SCIENTIFIC BITES®

Cancer research
e-learning platform

Mechanisms of resistance to anti-HER2 therapy in HER2+ disease

Joan Albanell
Hospital del Mar, Barcelona

by SOLTI

Outline of the presentation

I) Overview of mechanisms of action of anti-HER2 agents

II) Exploiting resistance related to HER2 downstream signaling

III) p95HER2 fragment: where are we going?

IV) Innate and adaptive (HLA) immunity and response to anti-HER2 MAbs

V) Role of fibroblasts

VI) Dissecting resistance to T-DM1

Outline of the presentation

I) Overview of mechanisms of action of anti-HER2 agents

Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185^{HER2} Monoclonal Antibody in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer

By José Baselga, Debasish Tripathy, John Mendelsohn, Sharon Baughman, Christopher C. Benz, Lucy Dantis, Nancy T. Sklarin, Andrew D. Seidman, Clifford A. Hudis, Jackie Moore, Paul P. Rosen, Thomas Twaddell, I. Craig Henderson, and Larry Norton

Table 4. Response Rate Obtained With rhuMAb HER2 in 43 Assessable Patients

Response	No. of Patients	%
Complete response	1	2.3
Partial response	4	9.3
Overall response	5	11.6
Minor response	2	4.6
Stable disease	14	32.6
Progression of disease	22	51.2

The observed activity of rhuMAb HER2 against advanced breast cancers that overexpress HER2 provides the first clinical evidence that anti–growth factor receptor-directed strategies may be useful in the treatment of human breast cancer. Therefore, continued research with this agent and other HER2-targeted treatment strategies appears warranted. The response to rhuMAb HER2 in a less heavily pretreated population and in those with less

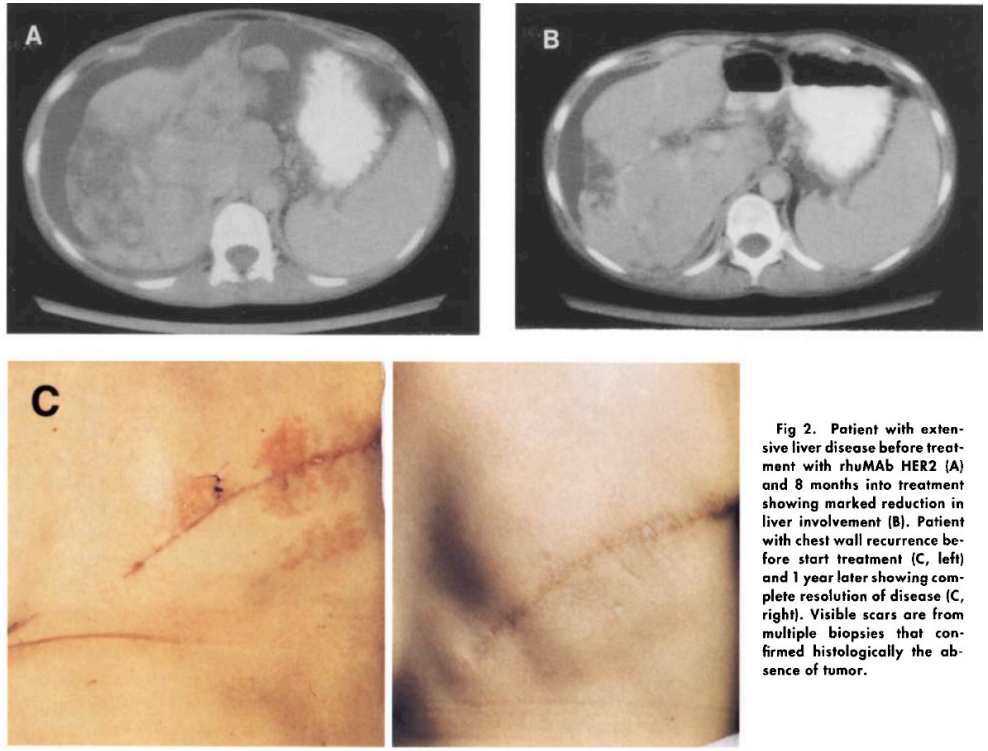


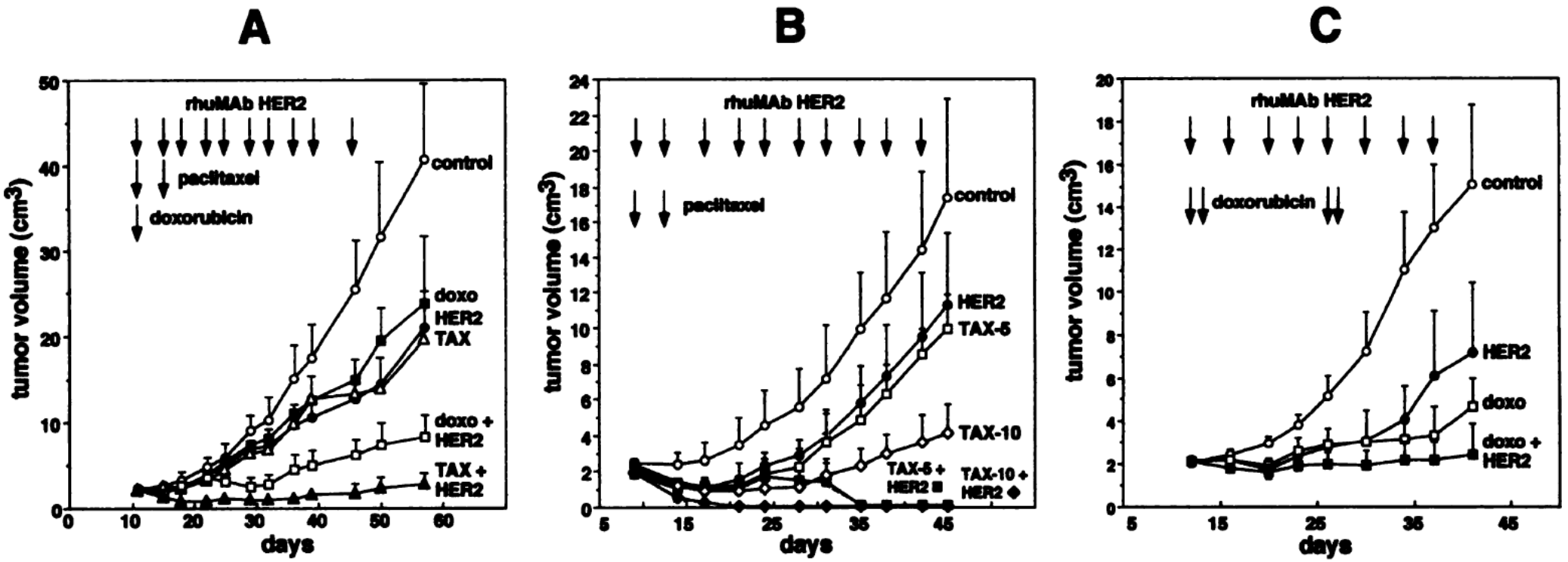
Fig 2. Patient with extensive liver disease before treatment with rhuMAb HER2 (A) and 8 months into treatment showing marked reduction in liver involvement (B). Patient with chest wall recurrence before start treatment (C, left) and 1 year later showing complete resolution of disease (C, right). Visible scars are from multiple biopsies that confirmed histologically the absence of tumor.

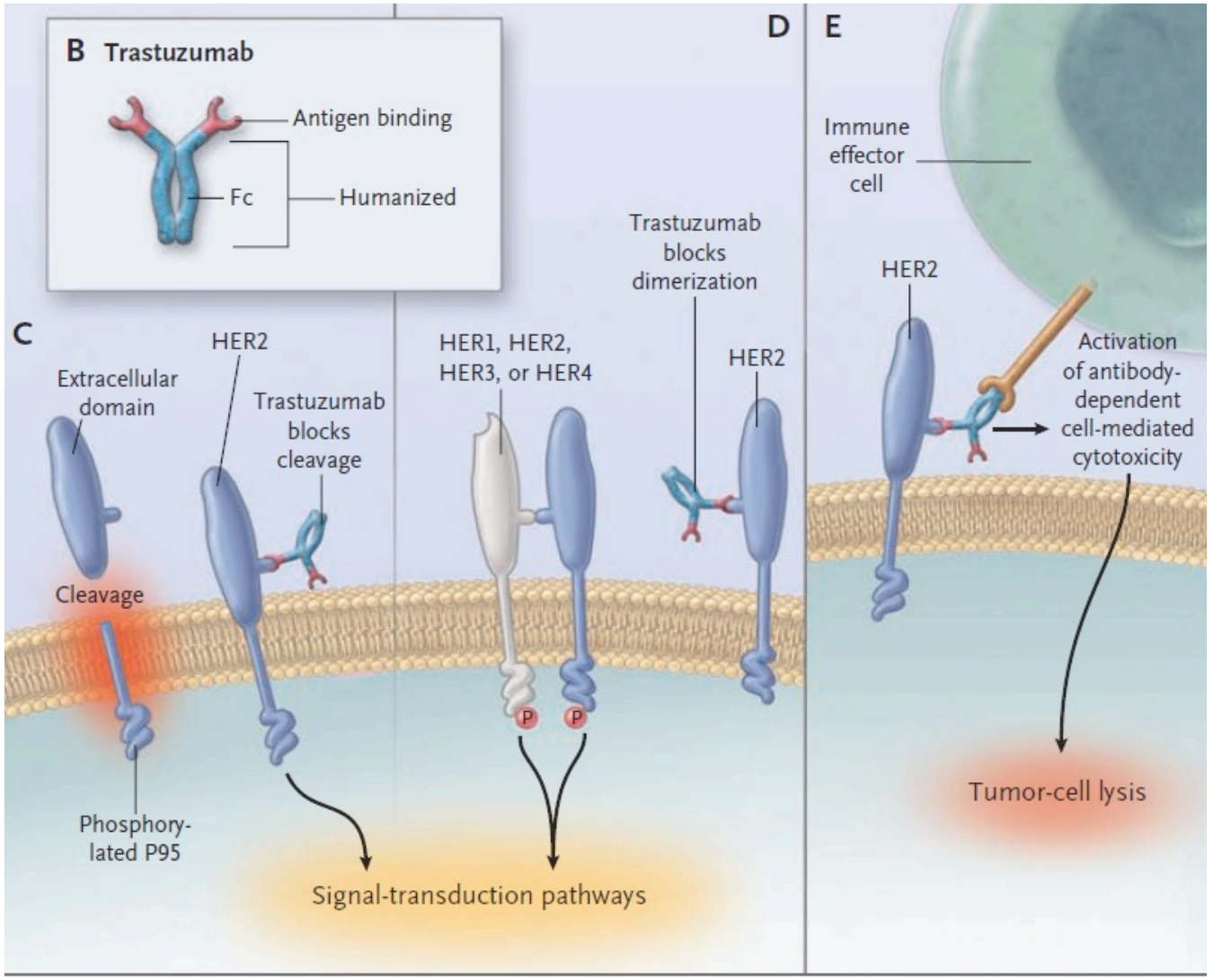
J Clin Oncol 14:737-744.

[CANCER RESEARCH 58, 2825–2831, July 1, 1998]

Recombinant Humanized Anti-HER2 Antibody (Herceptin™) Enhances the Antitumor Activity of Paclitaxel and Doxorubicin against HER2/*neu* Overexpressing Human Breast Cancer Xenografts[1]

Jose Baselga,[2] Larry Norton, Joan Albanell, Young-Mee Kim, and John Mendelsohn[3]





The NEW ENGLAND JOURNAL of MEDICINE

HER2-targeted agents: Different mechanisms of action

Small molecules

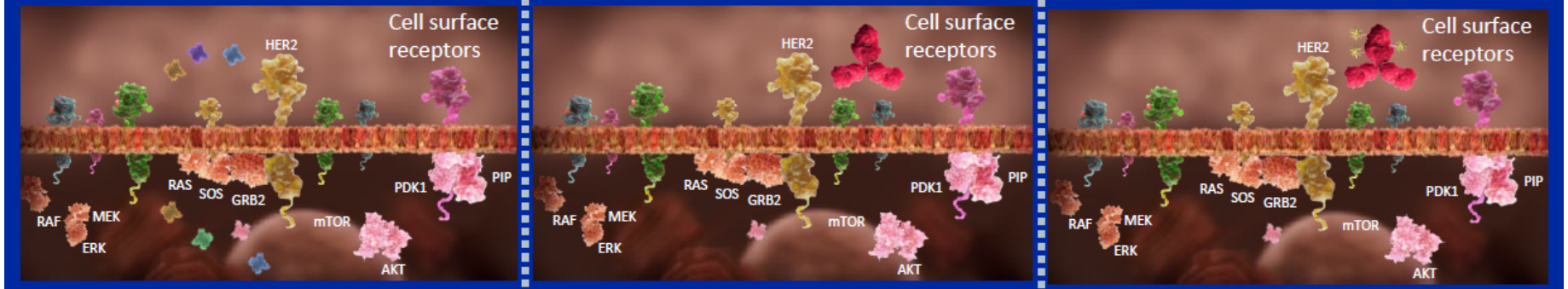
- Generally, chemical agents (~400 Da)
- Varying degrees of specificity
- Penetrate through the plasma membrane
- Cannot flag cells for destruction by the immune system

Antibodies

- Generally, large proteins (~150 000 Da)
- Highly specific
- Cannot freely penetrate through the plasma membrane
- Flag cells for destruction by the immune system (ADCC)

Antibody–drug conjugates

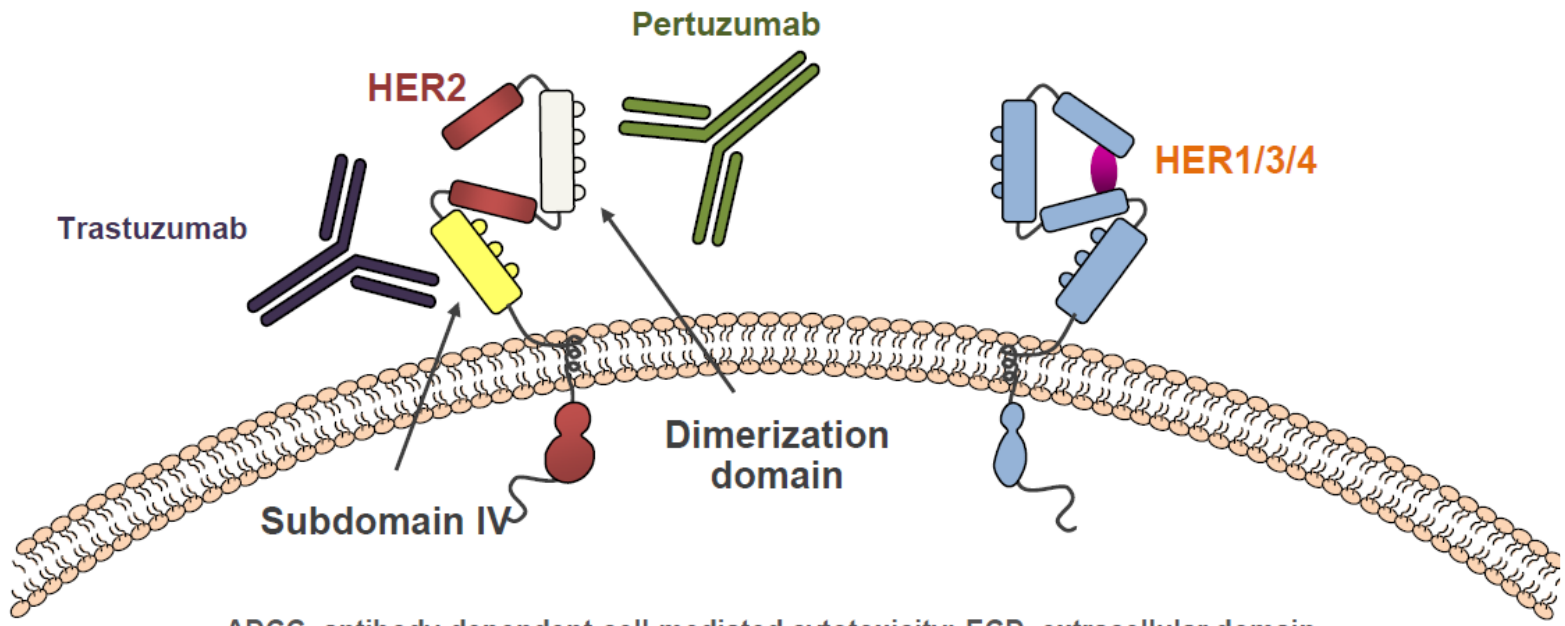
- Combine the properties of cytotoxic drugs and antibodies
- Allow targeted delivery of chemotherapy agents to tumour cells
- May initiate immune cell recruitment and ADCC



ADCC, antibody-dependent cellular cytotoxicity

1. El-Sahwi K, *et al. Br J Cancer* 2010; **102**:134–143;
2. Lewis Phillips G, *et al. Cancer Res* 2008; **68**:9280–9290.

Dual HER2 Blockade with two Antibodies: Pertuzumab and Trastuzumab



ADCC, antibody-dependent cell-mediated cytotoxicity; ECD, extracellular domain

Trastuzumab:

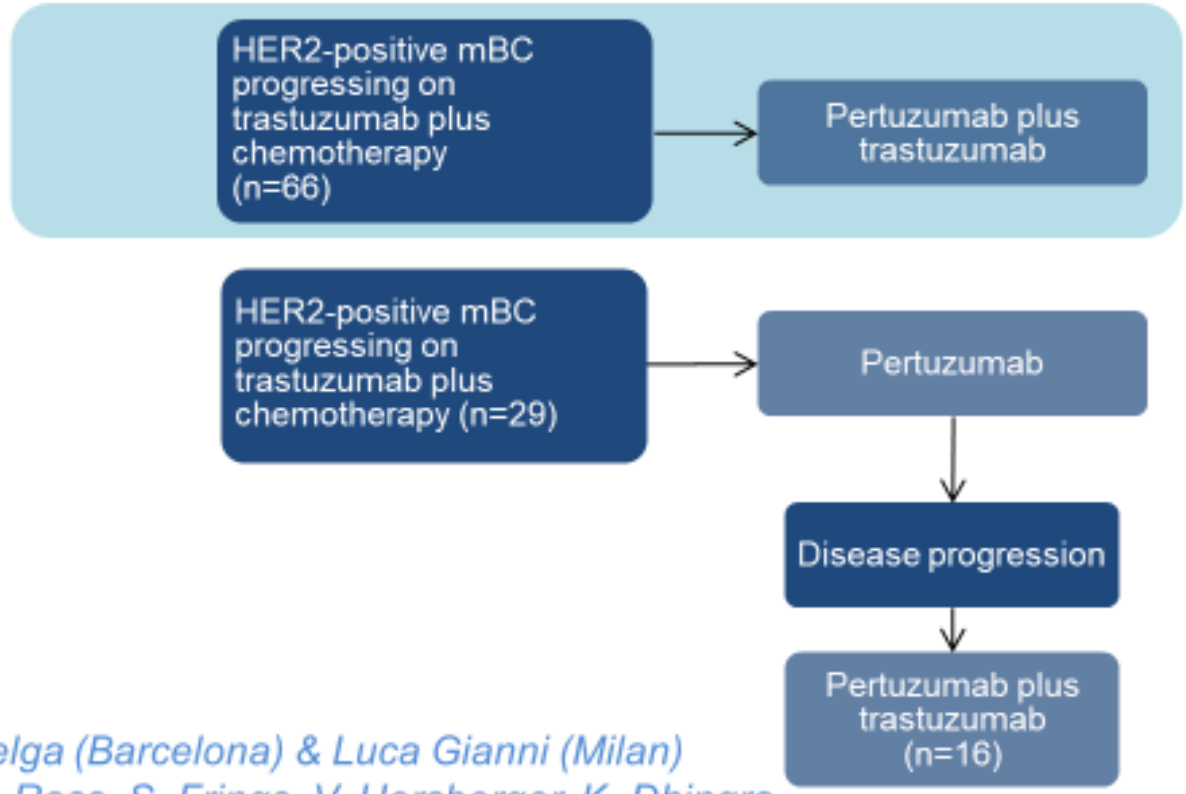
- Inhibits ligand-independent HER2 signaling
- Activates ADCC
- Prevents HER2 ECD shedding

Pertuzumab:

- Inhibits ligand-dependent HER2 dimerization and signaling
- Activates ADCC

Baselga, Nat Rev Cancer 2009

Pertuzumab and Trastuzumab Phase II Proof of Concept Study (BO17929)

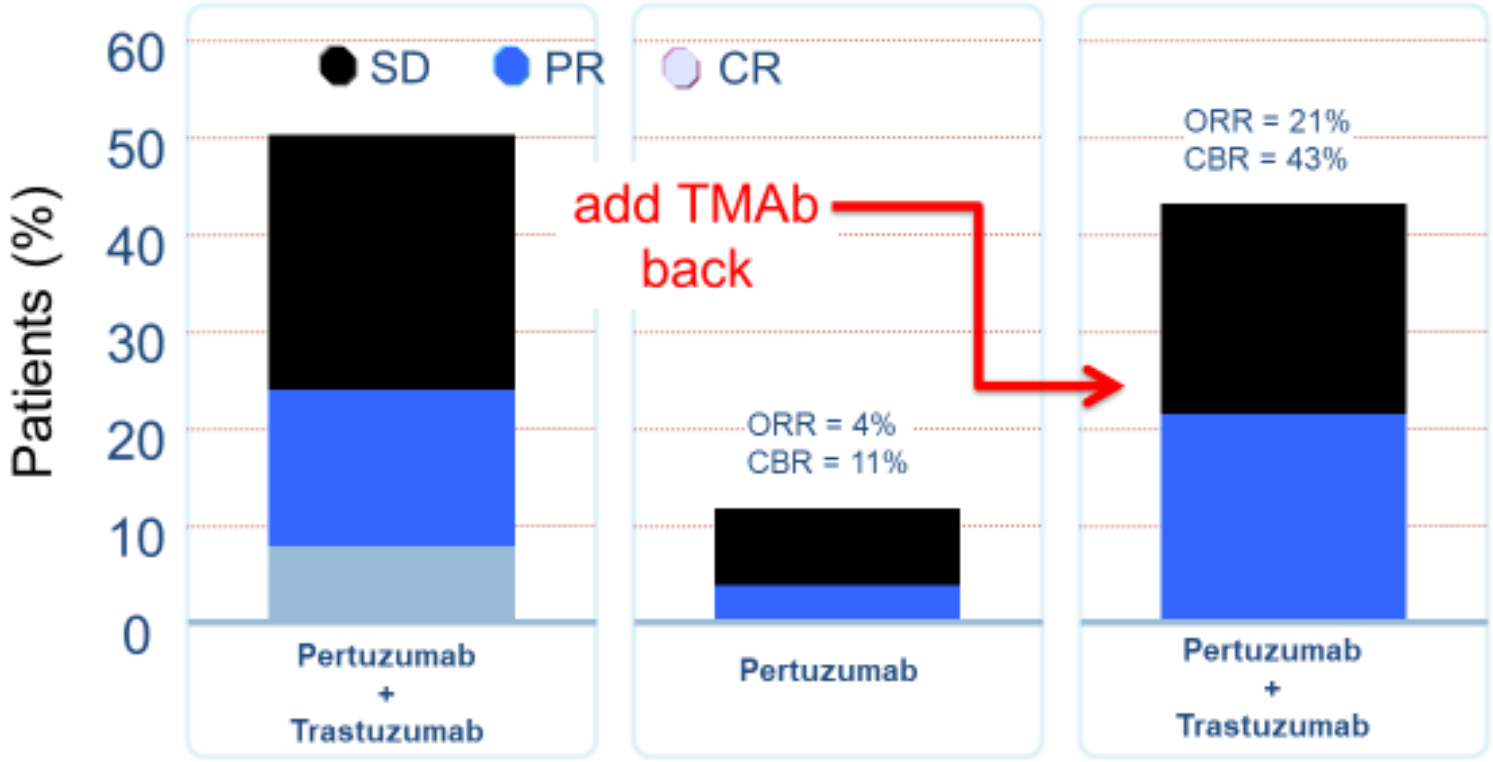


José Baselga (Barcelona) & Luca Gianni (Milan)
Roche: G. Ross, S. Frings, V. Hersberger, K. Dhingra

Baselga J, *et al. J Clin Oncol* 2010; **28**:1338–1144.

