International Neuroblastoma Pathology Classification: Pathologist's Role in Translational Research

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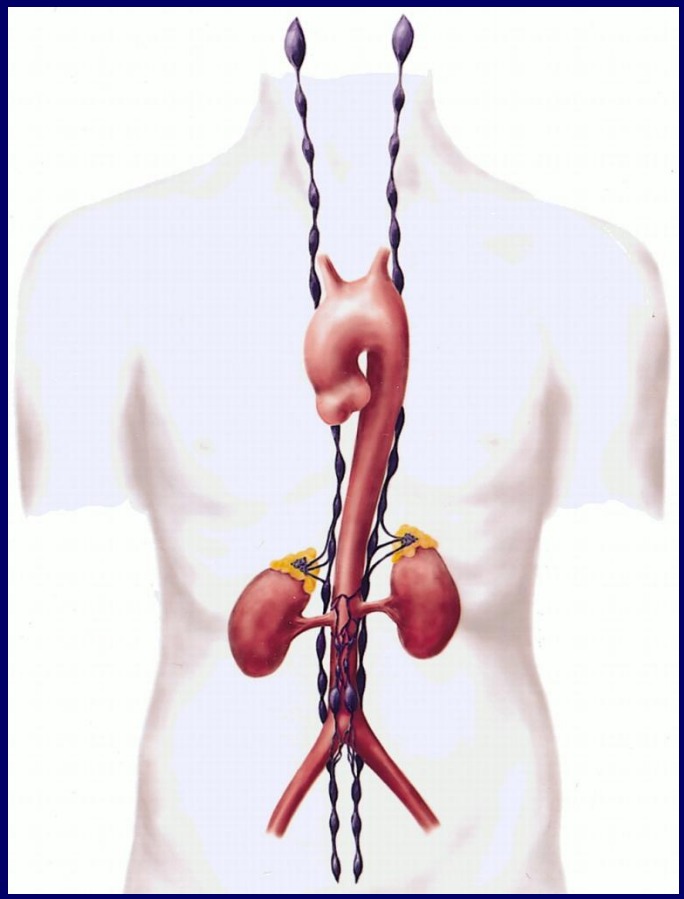
Peripheral Neuroblastic Tumors, Overview

- Neuroblastoma, Ganglioneuroblastoma, Ganglioneuroma
- Embryonal tumor
- Derived from post-ganglionic sympathetic neural precursors (neural crest cells)
- Half arise in adrenal, remainder occur in paraspinal ganglia
- Most common non-CNS solid tumor of children
- Prevalence: 1:7000 births, 700 USA cases/yr.
- 7-10% of all childhood cancers



Geneva Foundation for Medical Research

- Median age: 17 months, 75% dxed by age 4 yr., 98% by age 10 yr.

Peripheral Neuroblastic Tumors

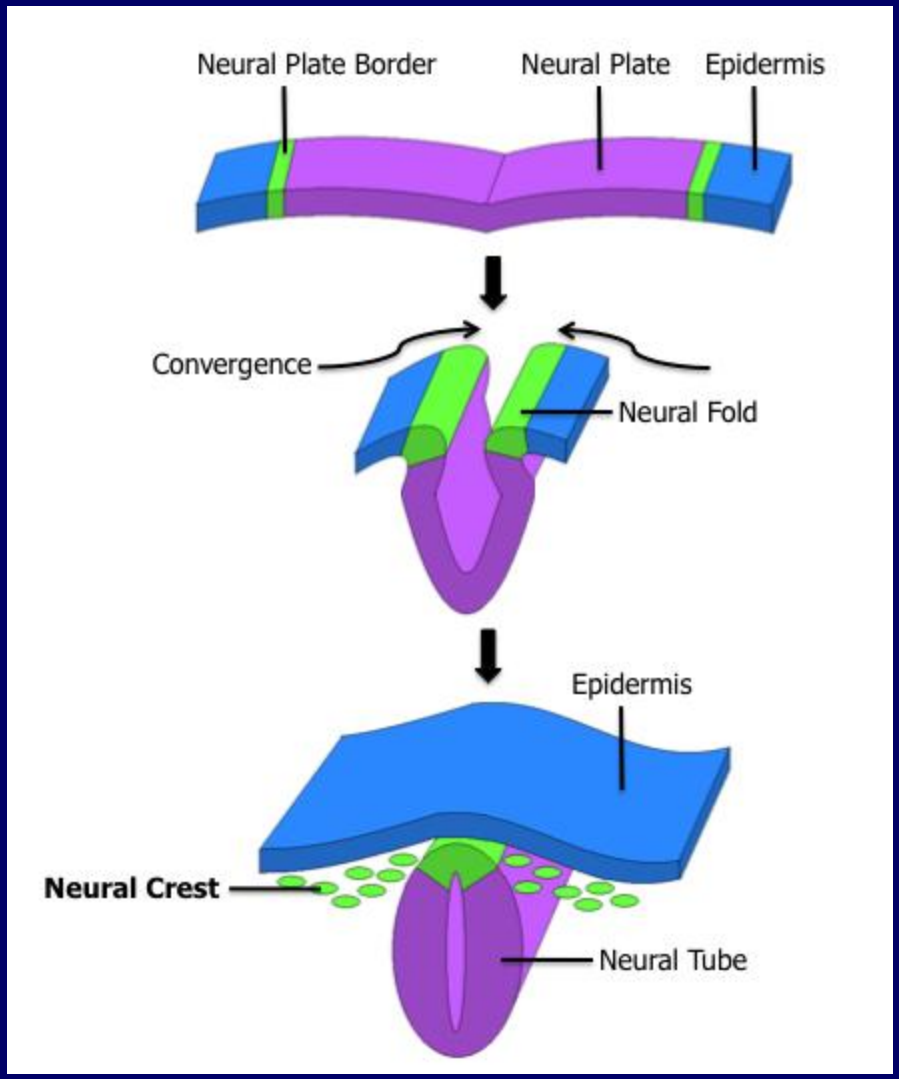
- **Phenotypic Diversity**
  - High percentage of cases show spontaneous differentiation or complete regression
  - One-third of patients cured with surgery alone
  - 50% of cases metastatic and highly malignant at diagnosis
  - Tumor genomics highly correlated with clinical phenotype
- **Important pediatric problem**
  - 10-15% of childhood cancer mortality
  - Chemoradiotherapy intensity has been maximized
  - Survivors with significant morbidity

