High Pregnane-X-Receptor (PXR) expression is correlated with poor prognosis in invasive breast carcinoma

Despoina Pouloudi[1], Constantinos Giaginis[2], Stefania Gourzi[1], Paraskevi Alexandrou[1], Gerasimos Tsourouflis[3], Artemis Michail[4], Nikolaos Tsoukalas[5], Samer Albesher[1], Panagiotis Politis[4], Lydia Nakopoulou[1] and Stamatios Theocharis[1]

[1] First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Greece

[2] Department of Food Science and Nutrition, University of the Aegean, Myrina, Lemnos, Greece

[3] 2nd Department of Propedeutic Surgery, School of Medicine, National and Kapodistrian University of Athens, Greece

[4] Section of Molecular Biology, Biomedical Research Foundation, Academy of Athens, Greece

[5] First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Greece

Pregnane-X-Receptor (PXR)

- Member of the nuclear receptor (NR) superfamily
- Modular protein with 4 domains:
  - a highly variable N-terminal domain,
  - a conserved DNA binding domain (DBD),
  - an H region (H) and
  - a C-terminal ligand-binding domain (LBD)
- Mainly and highly expressed in liver and intestine

PXR's activation

- **Ligand** dependent activation (endo- and xeno-biotics)
- Heterodimerization with the retinoid X receptor (**RXR**) and binding to its **transcriptional** gene targets

- Homeostasis of numerous endobiotics, such as glucose, lipids, steroids and bile acids
- Drug metabolism and drug–drug interactions
- Regulation of inflammation, vitamin D metabolism and bone homeostasis

- Involvement in IBD, liver steatosis and fibrogenesis

PXR is involved in various cancer types, including breast, pancreatic, endometrial, ovarian, prostate, colon, liver and esophageal cancer, by:

- directly affecting cell proliferation and apoptosis and/or
- inducing chemotherapy resistance, while
- its polymorphisms may also have clinical significance in certain cancer types and their treatment

Breast cancer is the most common malignancy and cause of cancer-related death for women, showing varying molecular signatures, morphology and clinical behavior.

Estrogen receptor (**ER**), Progesterone receptor (**PR**), Human Epidermal growth factor Receptor 2 (**HER2**) and **Ki67** index represent the minimum breast cancer pathology data set that defines prognosis and identifies tumors for targeted therapy.

Aim of the study

To evaluate PXR expression in invasive breast carcinoma and **to correlate** it with:

- Multiple clinicopathological characteristics
- ER, PR and HER2 expression,
- Tumor cells' proliferative capacity
- Overall and disease-free patients' survival

Patients

148 invasive breast carcinoma specimens from equal number of patients who underwent surgical resection, without pre-operative radiation or chemotherapy.

