SOCIETA' ITALIANA DI FARMACIA
OSPEDALIERA E DEI SERVIZI FARMACEUTICI
DELLE AZIENDE SANITARIE

XXXV CONGRESSO NAZIONALE SIFO

IL FARMACISTA: UNA RISORSA PER LA SALUTE.

RESPONSABILITA', APPROPRIATEZZA, SOSTENIBILITA'

LA MEDICINA PERSONALIZZATA: UN ESEMPIO IN ONCOLOGIA

ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI IRCCS

SILVIO GARATTINI

Montesilvano, 16 Ottobre 2014

MEDICINA PERSONALIZZATA

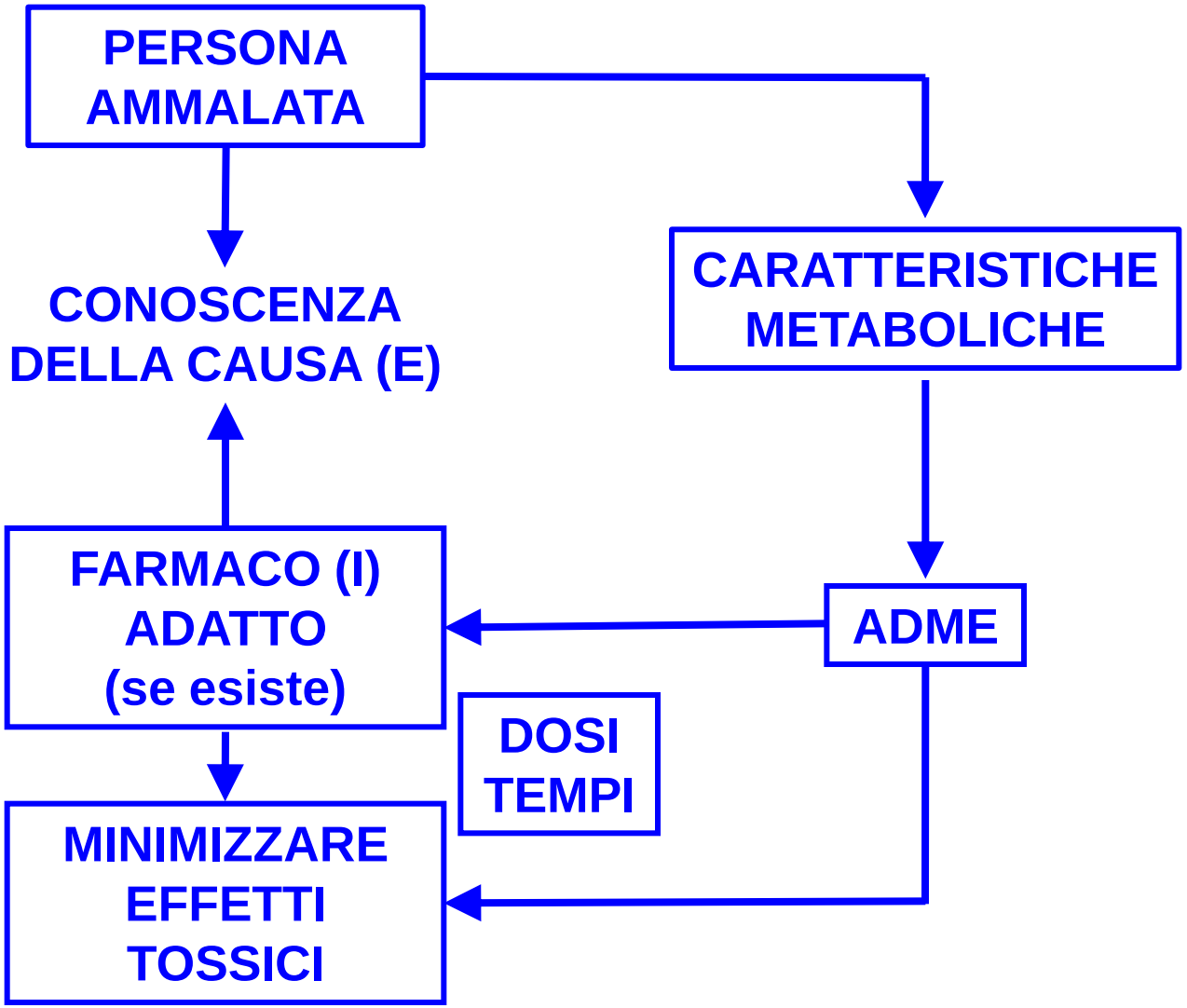
UN ESEMPIO APPLICATO

ALLA TERAPIA DEI TUMORI

MOSTRA LE DIFFICOLTA'

DA RISOLVERE

MEDICINA PERSONALIZZATA



L’eterogeneità è la caratteristica principale dei tumori ed è la base su cui si può costruire una terapia personalizzata.
Si distinguono due gruppi di eterogeneità

- Relativa al tumore
- Relativa al farmaco

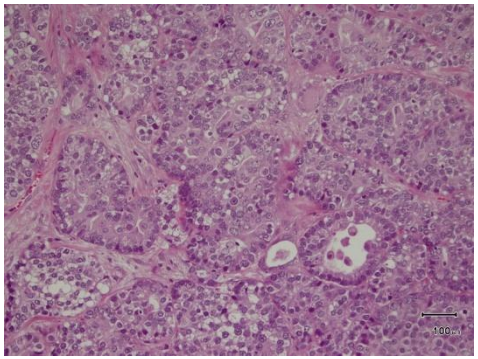
ETEROGENEITA’ MORFOLOGICA

TUMOR HETEROGENEITY

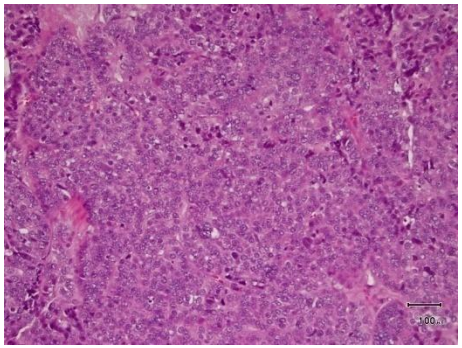
Ovarian Carcinoma

Case #135

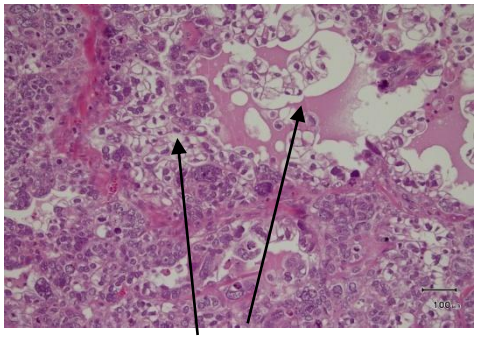
E/E



Gland structures with different differentiation grade

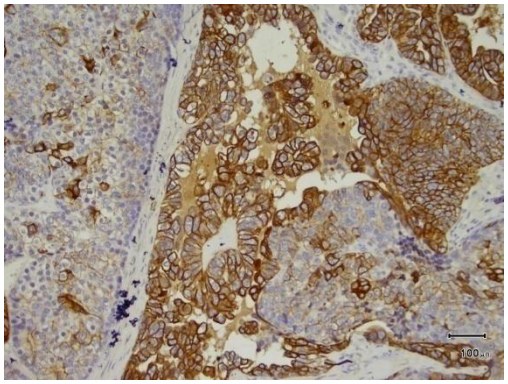


Sarcomatous area

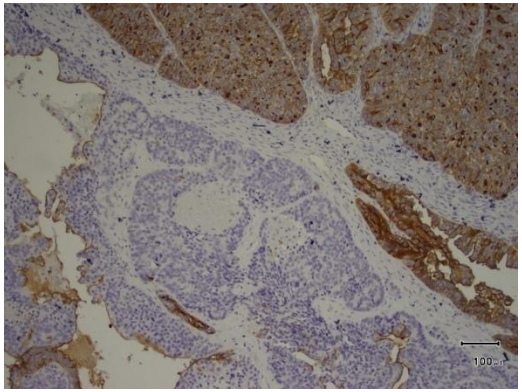


Condrosarcomatose component

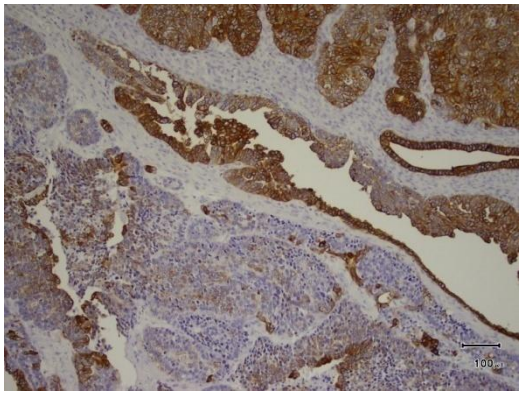
Cytokeratin pool



CA125



Citokeratin 7



ETEROGENEITÀ DEI TUMORI SULLA BASE DEI MARCATORI BIOLOGICI

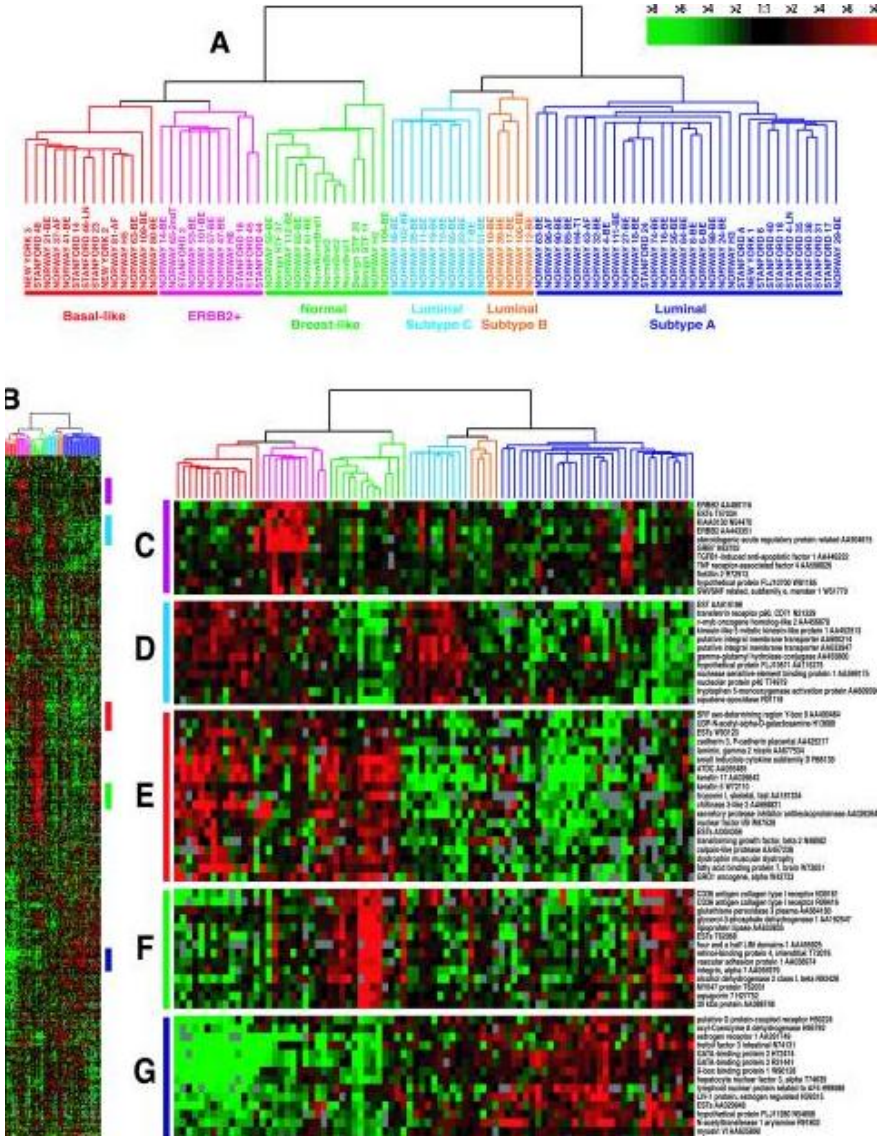
Traditional classification of mammary carcinoma