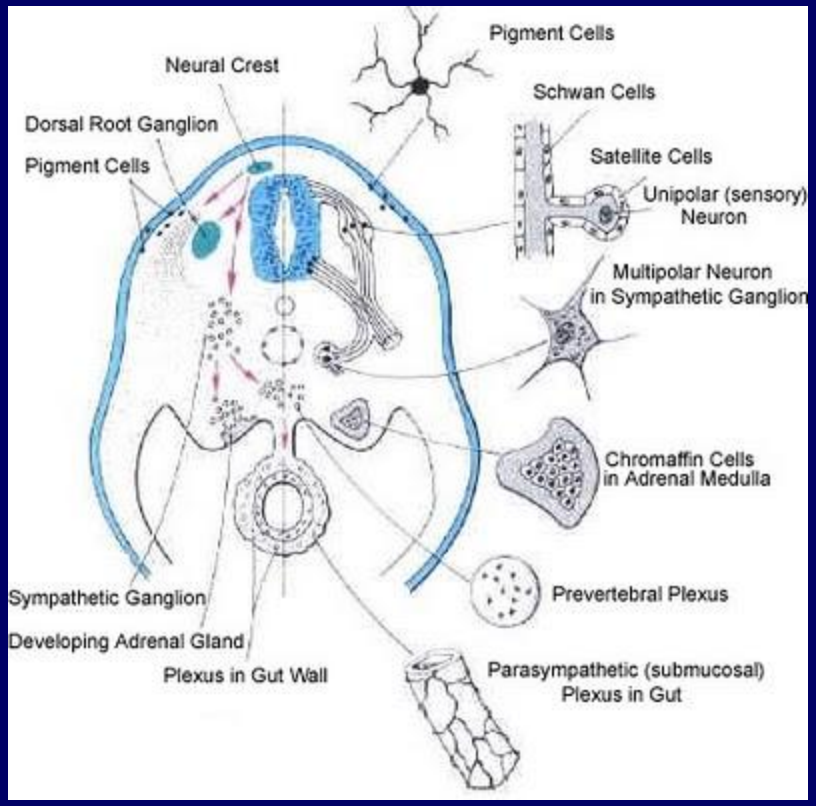
Stage 4S

Stage 4

Images: John Maris, MD

Neural Crest

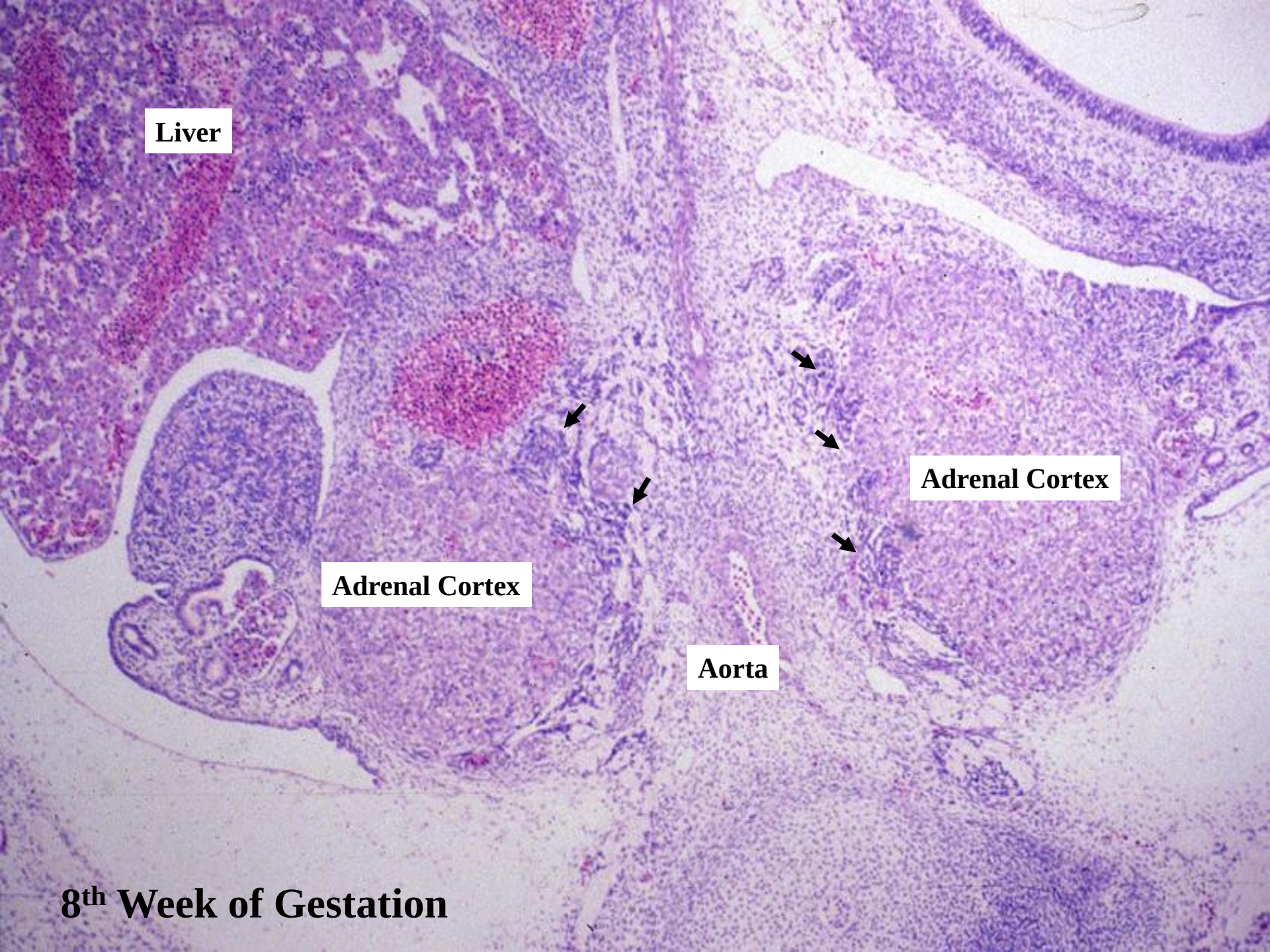




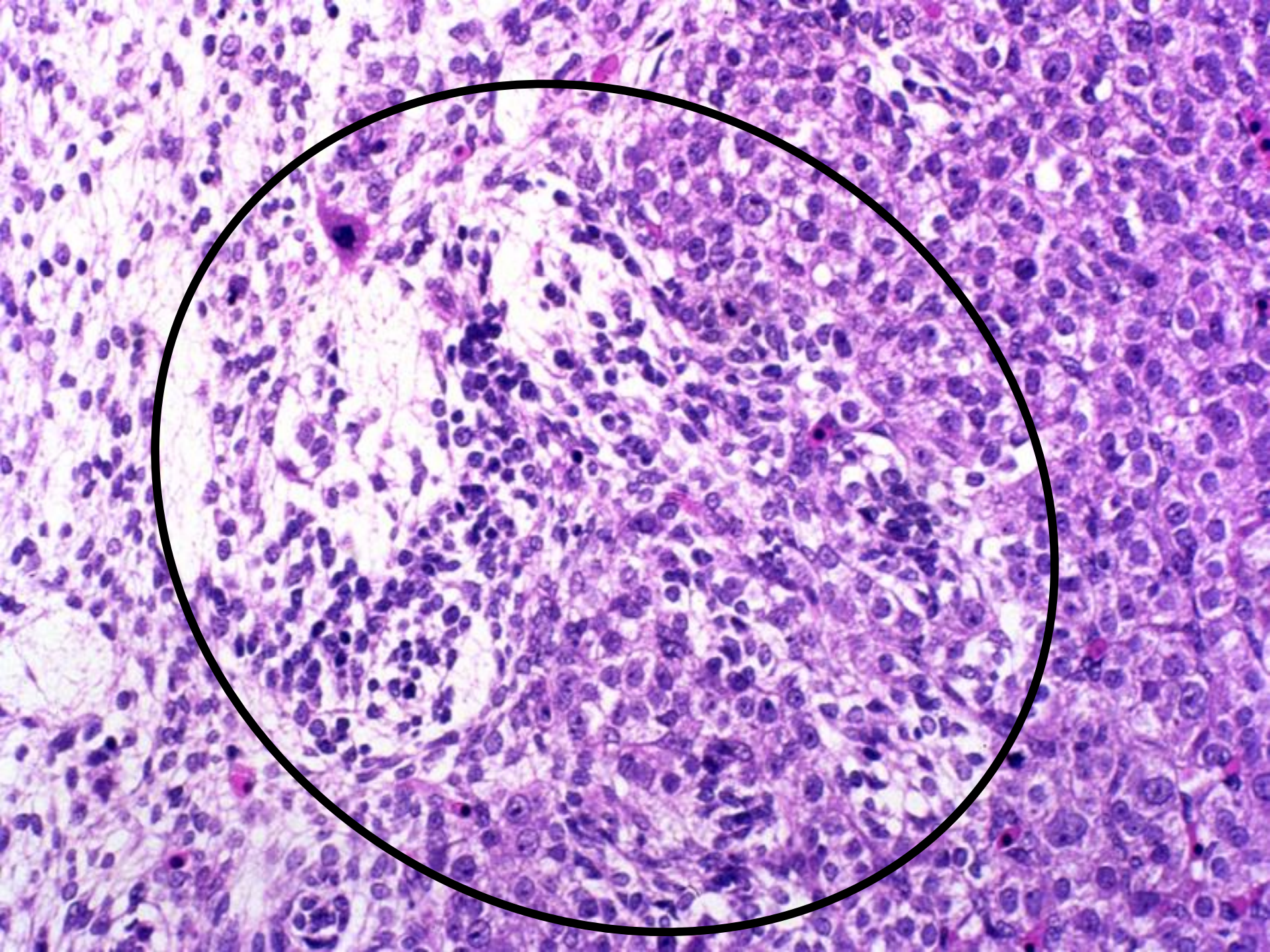
Sympathetic Nervous system

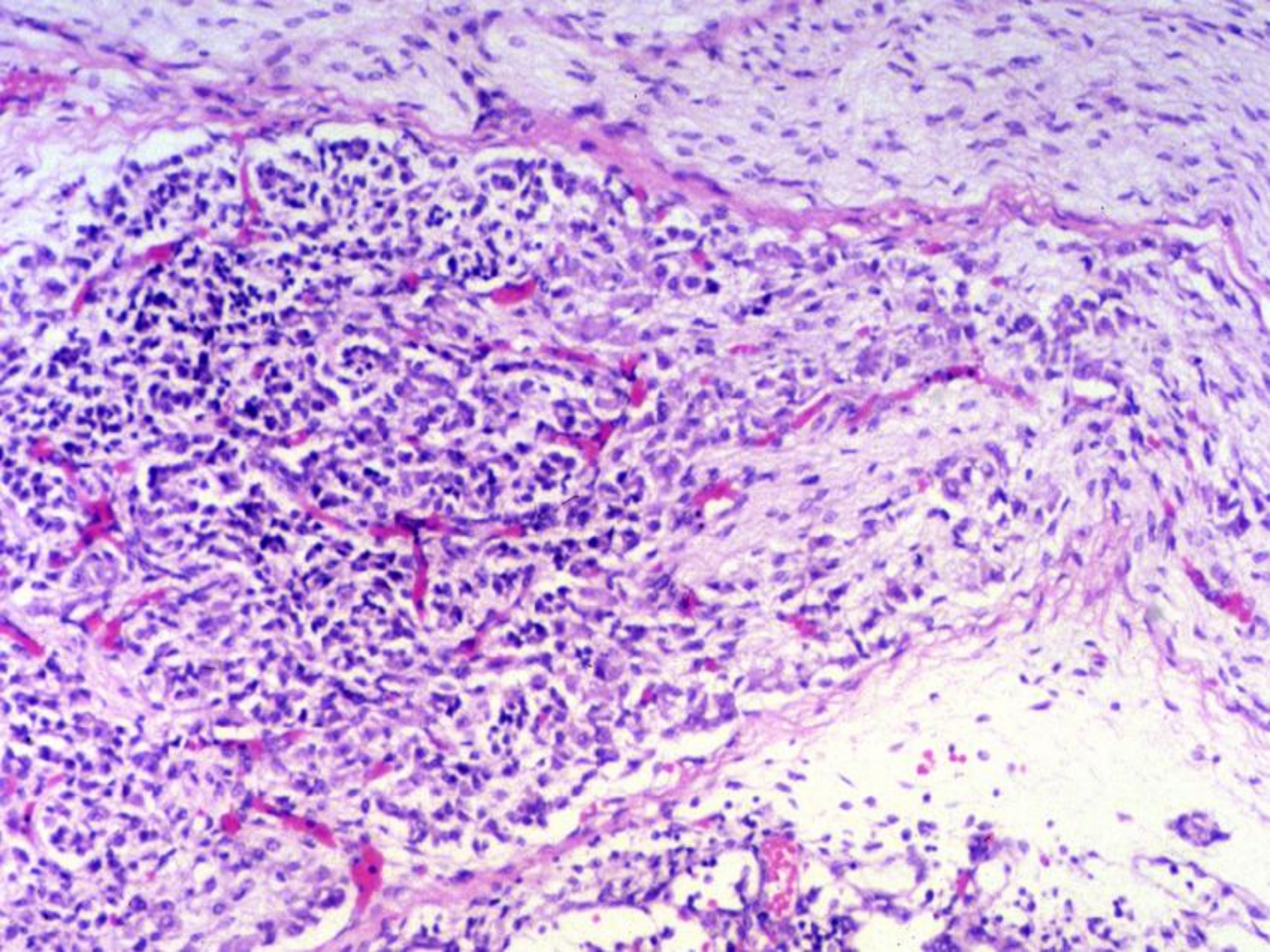
Neuroblastoma

Adrenal Medulla



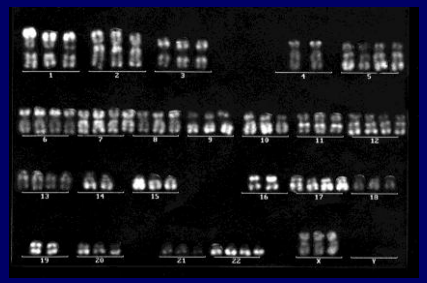
8th Week of Gestation





Peripheral Neuroblastic Tumors
Genomic/Molecular Characteristics

Whole Chromosomal Gains/Losses



Segmental Chromosomal Alterations

***MYCN* Amplification (2p24)**

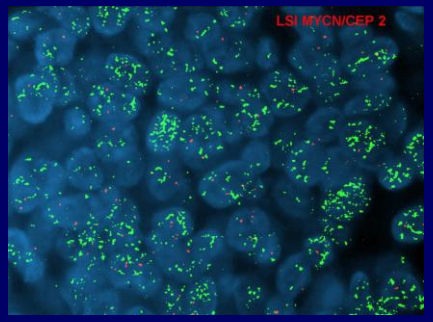
1p (1p36) Loss

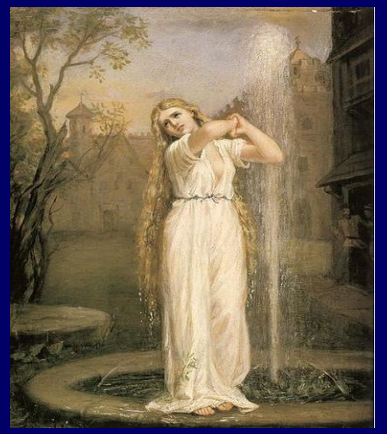
11q (11q23) Loss

17q (17q21) Gain

Others: Gain - 1q, 5q, 18q

Loss - 3p, 4p, 9p, 14q, 14p, 9q



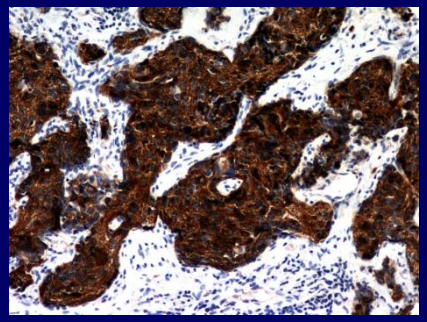


PHOX2B Mutation

ALK Mutation, Protein Expression

6p22 Abnormalities

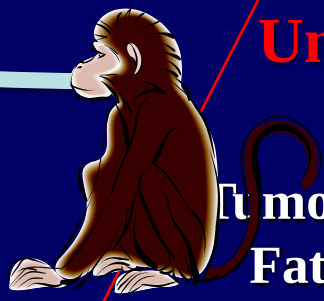
(FLJ22536 & FLJ4418 homozygous)



