

**Integrazione degli approcci molecolare e bioinformatico al profiling
molecolare delle linee cellulari di tumore della mammella estrogeno
responsivo**

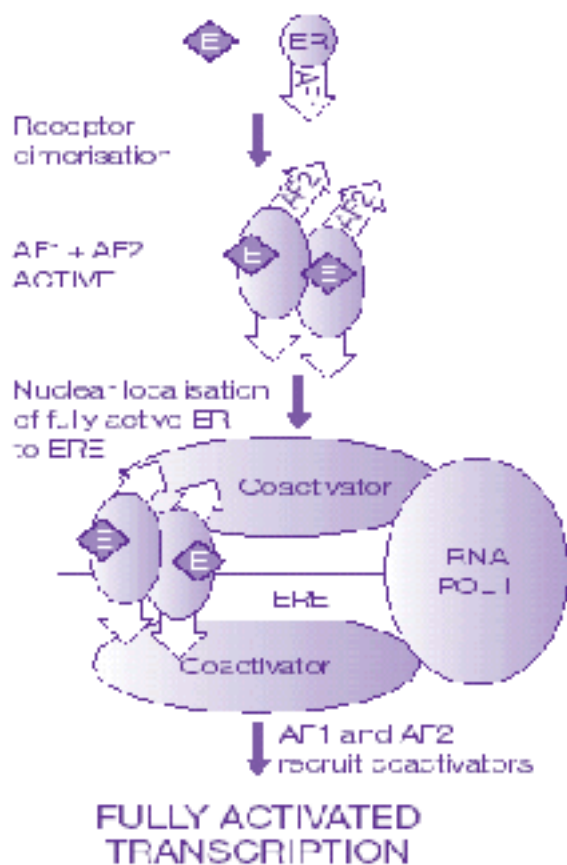
16 novembre 2004

*Studio comparativo dei profili di espressione genica indotti dalle
diverse classi di antiestrogeni nel carcinoma mammario ormono-
responsivo mediante microarrays a cDNA*

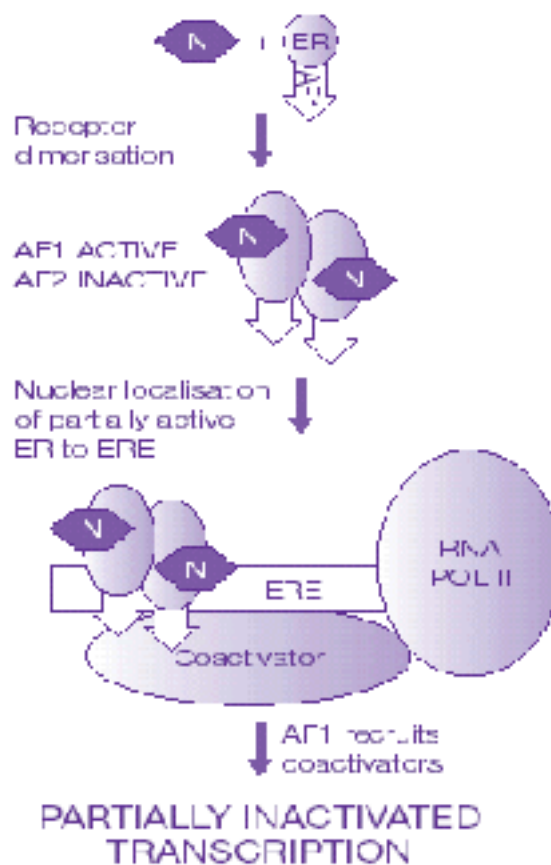
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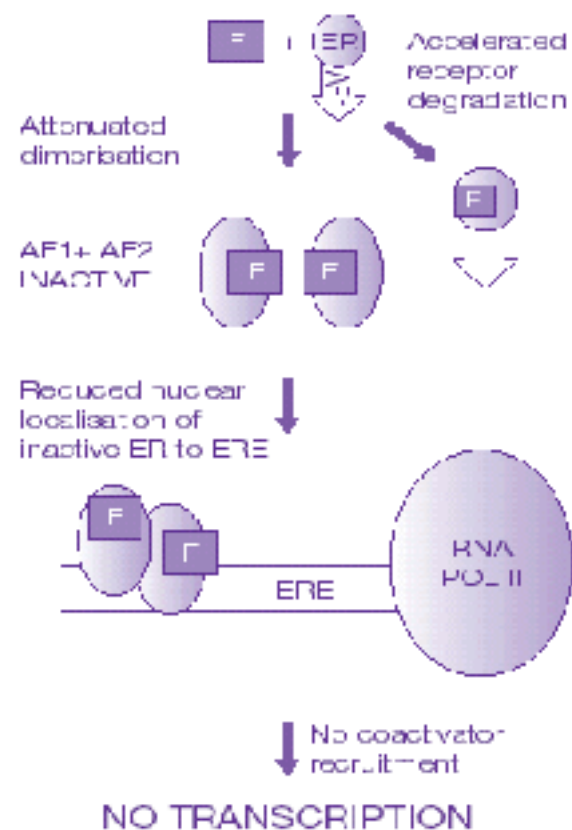
ESTRADIOL



