UNIVERSITÀ DI PISA

ANIMALI E UOMO CONTRO IL CANCRO: ATTUALITA' E NUOVE PROSPETTIVE

Giovedì 21 Marzo 2013

Tumori della mammella nell'uomo e nell'animale

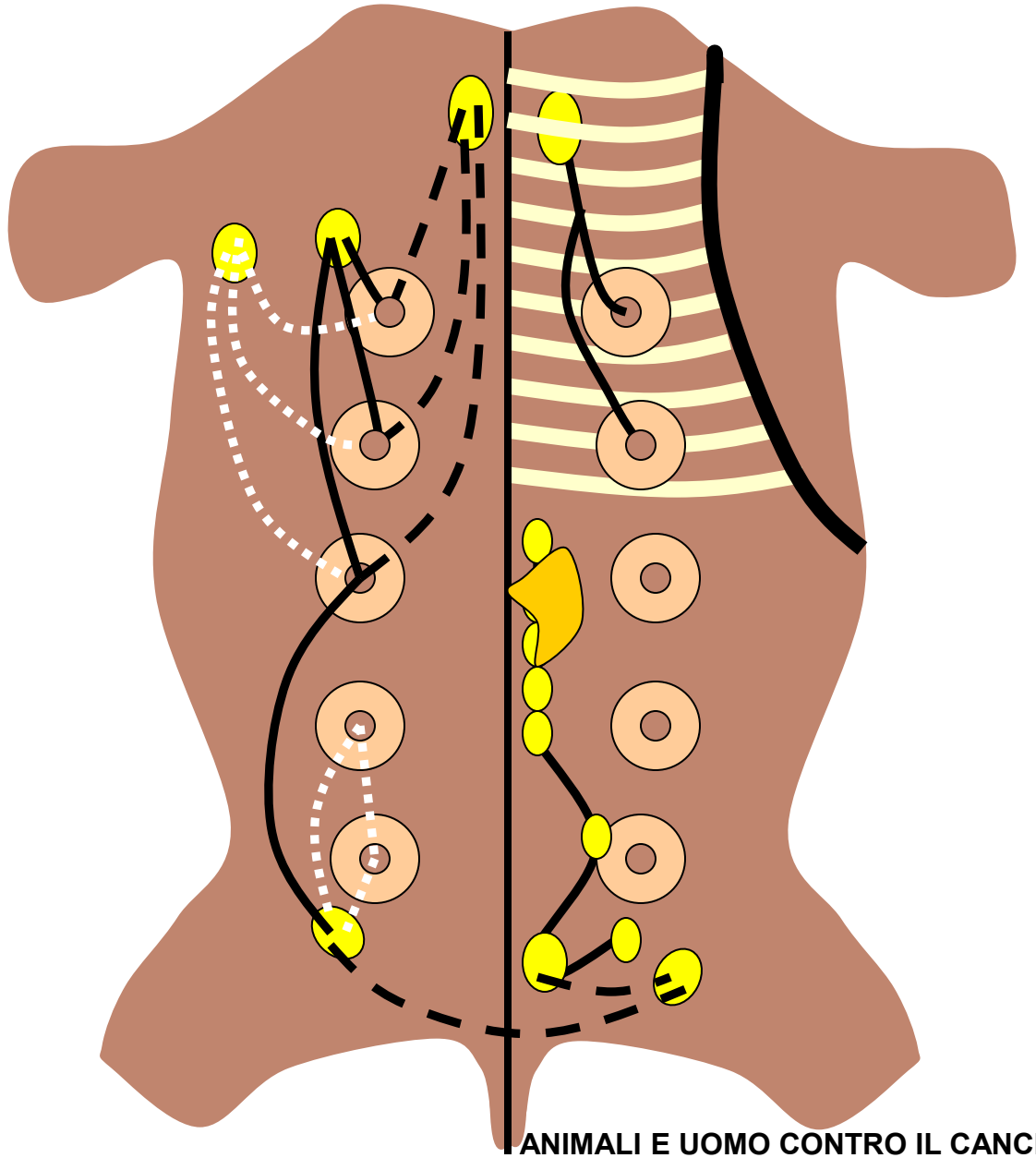
Prof. Alessandro Poli
Dipartimento di Scienze Veterinarie - Università di Pisa
apoli@vet.unipi.it

- Le malattie neoplastiche sono la seconda causa di morte nell'uomo e la prima nel cane e gatto

- Le neoplasie spontanee negli animali domestici interessante opportunità in oncologia comparata

- Nel cane e gatto:
  - Progressione più rapida della patologia neoplastica
  - Condivisione gli stessi fattori di rischio dell'uomo
  - Impiego di nuove terapie eticamente più accettabile negli animali che nell'uomo
  - Similitudini biologiche, anatomiche, istopatologiche, genetiche e molecolari

- Anatomia comparata della ghiandola mammaria
- Epidemiologia e Fattori di Rischio
- Aspetti clinici
- Aspetti istopatologici
- Fattori prognostici
- Aspetti molecolari



- Anatomia comparata della ghiandola mammaria
- Epidemiologia e Fattori di Rischio
- Aspetti clinici
- Aspetti istopatologici
- Fattori prognostici
- Aspetti molecolari

- Complessa serie di neoplasie riportata con elevata frequenza nel cane e gatto
- Solo raramente segnalate nelle altre specie
- La neoplasia più frequente nel cane
- La terza per frequenza nella gatta

	DONNA	CAGNA	GATTA
Incidenza delle neoplasie mammarie «Age—adjusted» /100.00 individui	300	381	264
Incidenza per apparato	32%	52%	17%
Importanza delle neoplasie mammarie	1° tumore nella donna	1° tumore nella cagna	3° tumore nella gatta dopo linfo-emopoietico e cute

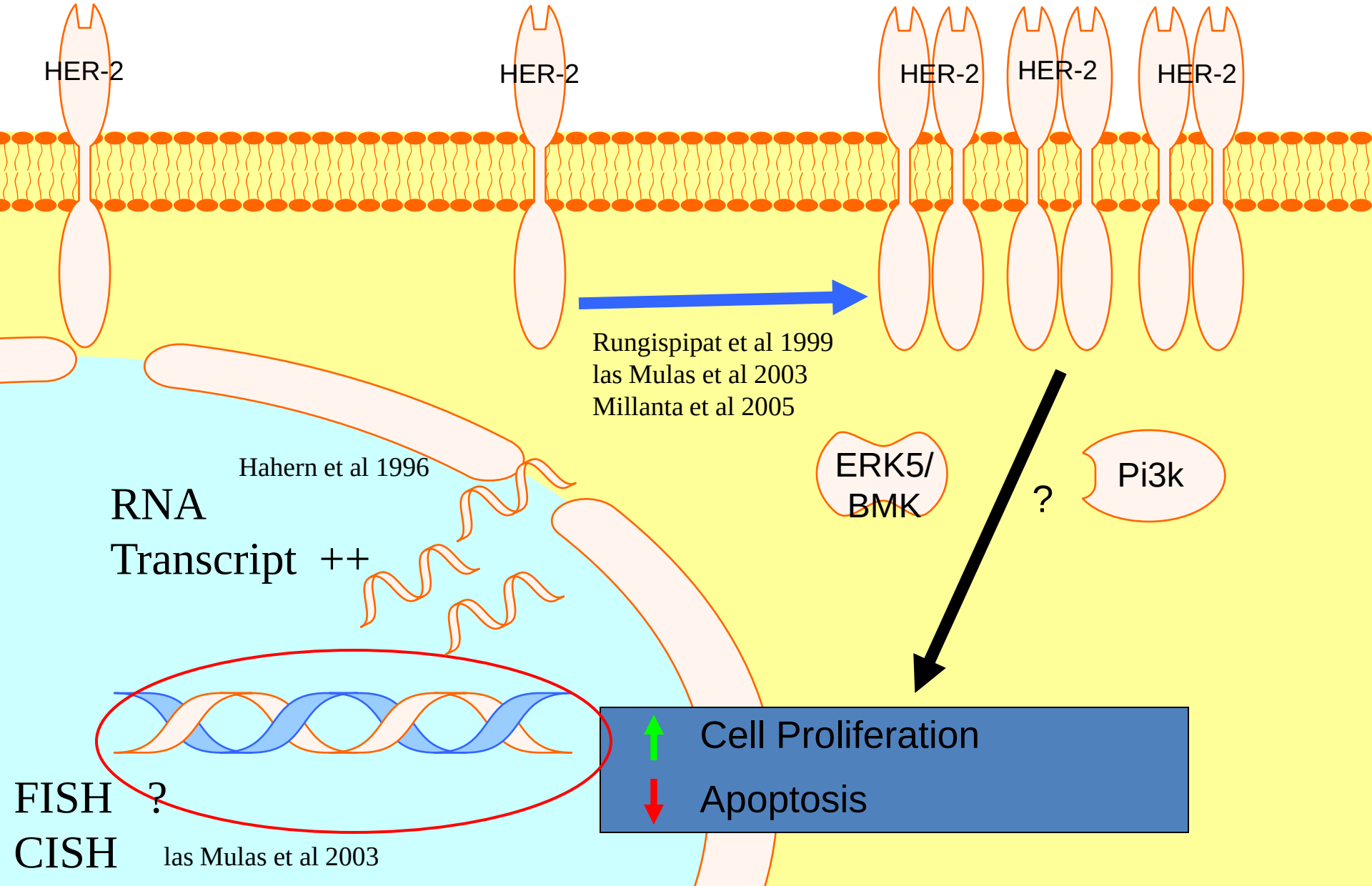
	CANE	GATTO
Età insorgenza	10-11 anni	10-12 anni
Predisposizione razza	Presente (Piccole/grandi 60/40)	Presente (Siamese)
Neoplasie maligne	41-53%	80-85%
Ormono-dipendenza	Presente	Assente (50% ancora PR +)

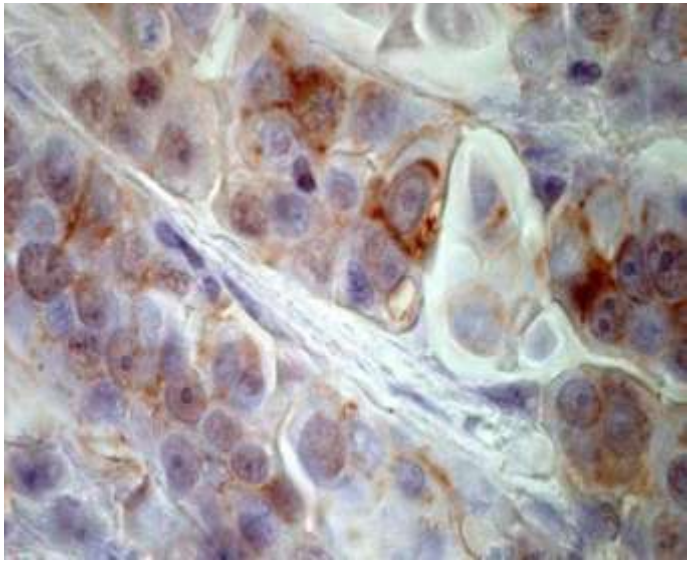
- Anatomia comparata della ghiandola mammaria
- Epidemiologia e Fattori di Rischio
- Aspetti clinici
- Aspetti istopatologici
- Fattori prognostici
- Aspetti molecolari

Aspetti molecolari e stato ormonale nelle neoplasie mammarie degli animali

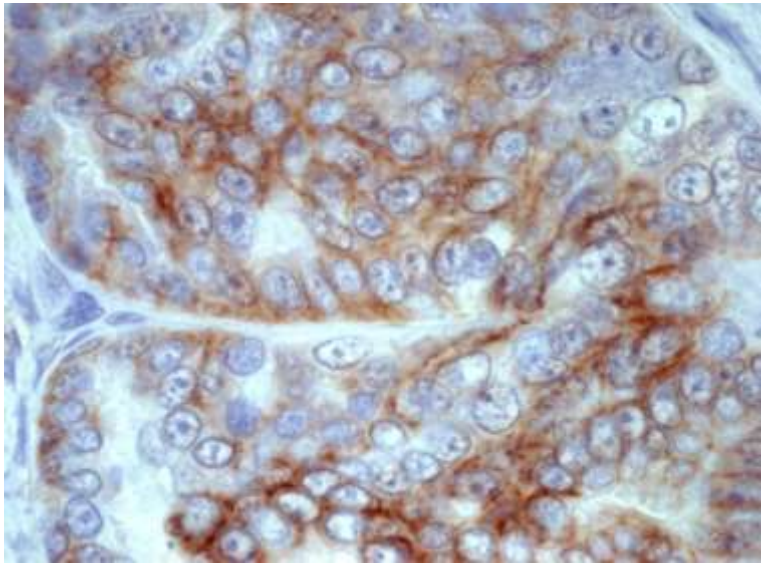
- HER-2
- Tyrosine chinase receptor family (MET e RON)
- Onco-soppressori (p53 and PTEN)
- Molecole di adesione (E-caderine e catenine)
- Molecole correlate alla proliferazione cellulare (Ki-67, cicline)
- VEGF
- Recettori per ormoni steroidei
- Recettori per le citochine
- Fenotipi molecolari

