	Lysine biosynthesis	8	0.001	Pre
2	Natural killer cell signaling	144	0.001	Pre
3	Fc Epsilon RI signaling	134	0.002	Pre
4	TREM1 signaling	71	0.002	Pre
5	B Cell Receptor Signaling	228	0.005	Pre
6	Role of NFAT in regulation of the immune response	272	0.006	Pre
7	Fc Receptor-mediated phagocytosis in macrophages and monocytes	153	0.007	Pre
8	IL-10 signaling	87	0.012	Pre
9	Dendritic cell maturation	188	0.018	Pre
10	p38 MAPK signaling	135	0.018	Pre
11	CTLA4 signaling in cytotoxic T lymphocytes	135	0.027	Pre
12	Sonic hedgehog signaling	36	0.027	Pre
13	IL-15 signaling	85	0.028	Pre
14	Role of PKR in IFN induction and antiviral response	69	0.030	Pre
15	IL-8 signaling	217	0.033	Pre
16	Fc_RIIB signaling in B lymphocytes	54	0.037	Pre
17	CD28 signaling in T helper cells	199	0.041	Pre
18	T Helper cell differentiation	57	0.044	Pre
19	Cytotoxic T lymphocyte-mediated apoptosis of target cells	51	0.048	Pre
20	NF-_B Signaling	183	0.048	Pre

Expression of 200/600 genes changed during NAT

Focus on tumor cells

Gonzalez-Angulo AM et al, Clin Cancer Res 2012 (PMID 22235097)
The MDACC team

Nonbasal samples ($n = 12$)				
1	Circadian rhythm signaling	37	0.005	Post
2	Notch signaling	44	0.006	Post
3	O-Glycan biosynthesis	26	0.011	Post
4	Chondroitin sulfate biosynthesis	38	0.017	Post
5	Caveolar-mediated endocytosis	119	0.027	Post
6	Thrombopoietin signaling	84	0.029	Post
7	Integrin signaling	280	0.034	Post
8	Actin cytoskeleton signaling	267	0.035	Post
9	Fatty acid biosynthesis	17	0.041	Post
10	PPAR signaling	124	0.043	Post
11	Semaphorin signaling in neurons	73	0.044	Post
12	Sonic hedgehog signaling	36	0.003	Pre
13	Role of RIG1-like receptors in antiviral innate immunity	54	0.020	Pre
14	Phototransduction pathway	49	0.026	Pre
15	BMP signaling pathway	116	0.031	Pre
16	Airway inflammation in asthma	3	0.044	Pre

- **BASAL TNBC RT :** PI3K, small G proteins, energy metabolism pathways ⬆
  Hedgehog signaling, immune pathways ⬇

- **NON-BASAL TNBC RT :** Notch signaling, energy metabolism ⬆
  Hedgehog signaling, immune pathways ⬇

No change in EMT genes

TNBC

RESEARCH ARTICLE

Molecular Profiling of the Residual Disease

ARTICLES

nature medicine

Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance

Justin M Balko[1], Rebecca S Cook[2,3], David B Vaught[2], María G Kuba[4], Todd W Miller[2,3], Neil E Bhola[1], Melinda E Sanders[3,4], Nara M Granja-Ingram[4], J Joshua Smith[5], Ingrid M Meszoely[3,5], Janine Salter[6,7], Mitch Dowsett[6,7], Katherine Stemke-Hale[8], Ana M González-Angulo[8,9], Gordon B Mills[8], Joseph A Pinto[10], Henry L Gómez[11] & Carlos L Arteaga[1–3]

Natasha Morse, Nerissa Therese Viola-Villegas, Ana Bosch, Dejan Juric, Saswati Hazra,[10] Sharat Singh,[10] Phillip Kim,[10] Anna Bergamaschi,[11] Shyamala Maheswaran,[1] Tony Ng,[5,12] Frédérique Penault-Llorca,[3,4] Jason S. Lewis,[9] Lisa A. Carey,[13] Charles M. Perou,[14] José Baselga,[2†] Maurizio Scaltriti[2†]

TNBC : IHC Signature

Sohn J et al, Annals of Oncology 2013 (PMID 23925999)

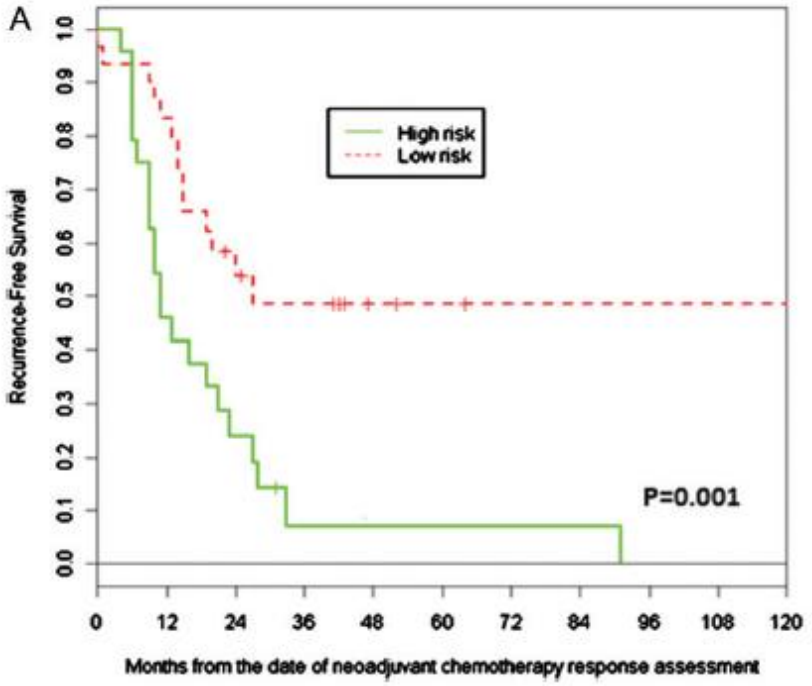
The MDACC team

74 TNBC, RT post-NT (uniform taxane-anthracycline NAT)

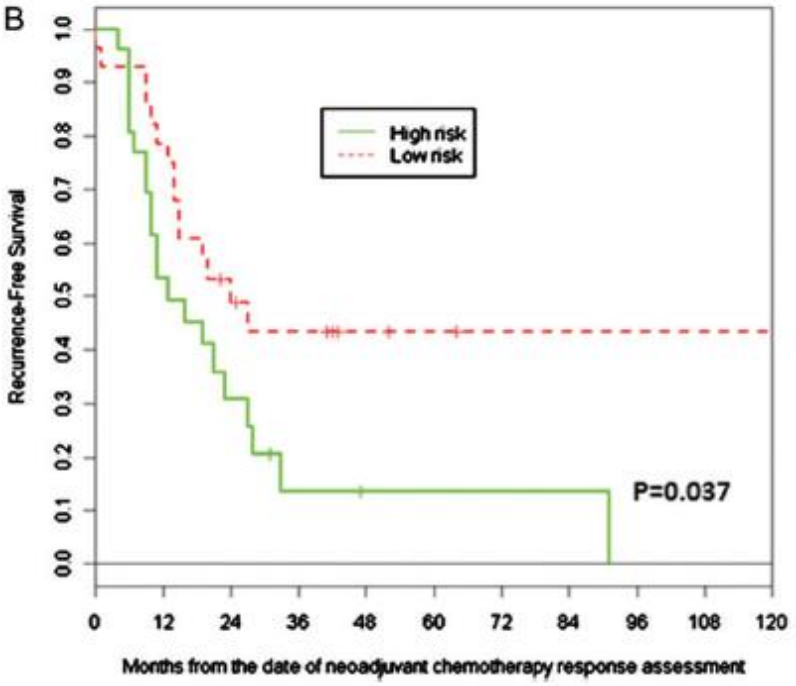
protein expression study : 76 antibodies used

5 proteins selected : AKT, IGFBP2, LKB1, S6, STATHMIN

expressions combined into 2 recurrence risk scores : High RR, Low RR



1st validation analysis



2nd validation analysis

(leave-one-out cross validation)

5-protein RR is independent predictor of RFS at 3 yrs

TNBC : 7-gene signature

Yu KD et al, Clin Cancer Res 2013 (PMID 23925999)
The Vanderbilt University Team

111 TNBC RT post-NAT (discovery cohort from MDACC)
+ 25 TNBC RT post-NAT (validation cohort (Baylor)
+ 269 predicted chemoresistant TNBC adjuvant setting (extended validation cohort)

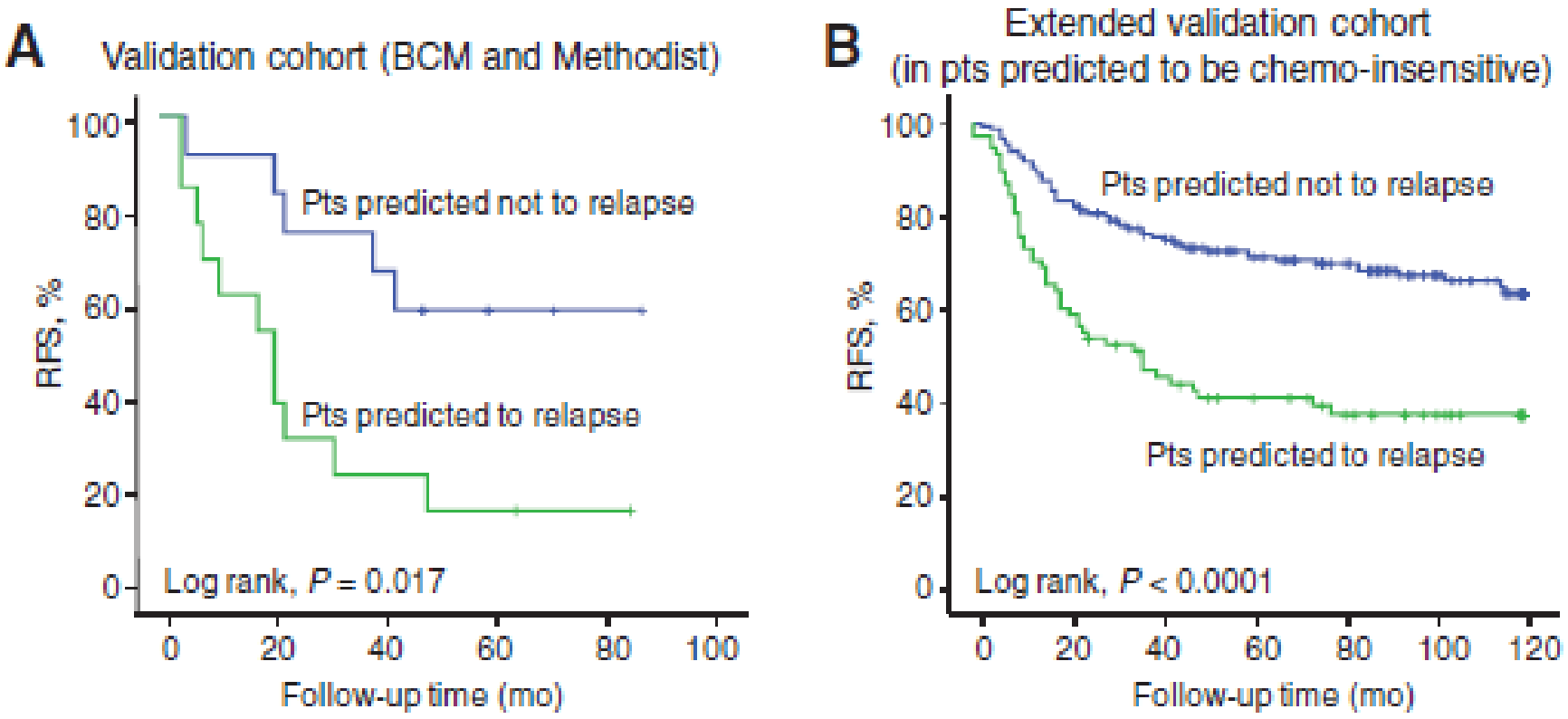
Gene expression study : a 7-gene signature discovered
AR, ESR2, GATA3, GBX2, KRT16, MMP28, WNT11