Peripheral Neuroblastic Tumors
Diversity of Genomic/Molecular Characteristics

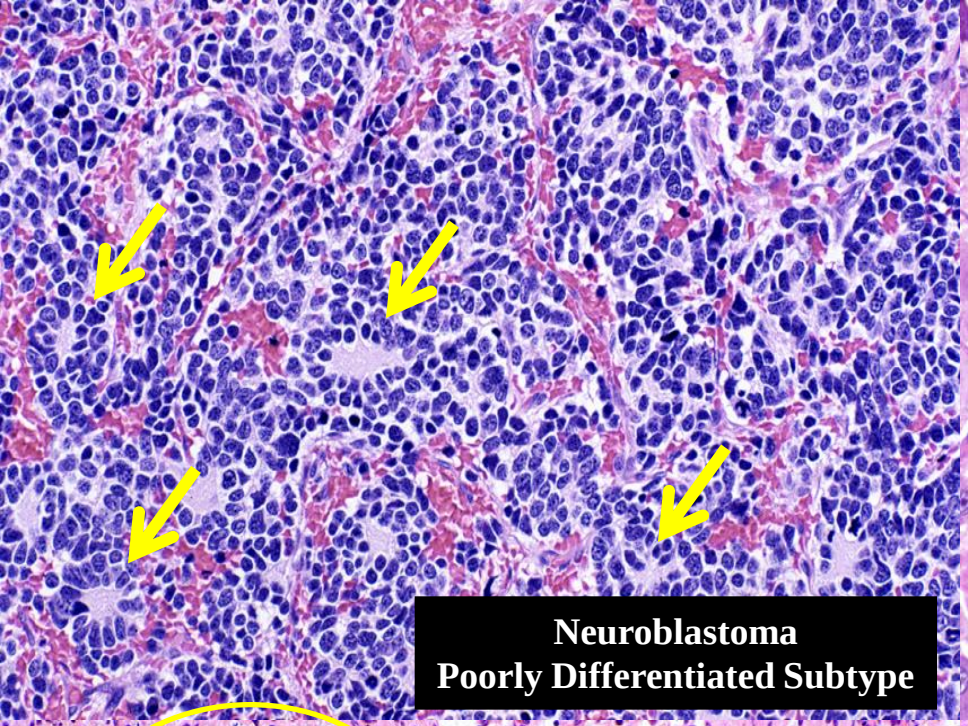
Spont. Regression
Tumor Maturation

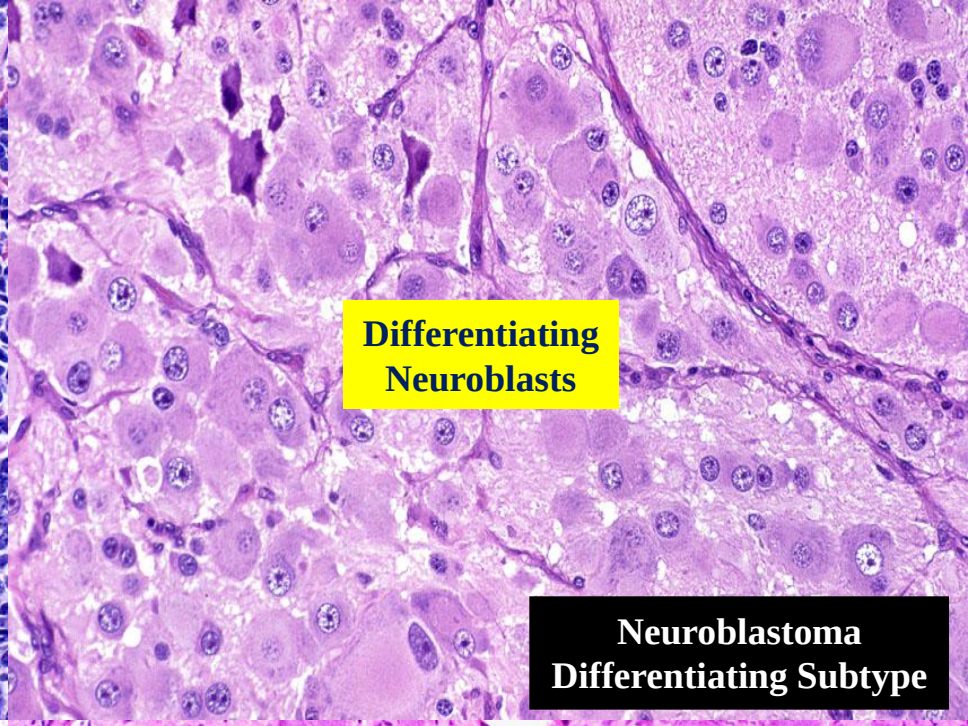
Favorable Biology

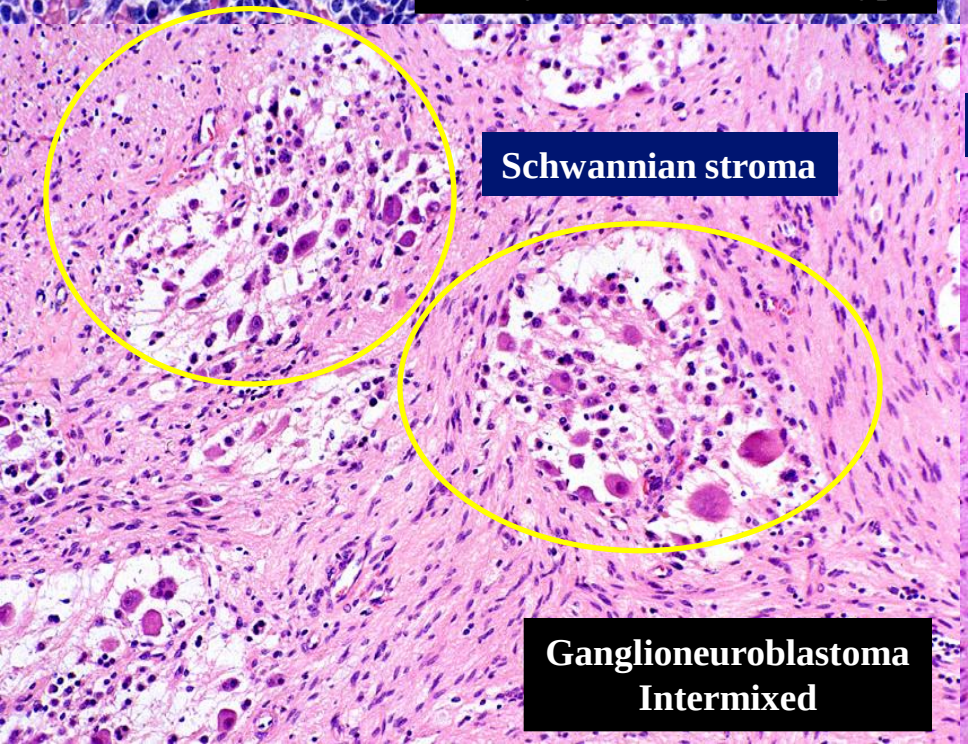


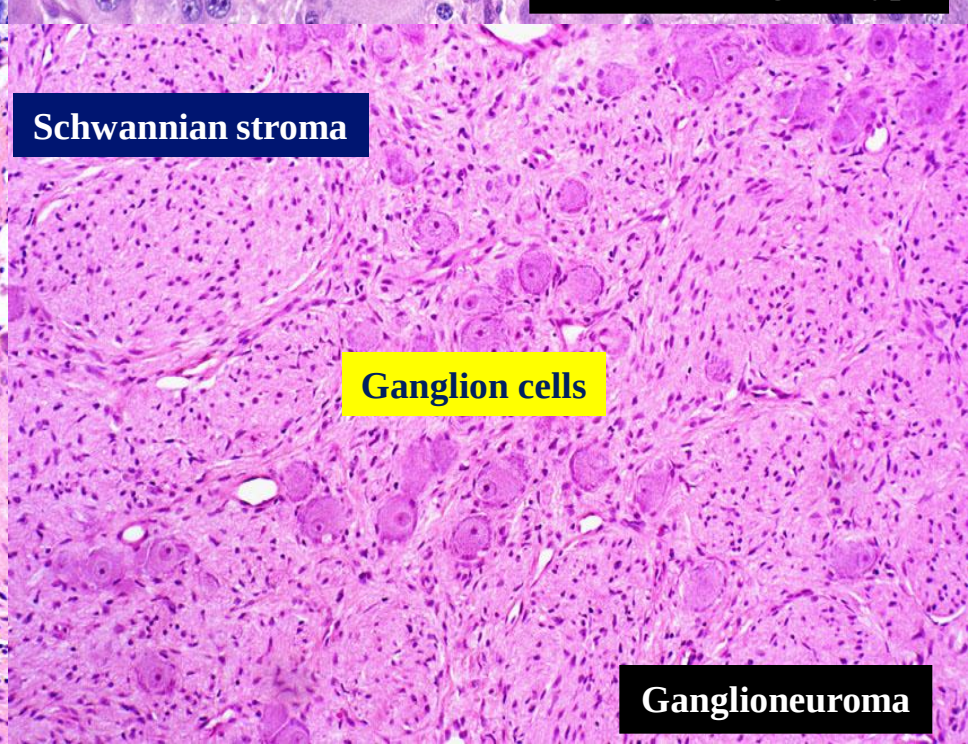
Unfavorable Biology

Tumor Progression
Fatal Outcome


Neuroblastoma
Poorly Differentiated Subtype


Differentiating
Neuroblasts
Neuroblastoma
Differentiating Subtype


Schwannian stroma
Ganglioneuroblastoma
Intermixed

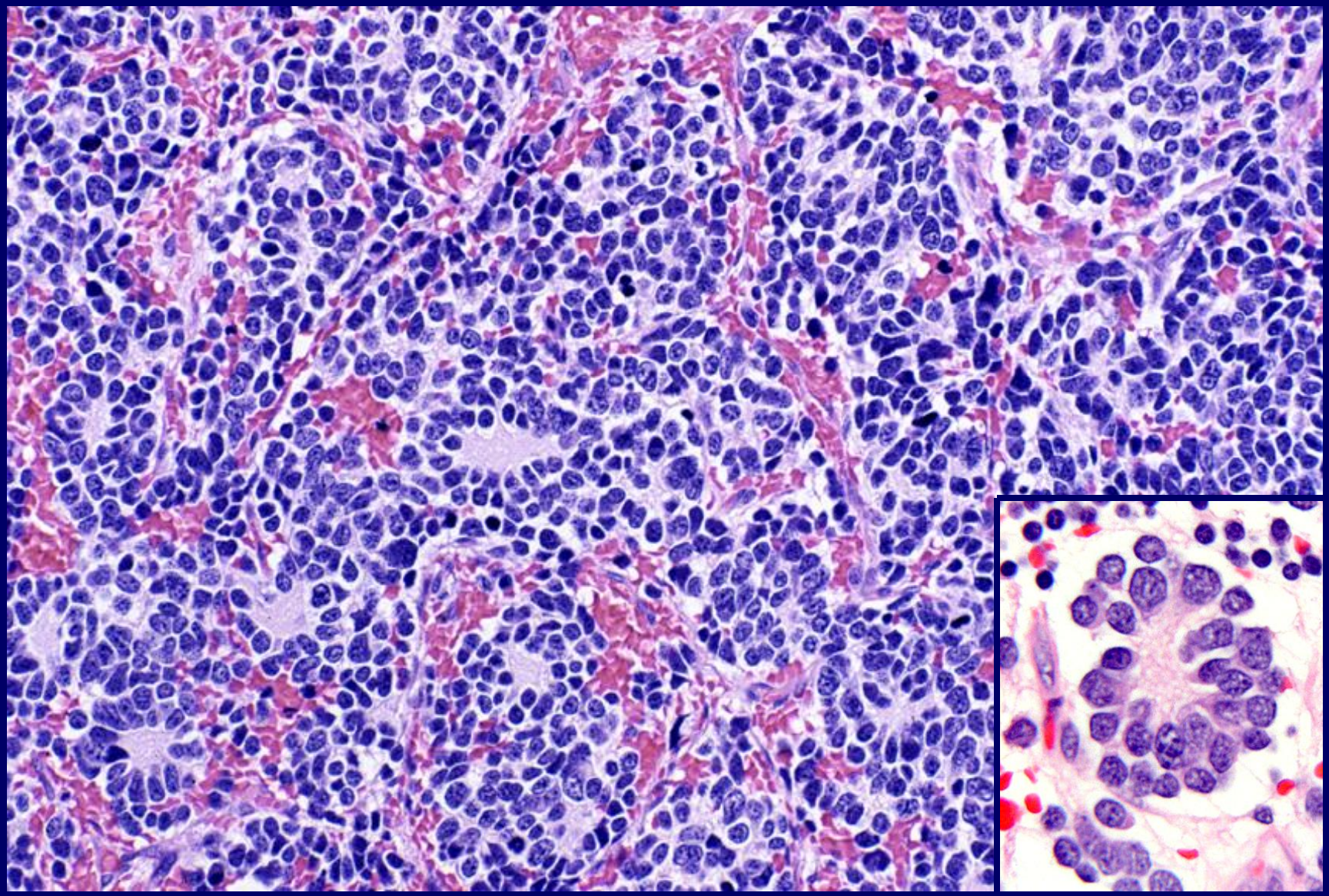

Schwannian stroma
Ganglion cells
Ganglioneuroma

INPC, Basic Concept

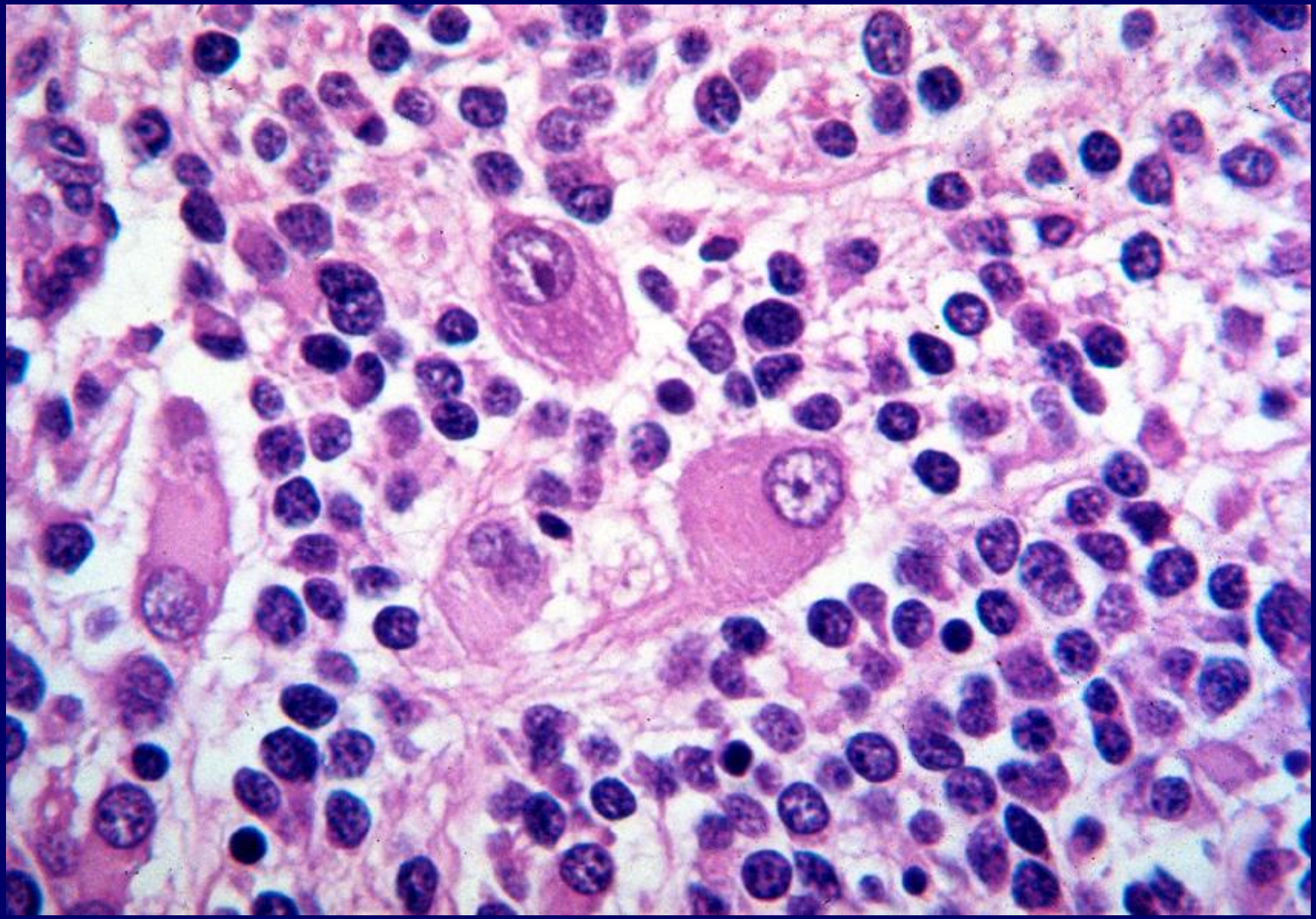
Peripheral Neuroblastic Tumors
One of the Best Models for Analyzing
Biologically Significant Relationship Between
Molecular/Genomic Alterations and Morphologic Manifestations

- **Crosstalk between Neuroblastic Cells and Schwannian Stromal Cells**
- ***Trk*AI/II (high-affinity NGF Receptor) Expression Critical for Neuroblastic Differentiation**
- ***MYCN* Oncogene Amplification, the Strongest Indicator of Aggressive Tumor Progression**

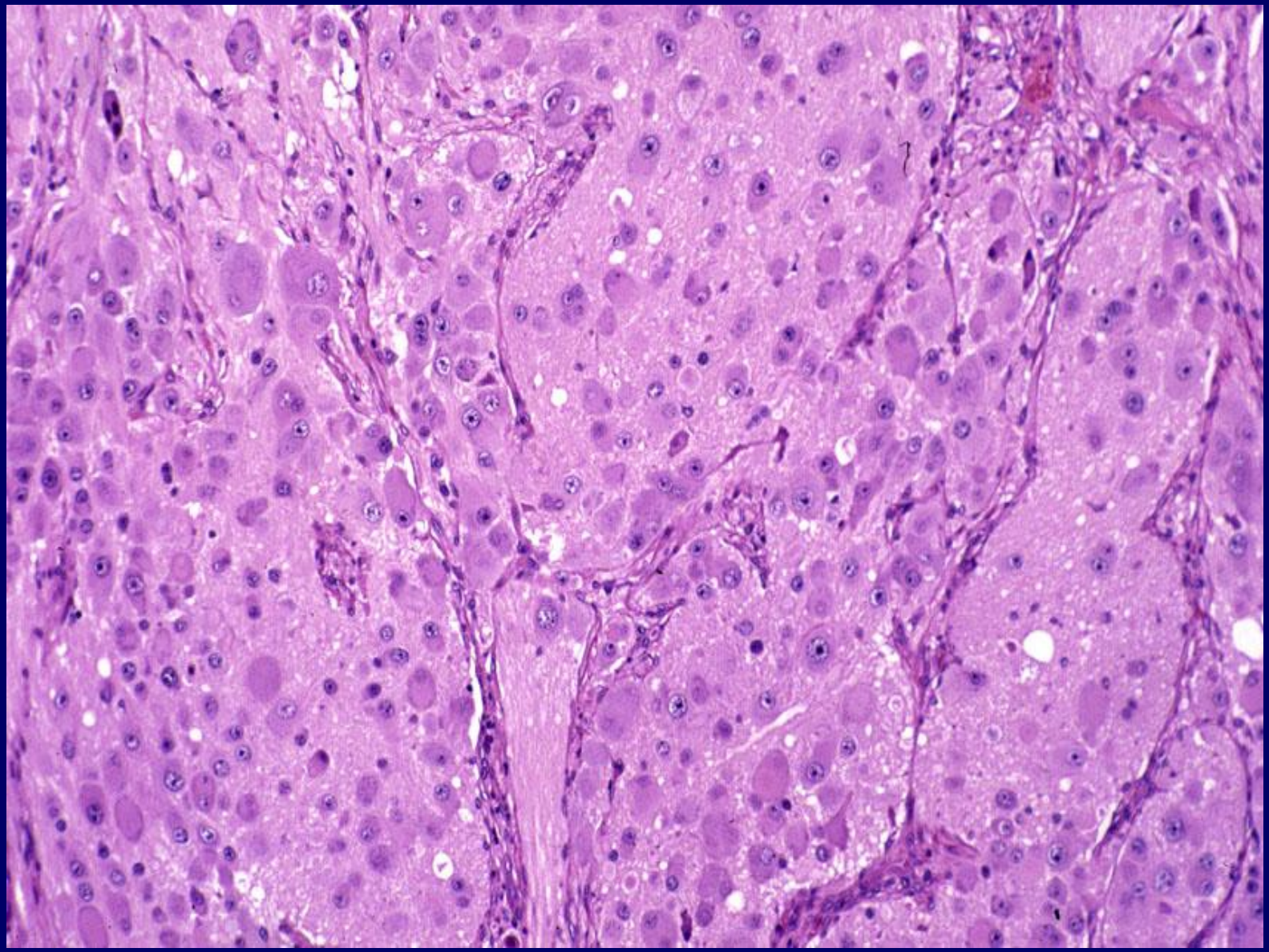
Neuroblastoma (Schwannian stroma-poor)
Poorly Differentiated Subtype



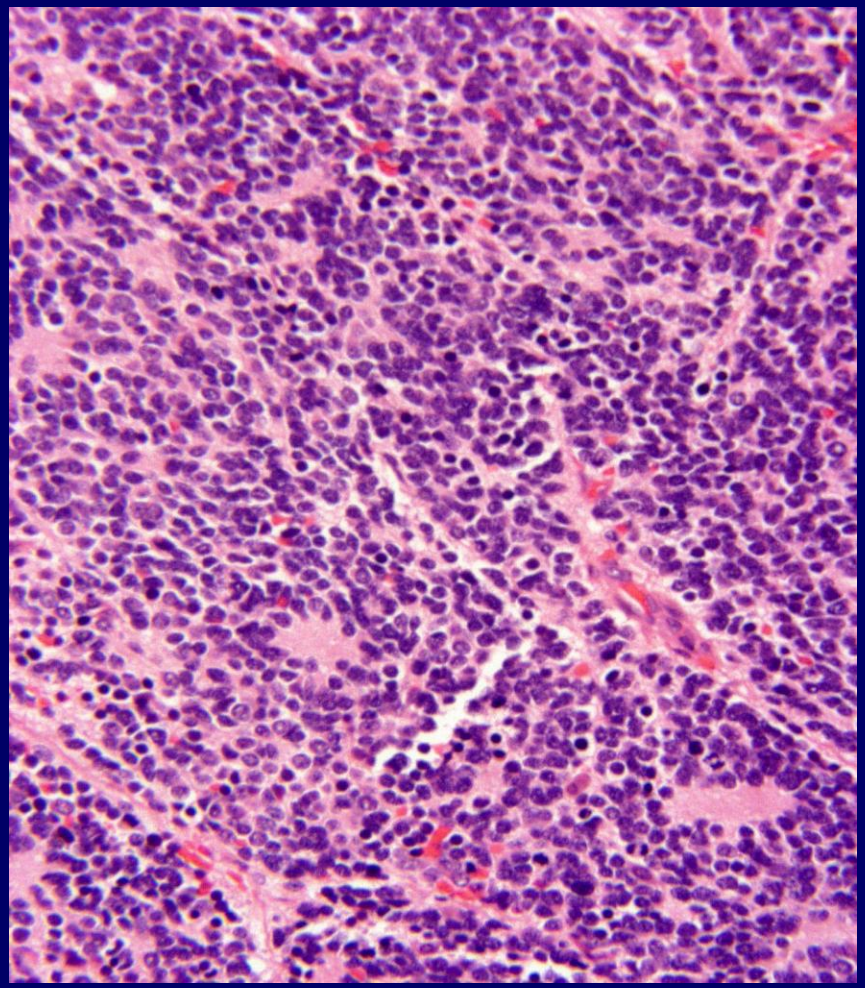
Differentiating Neuroblasts

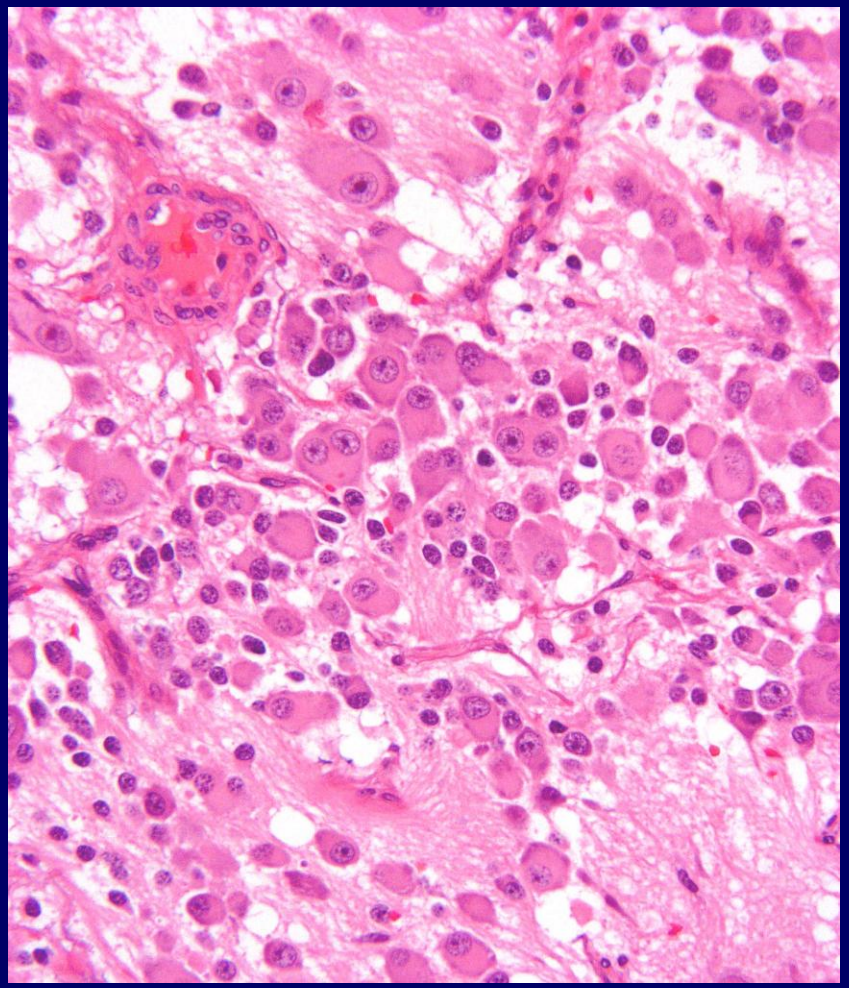


Neuroblastoma (Schwannian stroma-poor) Differentiating Subtype



Poorly Diff. Subtype to Differentiating Subtype





Neuroblastic Differentiation
Cellular Death

Neuronal Cells & Schwann Cells

Embryology

Neural Crest → Neuronal Cells

Neural Crest → Schwann Cells

Tumors of Neuroblastoma Group

Neural Crest → Neuroblasts (Tumor Cells)

Neuroblasts (Tumor Cells) → Schwann Cells (Stroma)

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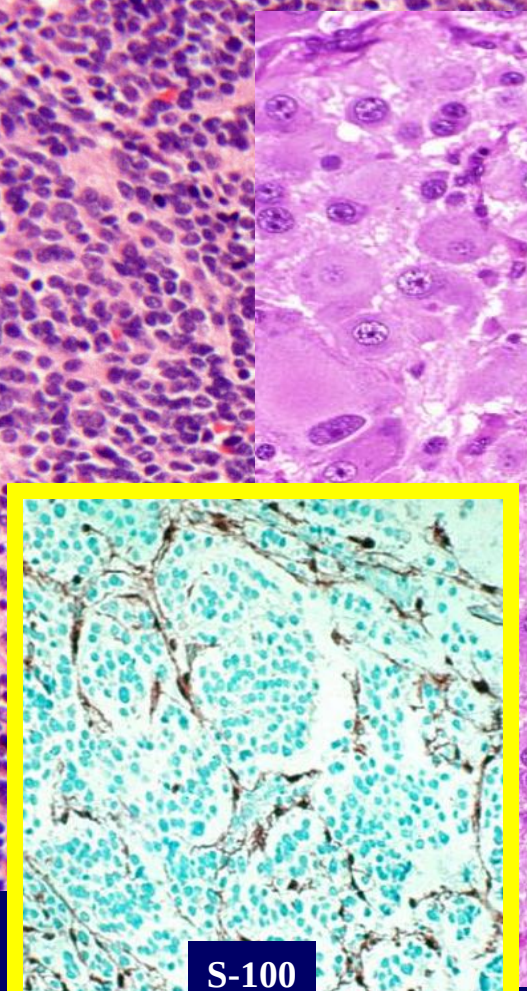
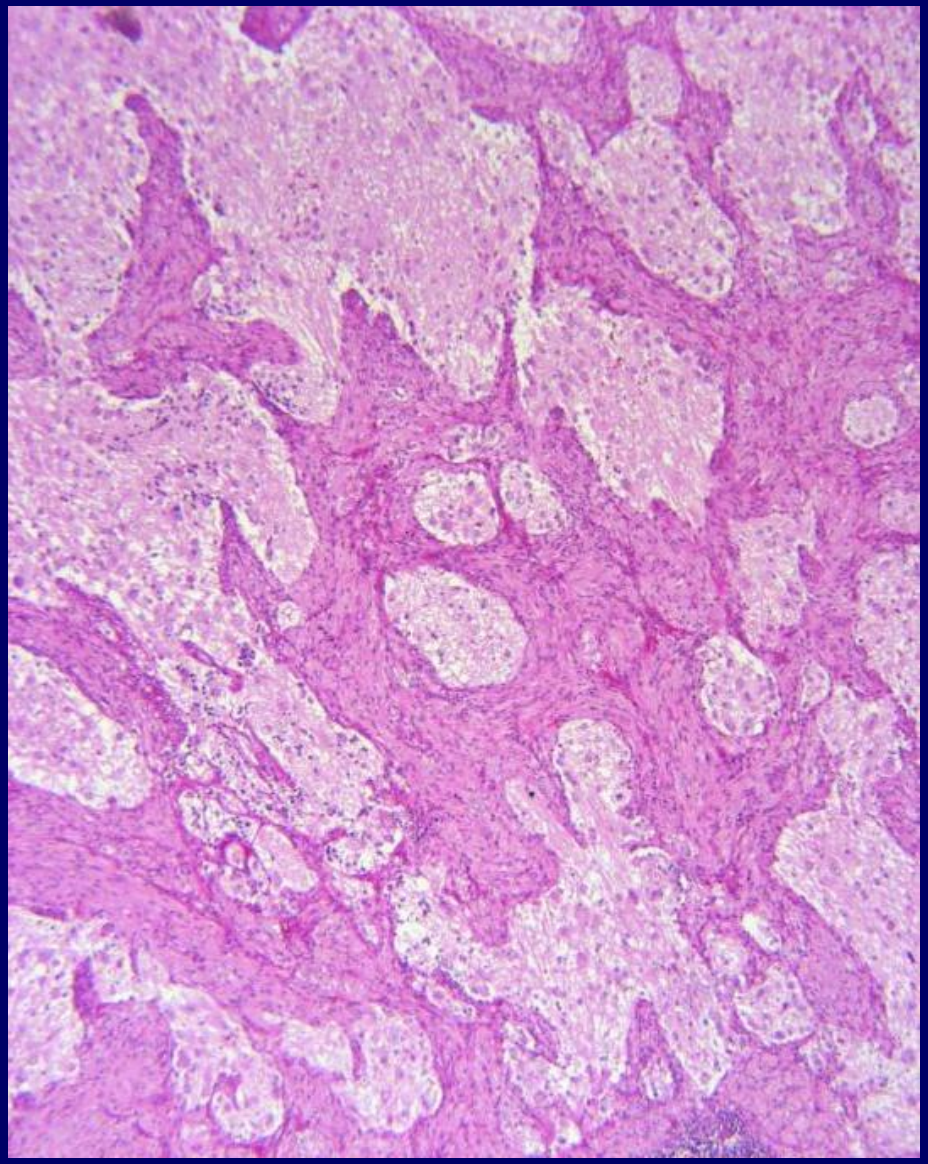
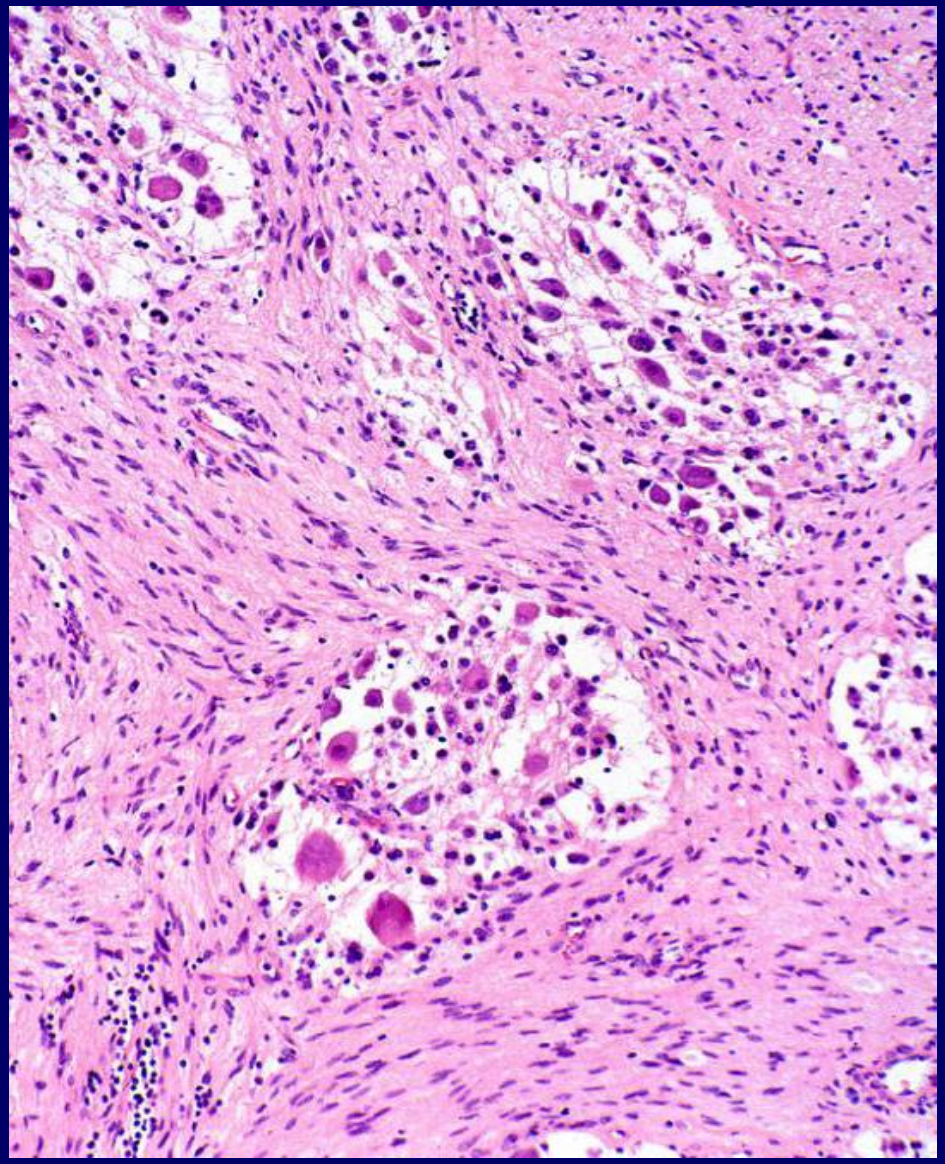
Transition
Tumor Maturation