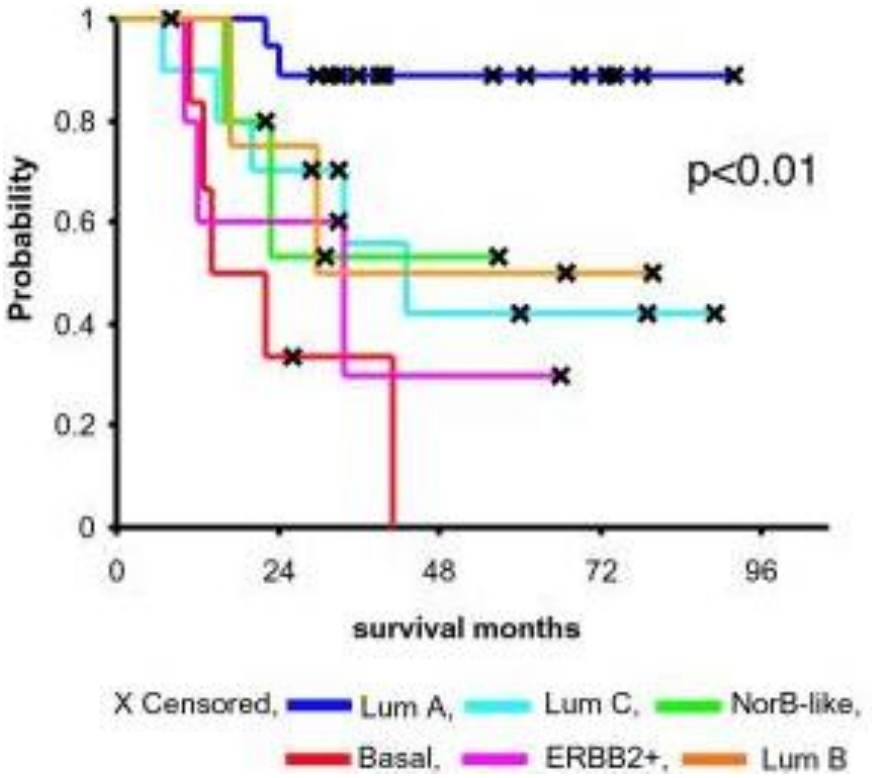
The mammary tumor is an heterogeneous neoplasia, indeed representing a collection of different diseases as to diagnosis, prognosis and treatment

Traditionally mammary tumors are classified in three different sub-groups:

- **ER^+** **(70%)**
- **$HER2^+$** **(20%)**
- **Triple negative** **(10%)**

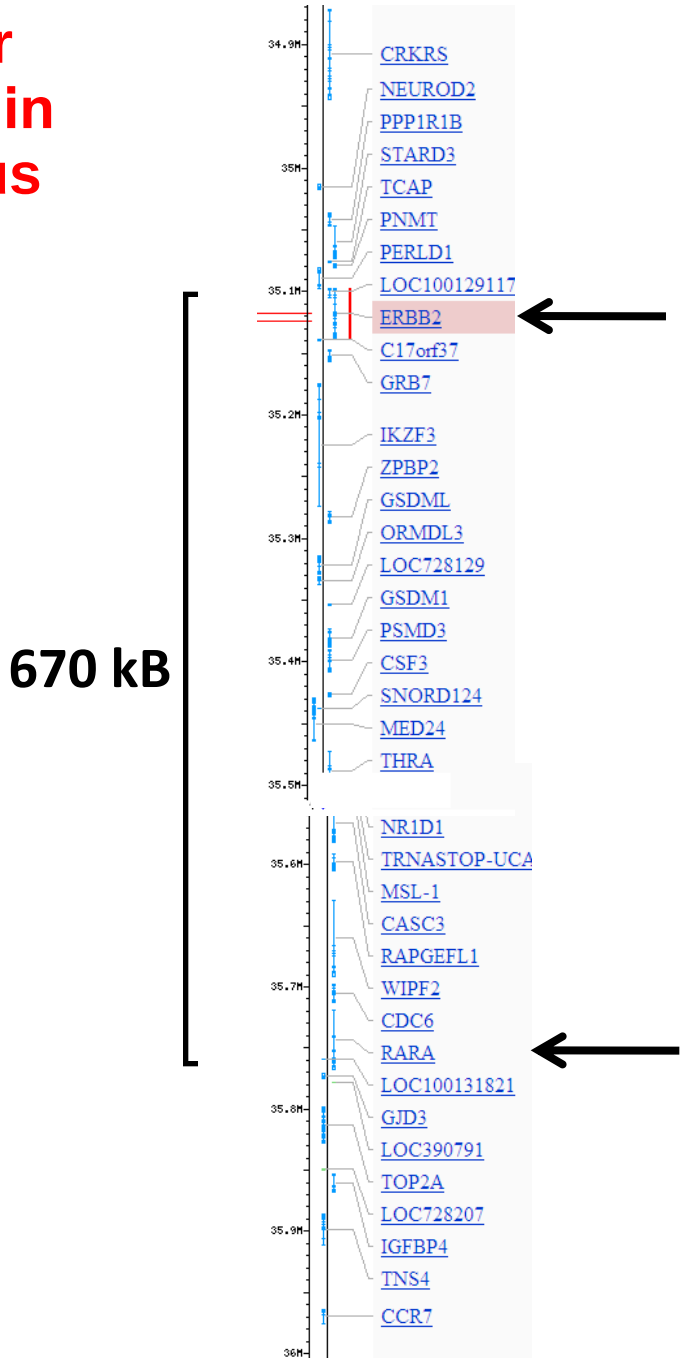
La classificazione del tumore mammario sulla base del profilo di espressione genica ha significato prognostico





Sorlie, M et al. Proc Natl Acad Sci U S A. 2001 98: 10869–10874

The gene coding for the nuclear retinoic acid receptor RARα lays in close proximity to the HER2 locus



670 kB

The situation in Italy

New cases: 35,000

↓

$HER2^{+}$: 11,000

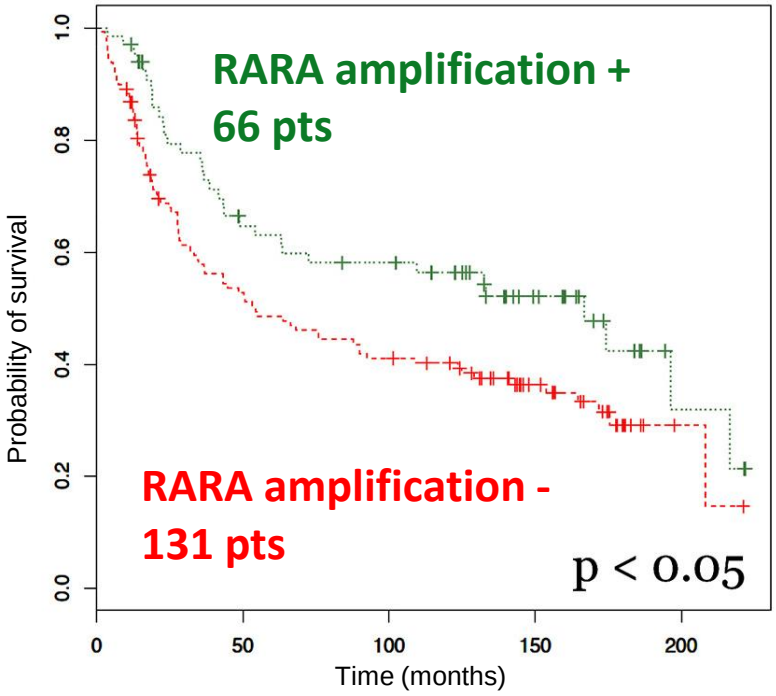
↓

$HER2^{+}/RARA^{+}$: 3,000

↓

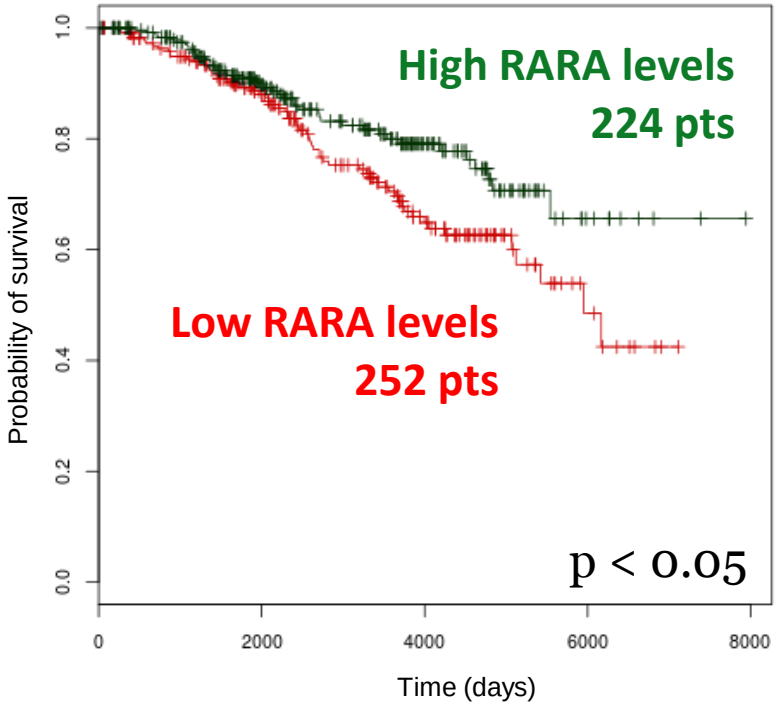
$ER^{-}/HER2^{+}/RARA^{+}$: 2,000

IMPACT OF *RARA* AMPLIFICATION AND LEVELS ON SURVIVAL IN BREAST CANCER PATIENTS



HER2 positive patients

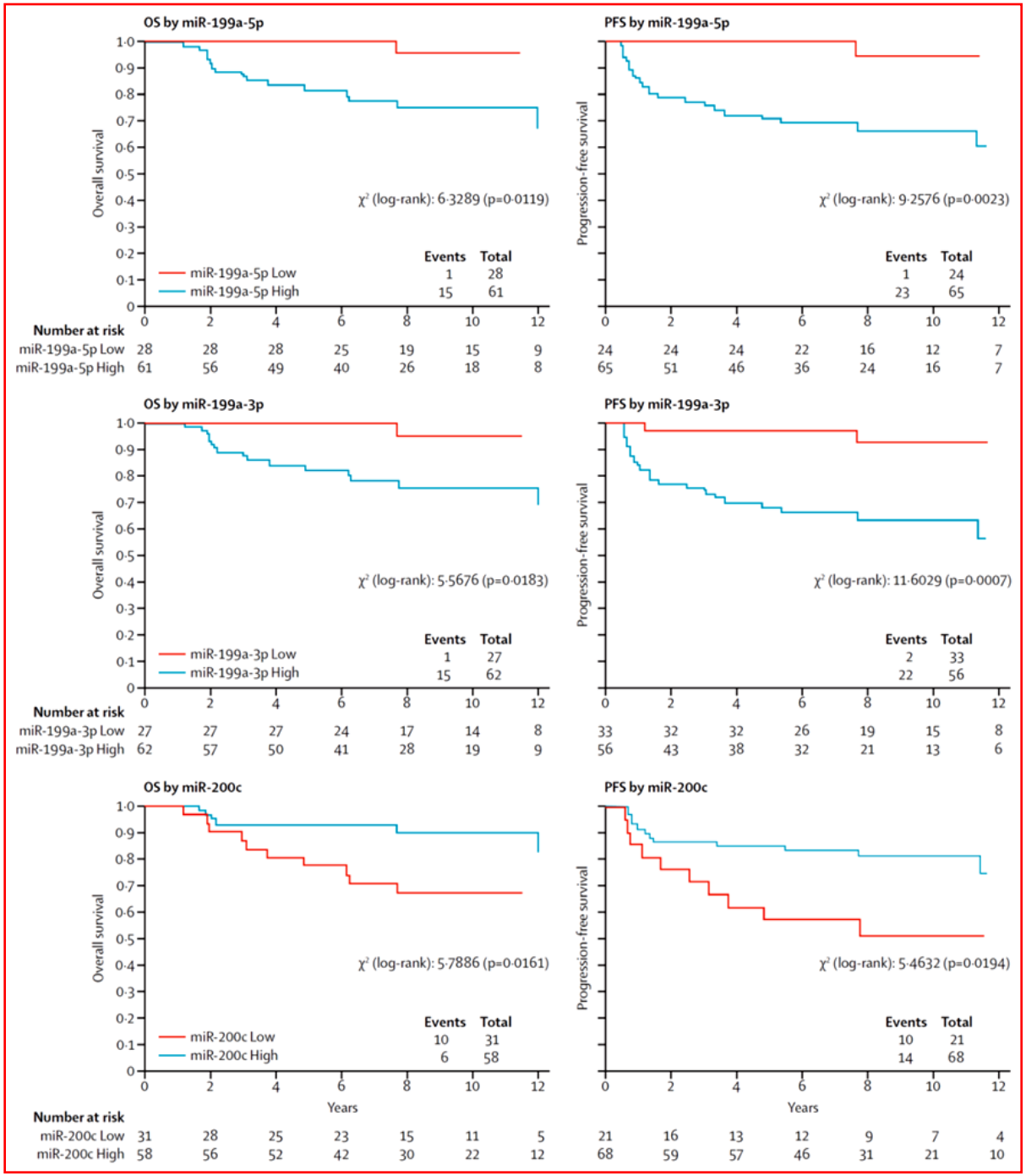
Analysis of a public dataset from:
Staal J et al. Breast Cancer Research 2010, 12:R25