Poor Prognosis subset defined by : KRT16 (basal marker)
WNT11 (stem cell marker)
MMP20 (EMT marker)

Good Prognosis subset defined by : AR + GATA3 (luminal markers)

TNBC : 7-gene signature predicts RFS (post NAT and Adjuvant setting)

Yu KD et al, Clin Cancer Res 2013 (PMID 23925999)



GOOD PROGNOSIS : LUMINAL GENES

POOR PROGNOSIS : STEM CELL GENES

ADJUVANT TH TARGETS

Identification of actionable targets

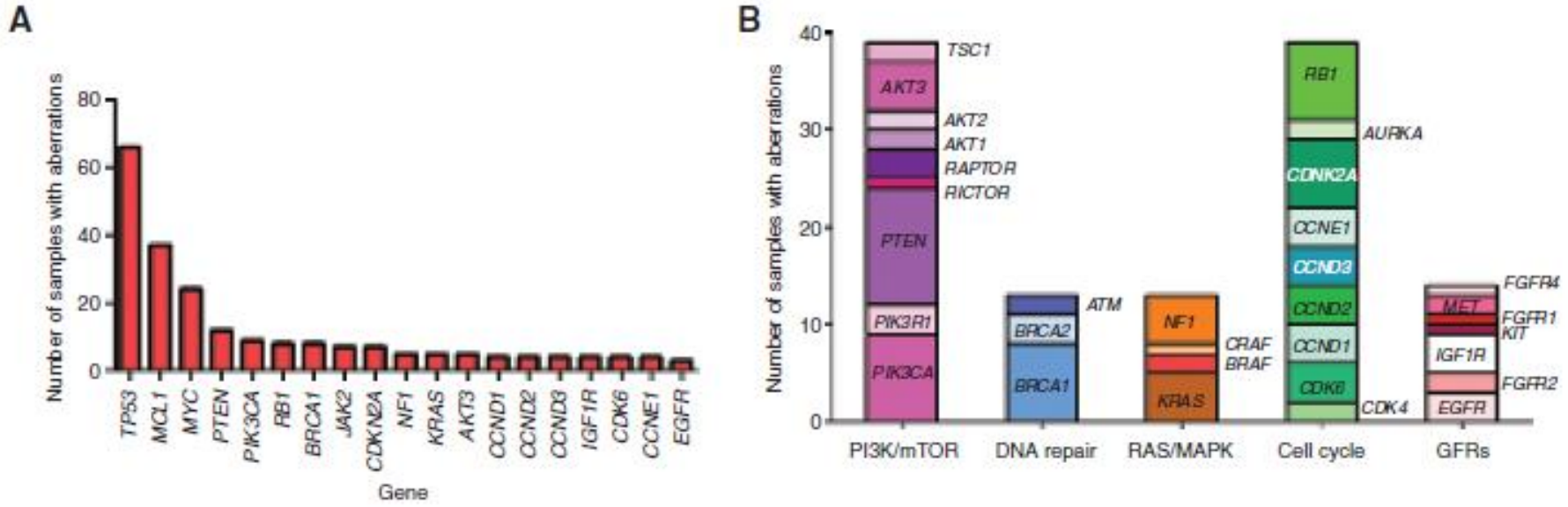
Balko J et al, Cancer Discovery 2014 (PMID 24356096)
The Vanderbilt University team

111 TNBC-RT : NGS and gene expression analysis

- **Ki67 index of RT was not prognostic in this cohort**

TARGETED NGS

85 FFPE samples : 74 TNBC
182 oncogenes/3320 exons
14 genes frequently rearranged in cancer/37 introns



➢ **90% pts (RT) : alteration in at least 1 targetable pathway**

TNBC : potential targets

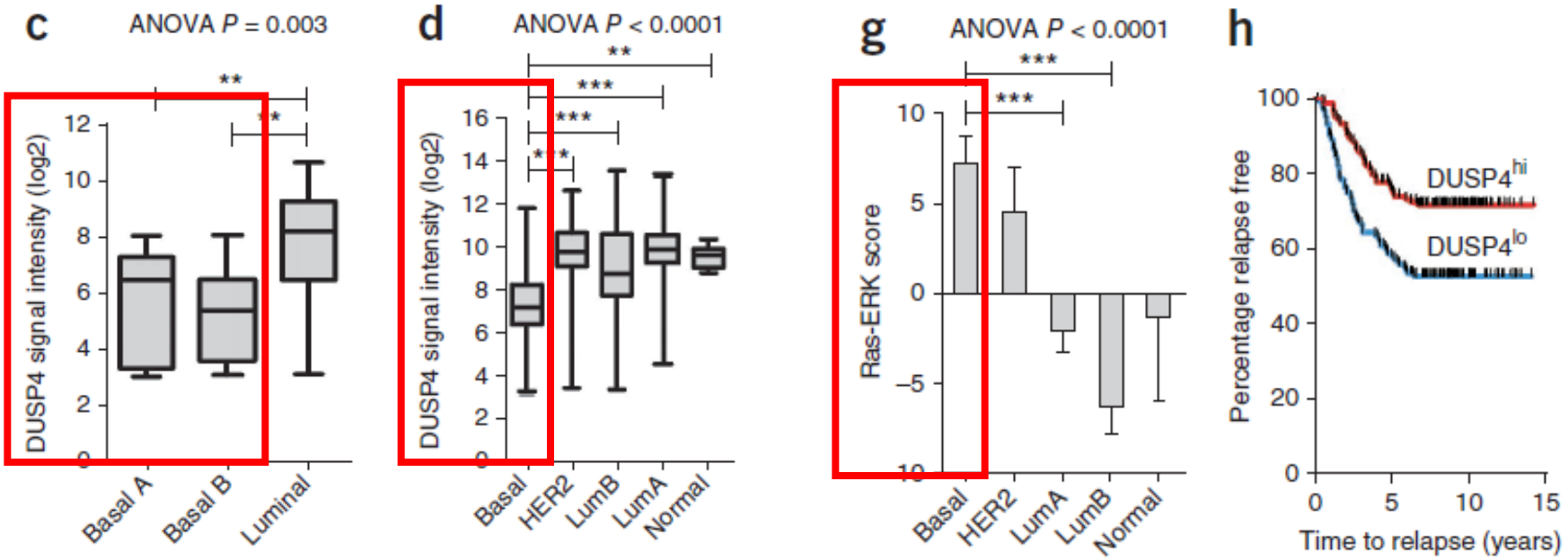
ACTIONABILITY OF LESIONS IDENTIFIED IN AT LEAST 3 POST-NAT SAMPLES

Gene symbol	# Altered	Category	Potential therapy
TP53	73	D	Prognostic (poor, potentially sensitive to WEE1 inhibitors, e.g., MK1775)
MCL1	40	C	Resistance to anti-tubulins, e.g., paclitaxel, MCL1 inhibitor in development
MYC	24	C	Aurora kinase inhibitors, e.g., MLN8237, AMG 900; possible sensitivity to CDK inhibitors
PIK3CA	13	B	PI3K/mTOR inhibitors, e.g., everolimus, temsirolimus, and others
PTEN	12	B	PI3K/mTOR inhibitors, e.g., GSK2636771, everolimus, temsirolimus, and others
BRCA1	9	B	PARP inhibitors, e.g., olaparib, CEP-9722, rucaparib, and others
RB1	9	D	Prognostic
JAK2	8	D	JAK2 inhibitors, e.g., ruxolitinib, and others
ERBB2	7	A	Herceptin, lapatinib, and others
CDKN2A/B	7	E	CDK4/6 inhibitors, e.g., PD0332991, LEE011, P276-00
NF1	5	C	MAPK/PI3K/mTOR inhibitors, e.g., MSC1936369B, everolimus, temsirolimus, and others
AKT3	5	C	AKT inhibitors, e.g., MK2206, PI3K/mTOR inhibitors, e.g., everolimus, temsirolimus
KRAS	5	A	Resistance to cetuximab, MEK inhibitors, e.g., MEK162
CCND1	5	C	CDK4/6 inhibitors, e.g., PD0332991, LEE011, P276-00
CCND3	4	C	CDK inhibitors, kinetin riboside
CCNE1	4	C	CDK2/4/6 inhibitors, e.g., ABT-888, PD0332991, LEE011, P276-00
CCND2	4	C	CDK inhibitors, kinetin riboside
CDK6	4	C	CDK4/6 inhibitors, e.g., PD0332991, LEE011, P276-00
IGF1R	4	C	IGF-IR inhibitors, e.g., AMG-479, BMS-754808, MK-0646, IMC A12, and others
LRP1B	3	E	Biologically relevant, presently no known targeted therapies
PIK3R1	3	C	PI3K pathway inhibitors
ATM	3	C	PARP inhibitors, e.g., olaparib, CEP-9722, rucaparib
BRCA2	3	B	PARP inhibitors, e.g., olaparib, CEP-9722, rucaparib, and others
EGFR	3	A	Cetuximab, panitumumab, and others
FBXW7	3	C	Resistance to anti-tubulins, potential sensitivity to PI3K/mTOR inhibitors
CDK4	3	C	CDK4/6 inhibitors, e.g., PD0332991, LEE011, P276-00
RPTOR	3	E	Biologically relevant, possible sensitivity to mTORC1 and mTORC2 inhibitors