Phase II Study in Advanced MBC: Demonstrates Need For Dual-Blockade

Objective Response (OR) = Complete Response (CR) + Partial Response (PR)
Clinical Benefit = OR + Stable Disease (SD) > 6 mo.



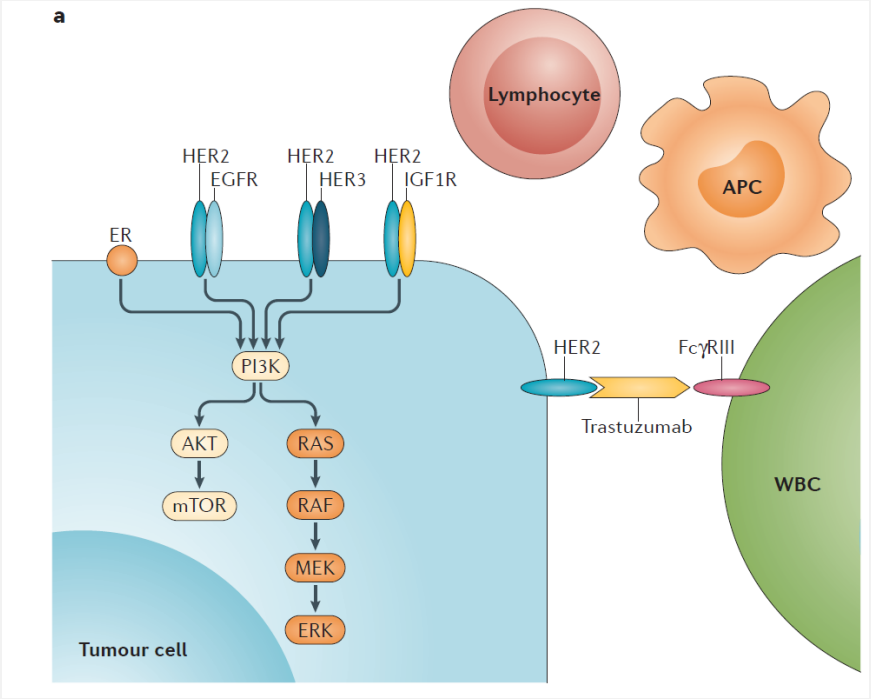
P+T median PFS 5.5 months

Cortés J, *et al. J Clin Oncol* 2012; **30**:1594–1600.

Table 1 | **Translational biomarker research efforts in HER2-positive breast cancer from 2005 to 2016**

HER2	Other receptors/ ligands	HER2 downstream pathways	Stroma	Host
• Copy number/FISH ratio • Polysomy • mRNA expression • Protein expression • HER2 protein domain expression • P95 HER2 • Serum ECD • HER2+ CTC • HER2-enriched subtype (PAM50)	• HER3 • EGFR (HER1) • IGFR • EGF • TNFα • Heregulin • ERα	• PIK3CA/PTEN • RhoA pathway	• TILs • Immune gene-expression signatures • pSTAT3	• FCγR polymorphism • *HER2* gene polymorphism

Abbreviations: CTC, circulating tumour cell; ECD, extracellular domain; ERα, oestrogen receptor α; FISH, fluorescence *in situ* hybridization; FCγR fragment C receptor γ; pSTAT3, phospho-signal transducer and activator of transcription 3; TILs, tumour-infiltrating lymphocytes.



Cingras et al. Nature Rev Clin Oncol 2017

HER2-positive breast cancer is lost in translation: time for patient-centered research

Isabelle Gingras[1], Géraldine Gebhart[2], Evandro de Azambuja[3] and Martine Piccart-Gebhart[4]

Table 5 | **Patient-centered biomarker research**

Patients needs	Biomarkers with clinical utility	Biomarkers with greatest 'promise' that require clinical validation
Advanced-stage disease		
• Can I be sure that the chosen therapy will truly help me? • Can I avoid therapies with marked side effects for long periods of time?	• NONE • NONE	• HER2 PET (± FDG–PET); ctDNA? • HER2 PET; ctDNA?
Early stage disease		
• Can I be confident that chemotherapy and single HER2 blockade is good enough for me? • Can I do as well with a simpler or shorter treatment? • Can I forego (aggressive) chemotherapy?	• NONE • NONE • NONE	• Immune-gene signatures: a/o TILs • To be validated across large adjuvant trials such as ALTTO/APHINITY (with a testing set and a validation set); • 8-Gene signatures (with high ESR1, intermediate HER2) to be tested in adjuvant trials of longer versus shorter trastuzumab duration; • PAM50 HER2-enriched subtype to be further validated with correlation to EFS

ctDNA, circulating tumour DNA; EFS, event-free survival; ESR1, oestrogen receptor; FDG, fluorodeoxyglucose; PAM50, Prediction Analysis of Microarray 50; TILs, tumour-infiltrating lymphocytes.

Cingras et al. Nature Rev Clin Oncol 2017

Understanding mechanisms of antiHER2 resistance in a patient-oriented manner

Identify mechanisms behind patients cured with current antiHER2 → Design rational de-escalation strategies to reduce toxicities and cost

Identify mechanisms behind patients dying despite current antiHER2 → Design rational strategies towards curation

Emerging Treatments for HER2+ Breast Cancer

Novel Anti-HER2 Antibodies (Abs)

- Margetuximab
- MCLA-128
- ZW25
- PRS-343
- BTRC4017A
- MM-111

New Antibody Drug Conjugates (ADCs)

- DS-8201a
- ZW49
- A166
- MEDI1426
- RC48
- SYD985
- XMT-1522
- ALT-P7
- ARX788

Potent Tyrosine Kinase Inhibitors

- Neratinib
- Pyrotinib
- Poziotinib
- Tucatinib

Promising Combinations

- Immune Therapies
- CDK4/6 Inhibitors
- Others
- PI3K Inhibitors

Outline of the presentation

II) Exploiting resistance related to HER2 downstream signaling

Cancer Cell. 2016 March 14; 29(3): 255–269. doi:10.1016/j.ccell.2016.02.006.

Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers With CDK4/6 Inhibitors

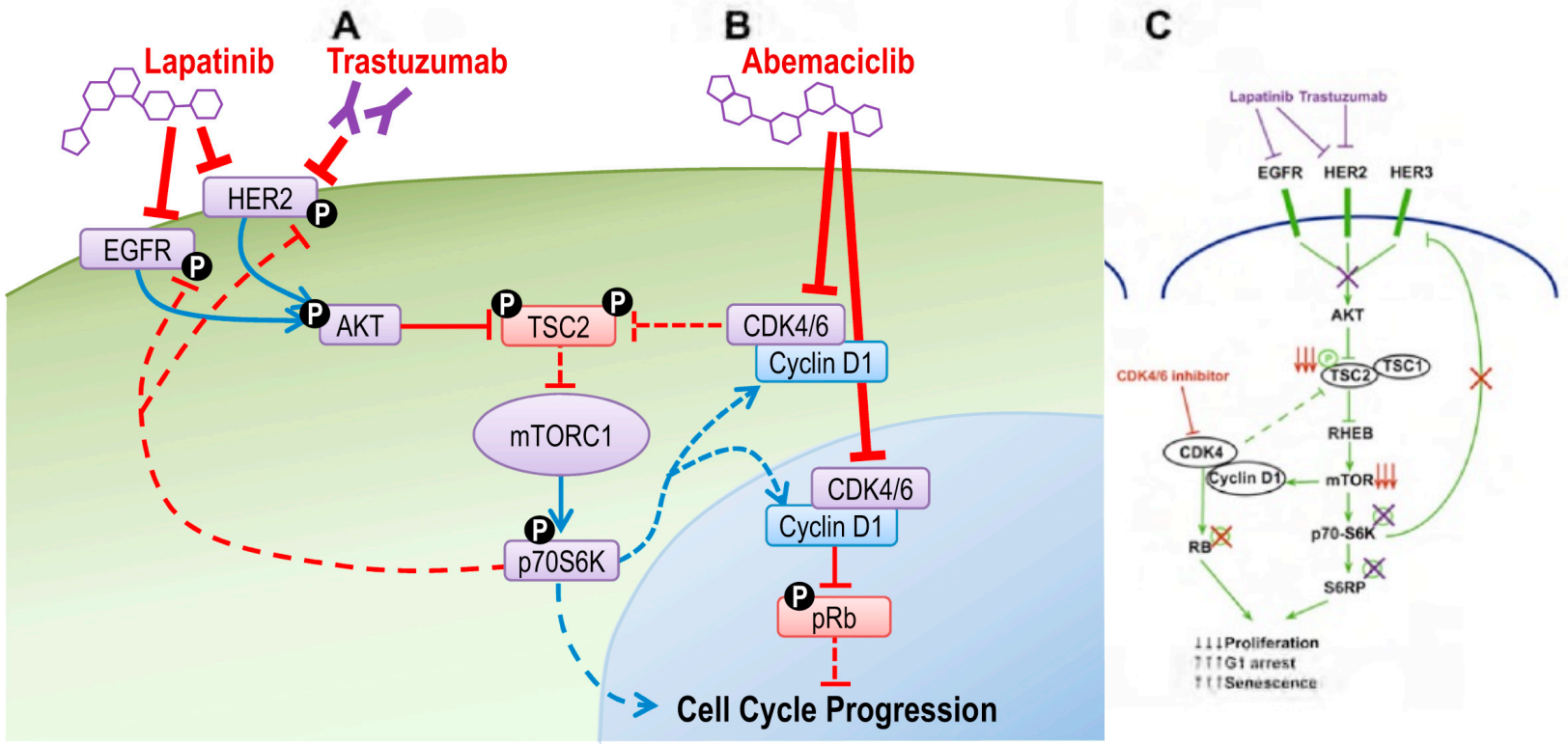
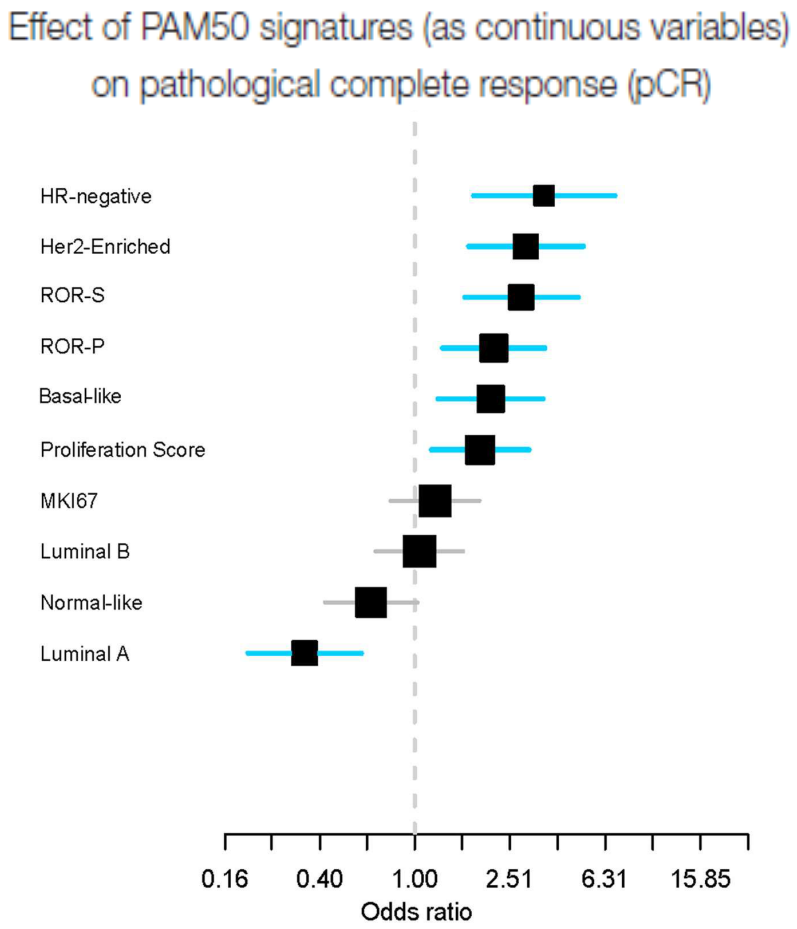


Figure 8. CDK4 inhibition re-sensitizes HER2-therapy resistant tumors to inhibition of EGFR-family kinases



PAM50 Subtypes in Baseline and Residual Tumors Following Neoadjuvant Trastuzumab-Based Chemotherapy in HER2-Positive Breast Cancer: A Consecutive-Series From a Single Institution

Sonia Pernas[1], Anna Petit[2], Fina Climent[2], Laia Paré[3], J. Perez-Martin[4], Luz Ventura[1], Milana Bergamino[1], Patricia Galván[3], Catalina Falo[1], Idoia Morilla[1], Adela Fernandez-Ortega[1], Agostina Stradella[1], Montse Rey[5], Amparo Garcia-Tejedor[6], Miguel Gil-Gil[1] and Aleix Prat[3]**

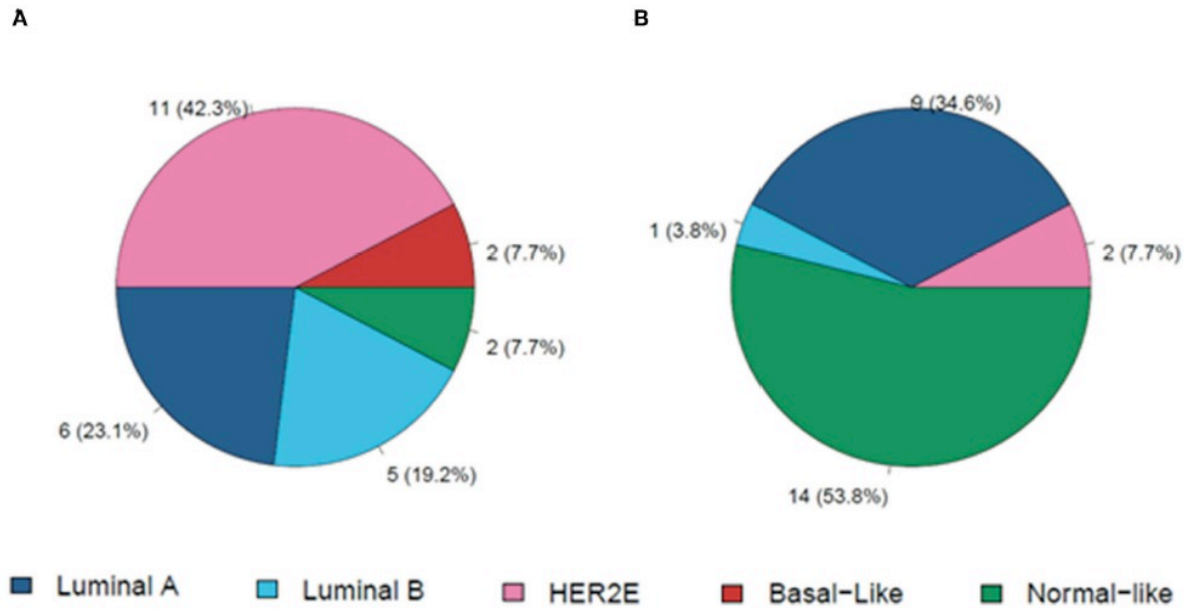


FIGURE 5 | Distribution of molecular subtypes in a cohort of patients with residual disease and paired baseline **(A)** and surgical specimens **(B)**. HER2E, HER2-enriched.

nature COMMUNICATIONS

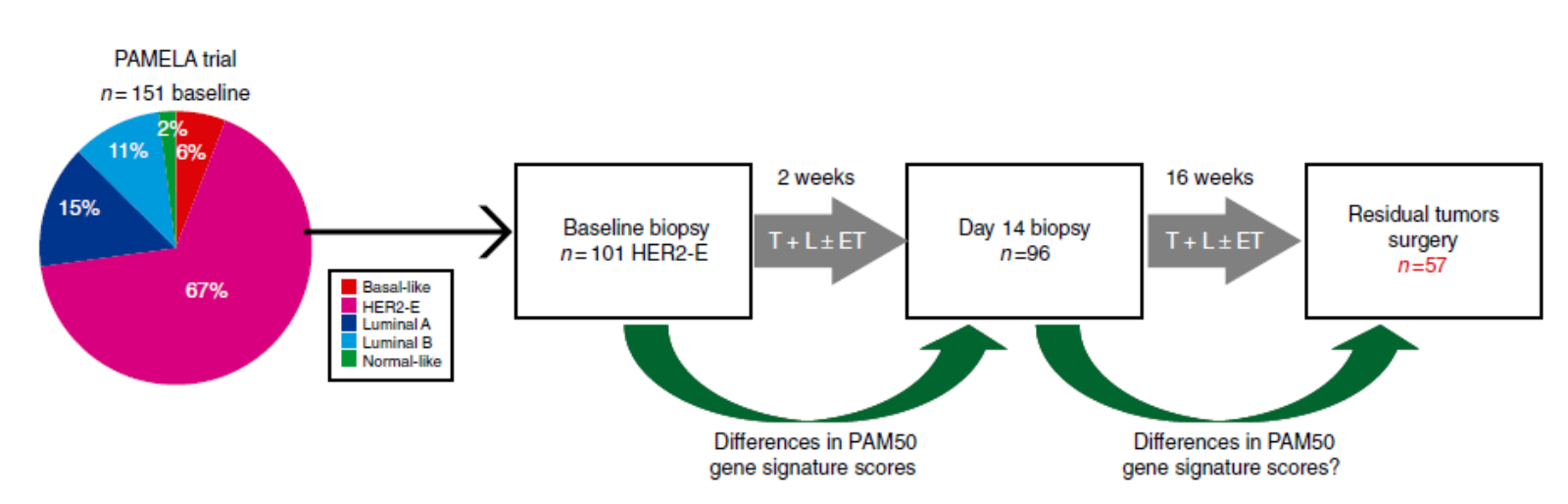
ARTICLE

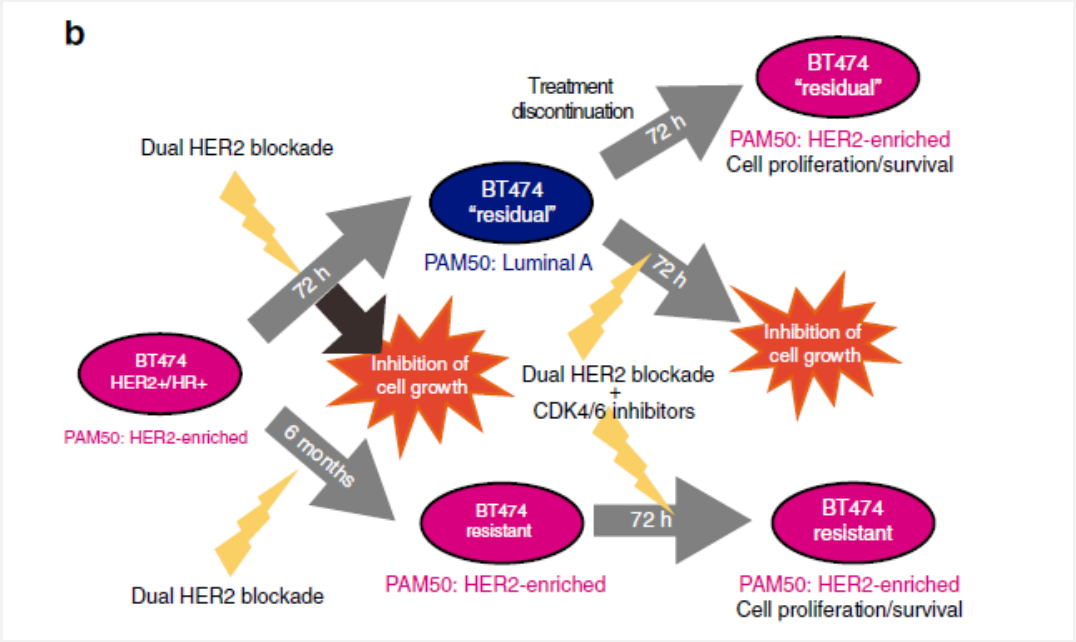
https://doi.org/10.1038/s41467-019-14111-3 OPEN

Phenotypic changes of HER2-positive breast cancer during and after dual HER2 blockade

Fara Brasó-Maristany[1,2], Gaia Griguolo[1,2,3,4], Tomás Pascual[1,2,5], Laia Paré[5], Paolo Nuciforo[6,7], Antonio Llombart-Cussac[8], Begoña Bermejo[9], Mafalda Oliveira[6,7], Serafín Morales[10], Noelia Martínez[11], Maria Vidal[1,2,5], Barbara Adamo[1,2], Olga Martínez[1,2], Sonia Pernas[5,12], Rafael López[13], Montserrat Muñoz[1,2], Núria Chic[1,2], Patricia Galván[1,2], Isabel Garau[14], Luis Manso[15], Jesús Alarcón[16], Eduardo Martínez[17], Sara Gregorio[18], Roger R. Gomis[18], Patricia Villagrasa[5], Javier Cortés[7,19], Eva Ciruelos[5,15] & Aleix Prat[1,2,5*]

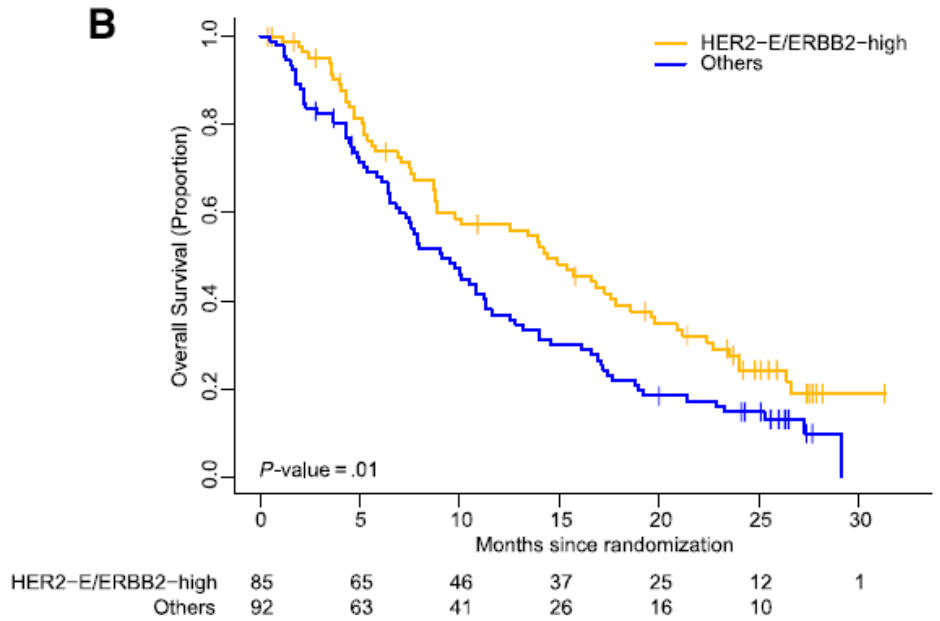
Shift to Luminal A sensitizes cells to anti-CDK4/6 treatment