ER positive patients

Analysis of a public dataset from:
Curtis C et al. Nature 2012, 18:346

E.Garattini et al., 2012



Marchini et al., 2011

ETEROGENEITA' DELLE CELLULE TUMORALI CIRCOLANTI

TUMOR CELLS ARE CONSTANTLY CHANGING AS

THE TUMOR PROGRESSES. CIRCULATING CANCER

CELLS MAY BECOME HER-2 POSITIVE WHILE THE

PRIMARY TUMOR WAS HER-2 NEGATIVE

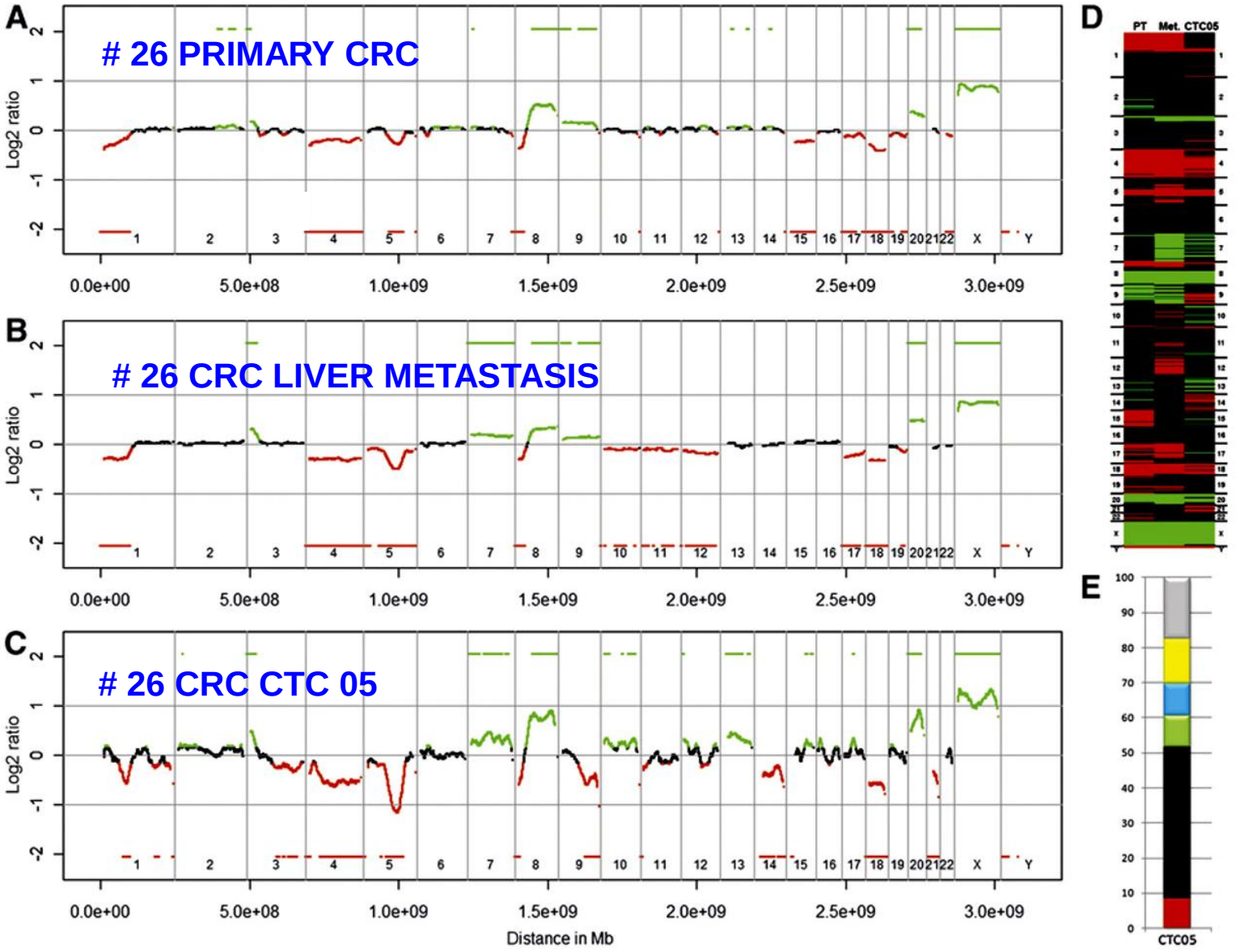
MENG et al., 2004

DISCREPANCY OF BREAST CANCER HER2 STATUS

- 29 % OF HER 2^+ PRIMARY TUMORS HAD HER 2^- CTC STATUS
- 9 % OF HER 2^- PRIMARY TUMORS HAD HER 2^+ CTC STATUS

Lightart et al., 2013

TUMOR SPECIFIC COPY NUMBER CHANGES



PC shared by P; MC shared by M; C unique

Heitzer et al., 2013

APC [p.R332X]
KRAS [p.G12V]
PIK3CA [p.E542K]

APC [p.R332X]
KRAS [p.G12V]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

CTC7

ABCA1 [p.P1209S]
APC [p.R332X]
KRAS [p.G12V]
LAMA1 [p.P1414S]
LAMA1 [p.H1002Y]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

CTC13

APC [p.R332X]
KRAS [p.G12V]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

CTC14

APC [p.R332X]
C10orf137 [p.A990E]
CACNA2D3 [p.G84S]
CTNNB1 [p.A149T]
GNAS [p.G869D]
GUCY1A2 [p.H439Y]
KRAS [p.G12V]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

Gene	Point mutations primary tumor	Point mutations cerebellar metastasis	Point mutations CTCs	Potentially clinically significant
APC	p.R332X	p.R332X	p.R332X	
KRAS	p.G12V	p.G12V	p.G12V	EGFR inhibitors
PIK3CA	p.E542K	p.E542K	p.E542K	PI3K inhibitors
TP53	Ø	p.R141C	p.R141C	

Heitzer et al., 2013

ETEROGENEITÀ FRA TUMORE PRIMARIO E METASTASI

N. PATIENTS	PRIMARY TUMOR/METASTASES		REFERENCE
	CONCORDANCE	DISCORDANCE	
21	13	8	Zidan et al., 2005
35	27	8	Santinelli et al., 2008
60	58	2	Gong et al., 2005

HER-2 STATUS IN METASTATIC BREAST CANCER

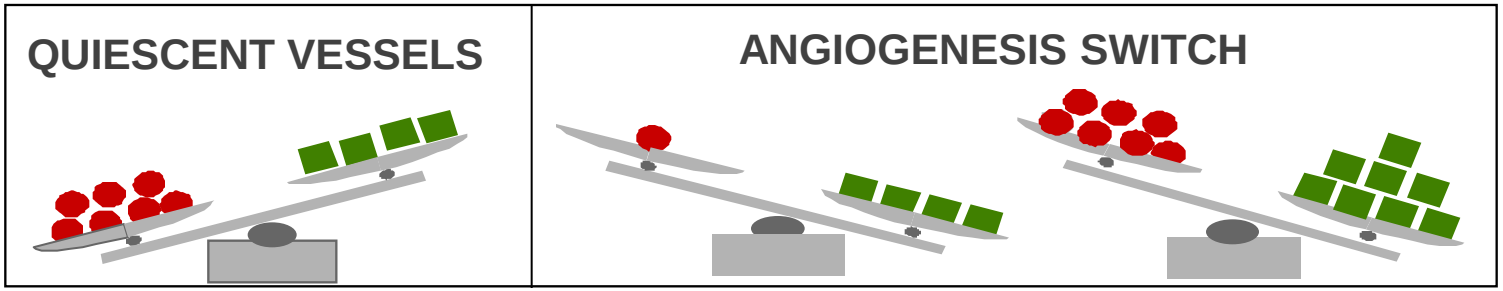
We identified a similar discordance rate in our recently published paper: 16.4%, 41.7%, 17.5% for ER, PgR and HER-2, respectively, as well as a trend toward poorer PRS in patients whose tumors showed a loss of ER expression

Farolfi et al., 2013

ETEROGENEITÀ DEL MICROAMBIENTE

- **VASCOLARIZZAZIONE**
- **INFILTRAZIONE DELLE CELLULE IMMUNITARIE**
- **COMPOSIZIONE DELLO STROMA**
- **RISPOSTA FIBROTICA**

Angiogenesis is regulated by a balance between angiogenic factors and inhibitors. Disruption of this balance and acquisition of angiogenic potential by tumor cells is called the "angiogenic switch"



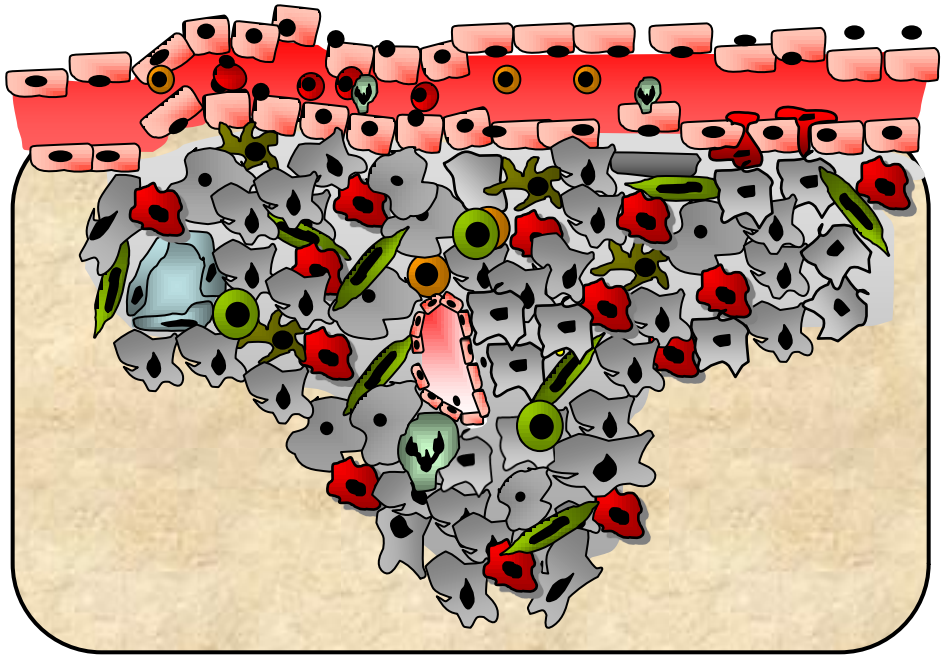
INHIBITORS

Angiostatin
Cartilage-derived
Endostatin
IFN-α
IFN-β
PAIs
PF4
Prolactin fragment
Proliferin-related protein
Protamine
Thrombospondin
TIMPs

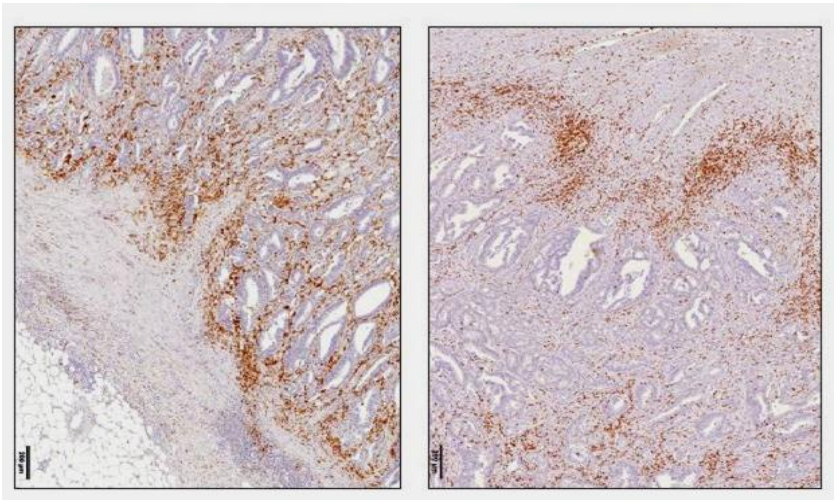
INDUCERS

Angiogenin
FGFs
G-CGF
IGF-1
Interleukin-8
PD-ECGF
Placenta growth factor
Pleiotrophin; Proliferin
Prostaglandins E1, E2
Scatter factor/HGF;
TAT
TGF-a, TGF-B
TNF-a
VEGF

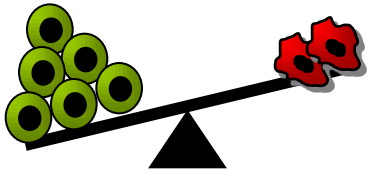
The types of immune cells and soluble mediators in the tumor microenvironment determine whether tumor-mediated immunosuppression or anti-tumor immunity will prevail