HER-2 Over-expression



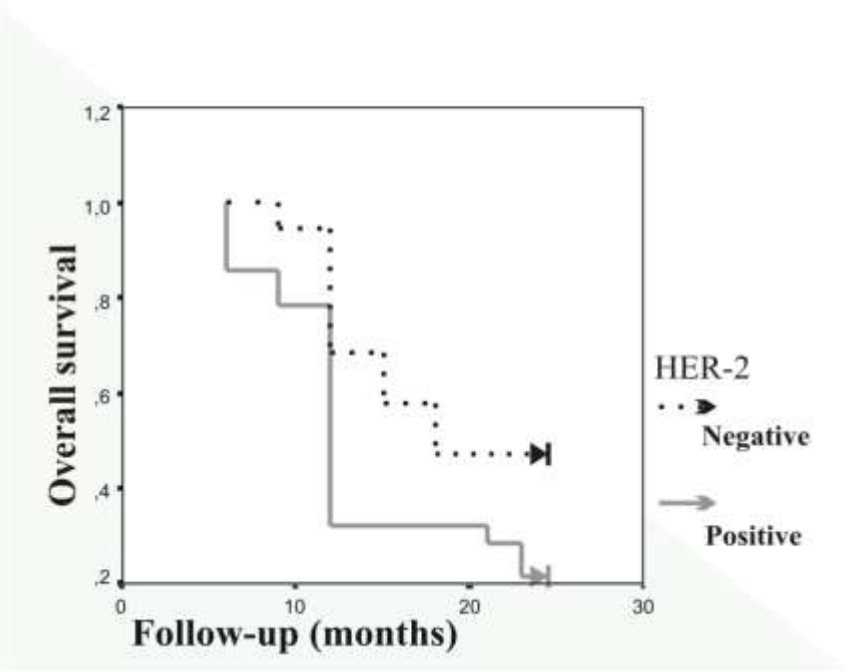


Gatto, HER-2 +2



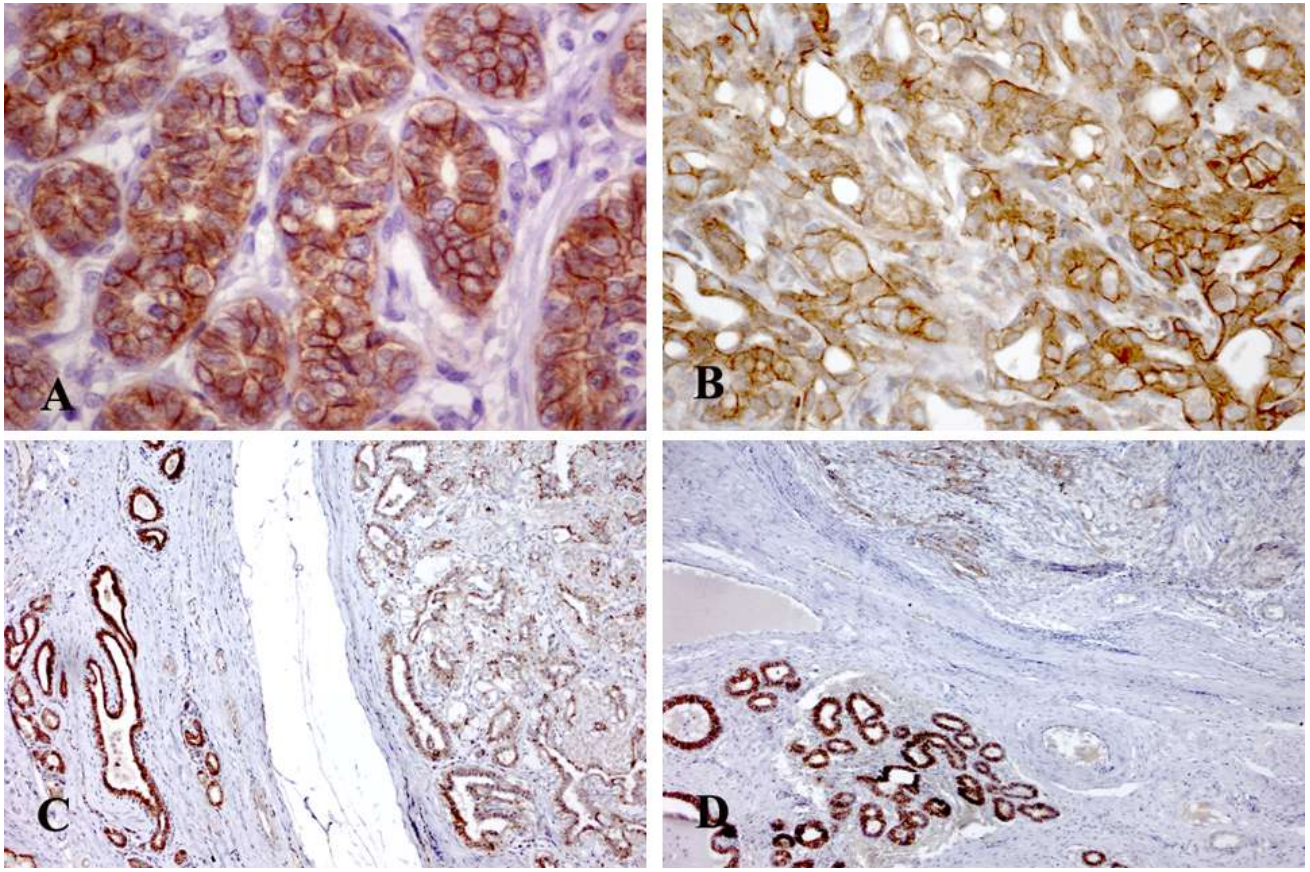
Gatto, HER-2 +3

Characteristic	P value
Histological type	>0.05
Tumour grading	>0.05
Lymphatic invasion	>0.05
Overall survival	0.02



Gatto, Correlazione tra HER-2 e OS

Millanta et al., Vet Pathol 42,30–34, 2005



Espressione di HER-2 nei carcinomi mammari della cagna, nelle diverse indagini varia tra il 15-30%

- Nella cagna, invece, l'over-espressione di HER-2 è associata ad una prognosi negativa quando legata a negatività per ER e l'espressione di uno dei marker per le cellule basali (P-caderina, p63 o citocheratina 5)
- In genere, quindi, non associato a prognosi peggiore

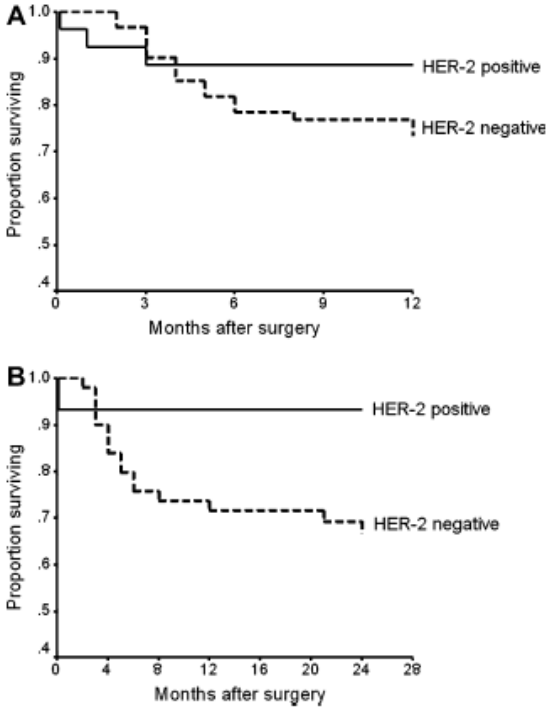
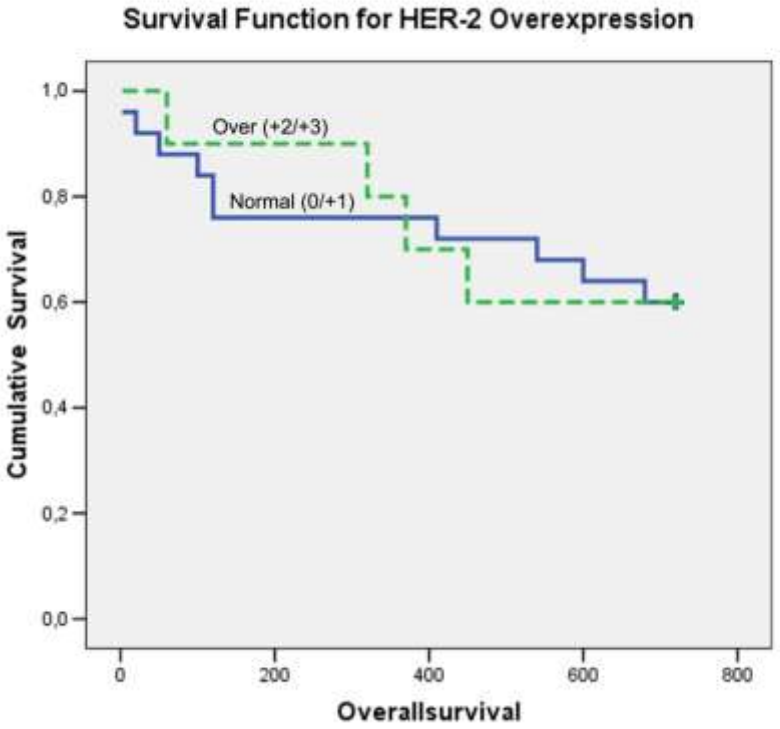


Fig. 3. Kaplan–Meier survival curves showing the relationship between HER-2 expression and (A) 1 year survival in 89 dogs; (B) 2 years survival in 66 dogs with malignant mammary tumours after surgery removal.

