Gene signature and response to chemotherapy in breast cancer : statistical artefact or reality ?

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Introduction (1)

- **Adjuvant chemotherapy** improves overall survival in breast cancer
- Newer chemotherapy **regimens containing taxanes** further improve survival compared to standard regimens but

  - are more toxic and more expensive

  - may benefit only a subgroup of patients
- Identification of **regimen-specific predictive factors or signatures** is a research priority.

Dowsett et al *Breast Cancer Res* (in press)

Introduction (2)

Trials aiming to identify predictive signatures

- **Neoadjuvant** chemotherapy
- **Clinical or pathological complete response** : surrogate for treatment efficacy
- Methods: **cDNA arrays** or quantitative RT-PCR
- Biostatistic: classic **supervised** methods on a test set of patients tumour samples.

Summary of these trials

Study	N	Chemo	Surrogate	Signature
Chang et al *(Lancet 2003)*	30	Docetaxel	Clinical resp	92 genes
Ayers et al *(JCO 2004)*	42	P -> FAC	pCR	74 genes
Iwao-Koizumi et al *(JCO 2005)*	70	Docetaxel	Clinical resp	85 genes
Hannemann et al *(JCO 2005)*	46	AC or ADx6	pCR	No
Gianni et al *(JCO 2005)*	171	AP -> w P	pCR	86 genes
Hess et al *(JCO 2006)*	133	P -> FAC	pCR	30 probes
Thuerigen et al *(JCO 2006)*	52	GE-D/GED	pCR	512 genes
Cleator et al *(BCRT 2006)*	40	AC	pCR	253 genes
Makris et al *(ASCO 2007)*	60	AC	pCR	82 genes

Promissing results
but 3 limitations:

- **Overfitting**

- small test sets
- small validation sets
- external validation ?

- **Cell type biais**

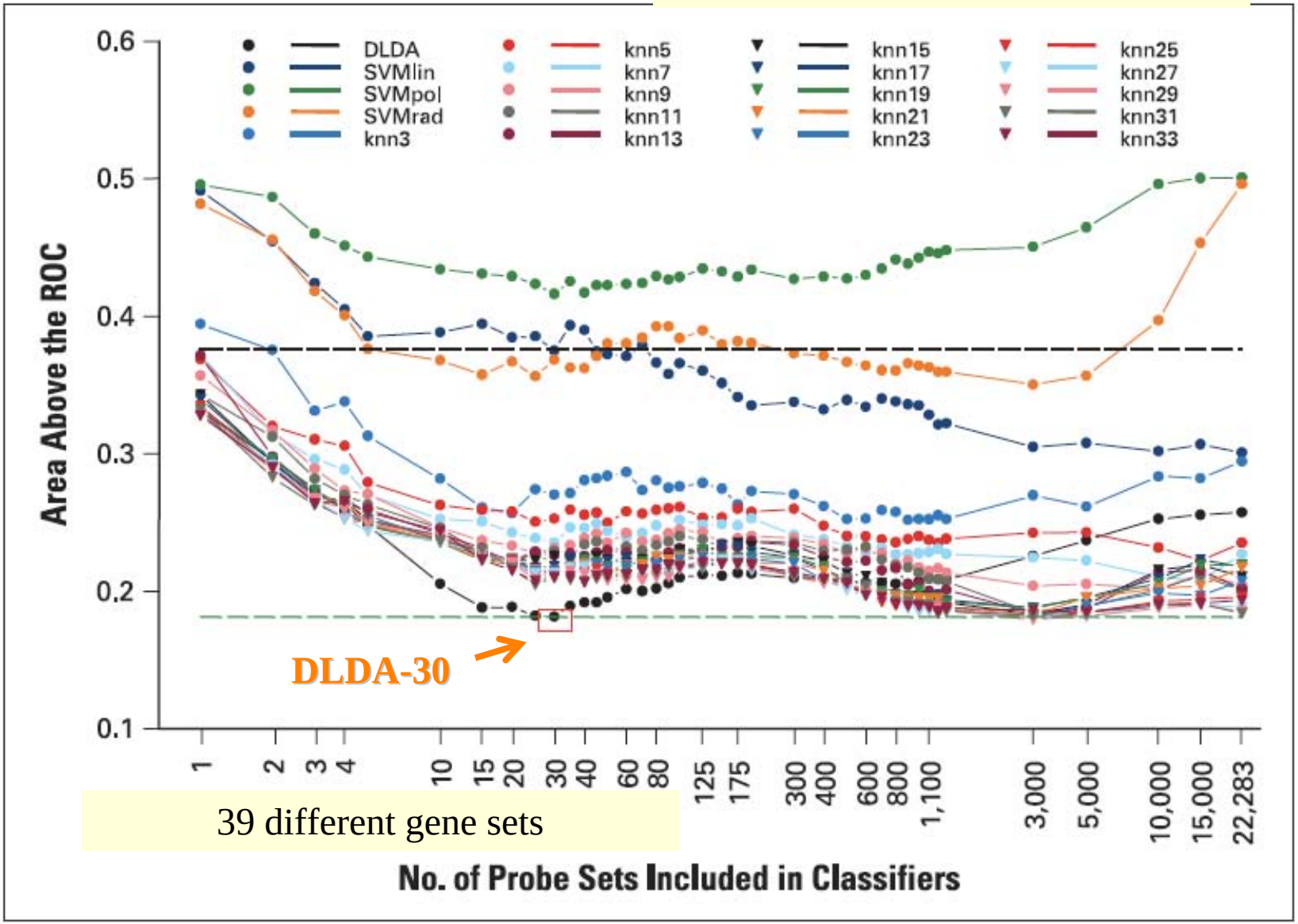
- ER pos and ER neg breast cancers

- **Single arm studies**

- prognostic or predictive signatures?