Balko J et al, Cancer Discovery 2014

Identification of actionable targets/prognostic

Balko J et al, Nature Medicine 2012 (PMID 22683778)



- DUSP4 LOSS IN BASAL-LIKE(BL) TNBC RT post-NAT
  - correlates with RAS-ERK (MEK) activation

- DUSP4 LOSS IS A RECURRENCE RISK FACTOR
- promotes SC-like phenotypes in BL-BC (PMID 23666295)

Identification of actionable targets

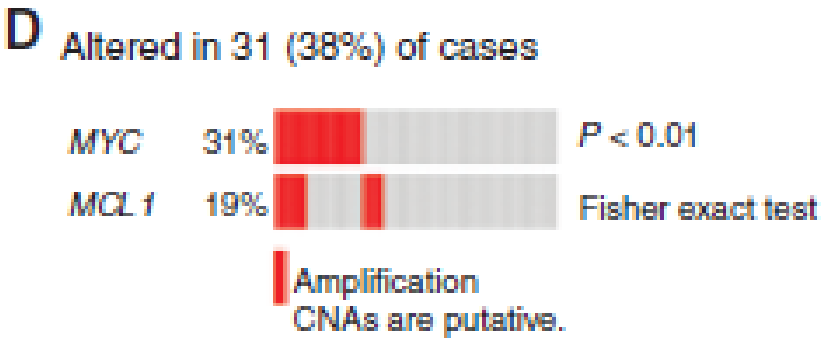
Balko J et al, Cancer Discovery 2014 (PMID 24356096)

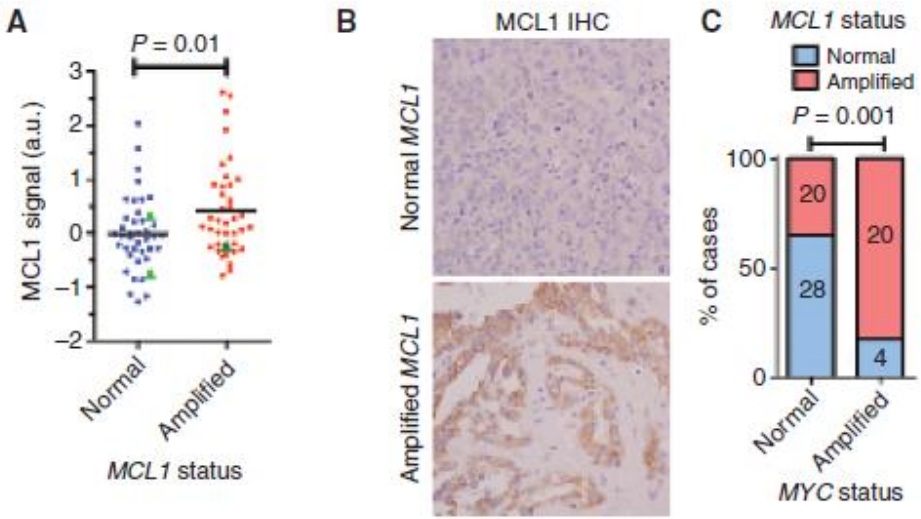
NGS : ENRICHMENT OF ALTERATIONS DURING NAT
20 paired pre-NAT + post-NAT samples

- alterations highly enriched post-NAT :

ATM mutations (R337H, R2443Q)
TP53 mutation (T253fs*11)
CDH1 splice deletions
KDM6A (L214fs*)
AR (A401V)
DPYD (S175W)

COAMPLIFICATION of MYC and MCL1

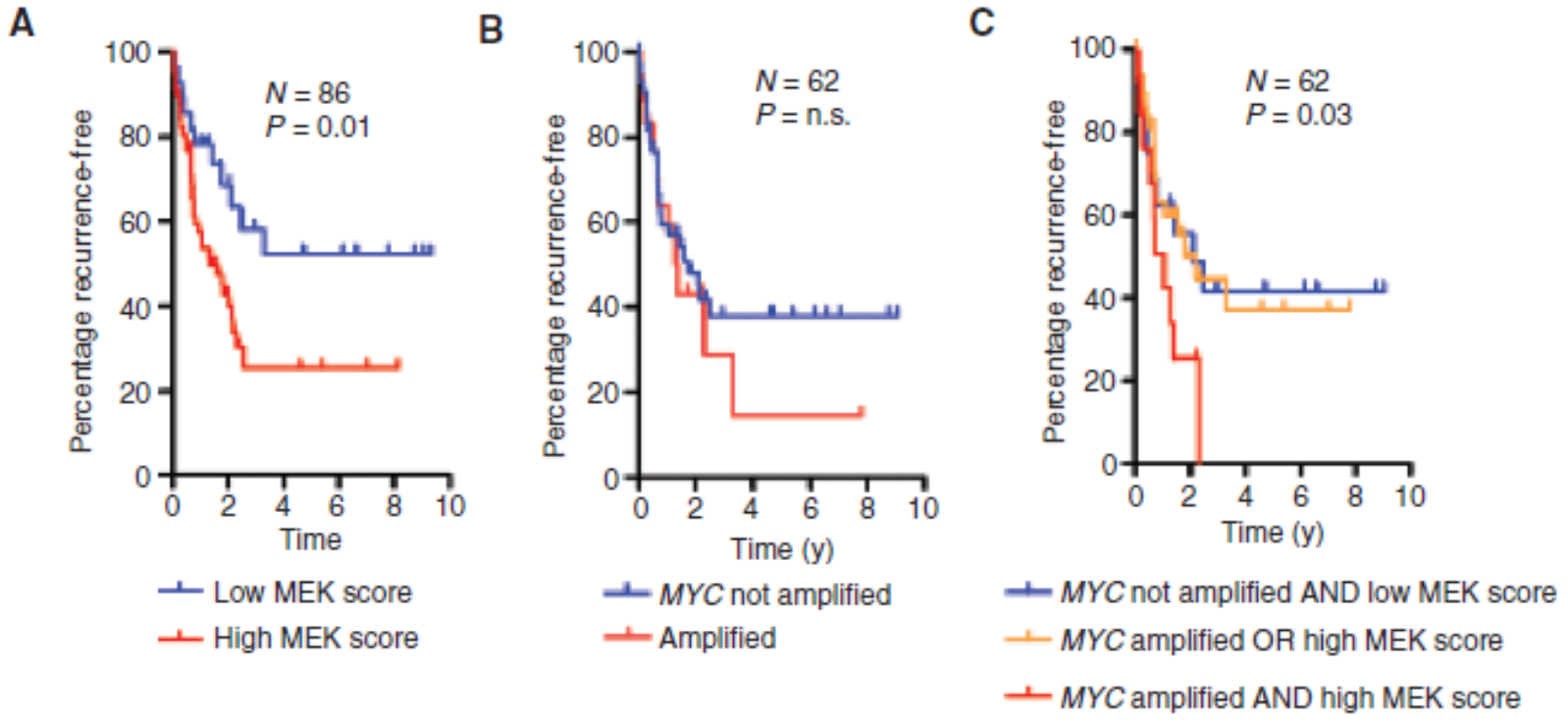




TNBC : MYC/MEK

Balko J et al, Cancer Discovery 2014 (PMID 24356096)

PROGNOSTIC INTERACTION OF MEK AMPLIFICATION AND MYC ALTERATION

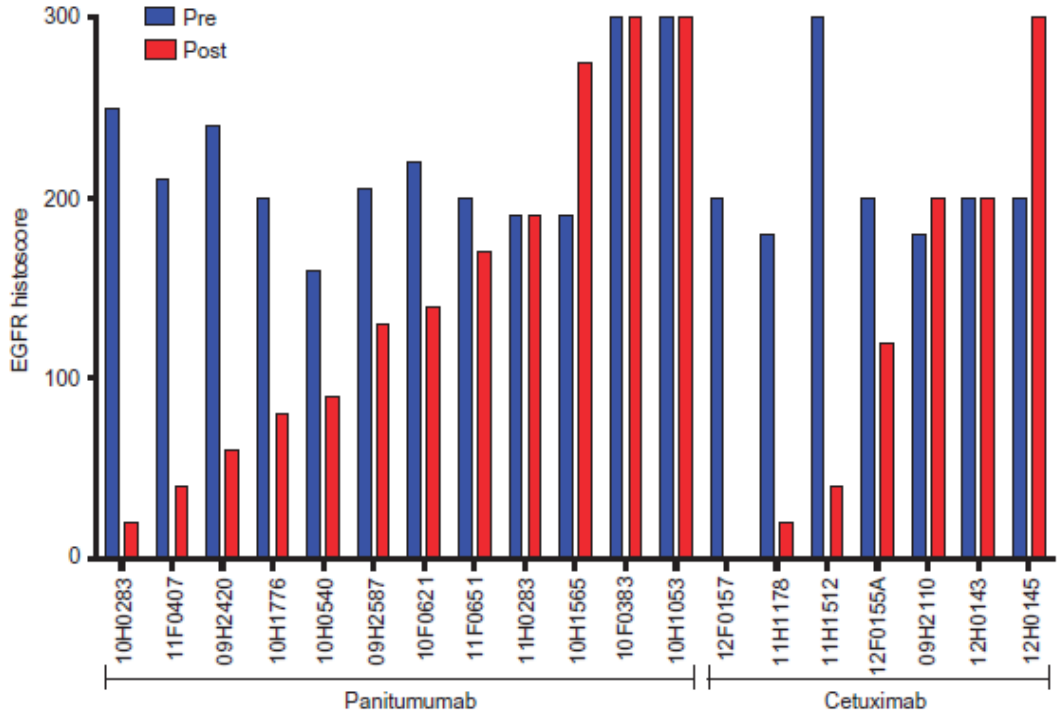


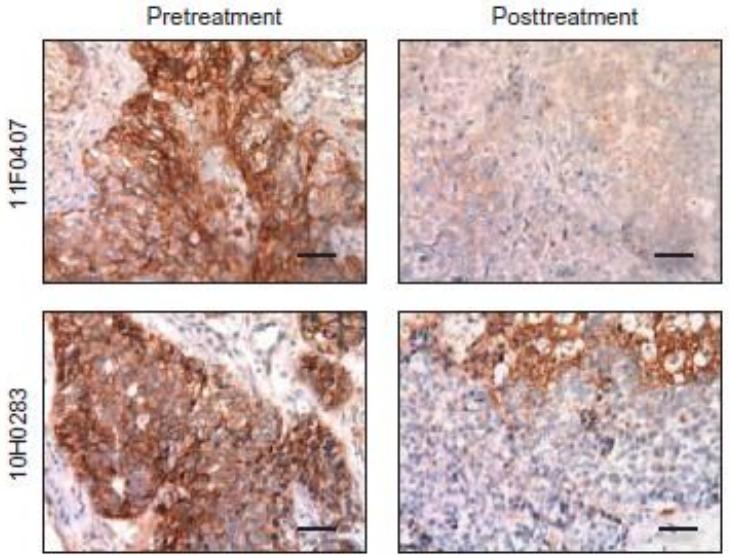
A SIGNIFICANT INTERACTION FOR RFS (p = 0.03) AND NOT OS (p = 0.83)

