

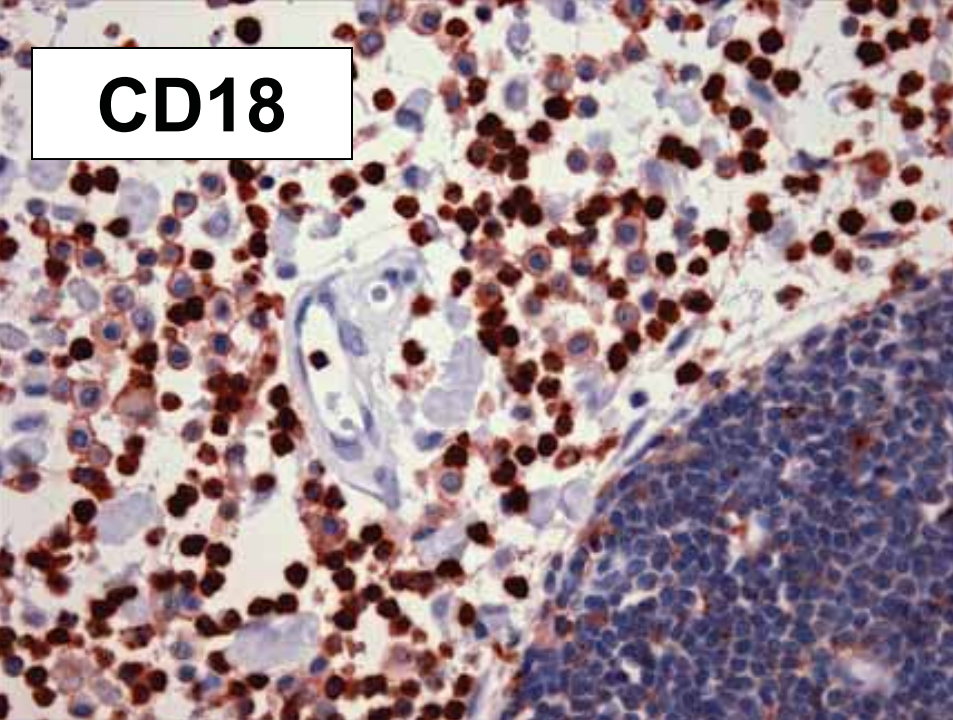
Cutaneous Mast Cell Tumor

Diagnostic Immunohistochemistry

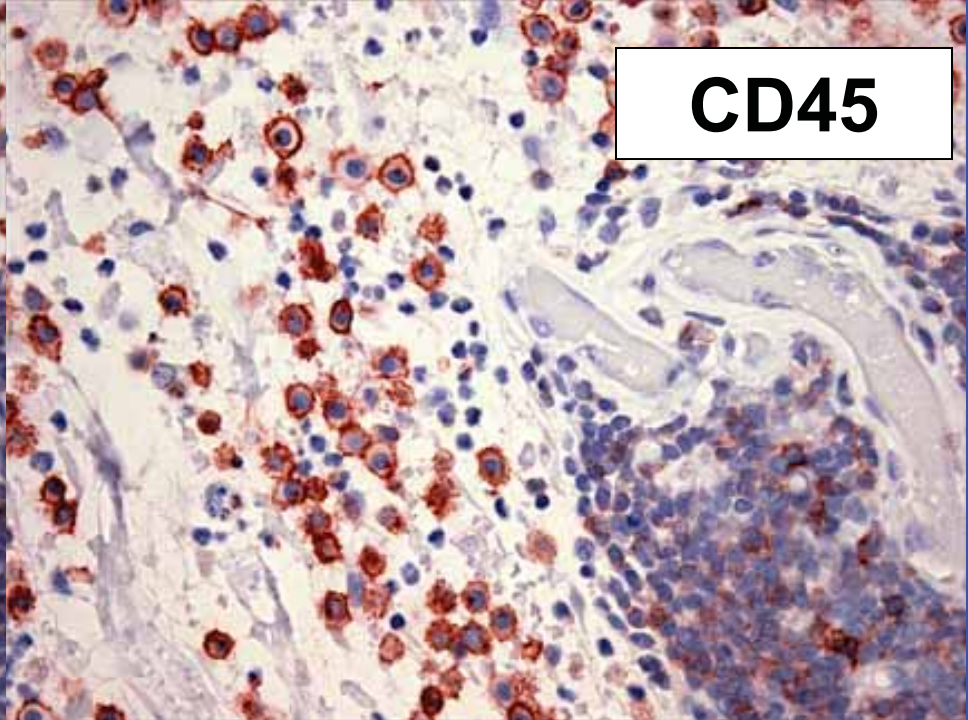


- **Immunohistology:** Canine Mast cells and MCT express CD18 (often), CD45 and CD45RA
- Canine mast cells are heterogeneous:
 - Contain **chymase** and/or **tryptase** :
 - **Tryptase** is detectable by IHC specific stain for mast cells
 - **Chymase** is sensitive to fixation with aldehydes - detection is by enzyme histochemistry
- **Polyclonal anti-KIT** antibody binds to canine *c-KIT*:
 - Mast cell development is influenced by SCF and its ligand *c-KIT* (a receptor tyrosine kinase)

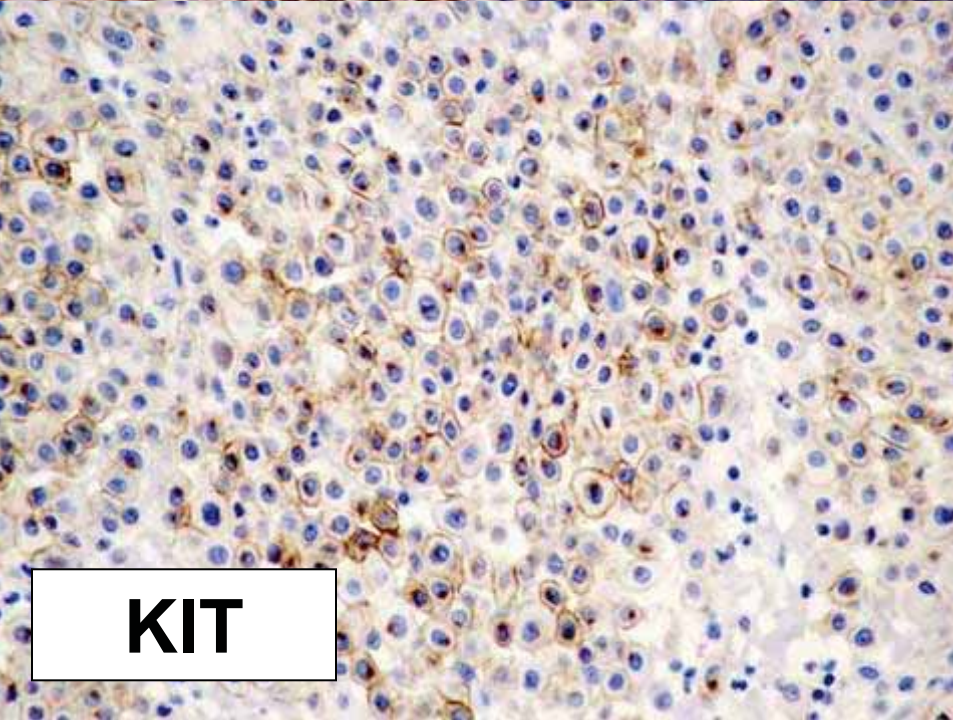
CD18



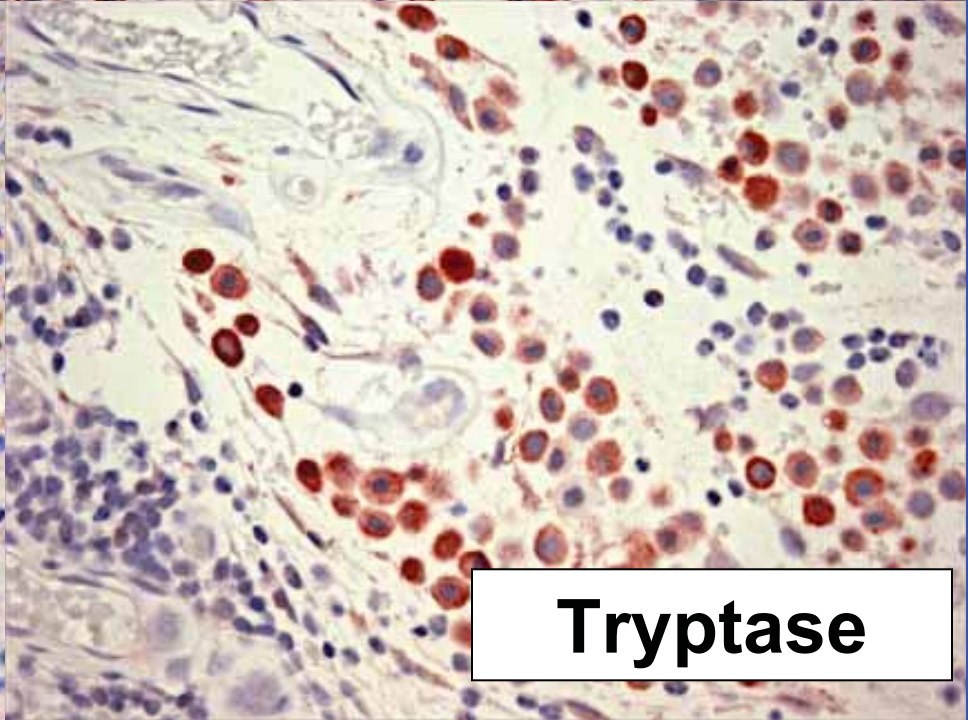
CD45



KIT

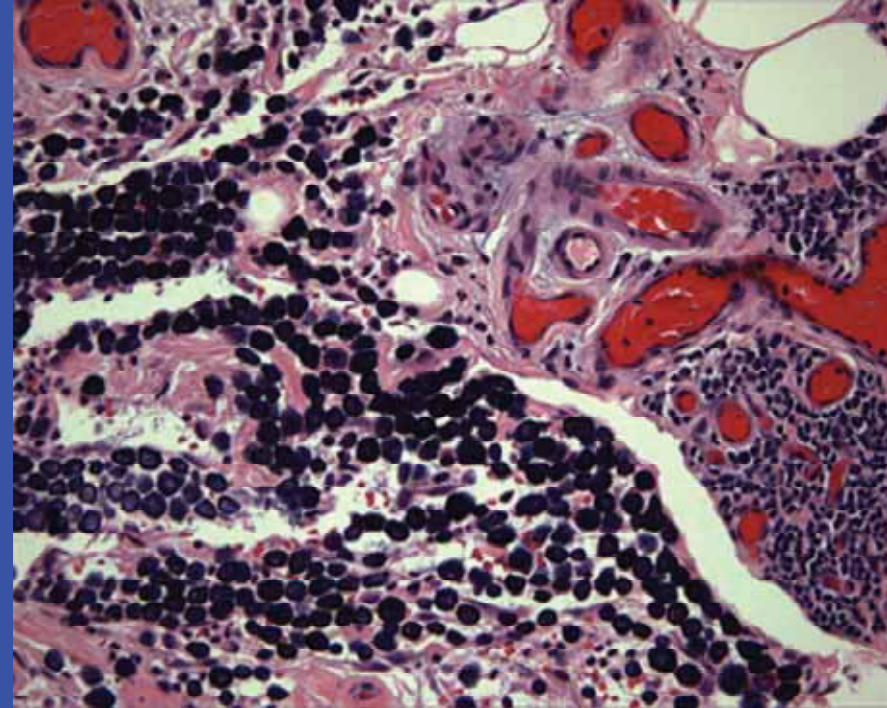


Tryptase



Canine MCT Prognostication

- Variable biologic behavior
 - Benign vs. malignant
- Several proposed parameters:
 - Tumor location
 - Tumor duration
 - Growth rate
 - Tumor stage
 - Tumor free margins
 - Proliferation markers: AgNORs, PCNA, Ki-67
 - Role of *c-KIT*
 - Histologic grade
- Variable predictive power

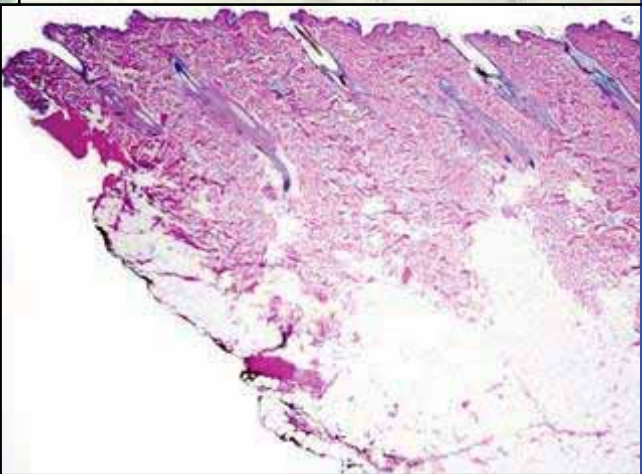
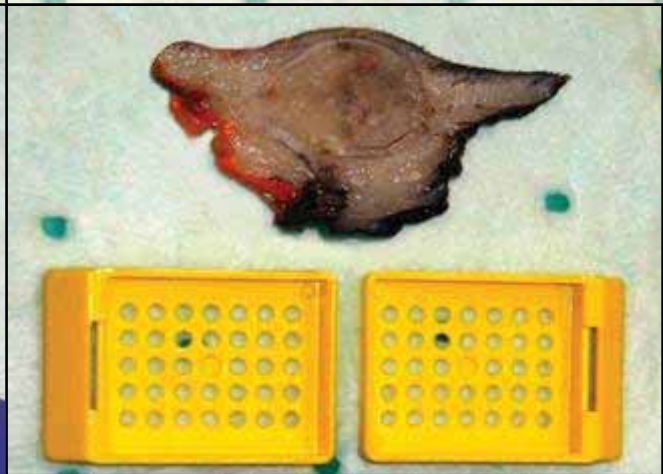
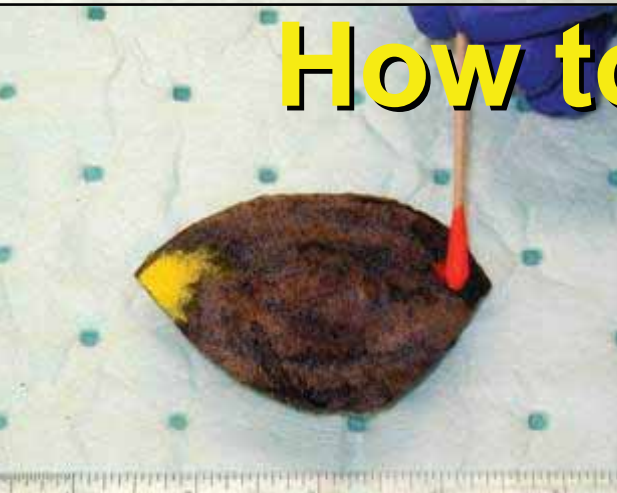


Therapeutic Concerns

- Know what you are treating before you treat it
- Variable reliability of prognostic indicators
 - Overall relevance of histologic grade?
- Difficulty of choosing treatment protocol
- Cost of treatment
- Need for more reliable prognostic indicators
- **Surgery, surgery, surgery!!**
 - Cut em' wide and cut em' deep
 - "3 cm" rule
 - 3 cm laterally, 3 cm margins deep



How to Ink Margins?



Treatment Options

- Surgery (wide margins)
- Radiation Therapy (incomplete removal, well tolerated)
- Chemotherapy
 - Steroids 20% response
 - Lomustine 42% mainly partial response
 - Myelosuppressive
 - Hepatotoxic
 - Vinblastine in combination
 - Prednison & Vinblastine
 - 33% complete
 - 14% partial response



Canine MCT Prognostication

• Clinical Features

- Signalment
- Location
- Multiple synchronous MCTs

• Histologic Features

- Grade
- Depth
- Tryptase immunostaining

• Proliferation Markers

- AgNORs
- Ki-67
- PCNA

• c-KIT Proto-oncogene

- Immunostaining: patterns, amount
- Activating mutations

(Kiupel M. Prognostic evaluation of canine cutaneous mast cell tumors: a pathologist's view. VCS News 29: 1-8, 2005)

Histologic Grading of Canine MCTs



- **Primary prognostic and therapeutic determinant**
 - **Bostock, 1973 (grade 3 to 1)**
 - **Patnaik et al., 1984 (grade 1 to 3)**
- **Grading criteria:**
 - **Cellular**
 - **Nuclear morphology**
 - **Nucleus : cytoplasm (Bostock)**
 - **Mitotic index**
 - **Cell density**
 - **Tumor depth/invasiveness (Patnaik)**
- **Significant association between survival and histologic grade**