SERMs e.g. 'NOLVADEX' (tamoxifen)



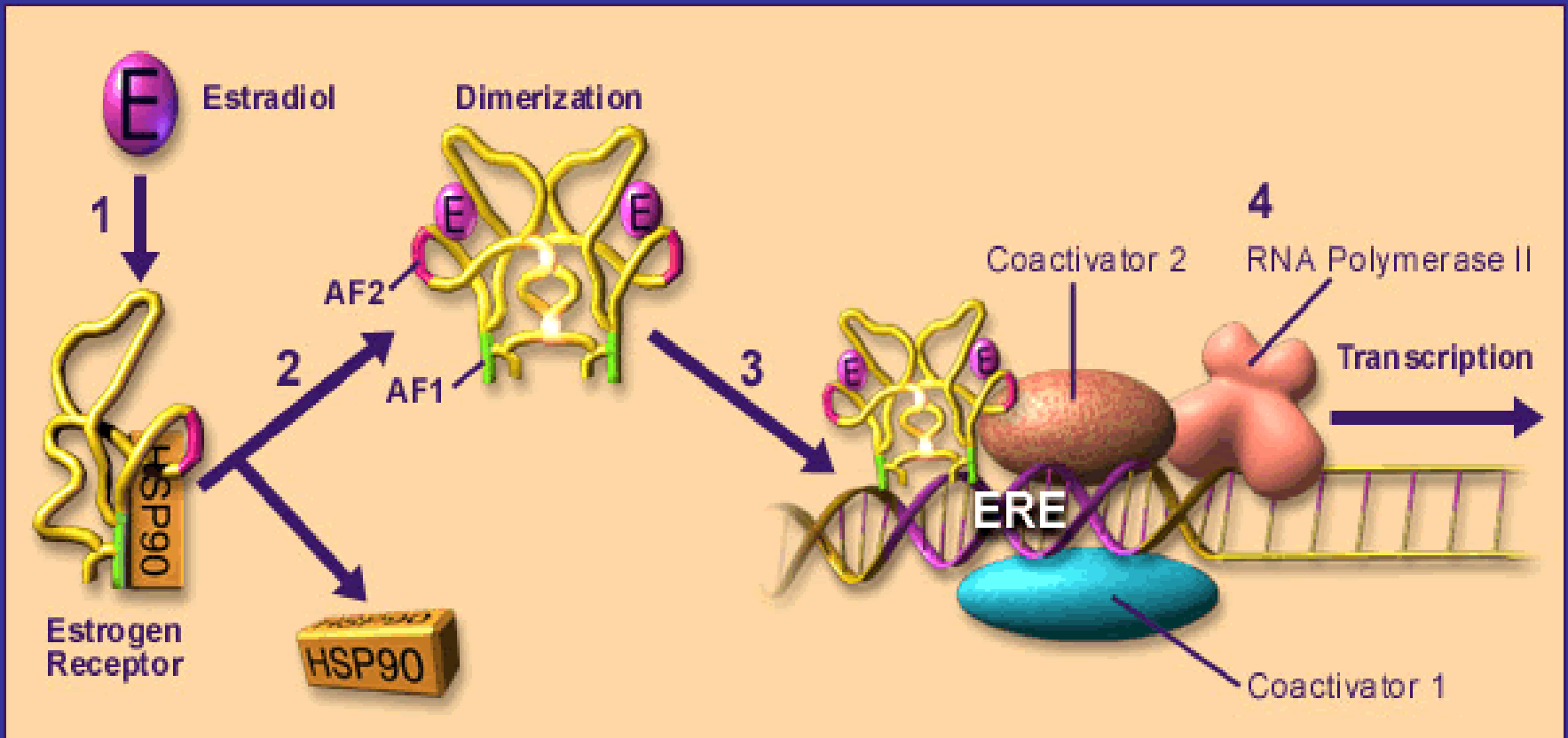
'FASLODEX'