T lymphocytes **Macrophages**

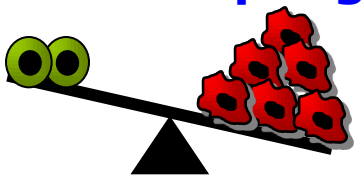


Adaptive immunity
T lymphocytes



TUMOR REGRESSION

Innate immunity
macrophages



TUMOR PROGRESSION

EFFECT ON NKT ON CT26 COLON TUMOR IN Balb/c

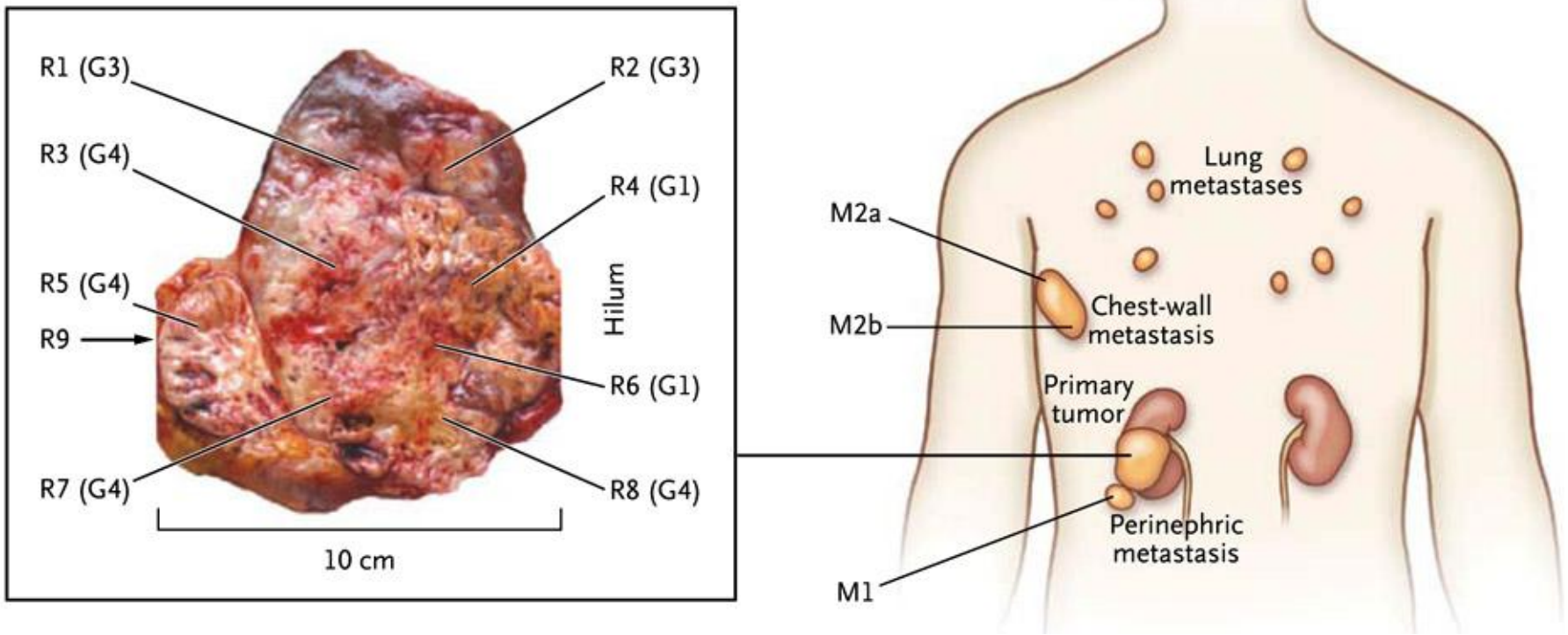
NKT-I		**NKT-II**
α-galactosylceramide	activator	sulfatide
↑	tumor growth	↓

BALANCE BETWEEN NKT SUBTYPES IS AN ELEMENT OF HETEROGENEITY

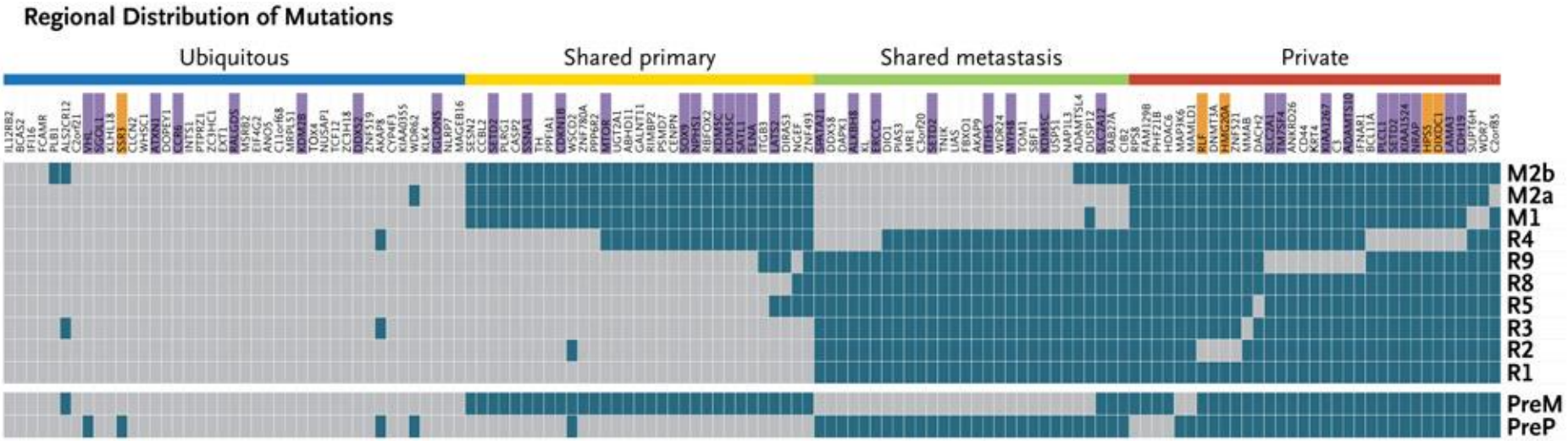
Izhak et al., 2012

ETEROGENEITA' NEL SINGOLO TUMORE

Biopsy Sites



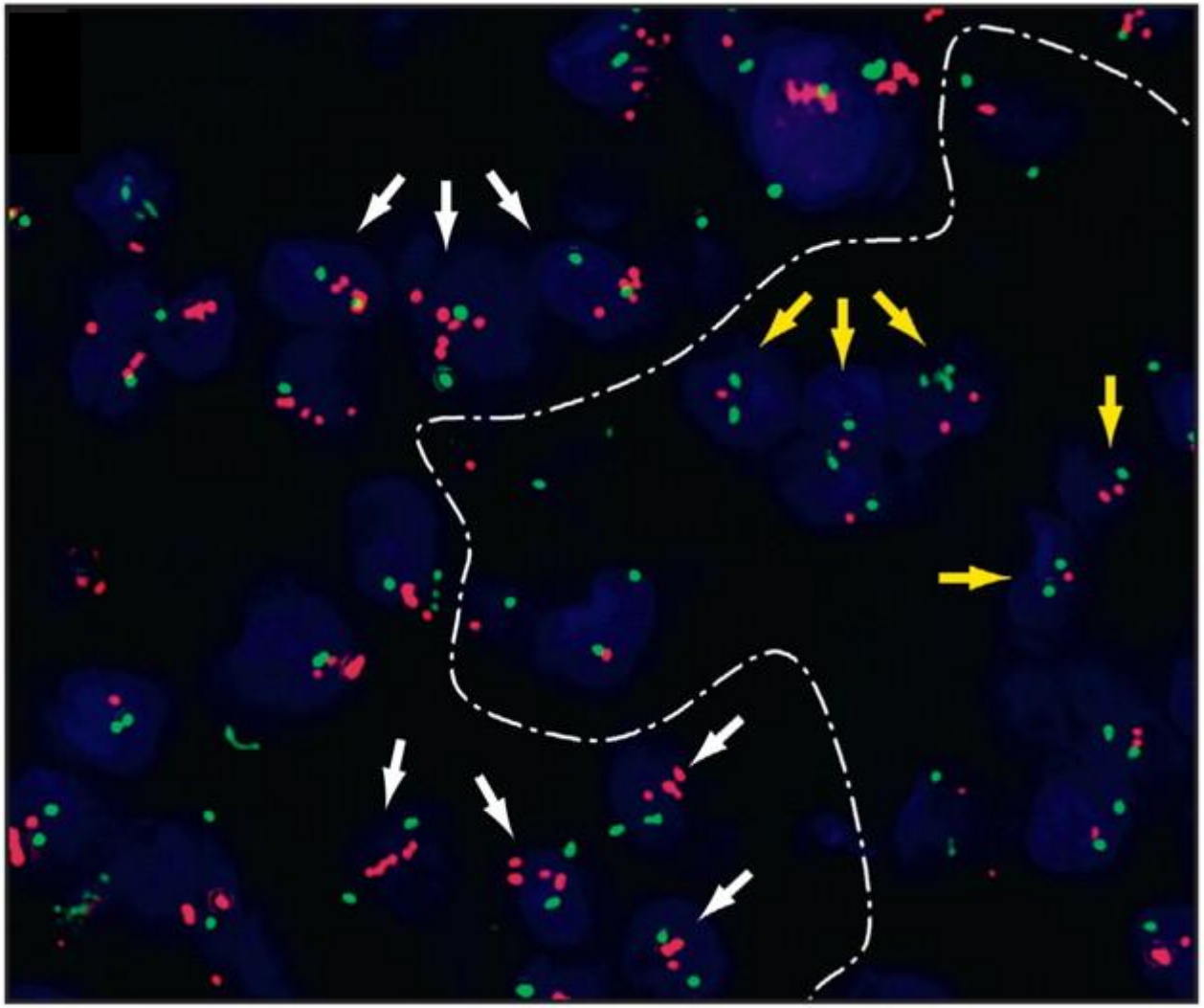
Gerlinger et al., 2012



(mutations are in grey)

Gerlinger et al., 2012

Intra-tumor heterogeneity in the amplification of HER2



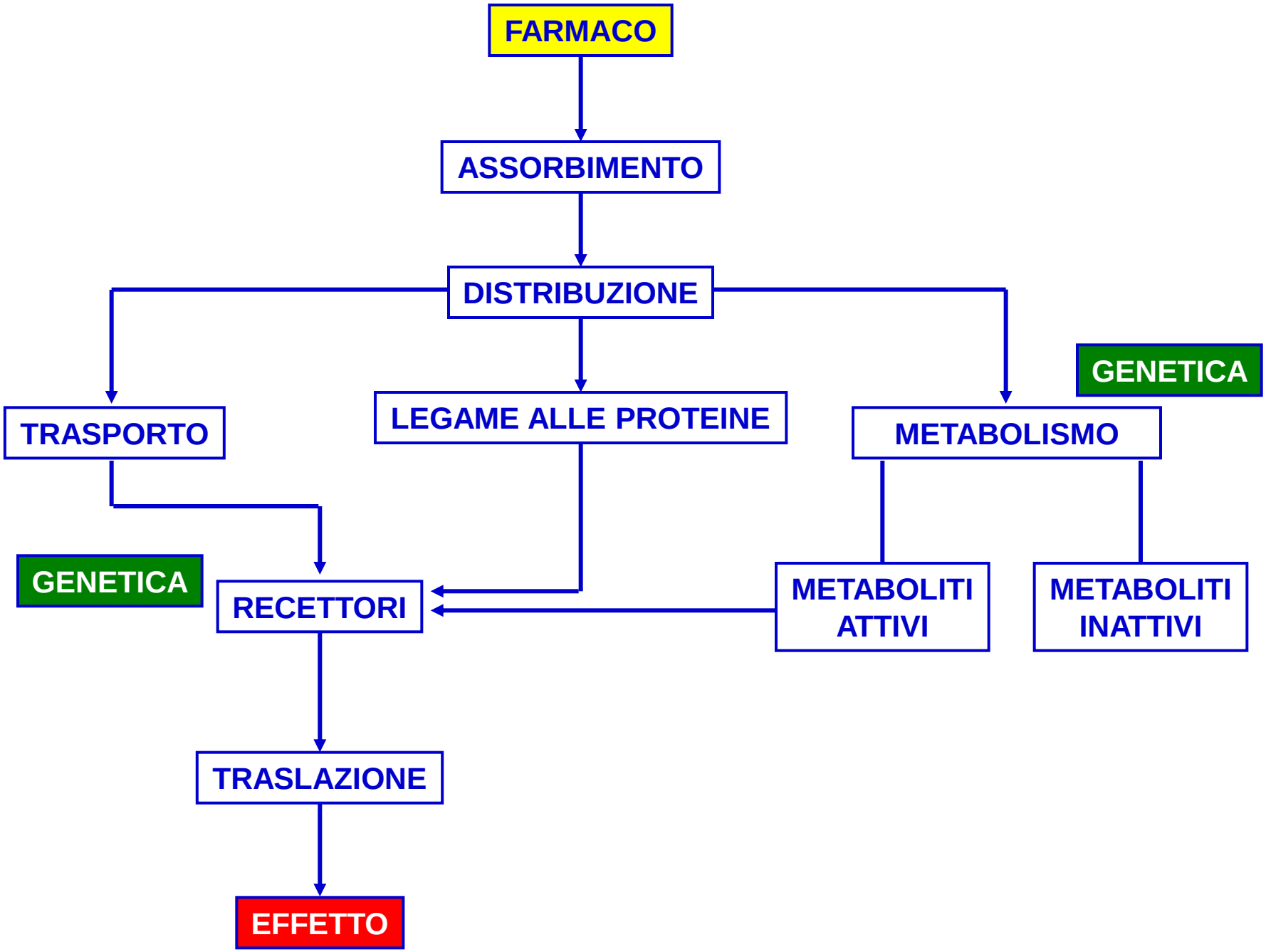
Starczynski J et al. Am J Clin Pathology, 2012

Yellow = cells with no amplification of the HER2 gene

White = cells with amplification of the HER2 gene

The tumor consists of two distinct cell populations: $HER2^+$ e l'altra $HER2^-$. Drugs like trastuzumab or lapatinib are likely to affect only the $HER2^+$ population