Prognostic Evaluation



Patnaik et al., 1984

83 dogs in study

Followed dogs for 1500 days

Recorded, sex, age, breed, survival

Wide surgical margins

No evidence of metastasis

Measured only total survival time

Used Kaplan-Meier and logrank test to compare survival times

No multivariate analysis

Did not account for cause of death

Bostock, 1974

114 dogs in study

Followed dogs for app. 910 days

Complete follow-up data

Only completely removed MCTs

No evidence of metastasis

Measured only total survival time

Used Student T-test to compare survival times

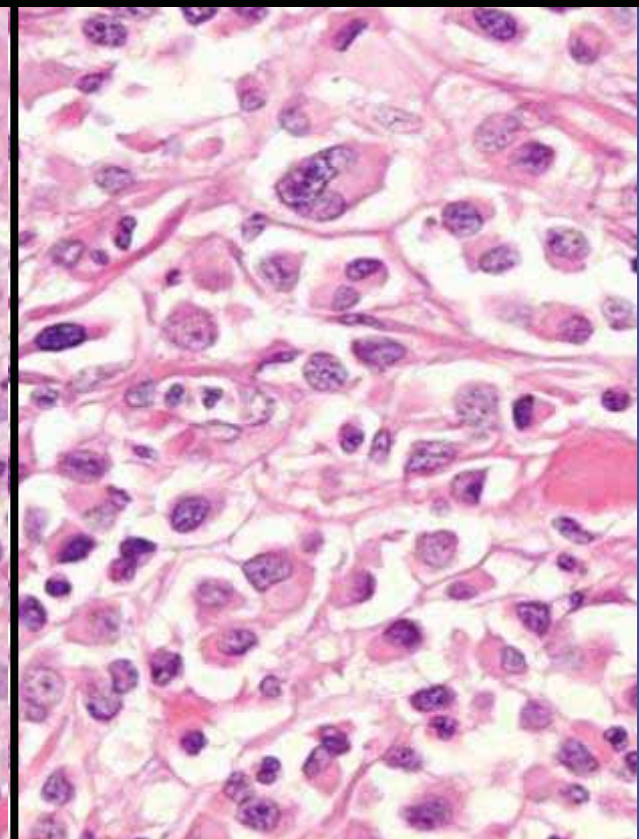
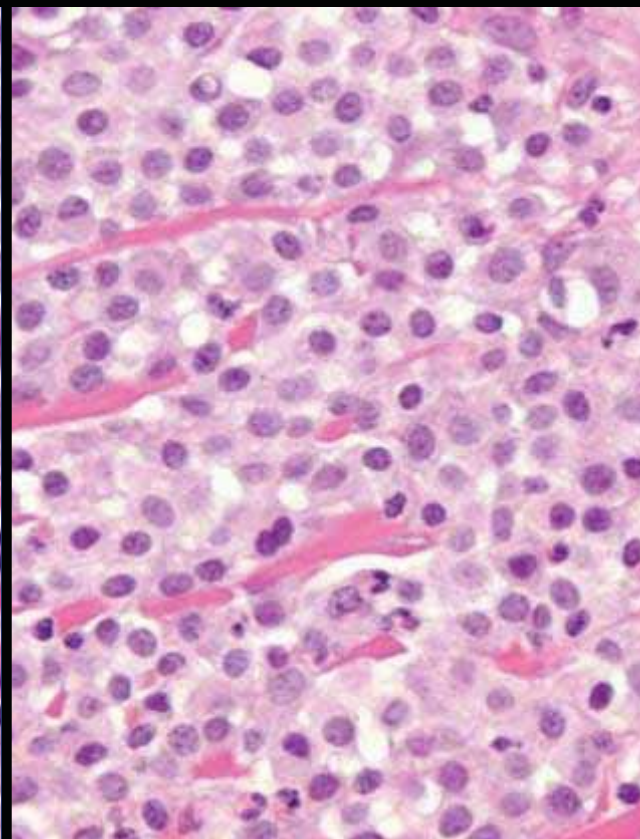
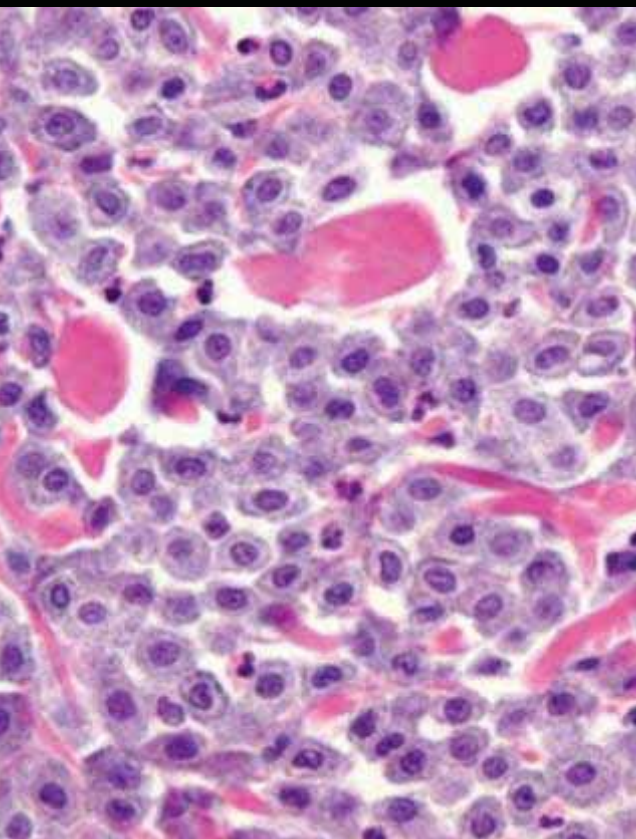
No multivariate analysis

Recorded cause of death, but did not account for it

Prognosis

Grade	Patnaik et al., 1984	Bostock, 1974
1 (3)	93% of dogs survived more than 1500 days 5% dead after 450 days	77% of dogs survived more than 210 days Mean survival: 357 days
2	47% of dogs survived more than 1500 days 60% dead after 600 days	45% of dogs survived more than 210 days Mean survival: 196 days
3 (1)	6% of dogs survived more than 1500 days 90% dead after 150 days	13% of dogs survived more than 210 days Mean survival: 126 days

Histologic Grading of Canine Mast Cell Tumors



Well-Differentiated
Patnaik I; Bostock III

Moderately-Differentiated
Patnaik II; Bostock II

Poorly-Differentiated
Patnaik III; Bostock I

Prognostic Classification of Canine Mast Cell Tumors



- **Retrospective study to evaluate the prognostic value of:**
 - Tumor location
 - Tumor depth
 - **Presence of multiple synchronous tumors for canine cutaneous MCTs**
(Kiupel et al., Impact of tumor depth, tumor location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumors. J Vet Med A 2005)
- **Multi-Institutional review of the histologic grading of canine cutaneous MCTs**
(Kiupel et al., Microscopic Grading of canine cutaneous mast cell tumors: A multi-institutional review. Vet Pathol 2004)

Study Population



- 95 cutaneous MCTs from 95 dogs:
 - Treated with surgery only
 - Total survival time and cause of death
 - Time to local or distant MCT development
- Age: 1-14 years (mean: 7.2 years)
- Breed: 22 breeds (23 Labrador retriever)
- Sex:
 - Male: 42
 - Female: 58

Tumor Location



Dorsal

- Inguinal
- Head/neck
- Trunk
- Extremities



Ventral

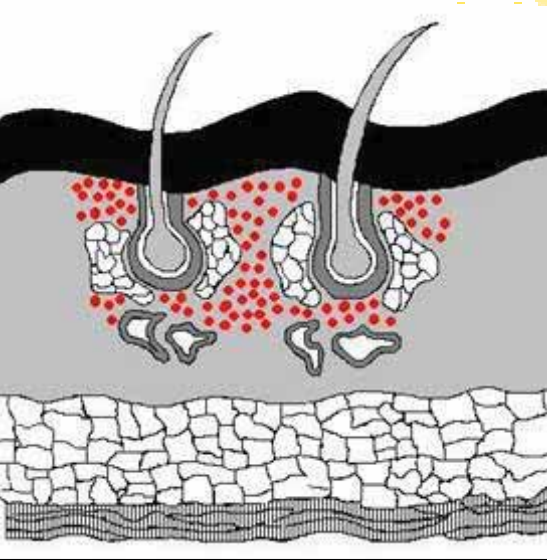
- Dogs with head/neck tumors appeared to have a decreased overall survival time, not significant ($p= 0.0613$)

Tumor Depth

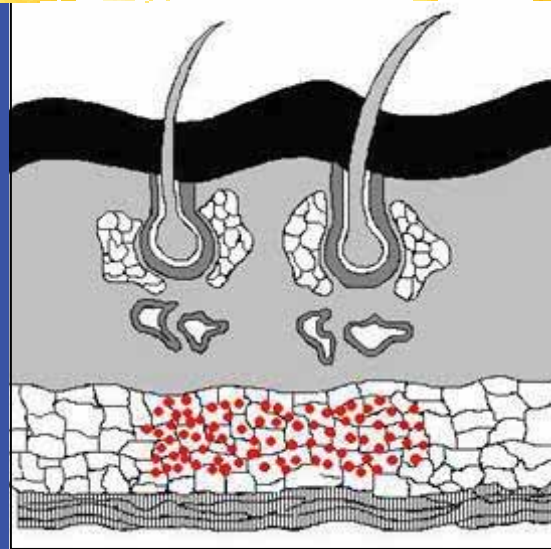


- Fascial plane infiltration
- Used for grading by Patnaik
- Possible measure of invasiveness and aggressive behavior
- Study including only completely excised tumors
 - Tumor may extend deeper than noted

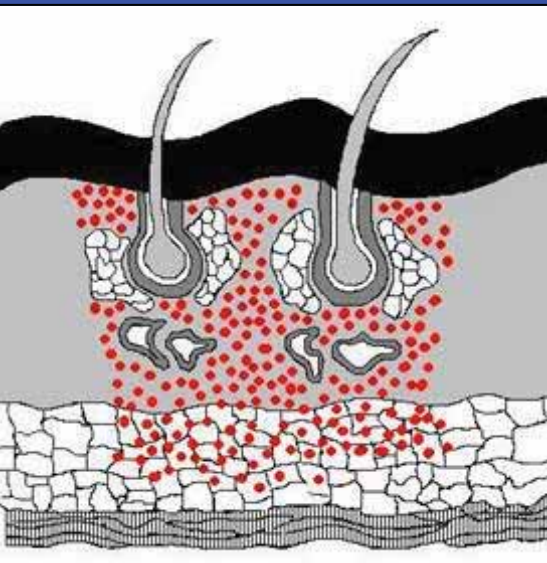
Tumor Depth



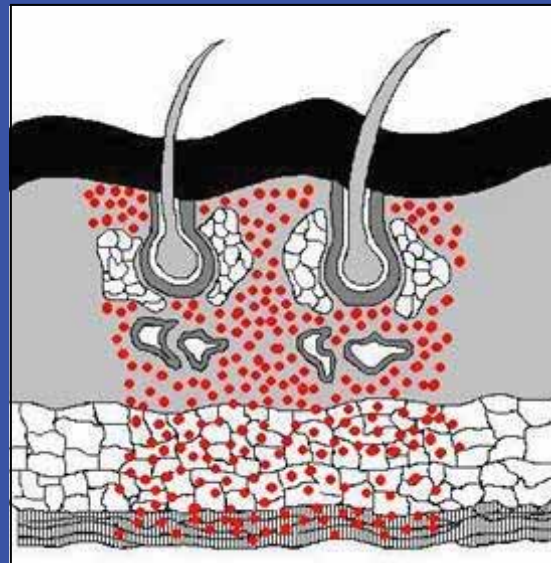
**Superficial
Dermis**



**Deep Dermis
Only**



**Superficial
and Deep
Dermis**

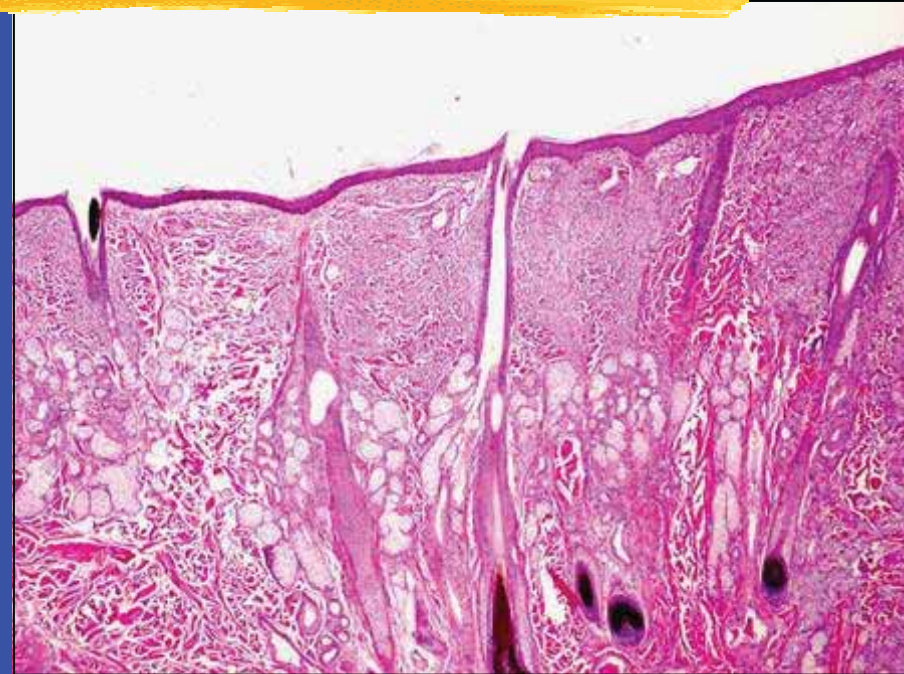


**Underlying
Musculature
Invasion**

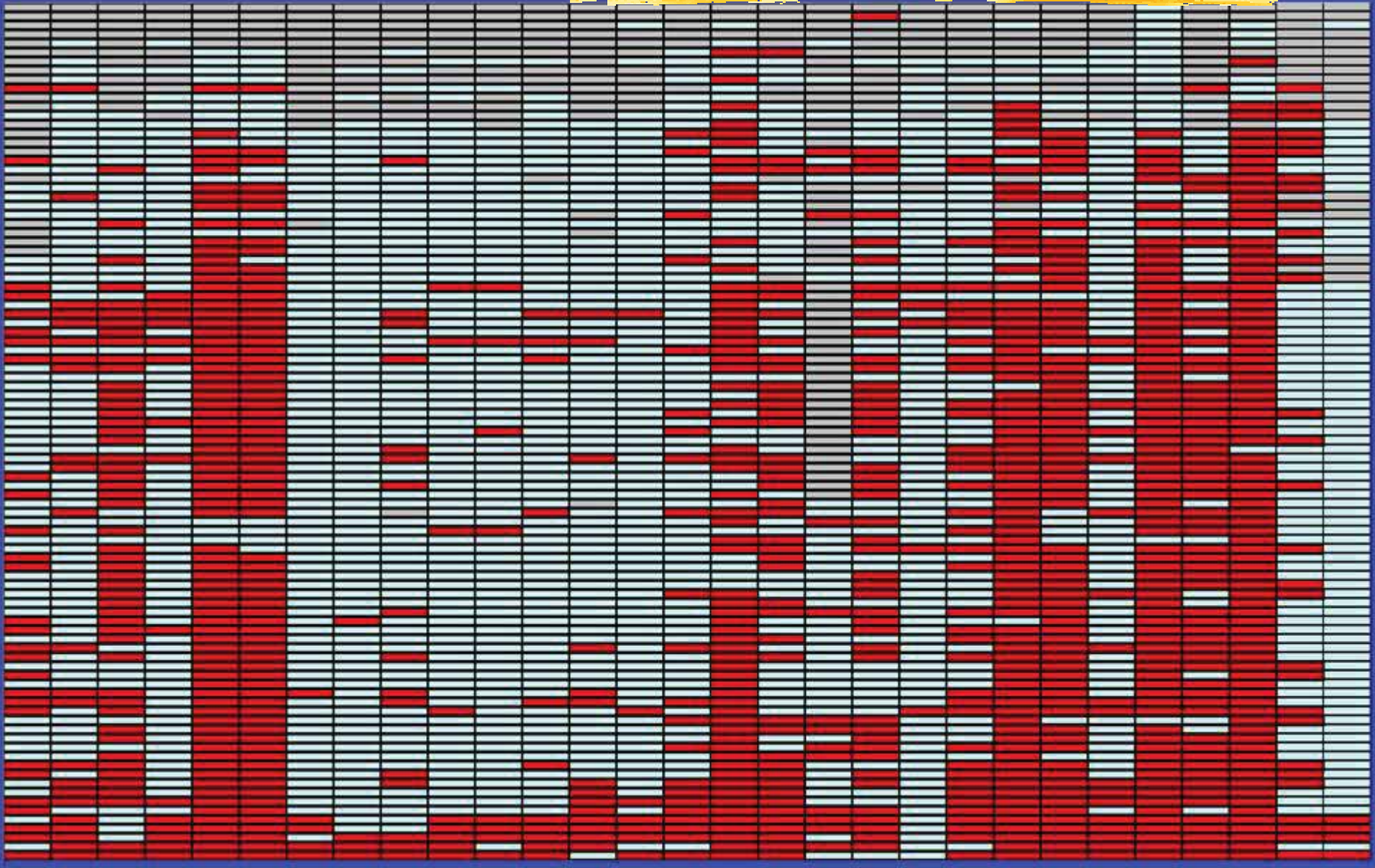
- MCT depth had no prognostic significance

Multiple Synchronous MCTs

- 10/100 cases
 - Histologic grade
 - Grade I: 0
 - Grade III: 1
 - Signalment:
 - Grade II: 9
 - Mean age: 6.85 y
 - Sex: 7M:3F
 - Breeds: Mixed (3), Boxer (3), Dalmatian (1), Cocker (1), Labrador retriever (1), Wheaten Terrier (1)
- Significantly decreased disease-free interval ($p=0.0013$)
- Significantly decreased survival duration ($p=0.0107$)



