

**Table 1:** Characteristics of the 48 ER $\alpha$ -positive tumors from patients with and without relapse

	ER $\alpha$ -positive tumors (n=48)			RFS <sup>a</sup>
	Number of patients	ER $\alpha$ -positive tumors with relapse (n=24)	ER $\alpha$ -positive tumors without relapse (n=24)	P-value <sup>b</sup>
<i>Age</i>				
≤70	26	13 (50%)	13 (50%)	NS
>70	22	11 (50%)	11 (50%)	(0.46)
<i>SBR histological grade<sup>c</sup></i>				
I + II	37	15 (62.5)	22 (91.7)	NS
III	11	9 (37.5)	2 (8.3)	(0.08)
<i>Lymph node status</i>				
Negative	31	13 (54.2)	18 (75)	NS
Positive	17	11 (45.8)	6 (25)	(0.09)
<i>Macroscopic tumor size</i>				
≤30mm	31	14 (58.3)	17 (70.8)	NS
>30mm	17	10 (41.7)	7 (29.2)	(0.28)

<sup>a</sup> Relapse-free survival

<sup>b</sup> Log-rank test. NS : not significant

<sup>c</sup> Scarff Bloom Richardson classification

**Table 2:** Characteristics of the 24 ER $\alpha$ -negative and 24 ER $\alpha$ -positive tumors.

	Tumors (n= 48)	
	ER $\alpha$ -negative tumors (24)	ER $\alpha$ -positive tumors (24)
<i>Age</i>		
$\leq 60$	14 (58.3%)	1 (4.2%)
$> 60$	10 (41.7%)	23 (95.8%)
<i>SBR histological grade</i> <sup>a</sup>		
I + II	5 (22.7%)	20 (83.3%)
III	17 (77.3%)	4 (16.7%)
<i>Lymph node status</i>		
negative	11 (45.8%)	0 (0%)
positive	13 (54.2%)	24 (100%)
<i>Macroscopic tumor size</i>		
$\leq 30$ mm	15 (62.5%)	12 (50%)
$> 30$ mm	9 (37.5%)	12 (50%)

<sup>a</sup>: Scarff Bloom Richardson classification. Information available for 48 patients.

**Table 3** : Oligonucleotide primer sequences used used for real-time RT-PCR

Gene	Upper primer sequence	Lower primer sequence	PCR product size (pb)
<b>ADAMTS3</b>	5' - GGT CCA TCG CAG CTT CTG TGA - 3'	5' - CAT TCT TCT GCT ACC CAG AGT GGA T - 3'	102
<b>ANKRD17</b>	5' - GCT CCA AGT CCC TCA GGT ATT GT- 3'	5' - GAA GCA AAG TTT CCC AAA TGT GTT - 3'	86
<b>ALB</b>	5' - AAG CTG CTT TTA CAG AAT GTT GCC A - 3'	5' - CAT CCC GAA GTT CAT CGA GCT TT - 3'	78
<b>RASSF6</b>	5' - CCA GTA CCC CTC TTG GAT CTT CA - 3'	5' - GTT TGC CAT CTT CAG TCT CTC CAT - 3'	143
<b>CXCL8</b>	5' - CAC CGG AAG GAA CCA TCT CAC TGT - 3'	5' - TCC TTG GCA AAA CTG CAC CTT CA - 3'	114
<b>CXCL6</b>	5' - GTT TAC GCG TTA CGC TGA GAG TAA A - 3'	5' - CGT TCT TCA GGG AGG CTA CCA - 3'	108
<b>CXCL4V1</b>	5' - CGC CAC CCG CCA GGA GAT - 3'	5' - CCC CAT CTT CTT CAG CTT CAG CTC T - 3'	89
<b>CXCL1</b>	5' - AAC CCC AAG TTA GTT CAA TCT GGA - 3'	5' - CAT GTT GCA GGC TCC TCA GAA - 3'	104
<b>CXCL4</b>	5' - AGC CGG GTT CTG CGC CTC A - 3'	5' - TTC AGC TTC AGC GCT GGC GAA - 3'	91
<b>CXCL7</b>	5' - GCA ACC AAG TCG AAG TGA TAG CCA - 3'	5' - ATC AGC AGA TTC ATC ACC TGC CAA - 3'	119
<b>CXCL5</b>	5' - CAT CGC CAG CGC TGG TCC T - 3'	5' - GGG ATG AAC TCC TTG CGT GGT CT - 3'	82
<b>CXCL3</b>	5' - GGA GCA CCA ACT GAC AGG AGA GAA - 3'	5' - ACC ACC CTG CAG GAA GTG TCA A - 3'	69
<b>CXCL2</b>	5' - TCA AAC CCA AGT TAG TTC AAT CCT GA - 3'	5' - GCT GAC ATG TGA TAT GTC ATC ACG AA - 3'	113
<b>EREG</b>	5' - GCT CTG CCT GGG TTT CCA TC - 3'	5' - CCA CAC GTG GAT TGT CTT CTG TC - 3'	125
<b>AREG</b>	5' - TGG AAG CAG TAA CAT GCA AAT GTC - 3'	5' - GGC TGC TAA TGC AAT TTT TGA TAA - 3'	116
<b>BTC</b>	5' - CTG AGG AAA ACT GTG CAG CTA CC - 3'	5' - TGA TGC AGT AAT GCT TGT ATT GCT T - 3'	90
<b>DKFZP56400823</b>	5' - CGC CAA ATA CCA GGC TAC CAT - 3'	5' - CTC TGT ACC CTC CAT CCT GCA GT - 3'	78
<b>CDKL2</b>	5' - AGA AAG TGA CGA TGA CAA AAT GGT T - 3'	5' - CAA GTT TTC ATG CCT AAG TTG CTT T - 3'	82
<b>CXCR1</b>	5' - CCT GGC CGG TGC TTC AGT TA - 3'	5' - ATC AAA ATC CCA CAT CTG TGG ATC T - 3'	89
<b>CXCR2</b>	5' - GCT CTG ACT ACC ACC CAA CCT TGA - 3'	5' - AGA AGA GCA GCT GTG ACC TGC TGT - 3'	81