ETEROGENEITÀ NELLA CINETICA DEI FARMACI ANTITUMORALI

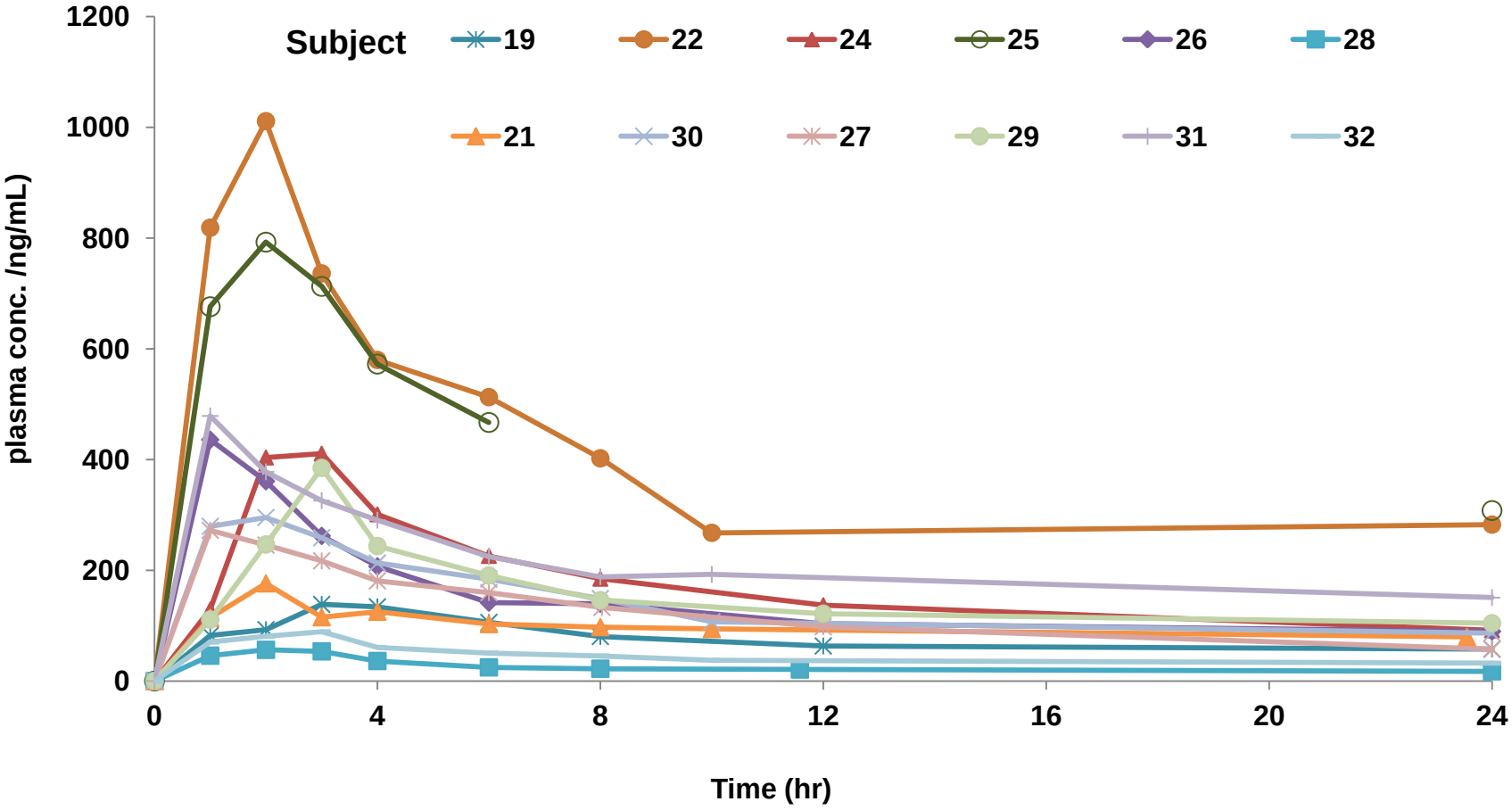

FARMACO
ASSORBIMENTO
DISTRIBUZIONE
TRASPORTO
LEGAME ALLE PROTEINE
METABOLISMO
GENETICA
GENETICA
RECETTORI
METABOLITI ATTIVI
METABOLITI INATTIVI
TRASLAZIONE
EFFETTO

VARIABILITY OF PLASMA LEVELS IN PATIENTS AFTER ORAL 30-35 mg/m^2 OF 4 DEMETHOXY DAUNORUBICIN

PATIENT NUMBER	AUC ng/ml x h
1	131
2	64
3	905
4	414
5	612
6	203
7	110
8	43
9	90

ZANETTE et al., 1990

INHIBITOR OF FGFR-1 and VEGF 1-3 (30mg oral)



Zucchetti et al., 2012

ETEROGENEITÀ NELLA DISTRIBUZIONE DEL FARMACO

Tumour concentration: time 4 hr after treatment on day five

TUMOUR TYPE	Tumor concentration (ng/g)	Mean (ng/g)	SD (ng/g)	*Ratio (T/Pl)
HEC1A (endometrial FGFR2 wt)	2587.7 2623.9 1349.9	**2187.2**	725.3	2.9
MNK45 (gastric FGFR2 wt)	1038.3 809.4 1180.8	**1009.5**	187.4	1.2
AN3CA (endometrial FGFR2 +)	2772.8 2135.3 2425.0	**2444.4**	319.2	2.5
MFE296 (endometrial FGFR2 +)	1718.0 1775.8 1519.6	**1671.1**	134.4	1.8
SNU16 (gastric FGFR2 +)	2828.8 4380.3 3481.0	**3563.4**	779.0	6.2

* Ratio: tumor concentration/plasma concentration

INHIBITOR OF FGF-1 and VEGF 1-3

Zucchetti et al., 2012

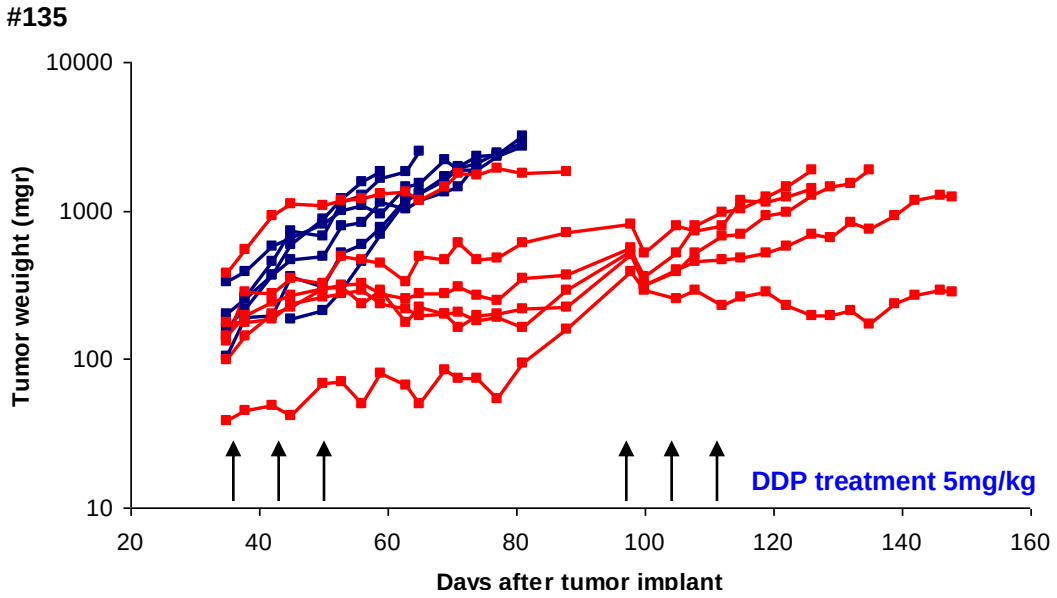
Levels of ADM, DM, CPA, MNU, etoposide, and teniposide in intramuscular primary 3LL and is pulmonary metastases

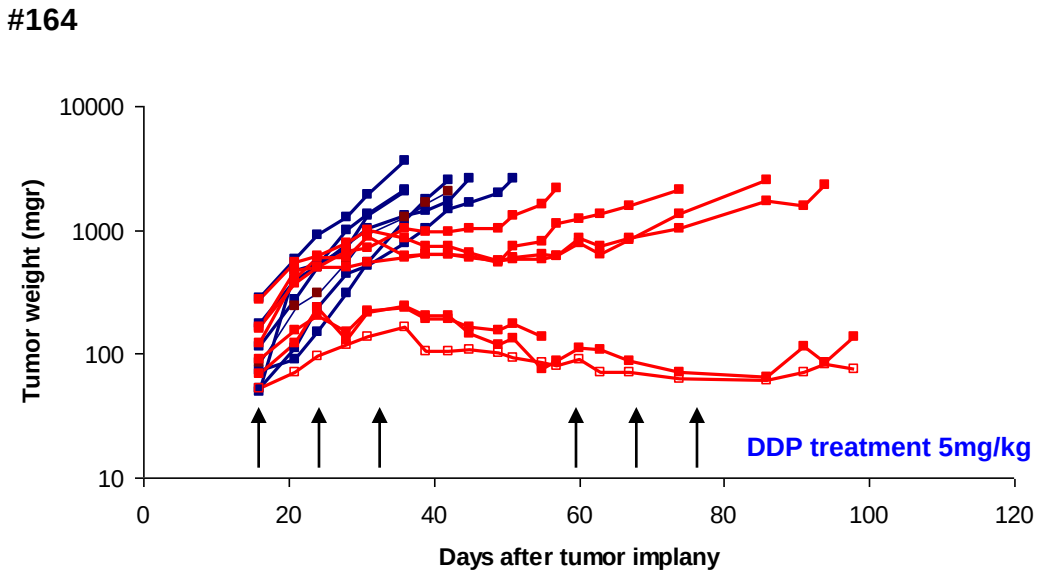
	Primary 3LL		Lung metastases	
	Cmax (µg/g)	AUC (µg/g x mins)	Cmax (µg/g)	AUC (µg/g x mins)
ADM	3.8 ± 0.3 (3 hrs)	3633 ± 365 (0-24 hrs)	16.6 ± 2.2 (5 mins)	11576 ± 1411 (0-24 hrs)
DM	2.9 ± 0.5 (3 hrs)	3515 ± 597 (0-24 hrs)	30.2 ± 2.1 (30 mins)	19286 ± 2236 (0-24 hrs)
CPA	9.7 ± 4.0 (15 mins)	1349 ± 579 (0-24 hrs)	66.4 ± 9.7 (5 mins)	1652 ± 172 (0-24 hrs)
HDU	95.2 ± 3.8 (3 hrs)	3122 ± 339 (0-60 mins)	233.1 ± 12.3 (1 mins)	11681 ± 528 (0-60 mins)
MNU	2.5 ± 0.5 (5 mins)	102 ± 13 (0-60 mins)	12.2 ± 0.6 (1 mins)	159 ± 14 (0-60 mins)
Etoposide	3.3 ± 0.5 (15 mins)	606 ± 20 (0-6 hrs)	10.9 ± 0.6 (1 mins)	1338 ± 318 (0-6 hrs)
Teniposide	6.1 ± 10 (15 mins)	999 ± 141 (0-6 hrs)	25.1 ± 5.0 (5 mins)	2692 ± 310 (0-6 hrs)

DONELLI et al., 1984

ETEROGENEITA' NELLA RISPOSTA AI FARMACI

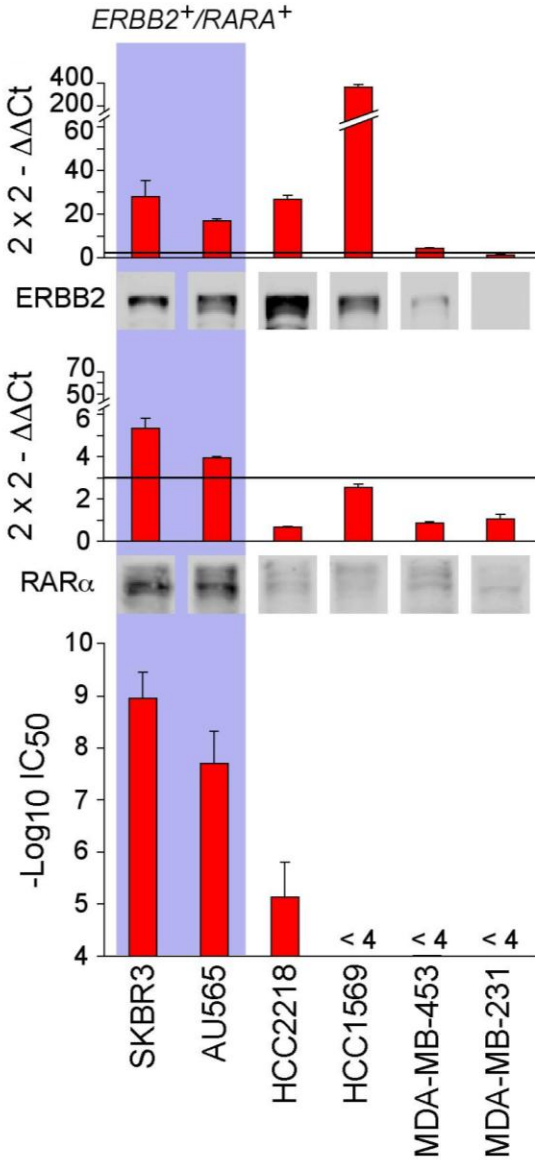
Does tumor heterogeneity influence response to treatment?