Multi-Institutional Grading



Consistency



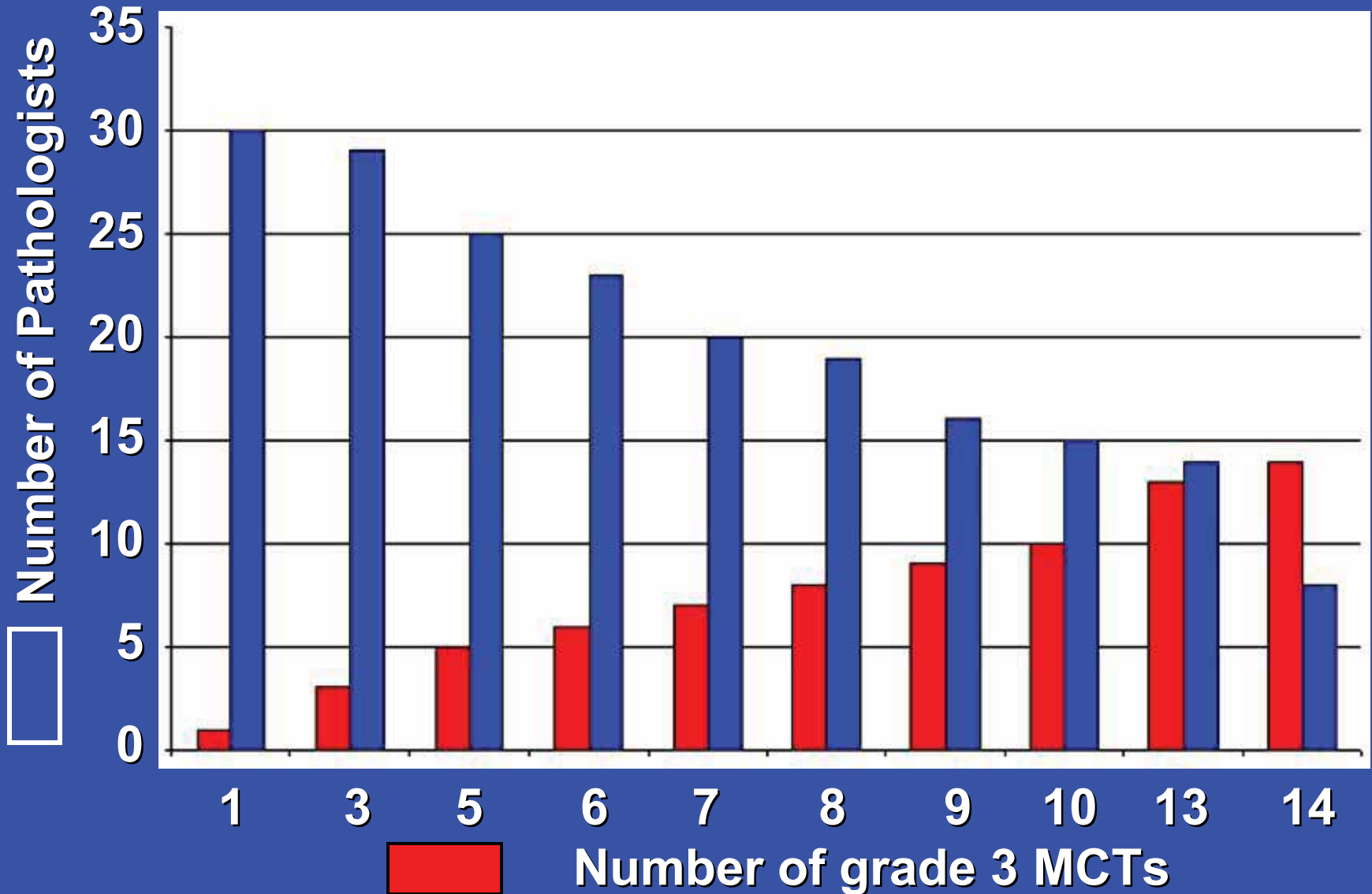
Cronbach alpha test

Grade 3: 74.6%

Grade 2: 63.0%

Grade 1: 63.1%

Grade 3



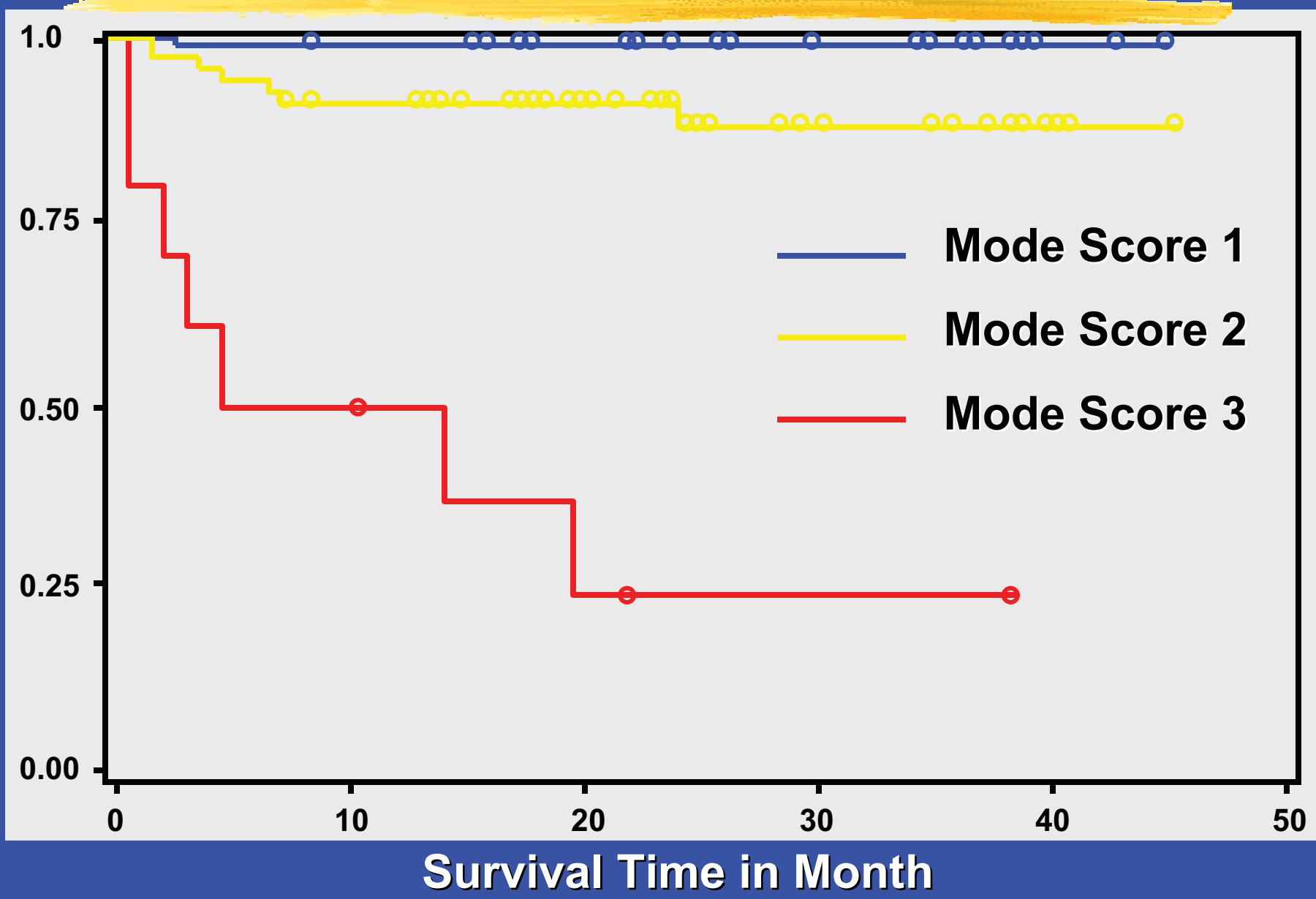
Numbers of cases and MCT-related mortality by % class for scores from 95 cases

Score	Class	N	X ²	p	Fisher's p	Odds Ratio*	
						Pt est	C.I.
3	≥ 90%	3	19.33	< 0.0001	0.0021	55.0	*
	≥ 80%	3	19.33	< 0.0001	0.0021	55.0	*
	≥ 70%	5	32.94	< 0.0001	< 0.0001	106.7	*
	≥ 50%	9	34.21	< 0.0001	< 0.0001	46.67	7.9 – 275.9
2	≥ 80%	5	9.48	0.0021	0.0174	12.0	1.8 – 80.7
	≥ 70%	18	11.81	0.0006	0.0024	7.53	2.1 – 26.6
	≥ 50%	75	3.97	0.0462	0.0635	8.86	*
1	≥ 80%	4	0.66	0.4183	1.0	0.65	*
	≥ 70%	5	0.83	0.3629	1.0	0.52	*
	≥ 50%	23	4.76	0.0291	0.0335	0.09	*

Time to metastasis by % class for scores from 95 cases

Score	Class	N	ANOVA		Wilcoxon Rank-Sum	
			F	p	X ²	P
3	≥ 90%	3	0.22	0.6391	1.72	0.1902
	≥ 80%	3	0.22	0.6391	1.72	0.1902
	≥ 70%	5	0.71	0.4023	6.90	0.0086
	≥ 50%	9	0.98	0.3254	7.90	0.0049
2	≥ 80%	5	1.28	0.2606	3.30	0.0695
	≥ 70%	18	0.01	0.9085	0.15	0.6951
	≥ 50%	75	0.03	0.8631	0.11	0.7434
1	≥ 80%	4	0.60	0.4390	0.08	0.7827
	≥ 70%	5	0.25	0.6155	0	1.0
	≥ 50%	23	0.06	0.8109	0.01	0.9391

Survival curves for MCT-related mortality, stratified by the mode of test scores



Numbers of cases and MCT-related mortality by class, for scores from 95 cases

Score	N	% mortality in this class	X ²	p	Fisher's p	Pt est	Odds Ratio C.I.
3	6	66.67	15.06	0.0001	0.0029	17.78	2.85 – 111.04
2b	4	25.0	0.45	0.5033	0.4506	2.19	0.21 – 22.85
2a	50	2.0	12.08	0.0005	0.0005	0.06	0.01 - .45
1	3	0	0.49	0.4857	1.0	0.84	0.04 – 17.22



Nuclear Morphology

Conclusions



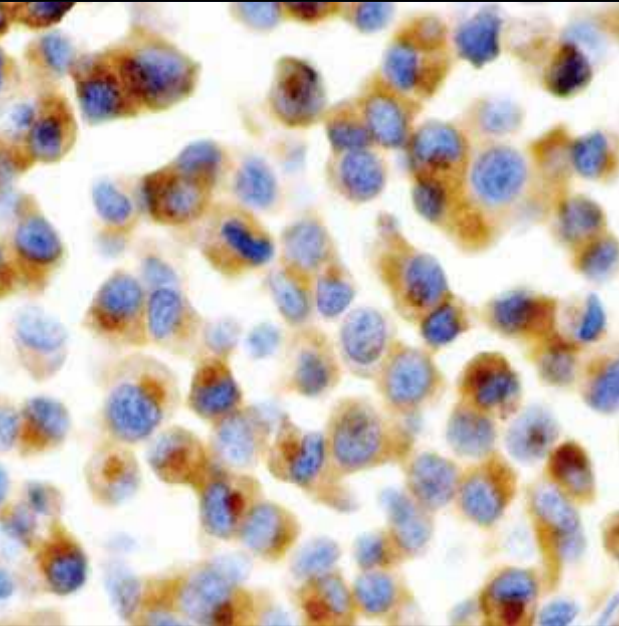
- Relative consistent grading of grade 3 MCTs
- Dogs with MCTs identified by 50% of pathologists as grade 3 have decreased survival
- **Inconsistent grading of grade 1 and 2 MCTs**
- **No prognostic significance for grade 1 MCTs**
- Subdivision into grade 2a and 2b MCTs of no prognostic significance
- **Nuclear and cell morphology are the primary criteria for determine prognosis**

Recommendation



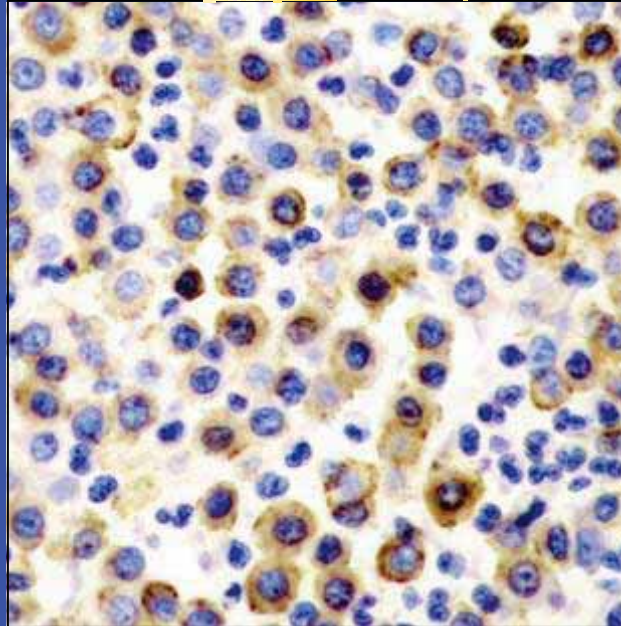
- Grade MCTs as high and low grade:
 - only two grades should be assigned
- **Each** of the following criteria warrants a grade 3:
 - marked anisocytosis
 - nuclear pleomorphism, anisokaryosis:
 - giant or bizarre nuclei
 - multiple nuclei
 - cleaved euchromatic nuclei with nucleoli
 - 2 or more mitotic figures/hpf

Tryptase Immunostaining Patterns



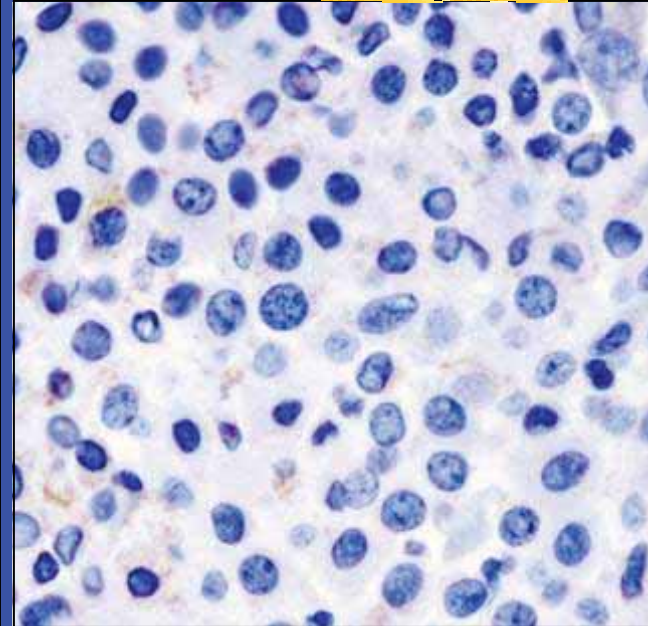
Tryptase Pattern 1

- Diffuse cytoplasmic staining



Tryptase Pattern 2

- Cytoplasmic stippling

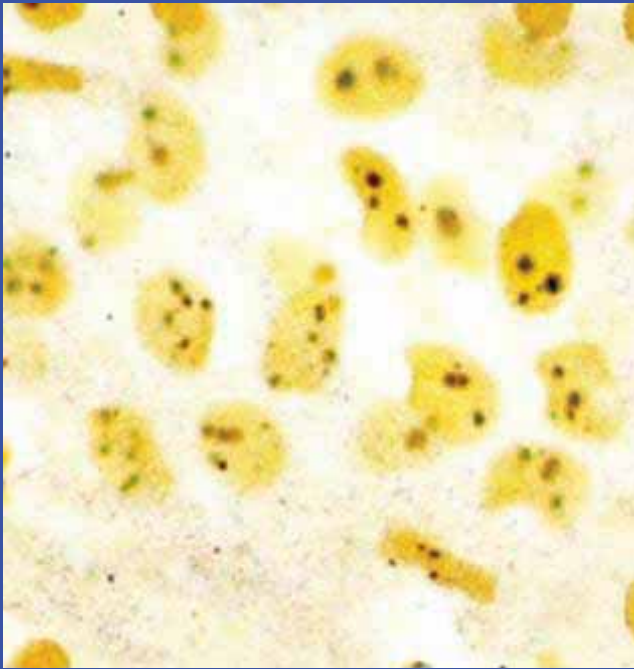


Tryptase Pattern 3

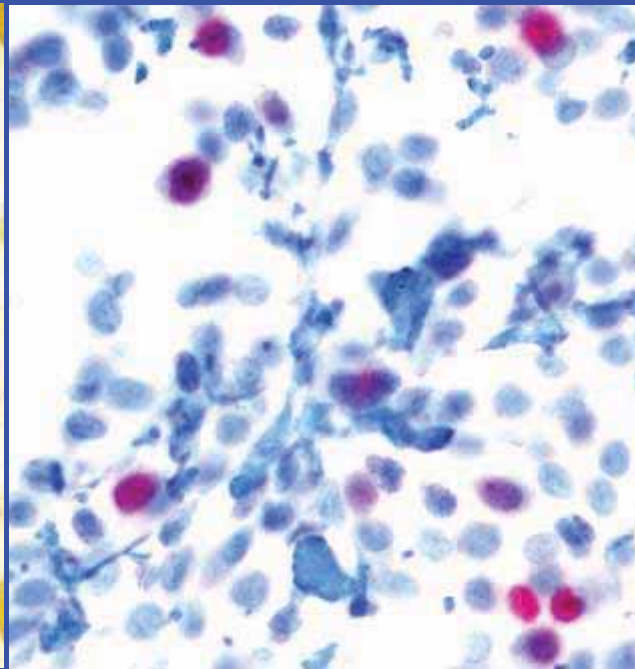
- Minimal cytoplasmic staining

- No significant associations with recurrence
- No significant association with survival