HER2-Enriched Subtype and ERBB2 Expression in HER2-Positive Breast Cancer Treated with Dual HER2 Blockade

Aleix Prat, Tomás Pascual, Carmine De Angelis, Carolina Gutierrez, Antonio Llombart-Cussac, Tao Wang, Javier Cortés, Brent Rexer, Laia Paré, Andres Forero, Antonio C. Wolff, Serafín Morales, Barbara Adamo, Fara Brasó-Maristany, Maria Vidal, Jamunarani Veeraraghavan, Ian Krop, Patricia Galván, Anne C. Pavlick, Begoña Bermejo, Miguel Izquierdo, Vanessa Rodrik-Outmezguine, Jorge S. Reis-Filho, Susan G. Hilsenbeck, Mafalda Oliveira, Maria Vittoria Dieci, Gaia Griguolo, Roberta Fasani, Paolo Nuciforo, Joel. S. Parker, PierFranco Conte, Rachel Schiff, Valentina Guarneri, C. Kent Osborne, Mothaffar F. Rimawi



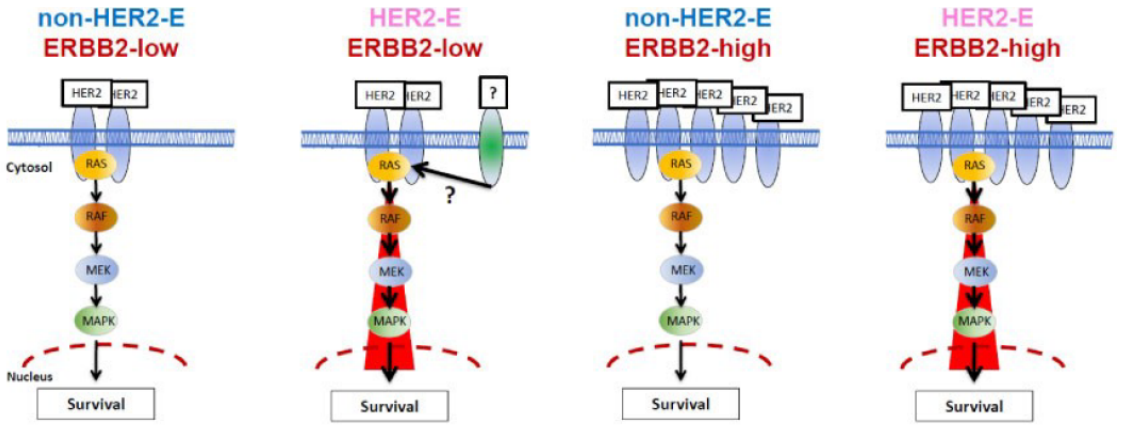
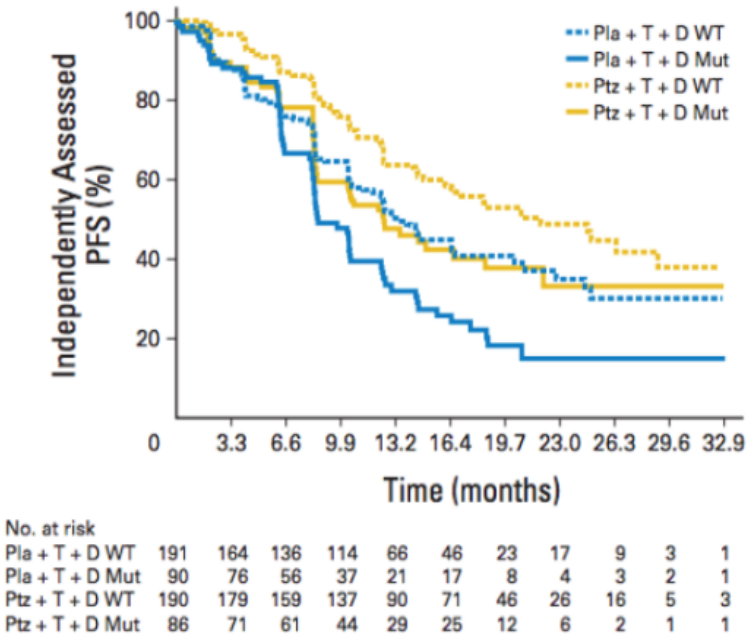


Figure 3. HER2-E/ERBB2-high biomarker in patients with HER2-positive breast cancer. **A)** Proportion of patients according to intrinsic subtype and ERBB2 levels. **B)** Schematic view of the four potential cell signaling scenarios (HER2 and its downstream signaling activation, such as the RAS/MAPK pathway) identified within HER2-positive breast cancer based on ERBB2 mRNA levels and intrinsic subtype (HER2-E vs non-HER2-E). In HER2-E/ERBB2-low disease, it is plausible that another transmembrane growth factor receptor with kinase activity such as FGFR4 is driving the HER2-E phenotype either alone or in combination with HER2. HER2-E = HER2-enriched; HER2-E/ERBB2 = HER2-E and ERBB2 group; HR + hormone receptor; MAPK = mitogen-activated protein kinase.

JNCI J Natl Cancer Inst (2020) 112(1)

Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer.



No. at risk

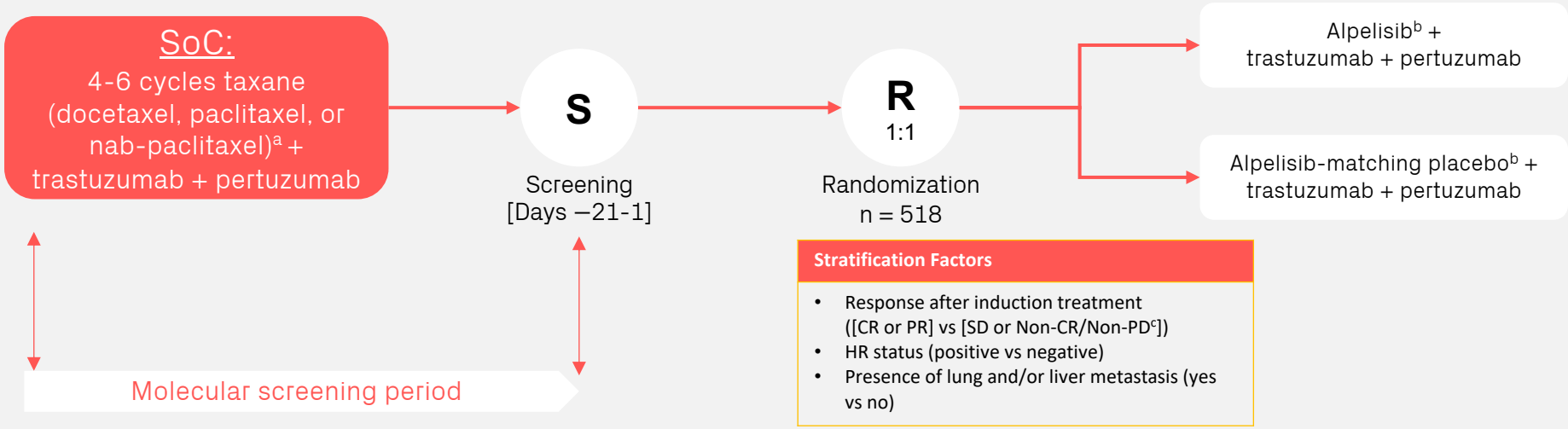
Pla + T + D WT	191	164	136	114	66	46	23	17	9	3	1
Pla + T + D Mut	90	76	56	37	21	17	8	4	3	2	1
Ptz + T + D WT	190	179	159	137	90	71	46	26	16	5	3
Ptz + T + D Mut	86	71	61	44	29	25	12	6	2	1	1

	PIK3CA mut	*PIK3CA* WT
Trastuzumab	8.6 months	13.8 months
Trastuzumab+ Pertuzumab	12.5 months	21.8 months

Baselga J, et al. JCO, 2014

EPIK-B2 (CBYL719G12301): Alpelisib + Trastuzumab + Pertuzumab in HER2+ ABC

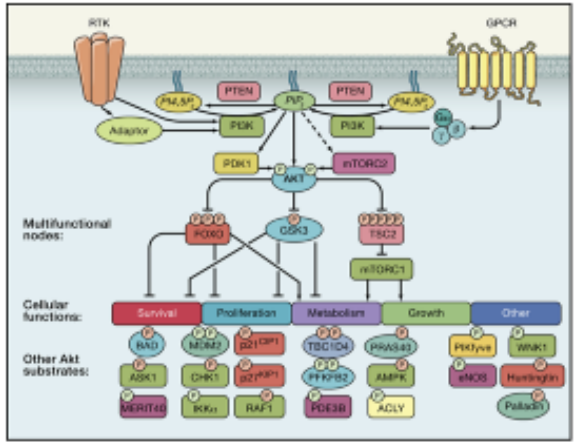
A Two-Part, Multicenter, Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Alpelisib + Trastuzumab and Pertuzumab as Maintenance Therapy in Patients With HER2+ PIK3CA-mutated ABC



Endpoints

- **Primary**: PFS per local investigator assessment according to RECIST 1.1
- **Key secondary**: OS
- **Secondary**: Safety and tolerability, exposure, ORR, CBR, DOR, TTR, deterioration of ECOG performance status

Breast cancer and the PI3K/AKT pathway



AKT can be activated by:

- Loss of function of negative regulators (PTEN, INPP4B, PHLPP, PP2A)
- Gain of function of positive regulators (PI3K, AKT, receptor tyrosine kinases [HER2])
- Therapy-induced survival response (chemotherapy, endocrine therapy)

PTEN = phosphatase and tensin homolog

Yap TA, et al. Curr Opin Pharmacol 2008; Manning BD and Toker A. Cell 2017

INVESTIGACIÓN

IPATHER

Código Clinicaltrial.gov:

Estado:
En inicio

Escenario:
Localmente avanzado o metastático

Subtipo tumoral :
HER2+

Fase:
Fase I

Fármaco/Intervención:
Ipatasertib Trastuzumab Pertuzumab

Estudio en fase Ib de ipatasertib, un inhibidor de AKT, en combinación con pertuzumab más trastuzumab en pacientes con cáncer de mama localmente avanzado o metastásico con mutación de PI3KCA y positividad de HER2

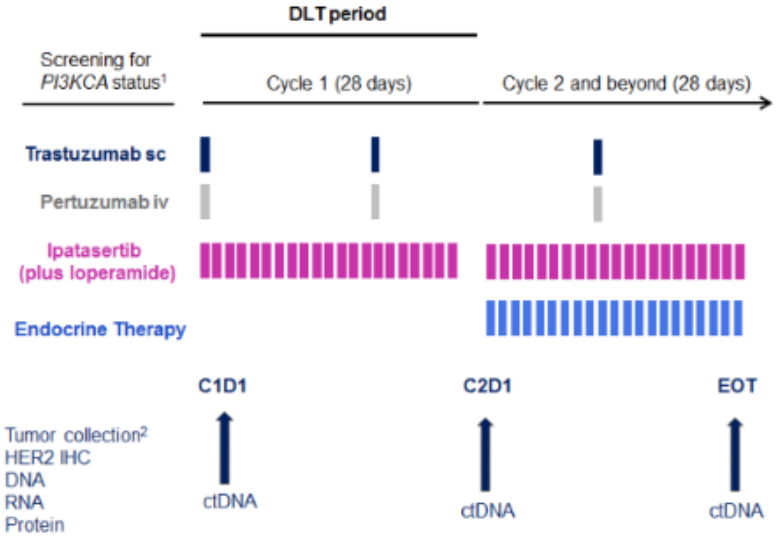
Promotor/es

SOLTI

Investigador/es principales

Mafalda Oliveira, MD, PhD
Hospital Universitario Vall d' Hebron, Barcelona

Cristina Saura, MD, PhD
Hospital Universitario Vall d' Hebron, Barcelona



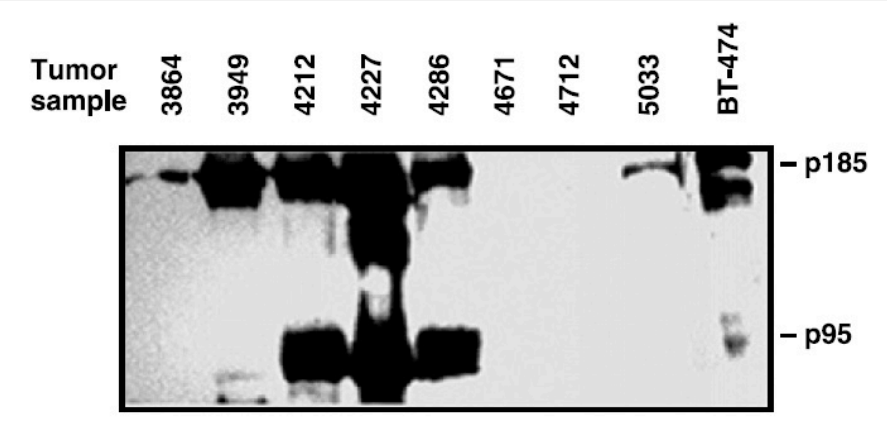
(1) Allowed during induction treatment with taxane
(2) Archival tumor tissue from primary or metastatic lesion

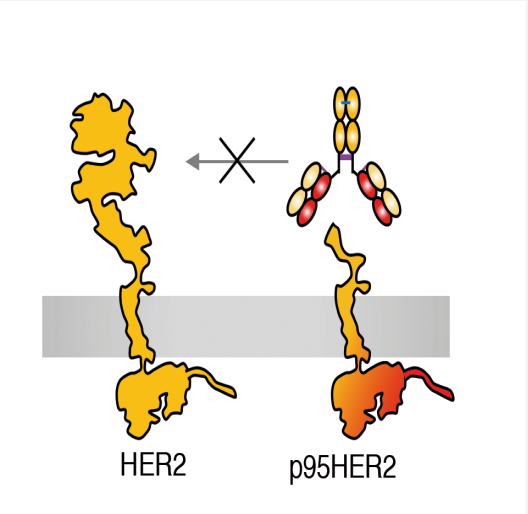
Outline of the presentation

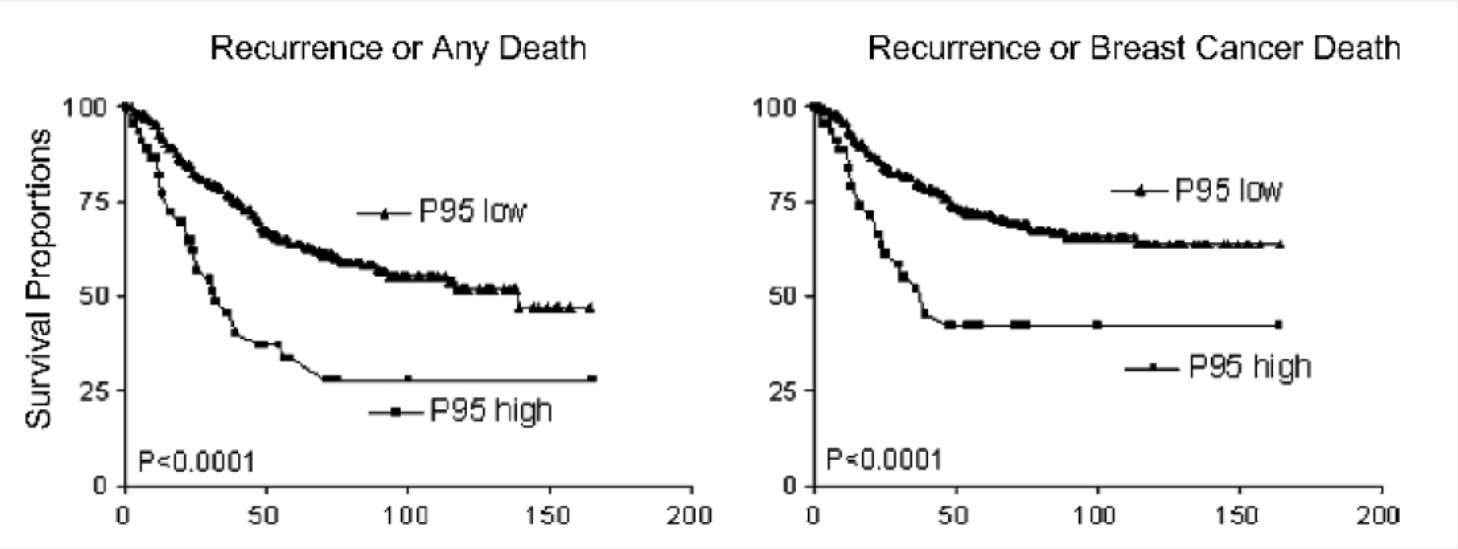
III) p95HER2 fragment: where are we going?

p95HER2 fragment: where are we going?

p95HER2 fragment relates to worse outcome in HER2+ breast cancer patients







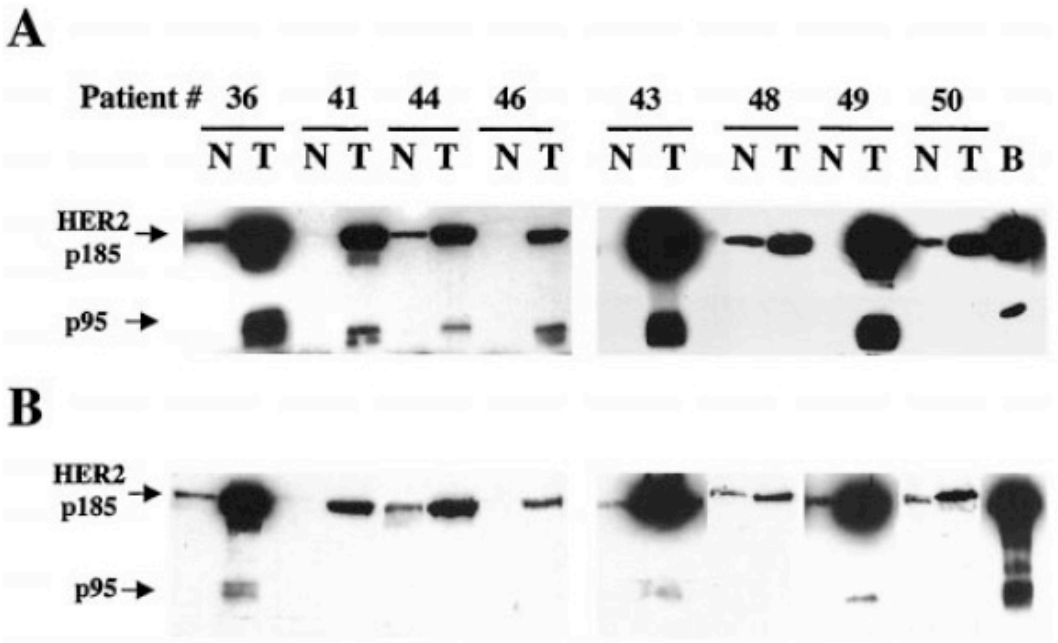
Saez et al Clin Cancer Res 2006

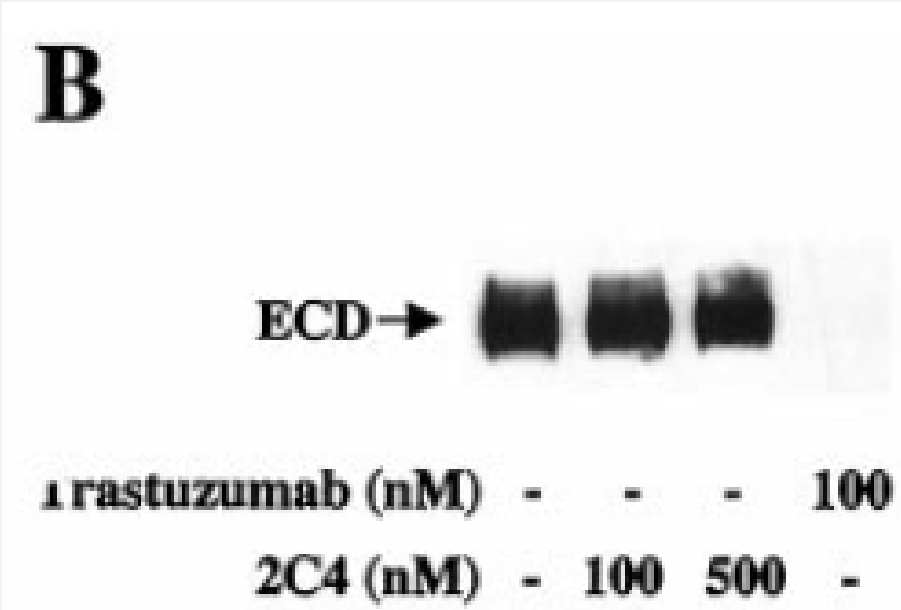
[CANCER RESEARCH 61, 4744–4749, June 15, 2001]

Trastuzumab (Herceptin), a Humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells[1]

Miguel A. Molina, Jordi Codony-Servat, Joan Albanell, Federico Rojo, Joaquín Arribas, and Jose Baselga[2]

Laboratory of Oncology Research, Medical Oncology Service [M. A. M., J. C-S., J. A., F. R., J. A., J. B.], and Universitat Autonoma de Barcelona [J. B.], Vall d'Hebron University Hospital, 08035 Barcelona, Spain

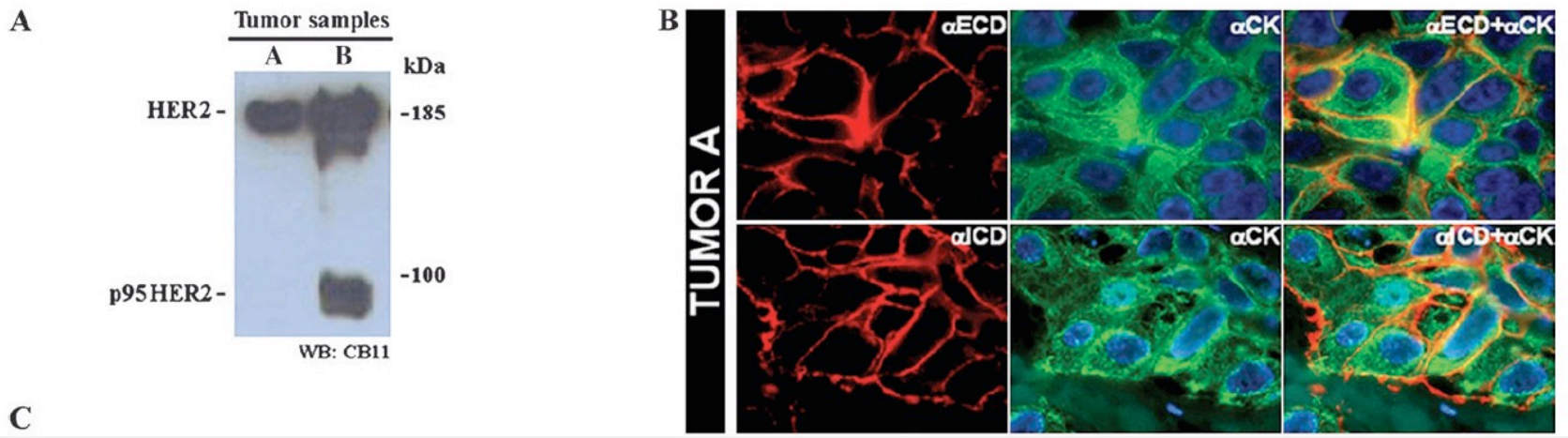




p95HER2 fragment: where are we going?