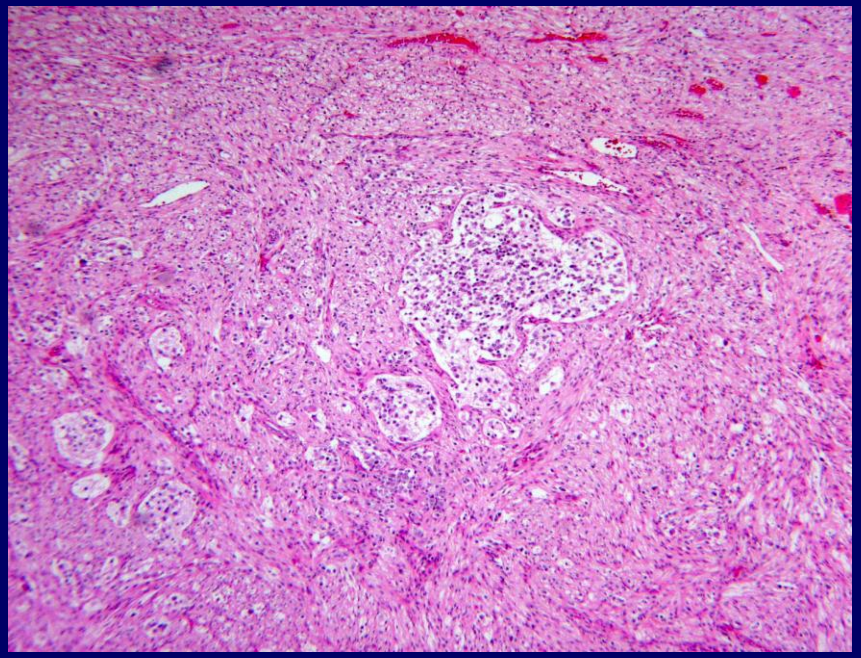


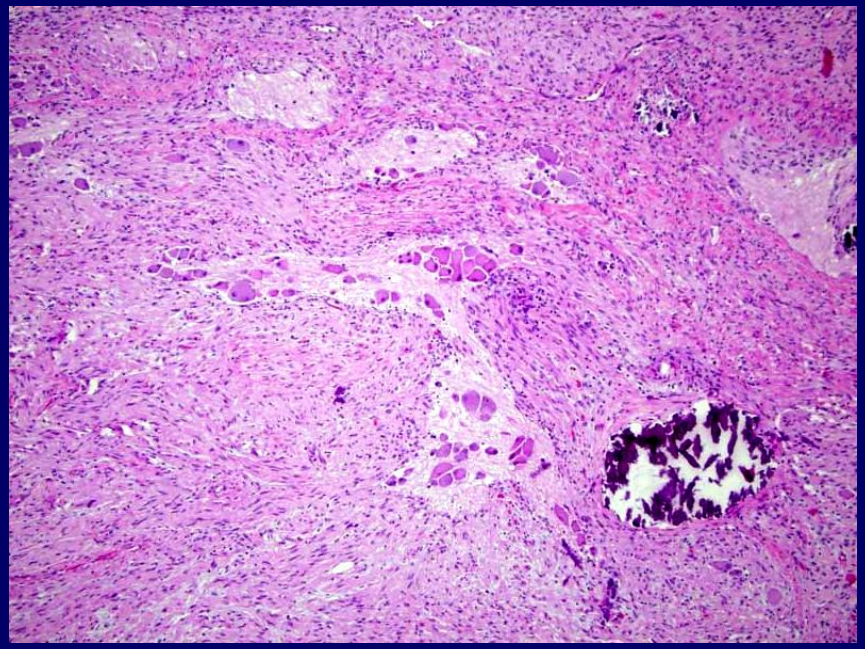
Neuroblastoma to
Ganglioneuroblastoma, Intermixed

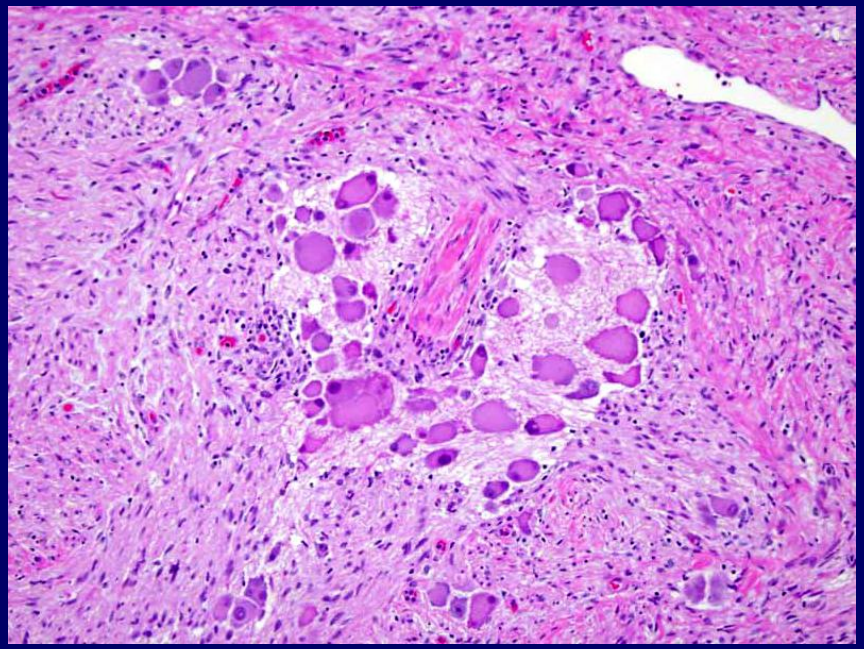
Ganglioneuroblastoma, Intermixed (Schwannian stroma-rich)





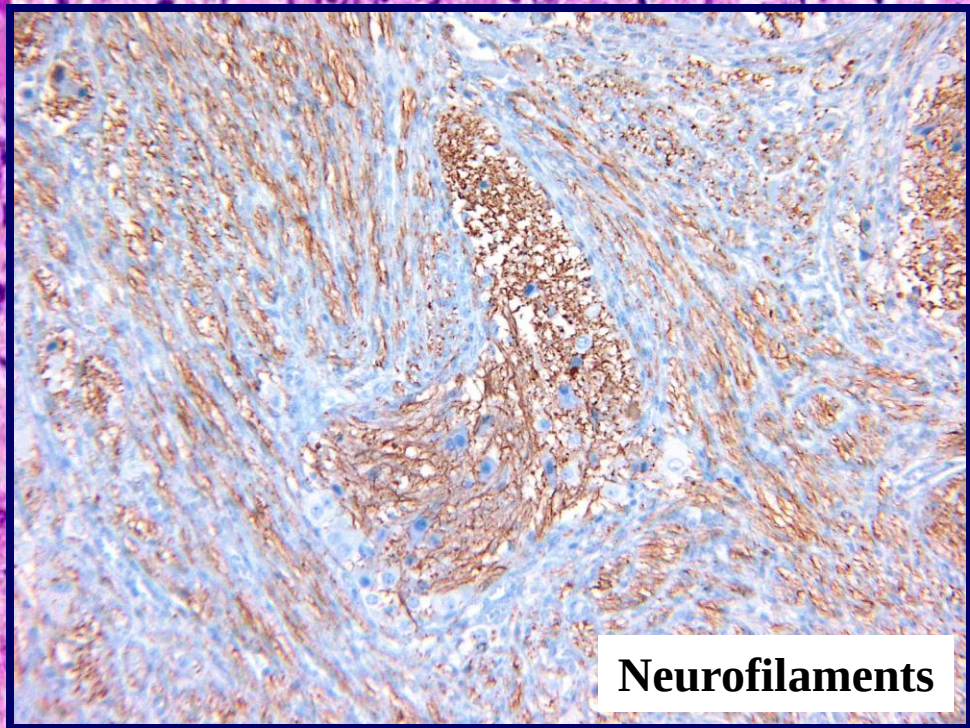




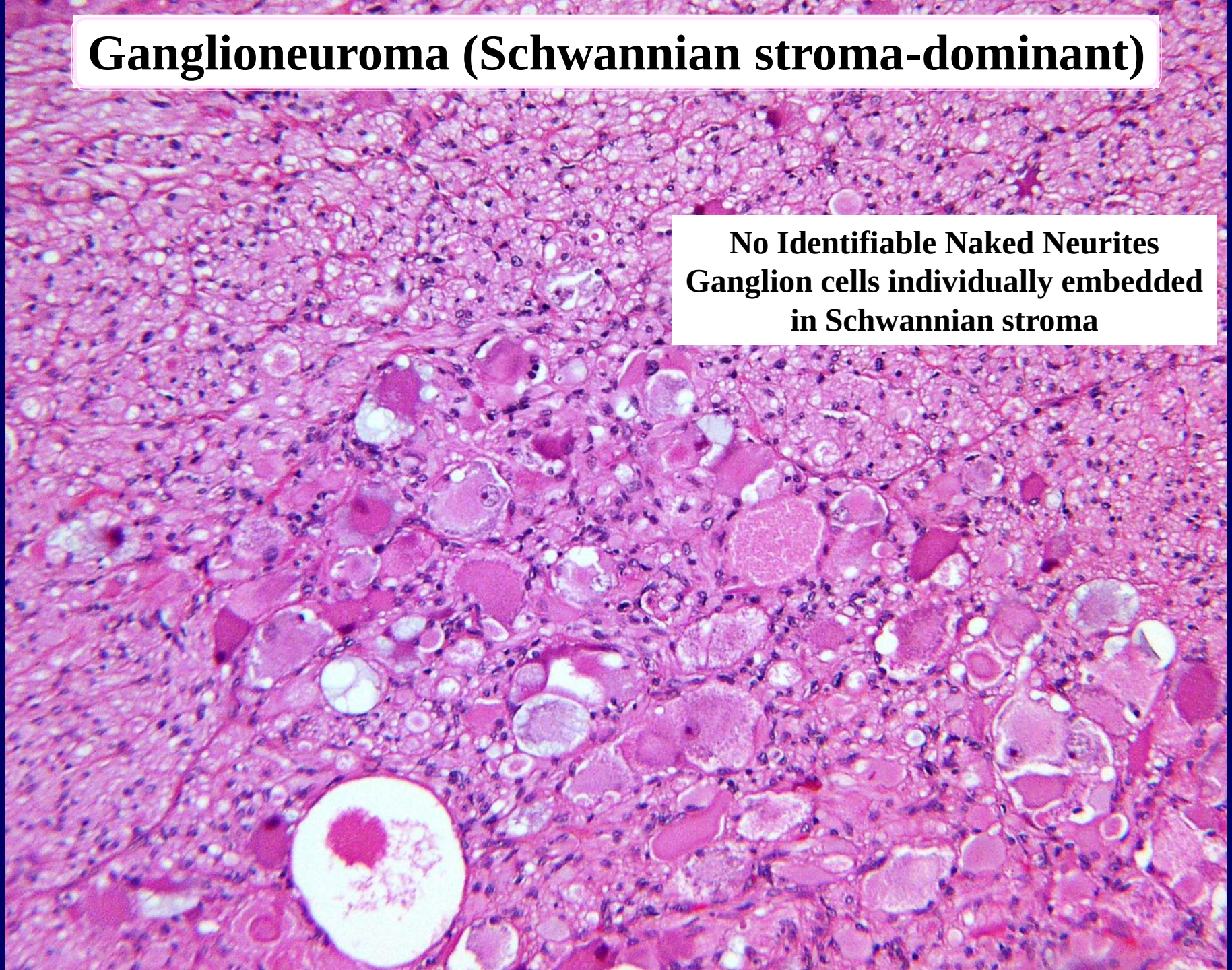


Ganglioneuroblastoma, Intermixed

Pockets of Naked (Not Covered by Schwann Cells) Neurites

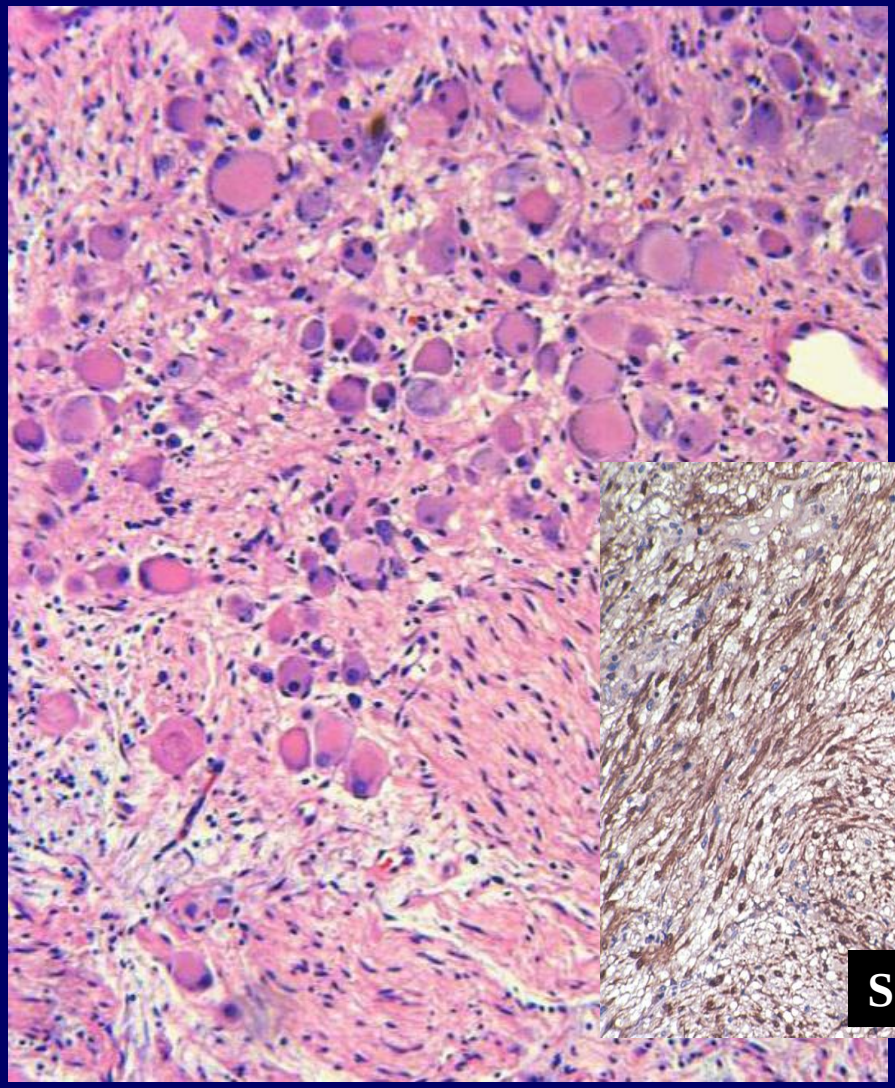

Neurofilaments

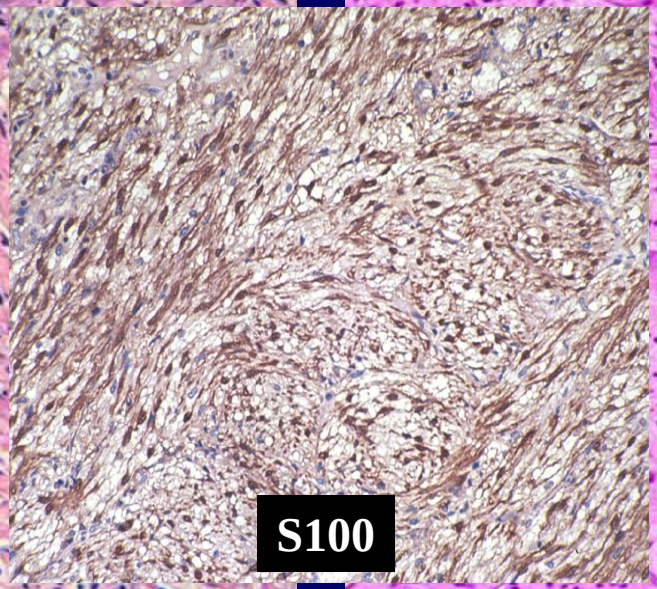
Ganglioneuroma (Schwannian stroma-dominant)