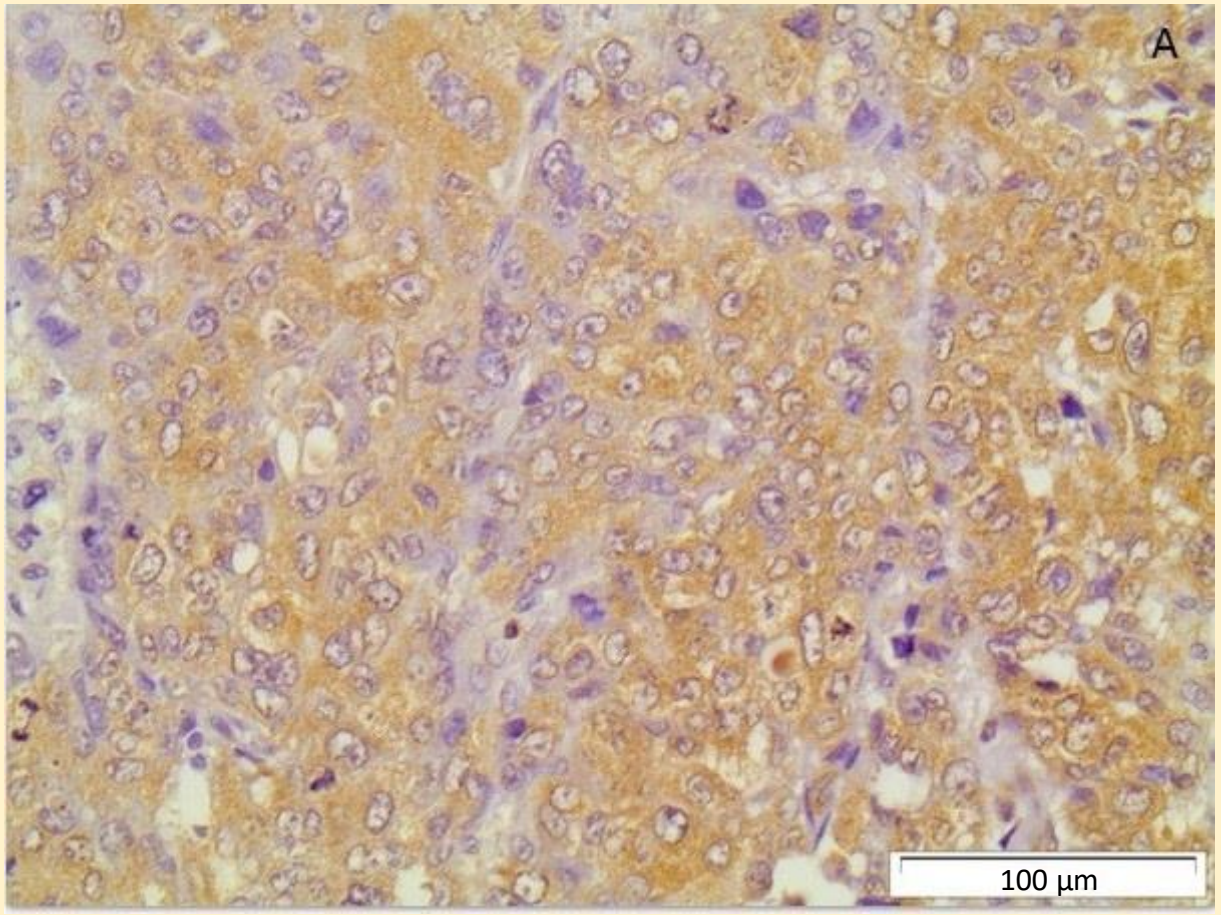
- Follow up for a time interval of 2 - 241 months
- All patients received conventional postoperative treatment when indicated.