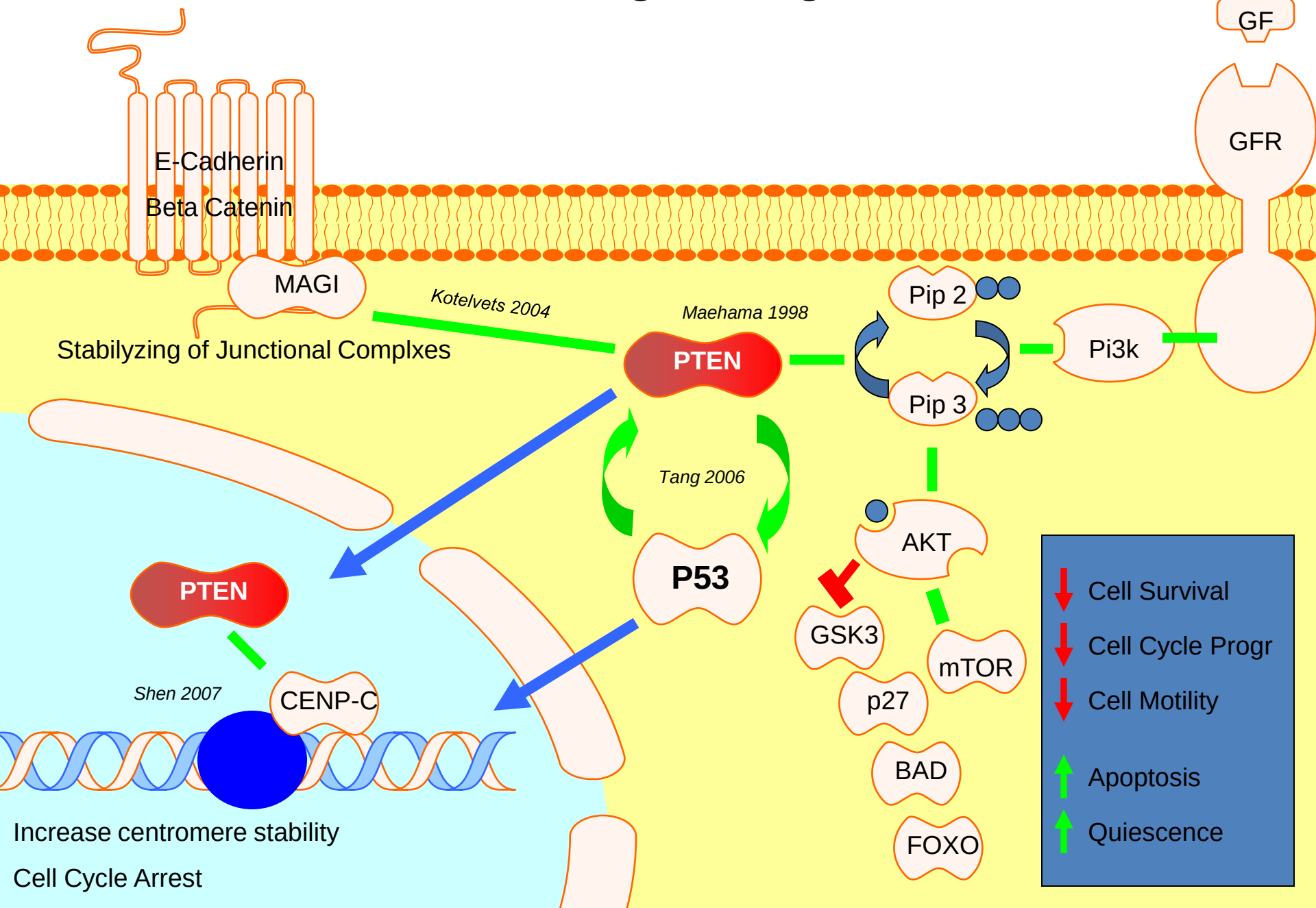
Hsu et al., 2007



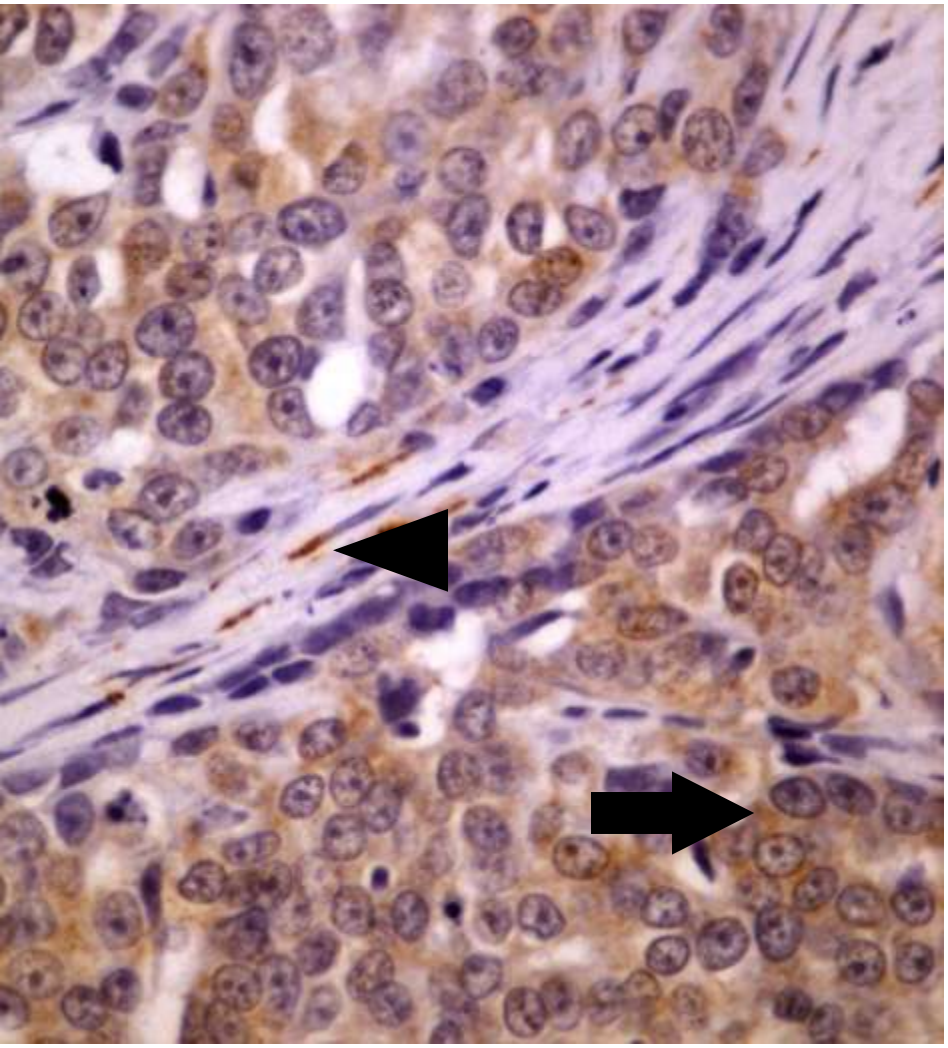
Poli et al., Res Vet Sci 2012

- Mutazioni della p53 sono frequentemente osservate nelle neoplasie mammarie della donna
- Over-espressione della proteine è stata segnalata nelle neoplasie mammarie feline
- 17.3% dei FMC presentano mutazioni della p53, mentre la proteina non modificata è sempre presente nelle forme benigne

Pten Signalling



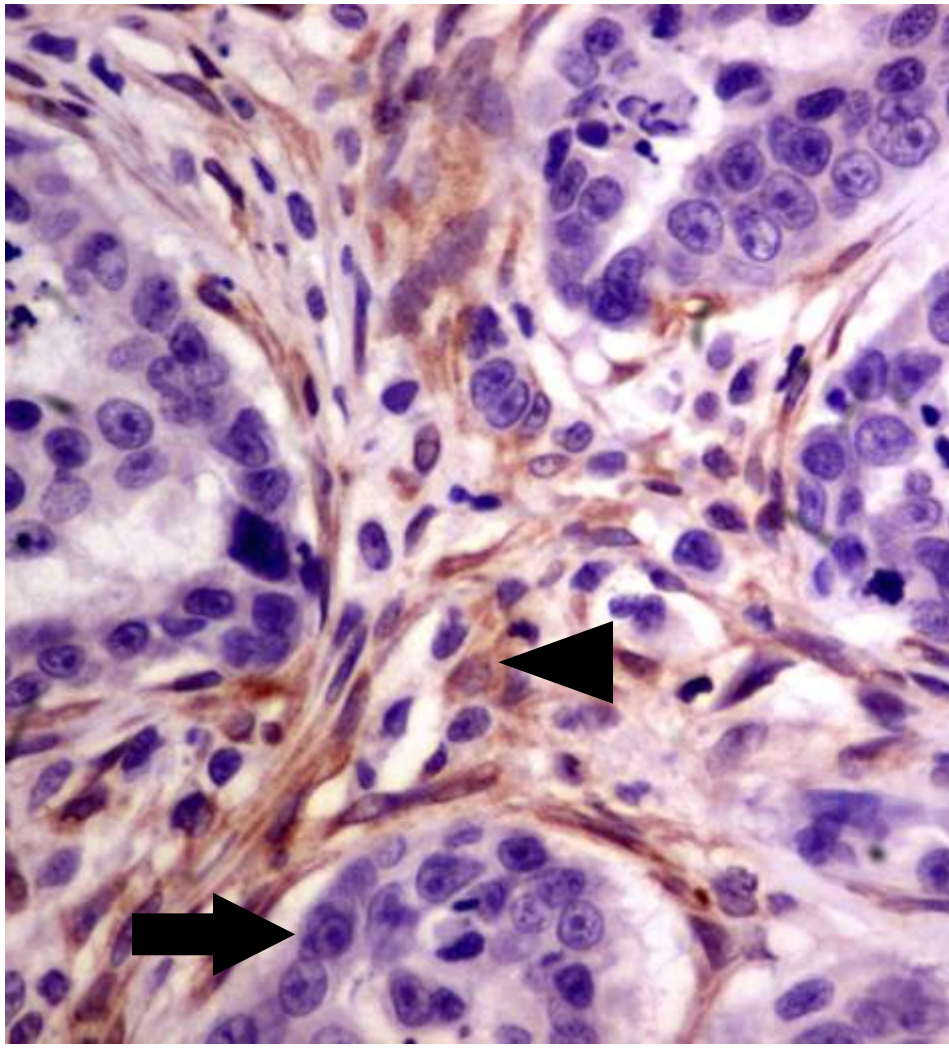
PTEN: Feline Mammary Carcinoma (FMC)



FMC

PTEN +

N = 4 (24%)



FMC

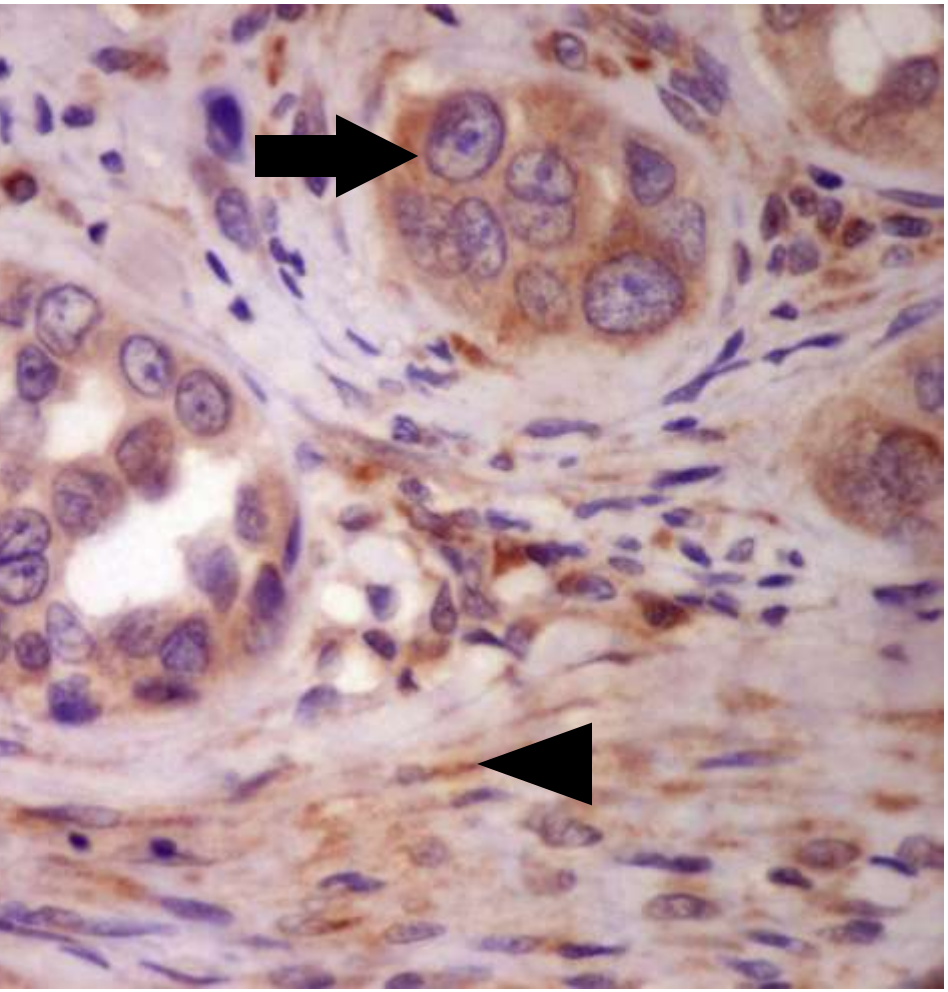
PTEN -

N = 13 (76%)