Table 1. p95HER2 expression in breast tumors and trastuzumab resistance*

p95HER2 status	Response n (%)	No response n (%)
Negative (n = 37)	19 (51.4)	18 (48.6)
Positive (n = 9)	1 (11.1)	8 (88.9)

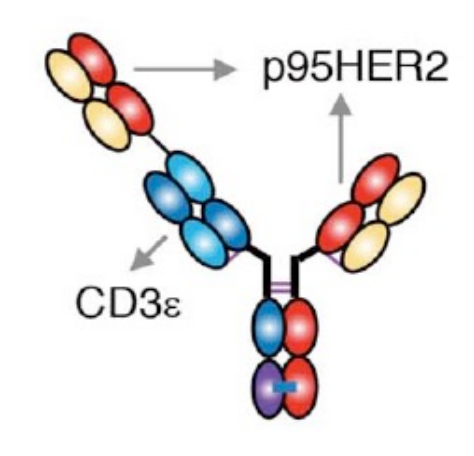


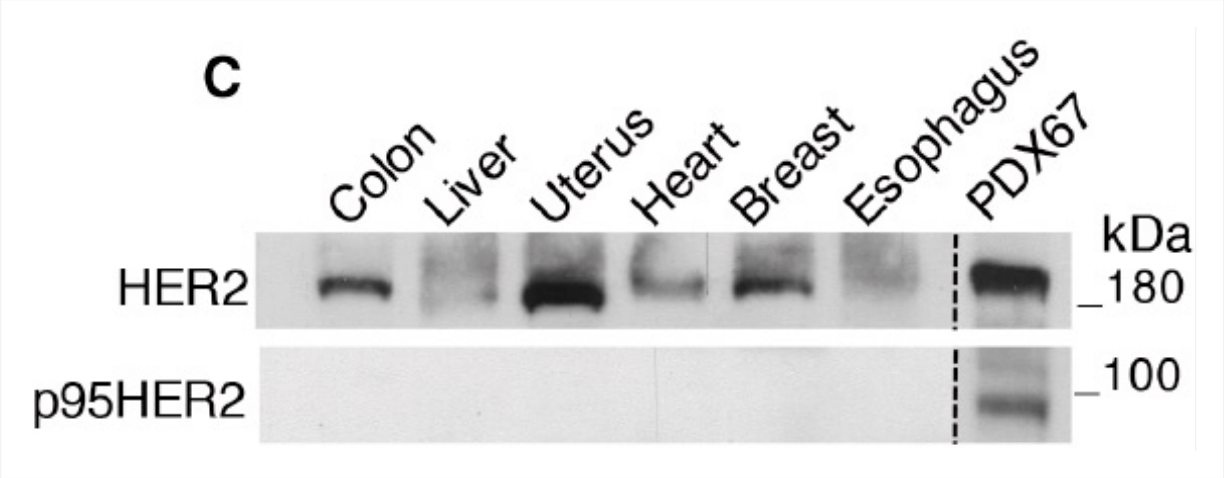
Scaltriti et al JNCI 2007

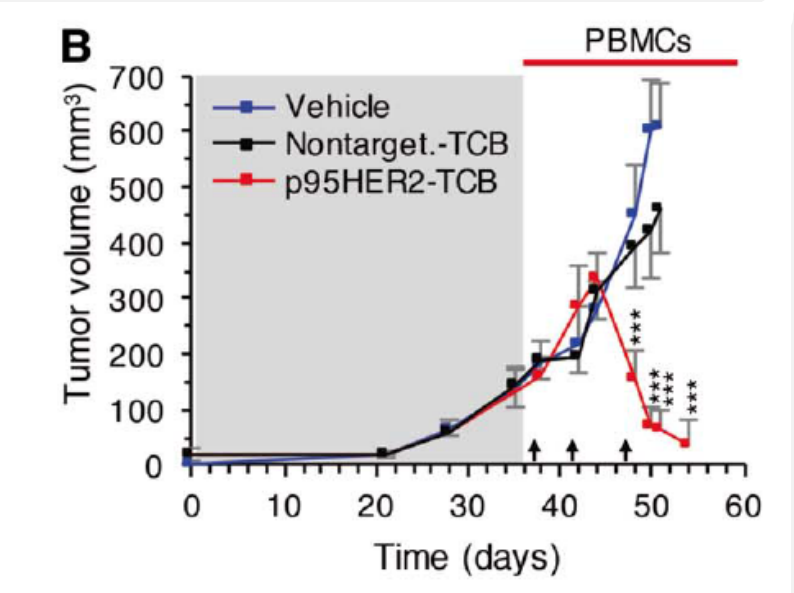
p95HER2 fragment: where are we going?

p95HER2 is a selective and promising target

for redirecting T lymphocytes to the tumor (Arribas J.)





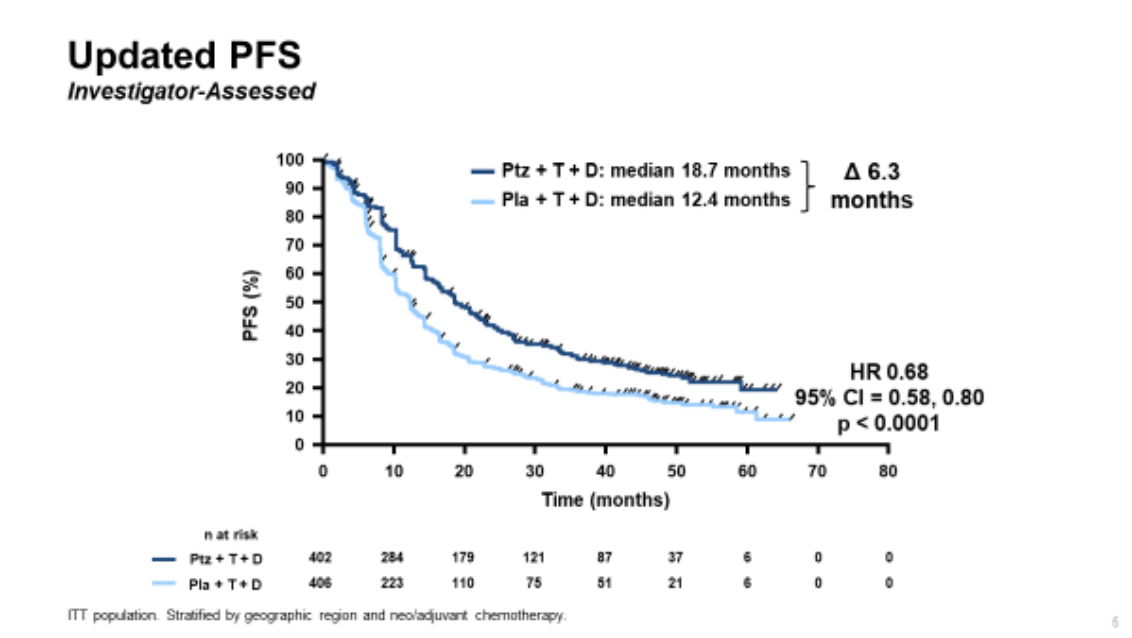


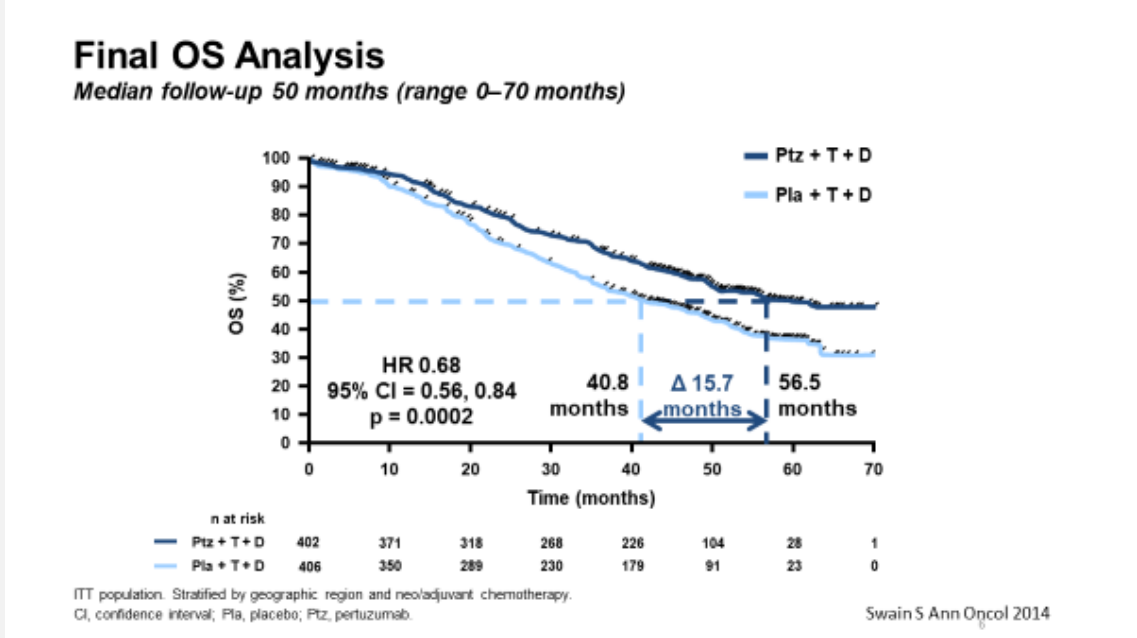
Rius Ruiz et al. Science Transl Med 2018

Outline of the presentation

IV) Innate and adaptive (HLA) immunity and response to anti-HER2 MAbs

Superior improvement of OS vs PFS in Cleopatra hard to explain Immune-mediated effects?





Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

The immune system and response to HER2-targeted treatment in breast cancer

Giampaolo Bianchini, Luca Gianni

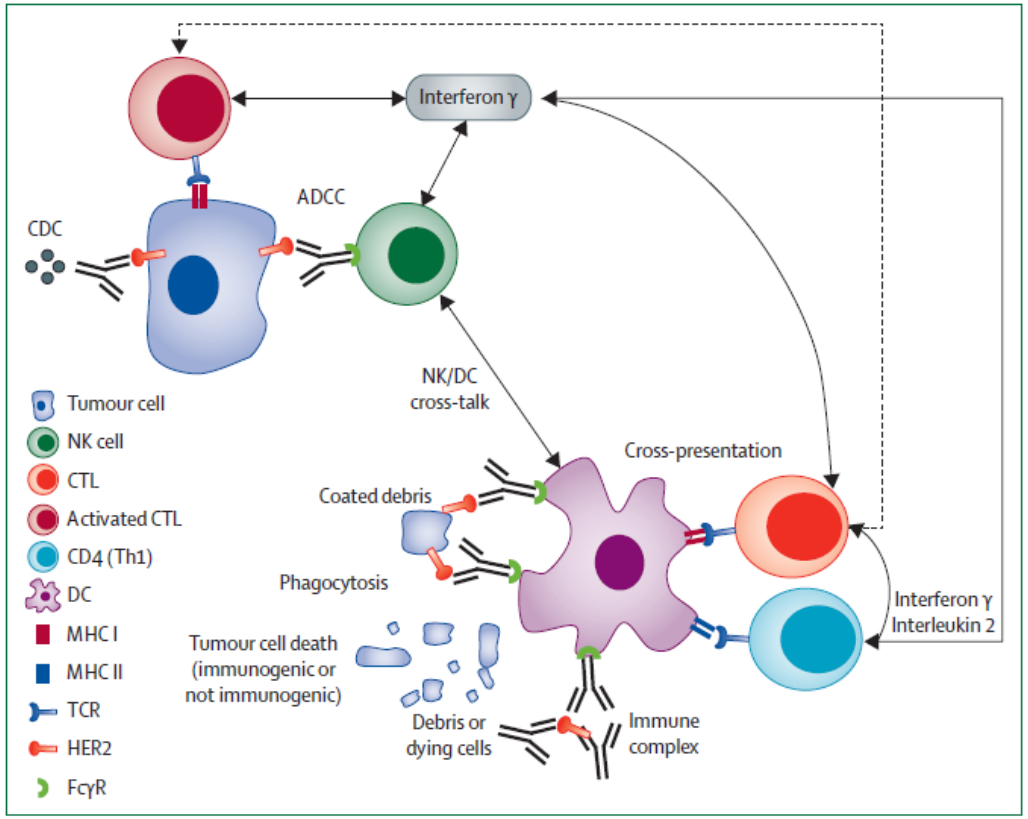
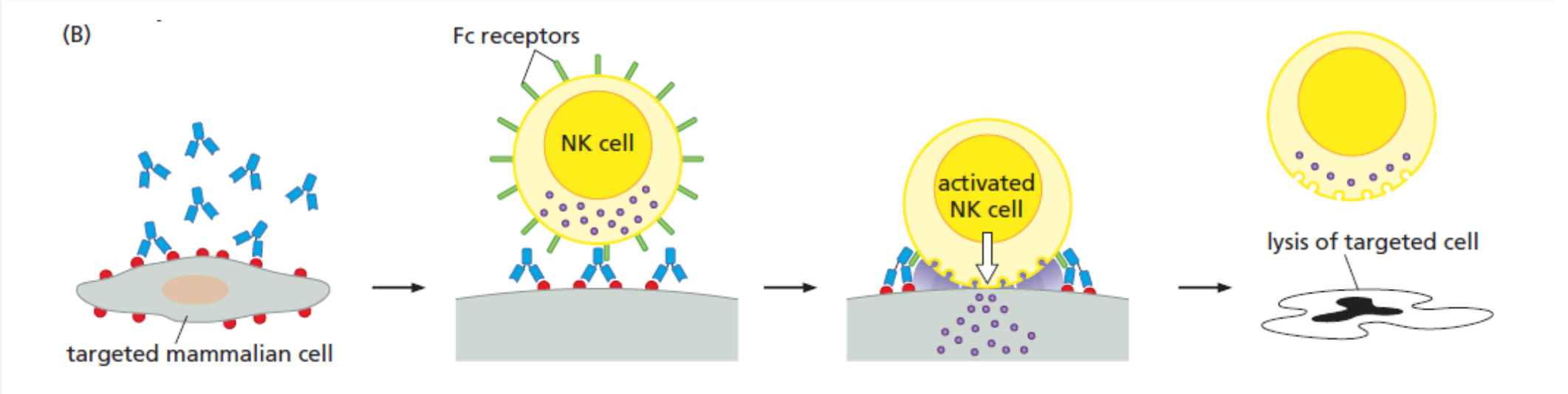


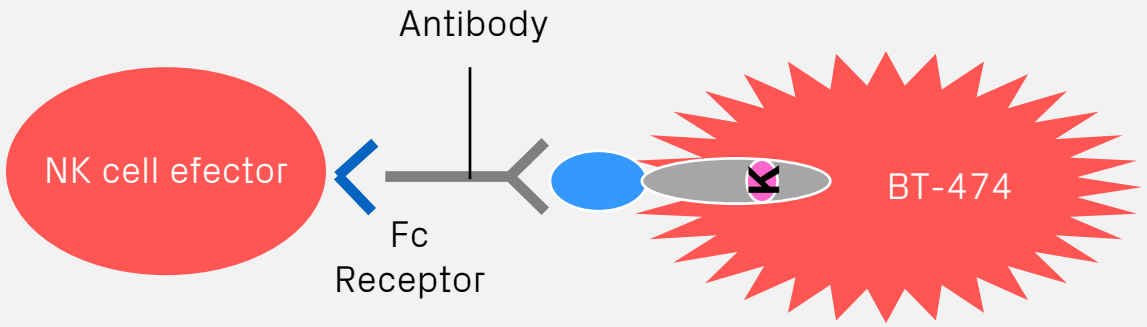
Figure 1: **Key mechanisms of involvement of the immune system in response to trastuzumab and other HER2-targeted monoclonal antibodies**

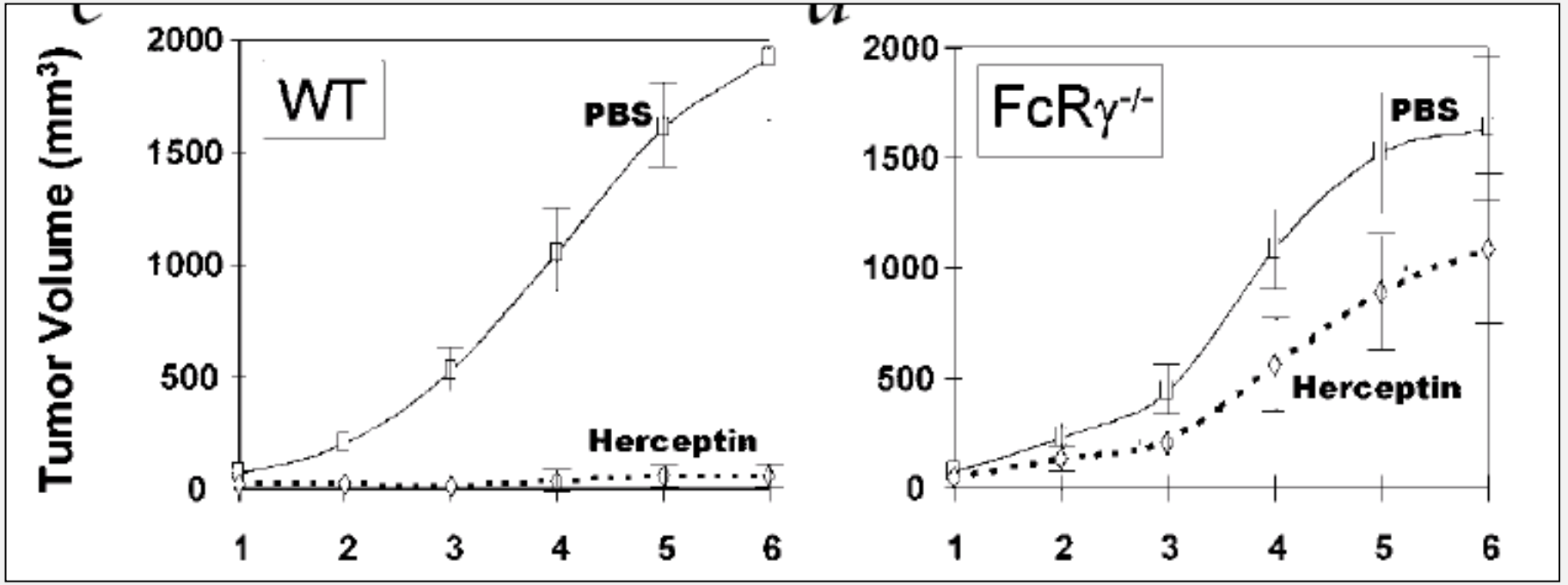
ADCC=antibody-dependent cell-mediated cytotoxicity. CDC=complement-dependent cytotoxicity. CTL=cytotoxic T lymphocyte. DC=dendritic cell. FcγR=Fcγ receptor. NK=natural killer. TCR=T-cell receptor. Th1=T helper 1 cell.