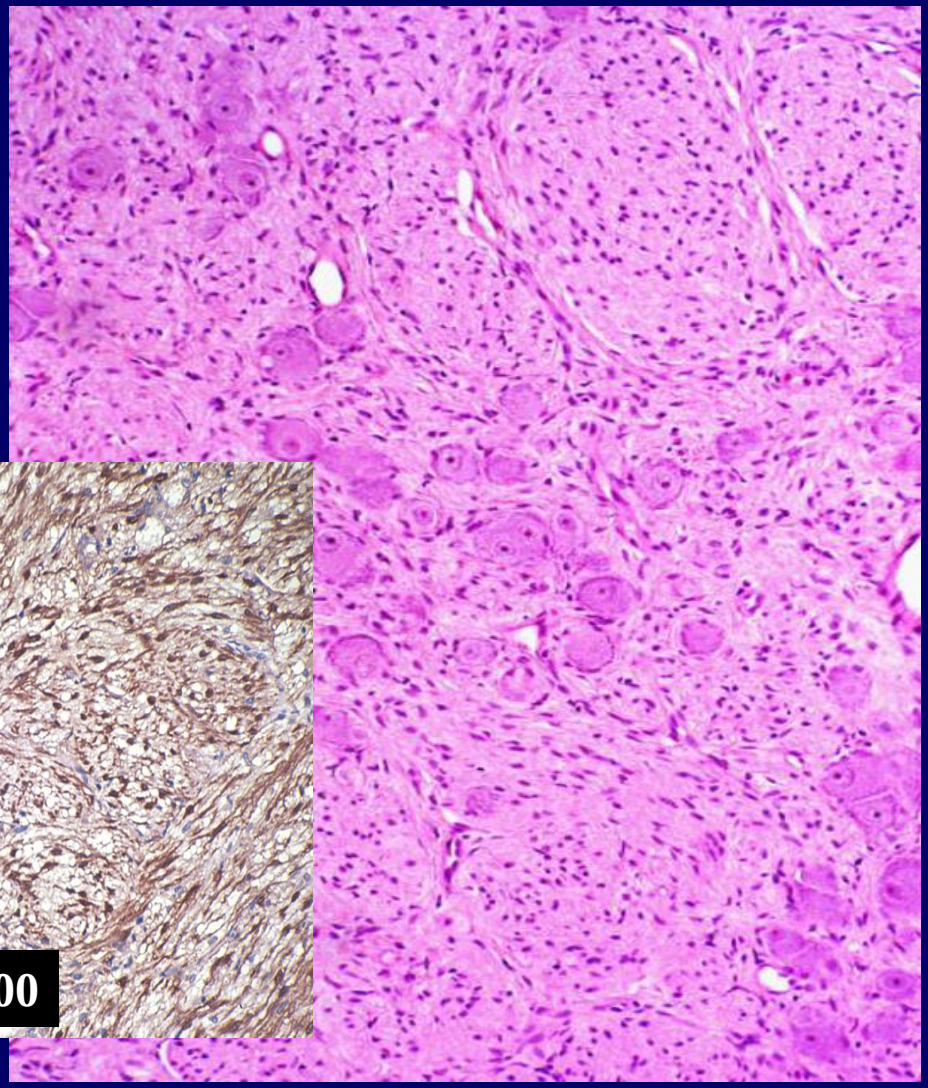
Ganglioneuroma (Schwannian stroma-dominant)

Maturing Subtype



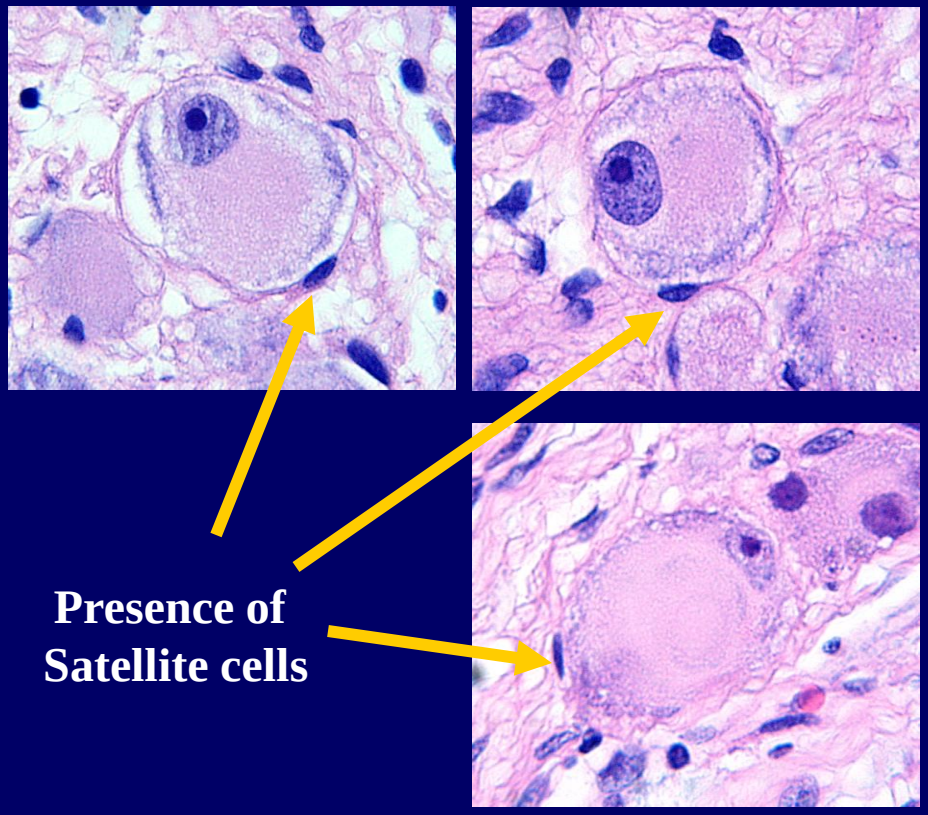


Mature Subtype



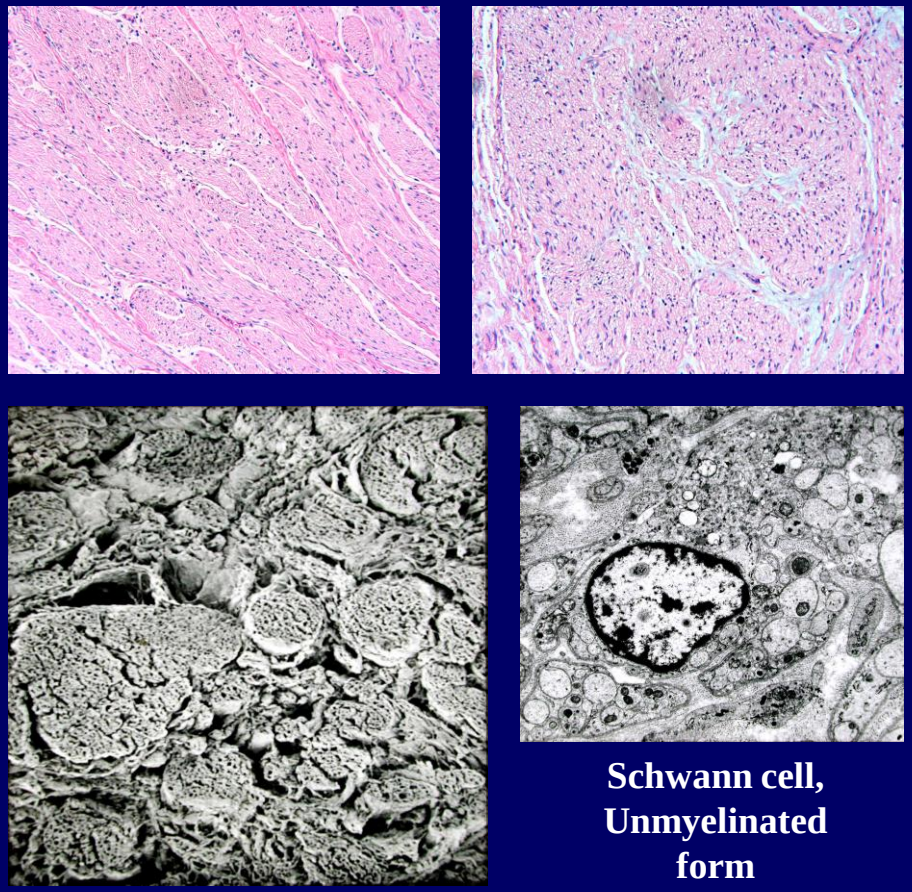
Ganglioneuromatous Tissue

Completely Mature Ganglion Cells



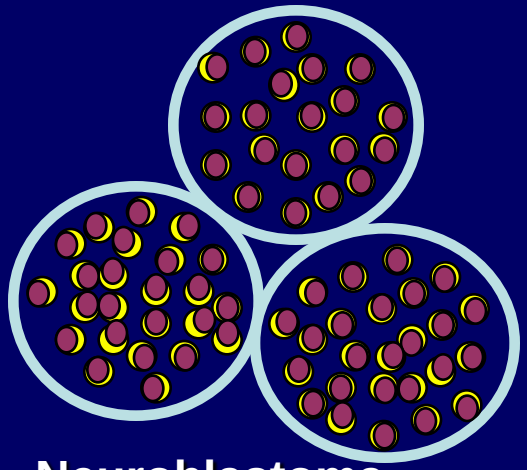
Presence of Satellite cells

Completely Developed Schwannian Stroma

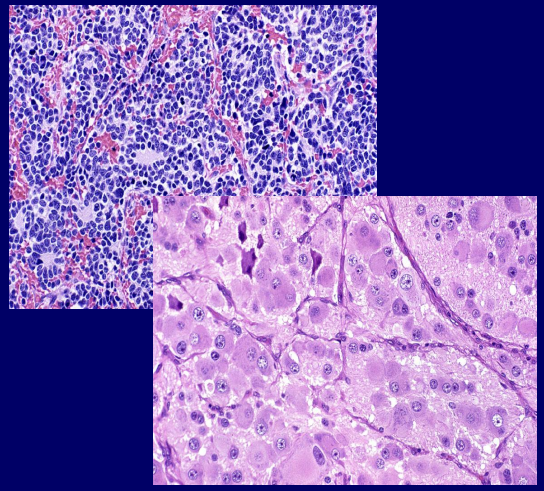


Schwann cell, Unmyelinated form

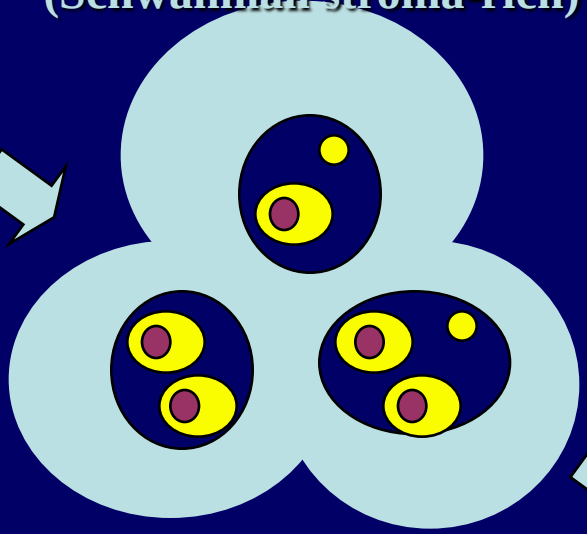
Peripheral Neuroblastic Tumors
Steps of Tumor Maturation

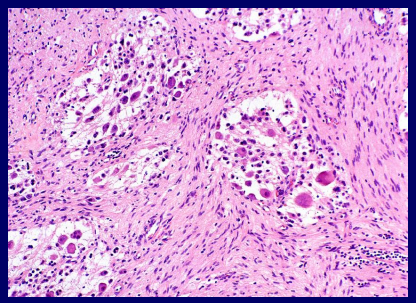


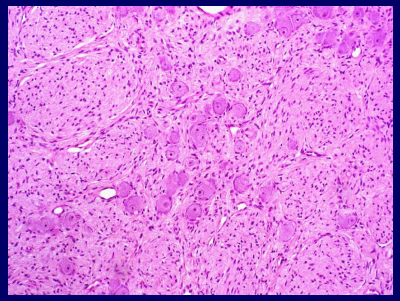
Neuroblastoma
(Schwannian stroma-poor)



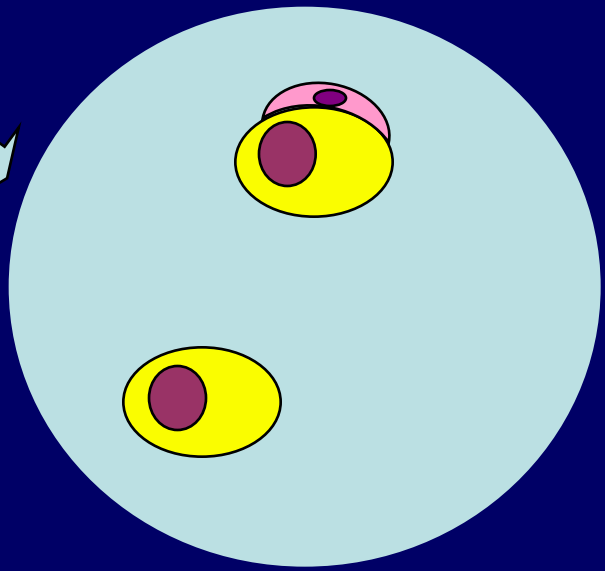
Ganglioneuroblastoma, Intermixed
(Schwannian stroma-rich)



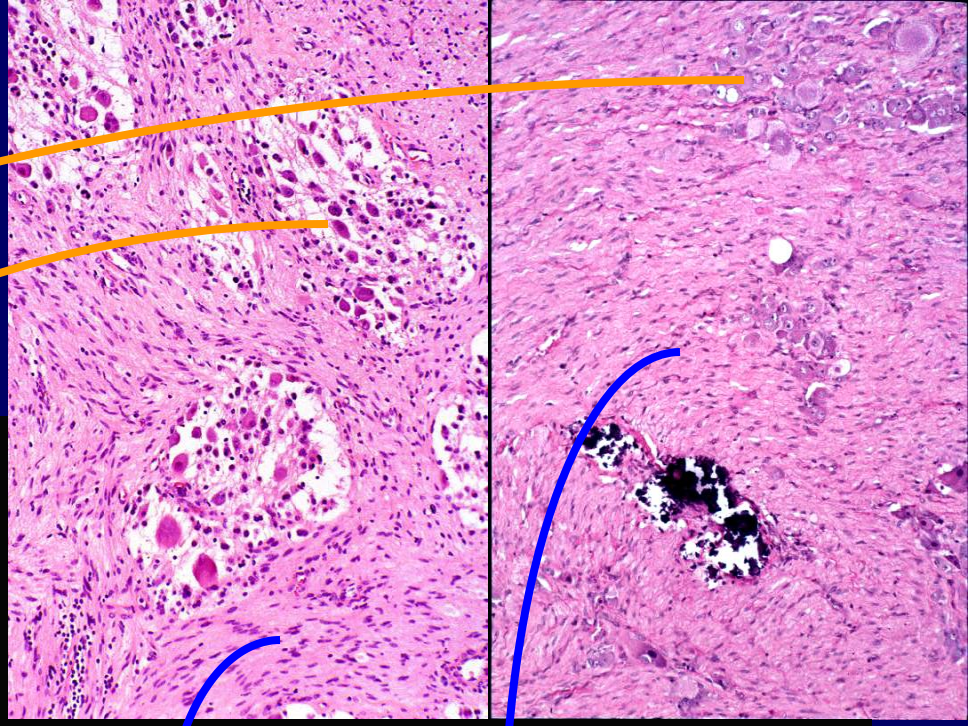


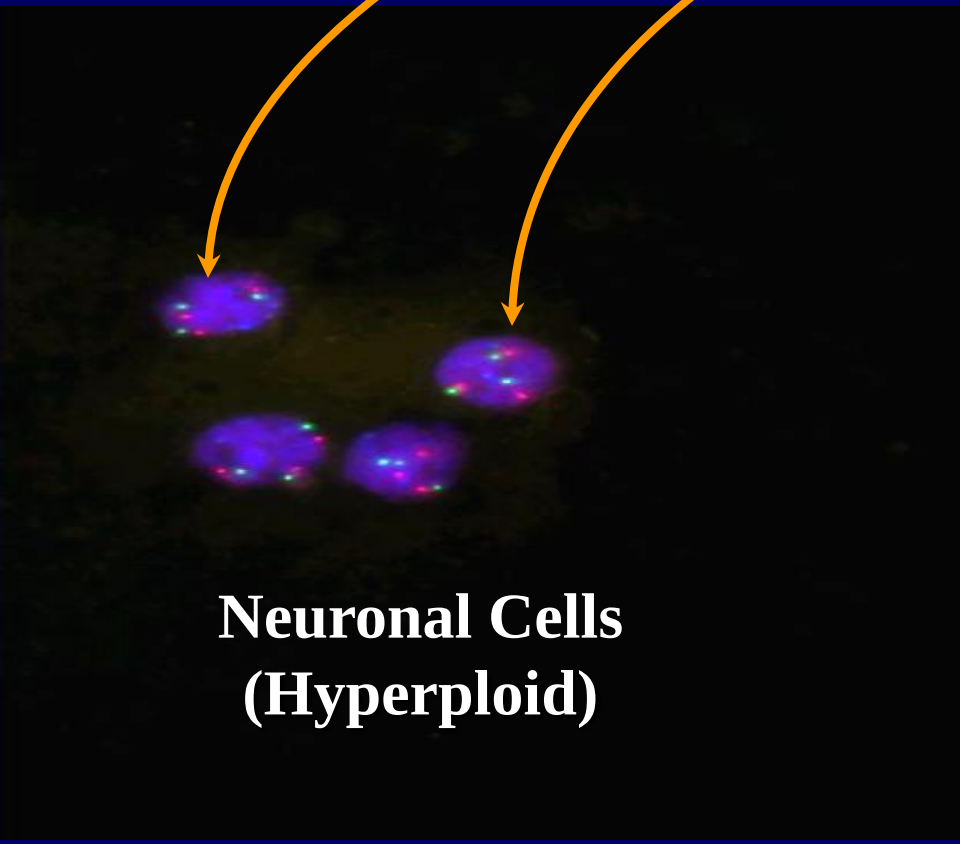


Ganglioneuroma
(Schwannian stroma-dominant)