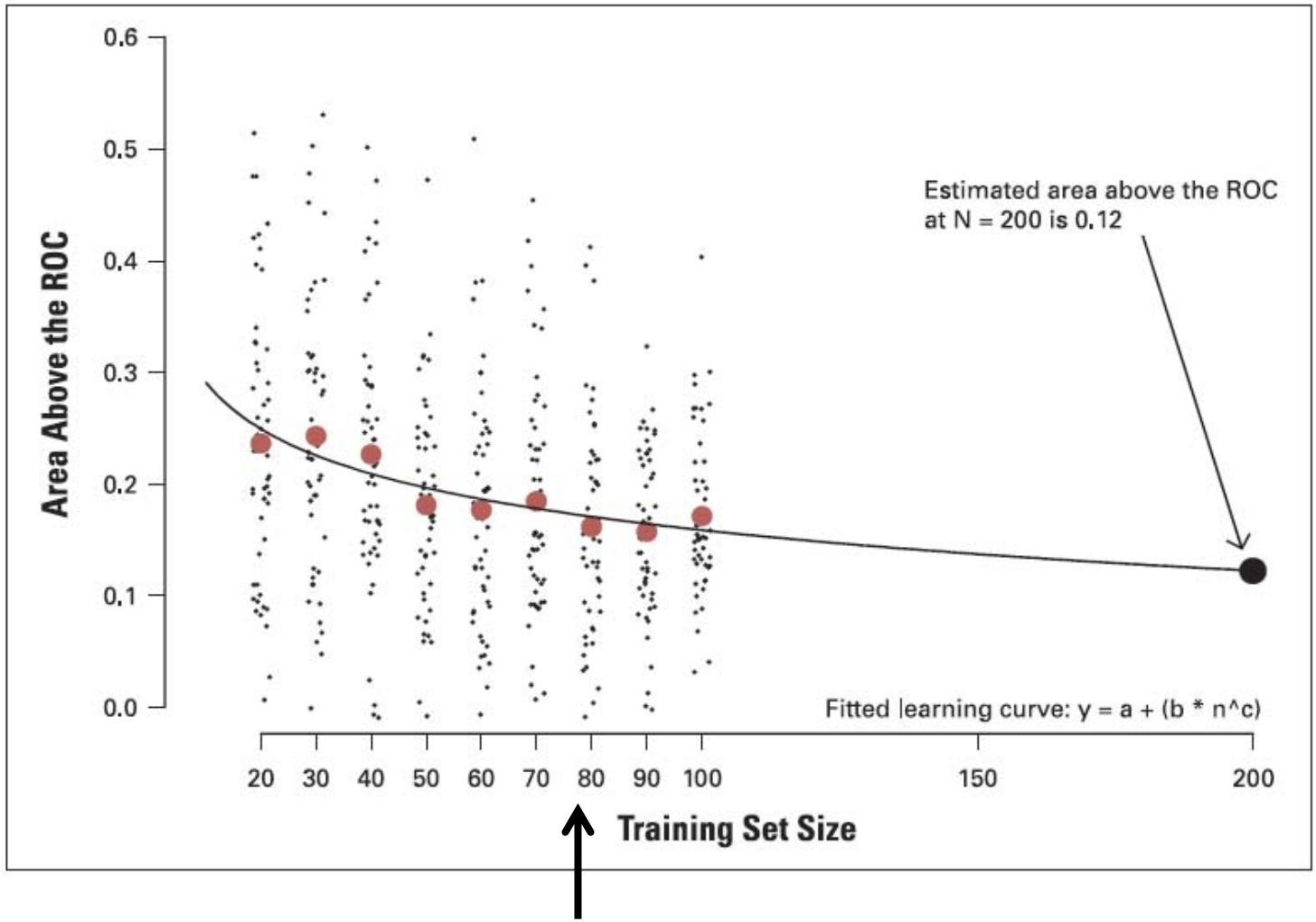
Performance (AAC) of 780 different class predictors

20 different classification algorithms



39 different gene sets

Performance (AAC) of DLDA-30 and size of the training set



Only a modest improvement of AAC when increasing the training set

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« Cell type biais »

- In microarrays studies ER status is the dominant factor (luminal versus basal)

Perou et al. Nature 2000

- ER neg tumours -> 20-30 pCR%

Bear et al JCO 2003; Colleoni et al. Clin Cancer Res 2004; Ring et al. Br J Cancer 2004; Guarneri et al 2006

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Previous studies: limitations