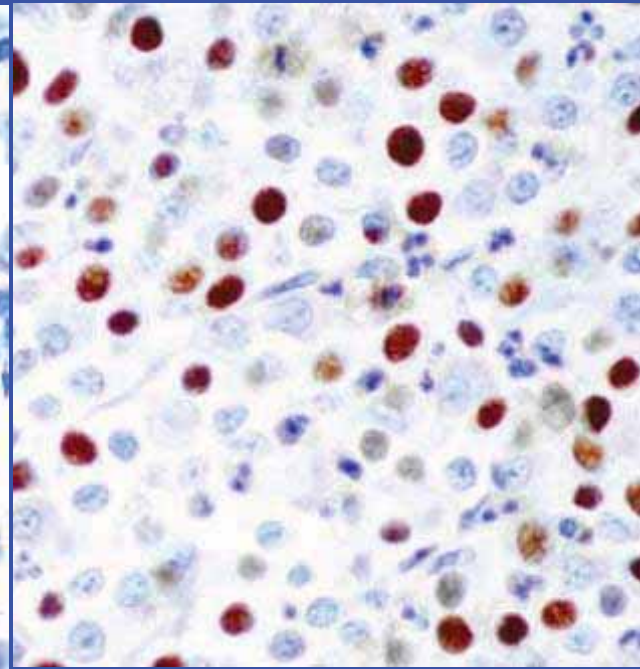
Proliferation Markers



AgNOR

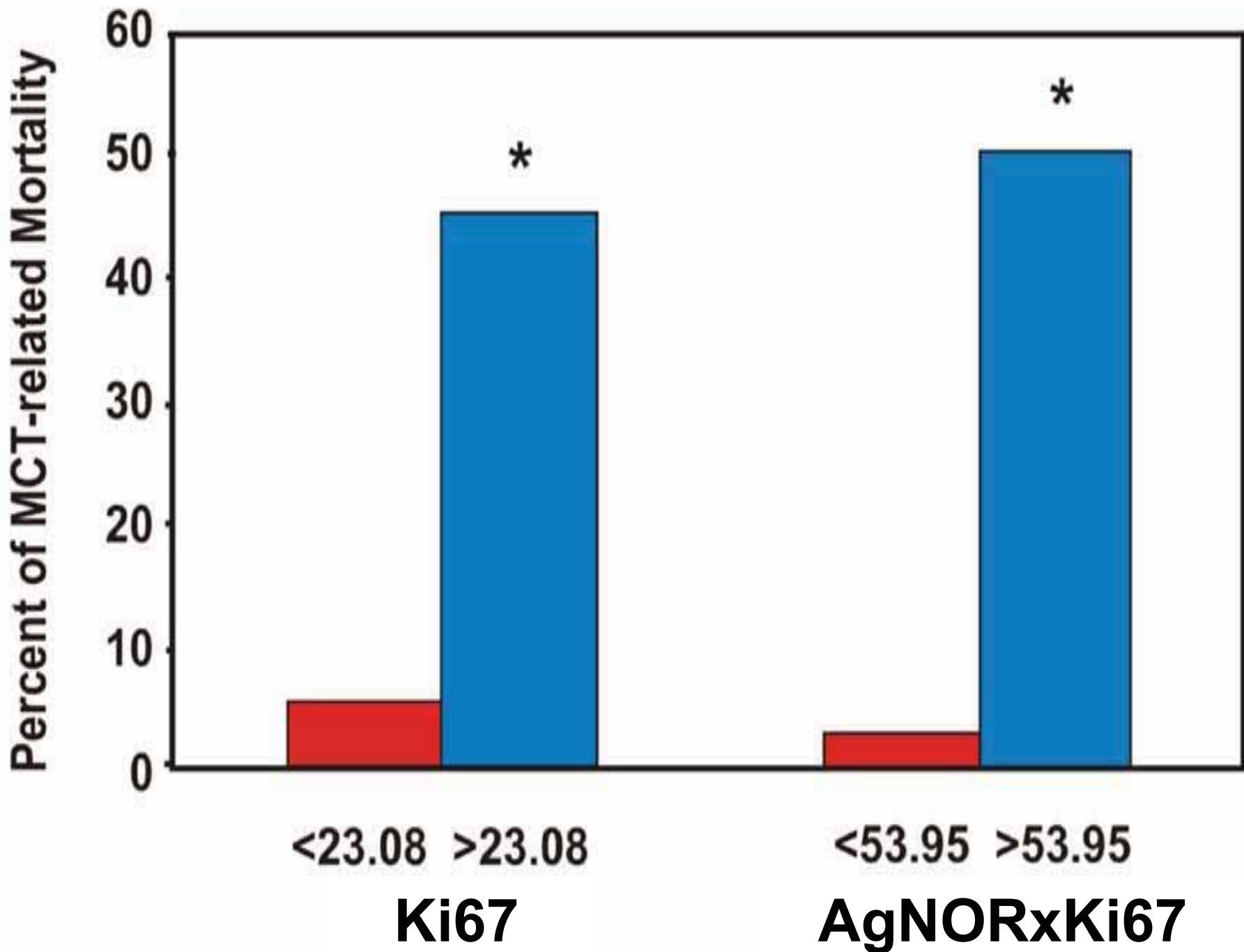


KI-67

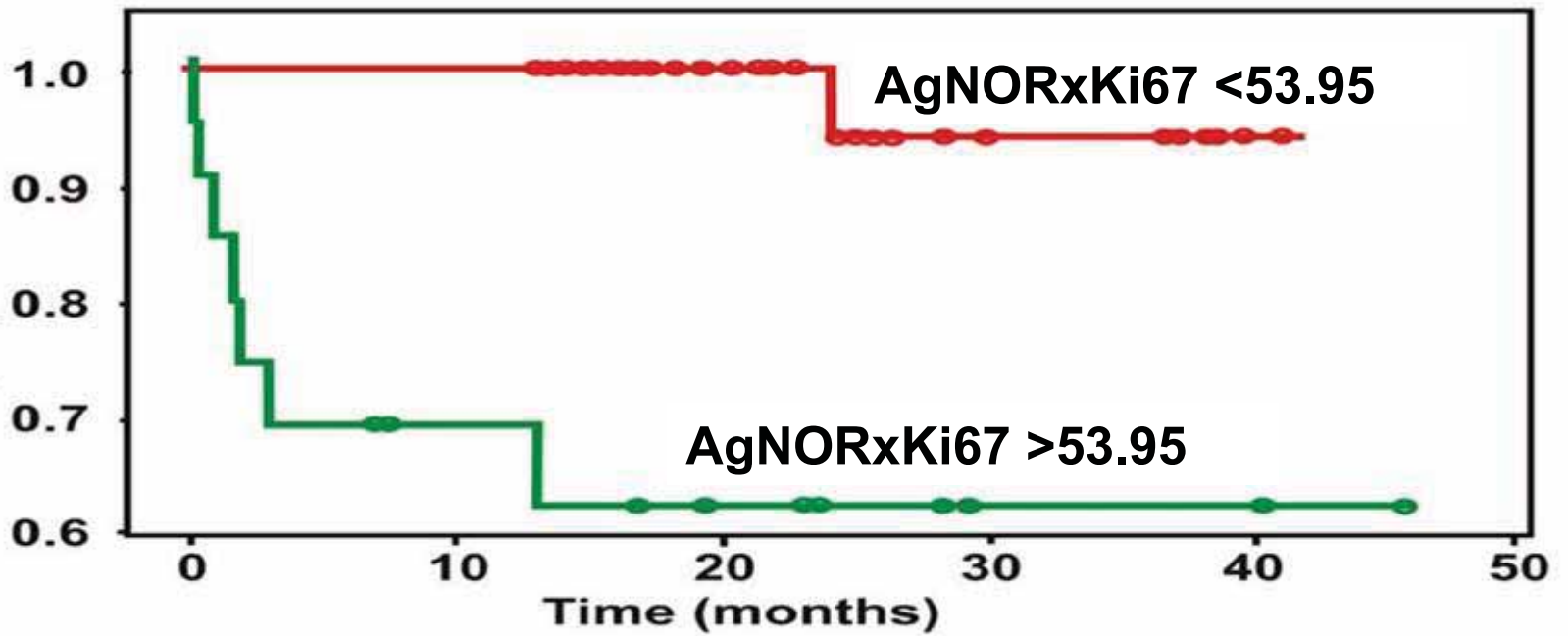
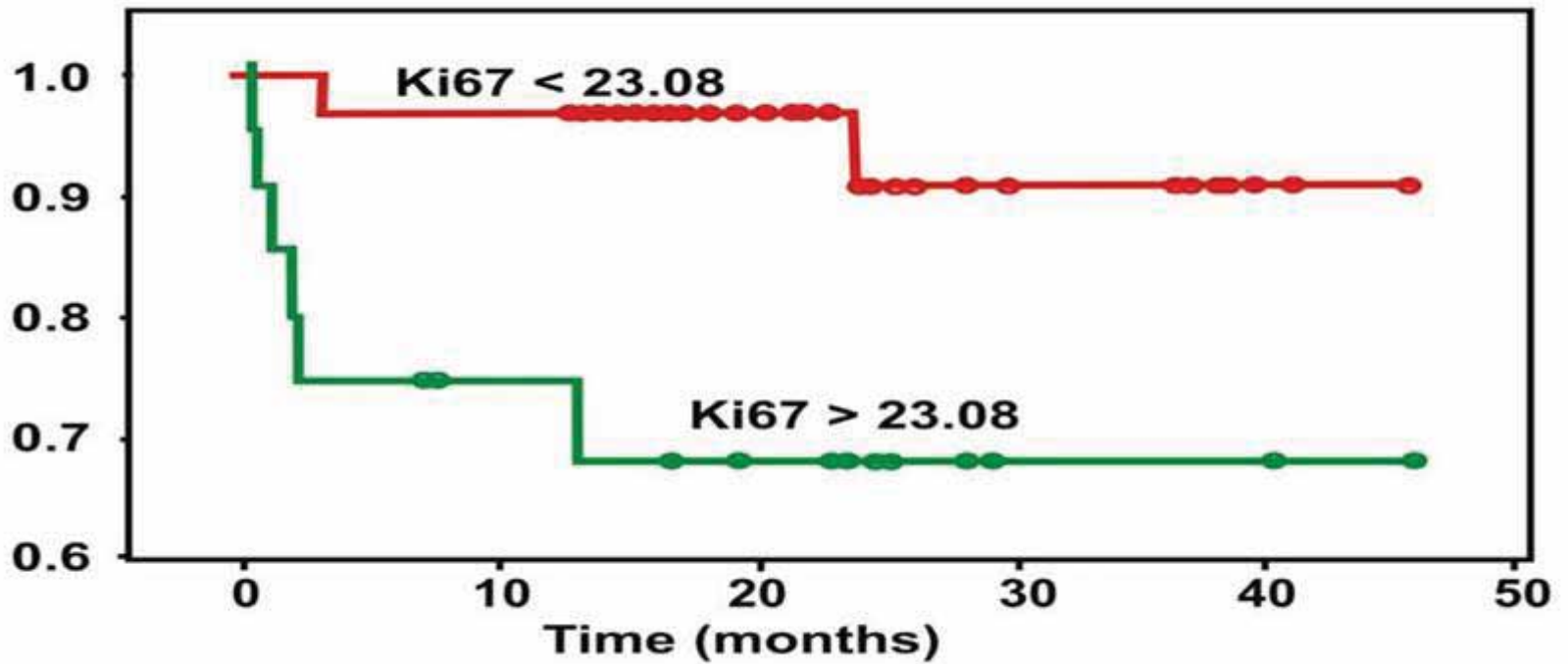


PCNA

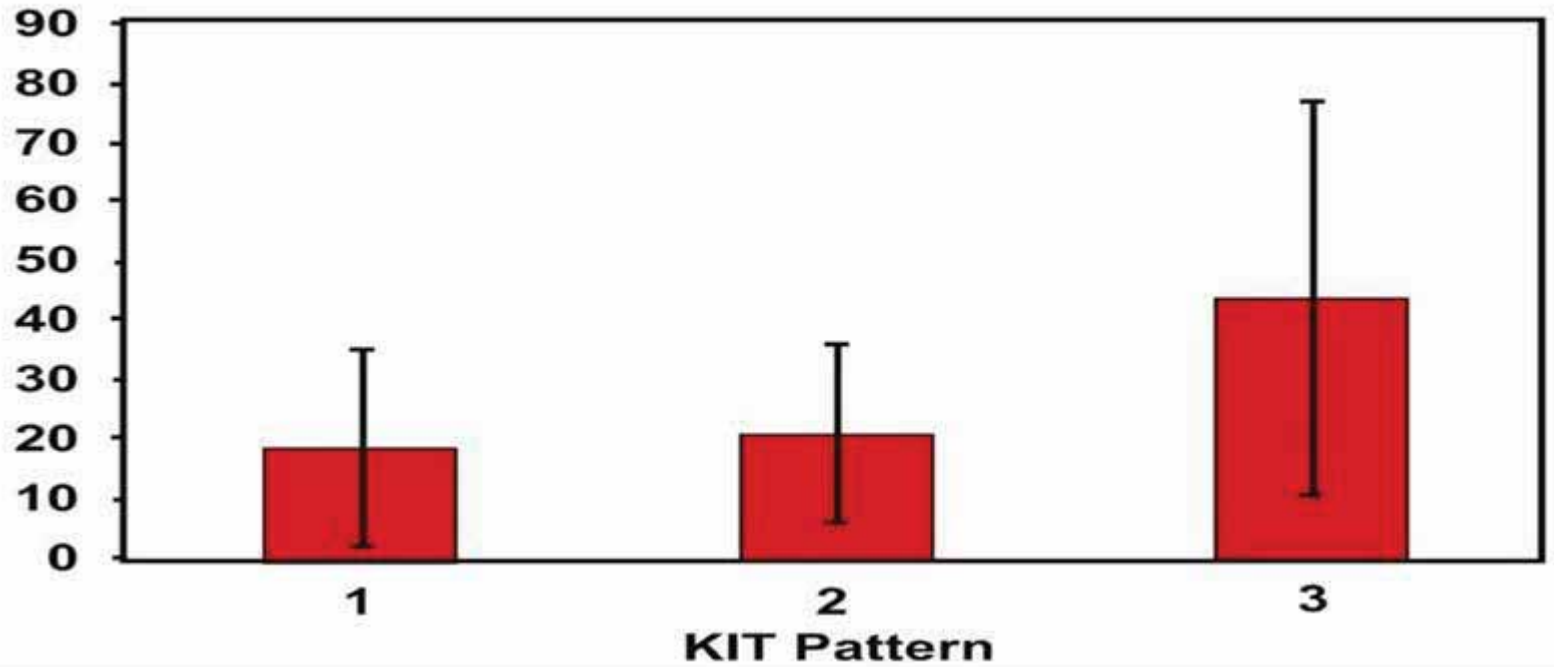
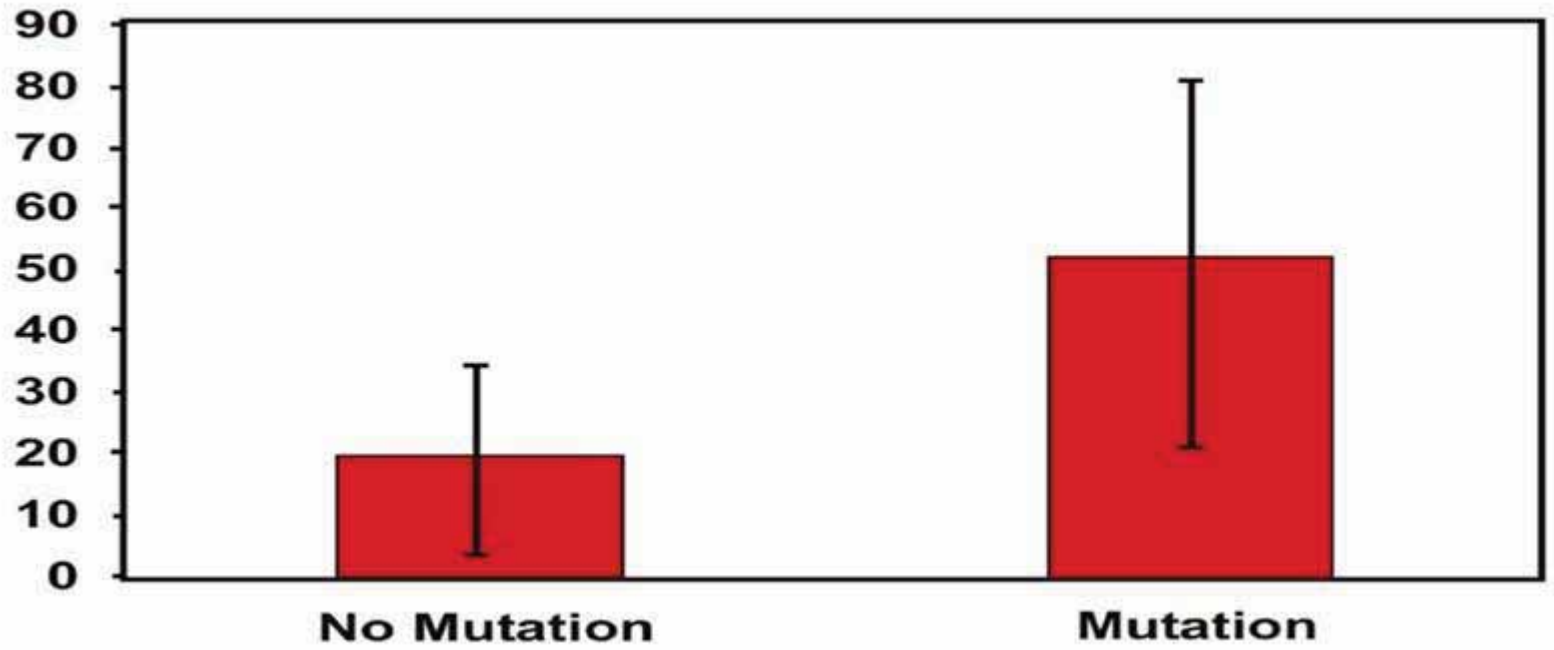
- Growth fraction: **KI-67**
- Cell cycle time: **AgNOR**
- Phase recognition: **PCNA**



Relative Frequency of Survival



Ki67 Index



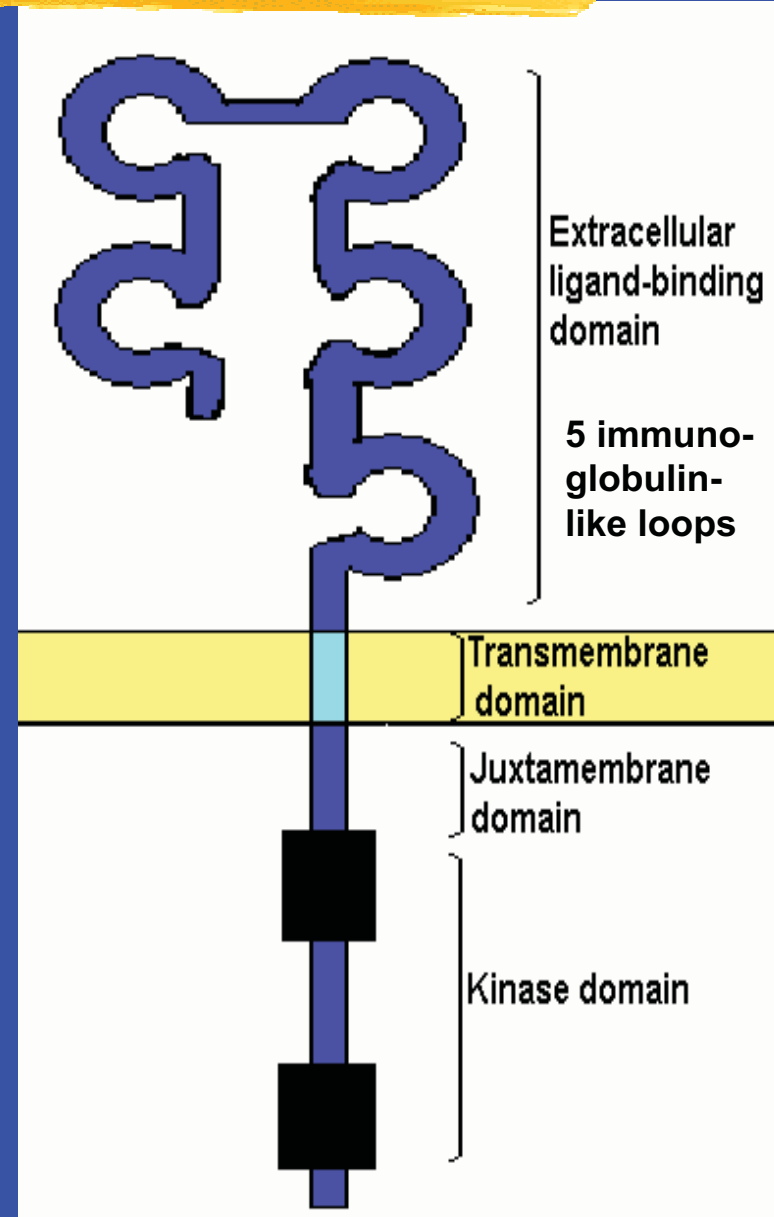
Conclusions



- **Assessment of cellular proliferation should be used in the prognostication of canine cutaneous MCTs**
 - **Proliferation Index:**
 - **Ki67: 23.08 cut point**
 - **Combination of AgNORs with Ki67**
 - **AgNORxKi67: 53.95 cut point**