Key:

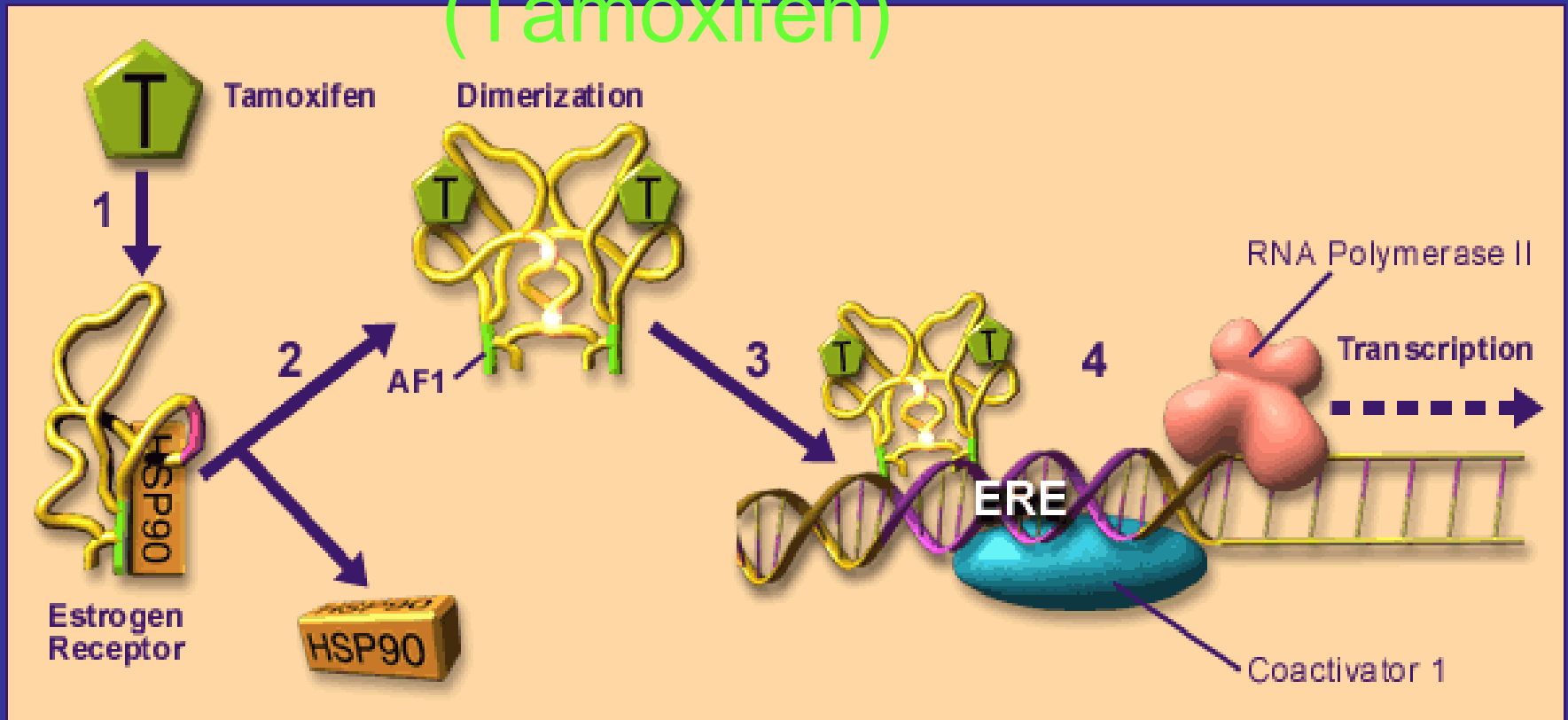
ER = estrogen receptor, ERE = estrogen response element, AF = activation function, RNA POL II = RNA polymerase II

Molecular Action of Estradiol



Adapted from Howell A, Osborne CK, Morris C, Wakeling AE. ICI 182, 780 (Faslodex®), development of a novel, "pure" antiestrogen. *Cancer* 2000; 89: 819.

Molecular Action of SERM (Tamoxifen)



Adapted from Howell A, Osborne CK, Morris C, Wakeling AE. ICI 182,780 (Faslodex®), development of a novel, "pure" antiestrogen. *Cancer* 2000; 89: 819.