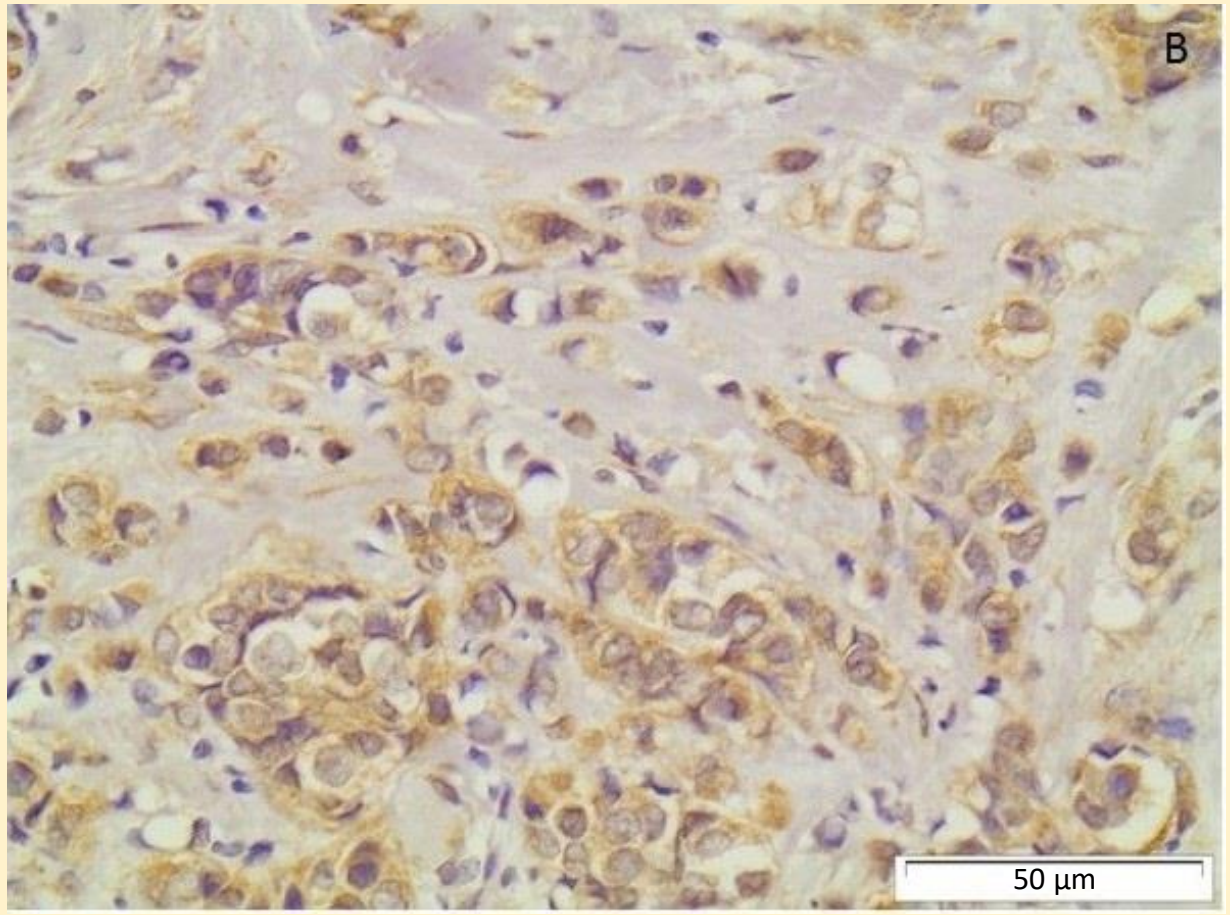
Methods

- Immunohistochemical staining for PXR, ER, PR, Her2 and Ki67
- PXR expression was also confirmed on breast cancer cell lines by Western Blot

Results

- PXR positivity (IHC score > 0) : 79/148 (53.4%)
- High PXR expression (IHC score ≥ 3): 48/148 (32.4%)
- PXR distribution: **mainly cytoplasmic** and occasionally nuclear
- Staining intensity
  - Mild: 36/79 (45.6%)
  - Moderate: 38/79 (48.1%)
  - Intense: 5/79 (6.3%)
- Normal areas adjacent to tumor: PXR (-) or mild nuclear staining