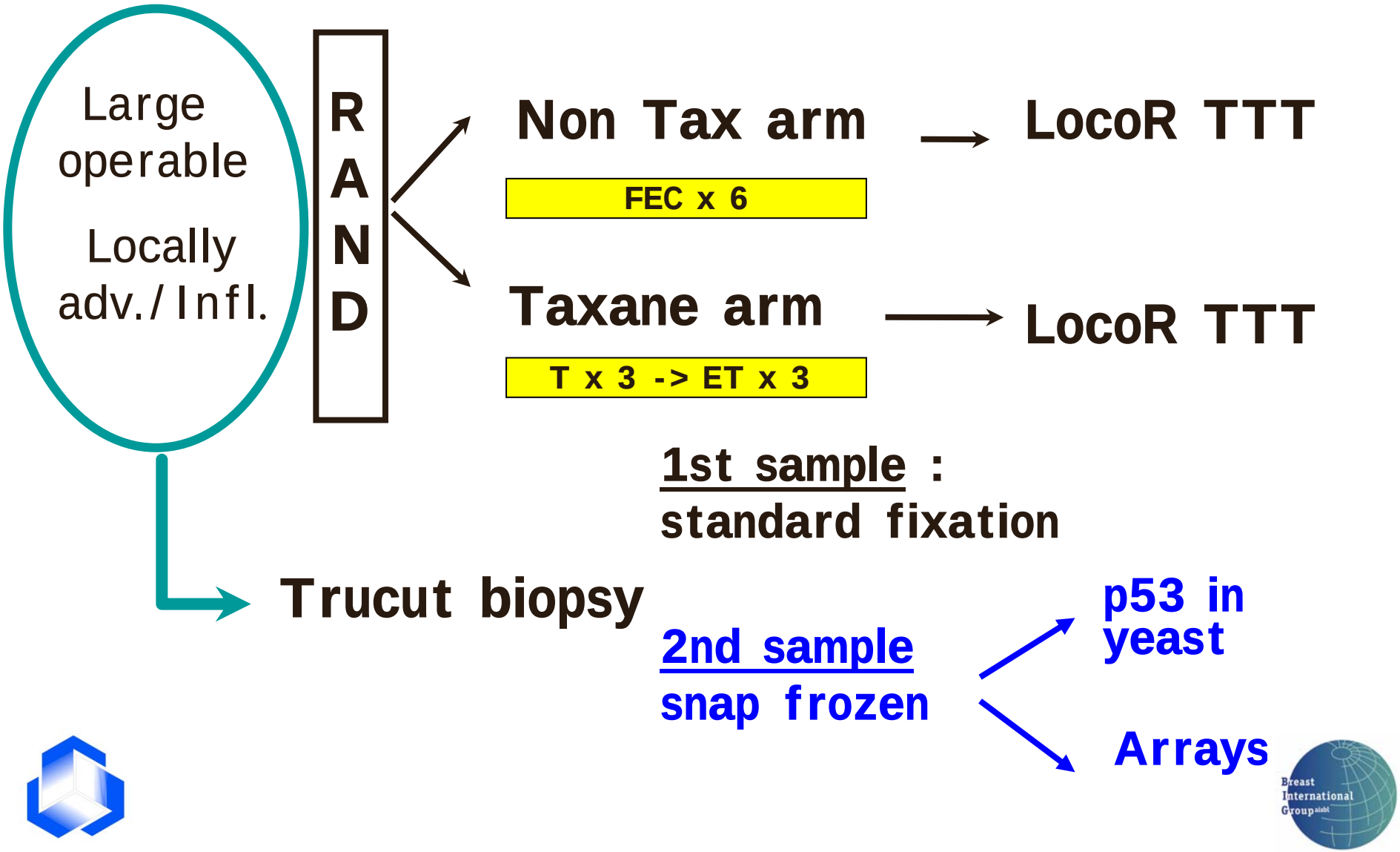
- **Overfitting**
  - small test sets
  - small validation sets
  - external validation ?
- **Cell type biais**
  - ER pos and ER neg breast cancers
- **Single arm studies**
  - prognostic or predictive signatures?

TGIF 2 study: potential advantages

- **Avoid overfitting**
  - test set = cell lines
  - valid. set = tmour samples in patients (external valid. set)
- **ER negative tumours**
- **Randomized trial**

Lancet Oncology 2007 (in press)

EORTC 10994/BIG 00-01 Study design



Methods (1): Patients selection, sample processing

- TGIF 2 study was restricted to cases evaluated at the EORTC Data Centre (1 April 2005), meeting the following criteria:
  - ER tumours negative
  - No major protocol violation
  - Non T4
  - Good quality and quantity of RNA

- Written informed consent
- Pathological complete response = outcome measure
- RNA hybridized to Affymetrix X3P chips