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs



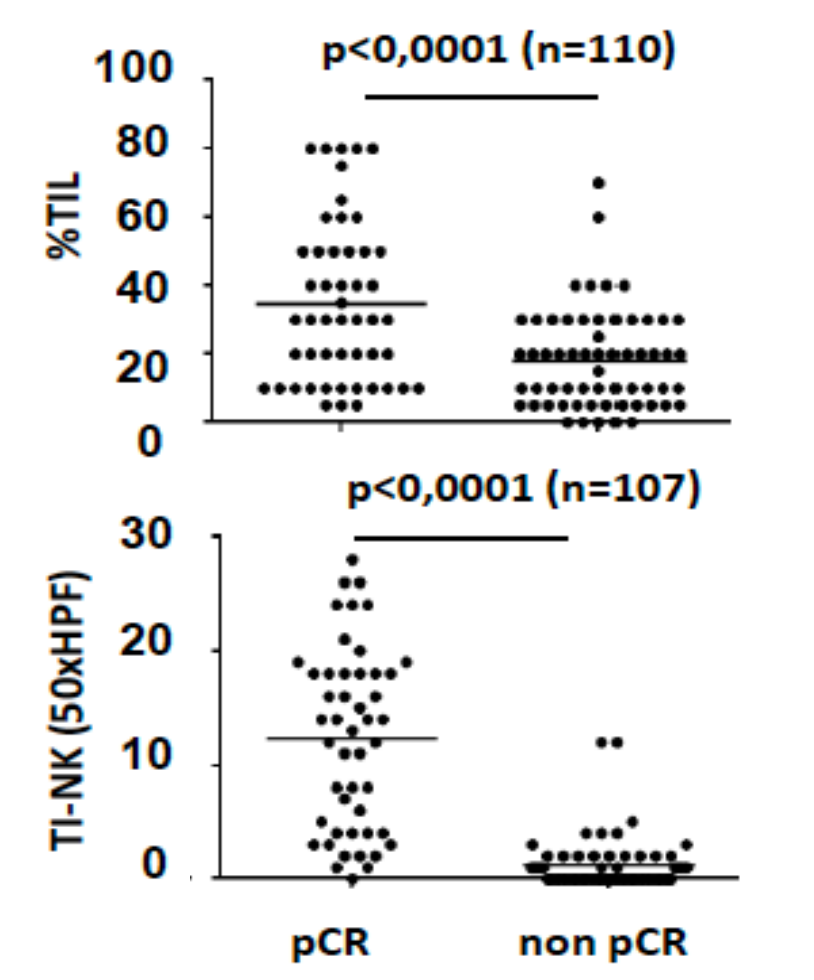
In vivo antitumor effect of trastuzumab: Role of antibody dependent cellular cytotoxicity

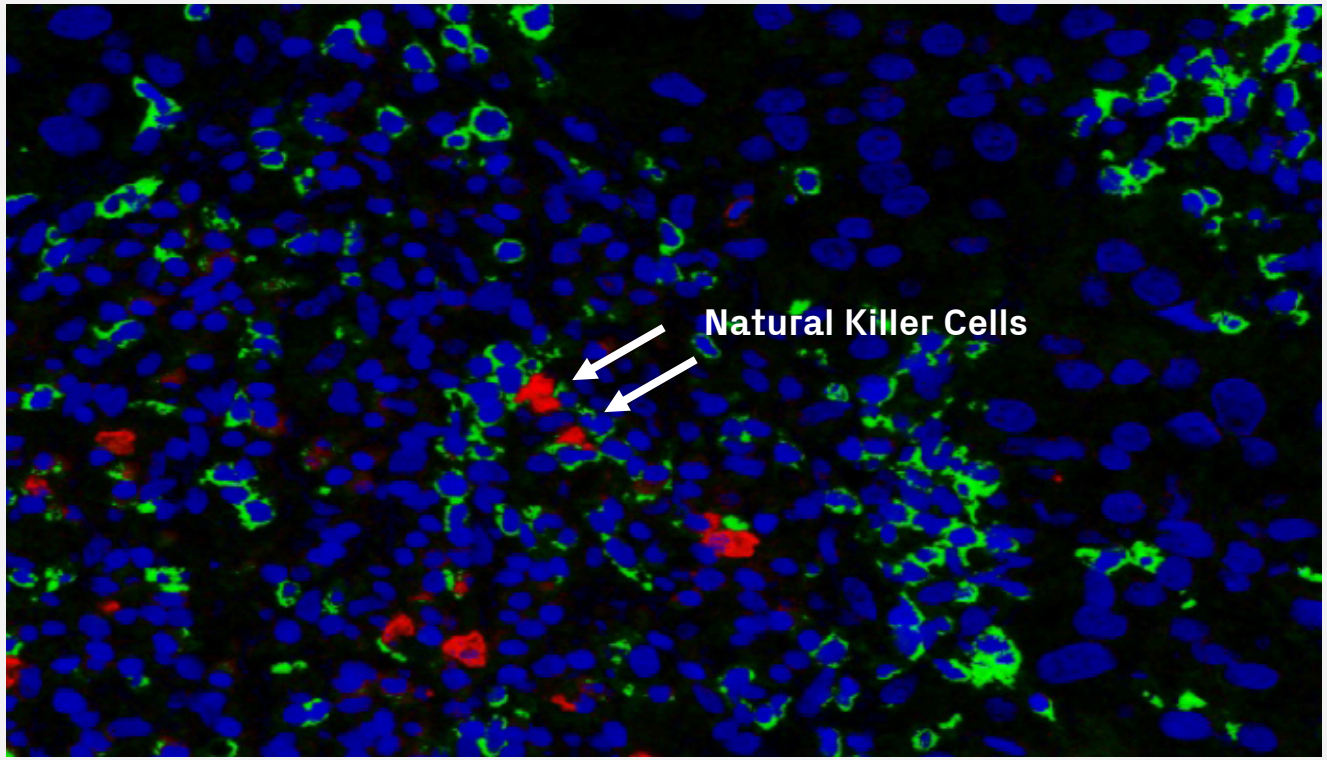




Clynes et al. Nature Medicine 6;443, 2000

Tumor infiltrating lymphocytes (TILs) and Natural Killer Cells in HER2 positive breast cancer predict pCR to neoadjuvant anti-HER2 based therapy

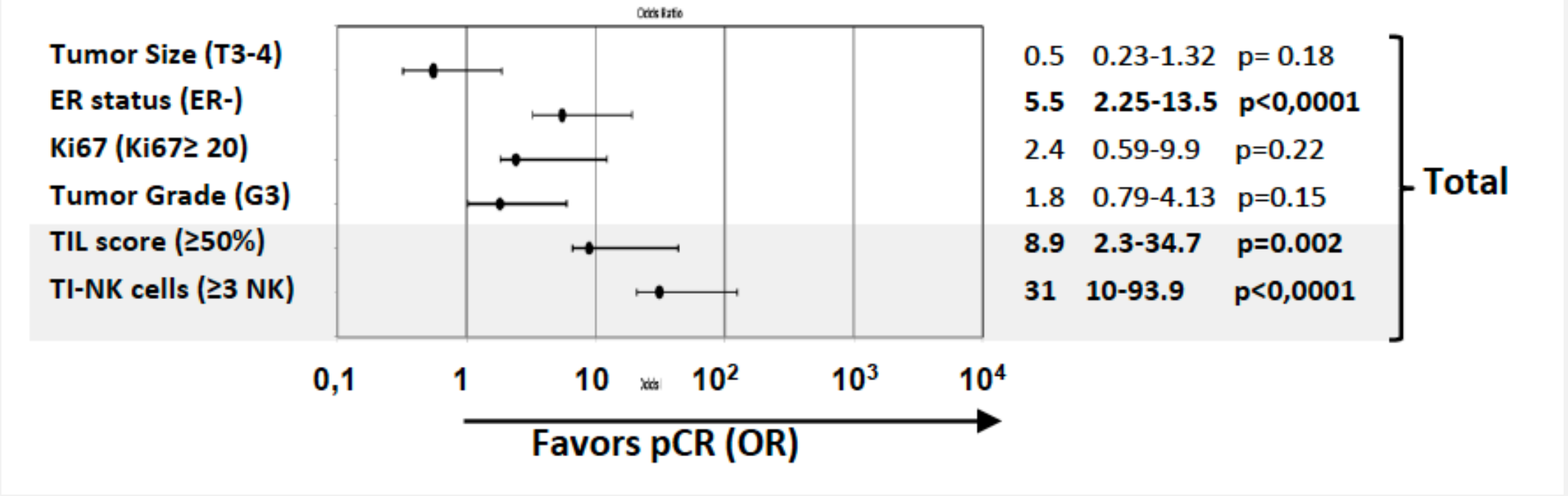




Muntasell et al. Clin Cancer Res 2019

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

Natural Killer Cell number independently predicted pCR



Muntasell et al. Clin Cancer Res 2019

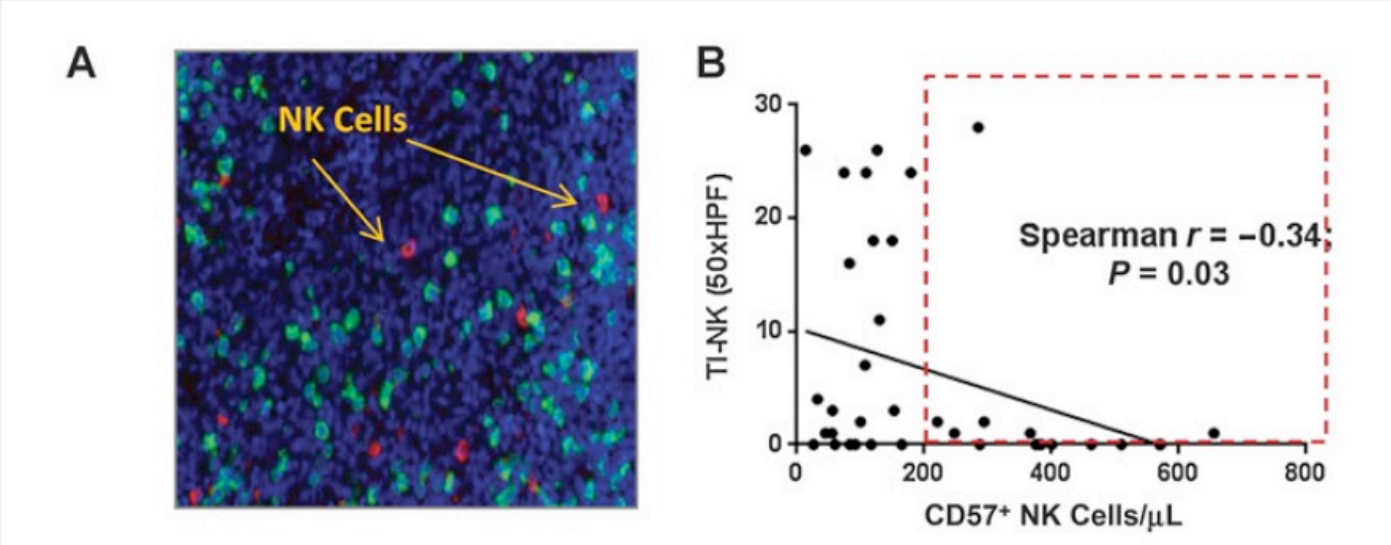
Research Article

Cancer Immunology Research

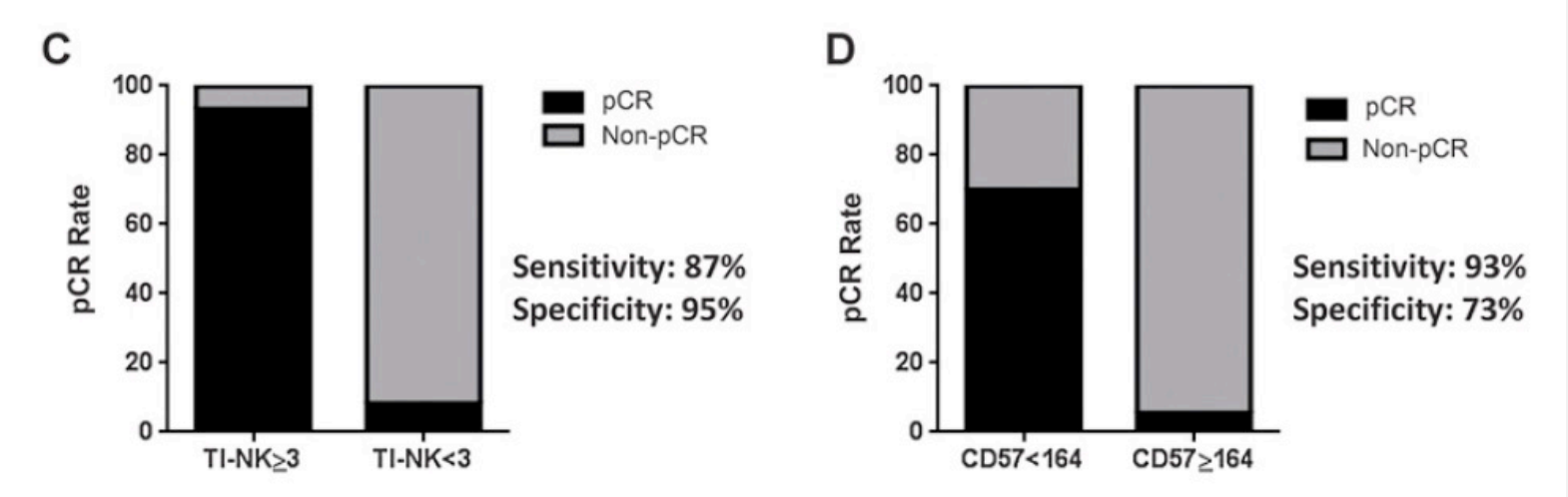
High Numbers of Circulating CD57$^+$ NK Cells Associate with Resistance to HER2-Specific Therapeutic Antibodies in HER2$^+$ Primary Breast Cancer

Aura Muntasell[1], Sònia Servitja[2,3], Mariona Cabo[1], Begoña Bermejo[4], Sandra Pérez-Buira[5], Federico Rojo[5], Marcel Costa-García[6], Oriol Arpí[2], Manuela Moraru[7], Laia Serrano[8], Ignasi Tusquets[2,3], María Teresa Martínez[4], Gemma Heredia[6], Andrea Vera[1], María Martínez-García[2,3], Laura Soria[1], Laura Comerma[8], Sara Santana-Hernández[1], Pilar Eroles[4,9], Ana Rovira[2,3], Carlos Vilches[7], Ana Lluch[4,9,10], Joan Albanell[2,3,6], and Miguel López-Botet[1,6]

Inverse relationship between CD57 NK Cells (a highly differentiated NK subpopulation) in Blood and Tumor Infiltrating NK Cells



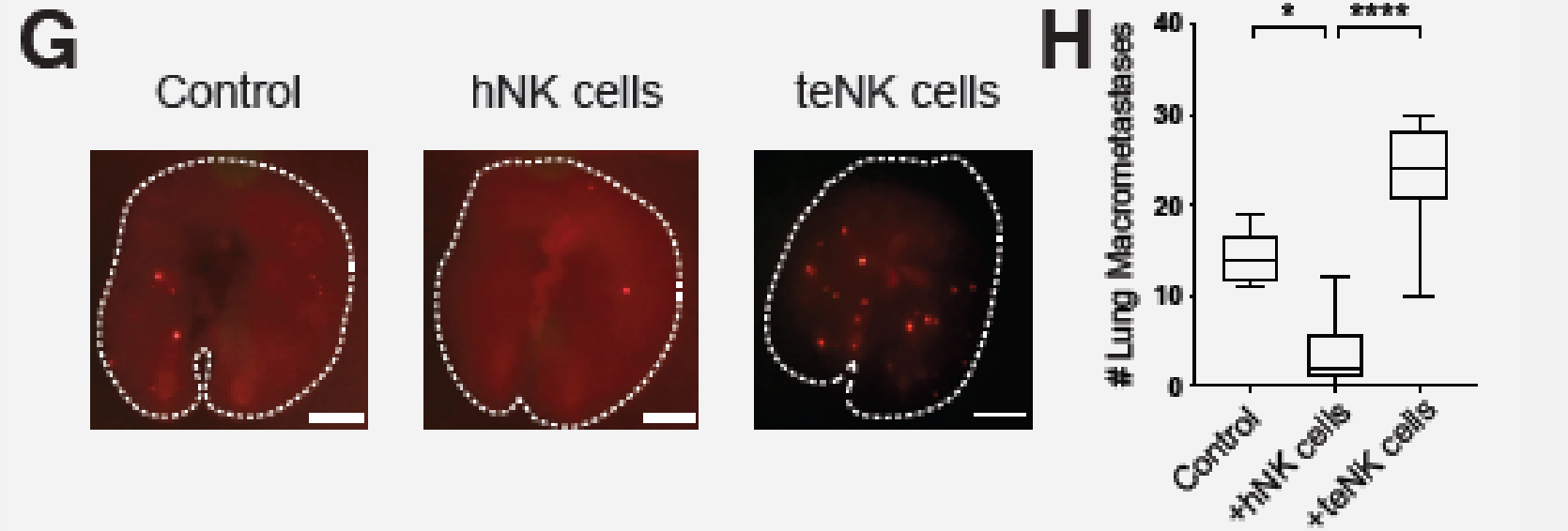
Low Peripheral CD57 NK Cells and High Tumor Infiltrating NK Cells Predict pCR



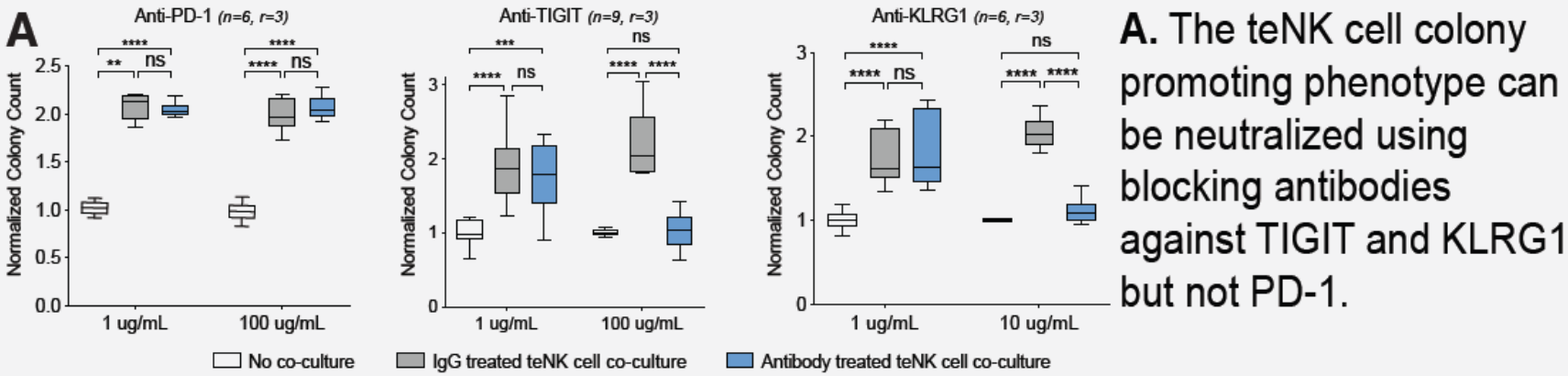
Breast cancer cells educate natural killer cells to a metastasis promoting cell state

Isaac S. Chan[1,2], Hildur Knútsdóttir[3], Gayathri Ramakrishnan[1,2], Veena Padmanaban[1,2], Manisha Warrier[3], Juan Carlos Ramirez[1,2], Joel S. Bader[3], Elizabeth M. Jaffee[1], Andrew J. Ewald[1,2]

[1]Cancer invasion and Metastasis Program, The Sidney Kimmel Comprehensive Cancer Center, [2]Department of Cell Biology and Center for Cell Dynamics, [3]Department of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD



The teNK cell phenotype can be reversed

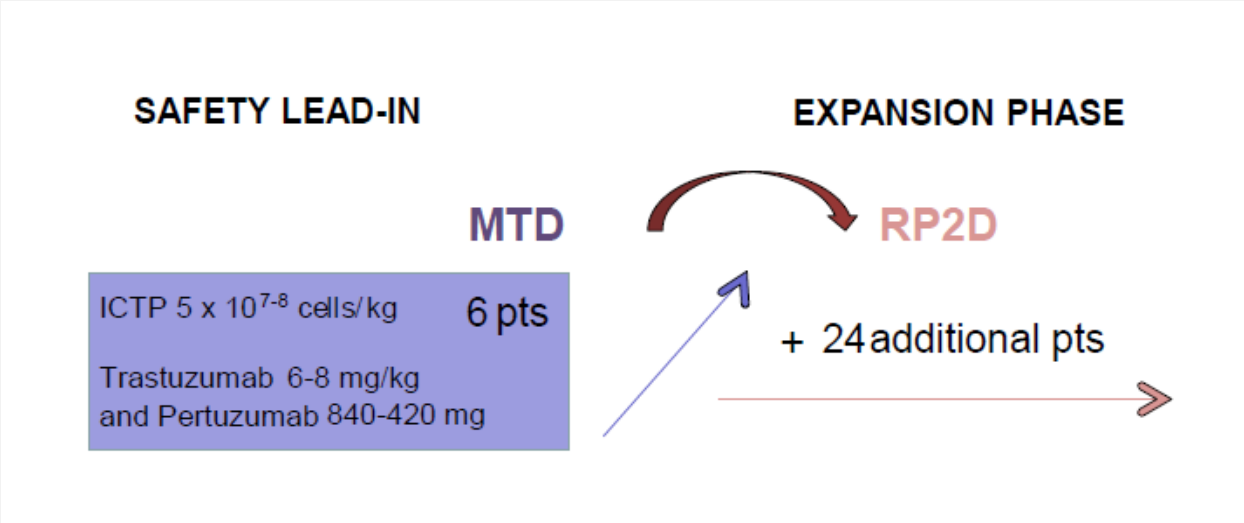


A. The teNK cell colony promoting phenotype can be neutralized using blocking antibodies against TIGIT and KLRG1 but not PD-1.

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

Plans for translating to patients

1. Could allogenic NK infusion + anti-HER2 MAbs be effective in HER2 resistant metastatic breast cancer?



2. Validation of the predictive value of NKs in clinical trials (transcriptomic signature of NK enriched tumors)

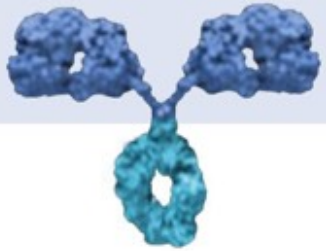
Move to early stage patients with cold tumors
Proposal under review
VHIO, Hospital del Mar, Pamplona

Margetuximab: Fc engineering Alters Fc Receptor Affinities

Trastuzumab

Fab:

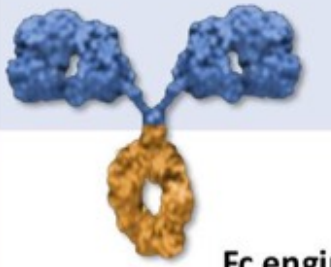
- Binds HER2 with high specificity
- Disrupts signaling that drives cell proliferation and survival



Fc:

- Wild-type immunoglobulin G1 (IgG1) immune effector domains
- Binds and activates immune cells

Margetuximab[1,2]



Fab:

- Same specificity and affinity
- Similarly disrupts signaling

Fc engineering:

- ⬆Affinity for activating Fcγ RIIIA (**CD16A**)
- ⬇Affinity for inhibitory Fcγ RIIB (**CD32B**)

CD16A genotypes

FF in 40% (low binding)

FV in 40-45% (low binding)

VV in 15% (high binding)

1. Nordstrom JL, et al. *Breast Cancer Res*. 2011;13(6):R123. 2. Stavenhagen JB, et al. *Cancer Res*. 2007;67(18):8882-8890.

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

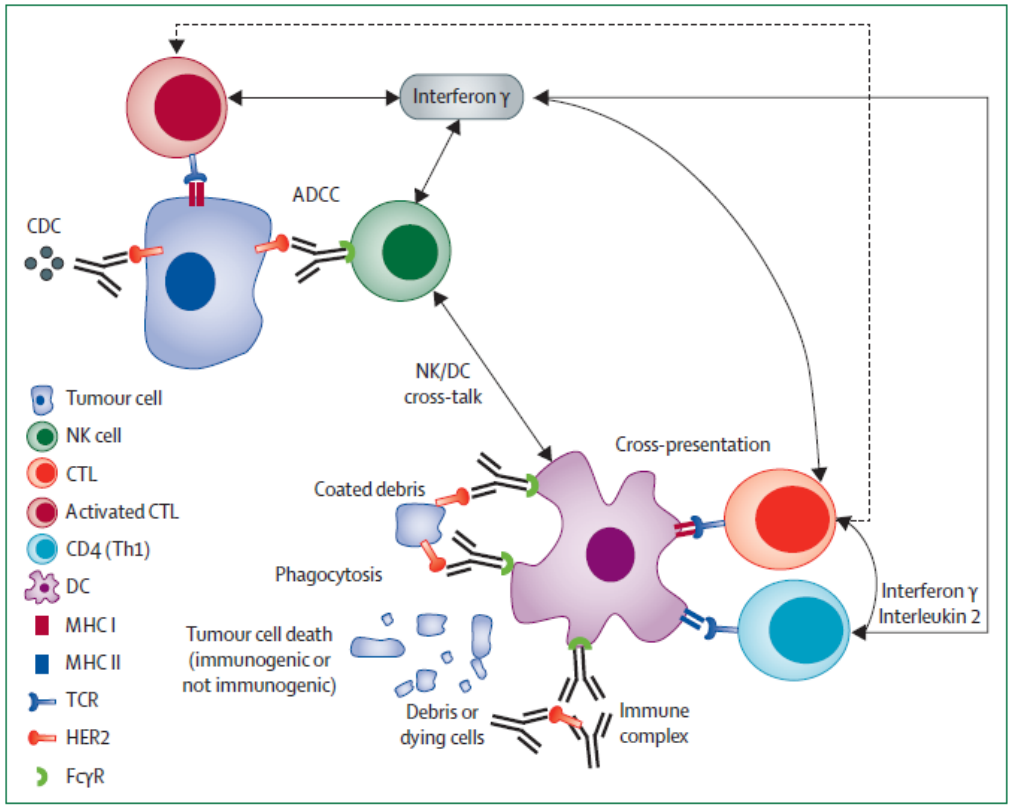


Figure 1: **Key mechanisms of involvement of the immune system in response to trastuzumab and other HER2-targeted monoclonal antibodies**
ADCC=antibody-dependent cell-mediated cytotoxicity. CDC=complement-dependent cytotoxicity. CTL=cytotoxic T lymphocyte. DC=dendritic cell. FcγR=Fcγ receptor. NK=natural killer. TCR=T-cell receptor. Th1=T helper 1 cell.