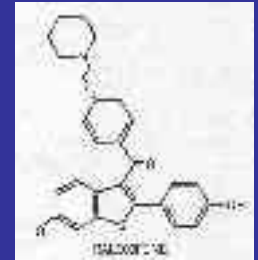
The co-factors expressed in the cell and their differential recruitment represent the molecular basis of the tissue specific activity of ER-TAM complex

Tamoxifen



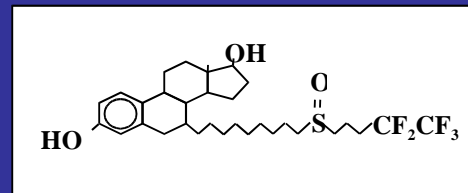
- Has many undesirable E / AE actions.
 - E in uterus – risk of End. Cancer.
 - Alleged as a carcinogen.
 - AE in vagina, CNS?
- Unsatisfactory safety/toxicity profile.
- Gave boost to the continued research for SERMs.
- Under evaluation-star trial-6/99, 22000 women for 5-10 yrs.

Raloxifene



- Originally approved (1998) for use for treatment and prevention of osteoporosis.
- Subsequently (1999) approved for breast cancer prevention after '*MORE*' study
- Improved safety profile than Tamoxifen
- Cardiovascular effects are unequivocal & under evaluation.

Faslodex

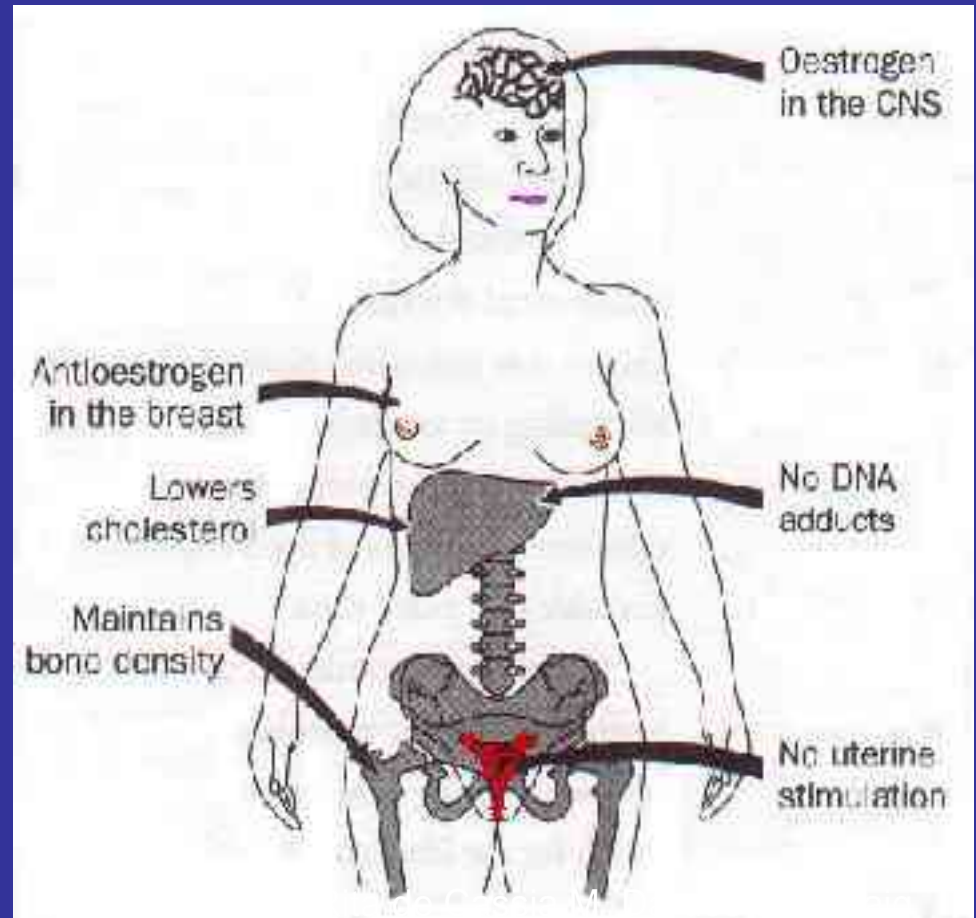


- New type of oestrogen receptor (ER) antagonist that downregulates cellular levels of the ER and has no known oestrogen agonist activity
- Fulvestrant has recently been granted U.S. FDA approval for the treatment of ER+, metastatic breast cancer in postmenopausal women progressing on prior antiestrogen therapy