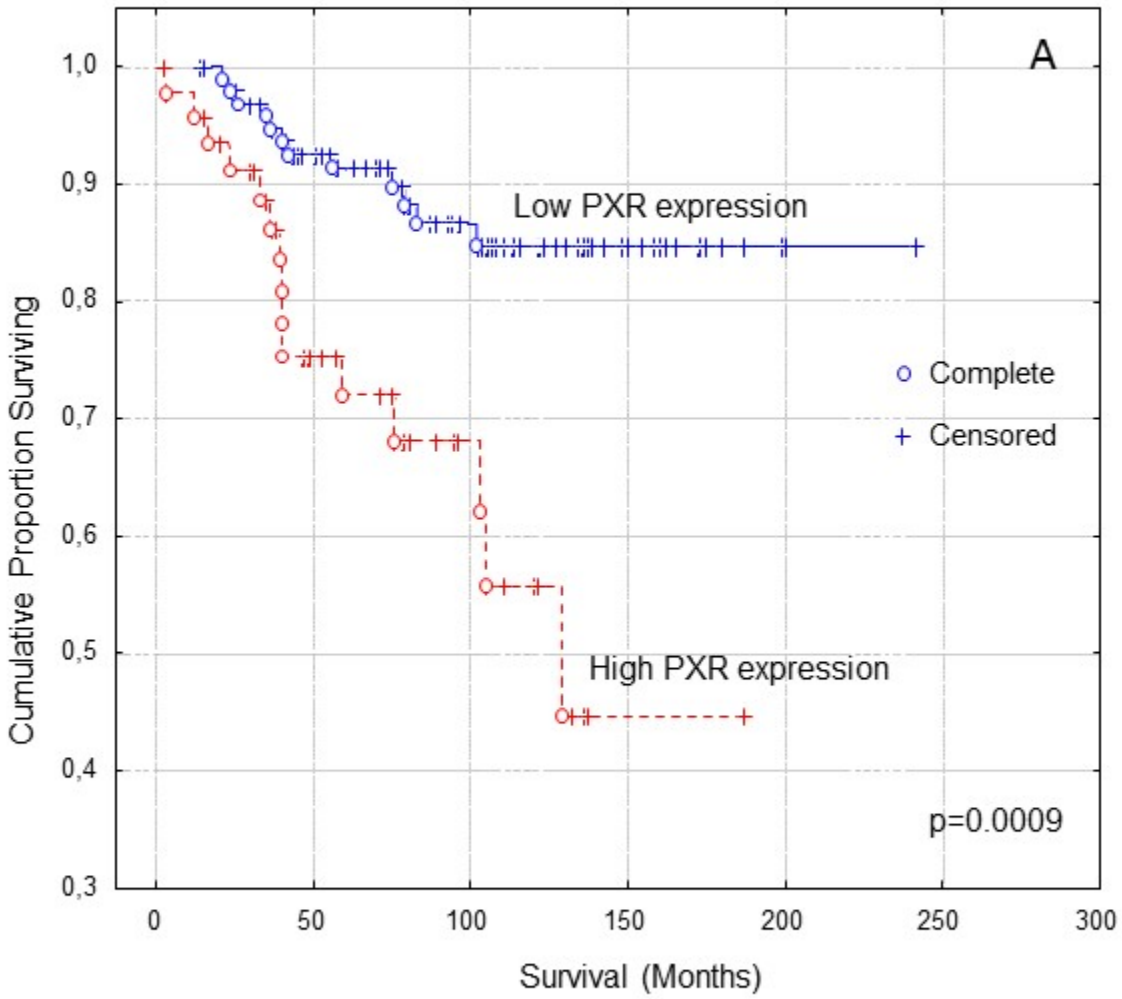

A
100 µm


B
50 µm

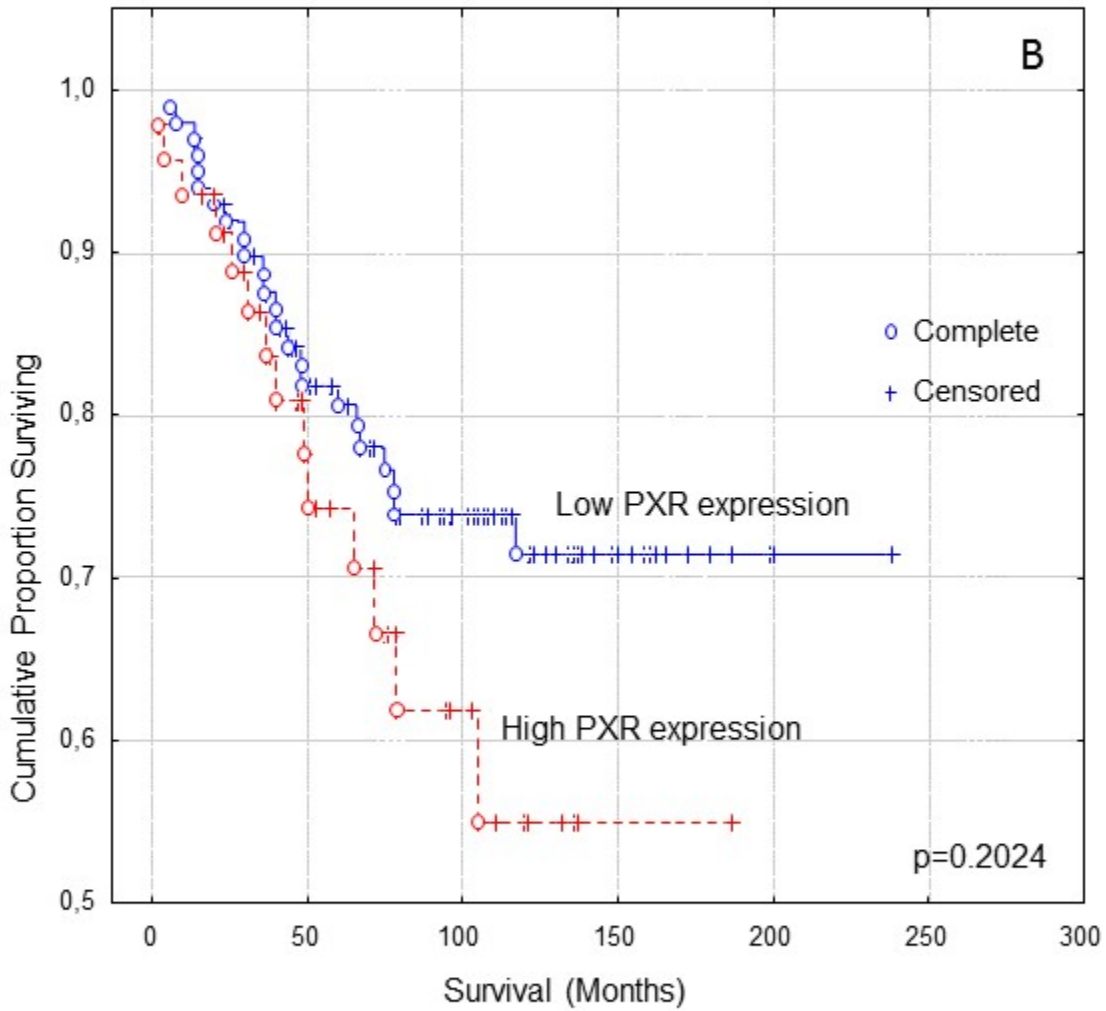
Clinicopathological parameters		PXR expression		p-value
		Low (%)	High (%)	
N=148		100 (67.6)	48 (32.4)	
Age (mean±SD;ys)	≤ 57.4±12.5 yrs	50 (33.8)	25 (16.9)	0.8124
	> 57.4±12.5 yrs	50 (33.8)	23 (15.5)	
Menopausal status	Premenopausal	33 (22.3)	20 (13.5)	0.3032
	Postmenopausal	67 (45.3)	28 (18.9)	
Histopathological type	Ductal	67 (45.3)	34 (23.0)	0.6391
	Lobular	33 (22.3)	14 (9.5)	
Histological Grade	Low	13 (8.8)	3 (2.0)	**0.0305**
	Intermediate	55 (37.2)	19 (12.8)	
	High	32 (21.6)	26 (17.6)	
Nuclear Grade	Low	51 (34.5)	12 (8.1)	**0.0112**
	Intermediate	26 (17.6)	19 (12.8)	
	High	23 (15.5)	17 (11.5)	
Molecular subtype	Luminal-A	48 (32.4)	11 (7.4)	**0.0295**
	Luminal-B	11 (7.4)	7 (4.7)	
	HER2	6 (4.0)	6 (4.0)	
	Triple negative	35 (23.6)	24 (16.2)	
Tumor size	pT1	34 (23.0)	13 (8.8)	0.6648
	pT2	55 (37.2)	30 (20.3)	
	pT3	11 (7.4)	5 (3.4)	
Lymph nodes	Non infiltrated	45 (30.4)	22 (14.9)	0.9240
	Infiltrated	55 (37.2)	26 (17.5)	
Histopathological stage	I	25 (16.9)	13 (8.8)	0.9054
	II	60 (40.5)	29 (19.6)	
	III	15 (10.1)	6 (4.0)	
ER expression	Negative	50 (33.8)	33 (22.3)	**0.0314**
	Positive	50 (33.8)	15 (10.1)	
PR expression	Negative	53 (35.8)	35 (23.6)	**0.0208**
	Positive	47 (31.8)	13 (8.8)	
HER-2 expression	Negative	89 (60.2)	44 (29.7)	0.6148
	Positive	11 (7.4)	4 (2.7)	
Ki-67 protein statement	Below median value	62 (41.9)	18 (12.1)	**0.0051**
	Over median value	38 (25.7)	30 (20.3)	

