Gene expression profiling can predict pathological complete response (pCR) to anthracycline-monotherapy in estrogen-receptor (ER) negative breast cancer (BC) patients

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Background

The TOP trial is an international study that aims to identify biological markers associated with pCR to neoadjuvant anthracycline chemotherapy (CT).

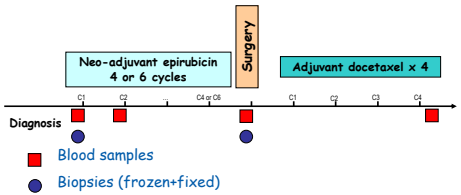
Its unique characteristics are:

1/Determination of the predictive factors of response to single agent epirubicin;

2/Evaluation of response in ER-negative pts only, eliminating the confounding effect of indirect ovarian suppression in ER+ BC.

3/Sample size calculation based on biological hypothesis

Study Design



Methods

1. Immunohistochemistry: ER (6F11-Novocastra), PgR (1A6-Novocastra), Ki67 (MB1-Dako), Her2/Neu (CB11-Novocastra), topoII (KiS1-Chemicon) et CK5/6 (D5/16B4-Dako).
2. Fluorescent In Situ Hybridization: Her2 and topo-II (Vysis).
3. Gene expression profiling with Affymetrix HG-U133Plus chip and Ingenuity Pathways was used for functional analysis.
4. CLUSTER and TREEVIEW were used to generate and visualize dendrograms.

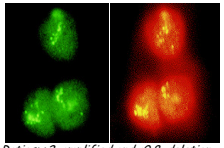
Patient's & Tumor Characteristics (n=95)

Age		*Tumor size*	
Median	46(27-68)	≤ 2cm	11
≤ 50 yrs	61	2-5 cm	66
> 50 yrs	34	> 5cm	4
		T4	14
Nodal Status		*Type of surgery*	
N0	48	Mastectomy	28
N+	47	Conservative	55
Treatment (n=82)		*Histological Grade*	
4 cycles	61	G1	-
6 cycles (dd)	21	G2	22
		G3	66
Histological Type		unknown	7
Ductal	88		
Others	7		

	ER	PgR	Ki67 (% cells)	Neu IHC	CK5/6 (% cells)	Topo-II (% cells)
Median	0	0	50	0	0	20
% pos (cutoff)	0 (>10%)	0 (>10 %)	79 (>25%)	33 (++/+++)	49 (>0 %)	60 (>10%)

Results

1. The observed pathological complete response rate, 16% (11/82) is in line with the rates reported in the literature.
2. No association was observed between pCR and age, size and the evaluated markers, except that 11/12 pCR pts had high Ki67.
3. Her2 & Topo-II amplification status (n=63):



Ratios: ≥2=amplified and ≤0,8=deletion

	Topo-II deleted	Topo-II "normal"	Topo-II amplif.
HER2 ampl.	*4/9 (44%)*	*5/46 (11%)*	*8/8 (100%)*
No pCR	7 (78%)	42 (91%)	6 (75%)
pCR	2 (22%)	4 (9%)	2 (25%)

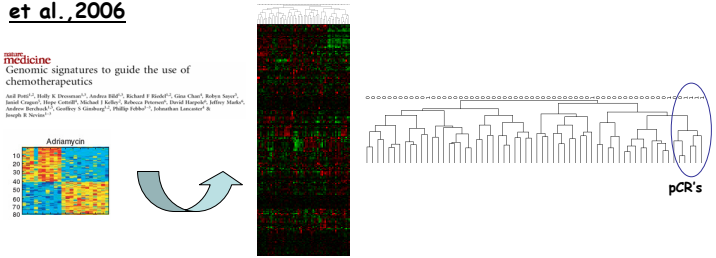
4. Class Comparison Analysis using gene expression data (n=62):

568 genes that were significantly associated with pCR

(student t-test, p<.001,probability of selecting these genes by chance was estimated to be p=0.005 after 1000 random permutations).

Main Functions	Genes
Protein Synthesis	ANAPC5, DHPS, EEF1A1, EIF2AK1, FCER1G, MRP63, NACA, PPP1CA, PSEN1, PTBP1, RPL3, RPL13, RPL28, RPL35, RPL18A, RPLP2, RPLP0, RPS3, RPS5, RPS6, RPS9, RPS10, RRBP1, SPG7, TPP1, UBA52, USP25, XPNPEP1
Cellular Assembly and Organization	ARHGDIB, CDK2AP2, CFL1, CRK, FCER1G, FHIT, FYN, GAPDH, LYN, NAPA, NDE1, PICALM, PRG1, RAC1, RAC2, RANBP9, RHOA, TUBB, UBTF, VAMP3, YKT6
Cellular Function and Maintenance	ANP32A, CDK2AP2, CFLAR, CSNK2B, FCER1G, FHIT, GAPDH, HLA-B, LYN, NAPA, NDE1, PFDN5, PICALM, RAC1, RAC2, RHOA, RXRB, SMC3, TIMP1, TUBB, VAMP3,
Cancer-related functions	B2M, BSG, C19ORF10, CD99, CFLAR, CREB1, CREM, CSF2RA,DYNLRB1, EIF2AK1, FHIT, FYN, KLF6, LYN, RAC1, RHOA, RNASE1, RRM1, S100A4, TIMP1, TXNIP
Gene expression	CREB1, CREM, CRSP3, FYN, GTF2F1, JAK1, MED6, PPIA, PSEN1, RXRB, SP110, THRAP5, TTF1, UBTF, ZNF148
Cell-to-Cell signaling	ANP32A, B2M, CFL1, CREB1, FYN, HLA-C, LYN, PSEN1, RAC1, RAC2, RHOA, S100A4, SMC3, TIMP1
Cell Death	B2M, CD99, CFLAR, CSF2RA, EEF1A1, FCER1G, FCGR3A, HLA-C, HLA-G, LYN, RAC2, RNASE1, TXNIP
Small Molecule Biochemistry	ALOX5AP, CYBA, DHPS, FCER1G, FYN, LYN, OAZ1, PSEN1, RAC1, RAC2, RHOA, UROD
Immune Response	B2M, CFL1, FCER1G, FYN, HLA-B, HLA-C, LYN, RAC1, RAC2, RHOA, TMSB4X

5. Clustering using the adriamycin predictor genes published by Potti et al.,2006



Conclusions

These results suggest that a group of genes can identify ER-negative BC pts likely to respond to epirubicin. Since 400 pts will be enrolled, these results will soon be tested on a larger cohort of patients.