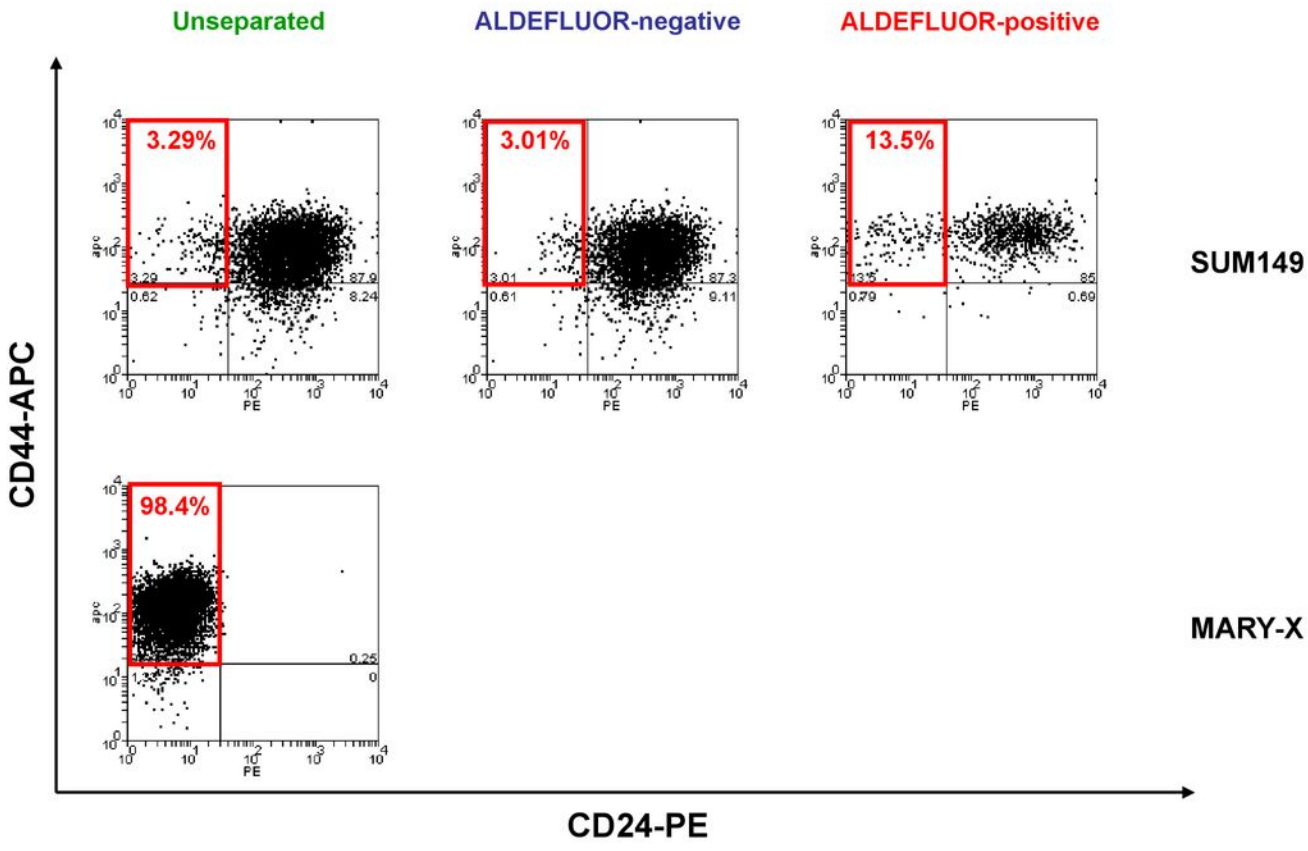
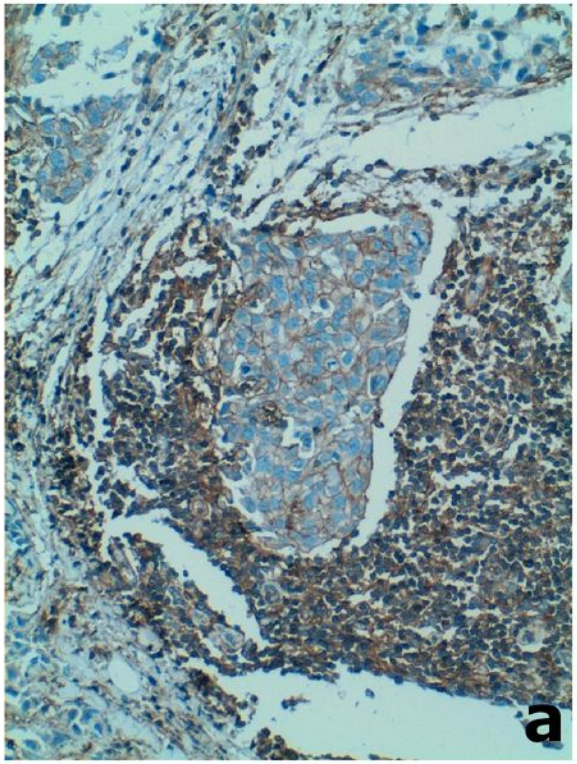