TNBC : EGFR/HER3 pathway

Tao J, Castel P, Radosevic-Robin N et al, Science Signaling 2014
MKSCC and University of Auvergne ERTICa teams

2 pilot NAT in TNBC clinical trials conducted by Jean Perrin Cancer Center
NAT : anti-EGFR Ab (panitumumab (PTMB)/cetuximab (CTX) + cytotoxics
44 RT (26 PTMB et 18 CTX)

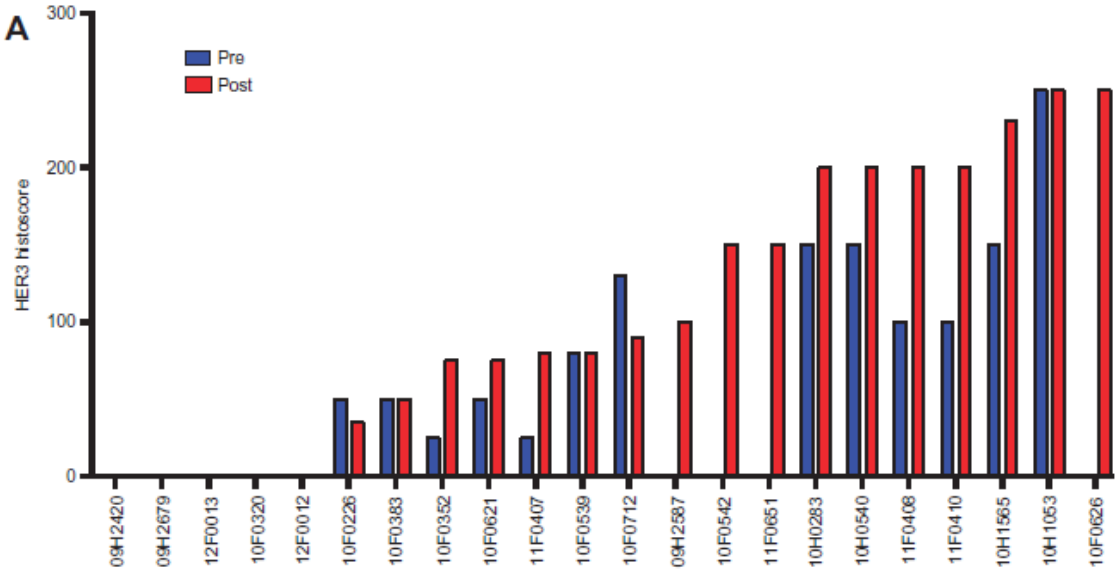


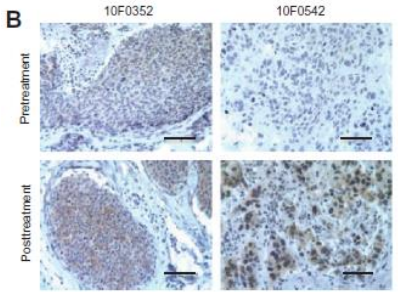


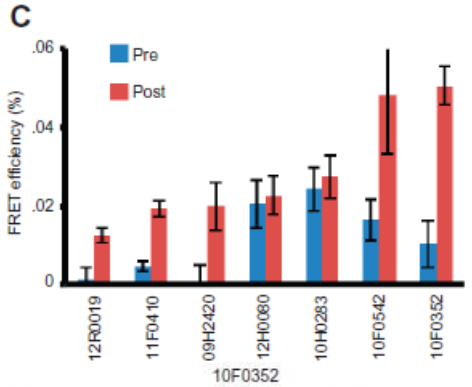
EGFR protein abundance (IHC) got reduced post-NAT in 12/19 RT with a high pre-NAT EGFR levels (EGFR IHC score > 150 [0-300])

TNBC : EGFR/HER3 pathway

Tao J, Castel P, Radosevic-Robin N et al, Science Signaling 2014

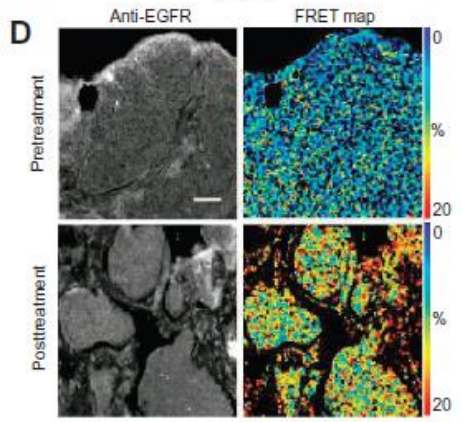






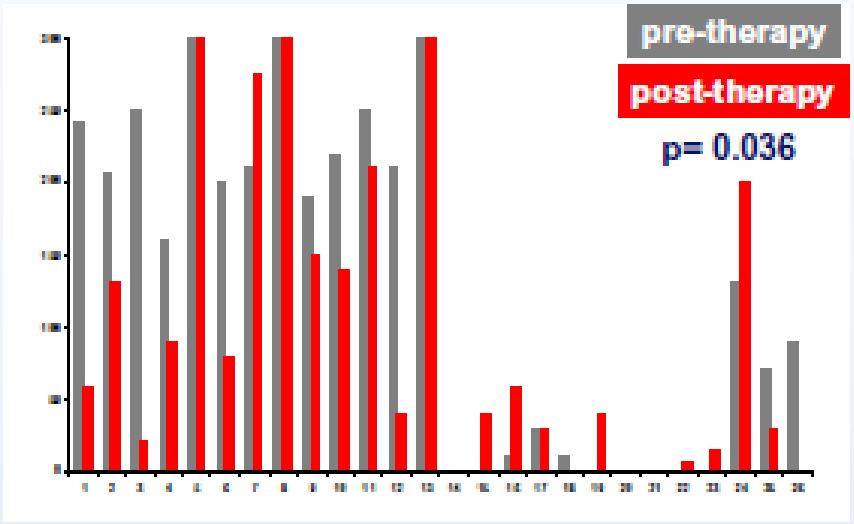
HER3 protein abundance (IHC) got increased in 25/42 RT

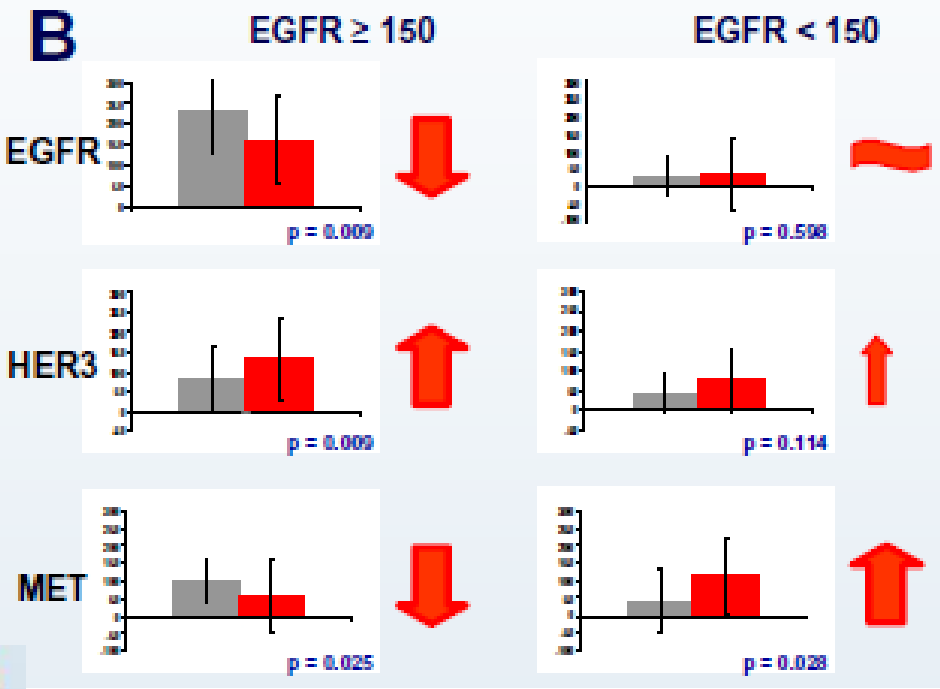
increase in EGFR/HER3 dimers post-NAT was demonstrated by FRET-FLIM