Methods (2

Conventi

Expressi

« in vitro

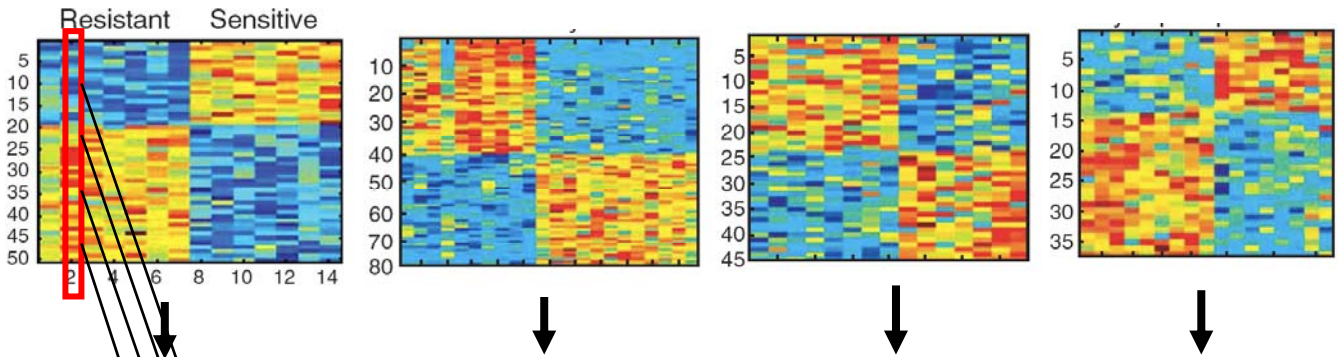
Methods (2): microarray data analysis

1st step: predictors of sensitivity to single agents from *in vitro* data

1. **Selection of cell lines from NCI-60 panel** (IC50, LC50)
2. **Affy express. Data**
3. **Supervised analys. Resist. vs Sensitive**

Predictors of Sensitivity to single agent

Docetaxel | Adriamycin | 5-FU | Cyclophos.

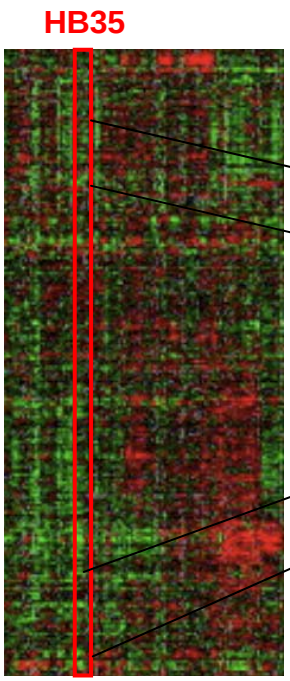


Docetaxel predictor | Adria. predictor | 5-FU predictor | Cyclo. predictor

Gene symbol	Intensities
ASPHD1	-
INPP5A	-
-	-
-	-
Docet. predictor	Score = 0.56

Potti et al *Nature Medicine* 2006

2nd step : from *in vitro* to clinic



Docetaxel predictor

Gene symbol	Intensities
ASPHD1	-
INPP5A	-
-	-
-	-
Docet. predictor	Score = 0.56

Adria. predictor

Gene symbol	Intensities
FAIM3	-
PMP22	-
-	-
-	-
Adria. predictor	Score = 0.09

5-FU predictor

Gene symbol	Intensities
CFL1	-
ATP1A2	-
-	-
-	-
5-FU predictor	Score = 0.20

Cyclo. predictor

Gene symbol	Intensities
RPS9	-
ANP32B	-
-	-
-	-
Cyclo. predictor	Score = 0.31

T->ET predictor

FEC predictor

3rd step : from single agents to multidrug regimens predictors (Duke)

multidrug regimens predictors are built combining probabilities of response to single agents
(theorem for combined probabilities described by William Feller)

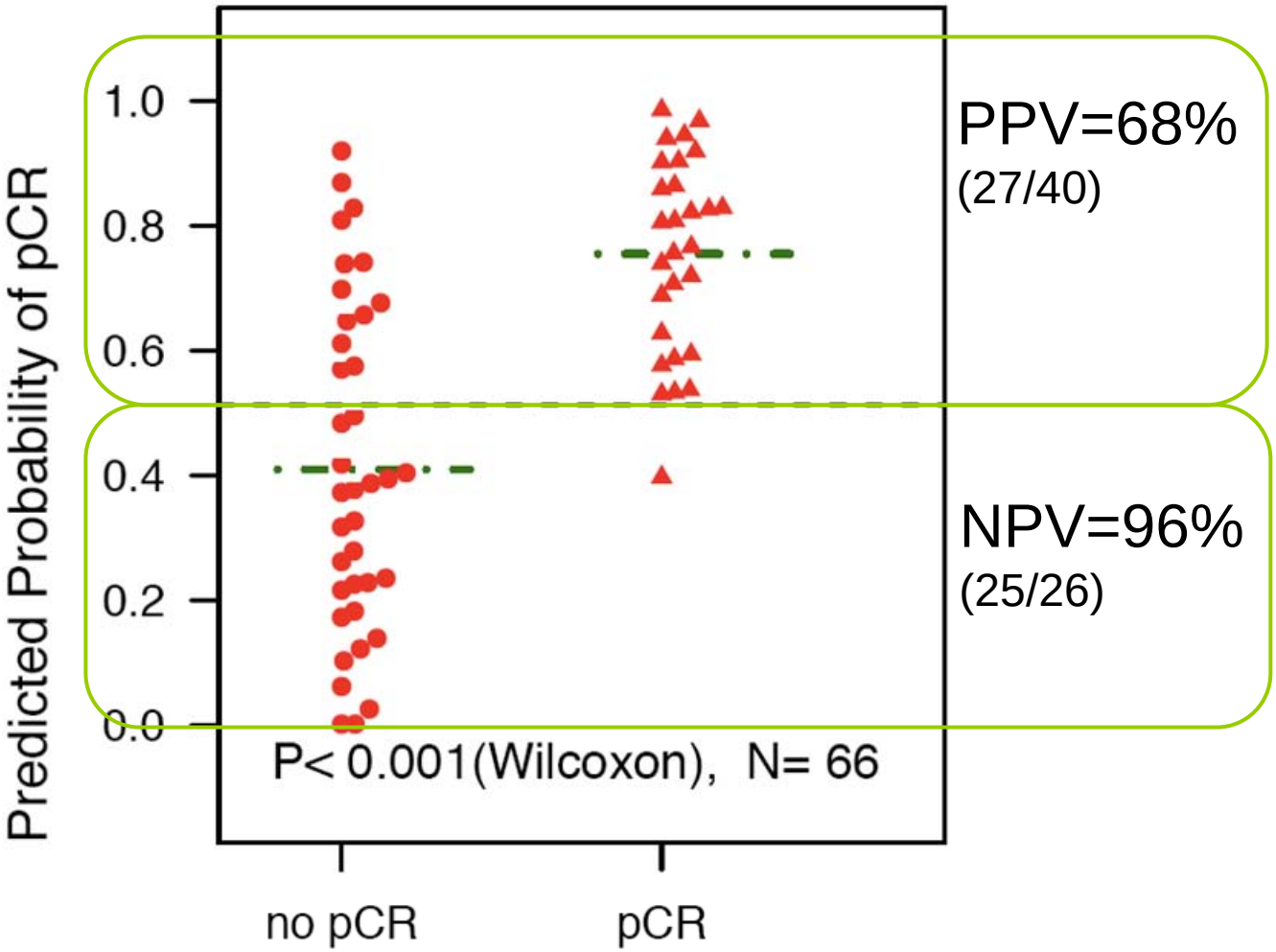
4th step : the statistical significance of the discrimination (pCR vs nonpCR) was assessed by Wilcoxon test