The Ideal Selective Oestrogen Receptor Modulator

The perfect SERM
The Search goes on

The ideal SERM is one that prevents bone loss, has no risk of uterine or breast cancer, a +ve effect on lipids & cardiovascular system, relieves PMS and maintains cognitive function of the brain

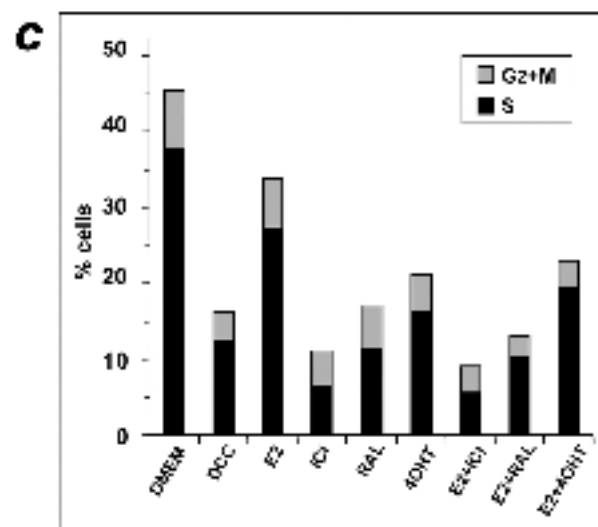
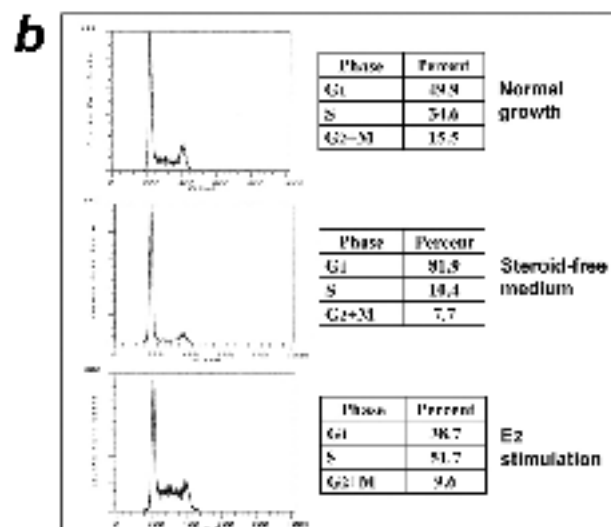
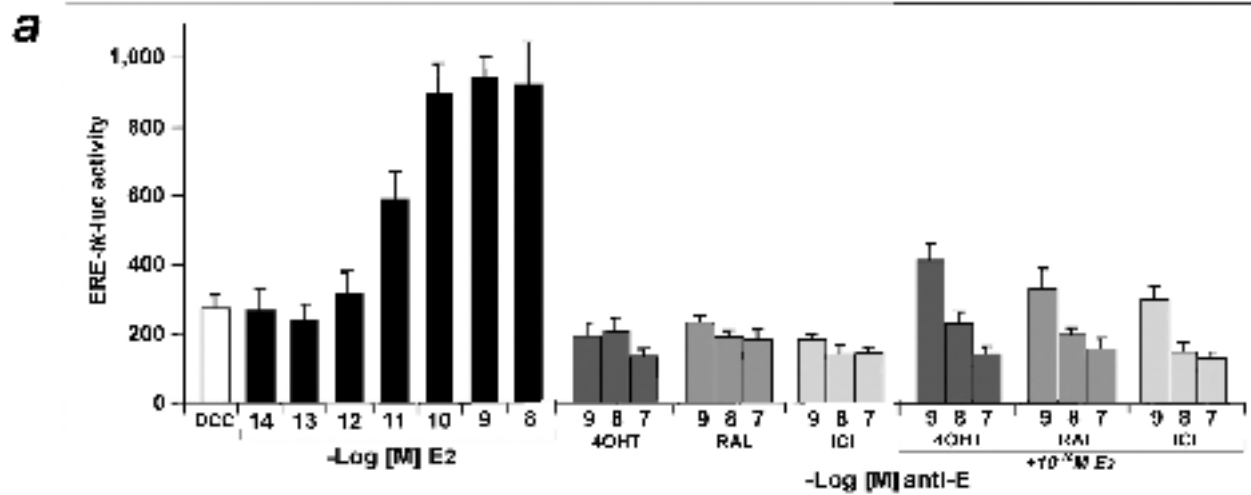