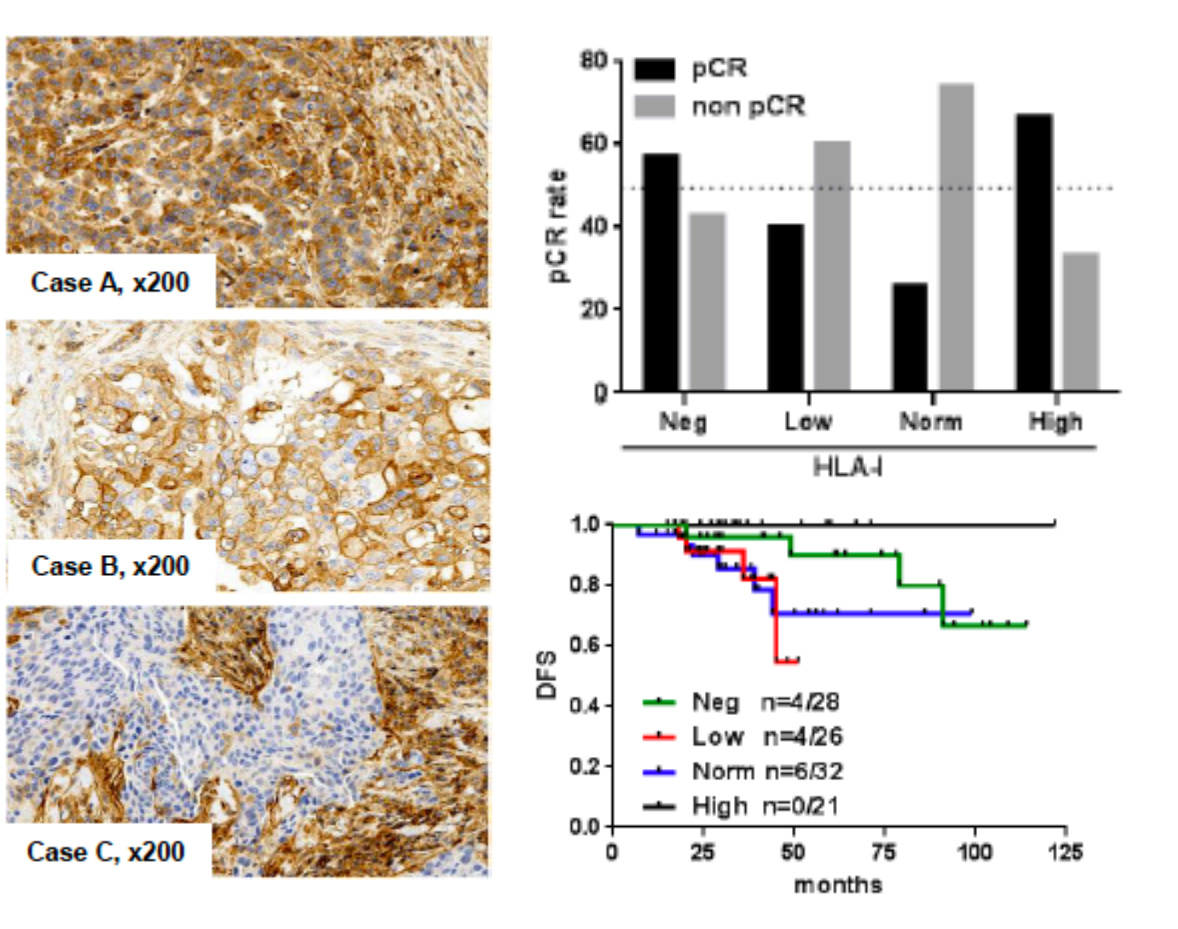
The immune system and response to HER2-targeted treatment in breast cancer

Giampaolo Bianchini, Luca Gianni www.thelancet.com/oncology **Vol 15 February 2014**

- AntiHER2 MAbs may also mediate adaptive immunity
- HLA class I (MHC) is necessary for antigen presentation to cytotoxic T lymphocytes
- Several studies support an important role for HLA class I in the response to various anticancer strategies (Chowell, Science 2018; De Groot BCRT 2018)

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

HLA-I expression was unrelated to Natural Killer Cells and pCR but:



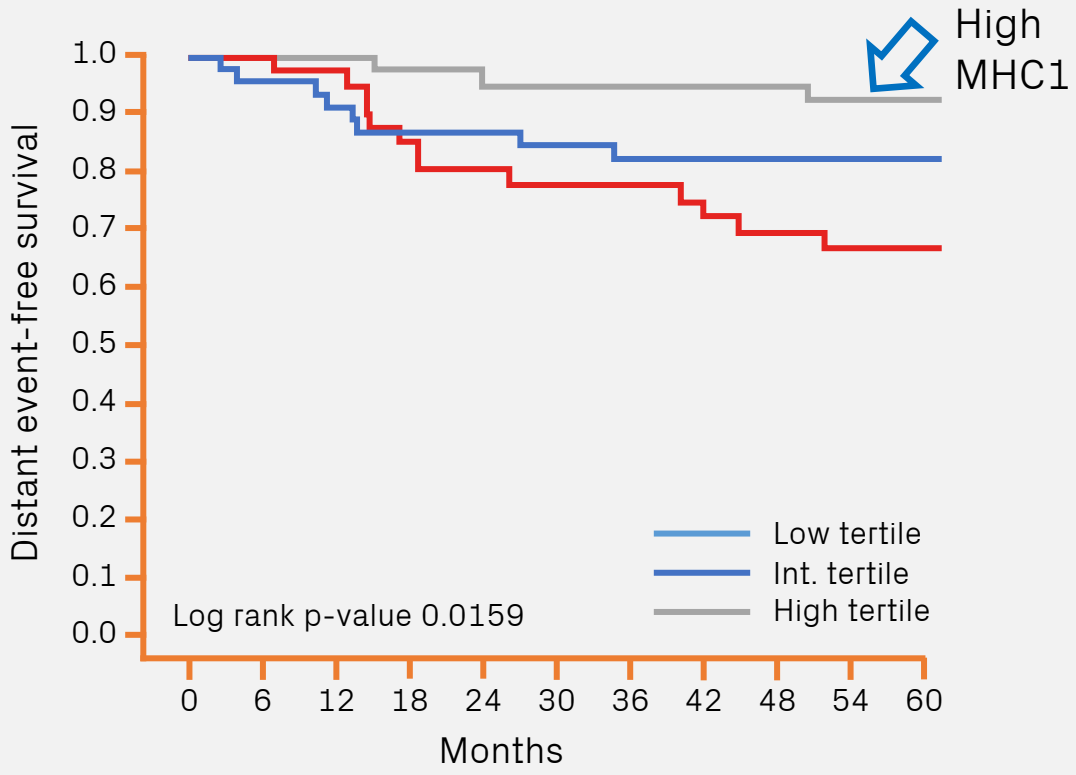
- HLA-I High: Excellent DFS regardless of achieving or not pCR
- HLA-I Negative/Low/Normal: Numerically (not statistically) more relapses in non-pCR patients

Muntasell et al. Clin Cancer Res 2019

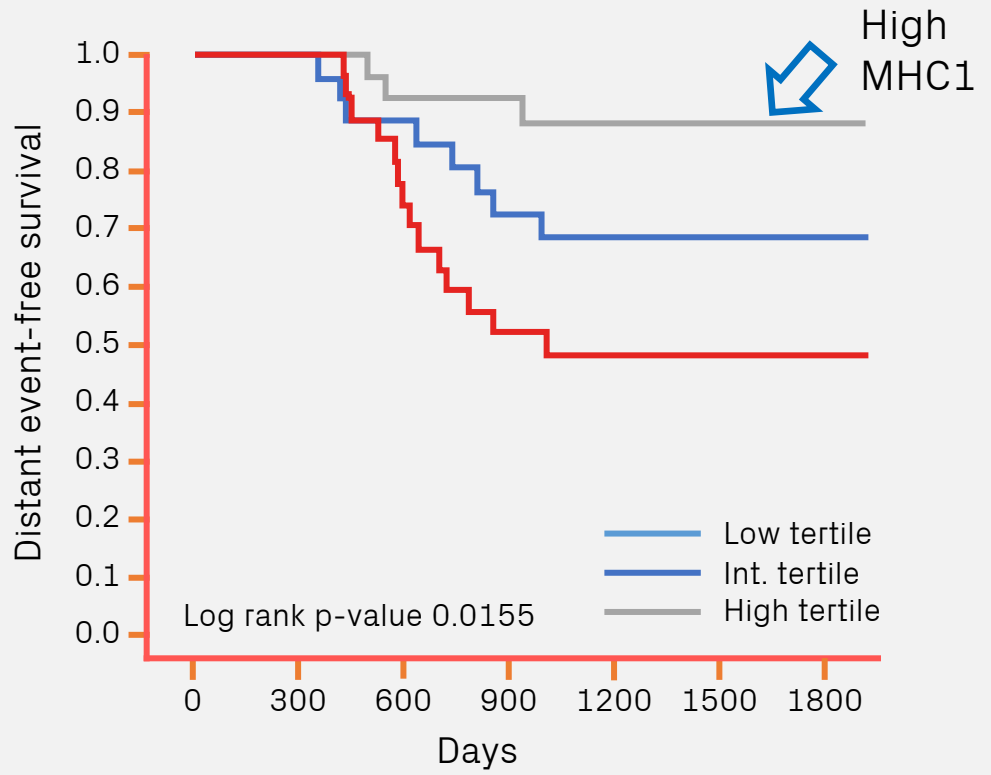
Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

High-risk ER-negative/HER2-positive patients with high MHC1 metagene have a low risk of relapse

HER2-positive/ER-negative (NeoSphere)*



HER2-positive/ER-negative (NOAH)



Bianchini G, et al. AACR 2016.

* Excluding PERJETA/Herceptin combination.

Innate and adaptive (HLA) immunity and response to anti-HER2 Mabs

DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer

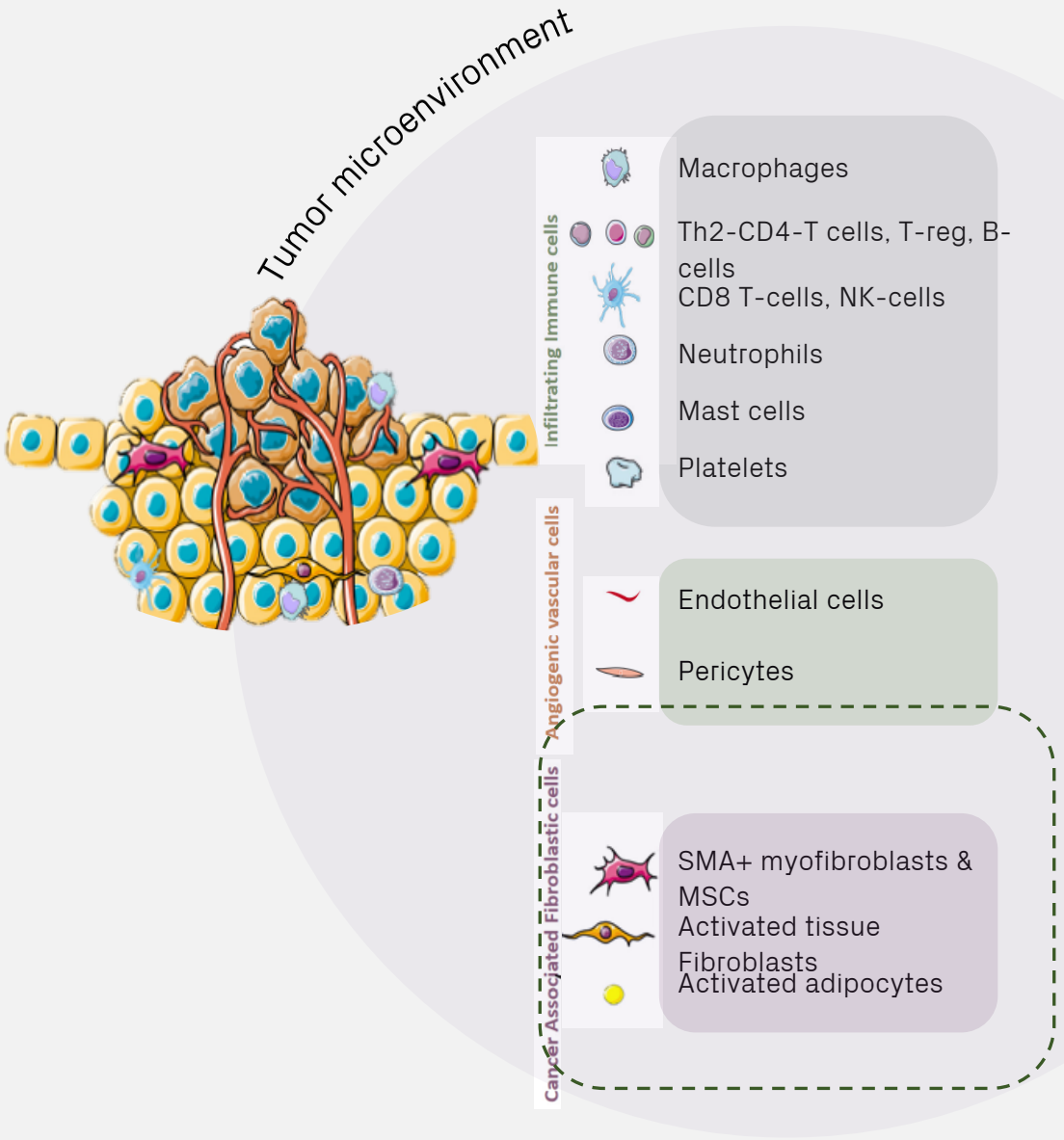
Na Luo[1,2], Mellissa J. Nixon[2], Paula I. Gonzalez-Ericsson[3,4], Violeta Sanchez[3,4], Susan R. Opalenik[2], Huili Li[5], Cynthia A. Zahnow[6], Michael L. Nickels[7], Fei Liu[7], Mohammed N. Tantawy[7], Melinda E. Sanders[3,4], H. Charles Manning[7,8] & Justin M. Balko[2,4,9]

NATURE COMMUNICATIONS | (2018)9:248

Outline of the presentation

V) Role of fibroblasts

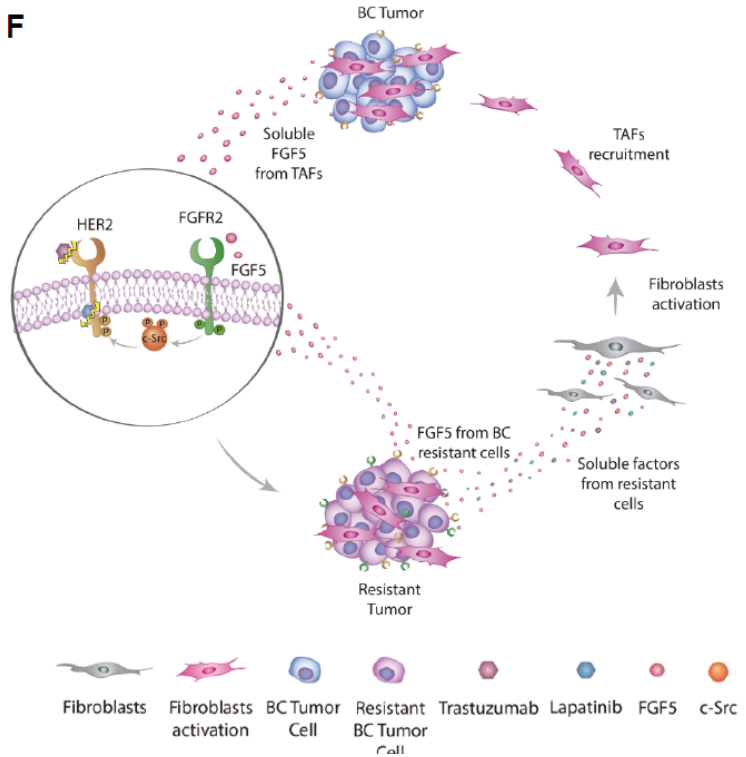
Role of fibroblasts

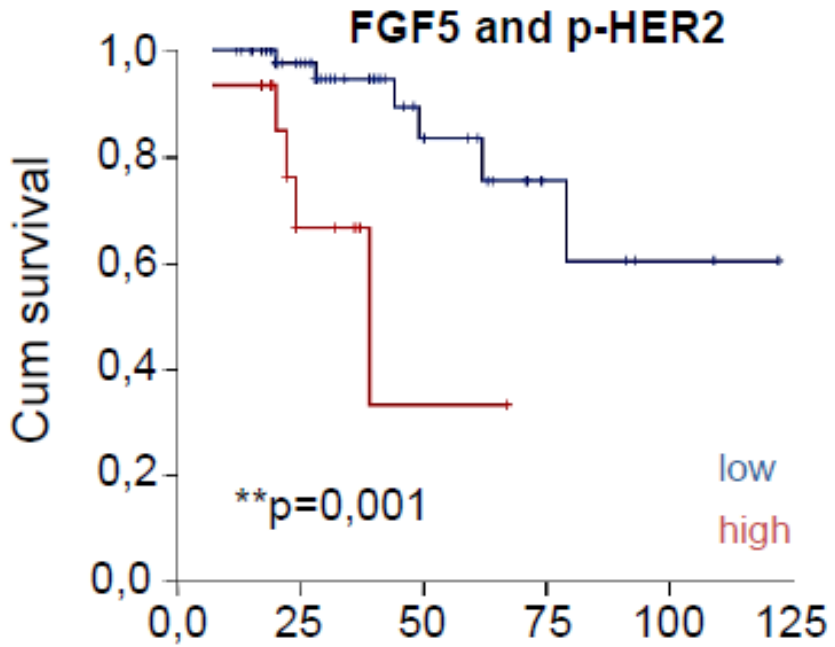


Adapted from Hanahan et al. 2012

Tumor Associated Fibroblasts Promote HER2-Targeted Therapy Resistance through FGFR2 Activation.

Patricia Fernández-Nogueira[1,5], Mario Mancino[1,5], Gemma Fuster[1], Anna Lopez-Plana[1], Patricia Jauregui[1], Vanesa Almendro[2], Estel Enreig[1,5], Silvia Menéndez[3], Federico Rojo[4], Aleix Noguera-Castells[1,5], Anke Bill[6], L. Alex Gaither[6], Laia Serrano[3], Leire Recalde-Percaz[1,5], Núria Moragas[1], Raul Alonso[1], Elisabet Ametller[1], Ana Rovira[7,8], Anna Lluch[9,10], Joan Albanell[7,8,11], Pere Gascon[1,5,12]*, Paloma Bragado[1,13]*

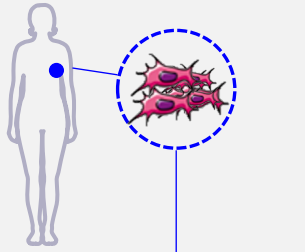




Clin Cancer Res 2019

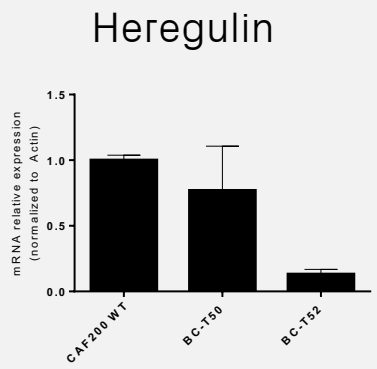
Role of fibroblasts

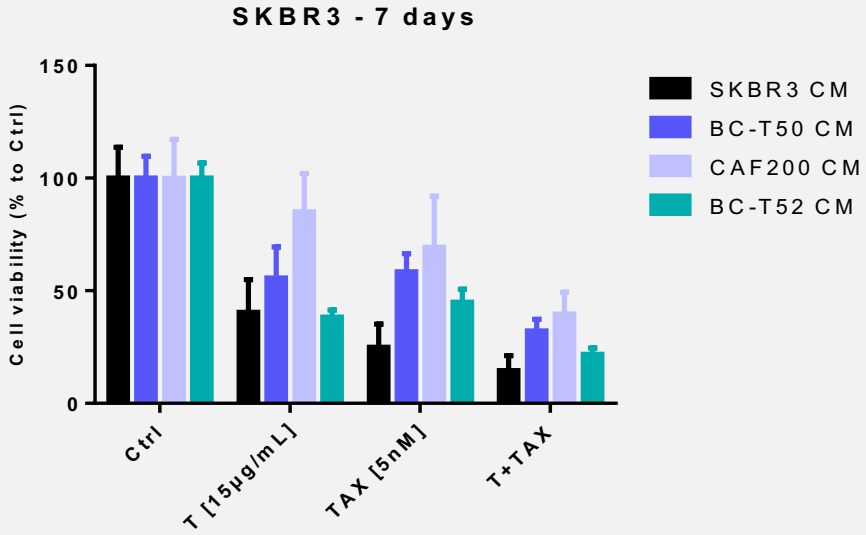
Media from fibroblasts expressing more heregulin, a HER3 ligand, mediated trastuzumab and paclitaxel resistance



Conditioned media (CM)

Anti HER2 and chemotherapy drug efficacy





- Pertuzumab reverts resistance

May be we should look at fibroblasts to understand what patients benefit the most from dual blockade

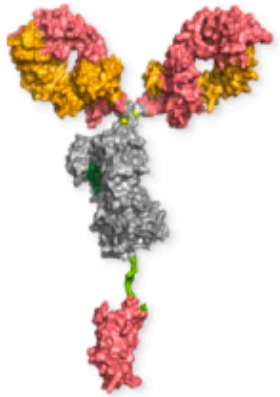
- Fibroblasts may mediate immune exclusion

(Guardia, Rovira, et al, manuscript in preparation)

Role of fibroblasts

Immunotherapy targeting stromal compartment: FAP-IL2v (Roche)

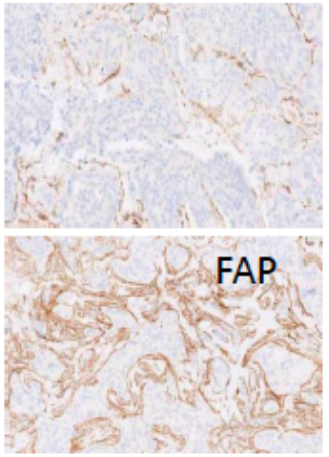
Tumor target
anti-FAP



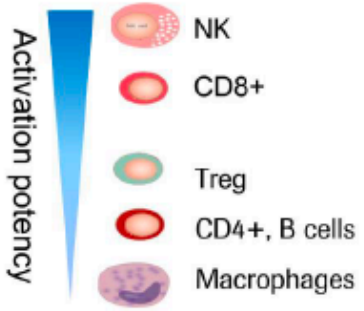
IL2v
cytokine

FAP (Fibroblast Activation Protein α)
= cancer associated fibroblast/stroma cell antigen