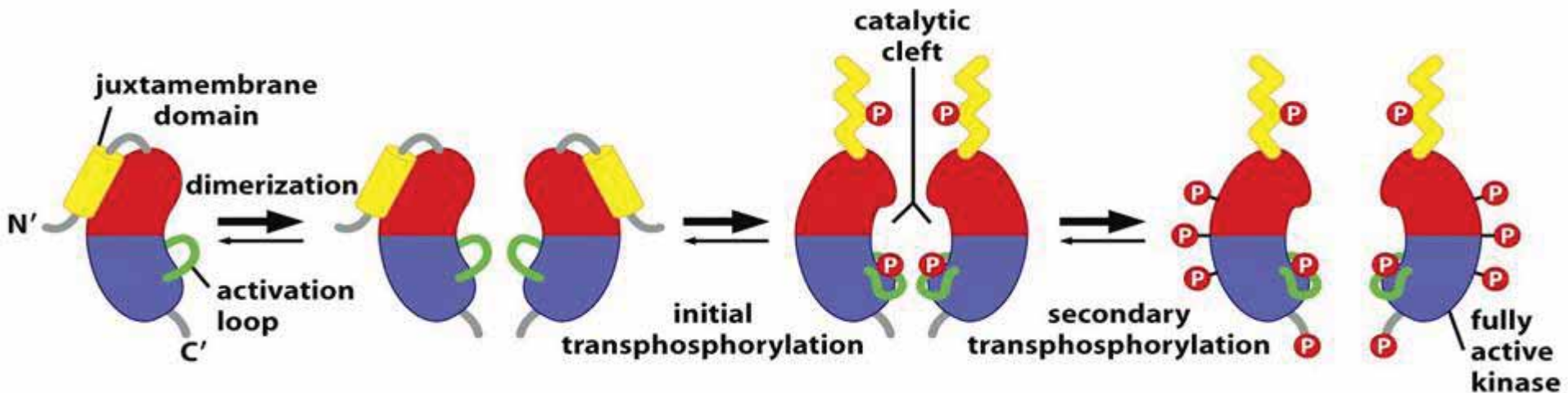
c-kit Proto-Oncogene

- Homology with *v-kit* : Hardy-Zuckerman 4-feline sarcoma viral genome
- Type III receptor tyrosine kinase
- Gene spans more than 70 kb of DNA and includes 21 exons, 20 are coding
- Cell specificity:
 - Mast cells
 - Hematopoietic progenitors
 - Neural crest-derived cells
 - Germ cells
- Ligand: Stem cell factor (SCF), steel factor, mast cell growth factor
- Cellular function
 - Hematopoiesis
 - Melanogenesis
 - Gametogenesis
 - Survival, proliferation, chemotaxis, and secretory activity of mast cells



KIT Receptor Activation

- Ligand binding results in dimerization and initial transphosphorylation of tyrosine residues in JMD
- Dissociates JMD from N-terminal lobe of two-lobed KIT tyrosine kinase (“moves out of the way”)
- Transphosphorylation of normally obstructing tyrosine residue in catalytic cleft
- Kinase activation and downstream signaling:
 - Phosphatidylinositol-3-kinase, Src family members, JAK/STAT pathway, Ras-Raf-MAP kinase cascade

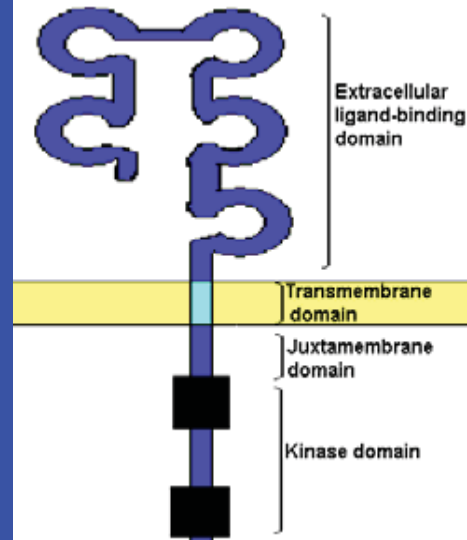


Loss of Function Mutations

- *c-kit*, KIT, CD117, SCFR, PTR, EC 2.7.10.1 3
- Terminology reflects historical mutations:
 - *W* locus in mice encodes for phenotype consisting of white-coat color, sterility, and anemia results from a 78-amino acid deletion that includes transmembrane of KIT, acts in dominant negative manner
 - Steel locus in mice, similar phenotype, is caused by mutations in the ligand for the c-KIT receptor, termed the steel ligand, KIT ligand etc.
 - Various coat color phenotypes in cattle and pigs can be attributed to mutations in KIT locus
 - Numerous human genetic diseases, including piebaldism, rare autosomal dominant disorder of melanocyte development

c-kit and Cancer

- *c-kit* activating mutations
 - Mastocytosis
 - Gastrointestinal stromal tumors (GISTs)
 - Germ cell tumors



5' 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 3'



Exons 1-9: Extracellular Domain

10: TM
11: JM

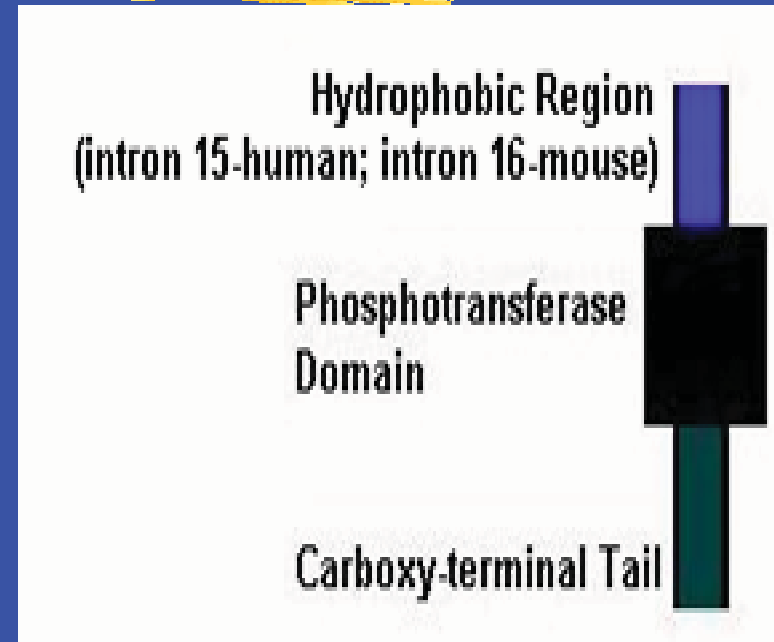
Exons 12-21: Kinase Domain

c-kit and Cancer

- Aberrant expression
 - Small cell lung cancer
 - Acute myeloid leukemia
 - Prostate cancer
 - Colon cancer

- Mechanism:

- Autocrine/paracrine signaling loops (SCF)
- Truncated KIT isoform (TR-KIT)



TR-KIT

***c-KIT* and Canine MCTs**

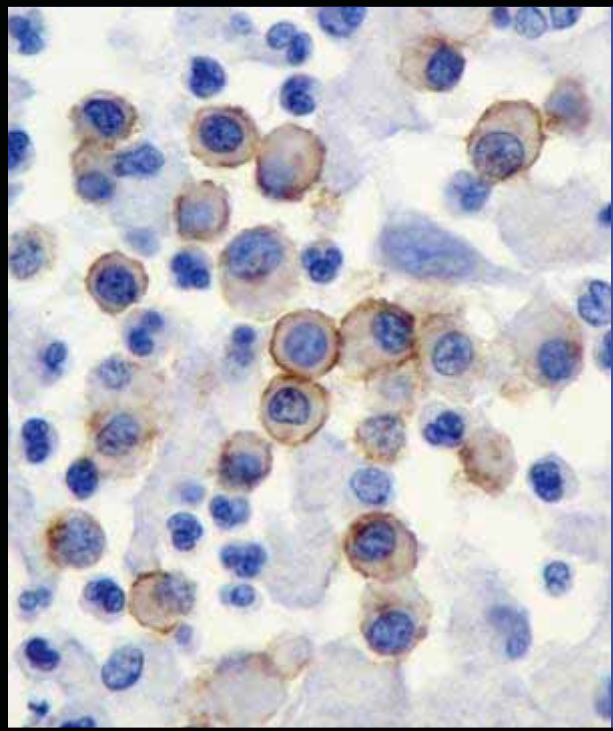


- Juxtamembrane domain mutations
 - **Internal tandem duplications (ITD)**
 - Prevalence: 15-20% of MCTs
 - Associated with higher histologic grade MCTs
 - Zemke *et al.*, 2001
 - No association with survival
 - Downing *et al.*, 2002
 - Deletions
 - Zemke *et al.*, 2001
 - Reguera *et al.*, 2002
- Kinase domain mutations: No mutations identified
- Aberrant KIT localization: Reguera *et al.*, 2000

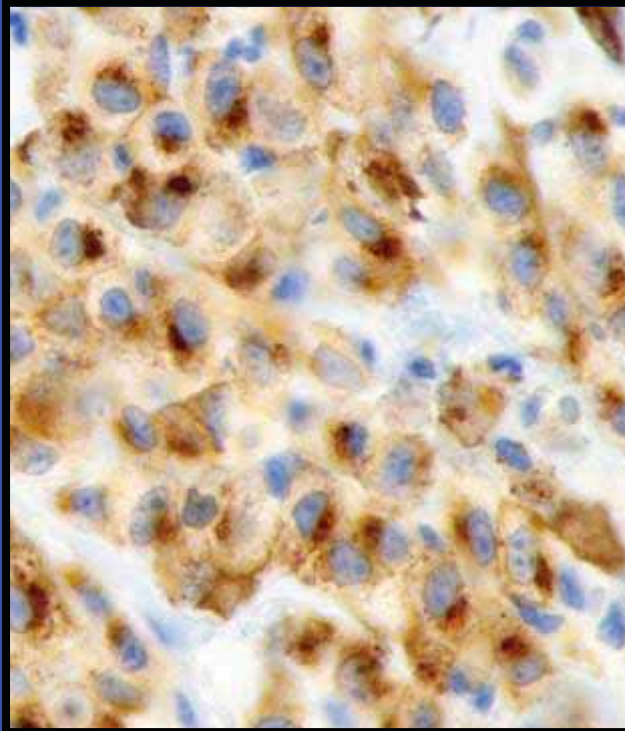
Role of *c-kit* in canine MCTs

- Characterize the biological significance of *c-KIT* in canine cutaneous MCTs:
 - Define the prognostic significance of
 - KIT staining patterns
 - *c-kit* mutations
 - KIT protein levels
 - Investigate the relationship between
 - KIT staining patterns
 - *c-KIT* mutations
 - KIT protein levels