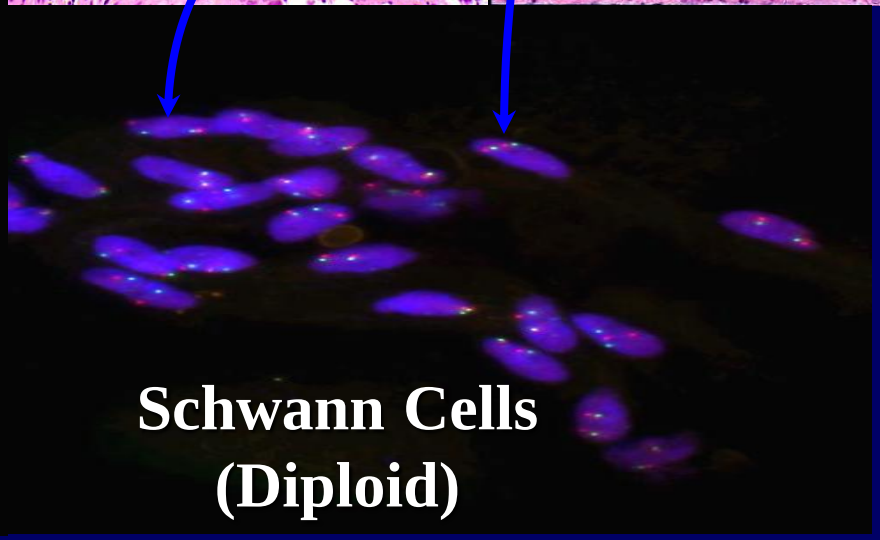


Neuronal Cells & Schwann Cells





Neuronal Cells
(Hyperploid)

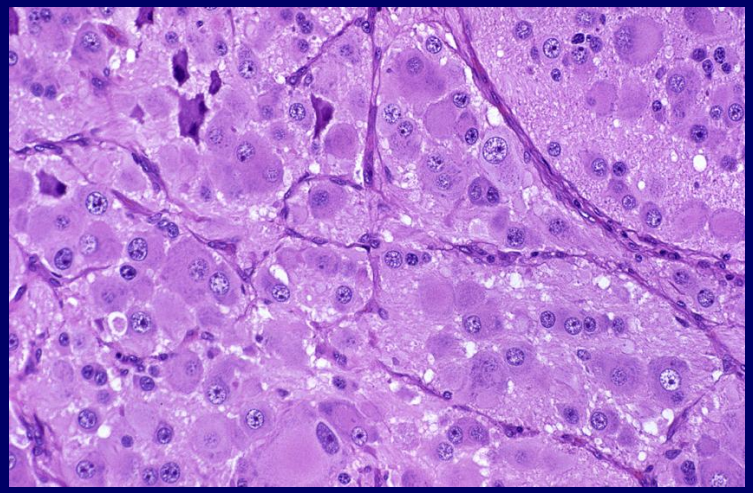


Schwann Cells
(Diploid)

Different Cellular Origins for Neuronal cells and Schwann Stromal Cells

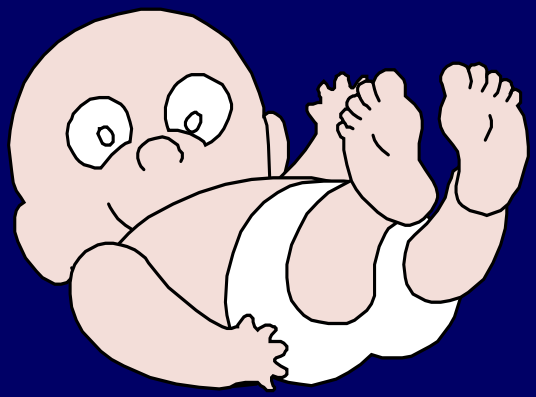
Historical Overview

Early 20th Century

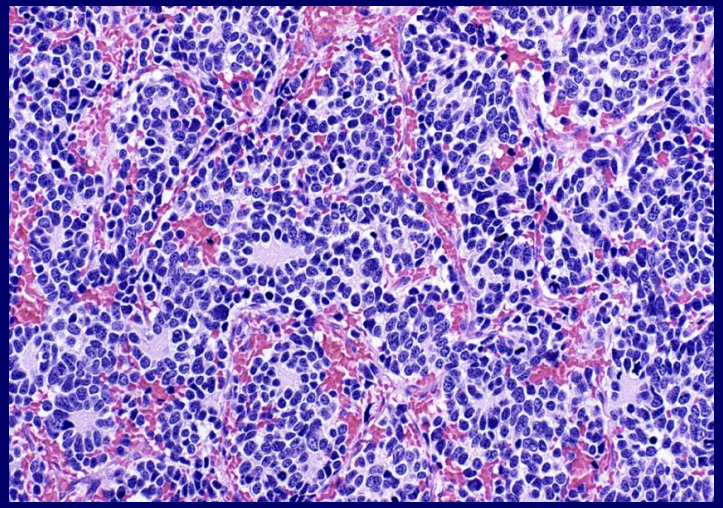


Diagnosed in Older Children Favorable Clinical Course

1950's



Favorable Clinical Course in Infants



Historical Overview

Neuroblastoma

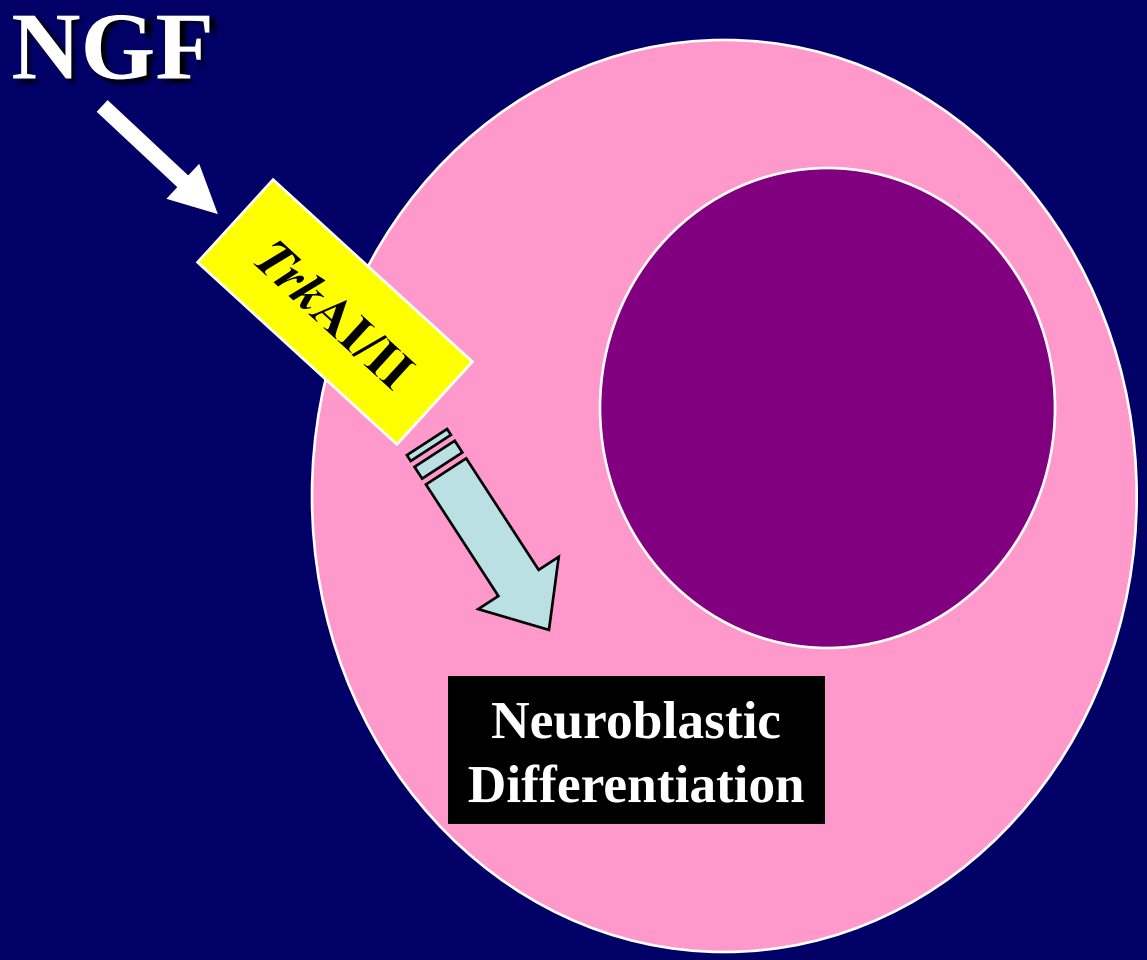
Newborns, Infants: Good Prognosis

Children>=5 years: Poor Prognosis

Ganglioneuroblastoma / Ganglioneuroma

Older Children: Excellent Prognosis

*Trk*AI/II Expression in Neuroblastoma



Tumors

high Expresser

low Expresser

better clinical outcome

poor clinical outcome

Neuroblastoma (Schwannian stroma-poor)
< 50% Schwannian Stroma/
Ganglioneuromatous tissue

Undifferentiated Subtype:

Totally Undifferentiated

Poorly Differentiated Subtype:

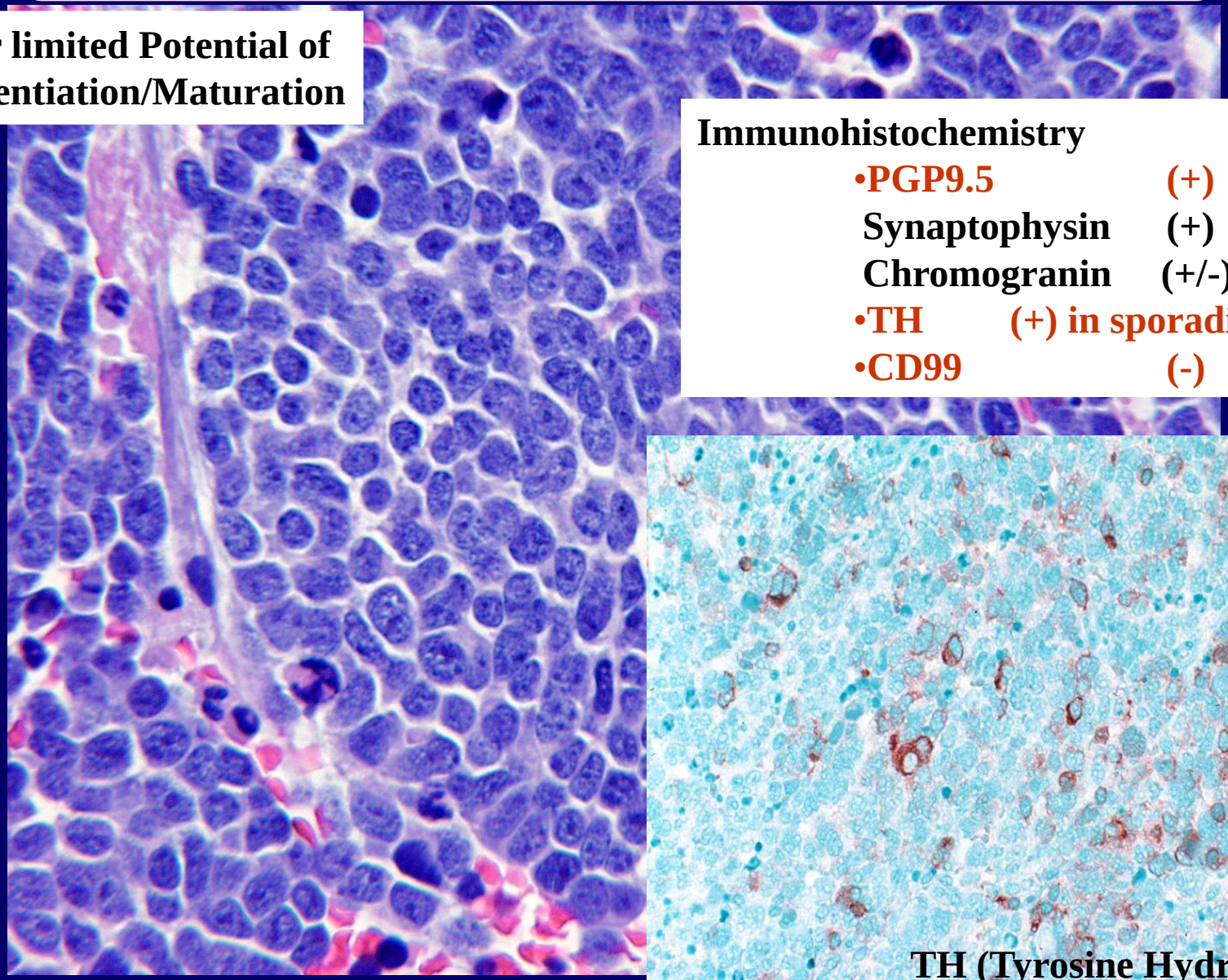
Neuropil Formation (+)

Differentiating Subtype:

>5% Differentiating Neuroblasts

Neuroblastoma (Schwannian stroma-poor)
Undifferentiated Subtype

No or limited Potential of Differentiation/Maturation



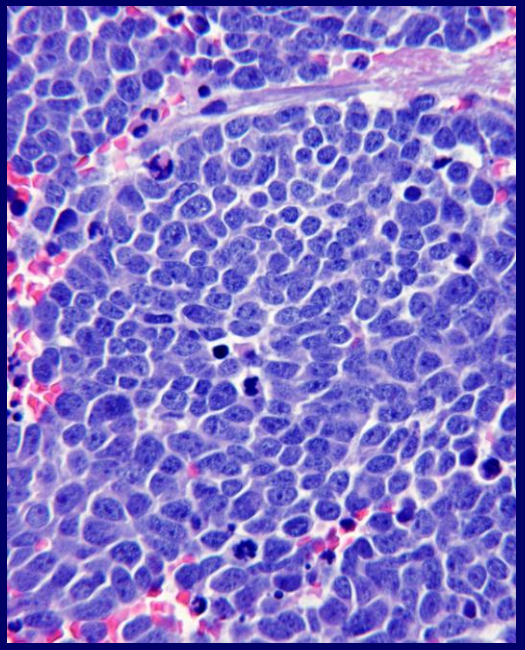
Immunohistochemistry

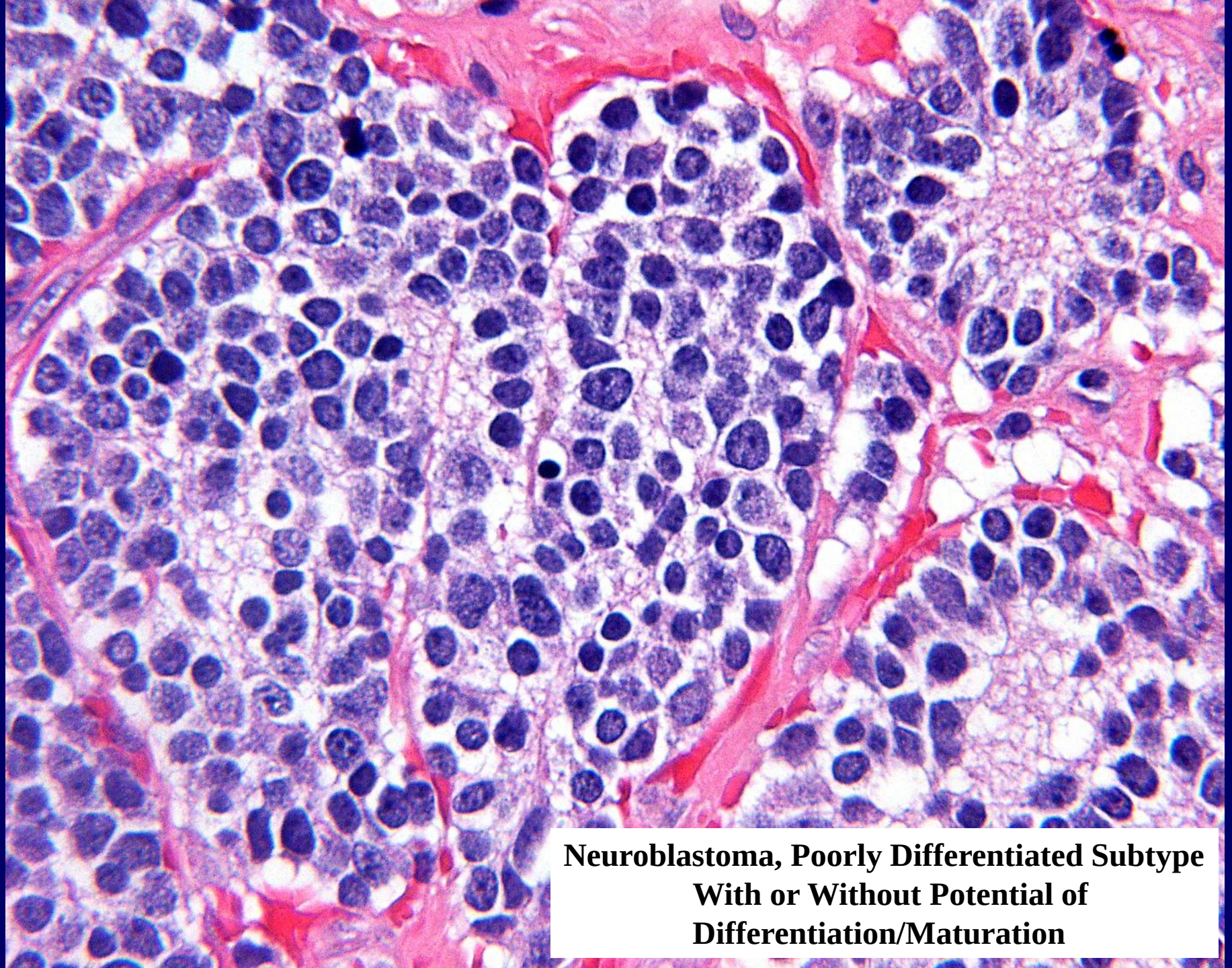
- **PGP9.5 (+)**
- **Synaptophysin (+)**
- **Chromogranin (+/-)**
- **TH (+) in sporadic cells**
- **CD99 (-)**

TH (Tyrosine Hydroxylase)