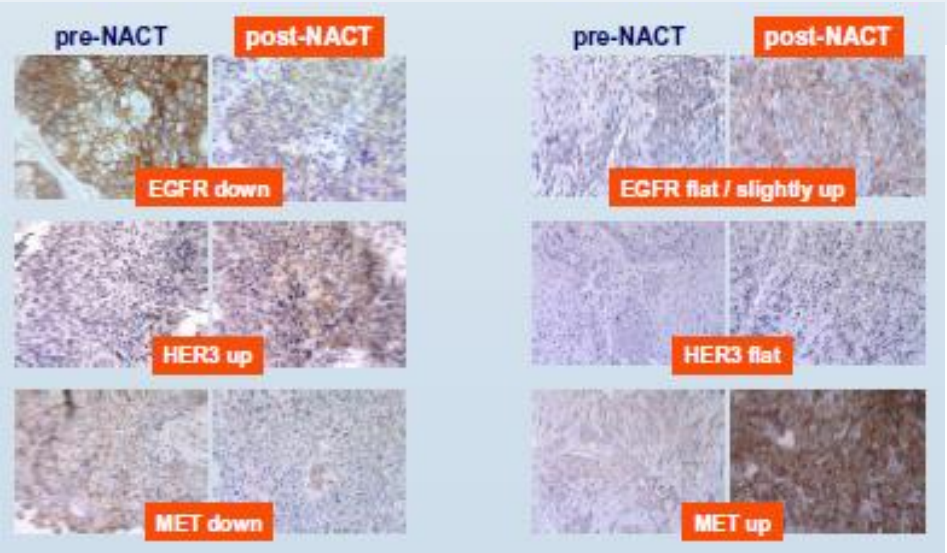


TNBC : EGFR/HER3/MET pathway

Radosevic-Robin N et al : AACR 2014, poster 1819





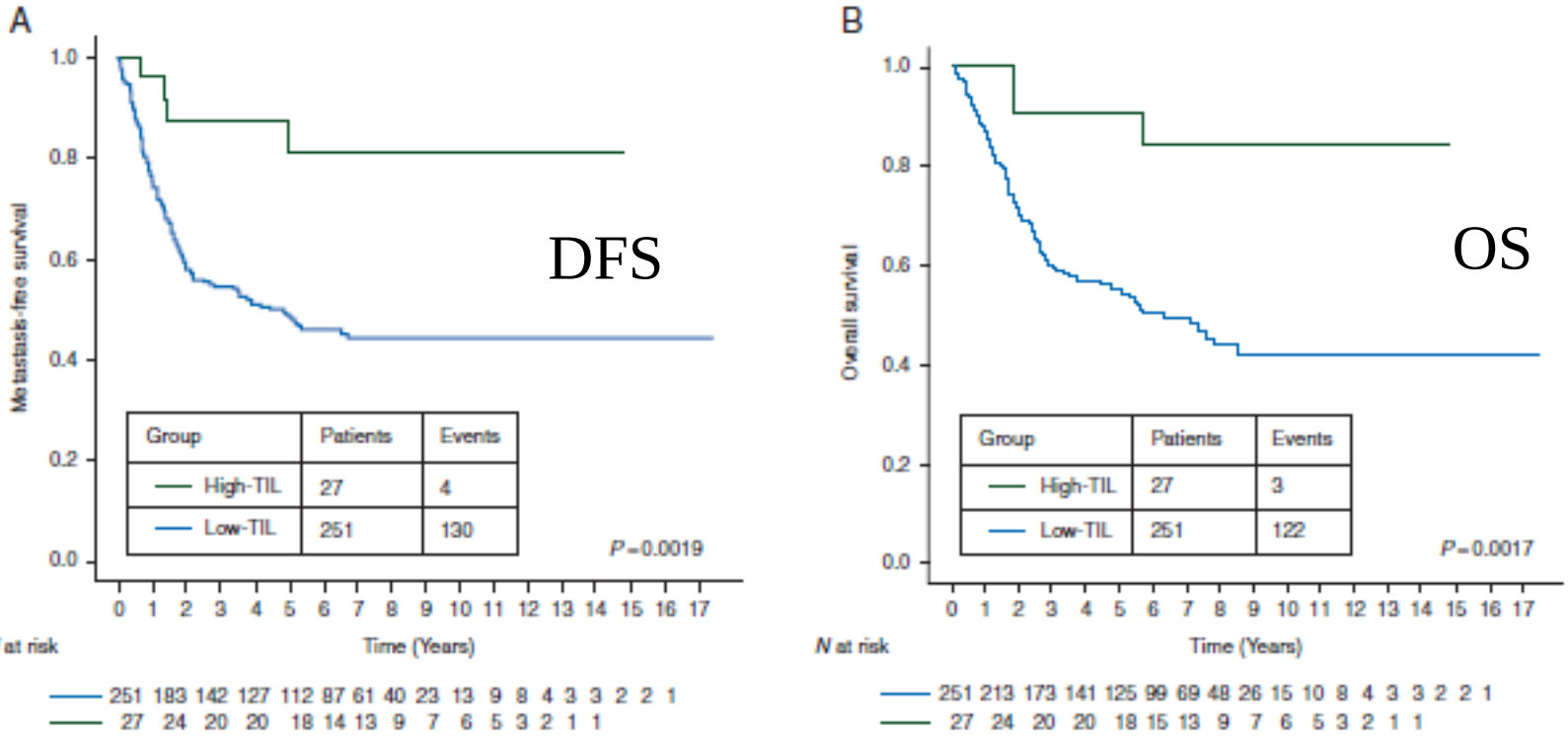


In a subset of 12/24 of post-NAT RT from the TNBC with a pre-NAT score < 150 MET protein abundance is increased

TNBC : post-NAT TILs

Dieci V et al, Annals of Oncology 2014 (PMID 24401929)
Universities of Padova and Modena, EIO Milan, IGR Paris

278 post-NAT TNBC RT analyzed for quantity of Tumor-Infiltrating Lymphocytes (TIL)

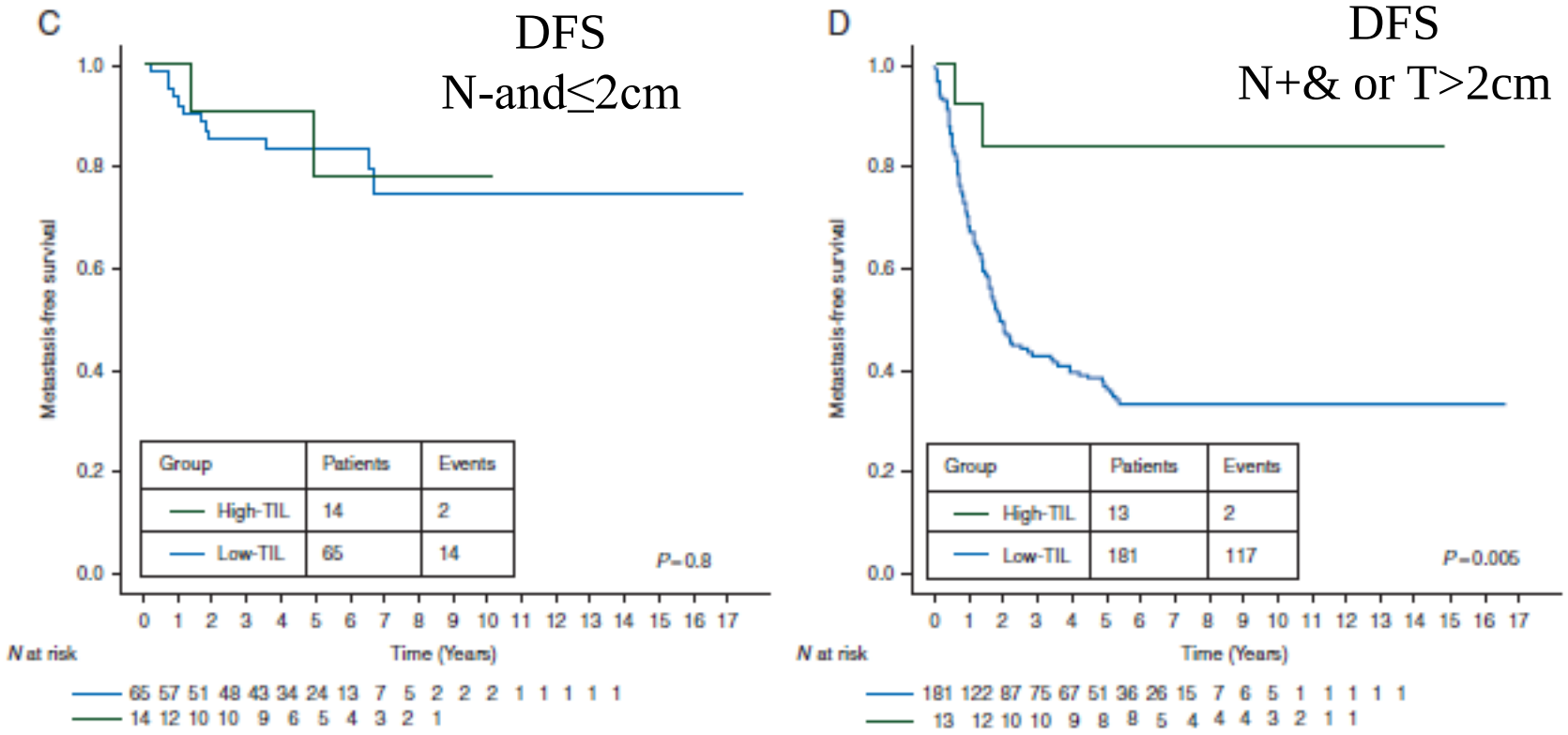


➢ **60% of stromal or intratumoral TIL : better metastasis-free and overal survival (5 yrs)**

TNBC : post-NAT TILs

Dieci V et al, Annals of Oncology 2014 (PMID 24401929)

278 post-NAT TNBC RT analyzed for quantity of Tumor-Infiltrating Lymphocytes (TIL)



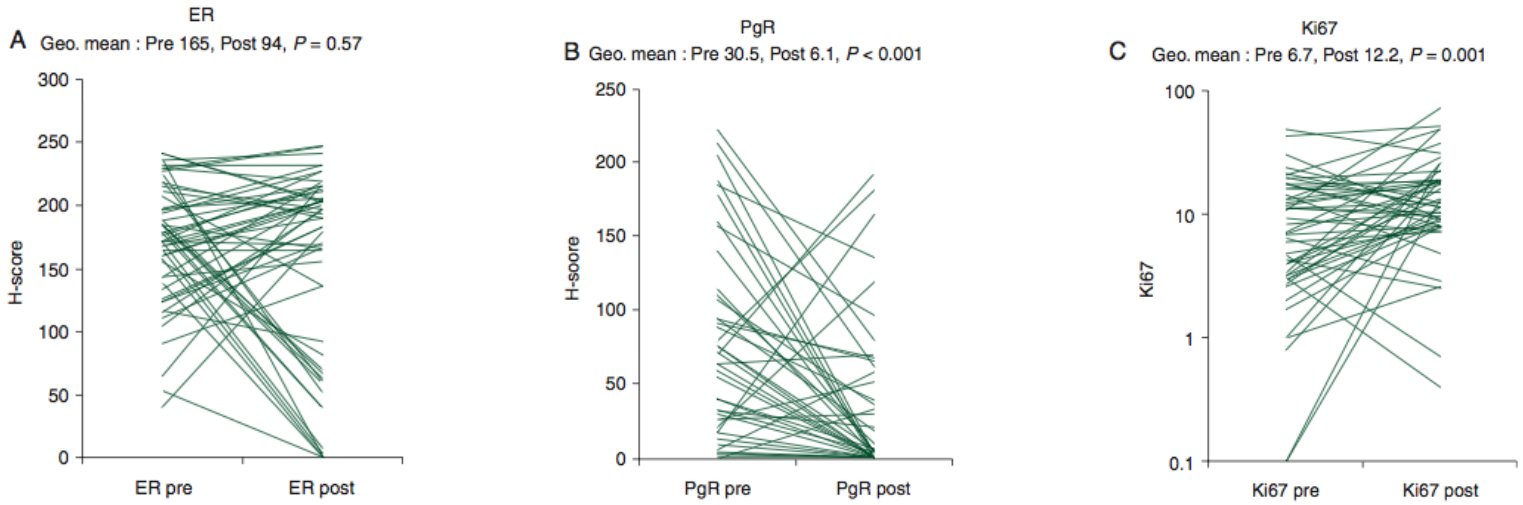
TILs have a prognostic value in pts
with a large tumor burden post-NACT (RT > 2 cm and/or N+)