**Table 4 : Genes tested**

<b>Genes<sup>a</sup></b>	<b>Alternate symbols</b>	<b>Genbank accession number</b>	<b>Location at 4q21 (Kb)</b>	<b>Orientation Sens (+) Antisens (-)</b>	<b>Description</b>
<b>Cen</b>					
<b>ADAMTS3</b>		NM_014243	<b>73 613 – 73 901</b>	-	Metalloprotease with thrombospondin type 1 motif 3
<b>ANKRD17</b>		NM_032217	<b>74 407 – 74 598</b>	-	Ankyrin repeat domain 17
<b>ALB</b>		NM_000477	<b>74 736 – 74 753</b>	+	Albumin
<b>RASSF6</b>		NM_177532	<b>74 905 – 74 953</b>	-	Ras association (RalGDS/AF-6) domain family 6
<b>CXCL8</b>	IL-8, GCP-1, SCYB8	NM_000584	<b>75 073 – 75 076</b>	+	Interleukin 8
<b>CXCL6</b>	GCP-2, SCYB6	NM_002993	<b>75 169 – 75 171</b>	+	Chemokine (C-X-C motif) ligand 6
<b>CXCL4V1</b>	PF4V1, SCYB4V1	NM_002620	<b>75 185 – 75 187</b>	+	Platelet factor 4 variant 1
<b>CXCL1</b>	GRO1, MGSA , SCYB1	NM_001511	<b>75 201 – 75 203</b>	+	Chemokine (C-X-C motif) ligand 1
<b>CXCL4</b>	PF4, SCYB4	NM_002619	<b>75 313 – 75 314</b>	-	Platelet factor 4
<b>CXCL7</b>	NAP-2, PPBP	NM_002704	<b>75 319 – 75 320</b>	-	Pro-platelet basic protein
<b>CXCL5</b>	ENA-78, SCYB5	NM_002994	<b>75 328 – 75 331</b>	-	Chemokine (C-X-C motif) ligand 5
<b>CXCL3</b>	GRO3, MIP2B, SCYB3	NM_002090	<b>75 369 – 75 371</b>	-	Chemokine (C-X-C motif) ligand 3
<b>CXCL2</b>	GRO2, MIP2A, SCYB2	NM_002089	<b>75 429 – 75 431</b>	-	Chemokine (C-X-C motif) ligand 2
<b>EREG</b>		NM_001432	<b>75 697 – 75 721</b>	+	Epiregulin
<b>AREG</b>		NM_001657	<b>75 777 – 75 787</b>	+	Amphiregulin
<b>BTC</b>		NM_001729	<b>76 130 – 76 178</b>	-	Betacellulin
<b>DKFZP564O0823</b>		NM_015393	<b>76 317 – 76 431</b>	+	DKFZP564O0823 protein
<b>CDKL2</b>	p56KKIAMRE	NM_003948	<b>76 963 – 77 014</b>	-	Cyclin-dependent kinase-like 2
<b>Tel</b>					
<b>CXCR1</b>	IL8RA	NM_000625	<b>2q35</b>		Interleukin 8 receptor, alpha
<b>CXCR2</b>	IL8RB	NM_001557	<b>2q35</b>		Interleukin 8 receptor, beta