- The antiestrogen classification can be based on their transactivating properties on the estrogen responsive genes.
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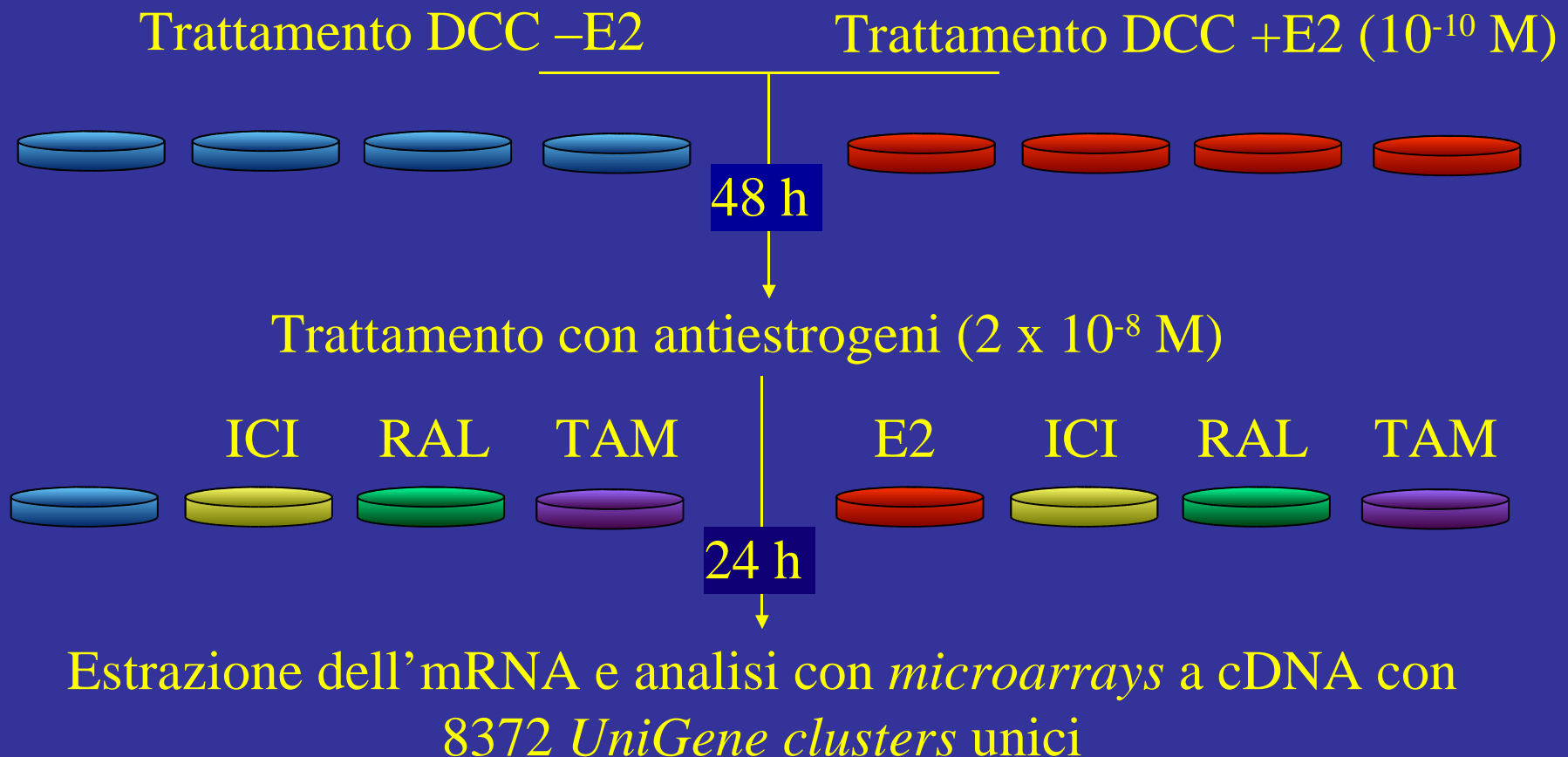
The study of the transcriptional programs regulated from the antiestrogen by microarray analysis will allow:

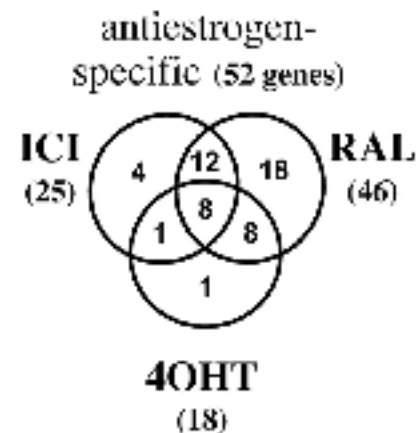
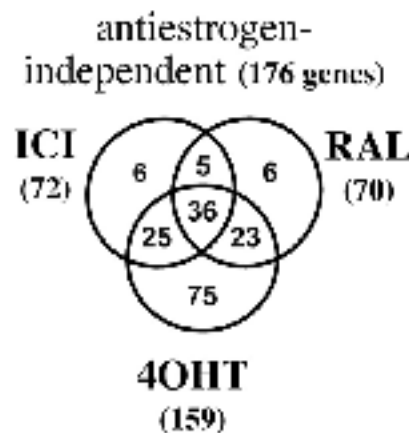
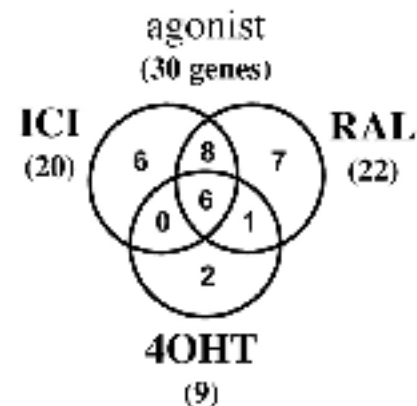
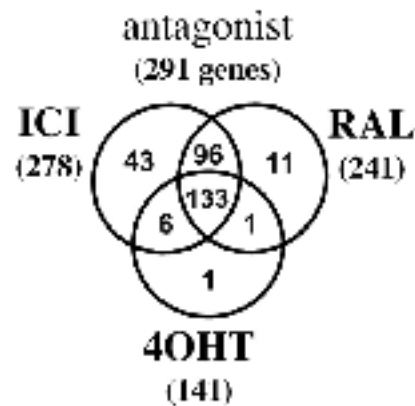
the identification of the molecular pathways specifically regulated by the three anti-estrogens or one of them;

the creation of an informative set of antiestrogen responsive genes useful for the classification of new antiestrogen

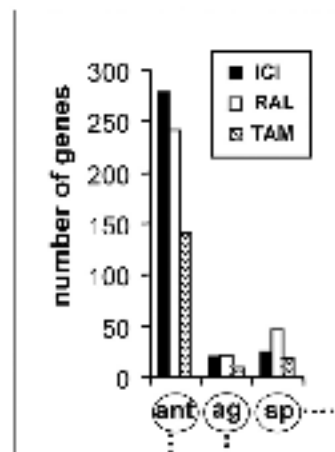


ANALISI DEI PROFILI DI ESPRESSIONE GENICA REGOLATI DA ANTIESTROGENI IN CELLULE DI CARCINOMA MAMMARIO ZR-75.1





	number of genes	%
protein metabolism	62	17.9
protein biosynthesis	19	5.6
protein catabolism	12	4.1
protein folding	8	2.1
protein targeting	8	2.1
other	12	4.1
cell communication	43	14.8
signal transduction	23	7.8
defense response	8	2.7
other	10	3.4
transport	27	9.3
small molecule transport	7	2.4
vesicle-mediated transport	9	2.9
protein transport	8	2.7
nucleocytoplasmic transport	10	3.4
other	2	0.7
DNA metabolism	23	7.9
DNA repair	8	2.7
DNA replication	9	3.0
other	10	3.4
development	22	7.8
reproduction	9	3.1
other	14	4.8
RNA metabolism	18	6.2
RNA processing	18	6.2
cell cycle	16	6.2
G phase of mitotic cell cycle	8	2.9
other	8	3.1
cell proliferation	15	5.2
positive regulation of cell proliferation	8	2.7
transcription	14	4.8
transcription from POUII promoter	9	3.1
regulation of transcription	9	3.1
other	2	0.7
apoptosis	10	3.4
response to stress	10	3.4
carbohydrate metabolism	8	2.8
cytoplasm organization and biogenesis	7	2.4
nuclear organization and biogenesis	5	1.7
TOTAL	291	



	number of genes	%
transcription	10	19.2
regulation of transcription	5	17.0
other transcription	2	2.6
development	10	19.2
cell communication	3	10.4
protein metabolism	6	11.5
transport	4	11.5
TOTAL	32	

	number of genes	%
cell communication	7	23.3
signal transduction	5	16.7
protein metabolism	7	23.3
TOTAL	32	