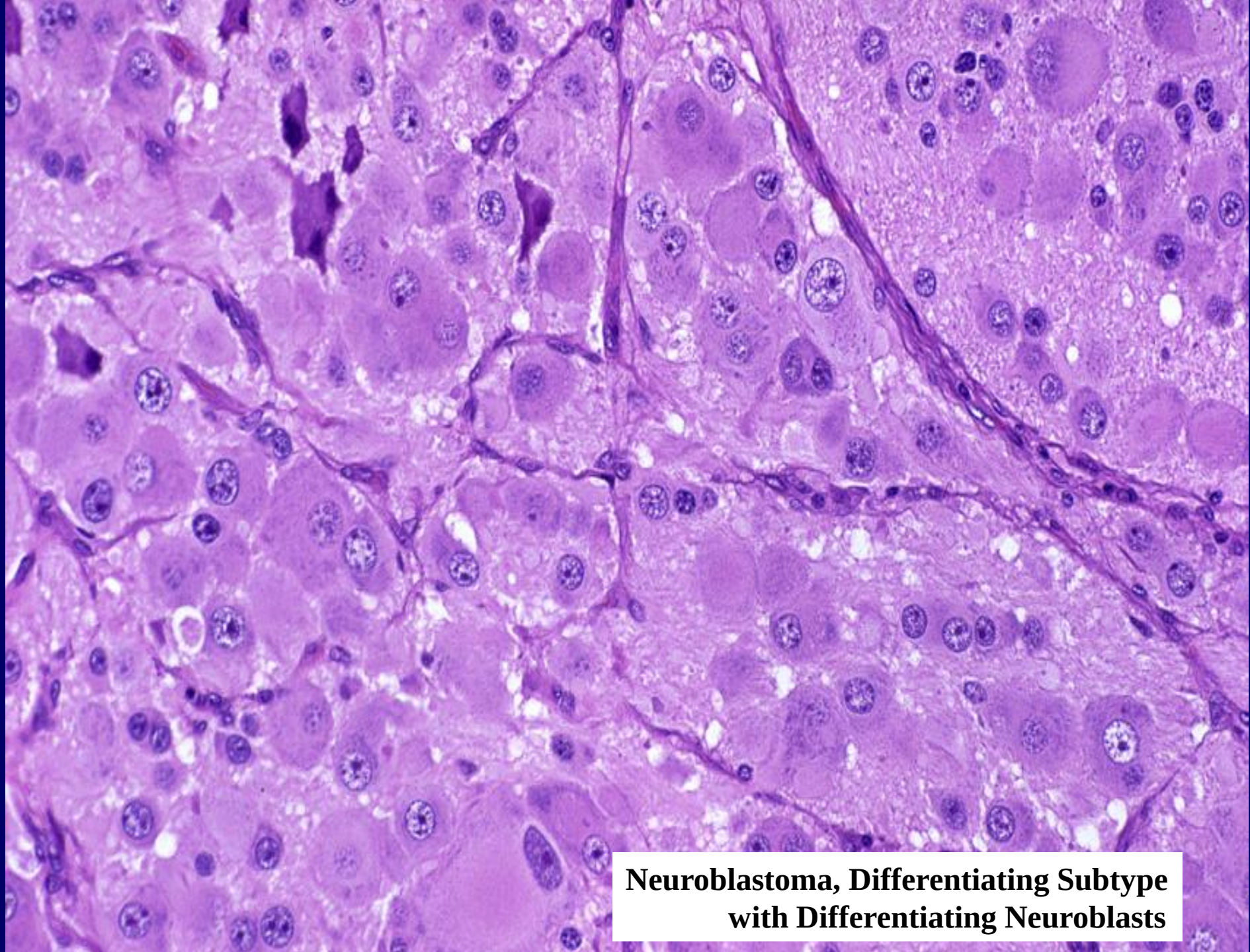
Neuroblastoma, Undifferentiated Subtype

- ✓Very Rare Subtype (<3%)
- ✓Very Low *trk*AI/II Expression
  - No or Limited Potential of Neuroblastic Differentiation
- ✓Extremely Poor Prognosis
- ✓High Incidence of MYC Protein Expression
  - *MYCN* Amplification with N-myc Protein Expression
  - C-myc Protein Overexpression



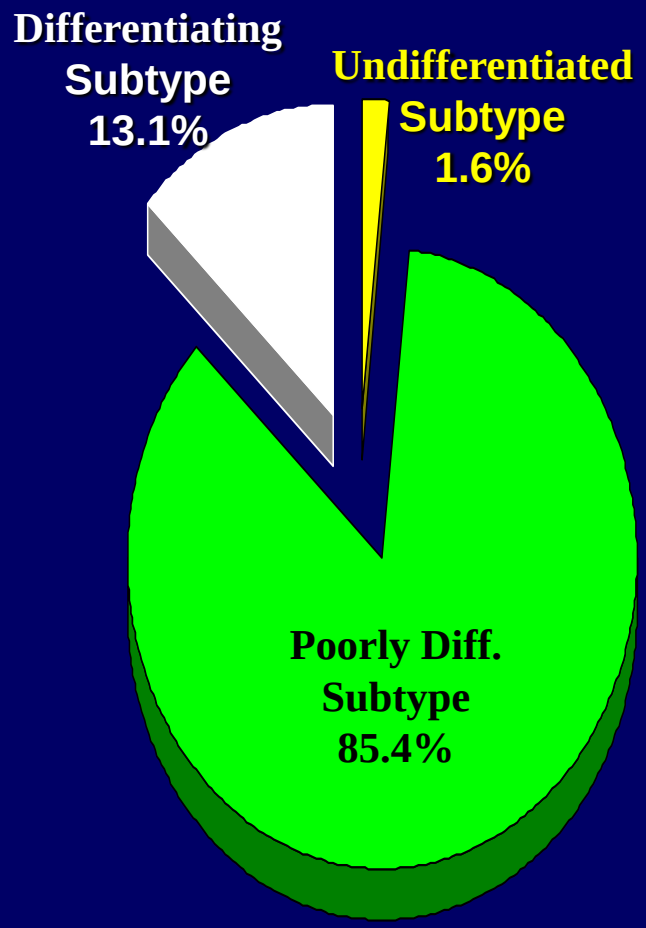


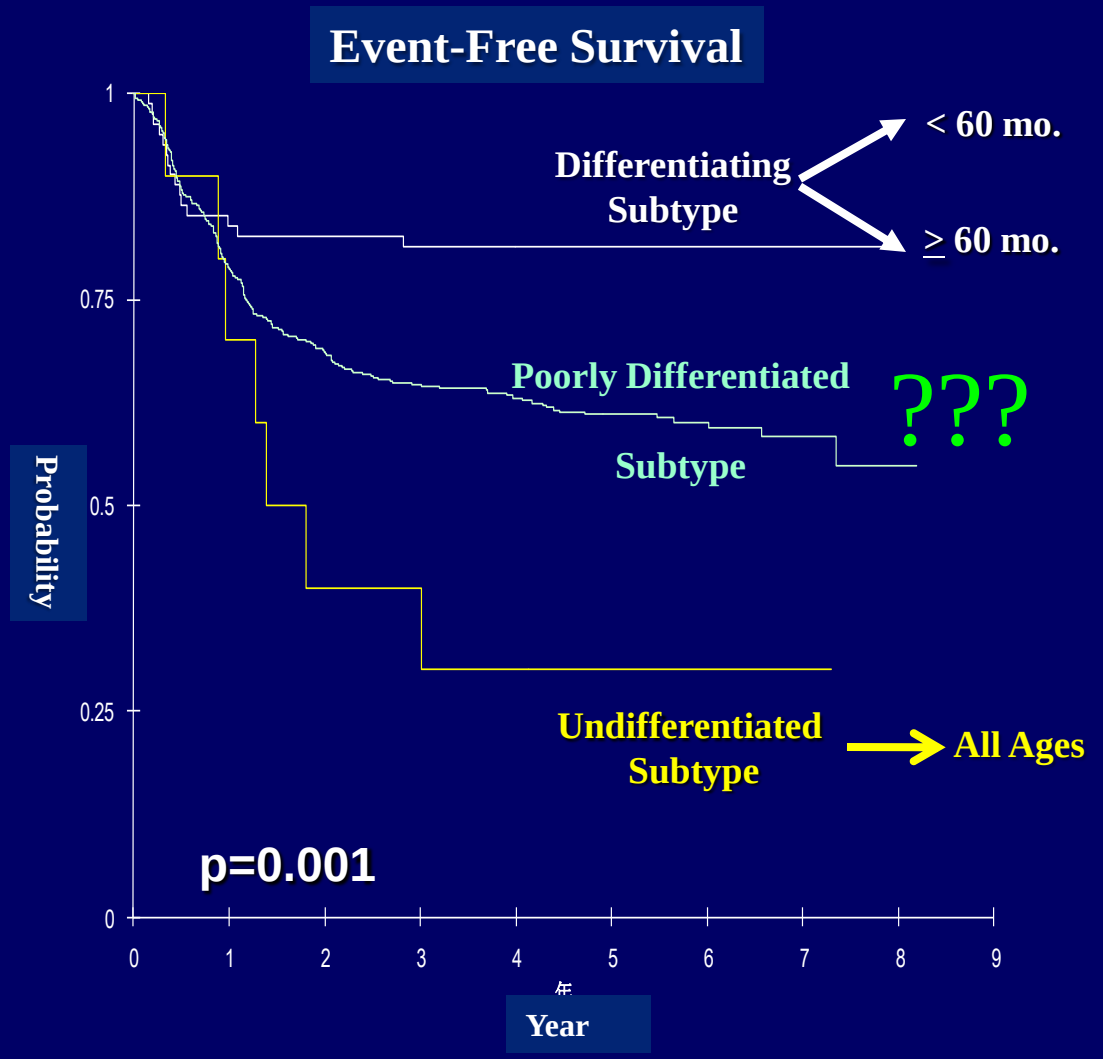
Neuroblastoma, Poorly Differentiated Subtype With or Without Potential of Differentiation/Maturation



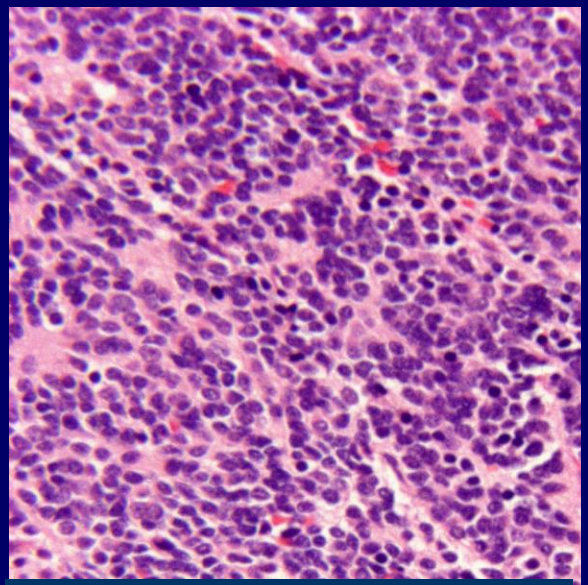
Neuroblastoma, Differentiating Subtype with Differentiating Neuroblasts

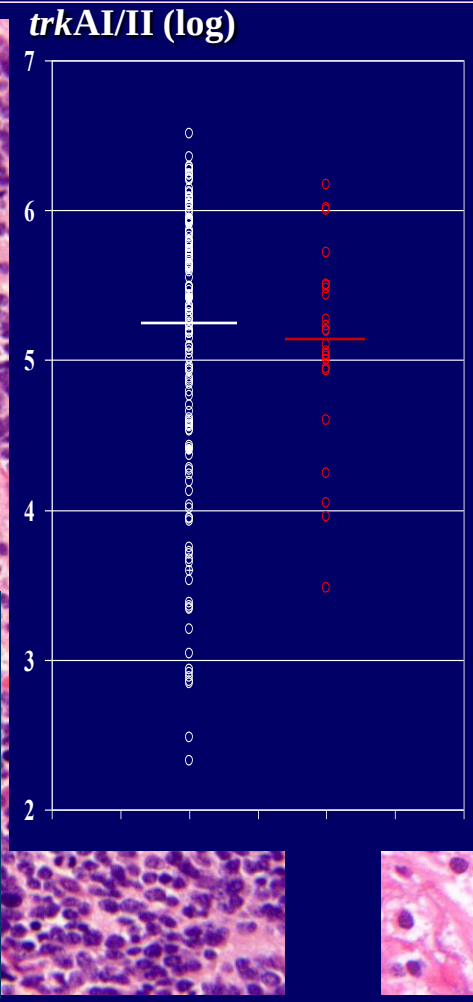
Neuroblastoma Patients - Survivals by Grade of Neuroblastic Differentiation (CCG -3881/-3891 Study)

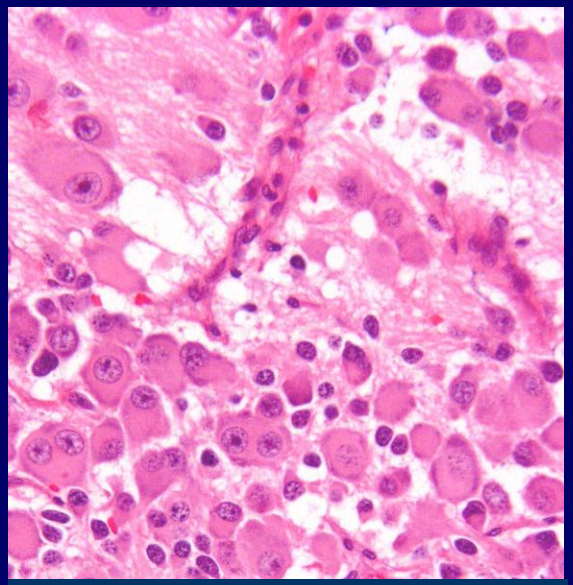


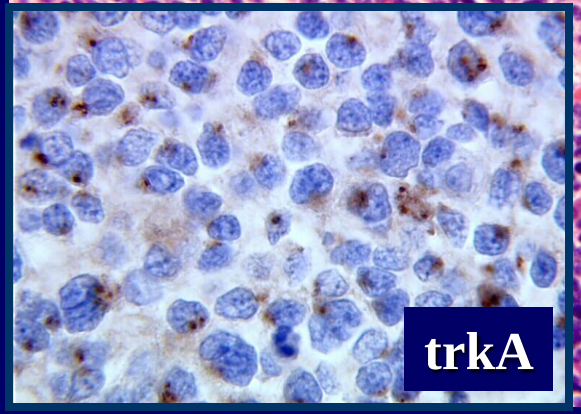


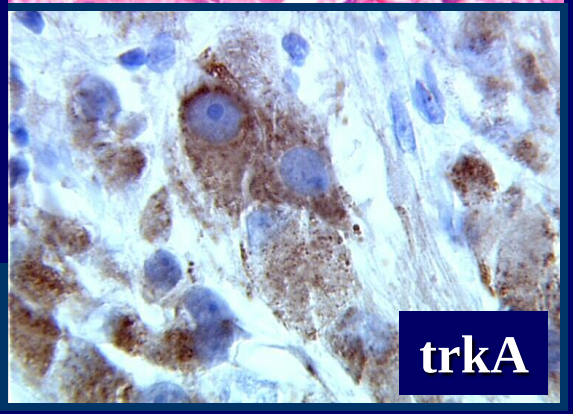
*Trk*AI/II Expression in Favorable Histology Neuroblastoma Tumors











***trk*A Expression: high**
***MYCN*: non-amplified**

Diagnosed at 5 months of age

***trk*A Expression: high**
***MYCN*: non-amplified**

Diagnosed at 2 years and one month of age

*Trk*AI/II Expression and Age-Appropriate Neuroblastic Differentiation in FH Neuroblastoma Tumors

Poorly Diff.
Differentiating

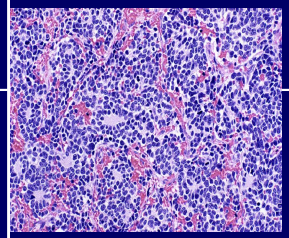
*trk*AI/II

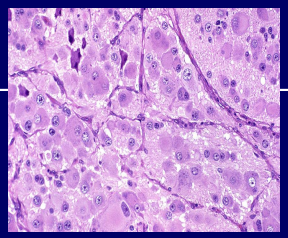
7
6
5
4
3
2

Same Molecular Properties
MYCN non-amplified
*Trk*A high expression

Age-Appropriate Neuroblastic Differentiation

Excellent Prognosis





0 18 40 60 100 180

Age at Dx. (mo.)