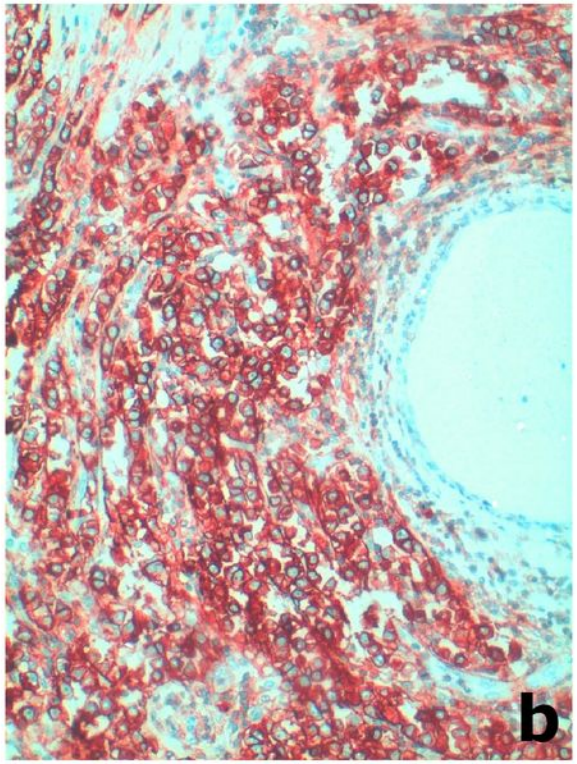
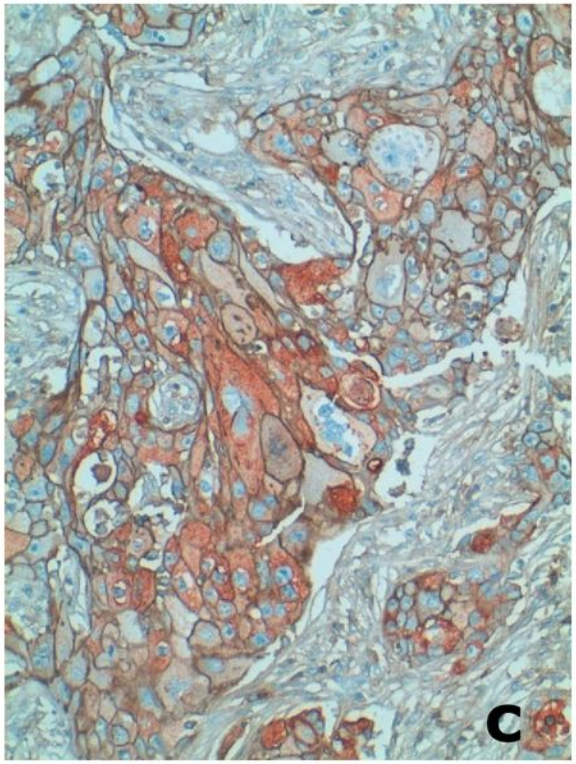