= Tumour

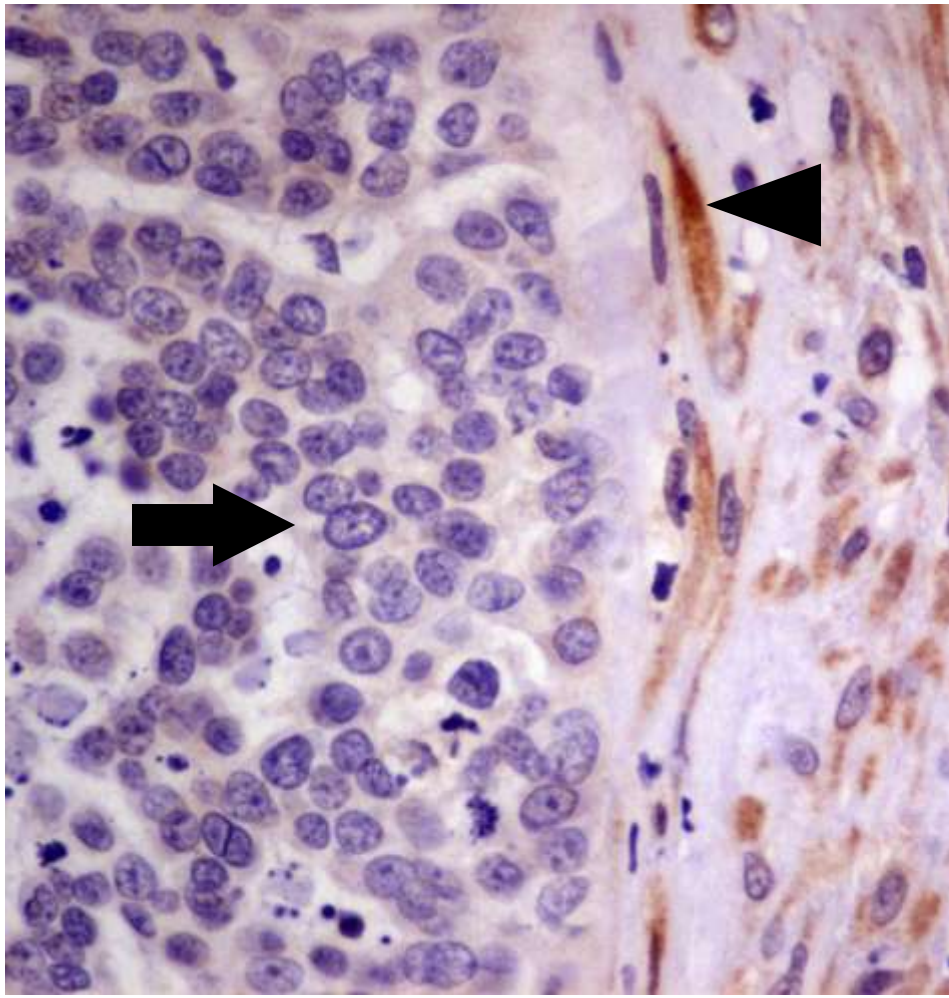
= Surrounding stroma

PTEN: Canine Mammary Carcinoma (CMC)



CMC PTEN +

N = 16 (67%)



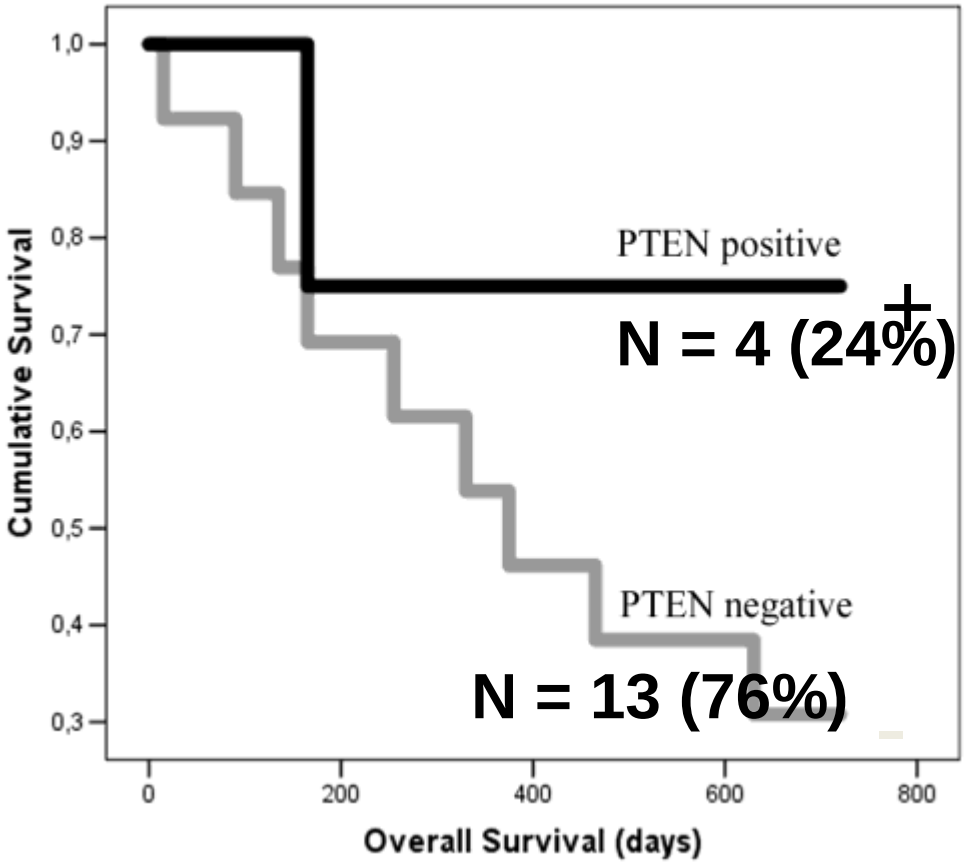
CMC PTEN -

N = 8 (33%)

= Tumour

= Surrounding stroma

Survival Curve for PTEN expression in n=37 FMCs



Ressel et al., Vet Pathol 46, 2009

- Le indagini per evidenziare una correlazione tra alti indici di proliferazione cellulare e maggiore malignità hanno dato esiti discordanti.
- Questo vale per:
  - Indice mitotico;
  - Indice di proliferazione (Ki-67);
  - Indice AgNOR
  - Espressione delle cicline

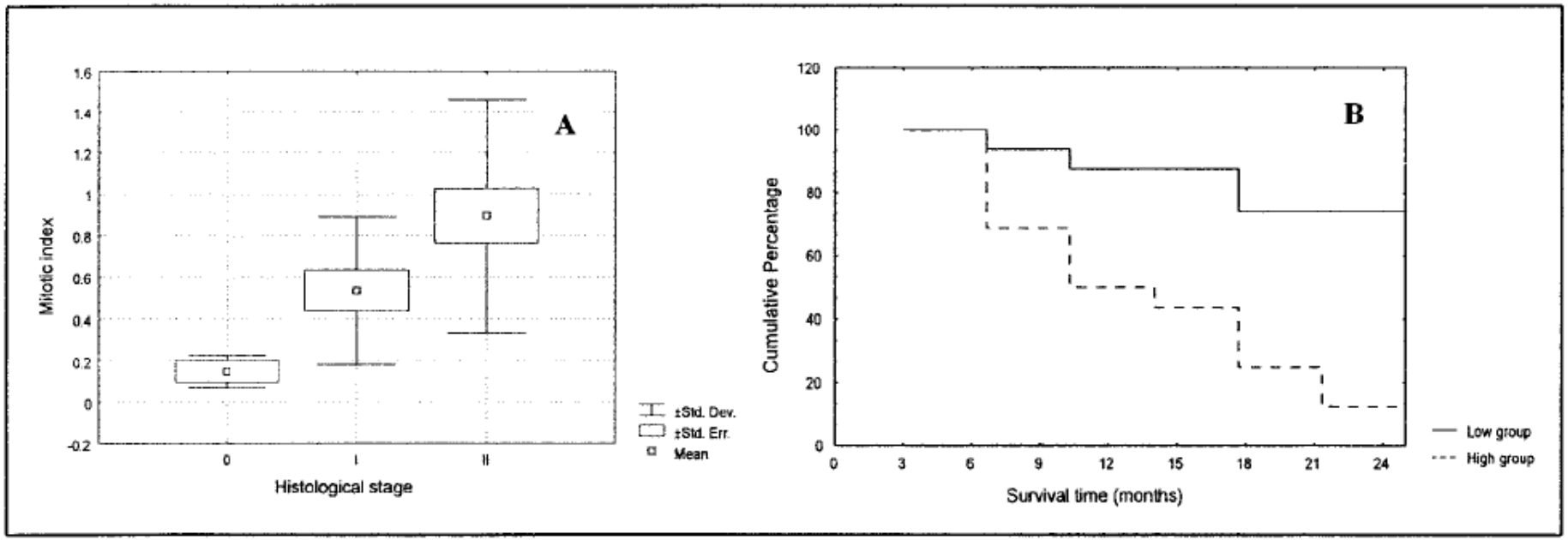
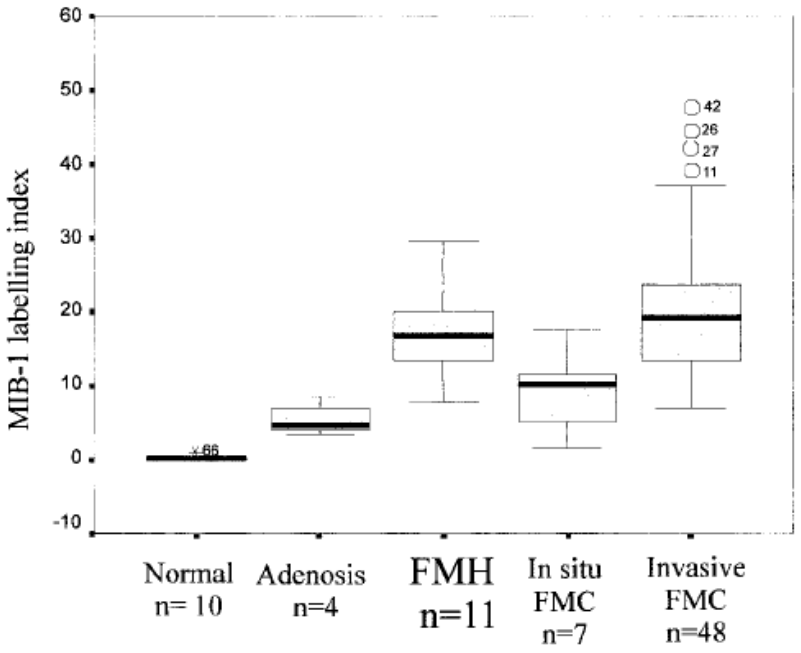


Figure 6. Relationship between mitotic index and histological stage and survival. Mitotic index reveals an ability **A**, to discriminate proliferative activity among the 3 histological stage groups (ANOVA, $P < 0.05$) and **B**, to be a prognostically useful variable. In the latter, cases are grouped by low and high apoptotic activity according to mitotic index median value, and the Kaplan–Meier estimated survival curves are compared (survival analysis, $P < 0.001$).



Tumor Characteristics	No. Cats	MIB-1 Index ≤18.7	MIB-1 Index >18.7	*P*
Histologic type[9]				0.266
Tubular	8	6	2	
Papillary cystic	4	3	1	
Papillary	22	11	11	
Solid	14	4	10	
Grading[5]*				0.824
WDC	16	10	6	
MDC	23	10	13	
PDC	9	4	5	
Lymphatic invasion				0.048
Yes	19	7	12	
No	29	17	12	
Tumor size				0.812
<2 cm	28	14	14	
≤2 cm	20	10	10	

* WDC = well-differentiated carcinoma; MDC = moderately differentiated carcinoma; PDC = poorly differentiated carcinoma.

Millanta et al., Vet Pathol 39,120–126, 2002

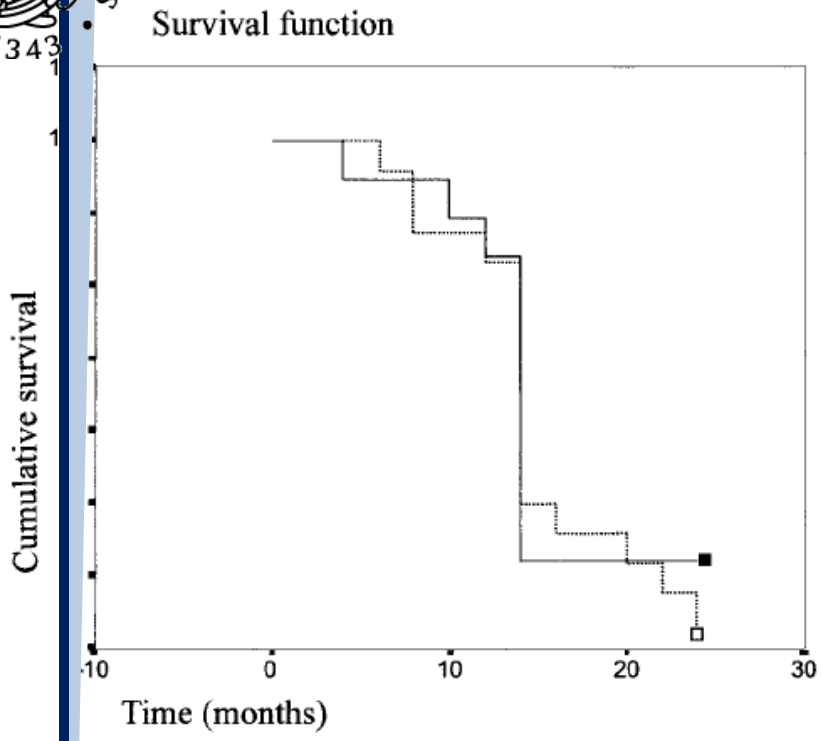


Fig. 6. Overall survival curves for the group of 48 queens with invasive carcinomas with low MIB-1 labeling index (≤18.7% □) and high MIB-1 labeling index (>18.7% ■) ($P = 0.0540$).