Luminal – Per treatment

ER+/HER2- BC : « CLASSICS »

Arnedos M et al, Annals of Oncology 2014 (PMID 24525703)
IGR Paris & The Royal Marsden Hospital team

- a protein expression (IHC) study on 55 pre-NAT/on-NAT (post-NAT)* BC samples
*** pts relapsed or progressed on AI-NAT (advanced metastatic /locally advanced setting)**
- neoadjuvant anastrozole (AI-NAT)



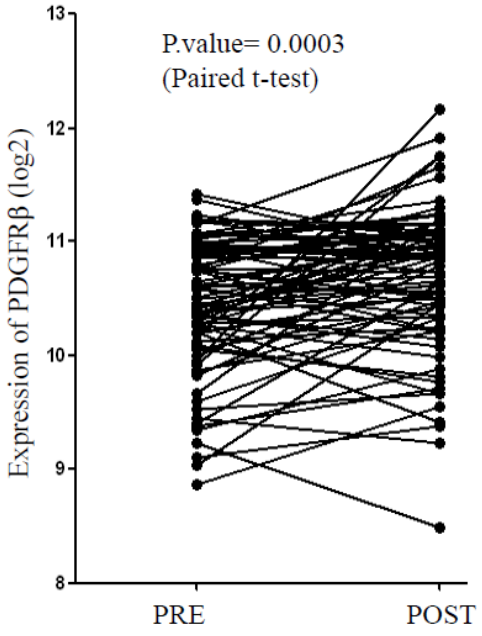
Changes after AI-NAT: **ER loss, PR decrease, IGF-1R decrease**
PTEN loss, HER2 gain (occasional cases)

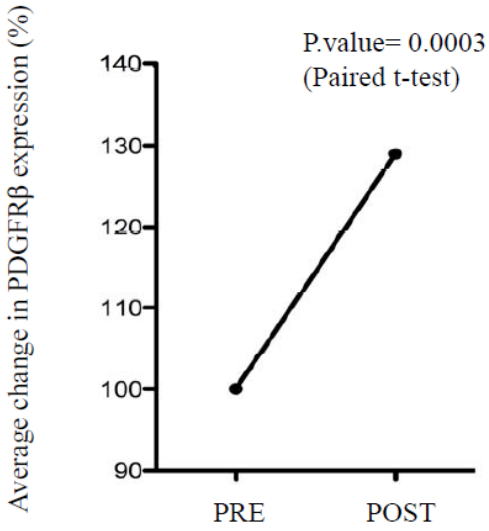
- **high intertumor heterogeneity**
- **multiple mechanisms of resistance on-/post-AI-NAT :**
  - **target loss/decrease**
  - **activation of growth factor signaling**
  - **activation of the PI3K-mTOR axis**

ER+/HER2- BC : NEW DISCOVERIES (1)

Weigel MT et al, Breast Canc Res 2012 (PMID 22608253)
The Royal Marsden Hospital team

- a preclinical study (cell lines) followed by GE analysis of 81 paired pre-NAT/on-NAT BC samples
- neoadjuvant anastrozole (AI-NAT)





PDGFR-B and PDGFR-L ARE UPREGULATED (1.2-fold, p = 0.0003 and 1.43-fold, p < 0.001, respectively) AFTER 2 WEEKS OF AI-NAT

CONFIRMED IN AN EXTERNAL VALIDATION STUDY (PMID 17885619)

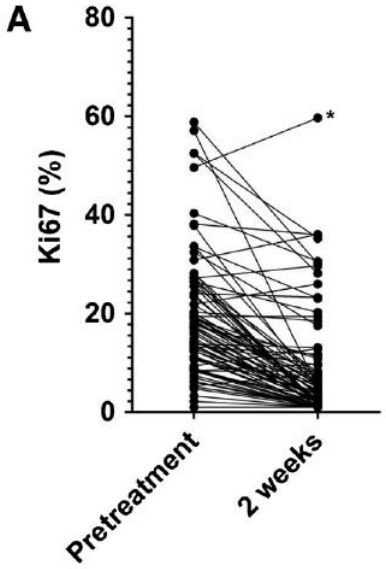
Marker of early resistance to AI

ER+/HER2- BC : NEW DISCOVERIES (2)

Dunbier AK et al, Clin Cancer Res 2013 (PMID 23493347)
The Royal Marsden Hospital team

- gene expression analysis of 81 paired pre-NAT/on-NAT BC samples
- neoadjuvant anastrozole (AI-NAT)
- 1327 genes diffentially expressed after 2 weeks of AI-NAT : 926 downregulated
  401 upregulated

a) AI-NAT INDUCES ANTI-PROLIFERATIVE RESPONSE



b) TUMORS ARE HETEROGENEOUS IN TRANSCRIPTIONAL RESPONSE TO AI-NAT

- no single gene up- or downregulated in all tumors
- most constantly altered : TOP2A (down in 94% of cases)

MOST FREQUENT CHANGES :

1) DOWNREGULATION OF ESTROGEN-RESPONSIVE GENES

2) DOWNREGULATION OF PROLIFERATION-ASSOCIATED GENES

3) UPREGULATION OF GENES FOR COLLAGENS AND CHEMOKINES

ER+/HER2- BC : NEW DISCOVERIES (3)

Dunbier AK et al, Clin Cancer Res 2013 (PMID 23493347)

The Royal Marsden Hospital team

Rank	Gene symbol	Description	Correlation coefficient	Parametric p-value or sco
Genes associated with poor response				
1	*SLAMF8*	Signaling lymphocytic activation molecule 8	0.520	3.0E-06
2	*P2RY6*	Pyrimidinergic receptor P2Y, G-protein coupled, 6	0.507	5.6E-06
3	*Hs.370503*	mRNA; cDNA DKFZp313O229	0.505	5.9E-06
4	*ZBED2*	Zinc finger, BED-type containing 2	0.492	1.11E-05
5	*PITPNM1*	Phosphatidylinositol transfer protein, 1	0.487	1.40E-05
6	*IL21R*	Interleukin 21 receptor	0.481	1.81E-05
7	*LAIR2*	Leukocyte-associated immunoglobulin-like R2	0.476	2.29E-05
8	*RGS19*	Regulator of G-protein signaling 19	0.474	2.54E-05
9	*HAVCR2*	Hepatitis A virus cellular receptor 2	0.465	3.75E-05
10	*IL32*	Interleukin 32	0.464	3.92E-05
11	*ADAM8*	ADAM metallopeptidase domain 8	0.464	3.84E-05
12	*PLCL3*	Phospholipase C, eta 1	0.464	3.77E-05
13	*FPRL2*	Formyl peptide receptor-like 2	0.462	4.25E-05
14	*LAG3*	Lymphocyte activation gene 3	0.461	4.31E-05
15	*SGK*	Serum/glucocorticoid regulated kinase	0.460	4.66E-05
16	*TNF*	Tumor necrosis factor	0.458	4.93E-05
17	*Hs.560728*	cDNA clone IMAGE:38786 3	0.457	5.12E-05
18	*CARD9*	Caspase recruitment domain family, member 9	0.457	5.24E-05
19	*TRAF3*	TNF receptor–associated factor 3	0.452	6.47E-05
20	*AKR1B1*	Aldo-keto reductase family 1, member B1	0.452	6.38E-05
24	*TNFAIP3*	Tumor necrosis factor, alpha-induced protein 3	0.438	0.000113
25	*CD53*	CD53 molecule	0.438	0.000112
33	*IRF8*	Interferon regulatory factor 8	0.427	0.000173
35	*CD86*	CD86 molecule	0.426	0.000177
36	*IL10RA*	Interleukin 10 receptor, alpha	0.426	0.000177
37	*CD84*	CD84 molecule	0.425	0.000184
40	*ITGAL*	Integrin, alpha L	0.423	0.000199
43	*IGSF6*	Immunoglobulin superfamily, member 6	0.420	0.000226
46	*ITGB2*	Integrin, beta 2	0.419	0.000228
48	*LPXN*	Leupaxin	0.418	0.000242
49	*SLAMF1*	Signaling lymphocytic activation molecule 1	0.417	0.000253
50	*TNFSF7*	CD70 molecule	0.417	0.000251

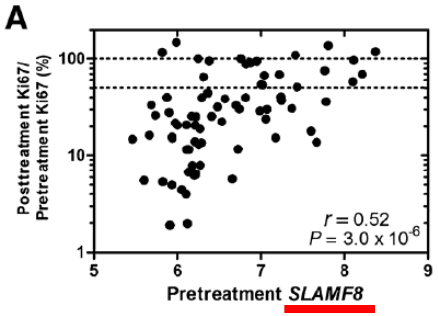
GENES ASSOCIATED WITH POOR RESPONSE TO AI-NAT :

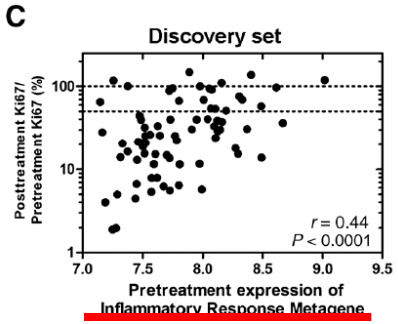
IMMUNE-RELATED (SLAM8, TNF, LAG3 etc.) Increasing during the duration of treatment

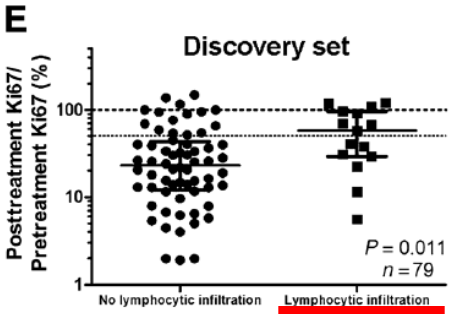
ER+/HER2- BC : NEW DISCOVERIES (4)

Dunbier AK et al, Clin Cancer Res 2013 (PMID 23493347)

The Royal Marsden Hospital team







THE OBSERVED ON-TREATMENT IMMUNE-RELATED CHANGES ARE LIKELY A CONSEQUENCE OF A PRE-TREATMENT TUMOR « IMMUNE STATUS »