Results (1): Patients and tumour characteristics (n=125)

	FEC arm (n=66)	T->ET arm (n=59)
Age (years)		
Median (range)	49 (26-70)	50 (34-70)
Histology		
Invasive ductal	56	57
Invasive lobular	4	2
TNM stage		
T1*	2	1
T2	38	38
T3	26	20
N0	27	22
N1	34	32
N2	5	5
Grade		
1	2	0
2	21	16
3	32	38
HER2		
amplified	13	20
Response		
pCR	28	27

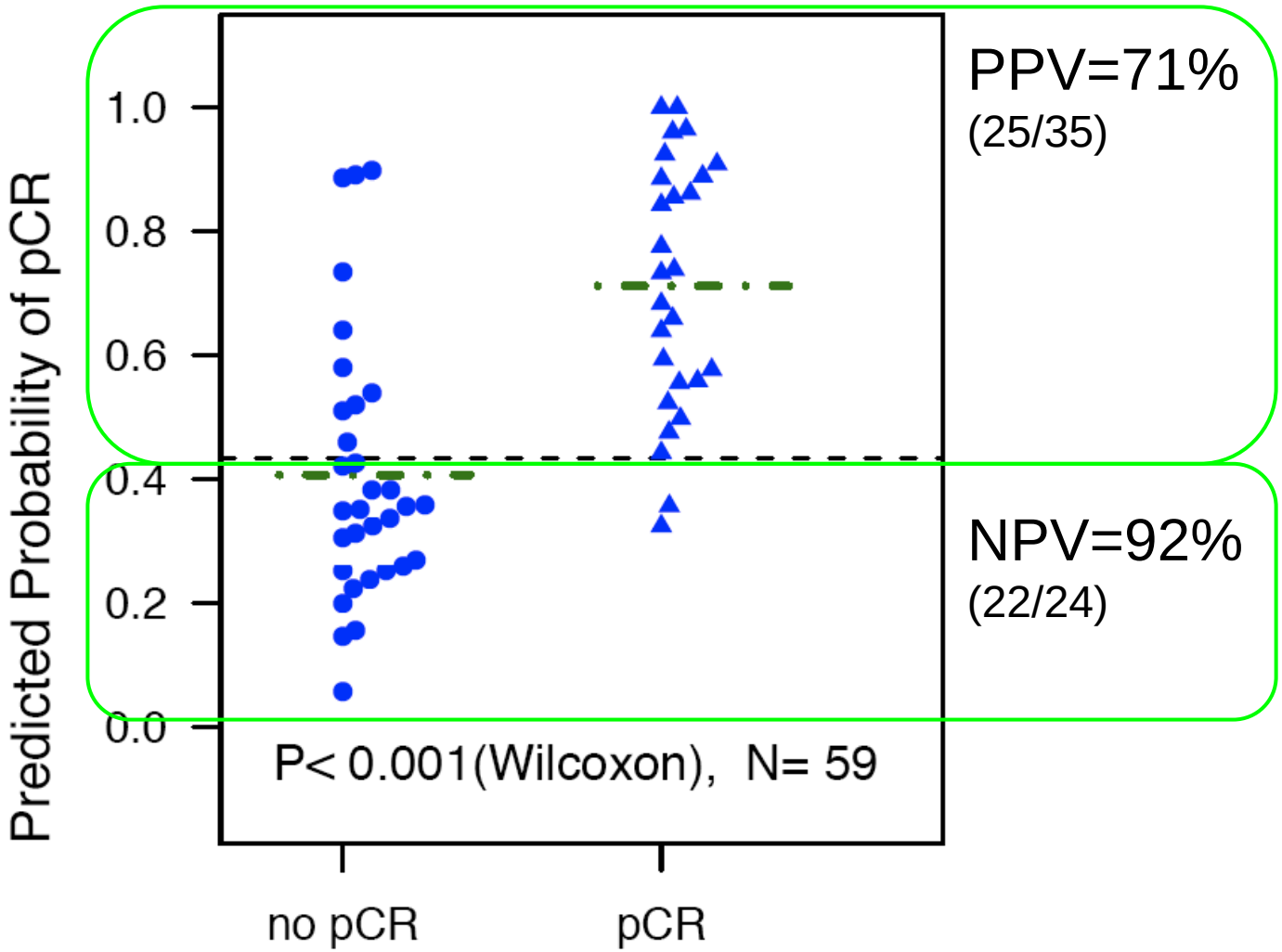
* These 3 cases were cN2

Results (2): Prediction of FEC signature in FEC arm



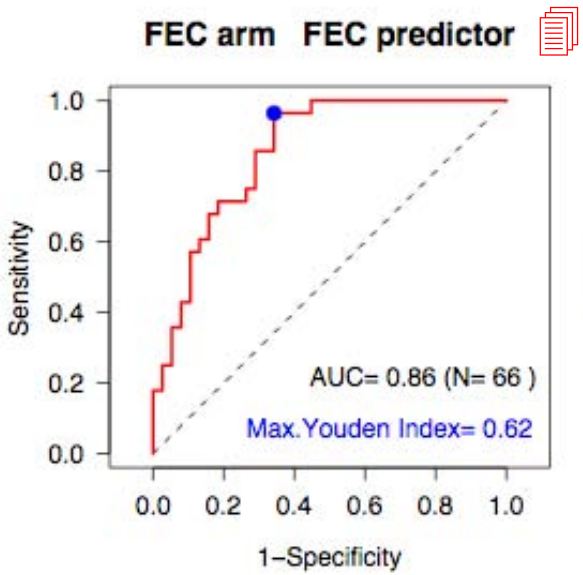
Accuracy	SENS	SPEC	NPV	PPV
79%	96%	66%	96%	68%

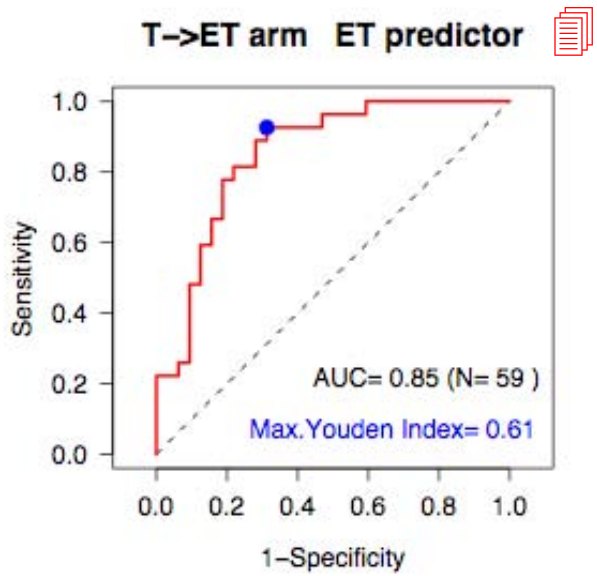
Results (3): Prediction of T->ET signature in T->ET arm