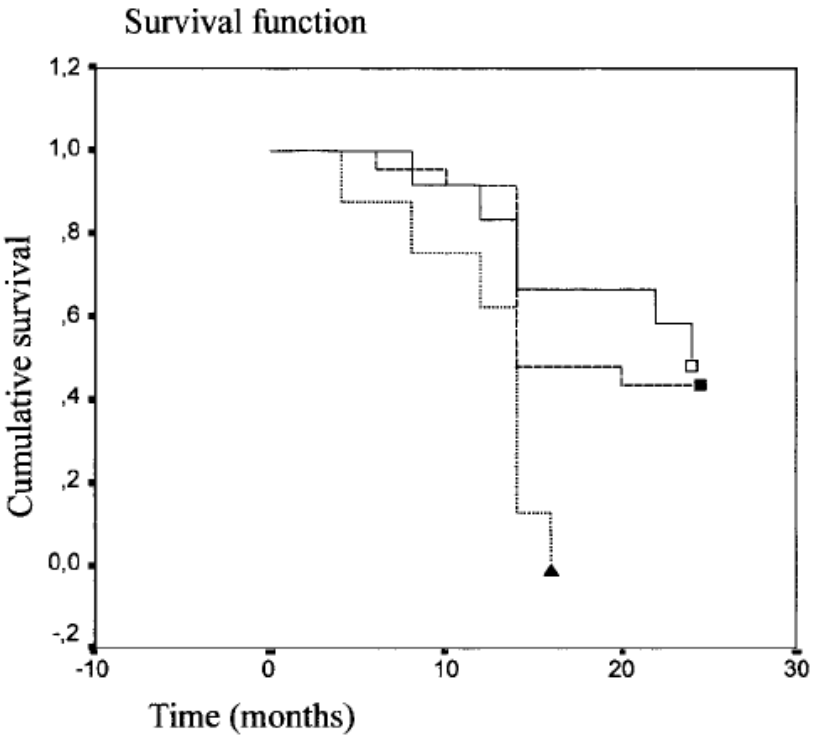


Fig. 7. Overall survival curves for the group of 48 queens with invasive carcinomas divided according to the tumor grade.[5] Queens had poorly differentiated carcinomas (▲), moderately differentiated carcinomas (■), or well-differentiated carcinomas (□) ($P = 0.0040$).

Millanta et al., Vet Pathol 39,120–126, 2002

33 gatte con carcinoma della mammella con follow up a 24 mesi

TABLE 2: Data on multivariate regression

	β	SE	t Value	Exponent β
Histological stage				
Stage I	1.36	0.54	2.50	3.89
Stage II				
Mitotic index				
$\leqslant$0.72	1.26	0.54	2.33	3.55
>0.72				
AgNOR index				
$\leqslant$3.19	1.17	0.56	2.07	3.22
$>$ 3.19				

Each of the three parameters showes a higher coefficient of regression (β) than its standard deviation, allowing to attribute them an independent prognostic significance.

Mitotic index

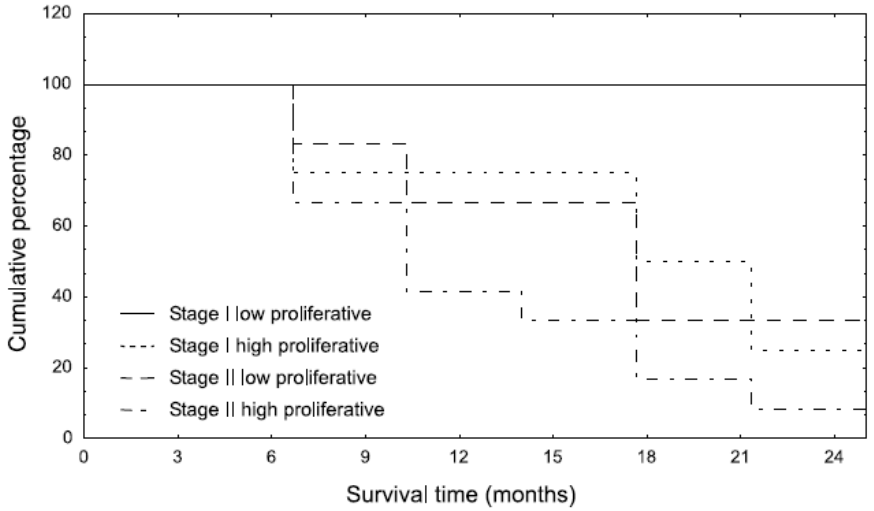
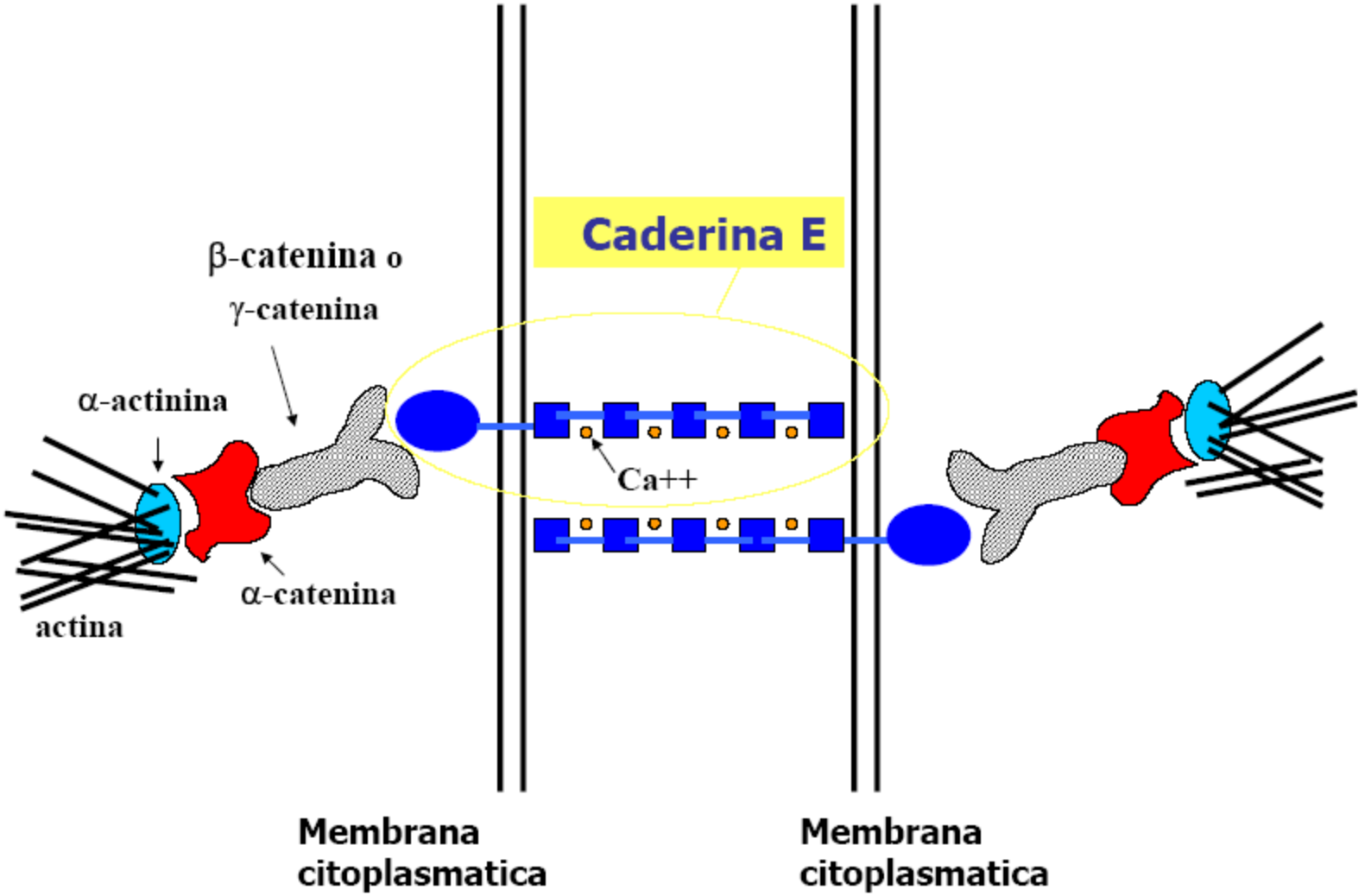


FIG 9: Kaplan and Meier survival curves of stage I and stage II cases each one grouped for low or high proliferative activity according to the median value of mitotic index. Survival analysis reveals a significant difference between the two stage I groups (log-rank test $P < 0.01$) and a non-significant post-surgical outcome in stage II groups (log-rank test $P = 0.062$).

Preziosi et al., Res. Vet. Sci., 73, 53–60, 2002

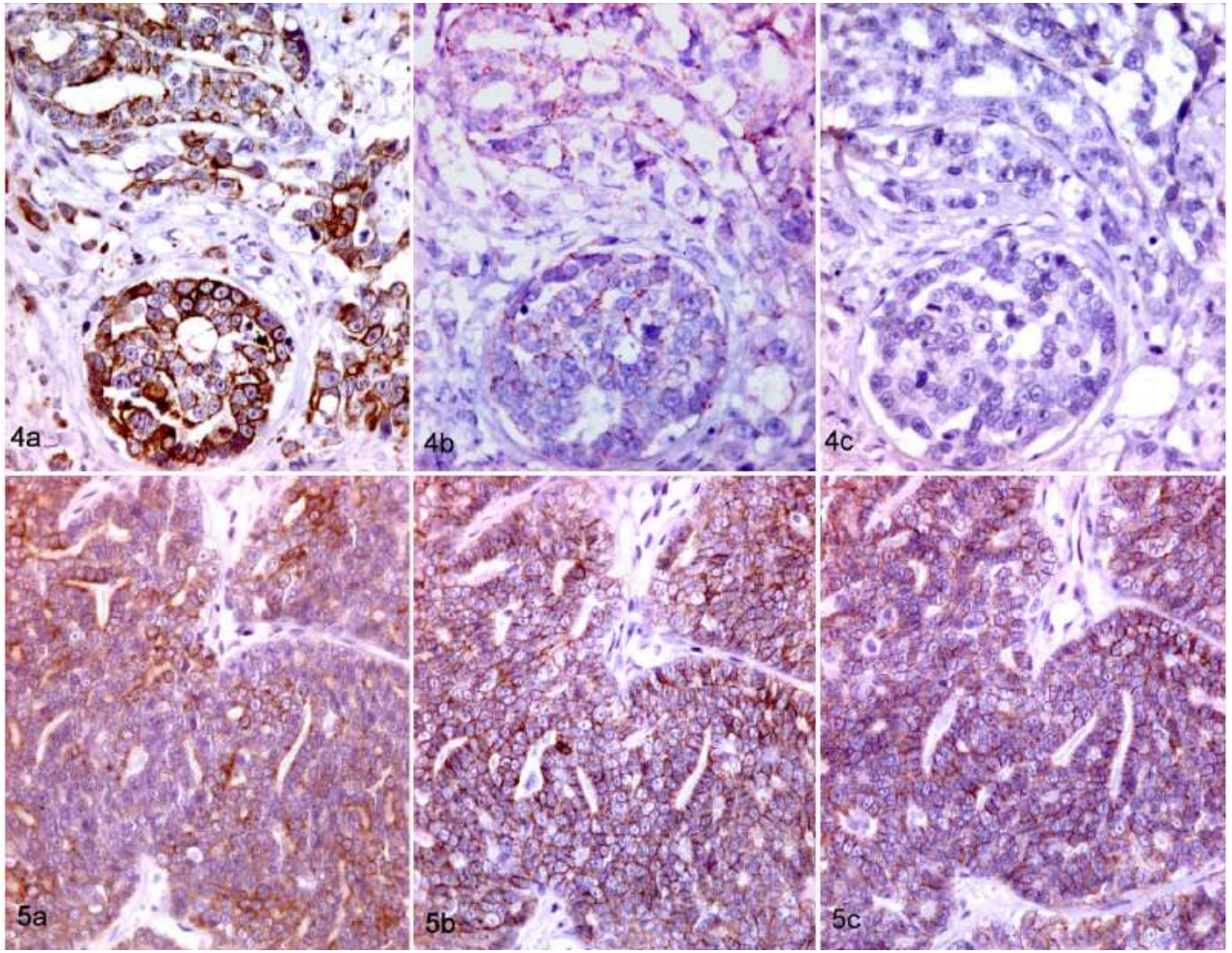

Caderina E
β-catenina o
γ-catenina
α-actinina
α-catenina
actina
Ca++
Membrana citoplasmatica
Membrana citoplasmatica