<sup>a</sup> Entrez Gene symbol

**Table 5:** Chemokine expression in breast cancer biopsies

	<b>CXCL8 -/+</b> (n = 24)		<b>CXCL8 ++</b> (n = 24)		<b>p<sup>a</sup></b>
	Med	range	Med	range	
<b>CXCL8</b>	95.9	(17.4 - 226.2)	833.5	(267.1 - 7 137)	<0.0000001
<b>ADAMTS3</b>	38.3	(4.0 - 173.7)	61.6	(4.0 - 262.3)	0.30 (NS)
<b>ANKRD17</b>	3.4	(1.1 - 7 802)	3.9	(1.6 - 13 121)	0.51 (NS)
<b>RASSF6</b>	165.9	(19.2 - 2 296)	397.0	(31.6 - 4 421)	0.14 (NS)
<b>CXCL6</b>	4.6	(0.0 - 128.6)	11.8	(0.0 - 376.6)	0.038
<b>CXCL1</b>	25.6	(1.0 - 226.1)	184.7	(7.8 - 1647)	0.00078
<b>CXCL5</b>	1.3	(0.0 - 196.6)	10.2	(0.0 - 2 511)	0.013
<b>CXCL3</b>	26.0	(8.8 - 132.0)	73.0	(10.9 - 342.5)	0.00028
<b>CXCL2</b>	75.0	(6.8 - 542.7)	118.9	(8.1 - 1 026)	0.15 (NS)
<b>EREG</b>	4.2	(0.0 - 343.3)	5.7	(0.0 - 3 993)	0.52 (NS)
<b>AREG</b>	134.3	(0.0 - 2114)	191.4	(0.0 - 9 484)	0.28 (NS)
<b>BTC</b>	100.0	(3.9 - 448.3)	85.9	(4.3 - 404.0)	0.56 (NS)
<b>DKFZP564O0823</b>	658.7	(74.6 - 5 062)	758.1	(30.0 - 6 190)	0.97 (NS)
<b>CDKL2</b>	15.0	(0.0 - 539.6)	10.0	(0.0 - 241.0)	0.70 (NS)
<b>CXCR1</b>	4.0	(0.0 - 121.5)	7.6	(0.0 - 68.3)	0.25 (NS)
<b>CXCR2</b>	9.4	(1.2 - 38.9)	11.6	(0.4 - 98.6)	0.31 (NS)
<b>ERA</b>	80.2	(367.0 - 989 875)	3.7	(107.0 - 952 849)	0.46 (NS)
<b>KI67</b>	3 986	(511.9 - 25 350)	5 185	(1 562 - 35 850)	0.13 (NS)
<b>HER2</b>	100.2	(35.7 - 371.4)	135.7	(21.4 - 3 135)	0.29 (NS)