- Specific marker of cancer-associated fibroblasts
- Limited expression in normal tissues



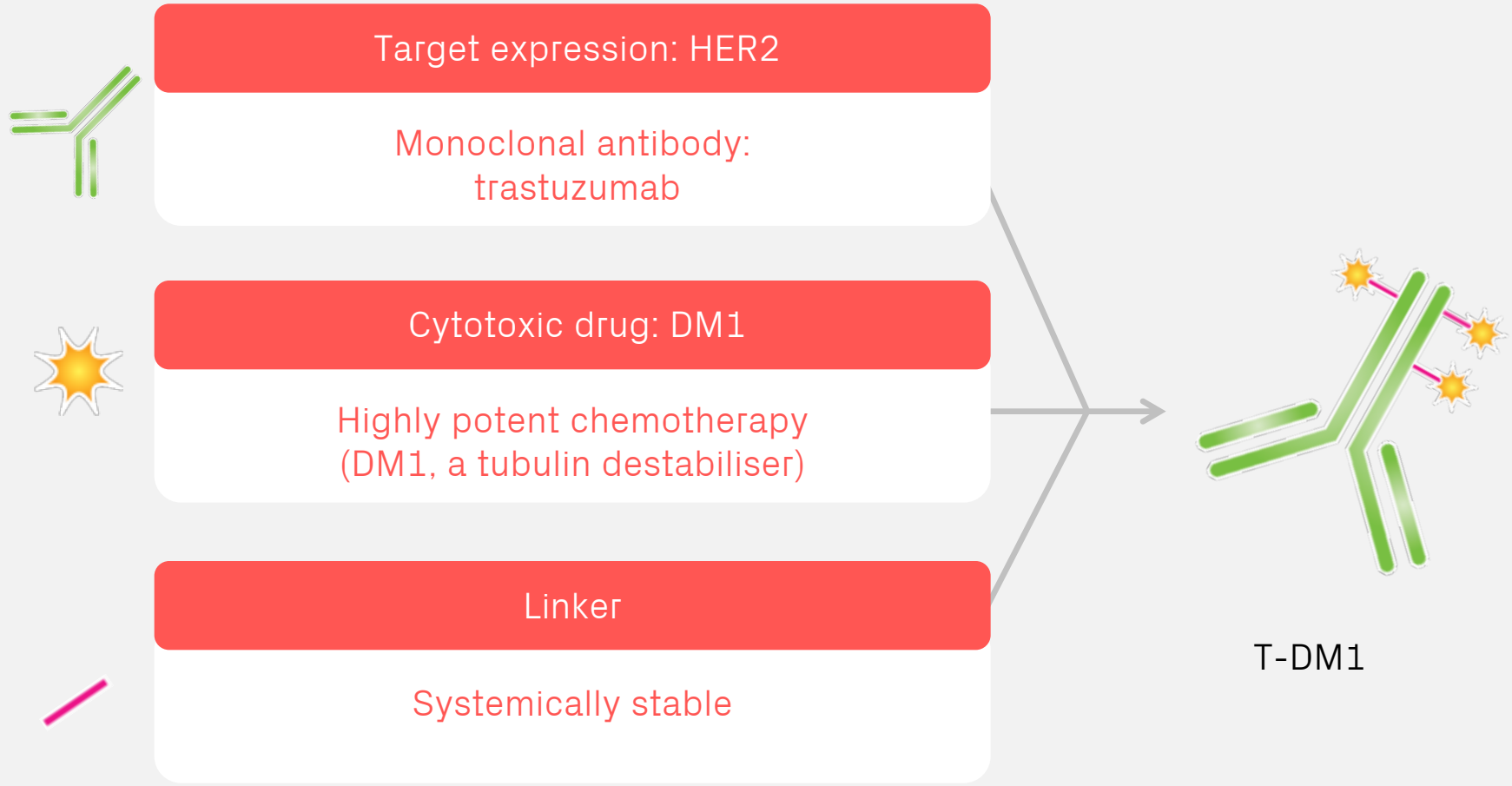
- **IL2v = IL-2 variant, IL-15 like cytokine**
  Promoting immune effector cells (CD8 T & NK
  over suppressor cells (Tregs &



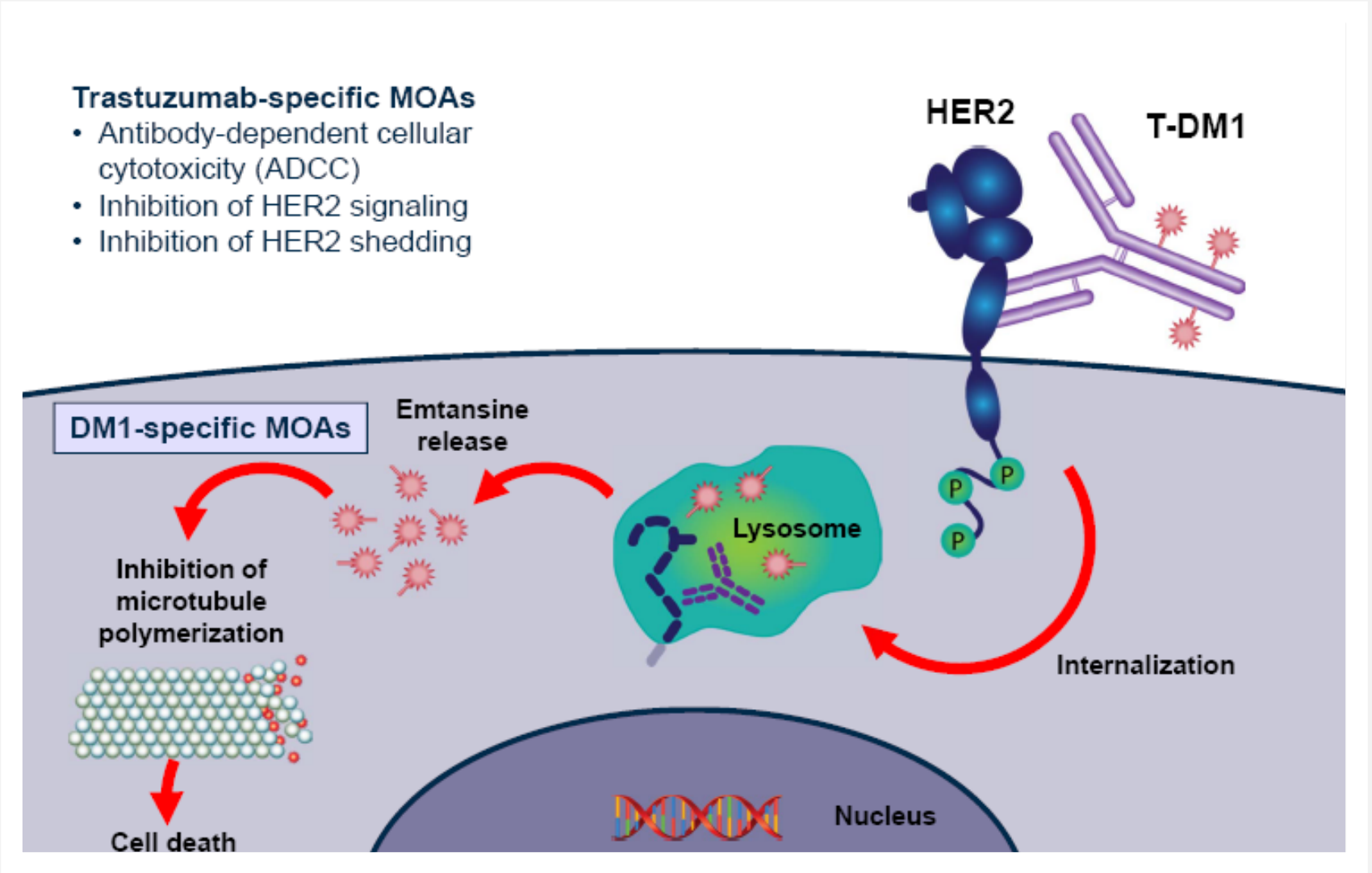
May FAP-IL2v recruit NK cells in cold tumors?

Outline of the presentation

VI) Dissecting resistance to T-DM1

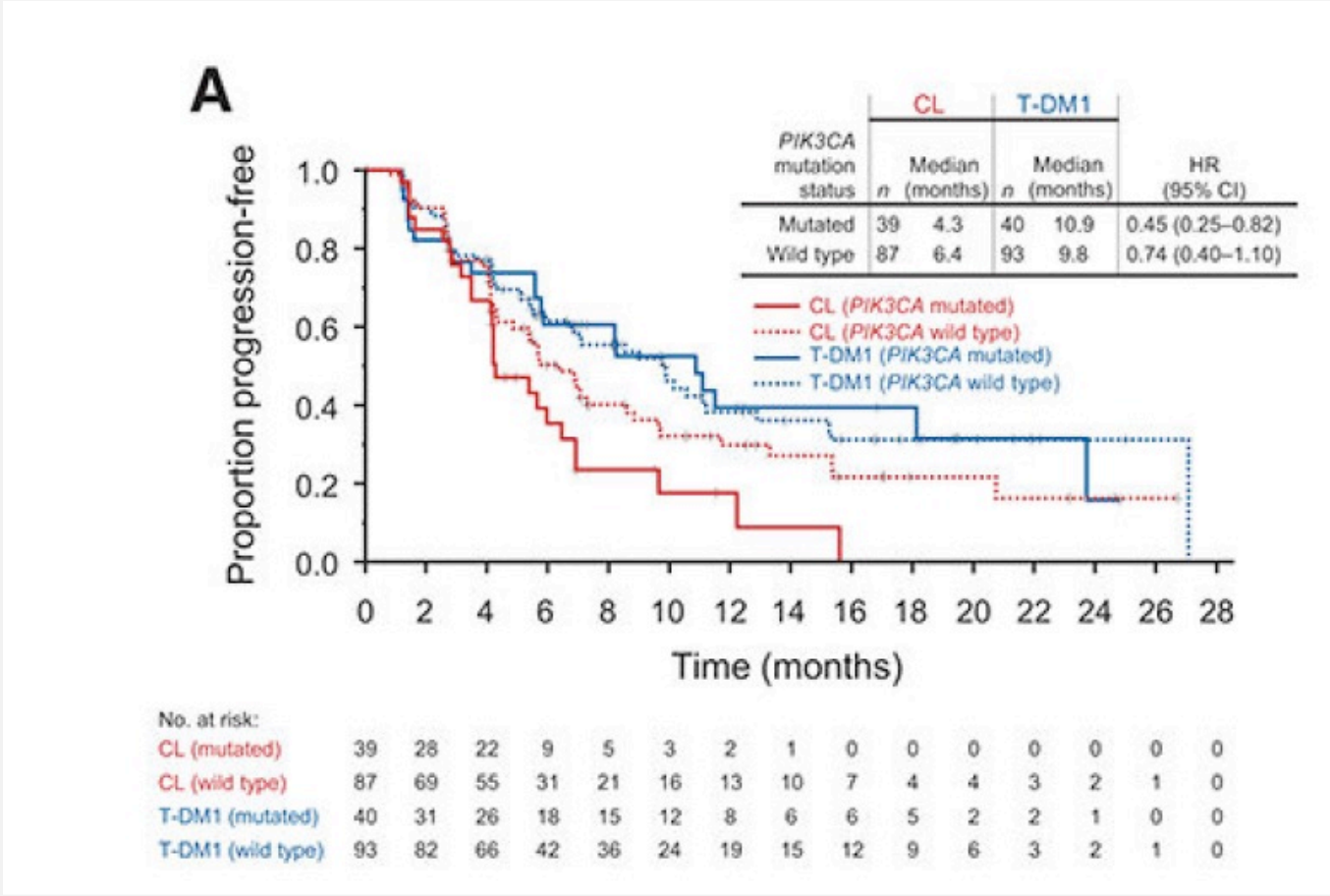

Target expression: HER2
Monoclonal antibody:
trastuzumab
Cytotoxic drug: DM1
Highly potent chemotherapy
(DM1, a tubulin destabiliser)
Linker
Systemically stable
T-DM1

Trastuzumab-emtansine (T-DM1): Mechanisms of action



Dissecting resistance to T-DM1

PIK3CA status **unrelated** to T-DM1 PFS

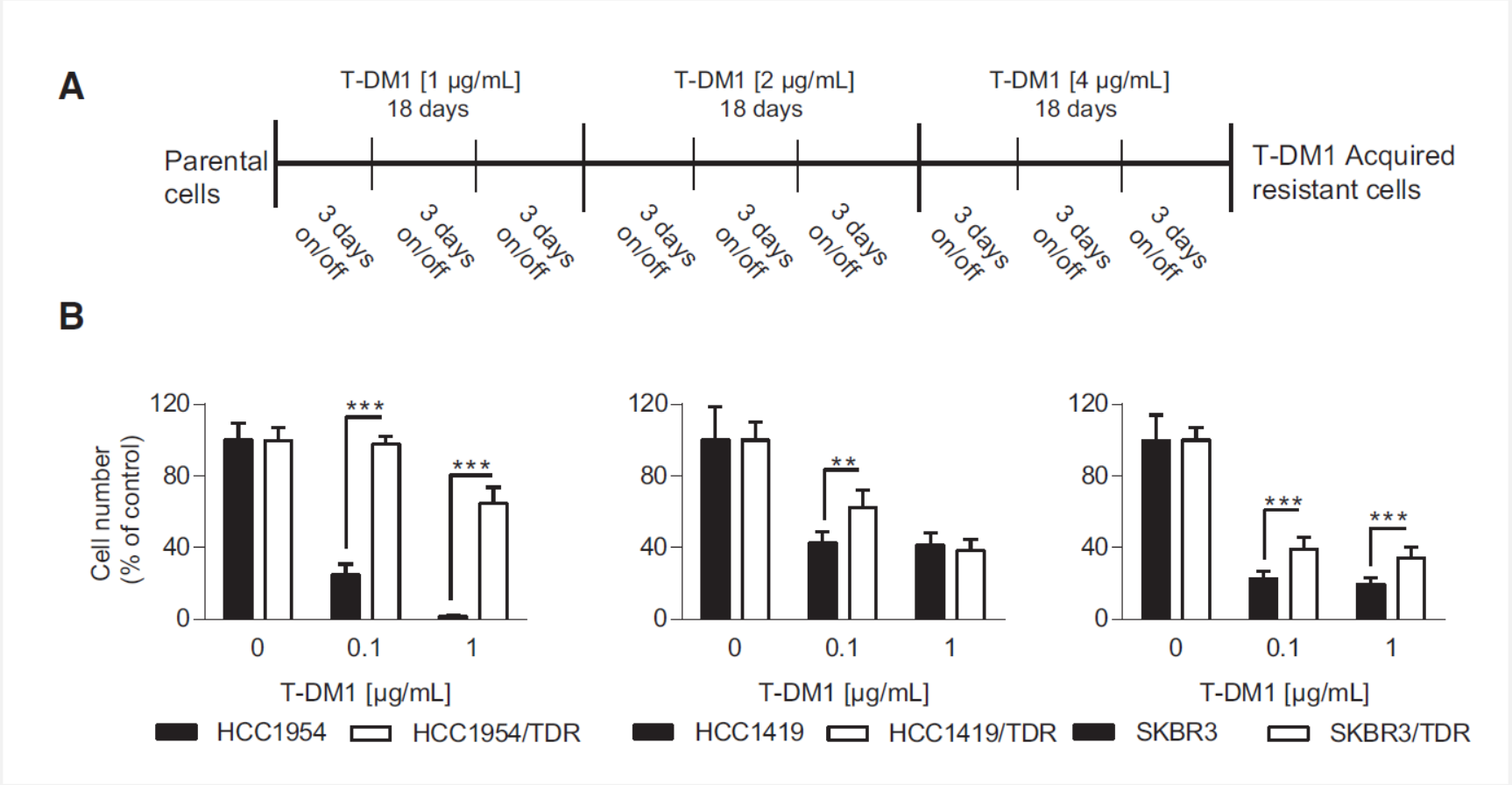


PIK3CA mutation status	CL n	CL Median (months)	T-DM1 n	T-DM1 Median (months)	HR (95% CI)
Mutated	39	4.3	40	10.9	0.45 (0.25–0.82)
Wild type	87	6.4	93	9.8	0.74 (0.40–1.10)

Baselga et al. Clin Cancer Res 2016

Dissecting resistance to T-DM1

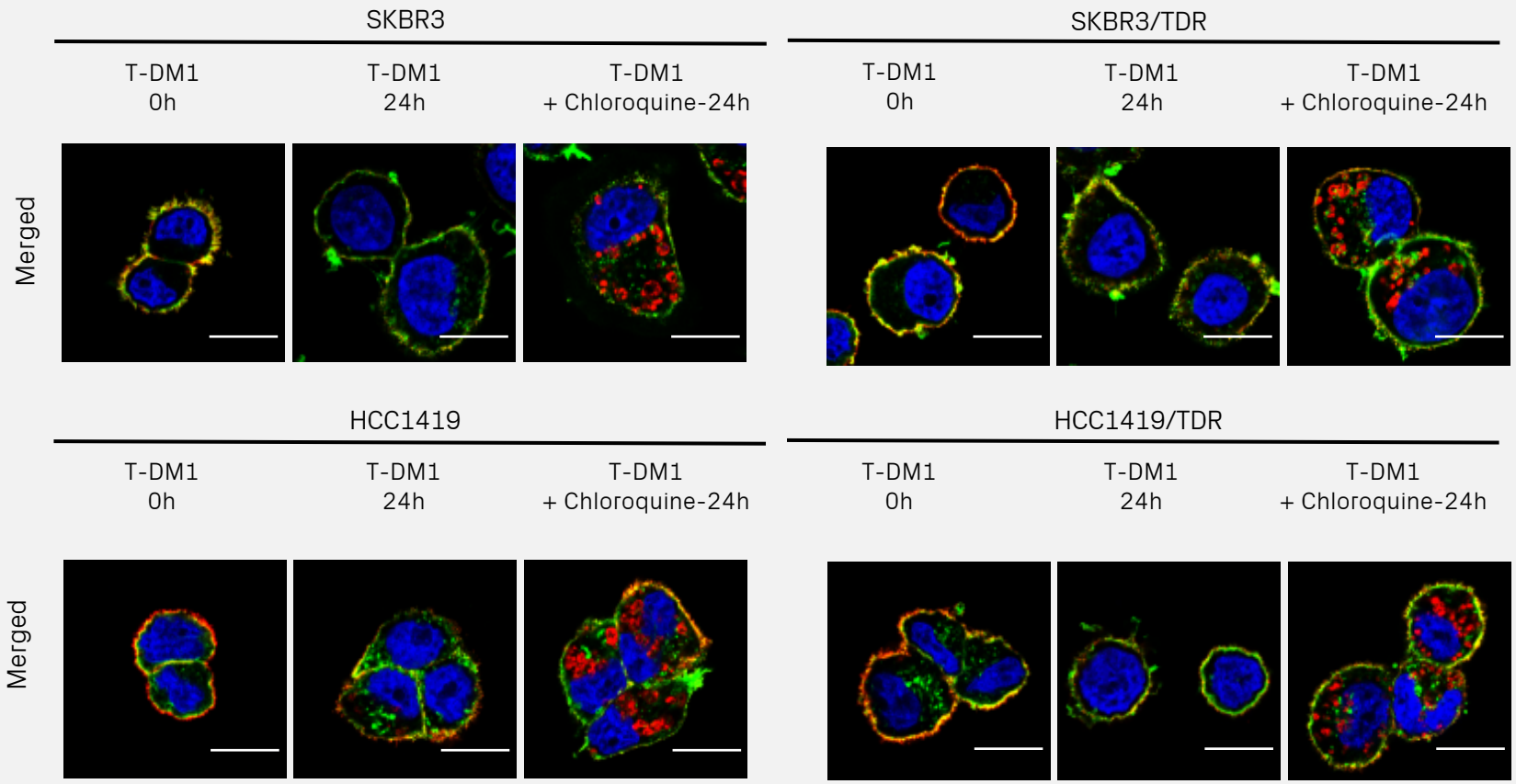
Generation of T-DM1 resistant (TDR) HER2+ breast cancer cells



Sabbaghi et al. Clin Cancer Res 2017

Dissecting resistance to T-DM1

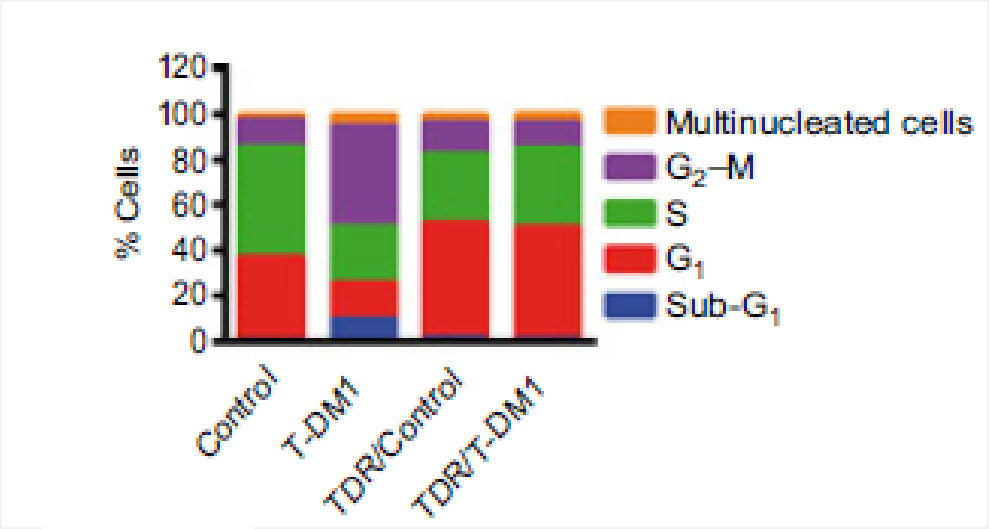
The magnitude of T-DM1 internalization and intracellular pattern were similar in parental and resistant cells