- Molecole ritenute coinvolte nella diffusione metastatica della neoplasia
- Indagini condotte su FMC hanno rielvato come i carcinomi metastatici presentino una perdita di entrambe le molecole di adesione

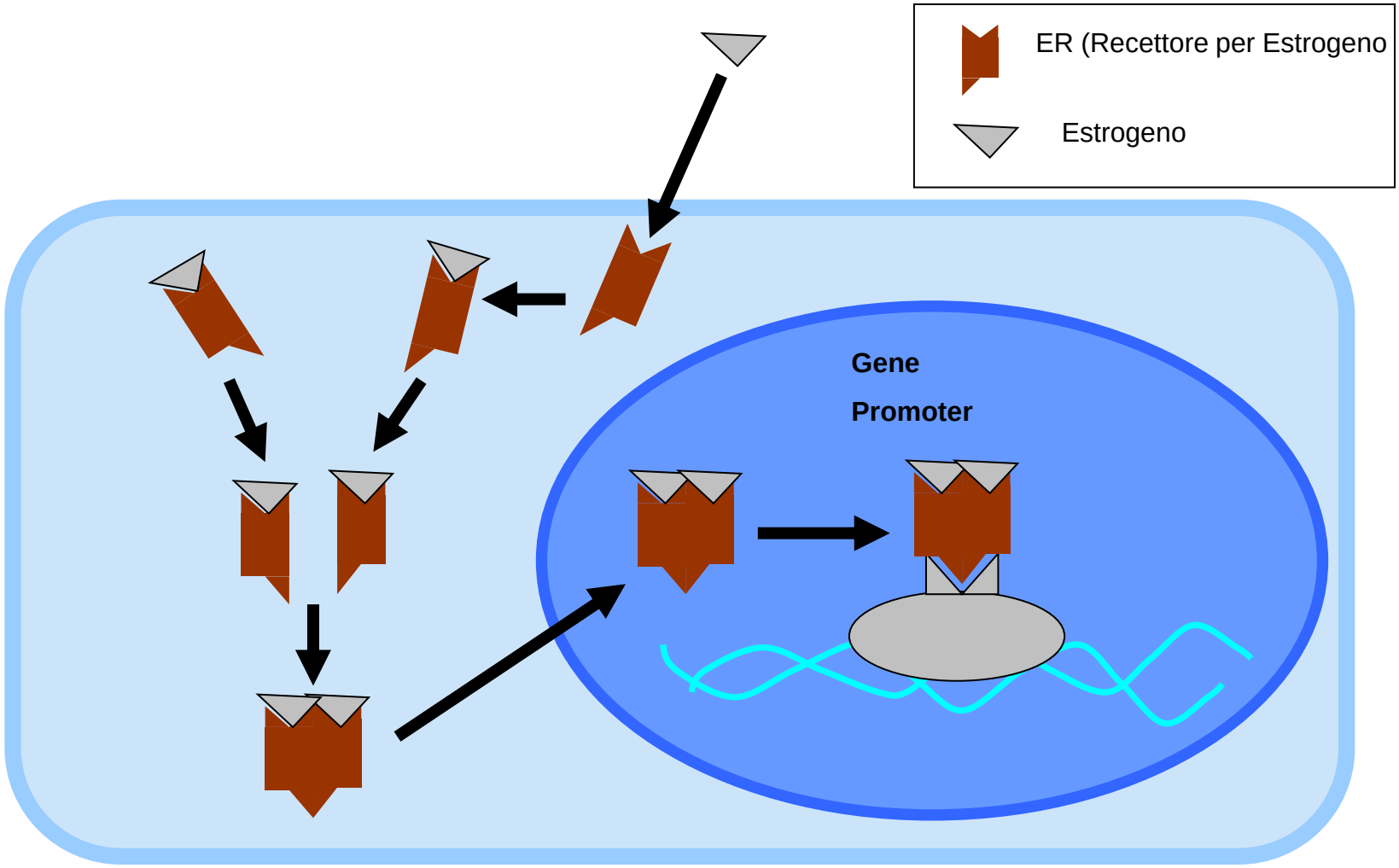
Table 2. Statistical Association Between Expression of E-cadherin, β-catenin, or Their Combination and the Existence Metastasis at the Time of Diagnosis

	Carcinomas, *n* (%)				
Expression	Metastasizing	Nonmetastasizing	*n*	*P*	Odds Ratio (95% Confidence Interval)
E-cadherin				.003	0.610 (0.43–0.85)
Preserved	29 (40.2)	43 (59.8)	72		
Reduced	44 (65.7)	23 (34.3)	67		
β-catenin				.001	0.424 (0.23–0.70)
Preserved	15 (31.9)	32 (69.4)	47		
Reduced	58 (63.1)	34 (36.9)	92		
E-cad/β-cat				< .001	0.335 (0.17–0.63)
Preserved	10 (27.0)	27 (73.0)	37		
Reduced	63 (61.8)	39 (38.2)	102		
Total	73	66	139		

Penafiel-Verdu et al., Vet Pathol 49, 2012

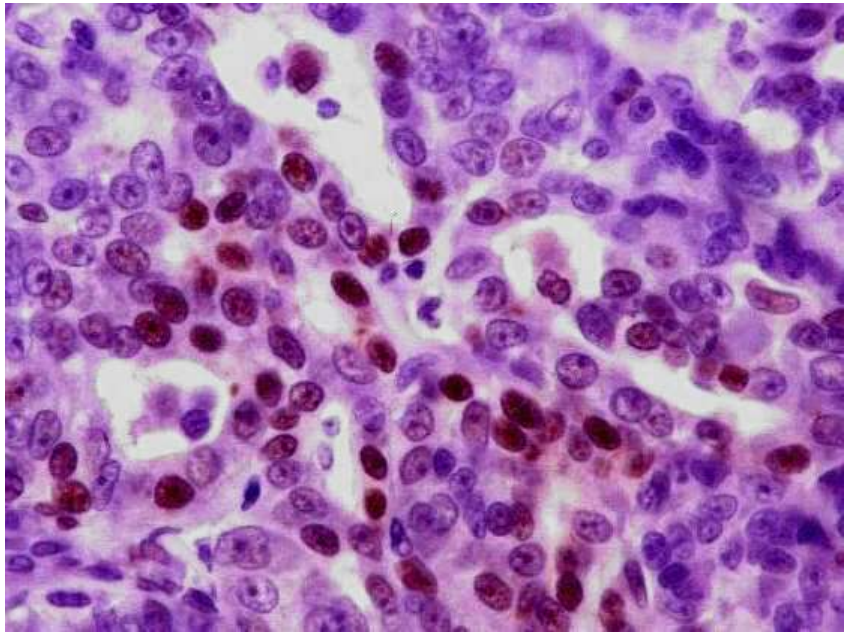


Espressione di CK 5/6 (a), E-caderina (b) e beta-catenina © in FMCs metastatizzanti (4) e non-metastatizzanti (5).
Penafiel-Verdu et al., Vet Pathol 49, 2012



ANIMALI E UOMO CONTRO IL CANCRO: ATTUALITA' E NUOVE PROSPETTIVE

Roma - 21 marzo 2013



Quantificazione: immunoistochimica (n° nuclei positivi/100 cellule in 10 campi a 40X)

Anti-PR, streptavidina-perossidasi

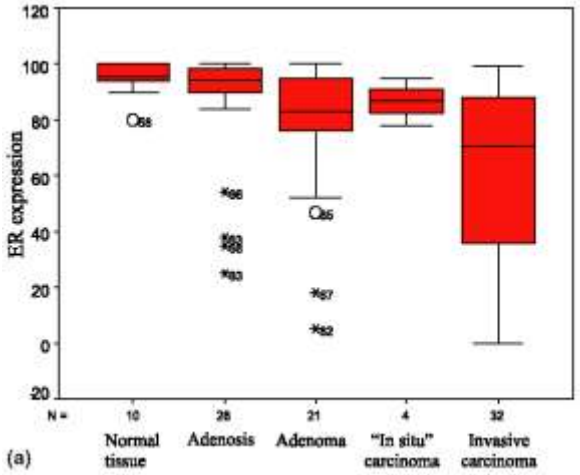
Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues

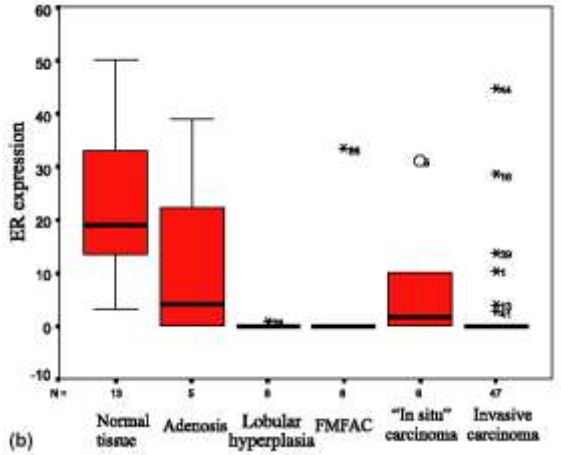
Research in Veterinary Science 79 (2005) 225–232

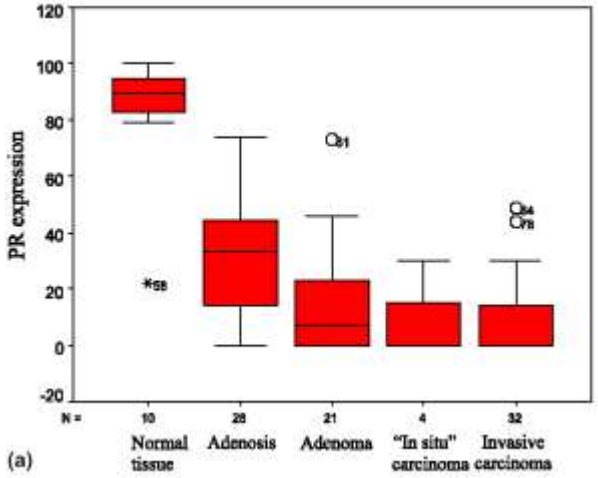
F. Millanta [a], M. Calandrella [a], G. Bari [a], M. Niccolini [a], I. Vannozzi [b], A. Poli [a,*]

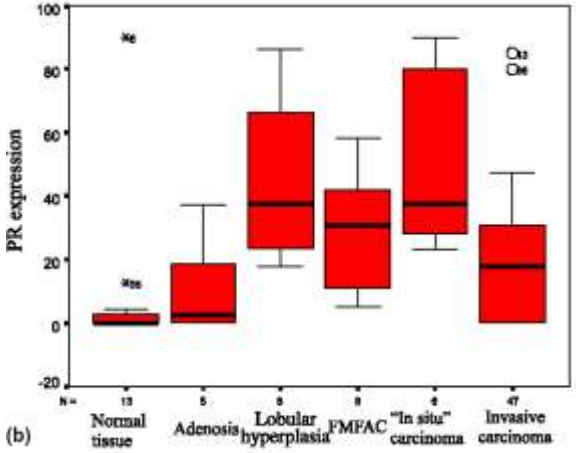
[a] Department of Animal Pathology, School of Veterinary Medicine, University of Pisa, Viale delle Piagge, 2, I-56124 Pisa, Italy
[b] Clinic Department, School of Veterinary Medicine, University of Pisa, I-56124 Pisa, Italy

cane **gatto**









	ER+PR+	ER+PR-	ER-PR+	ER-PR-
Cane (n tot=32)	10 (31.2%)	20 (62.5%)	0	2 (6.2%)
Gatto (n tot=54)	4 (7.4%)	1 (1.8%)	32 (59.2%)	17 (31.5%)

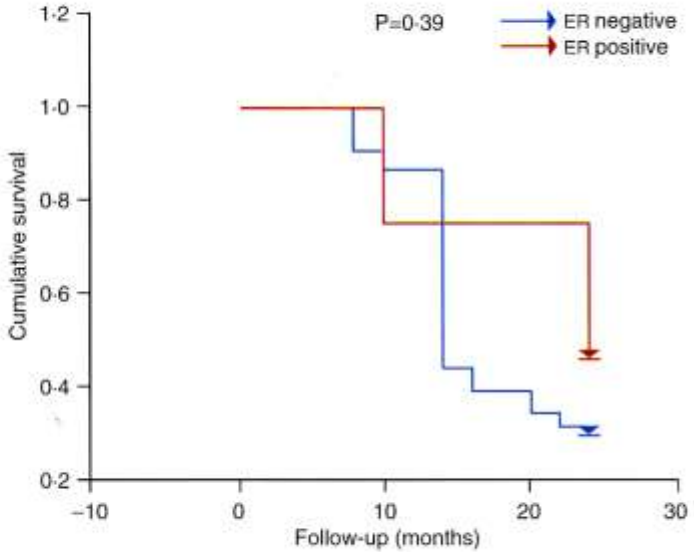


FIG 2: Overall survival curves for the cats with invasive mammary carcinomas either expressing (ER positive) or not expressing (ER negative) oestrogen-α receptors