It is possible to perform a statistical evaluation of the biological processes that are significantly regulated by the drugs: ONTO-Express analysis

Biological processes significantly affected in hormone-stimulated human breast cancer cells by the different antiestrogens tested

	ICI		RAL		TAM		E2
	Number of genes	P-value	Number of genes	P-value	Number of genes	P-value	
<i>A. Significant p-value for all three antiestrogens tested</i>							
RNA processing/modification	20	0,03928	17	0,00843	11	0,02863	23
Nuclear-cytoplasmic transport	8	0,04659	7	0,03663	7	0,00404	9
<i>B. Significant p-value for ICI 182,780 and raloxifene</i>							
RNA splicing	8	0,04659	7	0,03663	3	0,24427	9
<i>C. Significant p-value for ICI 182,780 only</i>							
Regulation of cell cycle	11	0,04010	8	0,00685	6	0,07363	13
<i>D. Significant p-value for raloxifene only</i>							
Oncogenesis	10	0,11901	10	0,02073	6	0,20772	10
DNA repair	5	0,23580	7	0,02015	4	0,07483	8
DNA replication	4	0,23163	5	0,04135	3	0,08850	6
Signal transduction	12	0,13396	6	0,02424	8	0,49977	31
<i>E. Significant p-value for 4-OHtamoxifen only</i>							
Protein amino acid phosphorylation	4	0,23163	2	0,45745	5	0,00713	6
Nucleocytoplasmic transport	4	0,11981	3	0,15783	4	0,01367	5
Other metabolism	7	0,02895	3	0,48184	7	0,00404	9
DNA synthesis	0	0,13798	0	0,08009	5	0,04160	9

Results of *in silico* analysis of estrogen- and antiestrogen-responsive gene promoters^a

Transcription factor	TRANSFAC ^b	+E2 ^d								-E2	
		+IUG		+RAL		+LIGN		+E1		+RAL	
		Up ^d	Down ^d	Up	Down	Up	Down	Up	Down	Up	Down
NRF-1	M00652	NS	3.5x10 ⁻⁴	NS	1.6x10 ⁻⁴	NS	1.7x10 ⁻⁴	4.6x10 ⁻⁵	NS	NS	NS
NF-Y	M00287	NS	3.2x10 ⁻⁵	NS	NS	NS	2.2x10 ⁻⁴	3.0x10 ⁻⁶	NS	NS	NS
ATF	M00330	NS	1.5x10 ⁻⁴	NS	8.8x10 ⁻⁶	NS	1.4x10 ⁻⁴	NS	NS	NS	NS
ERF-1	M00428/ M00940	NS	1.0x10 ⁻⁴	NS	NS	NS	NS	1.7x10 ⁻⁴	NS	NS	NS
LF-A1	M00646	2.8x10 ⁻⁴	NS	3.0x10 ⁻⁵	NS	NS	NS	NS	3.2x10 ⁻⁴	NS	NS
C-binding protein	M00730	21x10 ⁻⁴	NS	NS	NS	NS	NS	NS	1.6x10 ⁻⁵	NS	NS
EGR	M00807	81x10 ⁻⁵	NS	6.0x10 ⁻⁵	NS	NS	NS	NS	NS	NS	NS
Sp1	M00933	21x10 ⁻⁶	NS	1.6x10 ⁻⁵	NS	NS	NS	NS	NS	NS	NS
ETF-1	M00652	NS	NS	NS	NS	NS	NS	NS	NS	NS	9.5x10 ⁻⁵

^aNS, not significant; ^bTRANSFAC, transcription factor database; ^c10⁻⁴ and 10⁻⁵ are the P values for the chi-square test; ^dUp and Down are the number of promoters up-regulated and down-regulated, respectively.

Absence of any striking difference between pure antiestrogens and SERMs, or agonistic and antagonistic compound;

All the drugs shows both agonistic and antagonistic action;

The antiestrogen specific genes suggest the presence of ER independent and antiestrogen regulated pathways;

The biological functions and the transcriptional modification regulated by the three antiestrogens are partially overlapping.

The last point implies that the gene expression profile could reflect the clinical differences of the antiestrogens and serve to predict the BC hormone-responsiveness;

The tested drugs do not cover the whole spectrum of estradiol actions on BC cells.

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Gene Expression Group



<http://crisceb.unina2.it/geneexpression/>

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