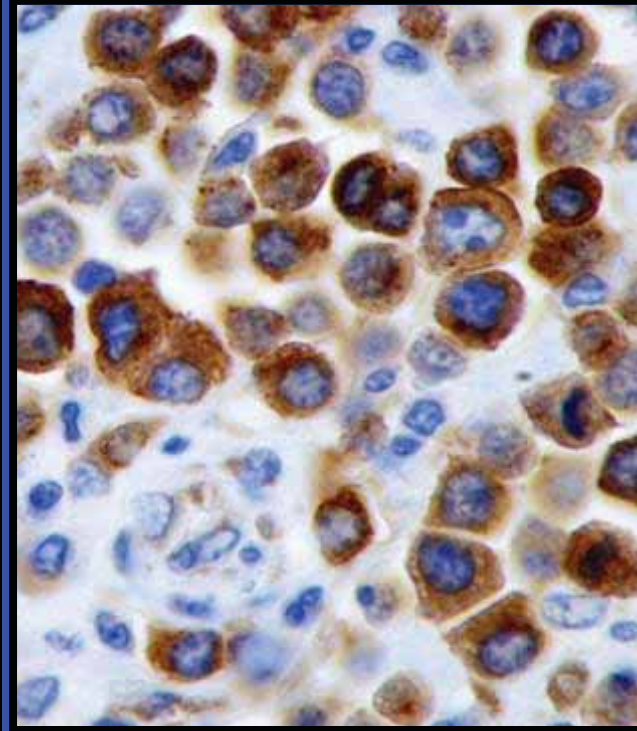
KIT Staining Patterns



**KIT Staining
Pattern 1**

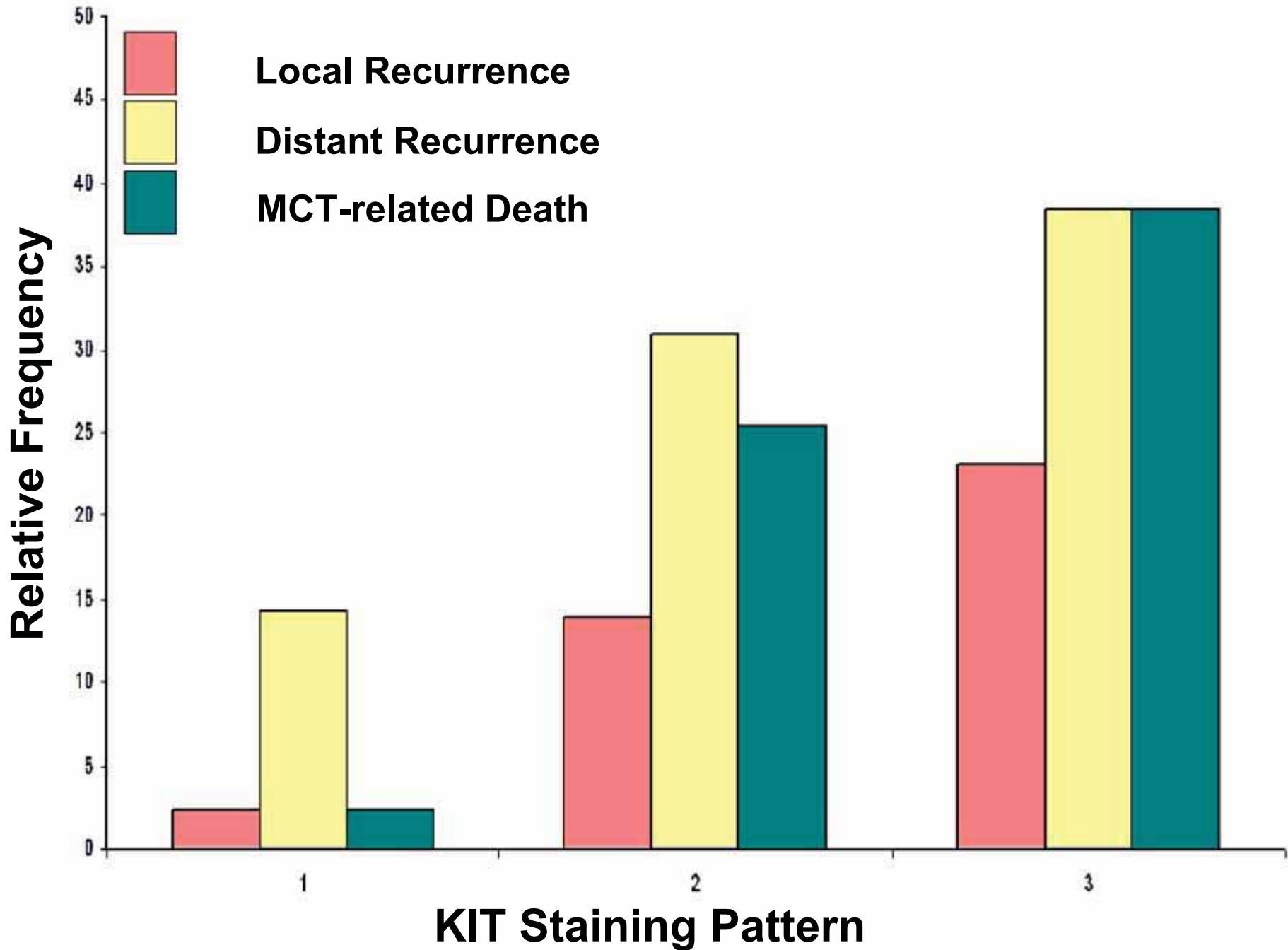


**KIT Staining
Pattern 2**

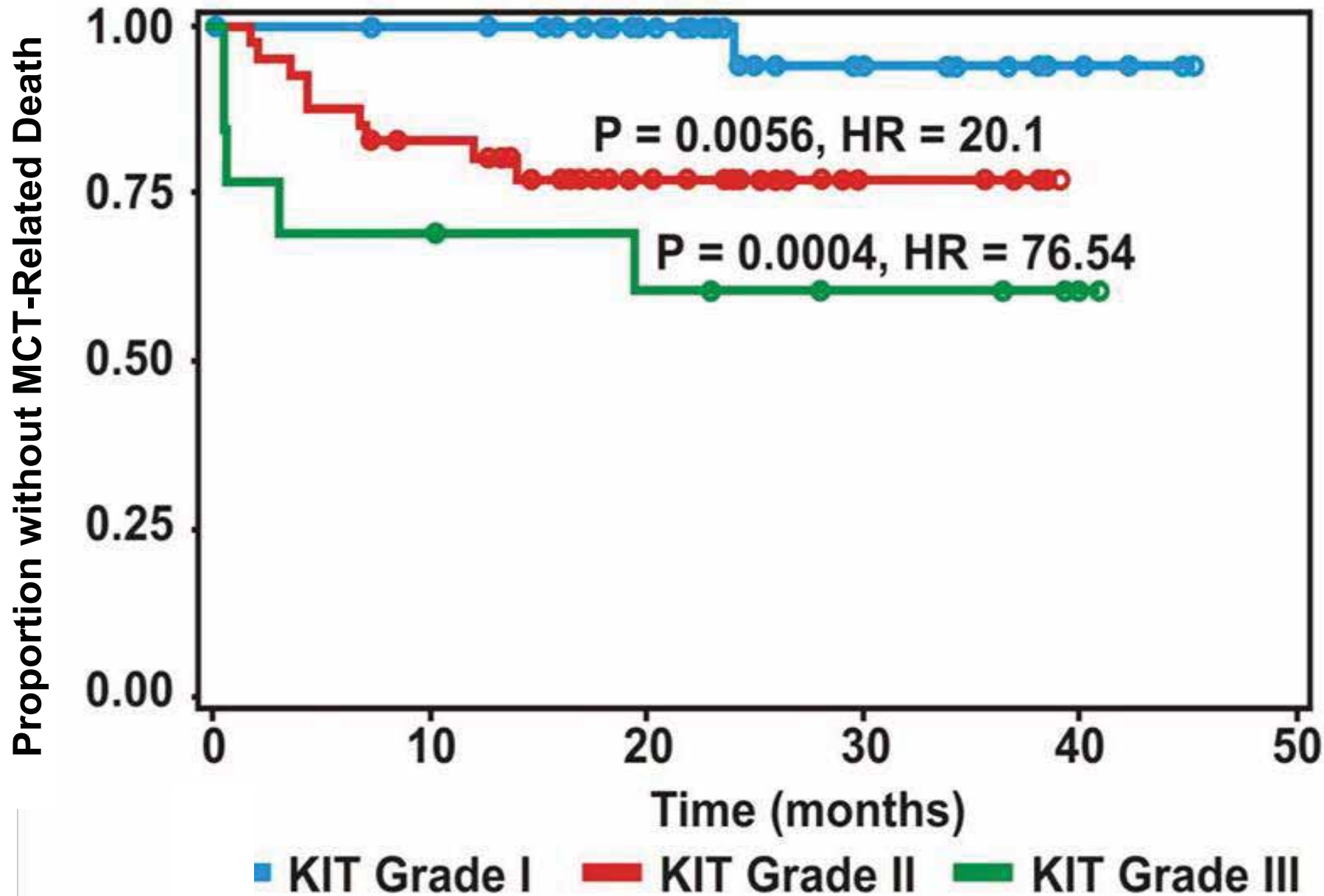


**KIT Staining
Pattern 3**

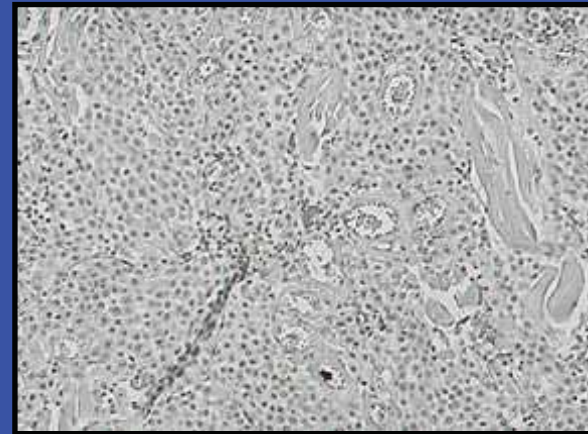
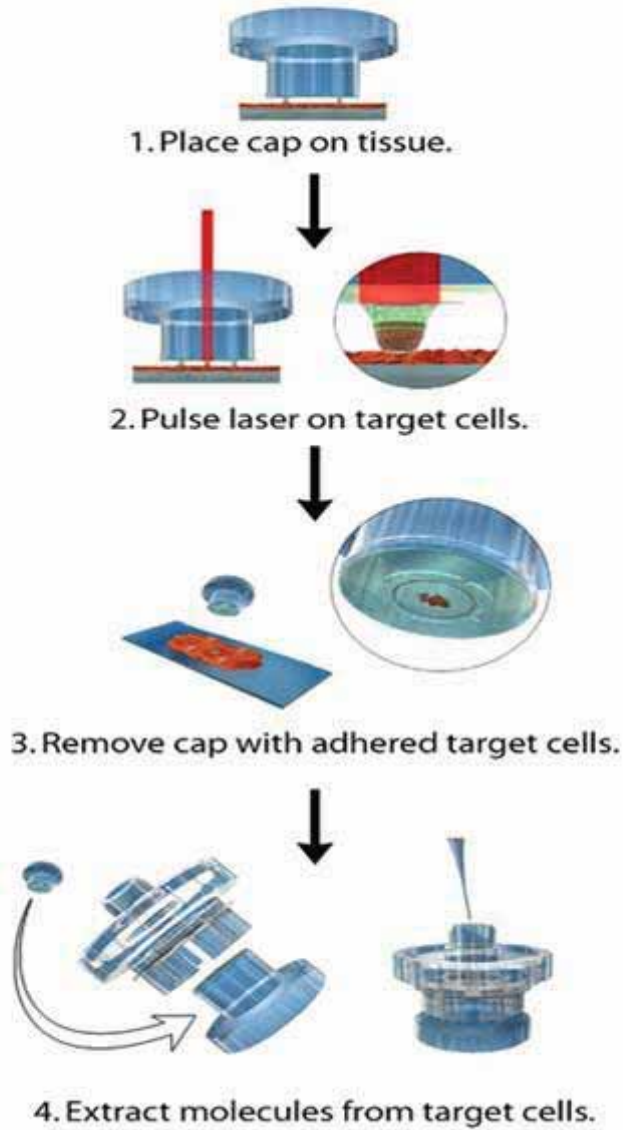
(Kiupel et. al., The Use of KIT and Tryptase Expression Patterns as Prognostic Tools for Canine Cutaneous Mast Cell Tumors. Vet Pathol 2004)



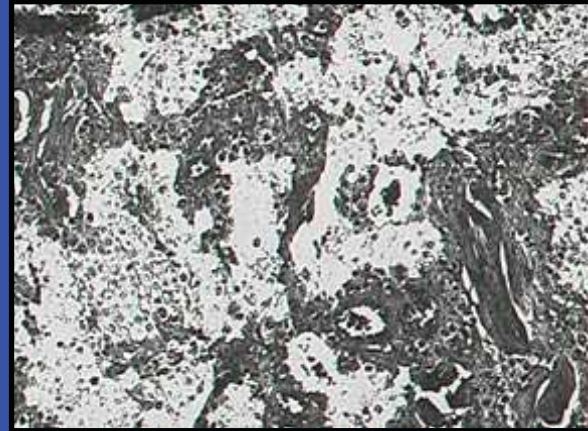
Kaplan-Meier Survival Plot: MCT-Related Death



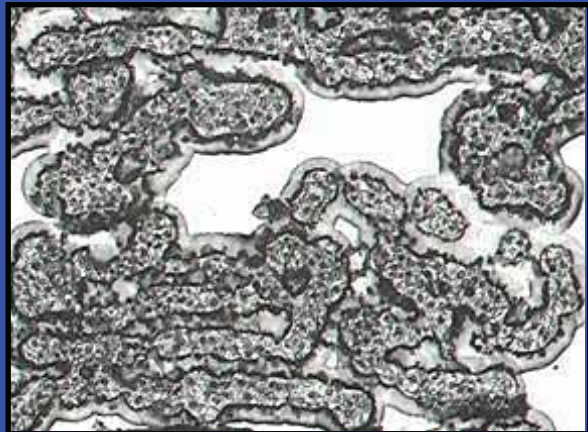
The Laser Capture Microdissection Process



Before



After



Cap

Detection of Internal Tandem Duplication Mutations

L= 100bp Ladder

N= Normal

M= Mutation

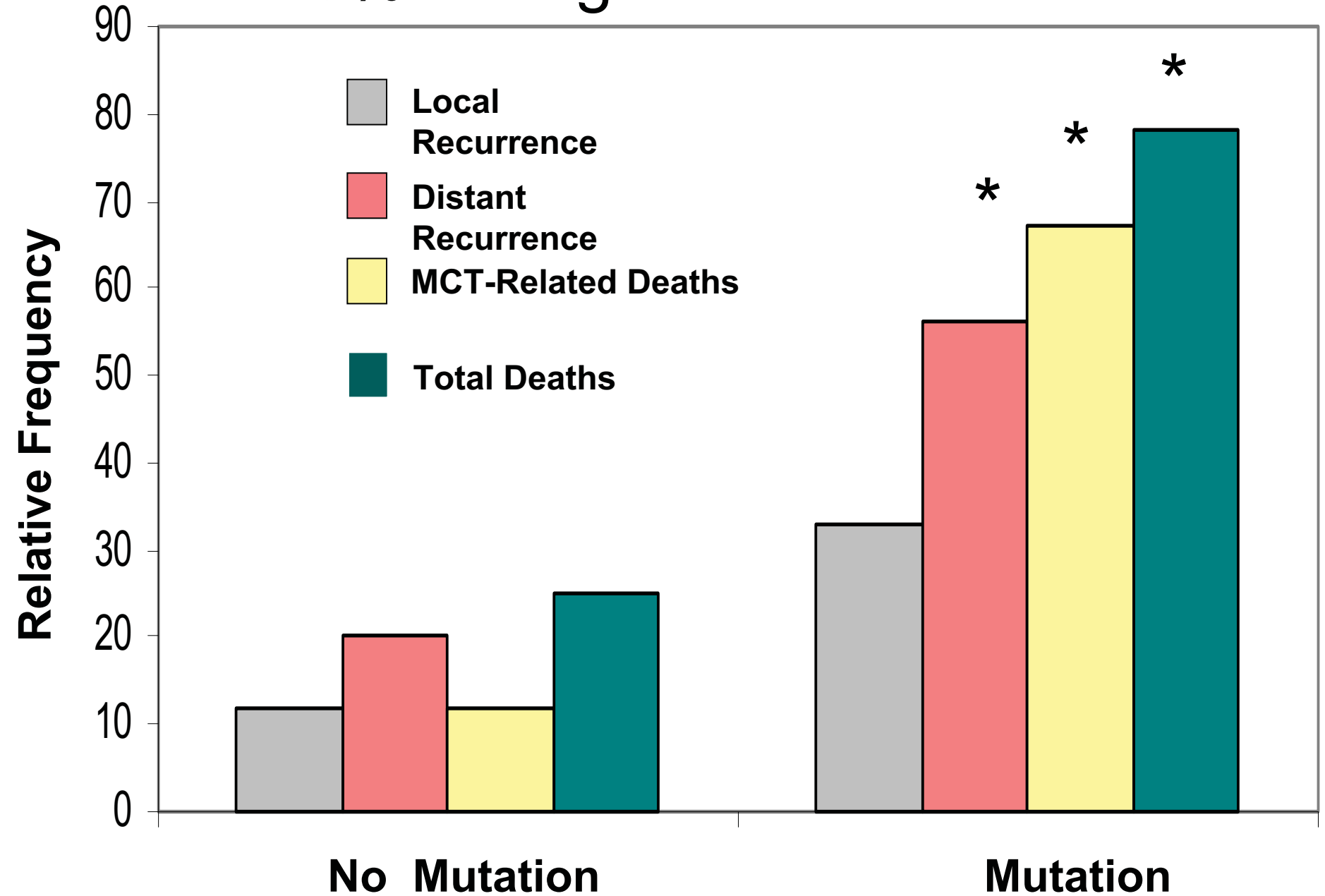
NC= Negative
Control

- PCR-amplified *c-kit* exon 11 and intron 11 from canine MCTs:
 - normal allele (191 bp)
 - mutant allele (250 bp), with an upper band representing heterodimerization of normal and mutant alleles

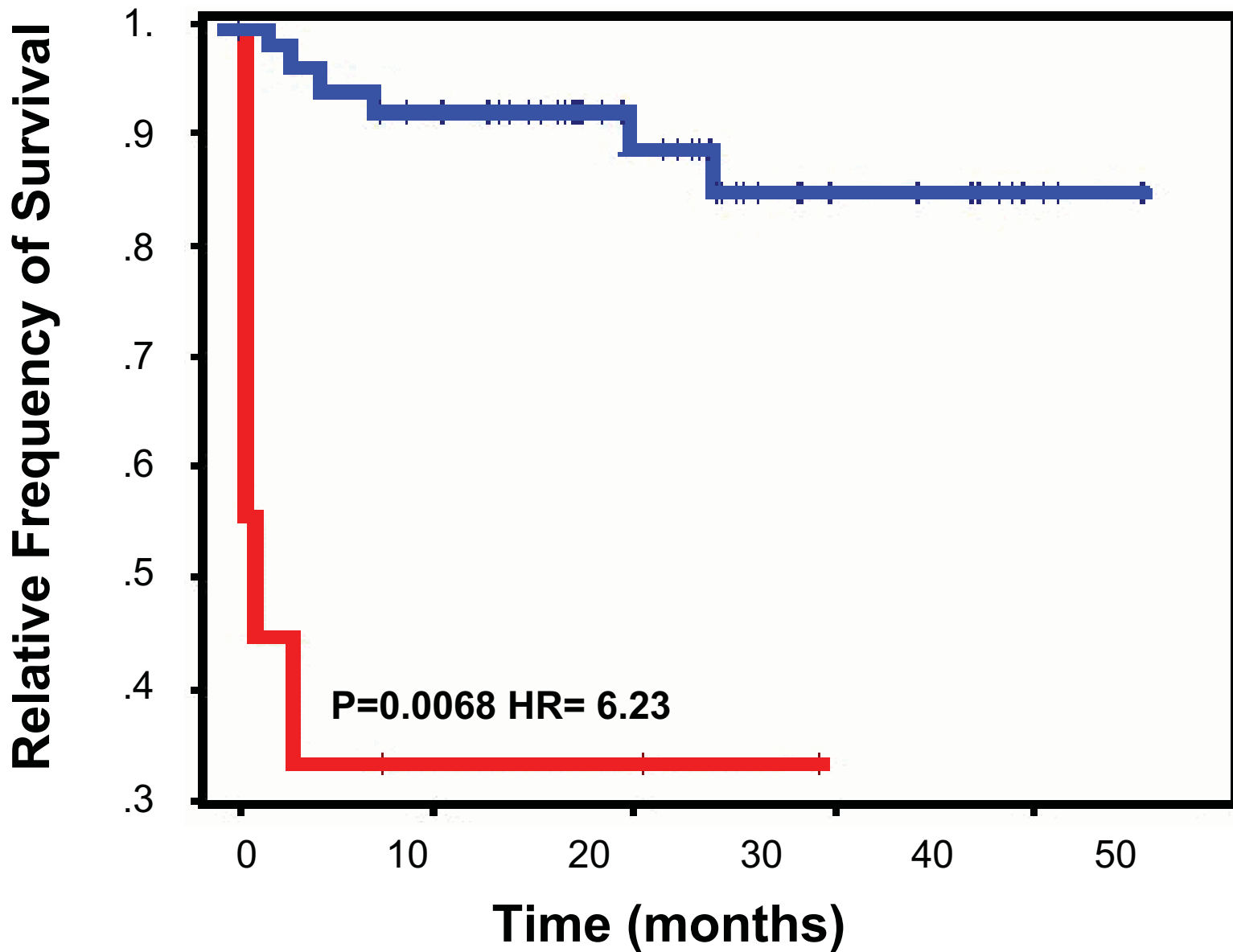


(Webster et. al., The Role of c-KIT in Canine Cutaneous Mast Cell Tumors: Evaluation of c-kit's Role in Tumorigenesis in a Spontaneous Canine Model. Neoplasia, 2006)

15% of dogs with *c-kit* mutation



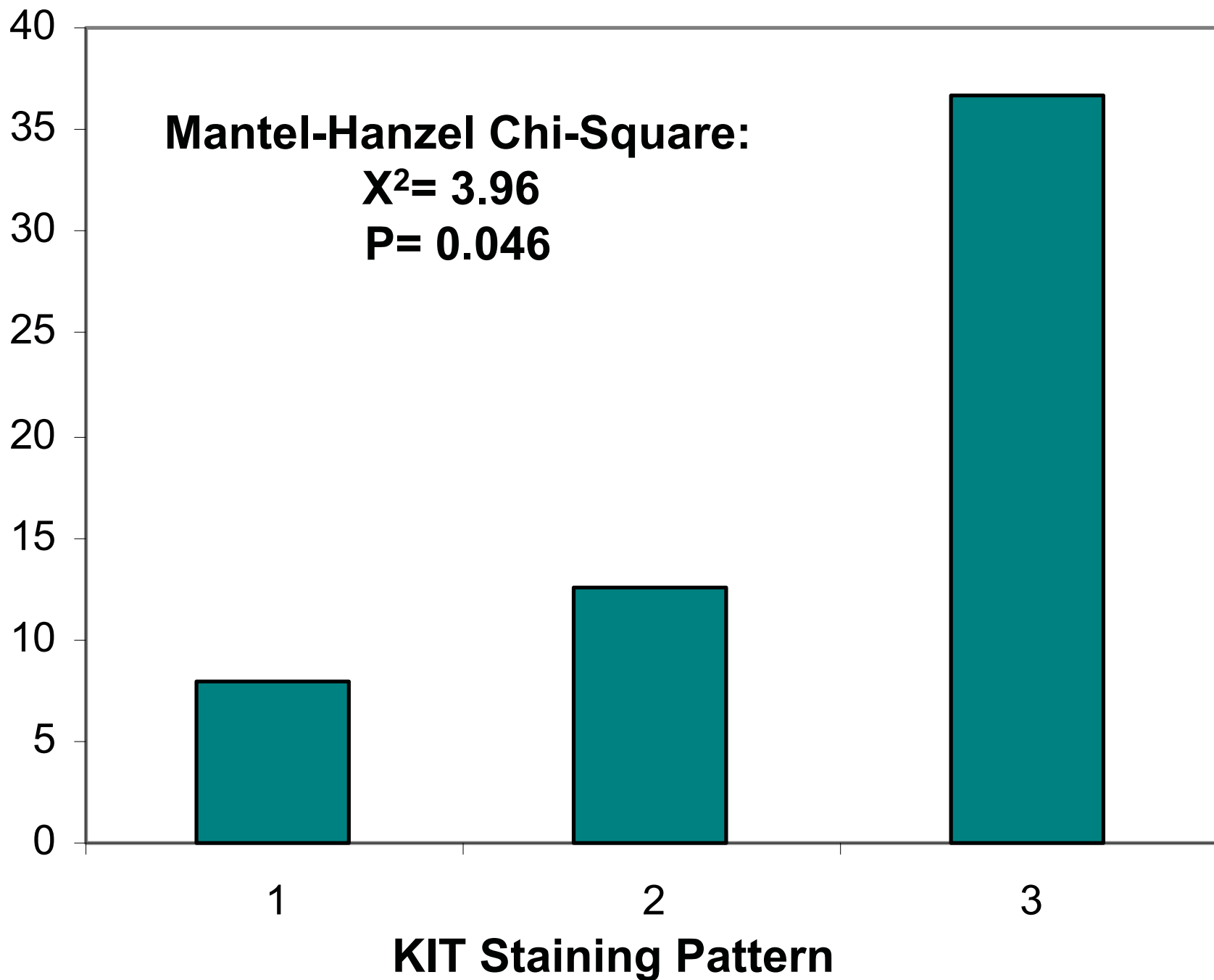
Kaplan-Meier Survival Plot: MCT-Related Death