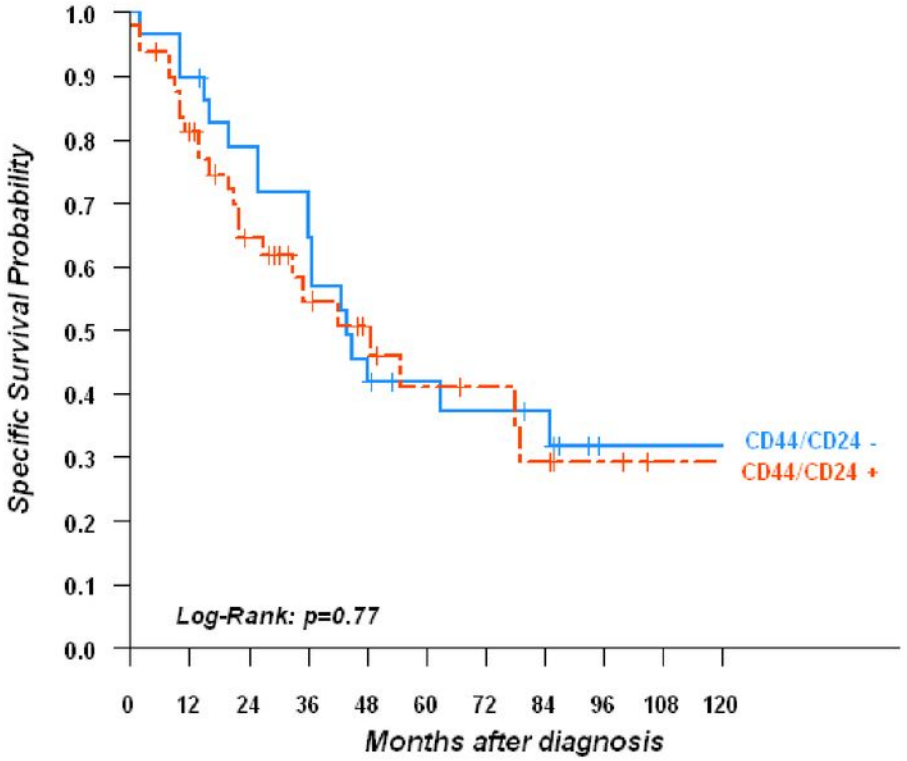
Supplementary Figure 1

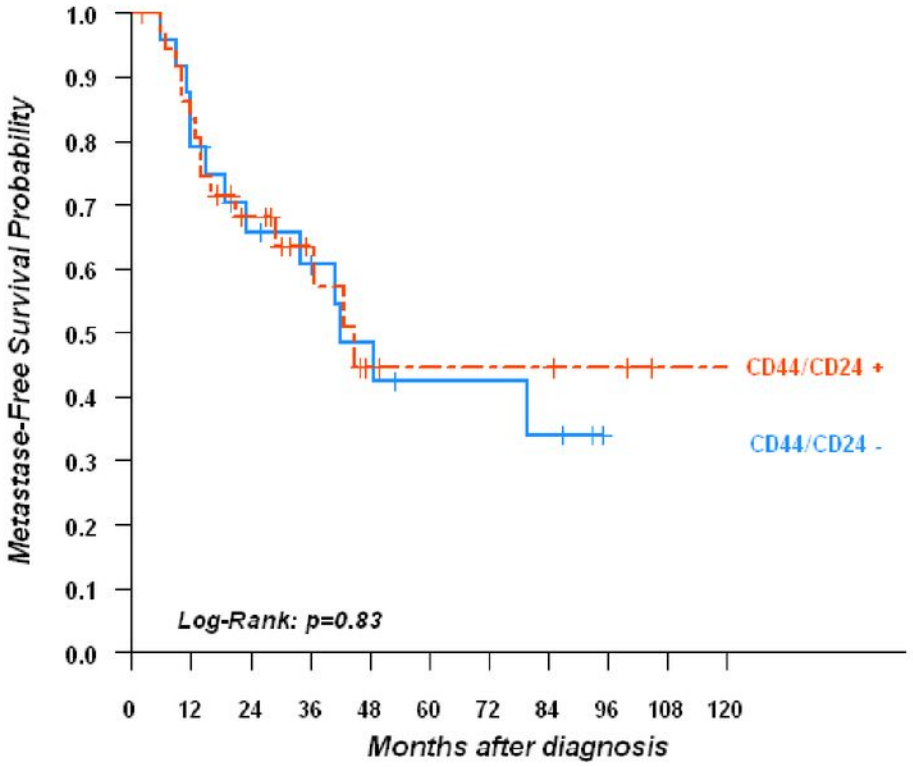






Supplementary Figure 2





Supplementary Figure 3

Supplementary Figure 1. Overlap between the cell populations identified by the ALDEFLUOR assay and the $CD44^+/CD24^-$ phenotype. ALDEFLUOR-negative and ALDELFUOR-positive cells from SUM149 were separated by FACS using the ALDEFLUOR assay. Cells were then fixed in RNA later, immunostained with a CD24–PE antibody, a CD44–APC antibody. Flow cytometry analysis of the SUM149 tumors showed that the ALDEFLUOR-positive population presented an enrichment of the CD44+/CD24- population, with 13.5% of CD44+/CD24- cells in the ALDEFLUOR-positive population compared to only 3.02% in the ALDEFLUOR-negative population. Because MARY-X cells presented more than 98% of cells with the CD44+/CD24- phenotype we did not investigated the overlap between the both phenotypes.