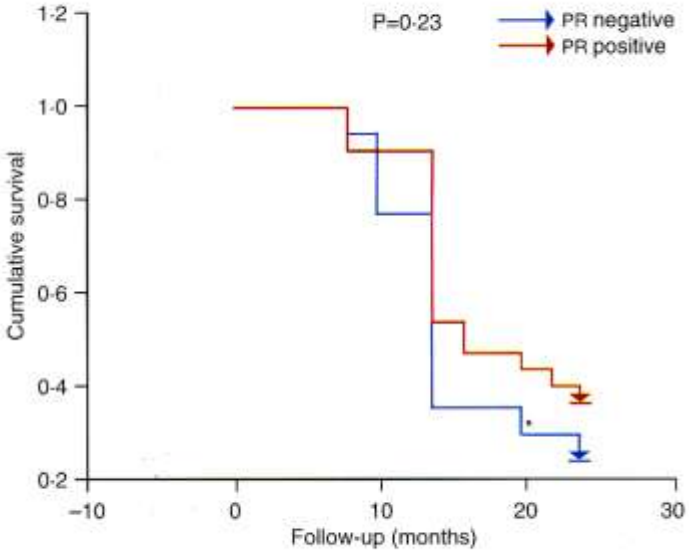


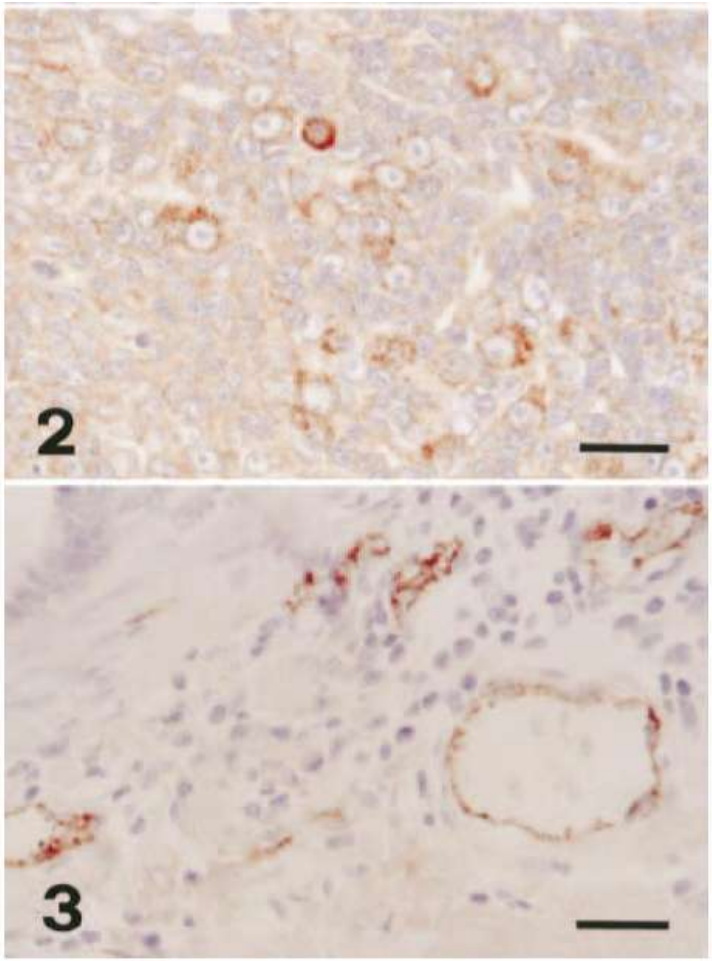
FIG 3: Overall survival curves for the cats with invasive mammary carcinomas either expressing (PR positive) or not

Millanta et al., Vet. Rec., 158, 821-824, 2006

- Tumori mammari della cagna: valido modello animale per lo studio dei tumori ORMONO-DIPENDENTI del seno

- I tumori mammari della gatta: valido modello animale per lo studio dei tumori ORMONO-INDIPENDENTI del seno

- L’espressione di citochine con attività pro-angiogenetica come il VEGF e la correlata «micro-vessel density» è stata valutata sia nelle neoplasie mammarie delle donna che della gatta e cagna
- Nelle diverse specie un over-espressione è risultata associata a neoplasia più aggressive ed una cattiva prognosi



Millanta et al., Vet Pathol 39, 2002

Table 1. Quartiles of VEGF values and deaths in 48 cats with invasive mammary tumors during the 2 years after surgery.

VEGF Value	No. of Cats	No of Deaths (%)
< 45.4	12	3 (25)
45.4–72.1	12	10 (83.3)
72.1–86.9	12	9 (75)
> 86.9	12	11 (91.7)

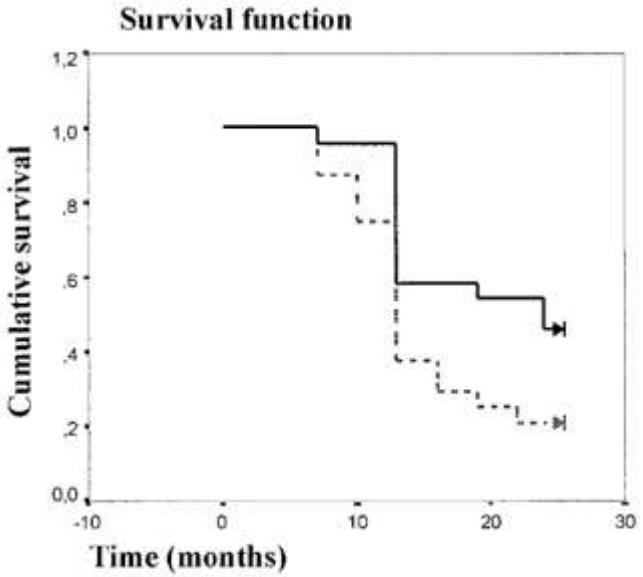


Fig. 4. Overall survival curves for the group of 48 queens with invasive mammary carcinomas with low VEGF values (≤ 72.1%; ——) and high VEGF values (> 72.1%; - - -) (P = 0.03).

- L'azione della citochina può avvenire con meccanismi

- AUTOCRINI

  Recettore e ligando espressi nella stessa popolazione cellulare

- PARACRINI

  Recettore espresso in un comparto cellulare e ligando in altri

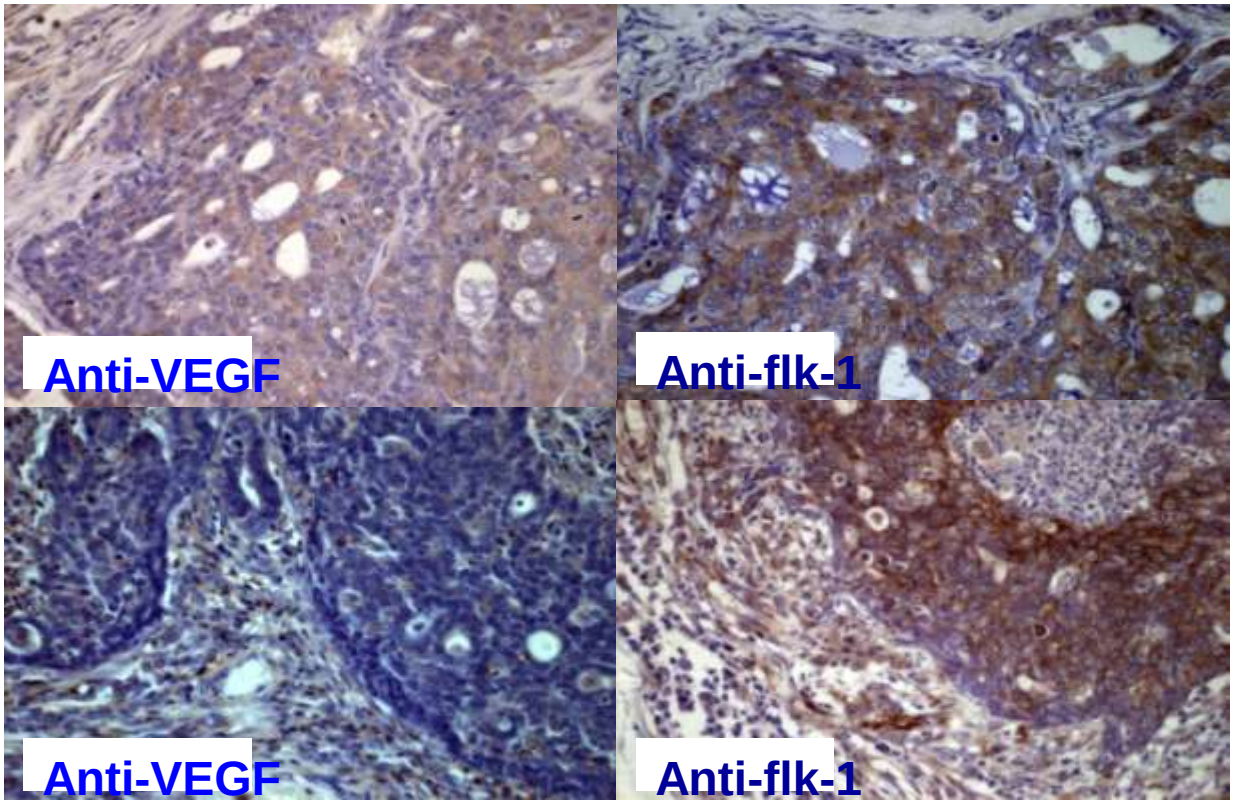


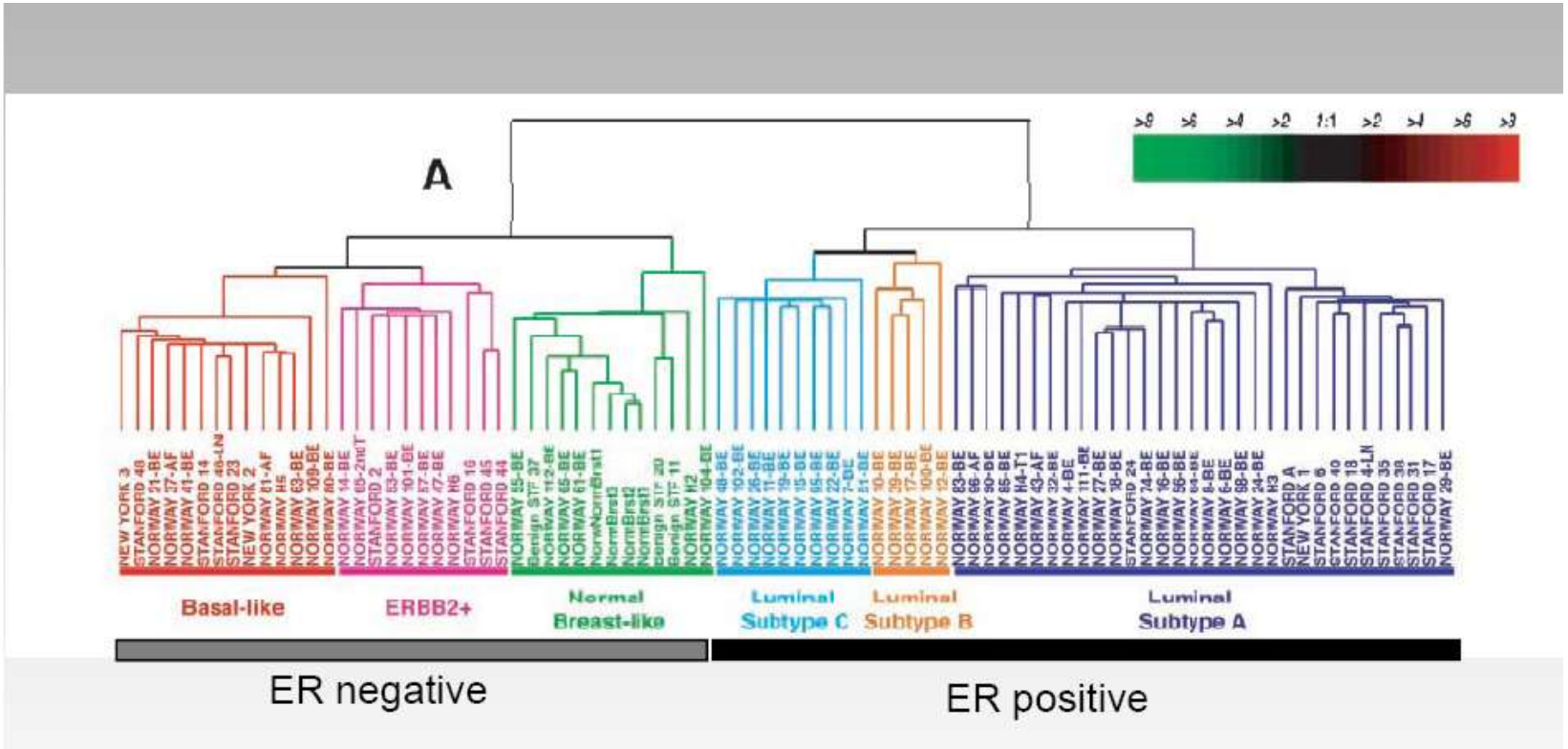
Table 2
Expression of vascular endothelial growth factor and its receptor Flk-1/KDR in the 29 cases of canine and the 48 feline mammary tumours

Antigen and ligand	Number of positive cases (%)					
	Epithelium		Endothelium		Stroma	
	Canine	Feline	Canine	Feline	Canine	Feline
VEGF	29/29 (100)	48/48 (100)	25/29 (86.2)	38/48 (79.1)	13/29 (44.8)	28/48 (58.3)
Flk-1/KDR	26/29 (89.6)	48/48 (100)	14/29 (48.3)	18/48 (37.5)	13/29 (44.8)	9/48 (18.7)

VEGF, vascular endothelial growth factor.