Accuracy	SENS	SPEC	NPV	PPV
80%	93%	69%	92%	71%

Results (4): predictors are regimen specific





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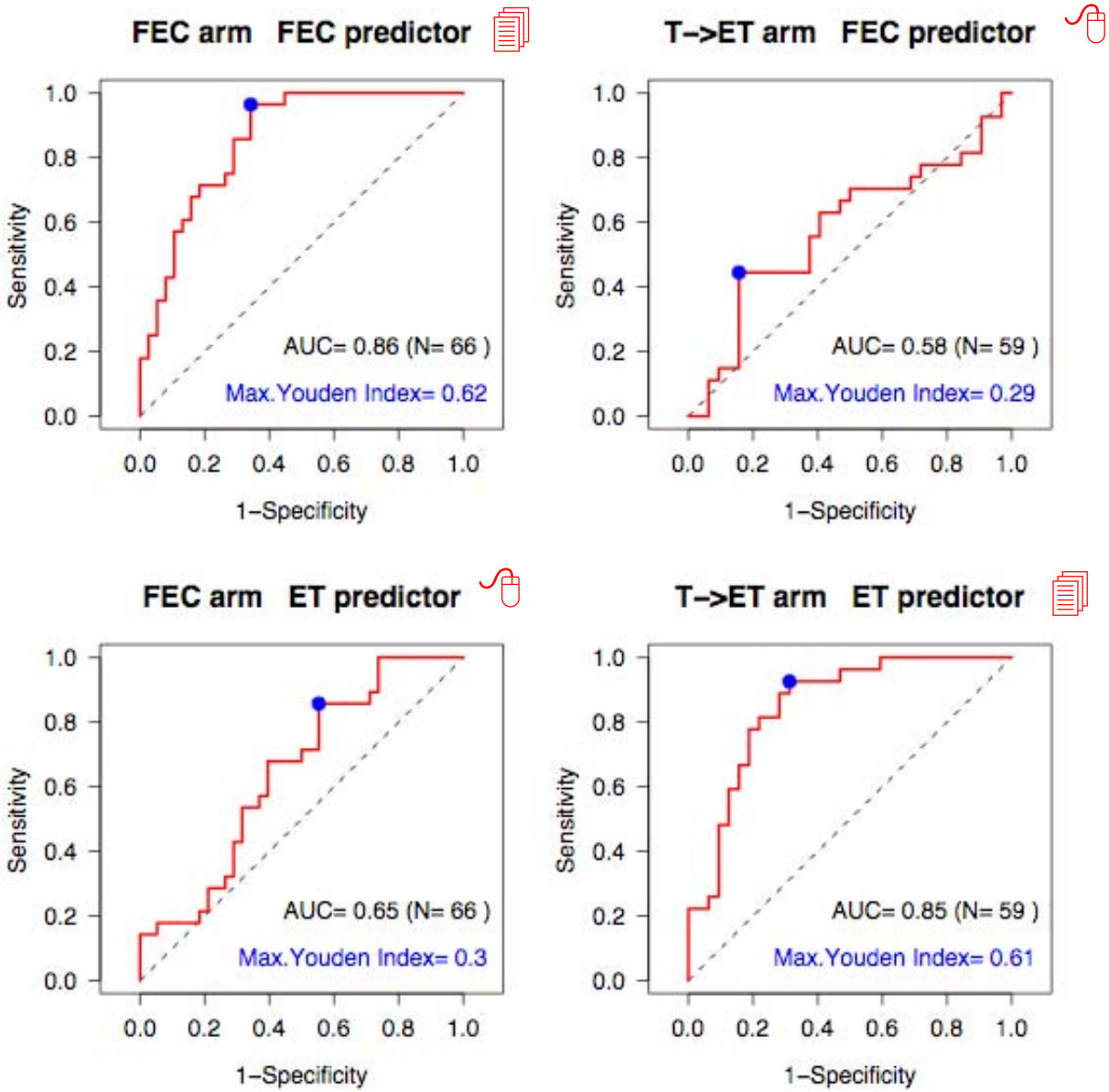
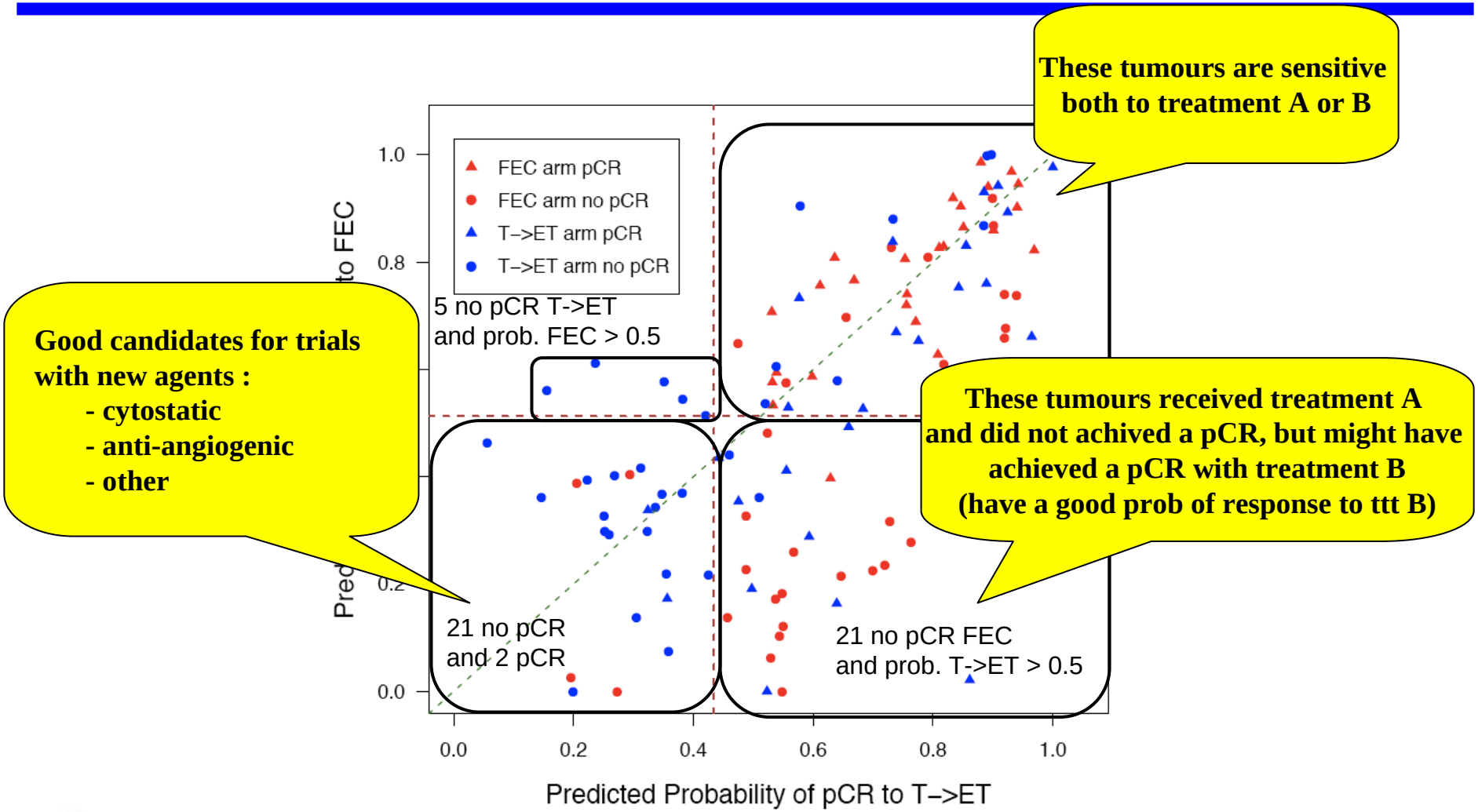