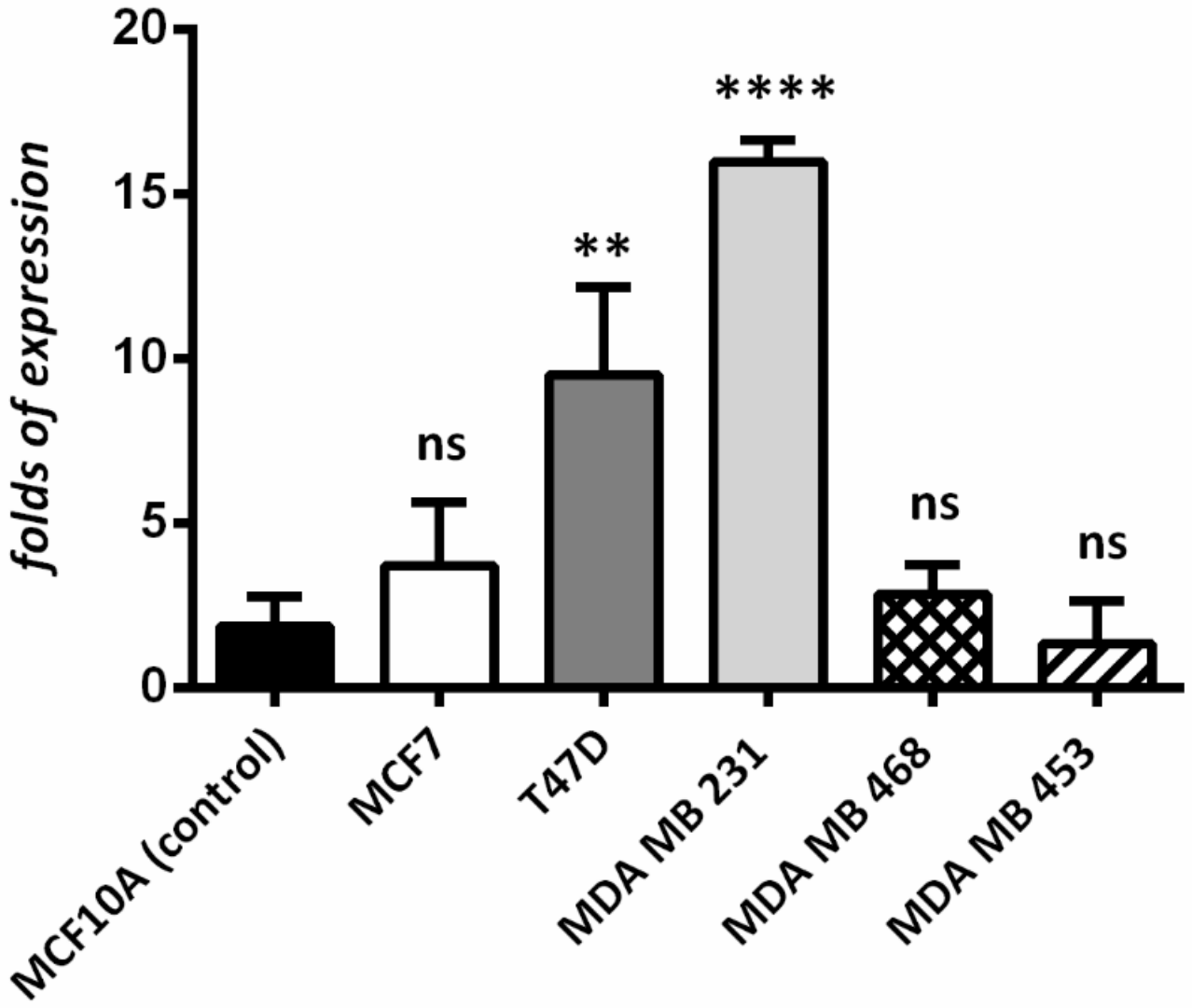
Clinicopathological	Overall survival	
Variables	**HR (95% CI)**	**p-value**
Histological type		
(Ductal / Lobular)	0.677 (0.067-2.985)	0.4351
Nuclear Grade		
(Low / Intermediate + High)	0.582 (0.093-2.076)	0.2972
Histopathological stage		
(I+II / III)	1.908 (0.839-3.985)	0.1787
Ki-67 statement		
(Below/over median value)	6.173 (3.219-9.444)	**0.0007**
PXR expression		
(Low / High)	0.183 (0.022-0.589)	**0.0056**