« IMMUNE » PARAMETERS

SLAMF8

inflammatory response metagene

lymphocytic infiltration

HAVE BOTH PREDICTIVE AND PROGNOSTIC VALUE

IMPORTANT TO EVALUATE THEM BEFORE AND AFTER NAT

ER+/HER2- BC : NEW DISCOVERIES (5)

Gao Q et al, Clin Cancer Res 2014 (PMID 24634384)

The Royal Marsden Hospital team

- 81 paired pre-NAT/on-NAT BC samples (69 with Ki67)
- neoadjuvant anastrozole (AI-NAT)
- GENE EXPRESSION : 26 PUBLICLY AVAILABLE GENE MODULES

- ESR1.1 , ESR1.2 : ER signaling and estrogen-regulated modules

- SET : sensitivity to endocrine therapy index

- ERG : estrogen-responsive genes

- IGF1, OBESITY, MAPK, SRC, BETA-CATENIN, RAS, ERBB2, VEGF, PIK3CA :

growth factor receptors and cytoplasmic signaling–related

MODULES

- PTEN LOSS

- AKT/mTOR

- MYC : cell cycle–related module

- CASP3 : apoptosis and cell survival signaling

- CIN70, GGI, AURKA, Gene70, E2F3 (13) : proliferation-based

- Stroma.1, Stroma.2-PLAU : tumor invasion

- Immune.1, Immune.2-STAT1 : immune response

ER+/HER2- BC : NEW DISCOVERIES (6)

Gao Q et al, Clin Cancer Res 2014 (PMID 24634384)

The Royal Marsden Hospital team

Table 2. Dynamic changes in expression of Gene modules and Ki67 in response to the 2 weeks' AI treatment

	All matched			PAGs excluded		
Module name	%Δ[a] of Geometric mean of intensities of a module of pre- and posttreatment	Two-sided unadjusted *P* value of Wilcoxon test	Adjusted *P*	%Δ of Geometric mean of intensities of pre- and posttreatment	Two-sided unadjusted *P* value of Wilcoxon test	Adjusted *P*
Ki67	−75.23	4.75E−12	4.77E−11	N/A	N/A	N/A
ERG	−28.36	1.40E−12	3.64E−11	−28.51	1.20E−12	3.64E−11
CIN70	−24.25	4.20E−12	4.77E−11	−11.34	1.60E−08	7.56E−08
GGI	−22.32	5.00E−12	4.77E−11	−7.82	3.90E−09	2.54E−08
AURKA	−12.34	5.50E−12	4.77E−11	N/A	N/A	N/A
Gene70	−8.37	8.60E−09	4.47E−08	−1.11	0.3400	0.3930
SET	−7.99	1.00E−05	3.25E−05	−8.20	6.70E−06	2.32E−05
PTEN	−7.22	2.80E−10	2.08E−09	−2.61	0.0002	3.85E−04
CASP3	−5.19	0.0114	0.0180	−4.45	0.0420	0.0642
E2F3	−4.40	4.40E−09	2.54E−08	−2.63	8.10E−05	1.91E−04
MYC	−4.32	1.80E−06	7.20E−06	−4.74	3.90E−07	1.69E−06
ESR1.2	−4.39	2.00E−04	3.85E−04	−4.53	2.00E−04	3.85E−04
ESR1.1	−4.18	0.0015	0.0027	−4.56	5.00E−04	9.29E−04
AKT/mTOR	−2.75	0.0052	0.0085	−2.07	0.0590	0.0877
IGF-I	−2.44	3.80E−05	9.88E−05	−1.94	2.00E−04	3.85E−04
VEGF	−1.80	0.1300	1.73E−01	−1.80	0.1300	0.1730
RAS	−1.14	0.1100	1.55E−01	−1.00	0.2500	0.3020
SRC	0.52	1.0000	1.0000	0.34	0.7700	0.7850
PIK3CA	0.47	0.3400	0.3930	0.28	0.4700	0.5200
Obesity	0.74	0.7100	0.7380	0.67	0.6100	0.6470
Betacatenin	1.01	0.3800	0.4300	0.73	0.4900	0.5310
ERBB2	1.62	0.1700	0.2100	2.12	0.0770	0.1110
Immune.2.STAT1	3.62	0.1400	0.1780	3.69	0.1400	0.1780
MAPK	5.03	4.80E−06	1.78E−05	4.50	2.20E−05	6.73E−05
Stroma.2.PLAU	7.57	0.0038	0.0066	7.72	0.0040	0.0067
Immune.1	13.45	3.20E−05	8.76E−05	13.45	3.20E−05	8.76E−05
Stroma.1	20.75	4.60E−05	1.14E−04	20.05	8.60E−05	1.94E−04

REDUCED BY ANASTROZOLE :

CIN70 **ESR1.1**
GGI **ESR1.2**
AURKA
Gene70 **SET**

signaling pathways :
PTEN, CASP3, E2F3, MYC, AKT/MTOR, IGF-1

UPREGULATED BY ANASTROZOLE :

PIK3CA
MAPK
Stroma2-PLAU
Stroma.1
Immune.1

Immune related genes are **highly predictive of poor antiproliferative response** to anastrozole

HER2+

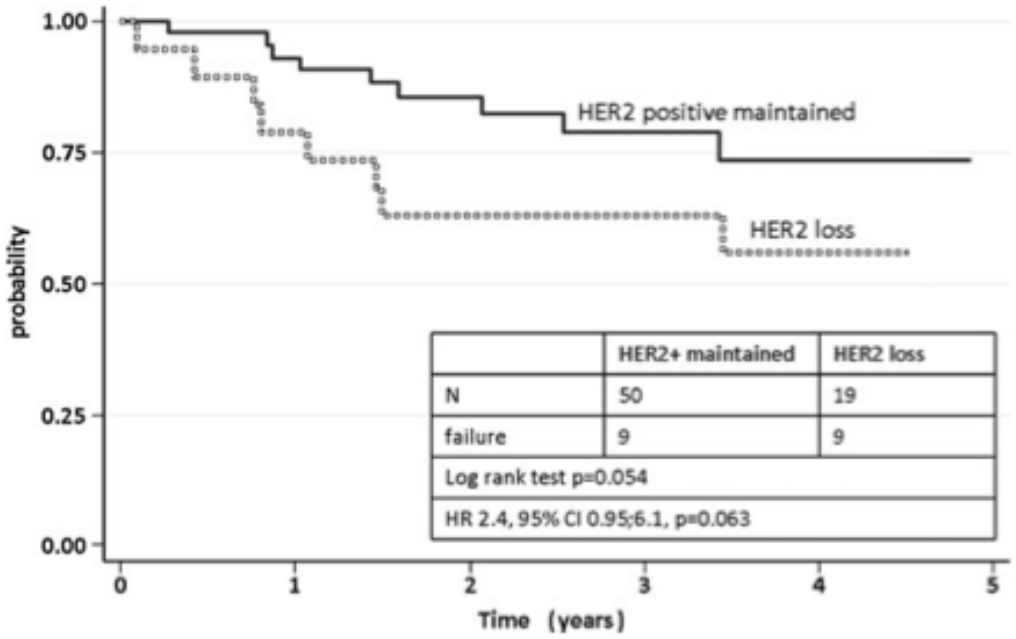
original article

Annals of Oncology 00: 1–5, 2013
doi:10.1093/annonc/mdt364

Loss of HER2 positivity and prognosis after neoadjuvant therapy in HER2-positive breast cancer patients

V. Guarneri[1]*, M. V. Dieci[2,3], E. Barbieri[2], F. Piacentini[2], C. Omarini[2], G. Ficarra[4], S. Bettelli[4] & P. F. Conte[1]

[1]*Istituto Oncologico Veneto IRCCS, University of Padova, Italy;* [2]*Department of Oncology, Hematology and Respiratory Diseases, University Hospital, Modena, Italy;* [3]*INSERM U981, Institut Gustave Roussy, Villejuif, Paris , France;* [4]*Department of Pathology, University Hospital, Modena, Italy*



	HER2+ maintained	HER2 loss
N	50	19
failure	9	9
Log rank test p=0.054		
HR 2.4, 95% CI 0.95;6.1, p=0.063		

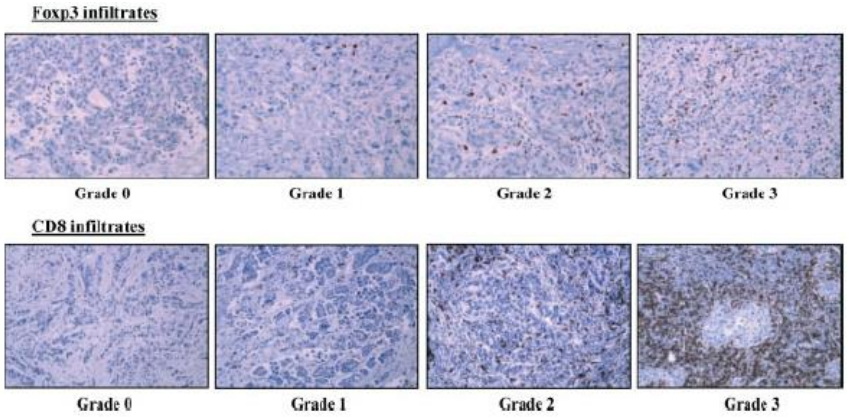
HER2+ BC : CD8/FOXP3 post treatment

Ladoire S et al, J Pathol 2011 (PMID 21437909)

French Cancer Centers

- 111 HER2+ BC treated by a NACT ; residual tumors

- analysis of tumor-infiltrating lymphocyte subpopulations by detecting CD8 or FOXP3 (IHC)

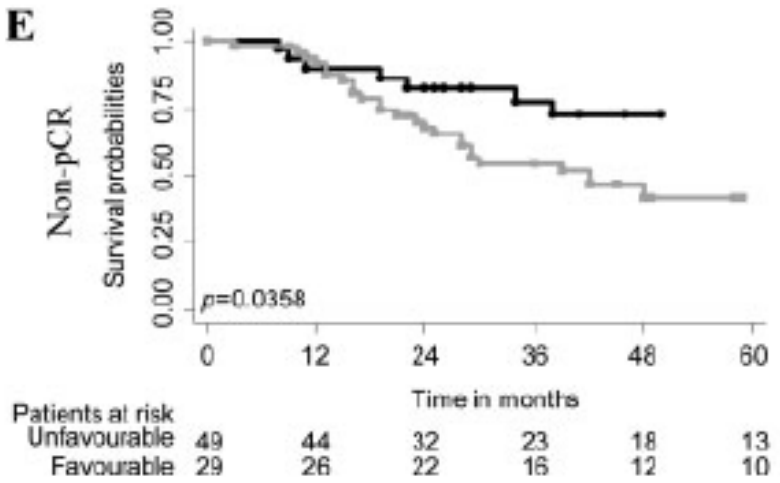


« The Dijon grading »
of TIL-CD8+ or TIL-FOXP3+ density

CD8/FOXP3 ratio

a) favourable (CD8high, FOXP3low)

b) unfavourable (CD8low, FOXP3high)