Millanta et al., Res Vet Sci 81, 2006

- APPROCCIO MOLECOLARE ALLA CLASSIFICAZIONE DEL CARCINOMA DEL SENO



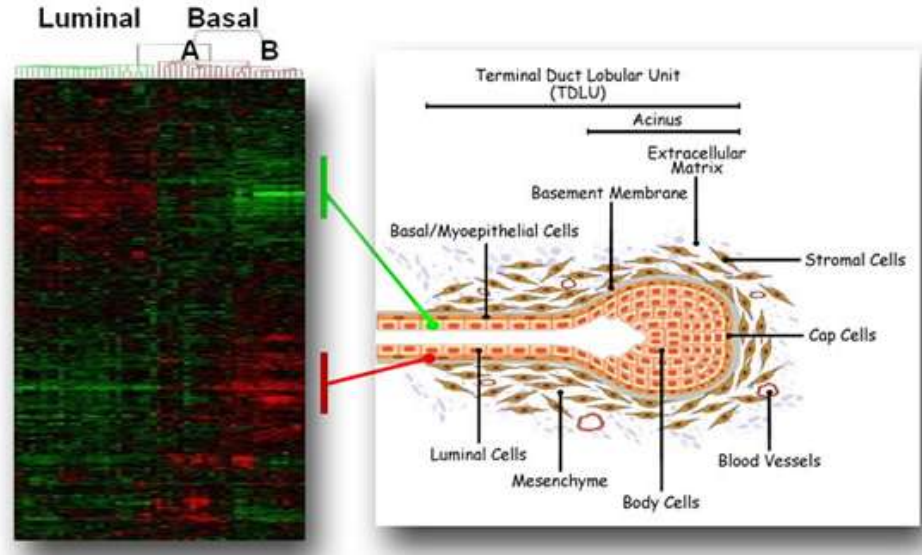
- APPROCCIO MOLECOLARE ALLA CLASSIFICAZIONE DEL CARCINOMA DEL SENO

Luminal-like

ER positive

Most favorable long term survival

Low intermediate grade



Basal-like

ER negative

High grade

Worse prognosis

Higher sensitivity to chemotherapy

BRCA1 mutation

HER-2 positive

Higher sensitivity to chemotherapy

- CLASSIFICAZIONE MOLECOLARE DEL CARCINOMA NEL CANE E GATTO

Virchows Arch (2008) 453:123–132

Table 3 Frequencies of immunohistochemically defined subtypes of canine mammary carcinomas (n=96)

Subtype	ER	HER2	P-CD and/or p63 and/or CK5	Frequency [n (%)]
Luminal A	Positive	Negative	Positive/negative	43 (44.8%)
Luminal B	Positive	Positive	Positive/negative	13 (13.5%)
Basal	Negative	Negative	Positive	28 (29.2%)
HER2-overexpressing	Negative	Positive	Positive/negative	8 (8.3%)
Negative/null phenotype	Negative	Negative	Negative	4 (4.2%)

Gama et al., Virchows Arch (2008) 453:123–132

- CLASSIFICAZIONE MOLECOLARE DEL CARCINOMA NEL CANE E GATTO

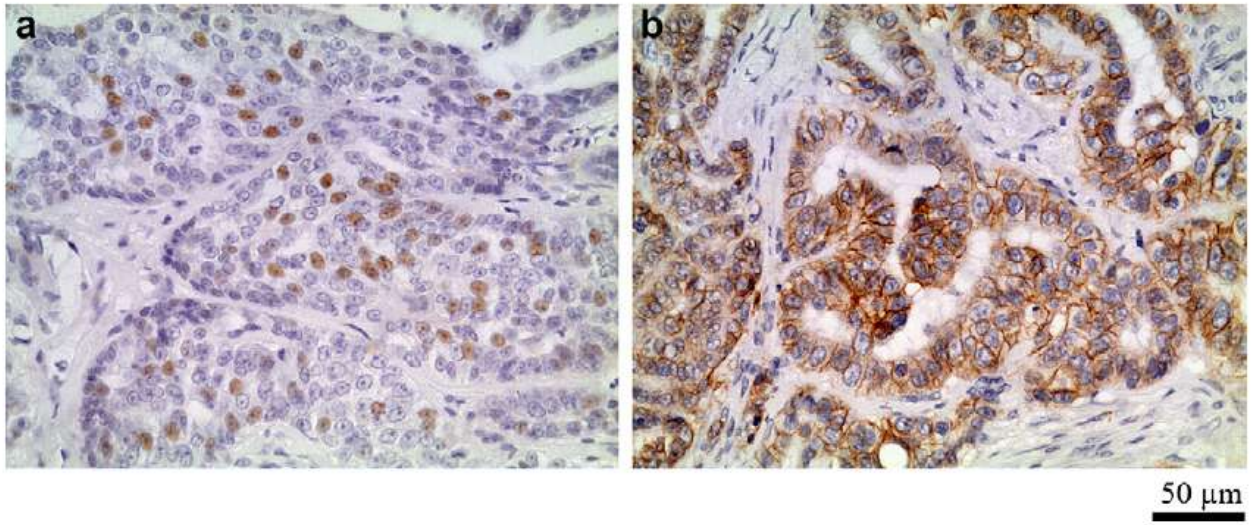


Fig. 1. Case 3. Luminal B phenotype. (a) OR expression and (b) c-erbB-2 expression. To be classified as luminal B phenotype, a tumour requires positivity for OR and/or PR and for c-erbB-2, while the results of labelling with other antibodies (CK5/6±, CK14±, p63±) are irrelevant. IHC.

Brunetti et al., J. Comp. Path. 2012, Vol. -, 1e8

- CLASSIFICAZIONE MOLECOLARE DEL CARCINOMA NEL CANE E GATTO

Table 3
Molecular phenotypes

Sample number	*Phenotype*		*Phenotypic relationship*
	Primary tumour	*Lymph node metastasis*	
1	Luminal B	Luminal B	Concordance
2	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance *
3	Luminal B	c-erbB-2 overexpressing	Discordance
4	Luminal B	c-erbB-2 overexpressing	Discordance
5	Luminal B	c-erbB-2 overexpressing	Discordance
6	Luminal B	c-erbB-2 overexpressing	Discordance
7	Basal-like	Basal-like	Concordance
8	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
9	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
10	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
11	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
12	Luminal B	Basal-like	Discordance
13	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
14	Luminal B	c-erbB-2 overexpressing	Discordance
15	Luminal B	c-erbB-2 overexpressing	Discordance
16	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
17	Luminal B	c-erbB-2 overexpressing	Discordance
18	Luminal B	c-erbB-2 overexpressing	Discordance
19	c-erbB-2 overexpressing	c-erbB-2 overexpressing	Concordance
20	Luminal B	Luminal B	Concordance
21	Basal-like	Basal-like	Concordance

Brunetti et al., J. Comp. Path. 2012, Vol. -, 1e8

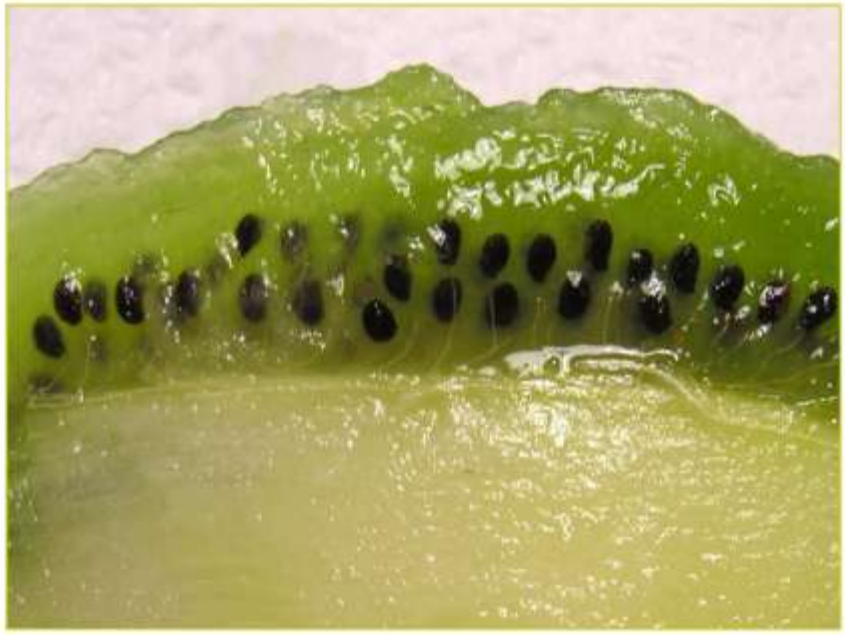
* Discordanza 43%

- Sulla base di queste considerazioni si può concludere:
  - neoplasie con ***aspetti istopatologici*** simili possono avere un differente comportamento clinico
  - una dettagliata ***analisi molecolare*** può invece dare informazioni importanti per migliorare la prognosi e indirizzare la terapia della malattia neoplastica

Path & Art

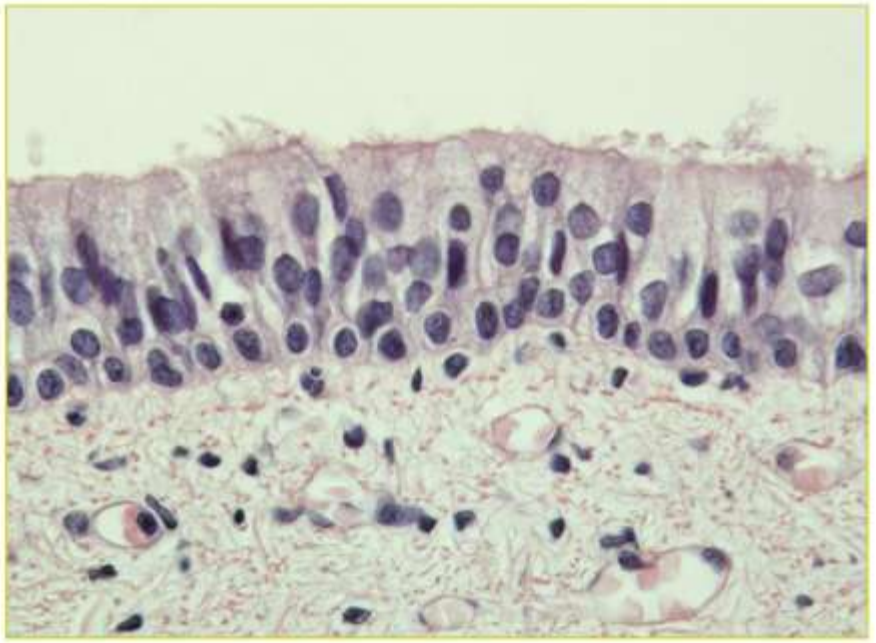
by L.Ressel
lressel@vet.unipi.it

Thank you for Attention



Fetta di Kiwi (***Actinidia chinensis)*** su scottex

Fotografia
Lorenzo Ressel



Cane, Trachea" *Epitelio pseudostratificato ciliato normale* ".
HH, (400x)
Dipartimento di Patologia Animale, Facoltà di Medicina Veterinaria, Università degli Studi di Pisa