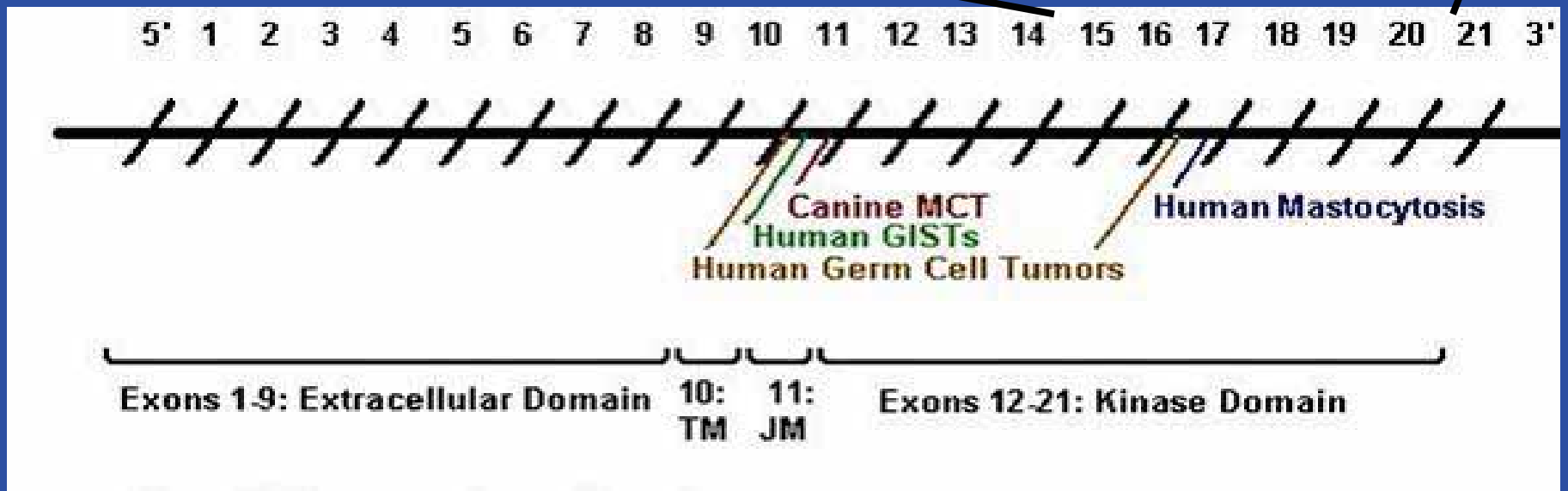
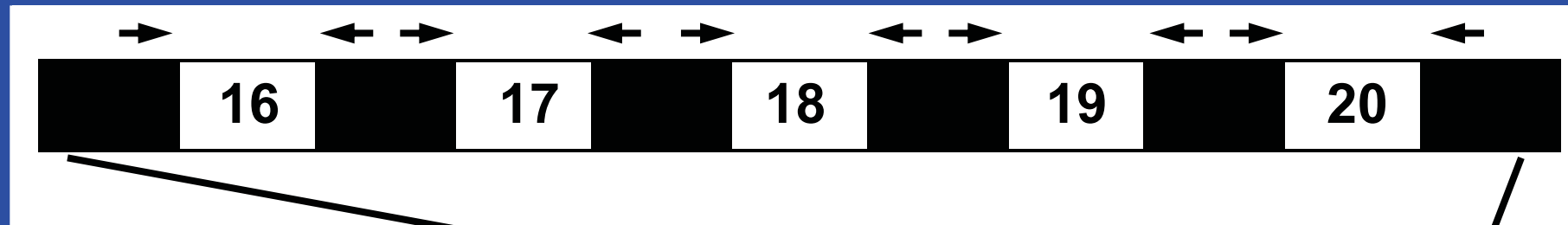
■ No *c-KIT* mutations

■ *c-KIT* mutations

Relative Frequency of Mutations



c-kit Kinase Domain



(Webster et. Al., Evaluation of Kinase Domain c-KIT Mutations in Canine Cutaneous Mast Cell Tumors. BMC Cancer, 2006)

Immunofluorescence

- Immunofluorescence
 - 42 MCTs
 - Anti-KIT antibody
 - Tissue microarray
 - Quantification:
Perkin Elmer Scan Array
- No correlation between immunofluorescence and KIT staining patterns
- Trend but no significant association between immunofluorescence and *c-KIT* mutations
- No prognostic significance



Conclusions

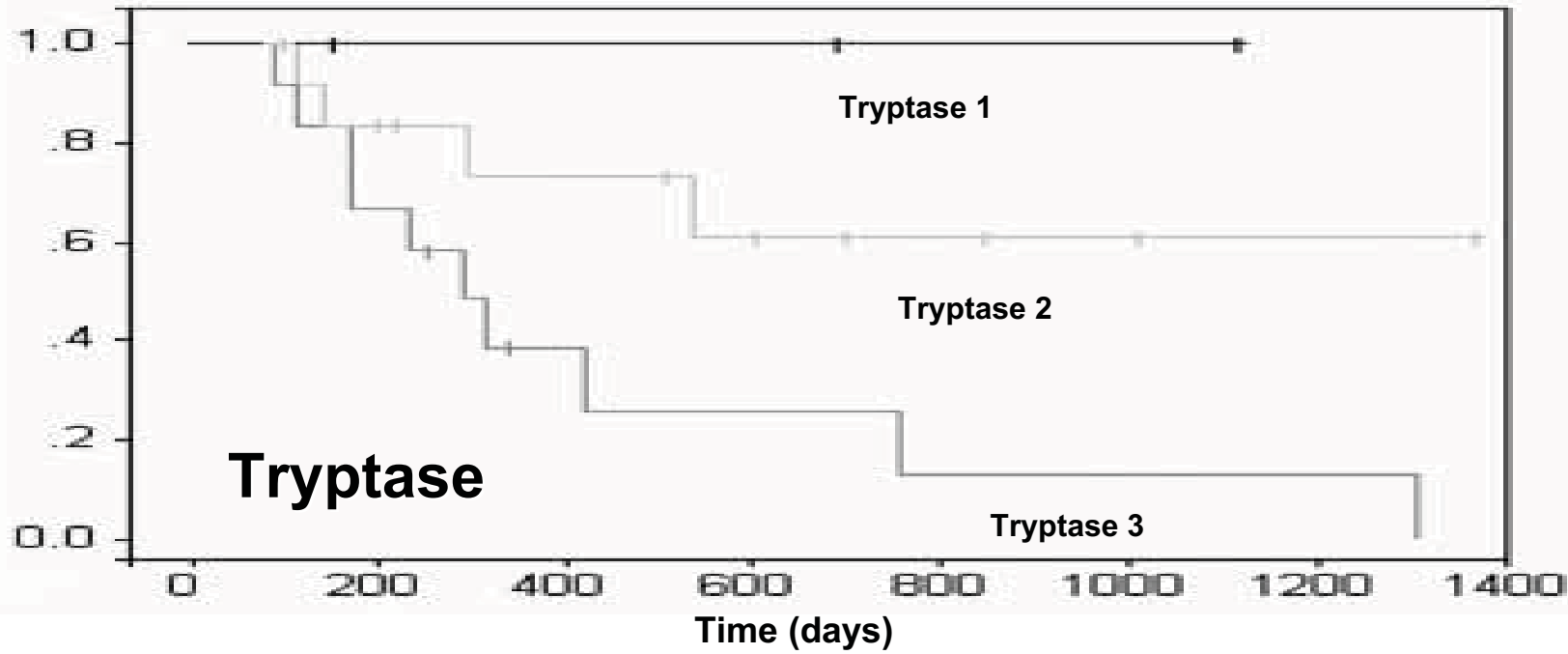
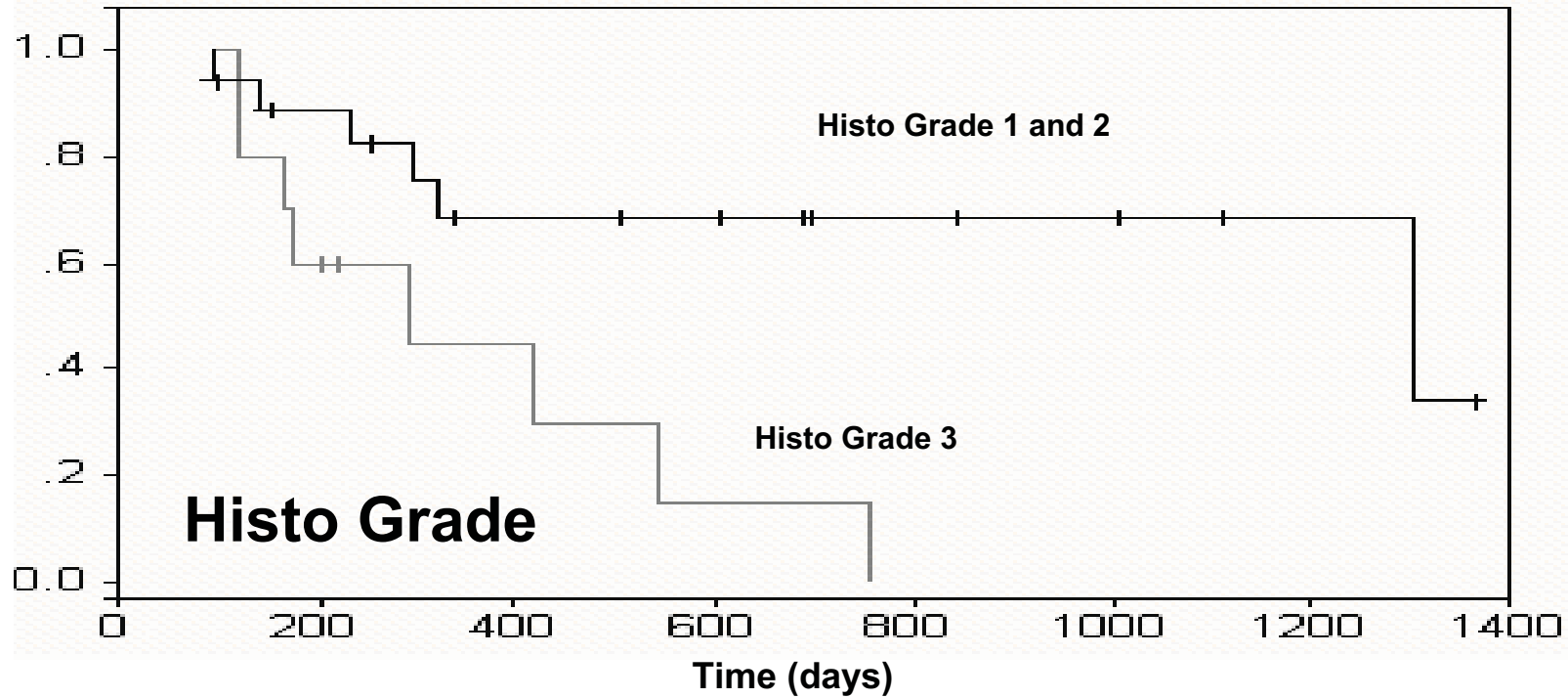


- **ITD *c-KIT* mutations**
 - **Constitutively active**
 - **15-20% canine MCTs**
 - **Associated with decreased survival and disease-free interval**
 - **Associated with aberrant KIT localization**
- **Kinase domain *c-KIT* mutations**
 - **Minimal importance in canine MCTs**
- **Aberrant cytoplasmic KIT localization**
 - **Associated with decreased survival and disease-free interval**

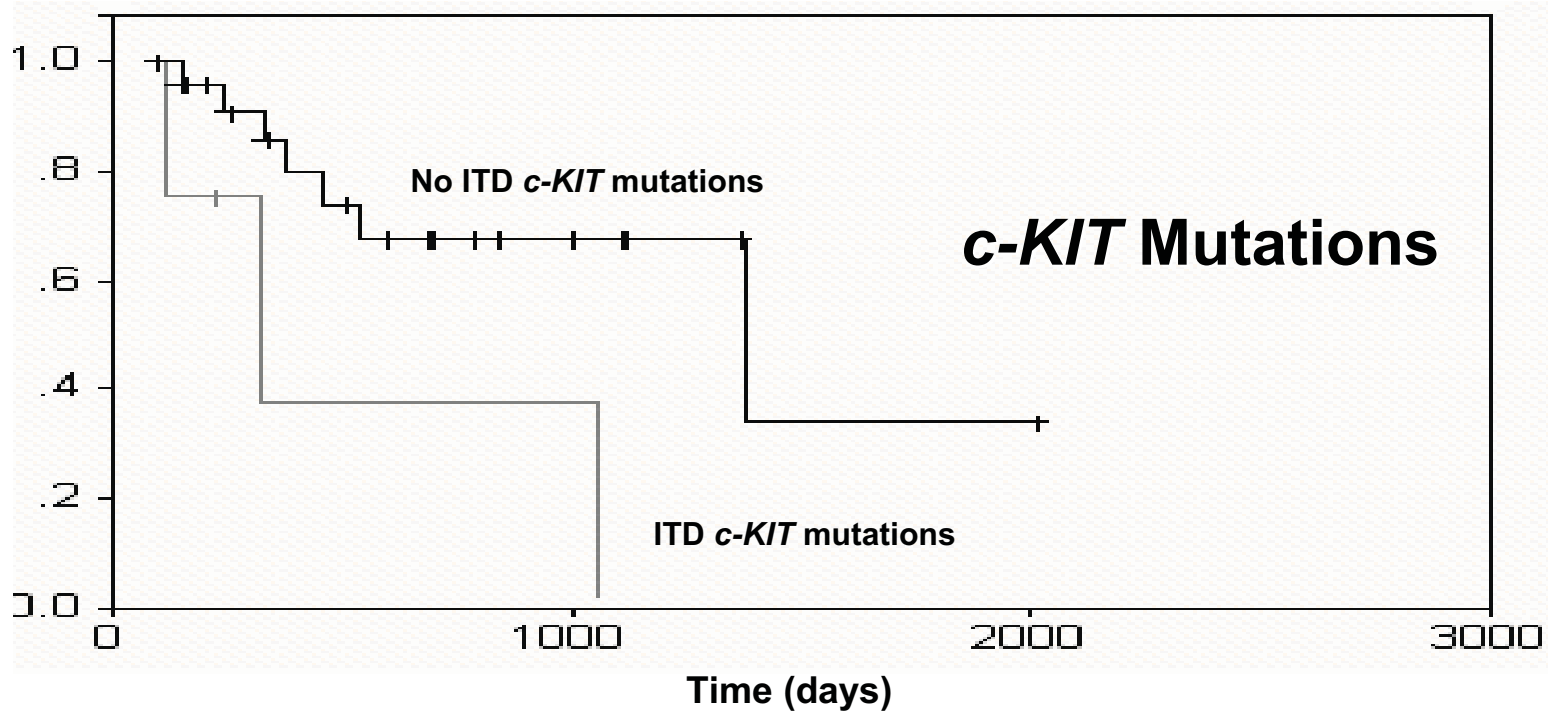
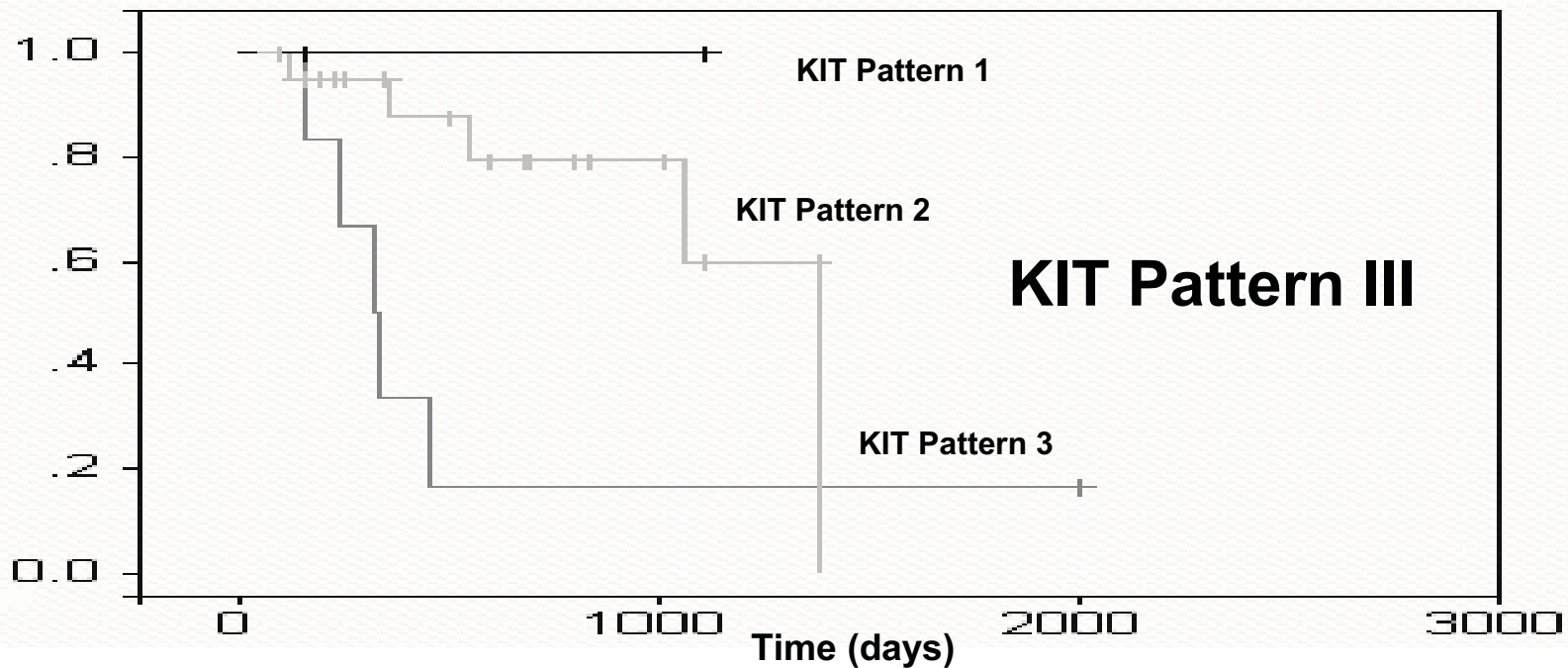
Evaluation of MCTs treated with Chemotherapy

- Confirmed diagnosis of canine cutaneous MCTs
- Treated with
 - Surgery +/- radiation
 - Vinblastine and prednisone
 - No other treatment
- Absence of measurable disease following surgery
- Absence of severe concurrent disease
- Complete staging
- Adequate tissue available for all studies