Supplementary Figure 2. The CD44/24 staining in IBC.

a.CD44 staining only at the membrane of tumoral cells. Note the staining in surrounding stromal lymphocytes. **b.**CD24 staining in cytoplasm of tumoral cells. **c.**CD24 and CD44 double staining in 80% of tumoral cells, the remaining 20% express only CD44 at the membrane.

Supplementary Figure 3. The $CD44^+/CD24^-$ phenotype in IBC patient tumors is not associated with the development of metastasis and with decreased survival.

Kaplan-Meier survival curves according to $CD44^+/CD24^-$ phenotype. The presence of $CD44^+/CD24^-$ cells in IBC tumors is not associated with decreased SS and MFS in IBC patients.

Supplementary material and methods

Animal model and test of tumorigenicity

Tumorigenicity of ALDELFUOR-positive, -negative and unseparated SUM149 and MARY-X cells was assessed in NOD/SCID mice. Fat pads were cleared at three weeks of age and humanized by injecting a mixture of irradiated and non-irradiated immortalized human fibroblasts (1:1 irradiated:non-irradiated, 50,000 cells/100µl Matrigel/fat pad) as described in Kupperwasser et al. (Kuperwasser et al., 2004). Irradiated fibroblasts (4Gy) support growth of cancer epithelial cells by secreting a variety of growth factors, collagen directly interacting with the epithelial cells (Orimo et al., 2005; Tlsty et al., 2001). Primary human mammary fibroblasts were immortalized by transfection with a retrovirus construct expressing telomerase. The fibroblast cell line is a generous gift from Dr. John Stingl and Dr. Connie Eaves (Terry Fox Laboratory, Vancouver, British Columbia, Canada). SUM149 or MARY-X cells were mixed with Matrigel (BD biosciences) (1:1) and implanted in the cleared humanized mammary fat pads 2-4 weeks later. Using a caliper, the size of palpable tumors were measured each week in animals and the two main dimensions were reported.The animals injected with SUM149 or MARY-X cell lines were euthanized when the tumors were approximately 1.2 cm in the largest diameter and a portion of each fat pad was fixed in formalin and embedded in paraffin for histological analysis. The animal studies were approved by the ULAM committee for research in vertebrate animals.

CD44/CD24 staining

CD44/CD24 staining was performed as previously described (Al-Hajj et al., 2003). Briefly, cells were sorted based on the ALDEFLUOR phenotype

(ALDEFLUOR-positive, ALDEFLUOR-negative) and fixed in RNA later. Then, sorted cells were stained with primary antibodies anti-CD44 labeled APC (dilution 1:10, BD Biosciences), anti-CD24 labeled PE (dilution 1:10, BD Biosciences. Each population was analyzed separately utilizing flow cytometry.

For immunohistochemistry, double staining was performed as described (Honeth et al., BCR 2008). Antibodies for the detection of CD44 (Clone 156-3C11,1:800) and CD24 (Clone SN3b, 1:400) were purchased from Neomarkers (Fremont, CA, USA). A tumor was considered as positive for the CD44+/CD24- phenotype when at least one cancer cell displayed the expression of CD44 and the absence of CD24 expression.

Invasion assay

Assays were done in transwell chambers with 8μm pore polycarbonate filter inserts for 12-well plates (Corning, NY). Filters were coated with 30 μl of ice-cold 1:6 basement membrane extract (Matrigel, BD-Bioscience) in D-MEM/F12 incubated at least 1 hour at 37°C to solidify the Matrigel layer. SUM149 cells were added to the upper chamber in 200 μl of serum-free medium. For the invasion assay, 5000 cells were seeded on the Matrigel-coated filters and the lower chamber was filled with 600 μl of full medium supplemented with 10% human serum (Cambrex). After 48 hours incubation, the cells on the underside of the filter were counted using light microscopy. Assays were done in triplicate or quadruplicate.

Patients and tissues

IBC patients were selected from computerized clinico-pathological databases of Institut Paoli-Calmettes between 1976 and 2003. IBC was clinically defined as T4d tumor (TNM, UICC); the presence of dermal lymphatic emboli was not mandatory for IBC definition. One hundred and nine patients with IBC with a median follow-up of 67 months were included. All samples were collected prior to chemotherapy on surgical biopsy or lumpectomy. All patients had received primary chemotherapy including anthracyclin in 89 cases, taxane in 19 cases and high-dose chemotherapy with hematopoietic stem cell support in 60 cases. After chemotherapy, 68 patients underwent mastectomy. External beam radiation therapy and tamoxifen was delivered in 95% and 50% cases, respectively.