**Table 6:** Basal chemokine expression in cell lines

<b>CXCL8 +/-</b>	<b>ADAMTS3</b>	<b>CXCL8</b>	<b>CXCL6</b>	<b>CXCL1</b>	<b>CXCL5</b>	<b>CXCL3</b>	<b>CXCL2</b>	<b>EREG</b>	<b>CXCR1</b>	<b>CXCR2</b>	<b>ER<math>\alpha</math></b>
<b>MDA-MB468</b>	0.01	12.86	0.00	0.05	0.03	0.16	0.04	0.18	0.06	0.00	0.05
<b>SKBR-3</b>	0.25	1.72	0.00	0.02	0.01	0.10	0.01	0.00	0.06	1.76	0.00
<b>MCF-7</b>	0.05	11.67	0.00	0.10	0.01	0.14	0.00	0.00	0.00	0.02	19.09
<b>T47-D</b>	0.18	0.89	0.00	0.03	0.01	0.15	0.01	3.12	0.00	0.10	2.45
<b>MDA-MB361</b>	0.04	15.14	0.00	0.10	0.02	0.16	0.03	0.00	0.00	0.13	2.97
<b>HCC1500</b>	0.05	18.63	0.01	0.63	0.10	0.34	0.02	0.17	0.05	0.00	4.63
<b>Median</b>	0.05	12.27	0.00	0.07	0.02	0.15	0.02	0.09	0.02	0.06	2.71
<b>CXCL8 ++</b>											
<b>BT-20</b>	0.03	359.52	0.00	0.01	0.02	0.59	1.97	530.60	0.00	0.00	0.15
<b>MDA-MB157</b>	1.67	78.79	1.90	1.52	4.35	0.44	0.24	0.00	0.00	0.00	0.00
<b>MDA-MB231</b>	0.22	3396.69	0.00	33.13	0.89	4.44	1.55	10.42	0.00	0.01	0.02
<b>MDA-MB435</b>	5.19	24.33	0.00	0.00	0.01	0.09	0.00	0.00	0.00	0.00	0.01
<b>MDA-MB436</b>	0.26	6038.25	15.94	14.12	43.76	8.63	4.04	41.11	0.00	0.00	0.00
<b>ZR-75-1</b>	0.00	48.33	0.01	0.10	0.01	0.12	0.01	0.00	0.75	0.00	2.74
<b>Median</b>	0.24	219.15	0.01	0.81	0.45	0.52	0.89	5.21	0.00	0.00	0.01

**Table 7:** Chemokine levels in non cancerous cells

	<b>CXCL8</b>	<b>CXCL6</b>	<b>CXCL4V1</b>	<b>CXCL1</b>	<b>CXCL4</b>	<b>CXCL7</b>
<b>Blood Circulating Cells</b>	13.5	0.7	68.1	195.4	1112.8	284.0
<b>Endothelial cells</b>	289.0	1.2	0.0	52.0	0.2	0.0
<b>Intra-tumor Fibroblasts</b>	103.2	0.9	0.1	17.9	0.2	2.0
<b>Tumoral Epithelial cells</b>	726.6	7.7	0.4	75.0	2.9	0.2
	<b>CXCL5</b>	<b>CXCL3</b>	<b>CXCL2</b>	<b>CXCR1</b>	<b>CXCR2</b>	
<b>Blood Circulating Cells</b>	24.1	79.3	63.1	4389.9	3769.1	
<b>Endothelial cells</b>	1.7	18.5	39.7	0.3	0.1	
<b>Intra-tumor Fibroblasts</b>	16.3	10.3	9.2	0.2	0.8	
<b>Tumoral Epithelial cells</b>	153.3	22.8	1.1	0.9	0.3	

**Table 8:** Effect of TNF- $\alpha$  treatment on cytokine RNA expression in MCF-7 and MDA-MB-231 cells <sup>a</sup>

**MCF-7**

	<b>CXCL8</b>	<b>CXCL1</b>	<b>CXCL3</b>	<b>CXCL2</b>	<b>EREG</b>	<b>AREG</b>	<b>ADAMTS3</b>
C	1.0	1.0	1.0	1.0	0	1.0	1.0
20 min	2.9	18.7	60.9	54.3	0	1.3	0.9
1h	32.2	66.3	47.2	106.1	0	1.8	1.1
6h	45.7	28.9	10.3	16.4	0	1.6	2.9
18h	67.9	38.7	13.4	24.5	0	1.8	5.7

**MDA-MB231**

	<b>CXCL8</b>	<b>CXCL1</b>	<b>CXCL3</b>	<b>CXCL2</b>	<b>EREG</b>	<b>AREG</b>	<b>ADAMTS3</b>
C	1.0	1.0	1.0	1.0	1.0	1.0	1.0
20 min	17.4	28.2	60.5	56.5	1.0	1.0	1.1
1h	131.6	82.1	91.8	47.9	2.2	0.9	1.3
6h	53.8	31.1	14.0	12.2	1.4	0.8	2.7
18h	74.5	32.9	15.3	14.7	1.6	1.0	1.5

<sup>a</sup> MCF-7 and MDA-MB231 cells were treated with TNF- $\alpha$  as described in fig. 2 and RNA were collected at the indicated times to measure the RNA levels of CXCL8, CXCL1, CXCL3, CXCL2, EREG and AREG.