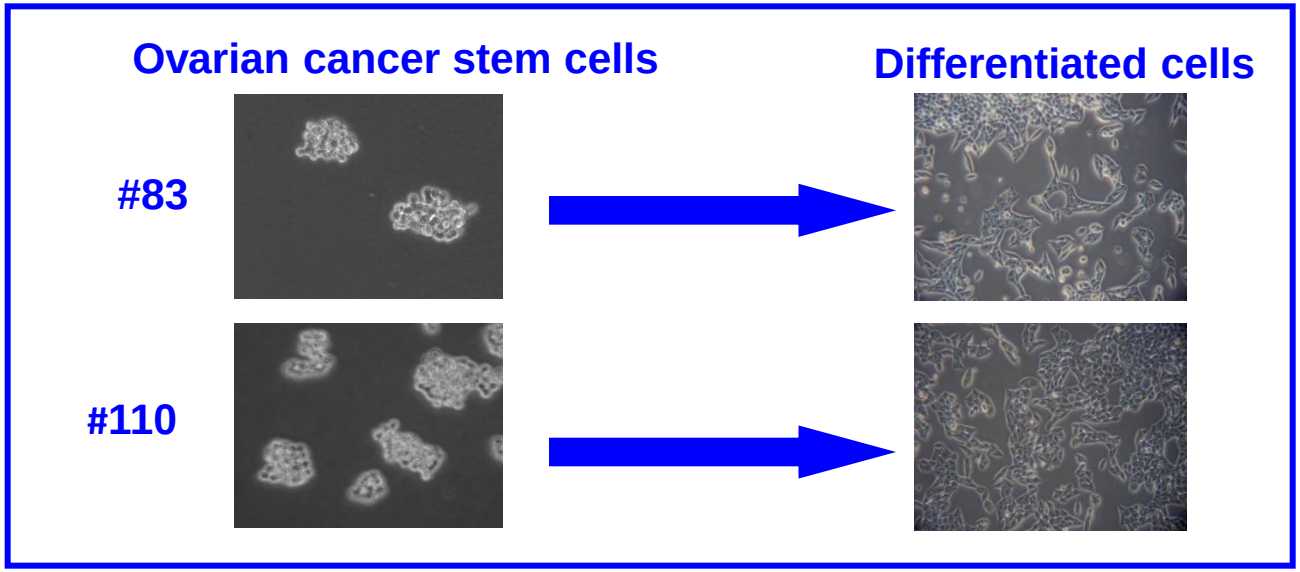
In strictly controlled experimental conditions, nude mice transplated with randomly selected s.c fragments of the same ovarian tumor respond in different way to therapy

The co-amplification of HER2 e RARα sensitizes ER$^-$ neoplastic cells to combinations of retinoic acid and lapatinib: an example of personalized therapy ?

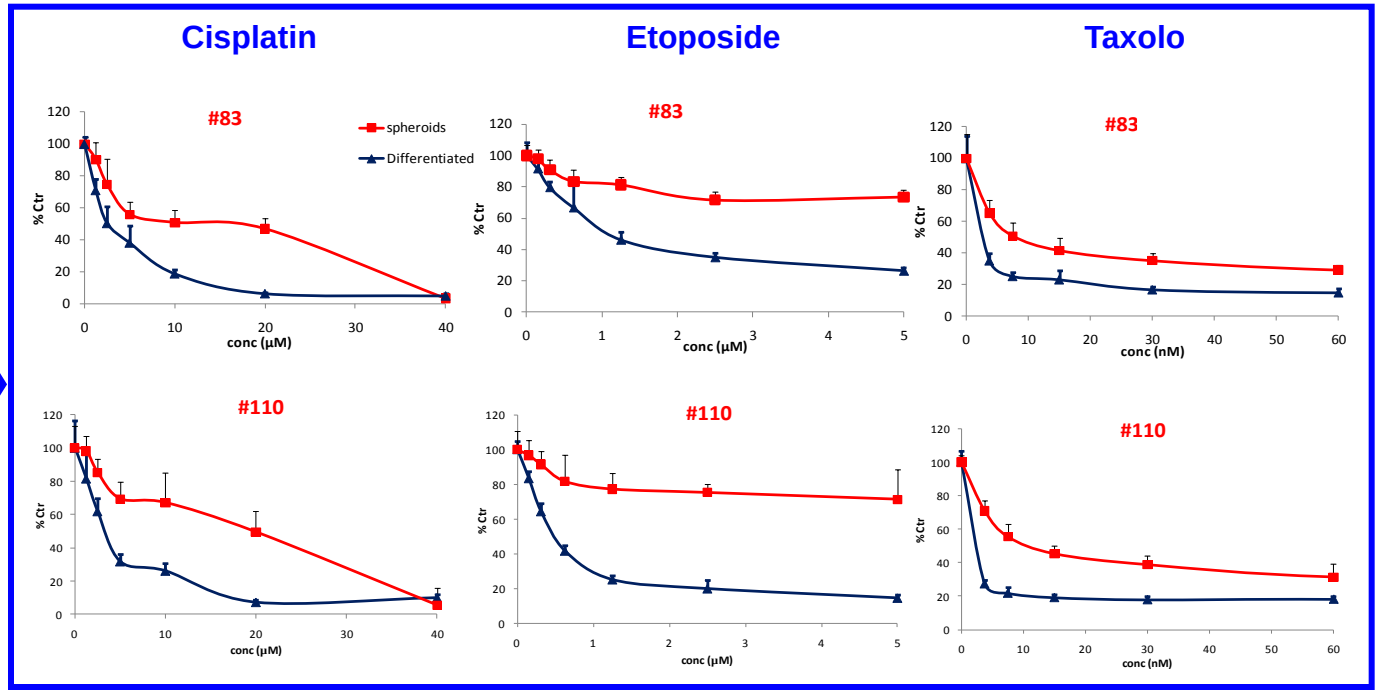


E. Garattini et al., 2012

Does tumor heterogeneity influence response to treatment?

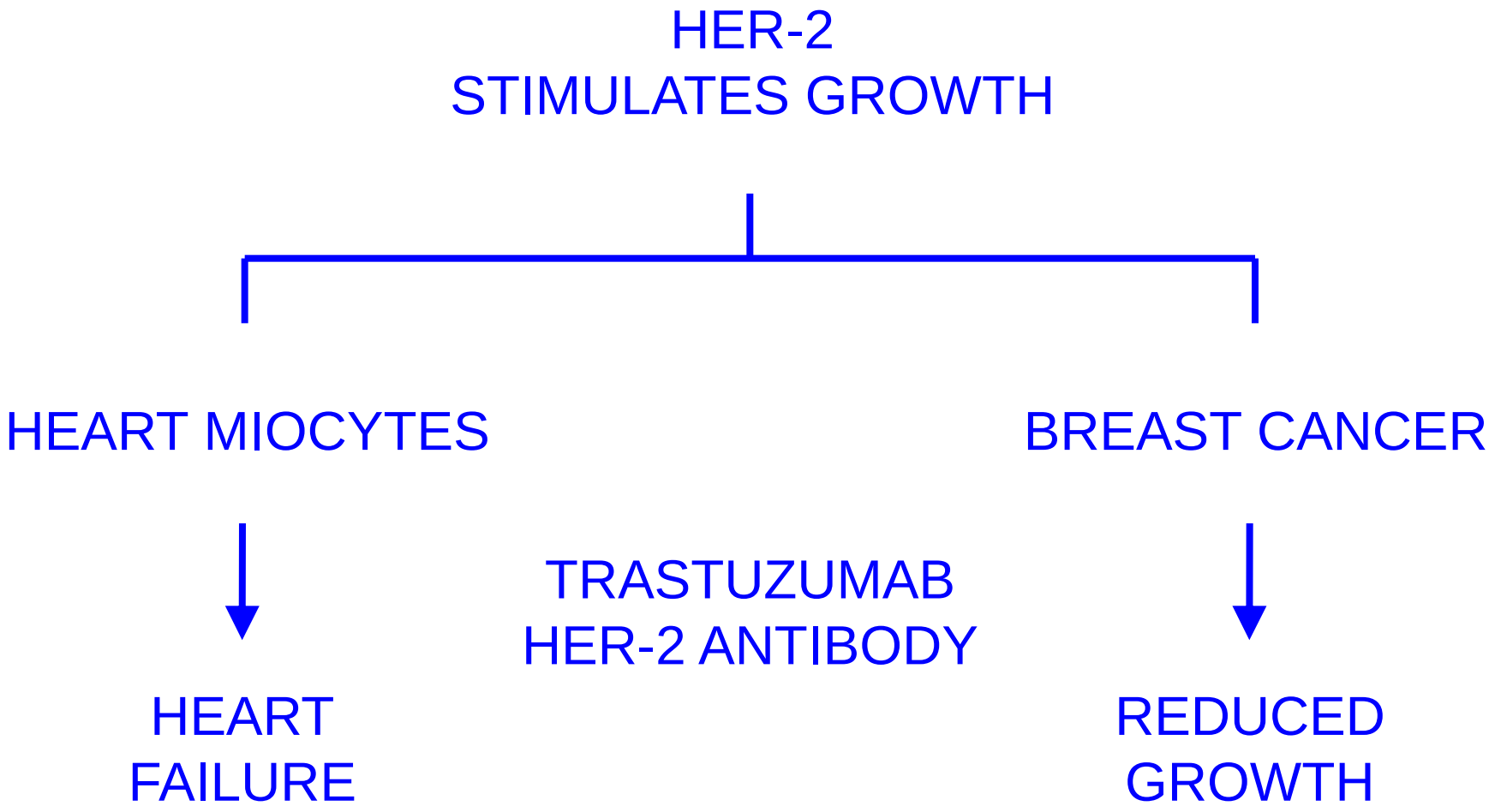


Ovarian cancer stem cells (red line) are more resistant than cancer non-stem cells to different drugs (blu line)



Ricci F et al; Cell Cycle. 2012 May 15;11(10):1966-76.

ETEROGENEITA' NELLA TOSSICITA' DEI FARMACI

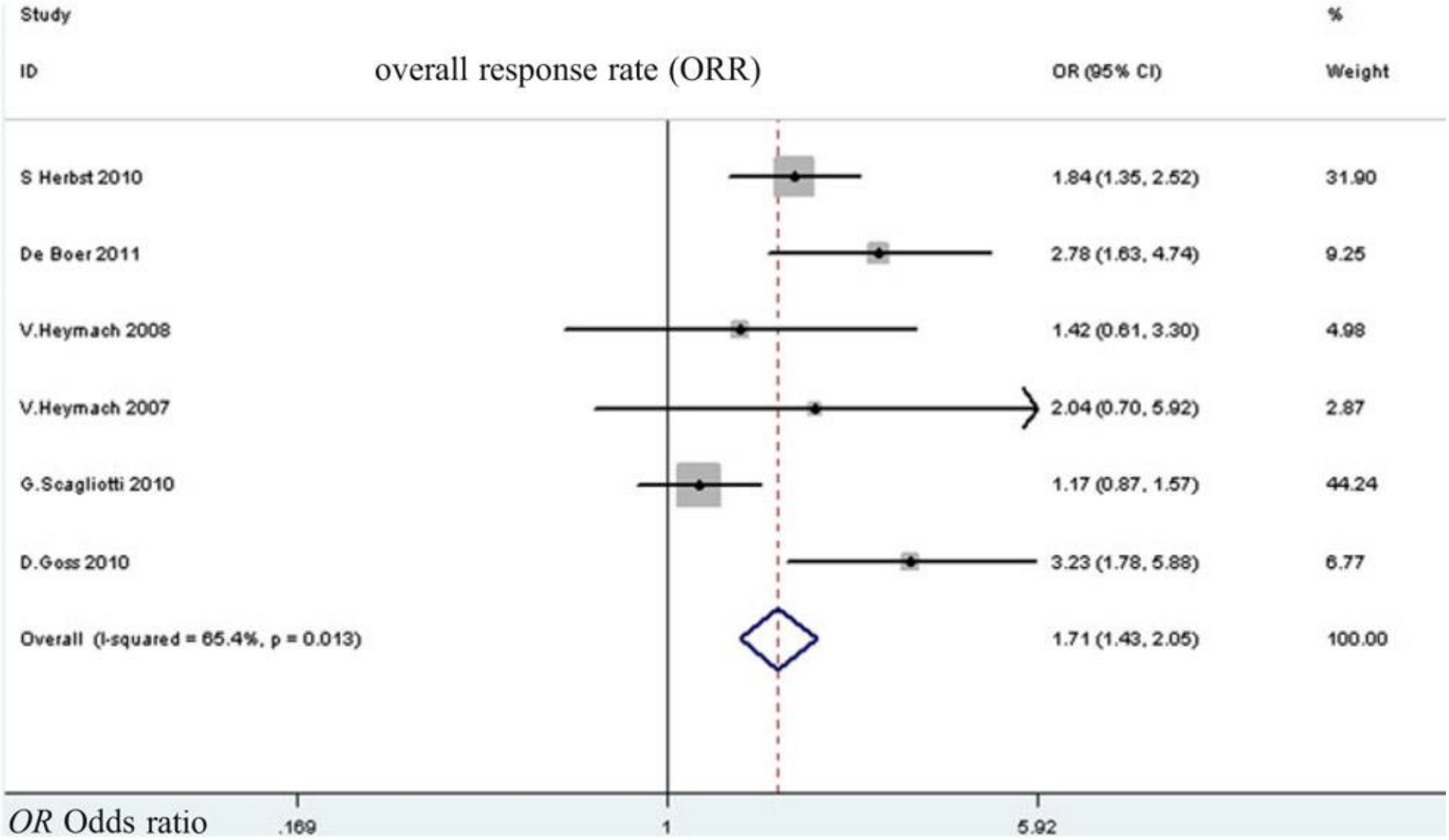

HER-2
STIMULATES GROWTH
HEART MIOCYTES
BREAST CANCER
TRASTUZUMAB
HER-2 ANTIBODY
HEART
FAILURE
REDUCED
GROWTH

Treatment-Related Toxic Effects.