Sample evaluation

Clinical data were obtained from medical records. Morphological criteria were reviewed independently by two pathologists (JJ, ECJ), according to guidelines established by the World Health Organization. The presence of dermal lymphatic emboli was noticed in 18 out of the 78 cases with available skin biopsy.

Immunohistochemistry and antibodies

Expression of ALDH1, BCL2, E-cadherin, ER, MIB1, ERBB2, MUC1, and PR was studied by IHC. The characteristics of the antibodies used and the details of the technique are listed in supplementary Table 1. IHC was done as previously described on 5 μm paraffin sections. Briefly, after deparaffinization, slides were pre-treated according to the supplier's recommendations (Table 1), transferred to a Dako autostainer except for ALDH1 and MUC1 which were stained manually. This was followed by the use of a streptavidin/biotin kit (Dako, Trappes 78196, France).

Diaminobenzidine (DAB) or 3-amino-9-ethylcarbazole (AEC) was used as chromogen. Sections counterstained with hematoxylin were evaluated by light microscopy by two independent observers. Immunoreactivities were scored by multiplying the percentage of positive cells by the intensity, i.e. by the so-called quick-score (Q) ($Q=P \times I$; maximum=300) with cut-off values in accordance with previous studies. For ALDH1, 80 out of 109 cases had tissue available for analysis with staining scored as positive if greater than 1% of cells displayed cytoplasmic staining.

Supplementary Table 1: List of Proteins Tested by Immunohistochemistry and Characteristics of the Corresponding Antibodies

Protein	Antibody	Origin	Clone	Pretreatment	Titer
Aldehyde dehydrogenase 1 ALDH1	Mmab	BD Biosciences	44	Citrate buffer (40 min, 98°C)	1/50
Anti-apoptotic BCL2	Mmab	Dako Corporation	124	Citrate buffer (40 min, 98°C)	1/100
Tyrosine kinase receptor ERBB2	Mmab	Novocastra Laboratory	CB 11	Citrate buffer (40 min, 98°C)	1/500
CD24	Mmab	Neomarkers	SN3b	Citrate buffer (40 min, 98°C)	1/400
CD44	Mmab	Neomarkers	3C11	Citrate buffer (40 min, 98°C)	1/800
E-cadherin (CDH1)	Mmab	Transduction Laboratory	36	Citrate buffer (40 min, 98°C)	1/2000
Estrogen receptor (ER)	Mmab	Novocastra Laboratory	6F11	Citrate buffer (40 min, 98°C)	1/60
MIB1 / Ki67	Mmab	Dako Corporation	Ki-67	Citrate buffer (40 min, 98°C)	1/100
MUC1	Mmab	Transgen	H23	Citrate buffer (40 min, 98°C)	1/1000
Progesterone receptor (PR)	Mmab	Dako Corporation	PgR 636	Citrate buffer (40 min, 98°C)	1/80

Supplementary Table 2.

	Primary Tumors/injections		
Cells injected/fat pad	**$5x10^4$**	**5,000**	**500**
SUM149			
ALDEFLUOR-negative	0/3	0/3	0/3
ALDEFLUOR-positive	3/3	3/3	3/3
Unseparated	3/3	---	2/3
MARY-X			
ALDEFLUOR-negative	0/3	0/3	0/3
ALDEFLUOR-positive	3/3	3/3	2/3
Unseparated	3/3	---	1/3

Supplementary Table 3. Correlations between presence of $CD44^+/CD24^-$ phenotype and histo-clinical factors in inflammatory breast carcinomas.

	$CD44^+/CD24^-$ absent	$CD44^+/CD24^-$ present	*p*-value
	No. of patients (%)		
Age (years)			
<45	7 (25)	11 (22)	NS
≥45	22 (75)	38 (78)	
Axillary lymph node status			
negative	1 (4)	23 (40)	**0.003**
positive	23(96)	34 (60)	
SBR grade			
I	0 (0)	3 (6)	NS
II	6(21)	9 (18)	
III	23(79)	37 (76)	
BCL2			
negative	17 (77)	25 (61)	NS
positive	5 (23)	16 (39)	
E-Cadherin			
negative	12 (55)	24 (75)	NS
positive	10 (45)	8 (25)	
ER			
negative	10 (50)	13 (53)	NS
positive	10 (50)	14 (47)	
ERBB2			
0-1	8 (42)	21 (58)	NS
2-3	11 (58)	15 (42)	
Ki67			
≤20	16 (80)	10 (38)	**0.01**
>20	4 (20)	16 (62)	
MUC1			
negative	1 (5)	6 (16)	**0.02**
positive	19 (95)	32 (84)	
PR			
negative	11 (58)	16 (59)	NS
positive	8 (42)	12 (41)	