**Table 9:** Correlations between CXCL8 and AP-1 and NF-kB pathways

	Target	<b>CXCL8 -/+</b> (n=8)	<b>CXCL8 ++</b> (n=8)	p <sup>a</sup>	ROC-AUC <sup>b</sup>
CXCL8	?	1,0 (0,35-2,39)	54,08 (15,08-103,97)	< 0,01	1,000
TNC	NFKB/AP1	1,0 (0,64-2,62)	8,76 (1,27-13,59)	< 0,01	0,969
MMP1	AP1	1,0 (0,00-42,39)	26,30 (2,68-547,09)	< 0,01	0,922
GADD45B	NFKB	1,0 (0,29-2,89)	2,62 (1,22-3,96)	< 0,01	0,922
ATF3	AP1	1,0 (0,46-2,52)	4,14 (0,93-12,25)	< 0,01	0,906
c-JUN	AP1	1,0 (0,19-1,62)	1,58 (1,22-3,86)	< 0,05	0,875
JUNB	AP1	1,0 (0,38-1,56)	1,63 (1,03-2,14)	< 0,05	0,859
MMP9	NFKB/AP1	1,0 (0,16-5,21)	4,25 (0,94-14,79)	< 0,05	0,828
BCL2A1	NFKB	1,0 (0,26-7,54)	3,78 (0,59-10,19)	> 0,05	0,781
IER3S	NFKB	1,0 (0,39-3,54)	2,39 (1,08-4,01)	> 0,05	0,750
VEGF	NFKB	1,0 (0,29-3,42)	2,09 (0,35-10,04)	> 0,05	0,719
NFKBIB	NFKB	1,0 (0,46-1,44)	1,12 (0,77-2,43)	> 0,05	0,695
FOS	AP1	1,0 (0,14-1,87)	1,60 (0,24-2,90)	> 0,05	0,672
MAP3K8	AP1	1,0 (0,63-2,50)	1,84 (0,69-4,03)	> 0,05	0,672
IKBKB	NFKB	1,0 (0,62-3,38)	1,41 (0,89-3,22)	> 0,05	0,672
NFKBIA	NFKB	1,0 (0,50-2,71)	1,54 (1,03-2,05)	> 0,05	0,672
JUND	AP1	1,0 (0,24-5,13)	1,73 (0,53-4,94)	> 0,05	0,641
DRT	AP1	1,0 (0,38-3,62)	1,35 (0,45-4,22)	> 0,05	0,641
CHUK	NFKB	1,0 (0,73-2,55)	1,48 (0,30-3,67)	> 0,05	0,625
FOSB	AP1	1,0 (0,11-2,59)	1,0 (0,15-8,08)	> 0,05	0,594
FOSL1	AP1	1,0 (0,56-1,69)	1,05 (0,32-3,46)	> 0,05	0,594
IKBKG	NFKB	1,0 (0,39-2,17)	1,10 (0,46-2,17)	> 0,05	0,570
CD40LG	NFKB	1,0 (0,16-5,08)	1,48 (0,06-2,61)	> 0,05	0,563
NLK	NFKB	1,0 (0,35-3,17)	1,08 (0,44-2,70)	> 0,05	0,531
IL12B	NFKB	1,0 (0,00-11,06)	1,41 (0,00-9,46)	> 0,05	0,508
CD40	NFKB	1,0 (0,42-5,73)	1,24 (0,25-1,90)	> 0,05	0,500

<sup>a</sup>Mann and Whitney's U Test

<sup>b</sup>ROC (Receiver Operating Characteristics) - AUC (Area Under Curve) analysis

<sup>c</sup>Median (range) of gene mRNA levels

**Table 10:** CXCL8 is highly expressed in distant metastases

	CXCL8	CXCL1	CXCL2	CXCL3	CXCL5	CXCL6
Grade I (n=11)*	0.91 (0.16 - 3.14)	0.38 (0.12 - 0.96)	0.04 (0.01 - 0.23)	0.19 (0.06 - 0.44)	0.13 (0.01 - 1.10)	0.10 (0.03 - 0.99)
Grade III (n=37)*	1.67 (0.01 - 125.29)	0.36 (0.04 - 18.68)	0.03 (0.01 - 0.40)	0.20 (0.02 - 4.94)	0.13 (0.01 - 8.68)	0.08 (0.02 - 1.40)
Metastases (n=26)*	11.34 (0.02 - 260.62)	1.11 (0.11 - 6.10)	0.22 (0.00 - 0.84)	0.54 (0.09 - 1.95)	0.49 (0.06 - 26.31)	0.64 (0.06 - 3.54)

\* = versus normal