Effect	Paclitaxel plus Bevacizumab (N=365)		Paclitaxel (N=346)		P Value
	Grade 3	Grade 4	Grade 3	Grade 4	
	percent				
Infection	8.8	0.5	2.9	0	<0.001
Fatigue	8.8	0.3	4.6	0.3	0.04
Sensory neuropathy	23.0	0.5	17.1	0.6	0.05
Hypertension	14.5	0.3	0	0	<0.001
Thrombosis or embolism	1.6	0.5	0.6	0.9	
Cerebrovascular ischemia	0.8	1.1	0	0	0.02
Headache	2.2	0	0	0	0.008
Proteinuria	2.7	0.8	0	0	<0.001

Miller et al., 2007

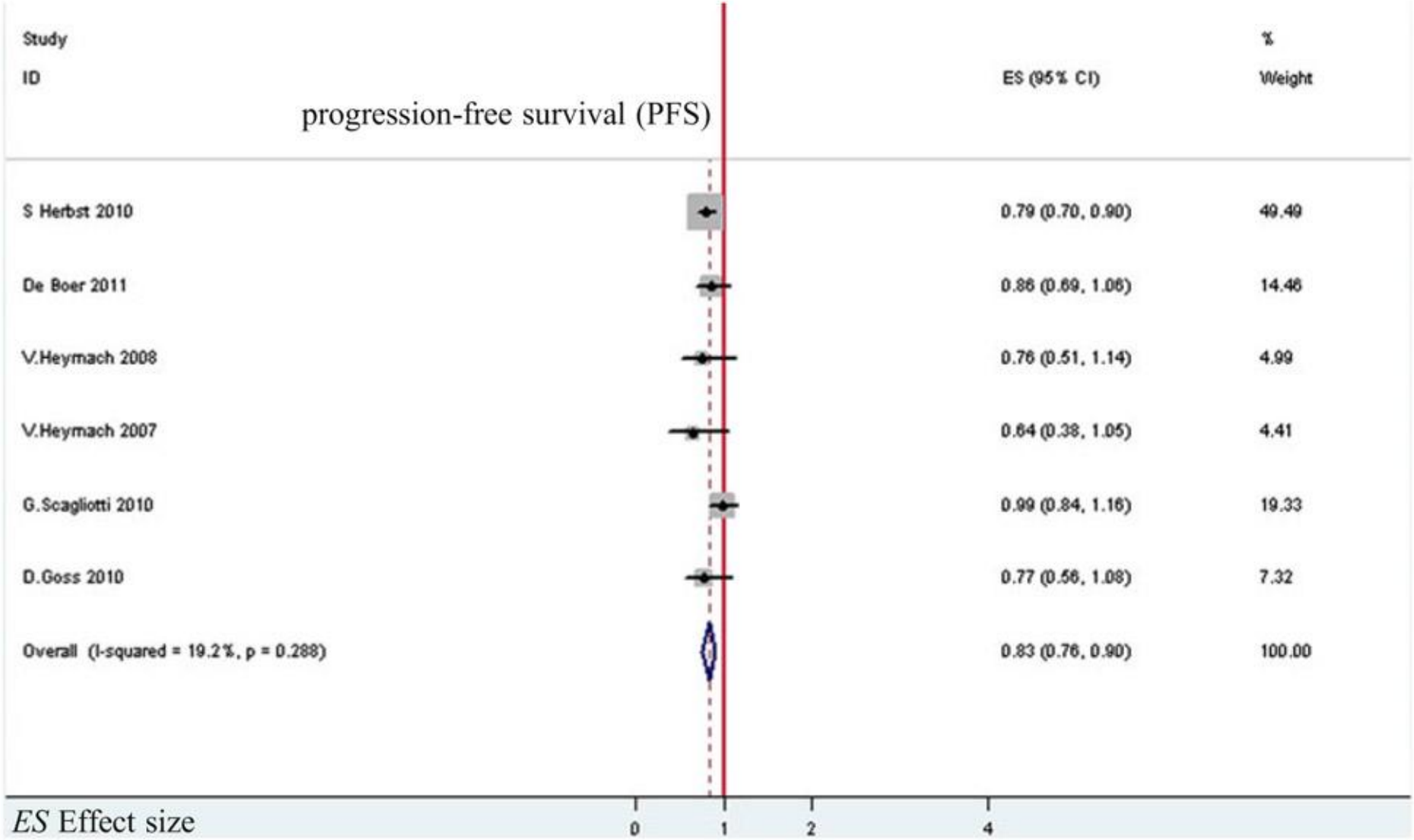
NSCLC and TKI



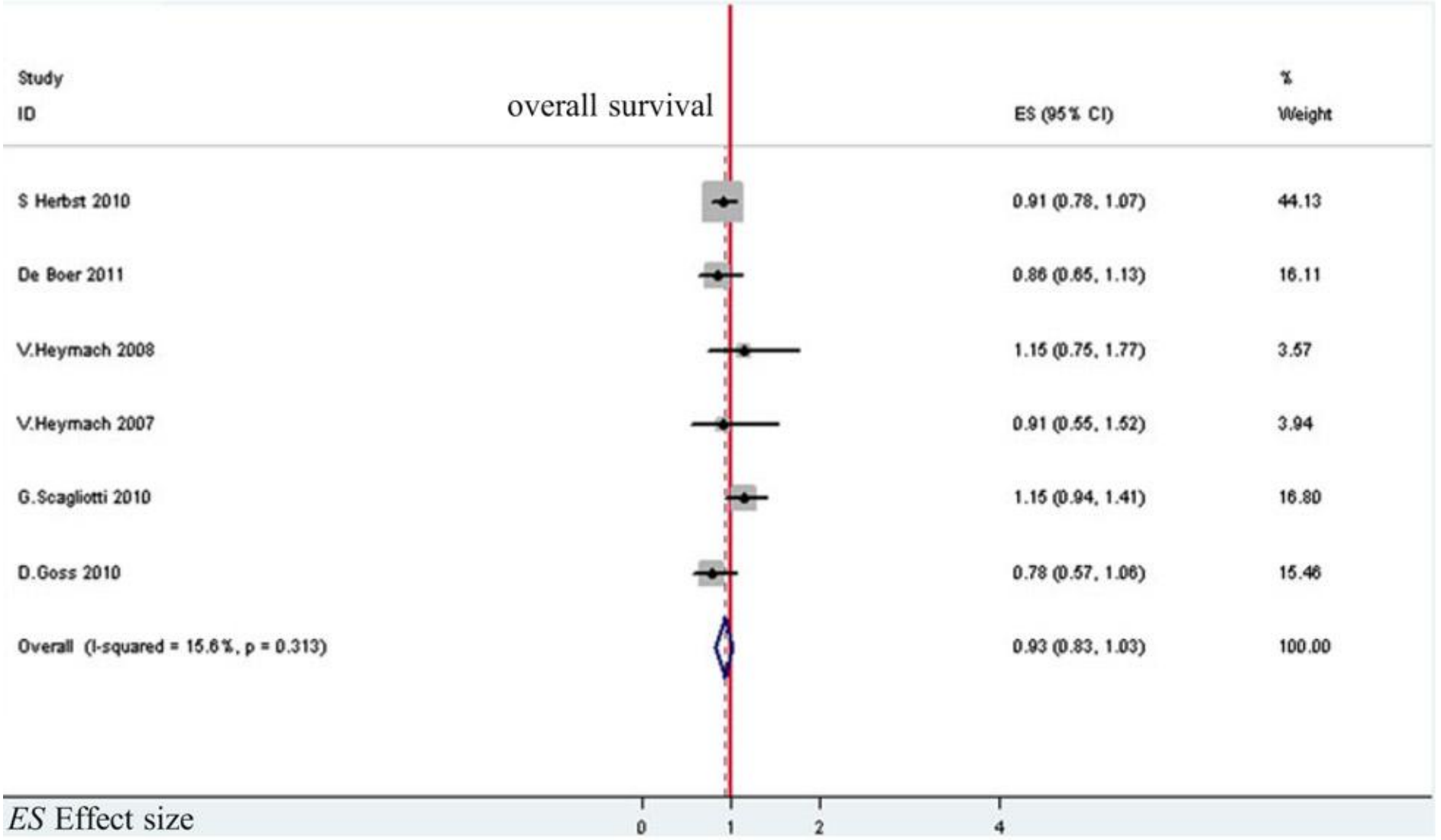
XIAO et al., 2012

patients with advanced non-small-cell lung cancer (NSCLC)

chemotherapy plus multitargeted antiangiogenic tyrosine kinase inhibitors (TKI)