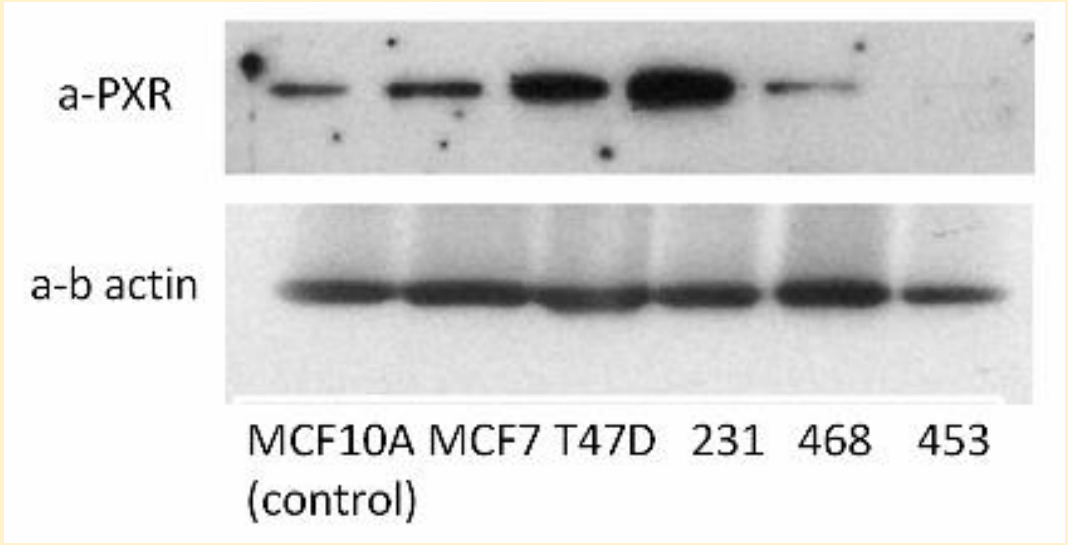
OS


A
Low PXR expression
High PXR expression
Complete
Censored
p=0.0009
Cumulative Proportion Surviving
Survival (Months)
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0
50
100
150
200
250
300

DFS


B
Low PXR expression
High PXR expression
Complete
Censored
p=0.2024
Cumulative Proportion Surviving
Survival (Months)
1,0
0,9
0,8
0,7
0,6
0,5
0
50
100
150
200
250
300





	ER	PR	HER 2
MCF10A (control cell line)			
MCF7	+	+	-
T47D	+	+	+
MDA MB 231	-	-	-
MDA MB 468	-	-	-
MDA MB453	-	-	-

Conclusions

In our study **PXR** expression in breast cancer

- was associated with crucial clinicopathological parameters for patients' management and prognosis
- proved to be an independent factor of poor prognosis

PXR may have a potential important role in the biological mechanisms governing breast malignant disease progression.

Thank you for your attention!