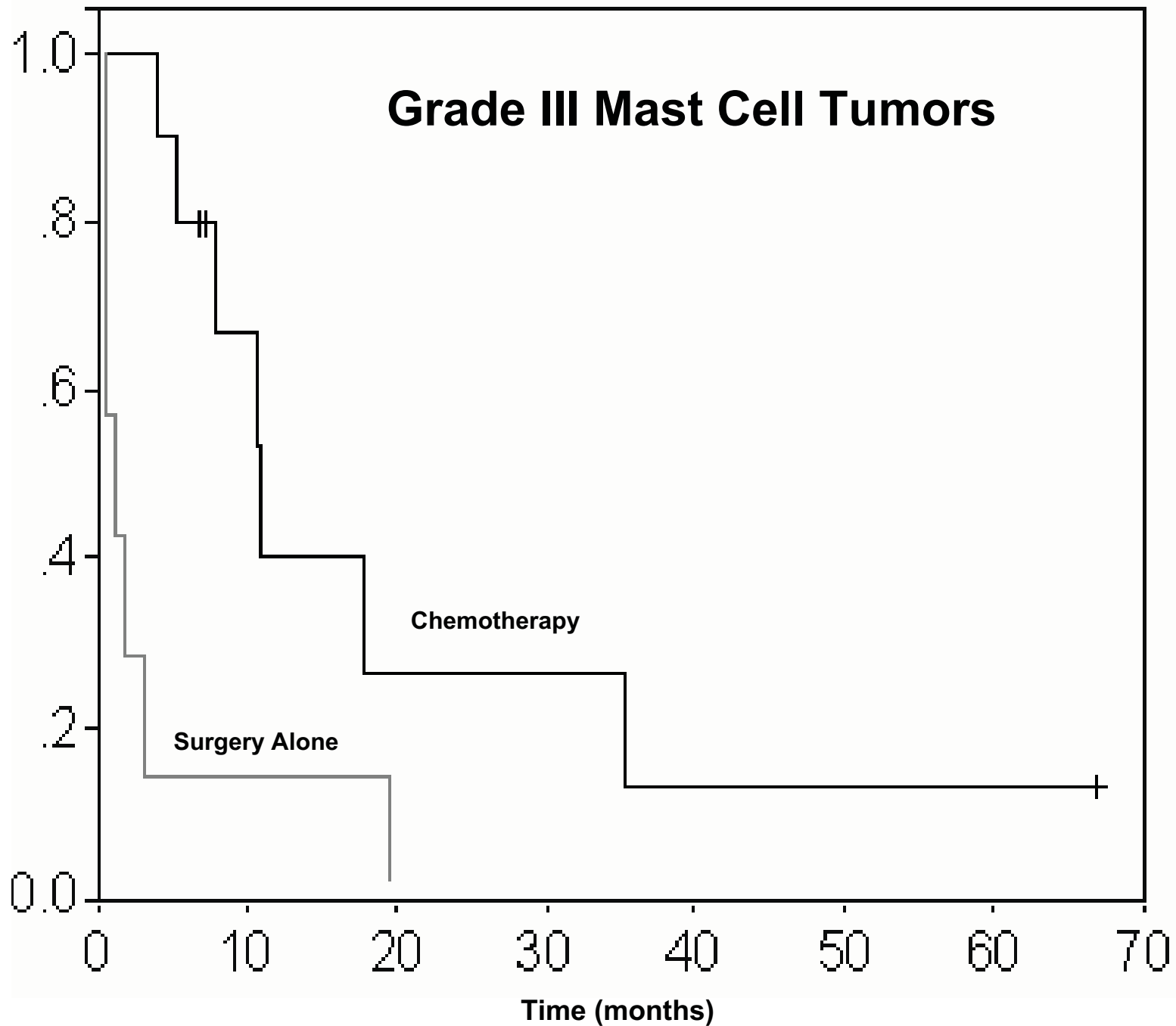
Relative Frequency of Survival



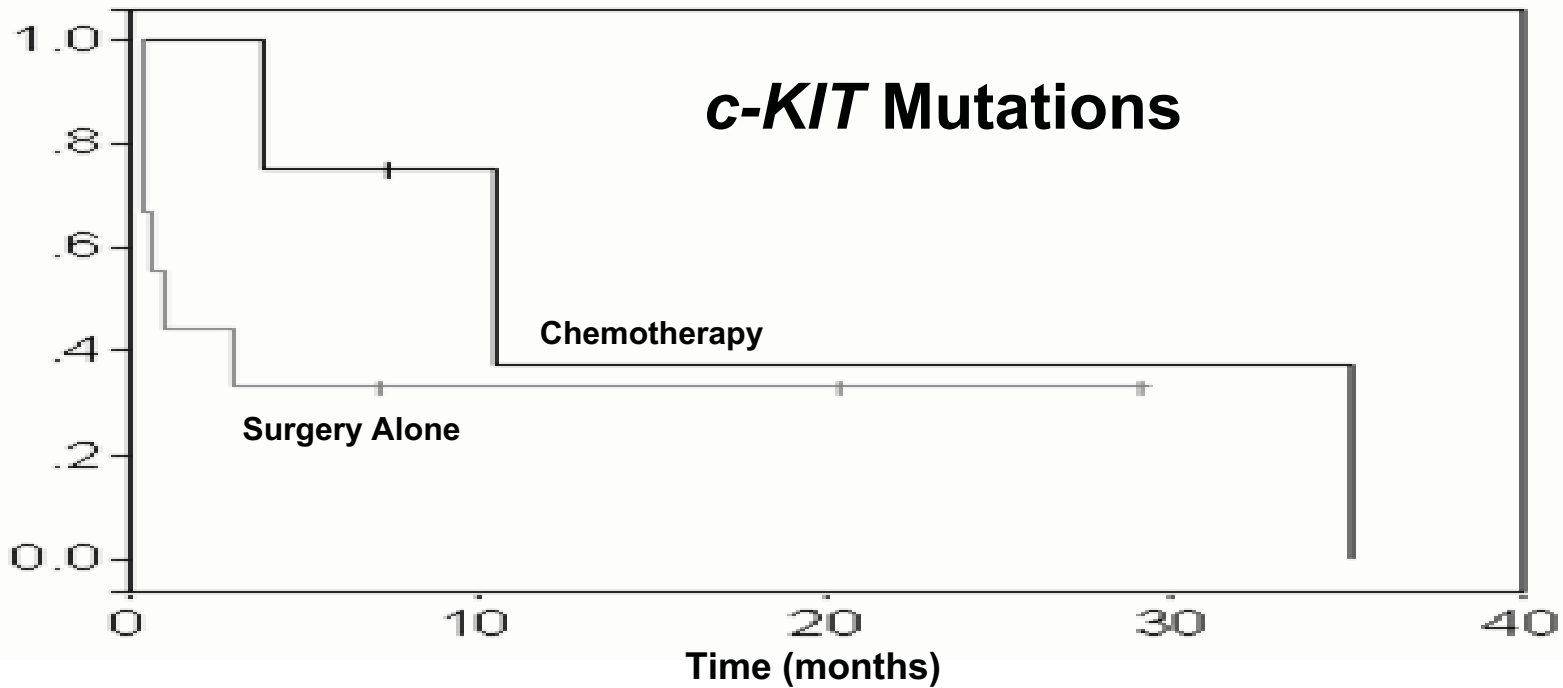
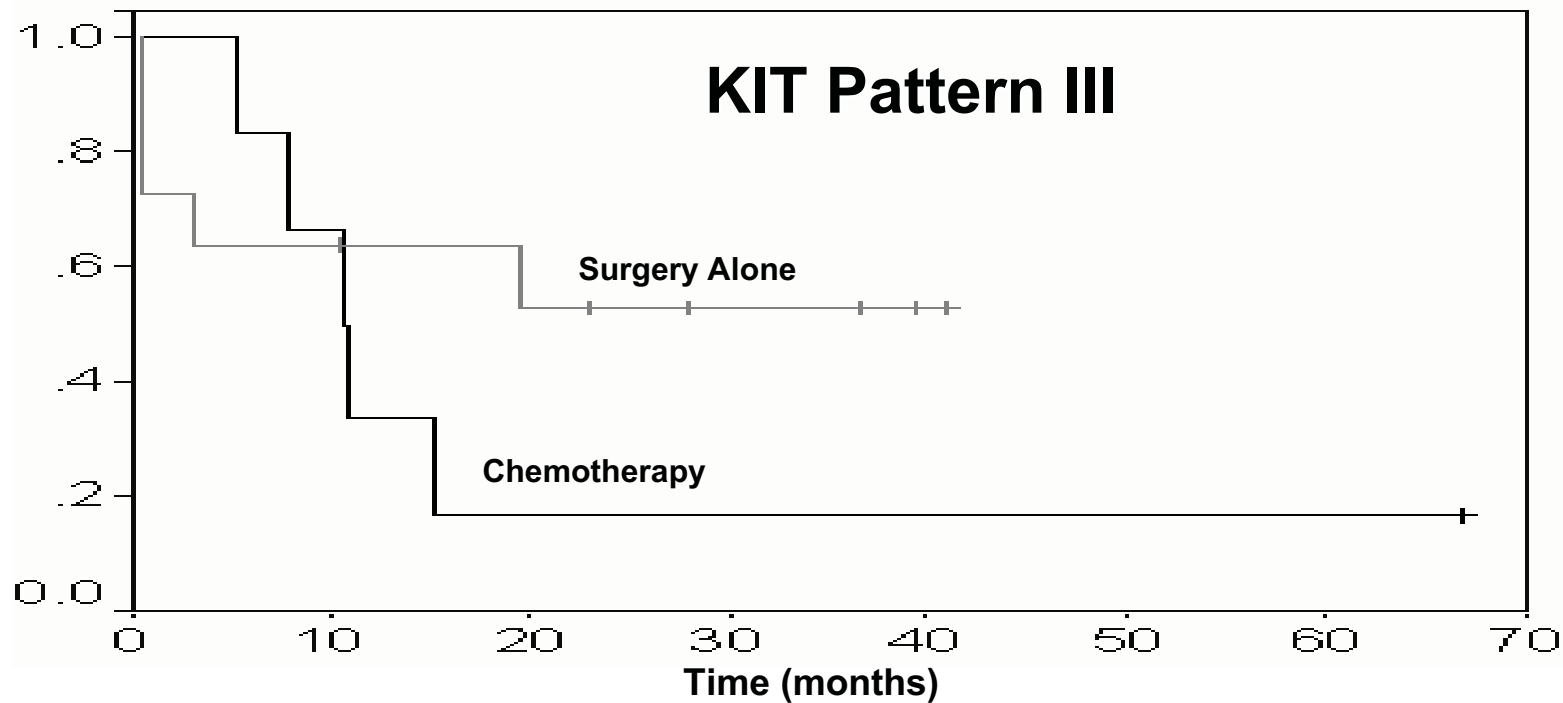
Relative Frequency of Survival



Relative Frequency of Survival



Relative Frequency of Survival



Conclusions



- **Significantly increased survival with adjunct chemotherapy**
 - **Histologic grade III MCTs**
- **Increased short-term survival with adjunct chemotherapy**
 - **ITD *c-KIT* mutations**
 - **KIT pattern III**

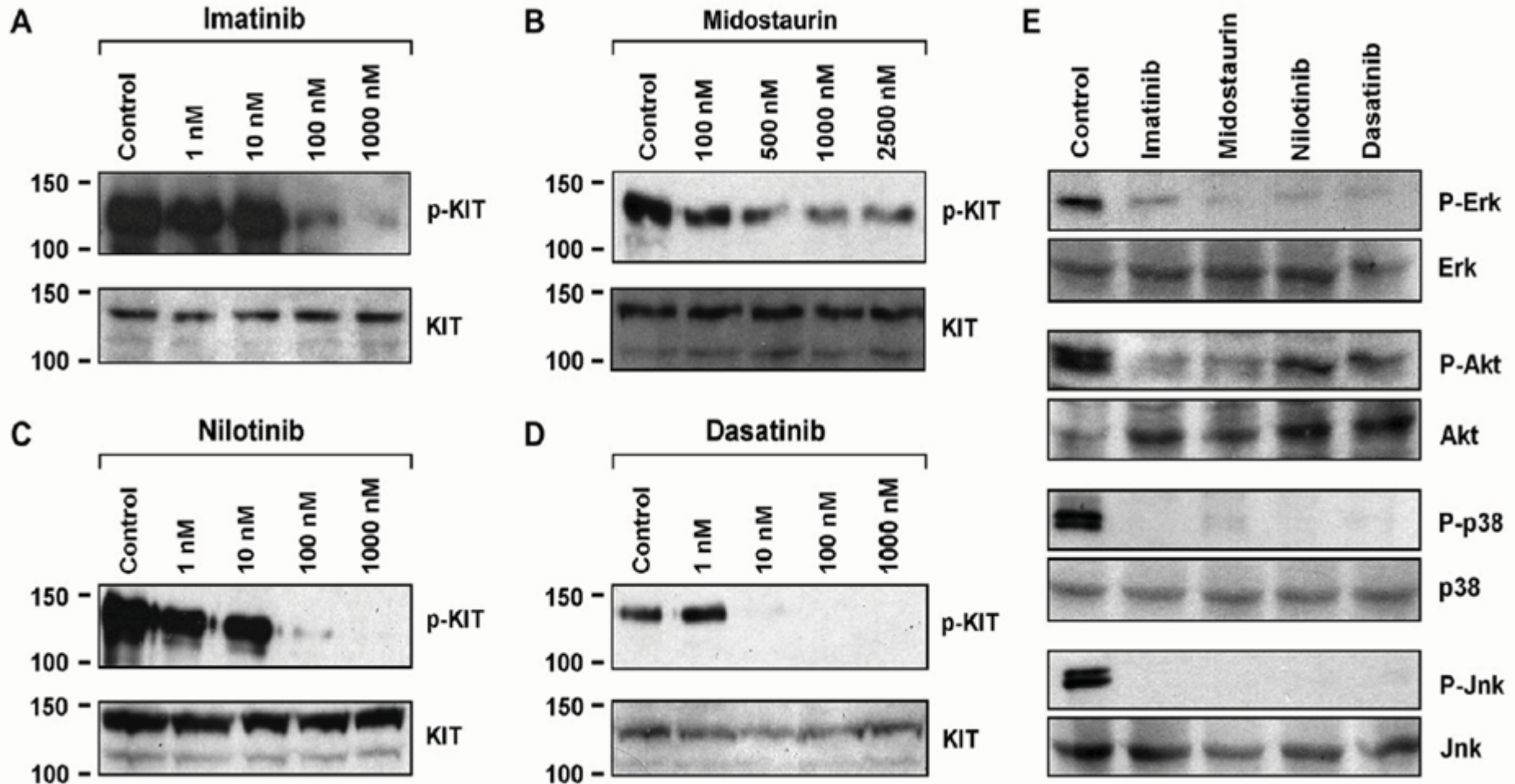
KIT as Target of Therapy



- **KIT is a Type III receptor tyrosine kinase**
- **Imatinib (Gleevec):**
 - **Tyrosine kinase inhibitor (TKI)**
 - **First successful targeted therapy**
 - **Effective in most CML patients**
 - **Chronic hepatotoxicity in dogs**
- **Many more TKIs under development**
 - **Dasatinib**
 - **Nilotinib**
 - **Midostaurin**
 - **Masitinib**

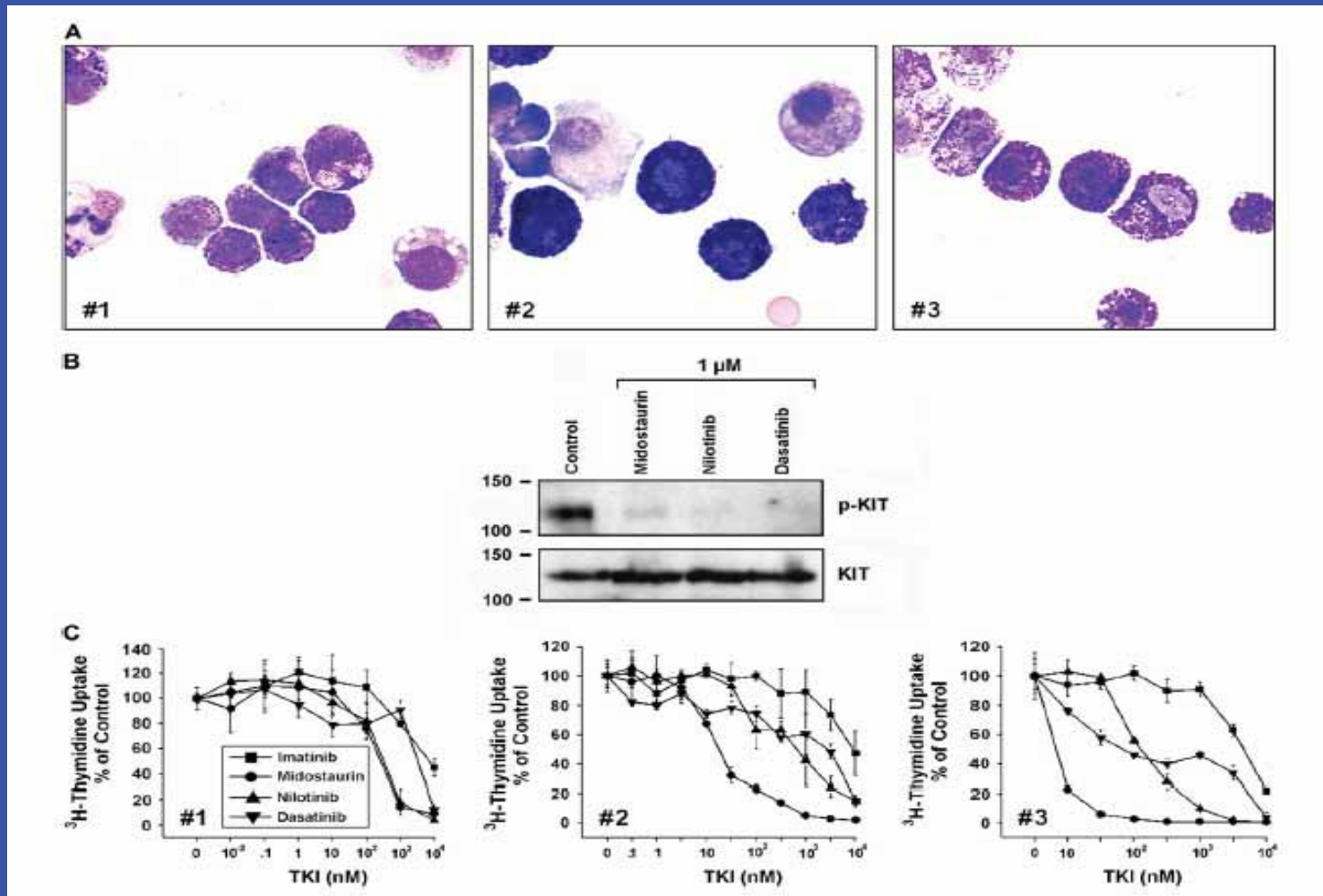
Inhibition of KIT Phosphorylation by TKIs

C2 MCT cell line with ITD mutation in exon 11



Inhibition of KIT Phosphorylation by TKIs

primary MCT cell line with no mutations



Induction of Apoptosis by TK Inhibitors

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