XIAO et al., 2012



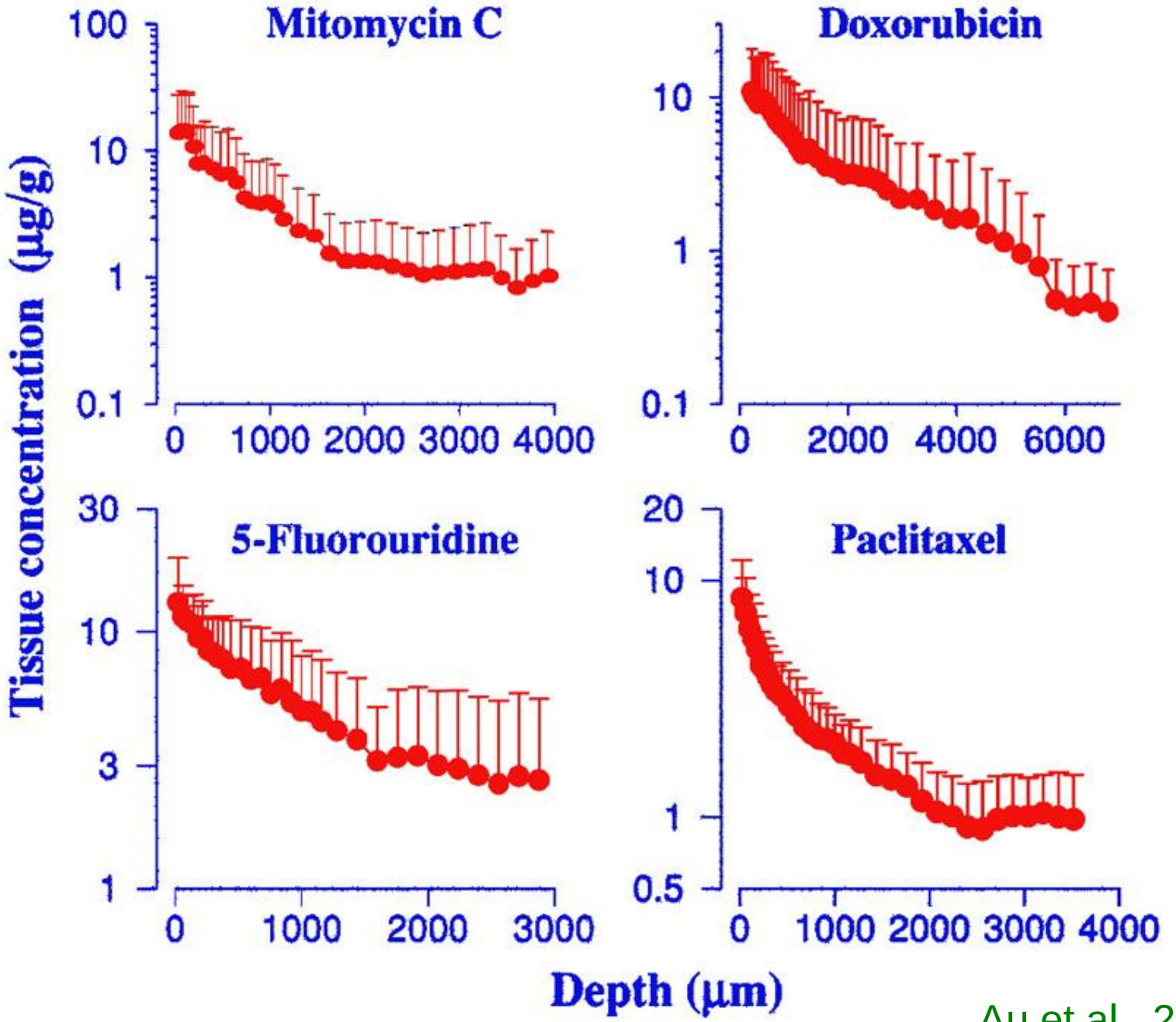
XIAO et al., 2012

ETEROGENEITA' NELLA DISTRIBUZIONE DEI FARMACI NEL SINGOLO TUMORE

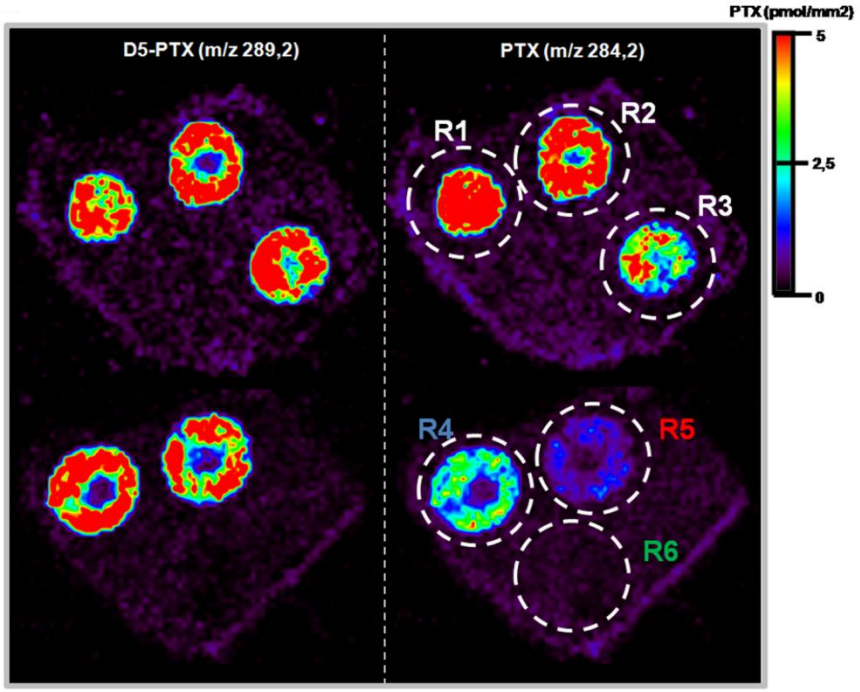
	DOXORUBICIN **	CYCLOPHOSPHAMIDE **	MNU **
WHOLE TUMOR	**557 ± 108**	**745 ± 52**	**54 ± 6**
VEGETATING PART	**2745 ± 205**	**2434 ± 386**	**266 ± 12**
NECROTIC PART	**< 1**	**< 0.1**	**< 1**

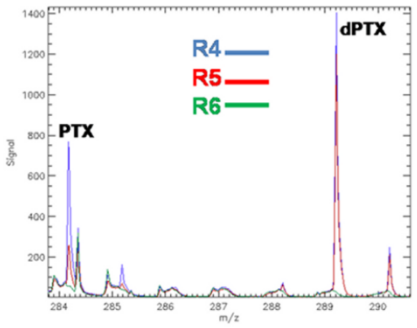
Auc after 60 min*or, 24 hr**

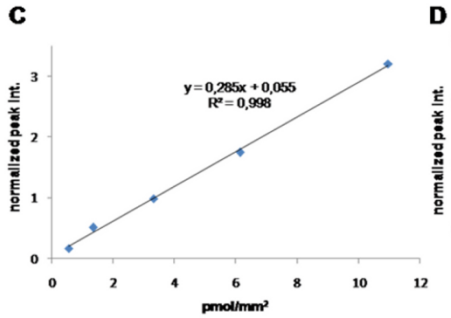
D'Incalci et al., 1977

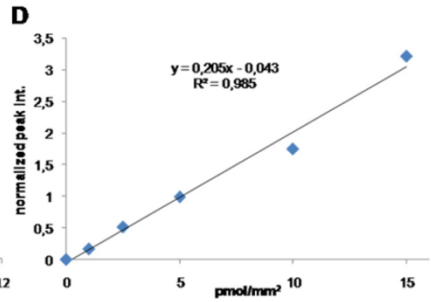

Mitomycin C
Doxorubicin
5-Fluorouridine
Paclitaxel
Tissue concentration (µg/g)
Depth (µm)
100
10
1
0.1
0
1000
2000
3000
4000
6000
30
3
20
0.5

Au et al., 2001









Quantitative MALDI imaging

A calibration curve is built directly on tissue

(A) with stable isotope labelling

(B) of the drug (PTX)

(C) control tissue (liver)

(D) cancer tissue (melanoma)

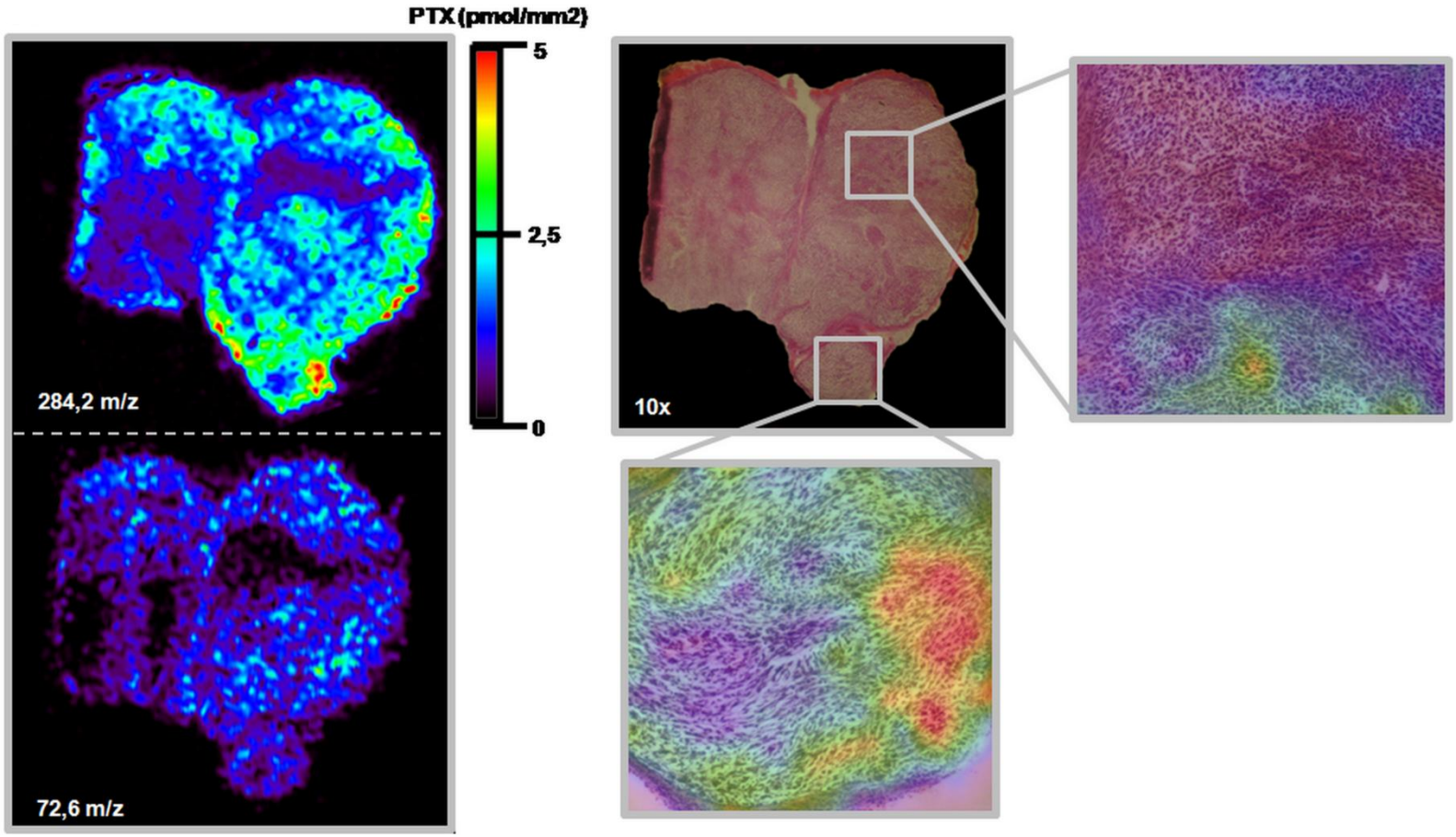
Overlapping MS, MS/MS and HE images of three adjacent slices of melanoma

treated with PTX 60 mg/kg **.**

A) MS distribution in treated melanoma reflects MS/MS

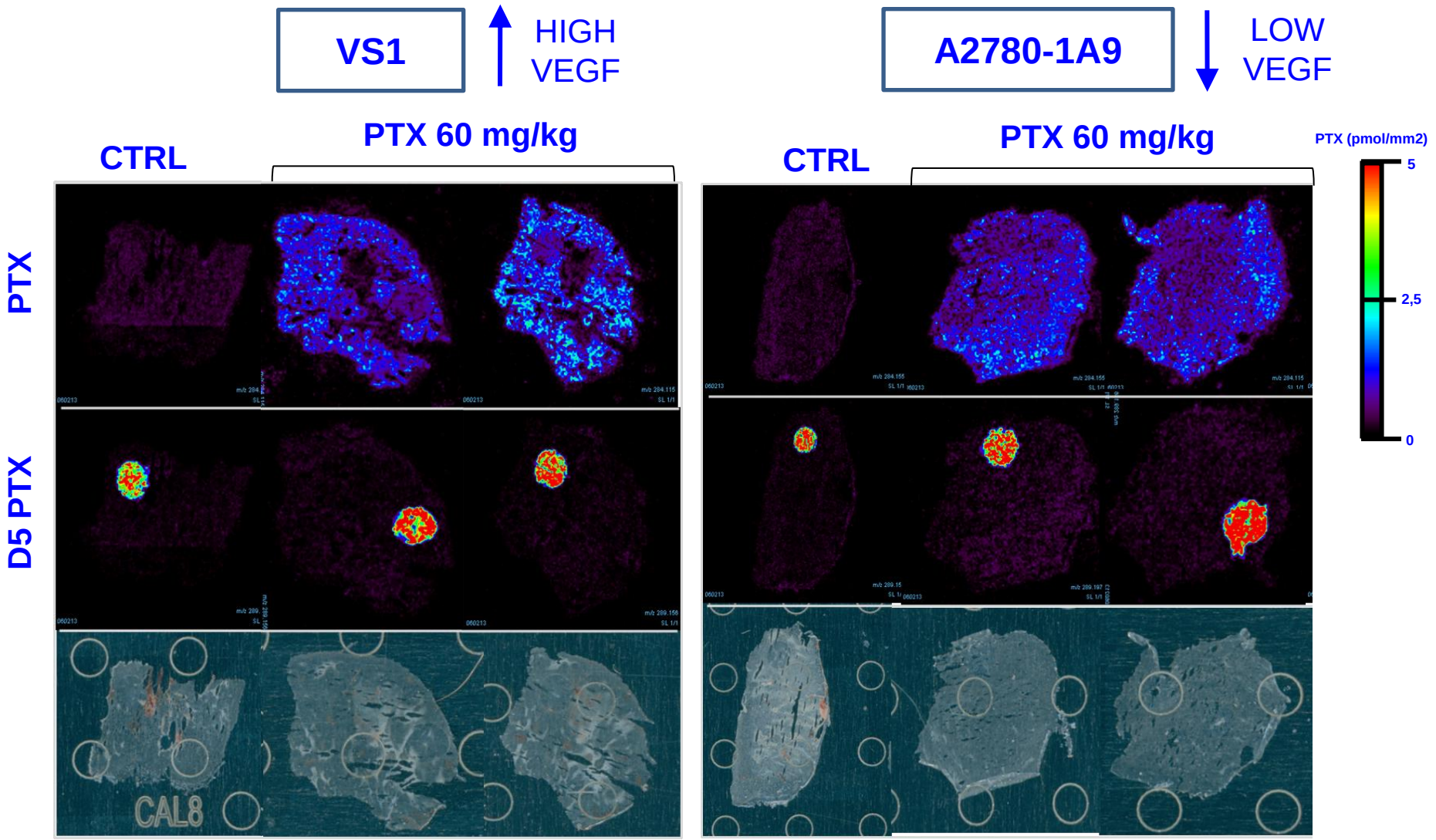
B) HE stained optical image shows that the cell compartment is uniform. The enlargements show superimposed histological and molecular data.

No differences in tissue histology can be seen in the areas where the drug is more concentrated.



Davoli et al., 2013

Drug distribution in ovarian tumor xenografts



Davoli et al., 2013

CONCLUSIONI

- L'ETEROGENEITÀ SI ARTICOLA NELL'OSPITE, NEL TUMORE E NEI FARMACI
- L'ETEROGENEITÀ È UN ELEMENTO FONDAMENTALE PER LO SVILUPPO DELLA RESISTENZA
- L'ETEROGENEITÀ È IL MAGGIOR OSTACOLO ALLA TERAPIA
- L'ETEROGENEITÀ RICHIEDE LO SVILUPPO DI NUOVE STRATEGIE TERAPEUTICHE

Capacities – Research Infrastructures
FP7-INFRASTRUCTURES-2011-1.1.5
ECRIN-PA

SOCIETA' ITALIANA DI FARMACIA
OSPEDALIERA E DEI SERVIZI FARMACEUTICI
DELLE AZIENDE SANITARIE

XXXV CONGRESSO NAZIONALE SIFO

IL FARMACISTA: UNA RISORSA PER LA SALUTE.

RESPONSABILITA', APPROPRIATEZZA, SOSTENIBILITA'

SEVENTH FRAMEWORK PROGRAMME