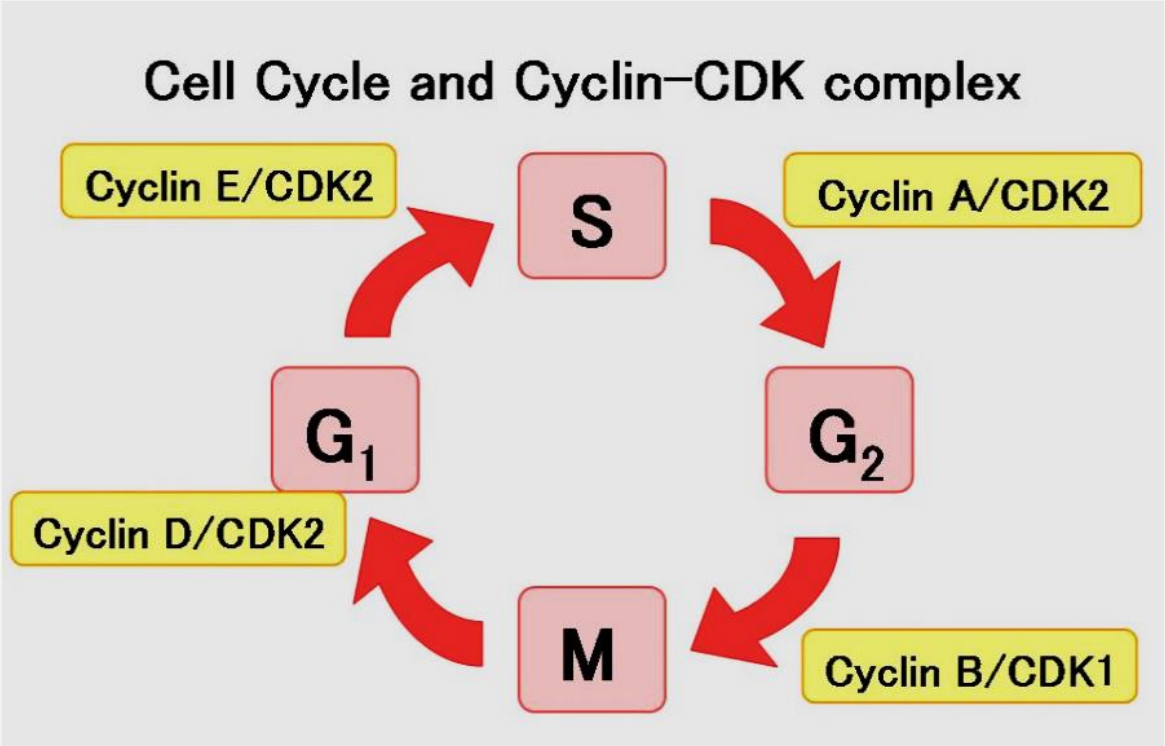
Sabbaghi et al. Clin Cancer Res 2017

Dissecting resistance to T-DM1

Cells with acquired resistance to T-DM1 do not undergo G2-M arrest



Cyclin B/CDK1 mediates G2M transition

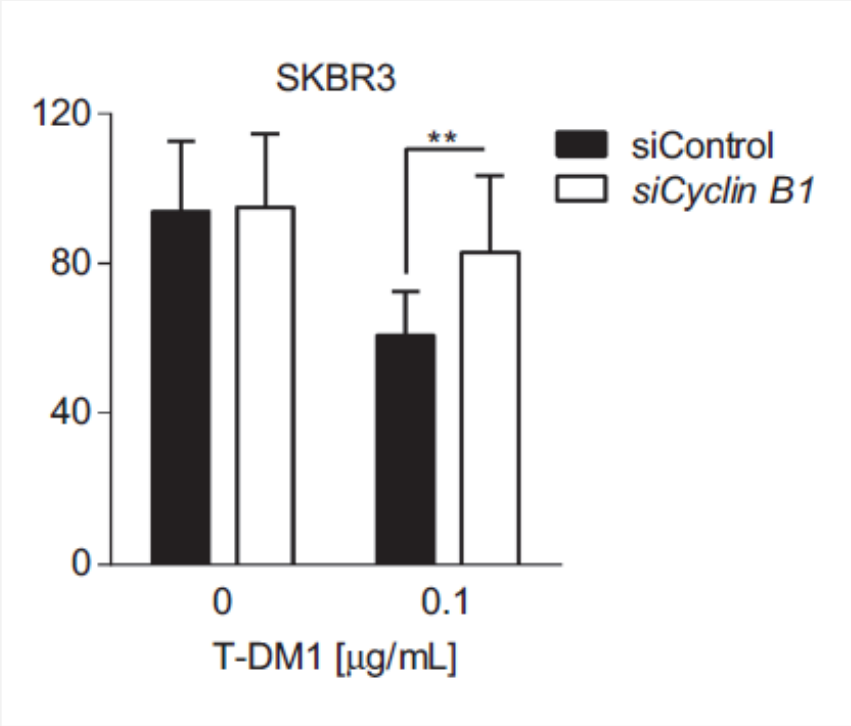


Sabbaghi et al. Clin Cancer Res 2017

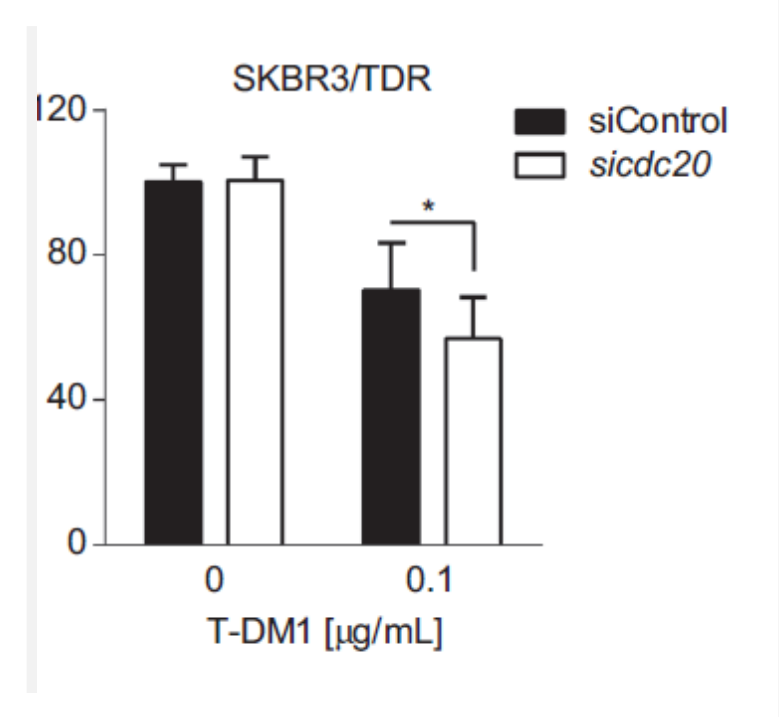
Dissecting resistance to T-DM1

Cyclin B1 deficiency mediates T-DM1 resistance

Depleting Cyclin B1 causes T-DM1 resistance in parental cells



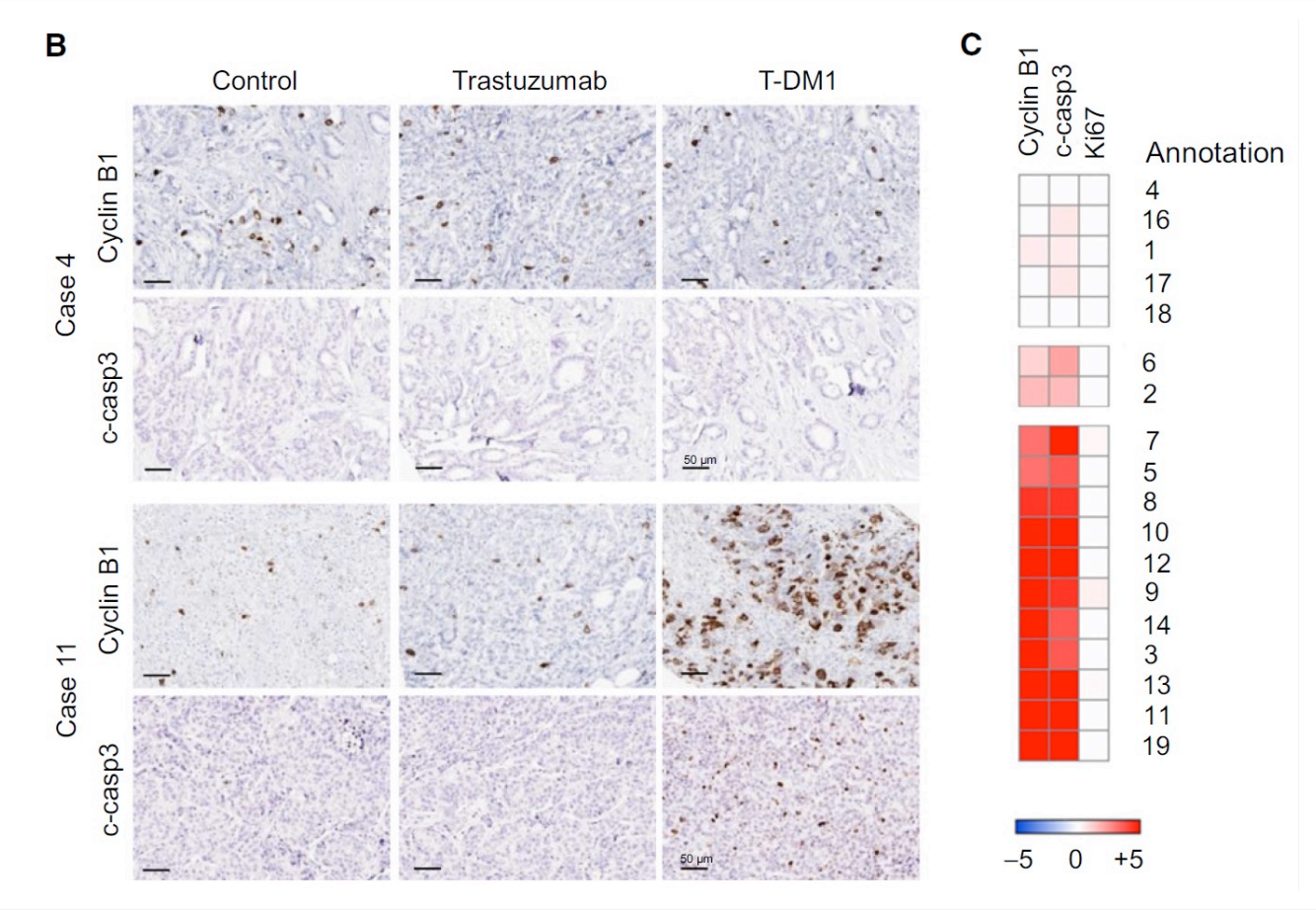
Overexpressing Cyclin B1 reduces T-DM1 resistance in TDR cells



Sabbaghi et al. Clin Cancer Res 2017

Dissecting resistance to T-DM1

In freshly cultured HER2+ breast cancer patient tumors, induction of cyclin B1 by T-DM1 correlates with apoptosis



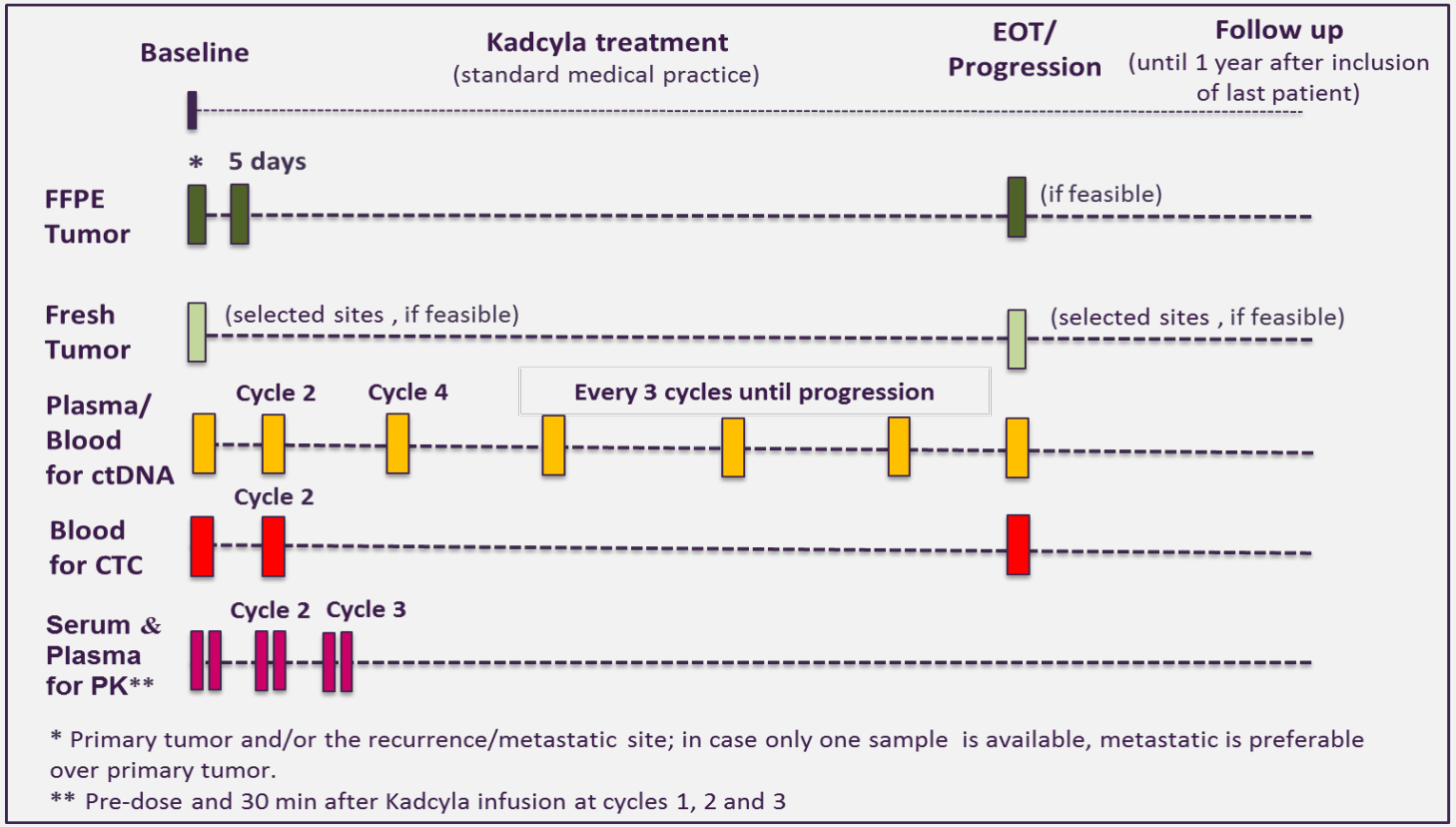
Sabbaghi et al. Clin Cancer Res 2017

Dissecting resistance to T-DM1

Study Population and Design

Advanced HER2+ BC patients planned to be treated with Kadcyla within the approved indication in Spain

(N=50;
24 months of recruitment)



Samples required for Foundation tests :

- F-One: 10 FFPE tumor (primary/metastases) slides sent to Foundation Medicine Lab on batches, e.g. every 5-6 months.

- F-ACT : 2 whole blood samples collected at baseline and at progression (prior to initiate a new therapy). If a patient ends Kadcyla treatment due to a reason different to progression (e.g. toxicity), two additional samples should be collected at end of Kadcyla.

Dissecting resistance to T-DM1

Mechanisms of Acquired Resistance to Trastuzumab Emtansine in Breast Cancer Cells

- Diverse targetable mechanisms

- KPL4 T-DM1 resistant: Decreased HER2 and upregulation of MDR1

- BT-474 T-DM1 resistant: PTEN deficiency

Li et al. Mol Cancer Res 2018

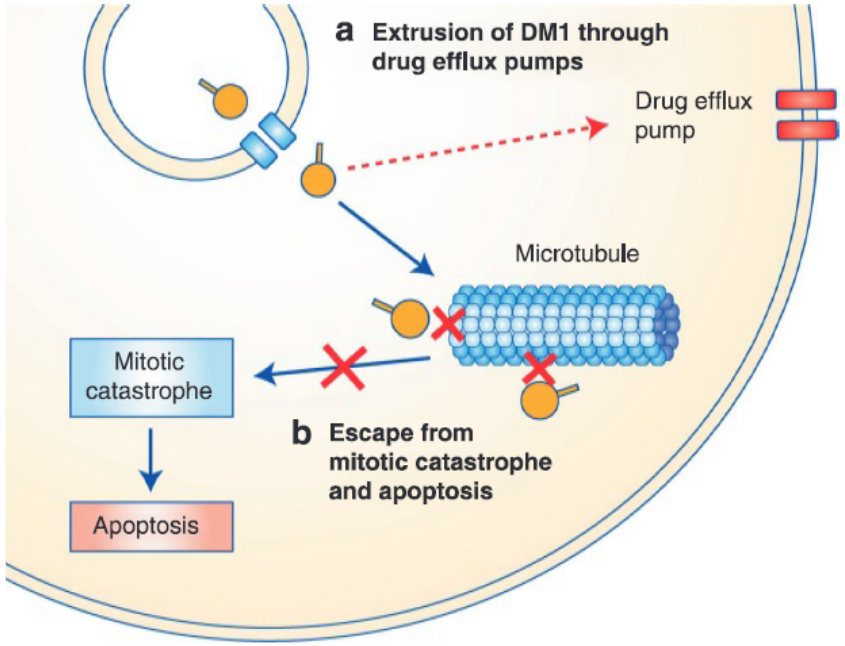


Fig. 5 T-DM1 resistance arising from impairment of DM1-mediated cytotoxicity. Increased expression of drug efflux transporters for which DM1 is a substrate might promote the efflux of lysine-MCC-DM1 from cells. Alternatively, cells might escape from DM1-mediated mitotic catastrophe through reduced induction of cyclin B1 or increased expression of polo-like kinase 1 (PLK1), allowing cells to complete mitosis and avoid apoptosis despite having an abnormal mitotic spindle.

British Journal of Cancer (2020) 122:603–612

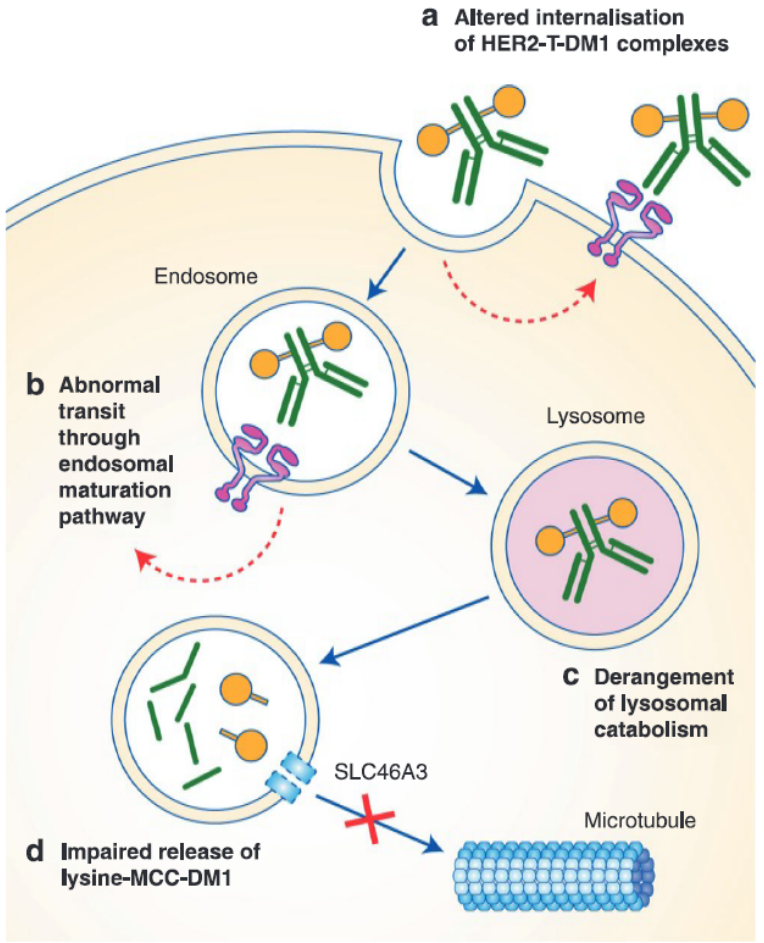
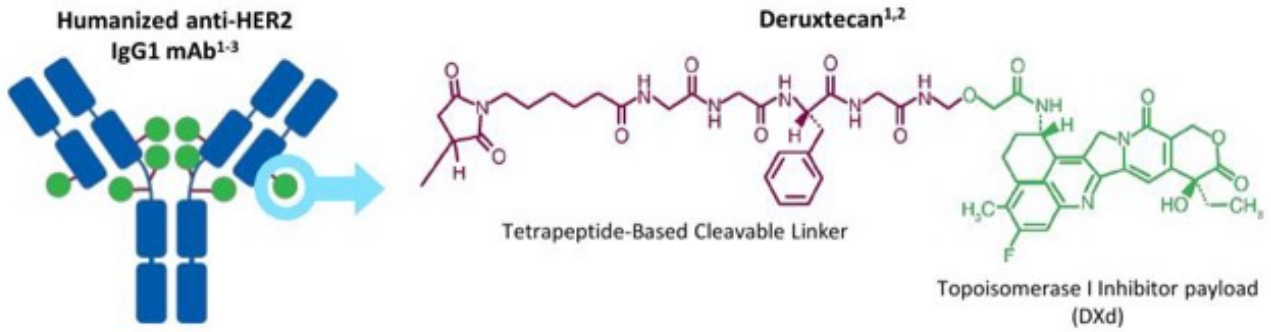


Fig. 4 T-DM1 resistance arising from dysfunctional intracellular trafficking and metabolism. HER2–T-DM1 complex internalisation might be reduced by enhanced recycling of HER2–T-DM1 complexes back to the plasma membrane, thereby promoting the efflux of T-DM1. Altered expression of certain endocytic and cytoskeletal proteins could impair normal transit of HER2–T-DM1 complexes through the endosomal maturation pathway. Altered lysosomal pH regulation resulting in decreased acidity of lysosomal vesicles can reduce catabolism of HER2–T-DM1 to lysine-MCC-DM1 and prevent the release of the active compound. Reduced expression of lysosomal transporter proteins, such as SLC46A3, might also impair the release of lysine-MCC-DM1 into the cytoplasm.

Trastuzumab Deruxtecan (DS-8201) is a Novel ADC Designed to Deliver an Optimal Antitumor Effect

Trastuzumab deruxtecan is an ADC composed of 3 components:

- A humanized anti-HER2 IgG1 mAb with the same amino acid sequence as trastuzumab
- A topoisomerase I inhibitor payload, an exatecan derivative
- A tetrapeptide-based cleavable linker



- Payload MOA: topoisomerase I inhibitor
- High potency of payload
- High drug to antibody ratio ≈ 8
- Payload with short systemic half-life
- Stable linker-payload
- Tumor-selective cleavable linker
- Membrane-permeable payload

The clinical relevance of these features is under investigation.

ADC, antibody-drug conjugate; MOA, mechanism of action.

1. Nakada T, et al. Chem Pharm Bull (Tokyo). 2019;67(3):173-185. 2. Ogitani Y, et al. Clin Cancer Res. 2016;22(20):5097-5108. 3. Trail PA, et al. Pharmacol Ther. 2018;181:126-142. 4. Ogitani Y, et al. Cancer Sci. 2016;107(7):1039-1046.

Take home messages

1. HER2 positive breast cancer is a complex ecological system where any member may play a role in resistance

2. Intrinsic subtypes have distinct biological properties that may be the basis for rational combinations of anti-HER2 plus biologicals.

3. Trials to overcome biological resistance with the combination of anti-HER2 plus Akti, PI3Ki and CDK4/6i underway.

4. A defective immune system may limit the activity of anti-HER2 MAbs and ways to restore/enhance it under study (*i.e*. margetuximab).

5. Antibody-drug conjugates overcome some limits of the MAbs but still face mechanisms of chemotherapy resistance. Novel ADCs are highly potent and without cross-resistance.

6. Understanding resistance mechanisms and their interplay to anti-HER2 therapy paves the way for the rational design of novel therapeutic strategies.

SCIENTIFIC BITES®

Cancer research
e-learning platform

Thank you

by SOLTI