In RT (non-pCR) post-NACT
unfavourable TIL CD8/FOXP3 score
predicts poor survival

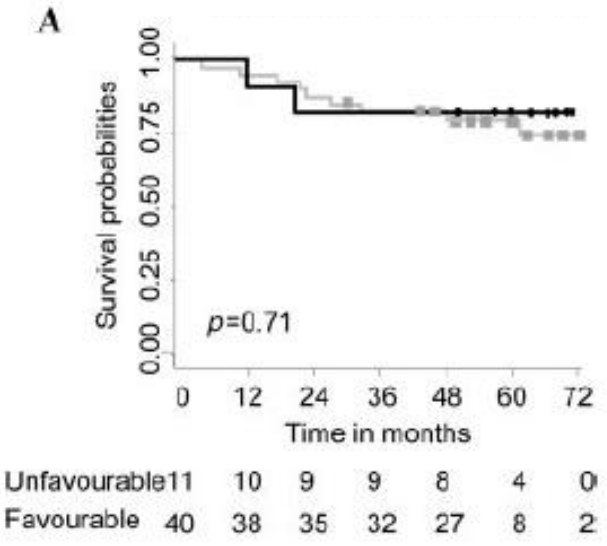
HER2+ BC : CD8/FOXP3 post treatment

Ladoire S et al, J Pathol 2011 (PMID 21437909)

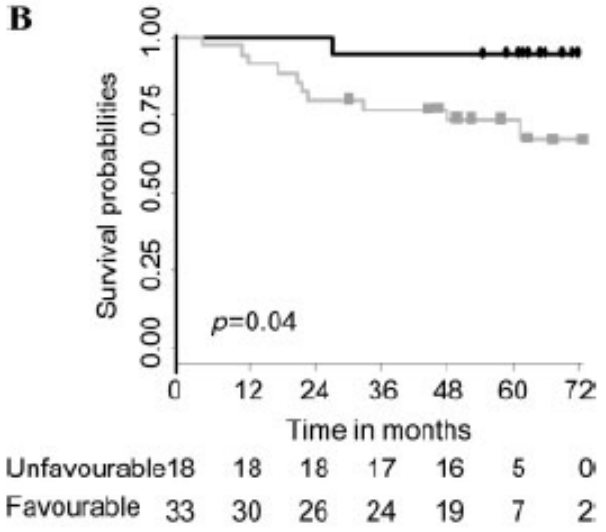
French Cancer Centers

- 111 HER2+ BC RT post-NACT

- analysis of tumor-infiltrating lymphocyte subpopulations by detecting CD8 or FOXP3 (IHC)



TIL CD8+/FOXP3 before NACT



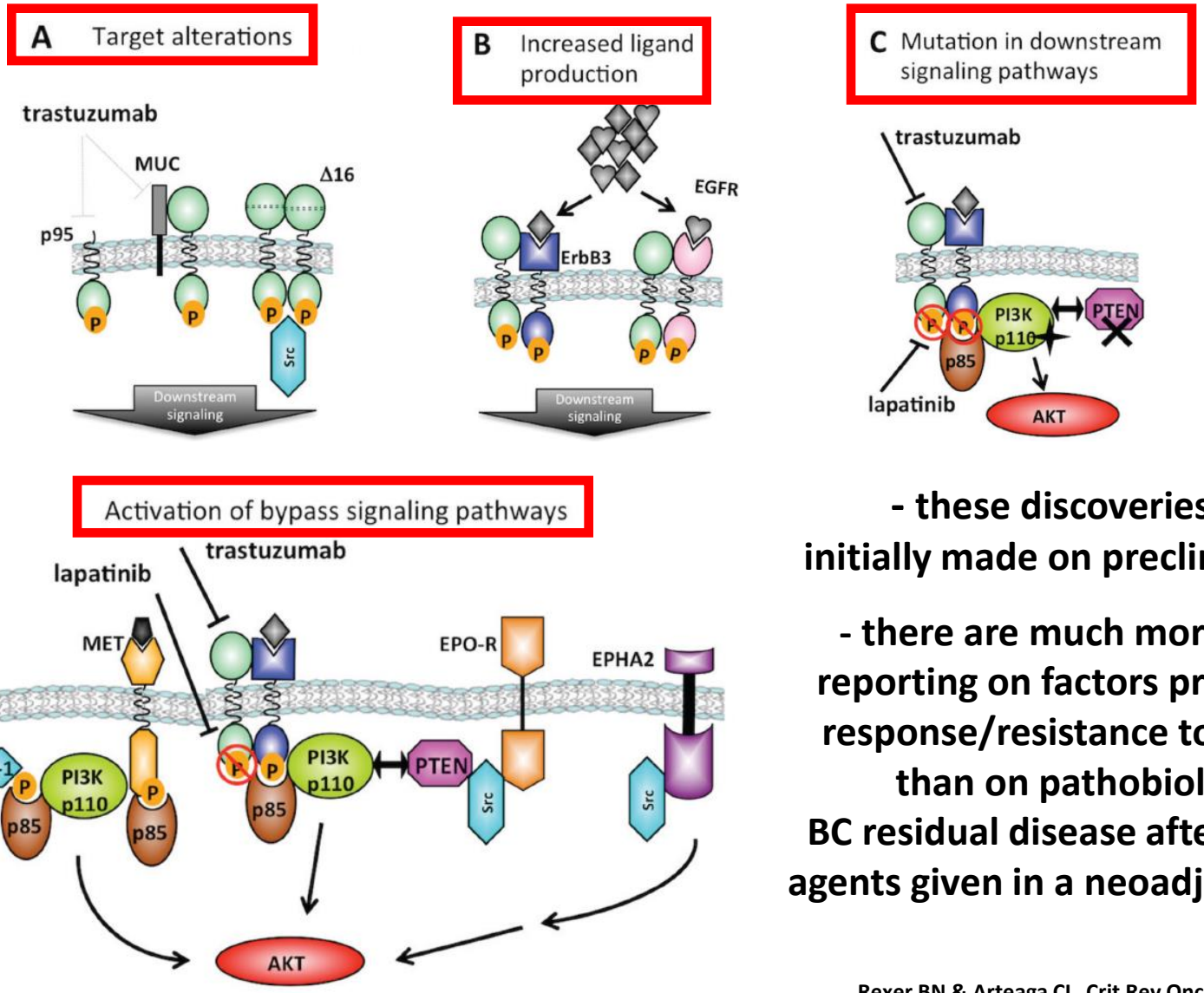
TIL CD8+/FOXP3 after NACT

Prognostic value of the ratio between TIL-CD8+ and TIL-FOXP3 count

Is more significant when evaluated on the post-NACT RT then on the pre-NACT sample

HER2+ BC : NEW DISCOVERIES (1)

MAJOR MECHANISMS OF RESISTANCE TO ANTI-HER2 AGENTS



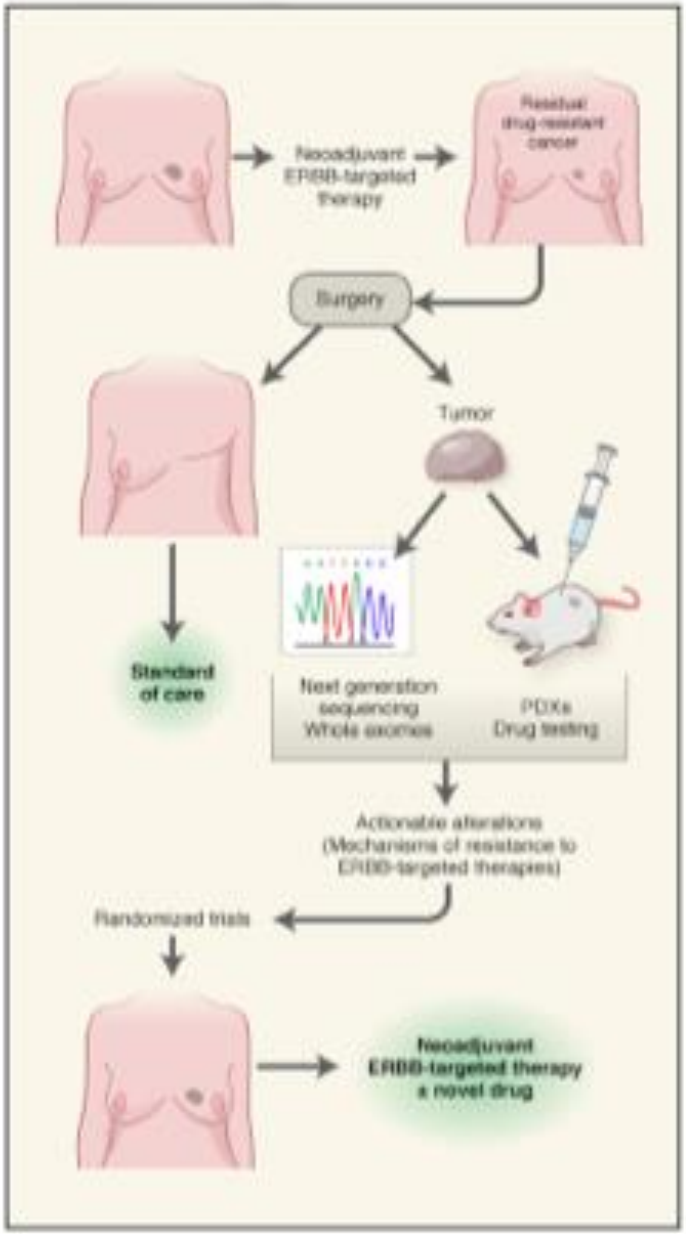
- these discoveries were initially made on preclinical models

- there are much more studies reporting on factors predictive for response/resistance to anti-HER2 than on pathobiology of BC residual disease after anti-HER2 agents given in a neoadjuvant setting

Rexer BN & Arteaga CL, Crit Rev Oncog 2012, PMID 22471661

CONCLUSION

- Post NAT Residual cancers have different prognostic value
- Post NAT (“drug-resistant”) Residual cancers harbor targetable genomic alterations causally associated with resistance to neoadjuvant therapy
- **Molecular profiling** of these residual tumors should identify these alterations.
- In addition, **patient-derived xenografts** generated with these residual cancers can be used to test novel combinations with activity against these drug-resistant cancers.



Arteaga1 CL & Engelman JA, Cancer Cell 2014