Results (5): plot of the predicted probabilities of pCR to the 2 regimens



Results (6):
clinical, pathological variables, and signatures (dicho.variables)

(Fisher's exact test)

	FEC arm			T->ET arm		
	OR	95%CI	P	OR	95%CI	P
T3 vs T1-2	0.8	0.3-2.3	0.6	0.3	0.06-0.1	0.03
G1-2 vs G3	0.4	0.1-1.4	0.1	0.7	0.2-2.5	0.6
N1-2 vs N0	0.9	0.3-2.7	0.8	0.6	0.2-1.9	0.4
HER2 Ampl. vs Non	1.8	0.4-7.3	0.4	1	0.3-3.2	1.00
Age (<49.5 vs >49.5)	1.6	0.6-5	0.5	1.4	0.5-4.5	0.60
FEC signature	8.7	2.6-34	<0.001	2.1	0.7-6.9	0.20
T->ET signature	2.7	0.9-8.6	0.08	14.8	3.8-70	<0.001

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Results (6):
clinical, pathological variables, and signatures (contin.variables)

(logistic regression model)

Univariate

	FEC arm			T->ET arm		
	OR	95%CI	P	OR	95%CI	P
FEC signature (0.1)	2	1.4-2.7	<0.001	1.1	0.9-1.4	0.30
T->ET signature (0.1)	1.4	1.0-1.9	0.03	1.9	1.4-2.5	<0.001

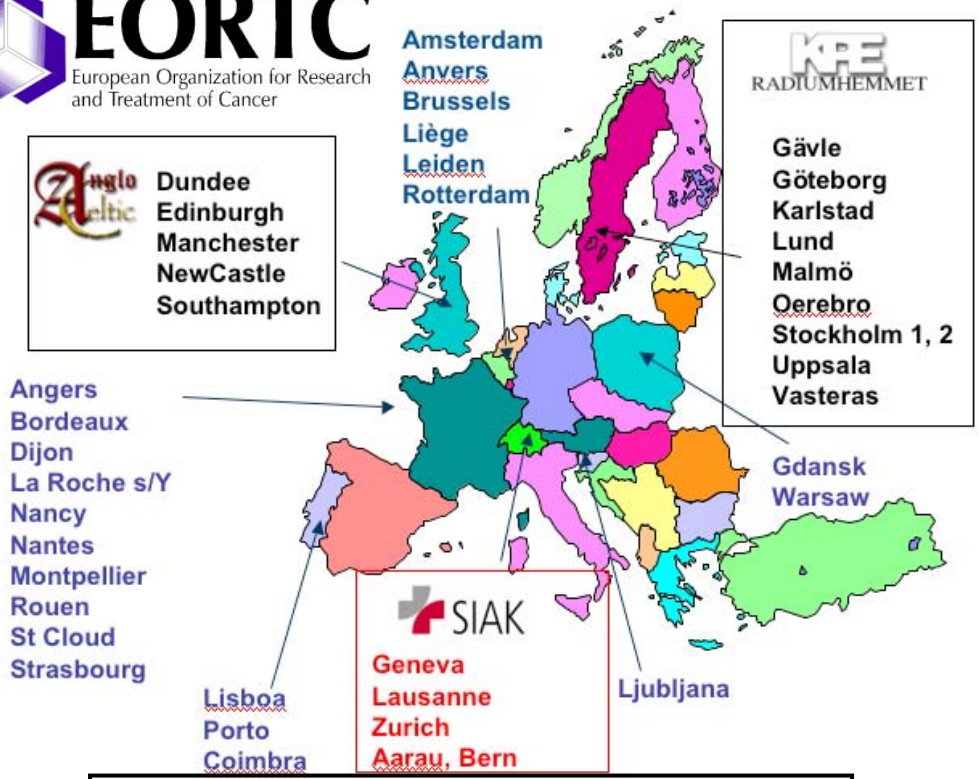
Multivariate

	FEC arm			T->ET arm		
	OR	95%CI	P	OR	95%CI	P
FEC signature (0.1)	2.3	1.5-3.5	<0.001	0.5	0.2-0.9	0.02
T->ET signature (0.1)	0.8	0.5-1.4	0.44	5.1	2-14	<0.001

Discussion

- We have validated **regimen-specific *in vitro* signatures** in the context of a randomised trial
- The high NPV (>90%) of both signatures has the potential to **select patients for trials with new agents**
- A positive test predicts for a 70% pCR (PPV 70%)
- **A validation study is planned** (approx. 180 patients with ER negative tumours)
- Longer follow-up will tell whether these signatures also predict for longer **survival.**

Acknowledgements



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