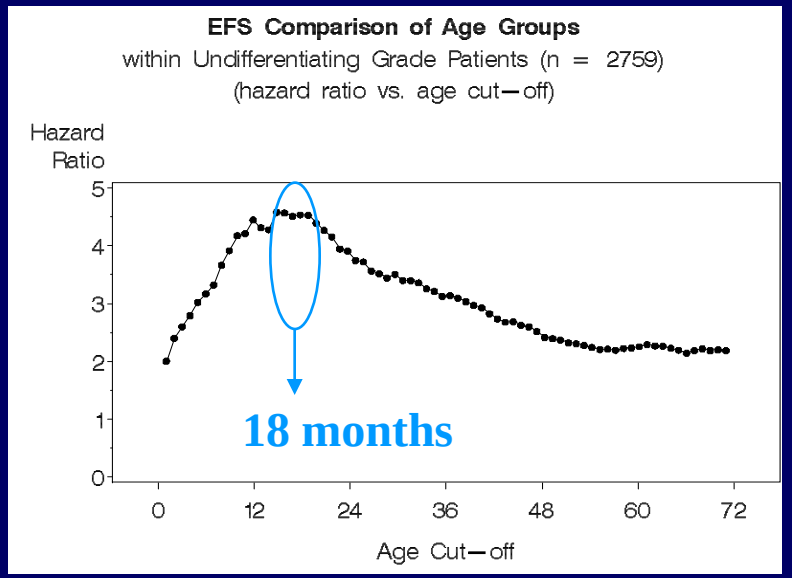
Neuroblastoma, Poorly Differentiated Subtype

*Trk*AI/II High Expresser

- Age-Appropriate Neuroblastic Differentiation
  - *In Vivo* Latent Period for Differentiation
- Newborns, Infants — Good Prognosis with This Subtype

*Trk*AI/II Low Expresser

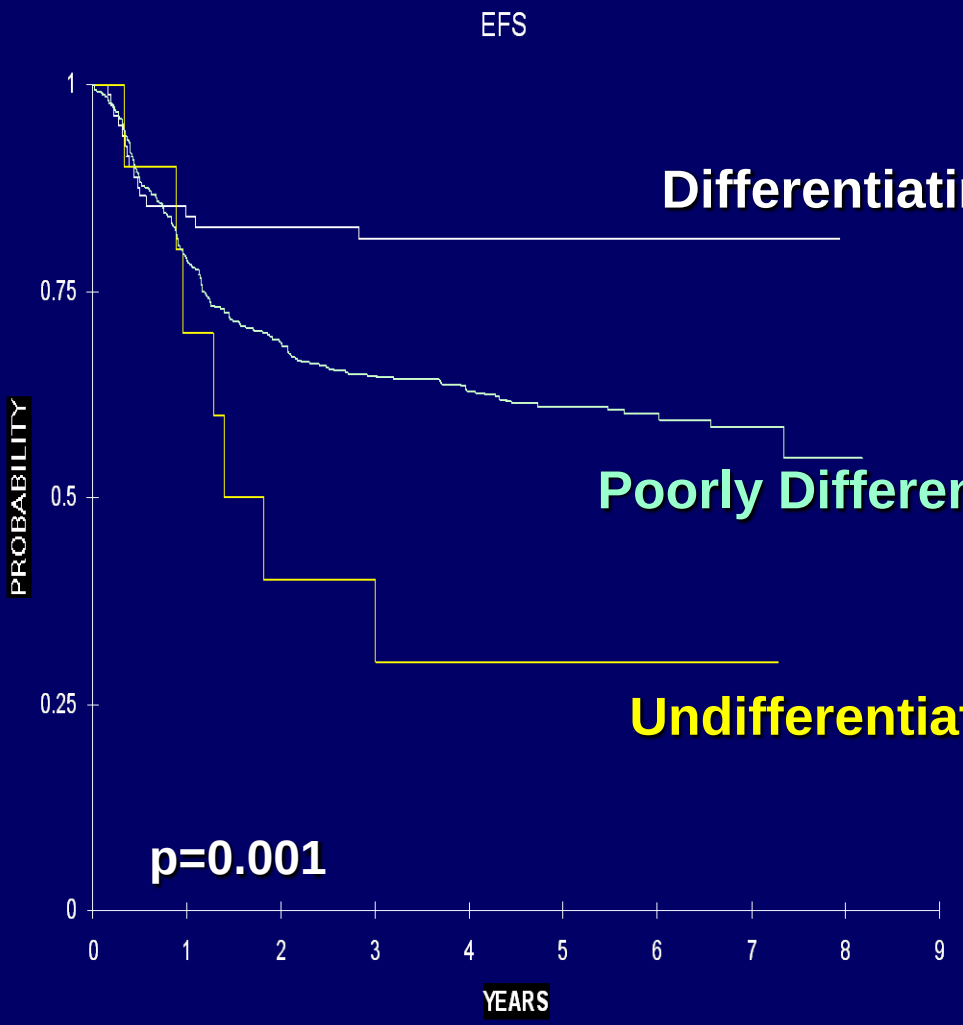
- No Potential of Neuroblastic Differentiation
- Older Children — Poor Prognosis with This Subtype



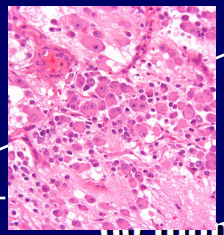
Distinction of Prognostic groups

- Based on the Hazard Ratio according to the Age of the Patients

Biological Heterogeneity of Neuroblastoma (Schwannian Stroma-Poor)



Differentiating



Furth ration

Gangli astoma

Age-Appropriate Differentiation

Poorly Differentiated

18 months

Undifferentiated

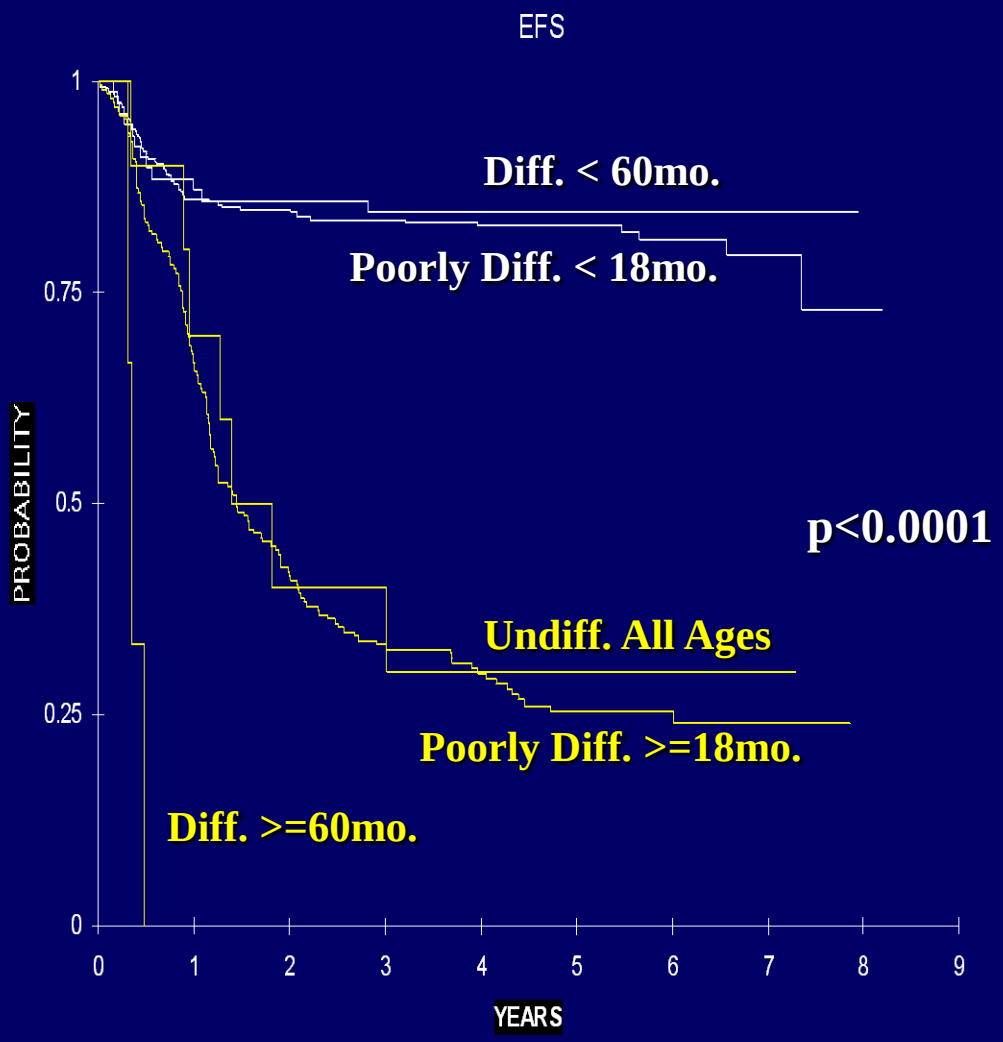
All Ages

No Potential of Differentiation

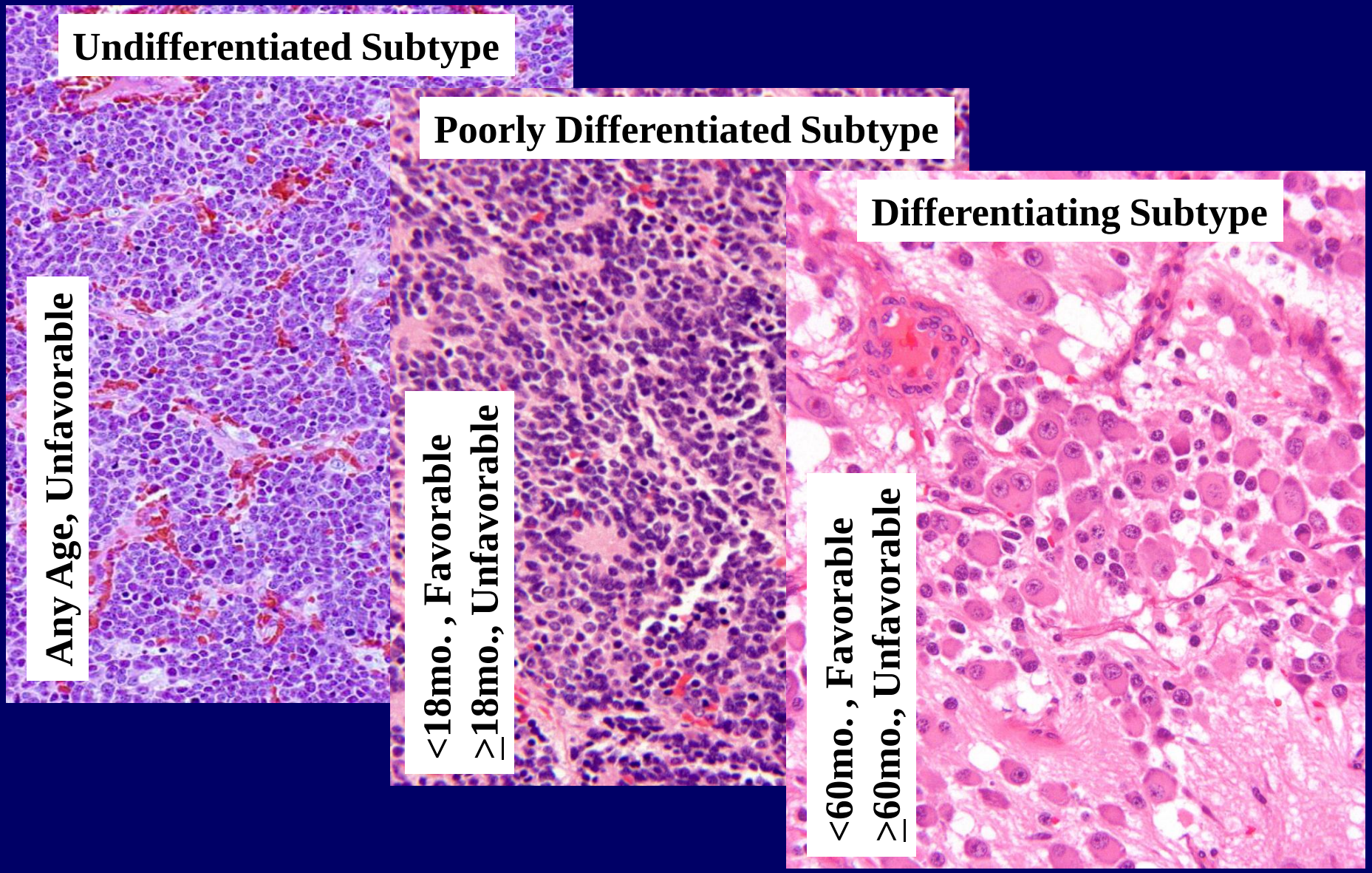
Grade of Neuroblastic Differentiation in Neuroblastoma Age-linked Prognostic Effects

Prognostic Grouping

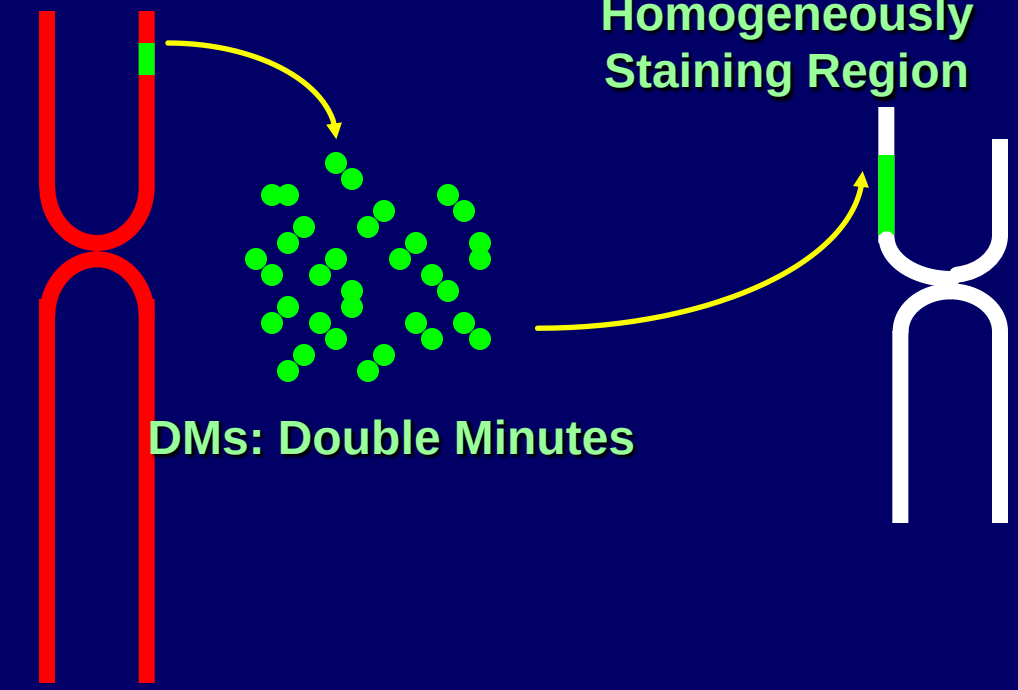
	<18mo.	18-60mo.	>=60mo.
Undiff.			
Poorly Diff.			
Diff.			

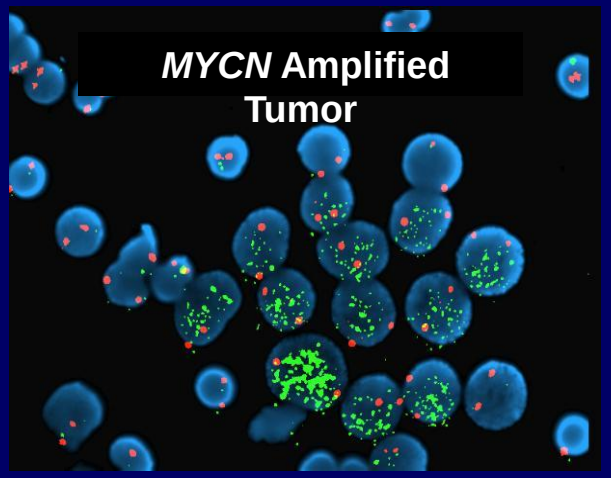


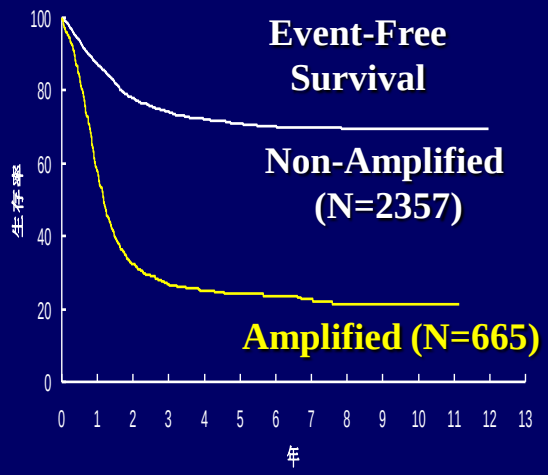
Neuroblastoma (Schwannian stroma-poor)



MYCN Oncogene Amplification In Peripheral Neuroblastic Tumors

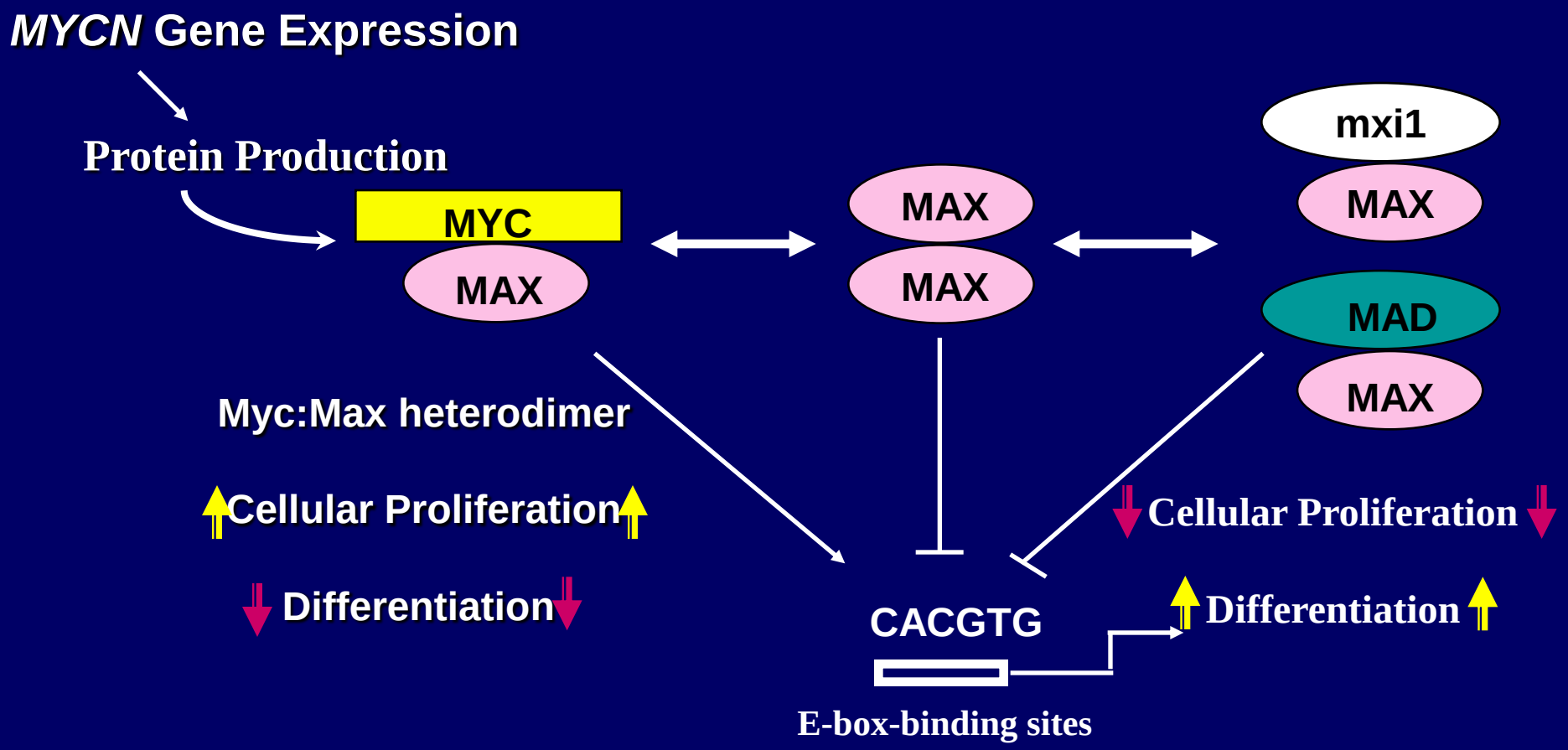






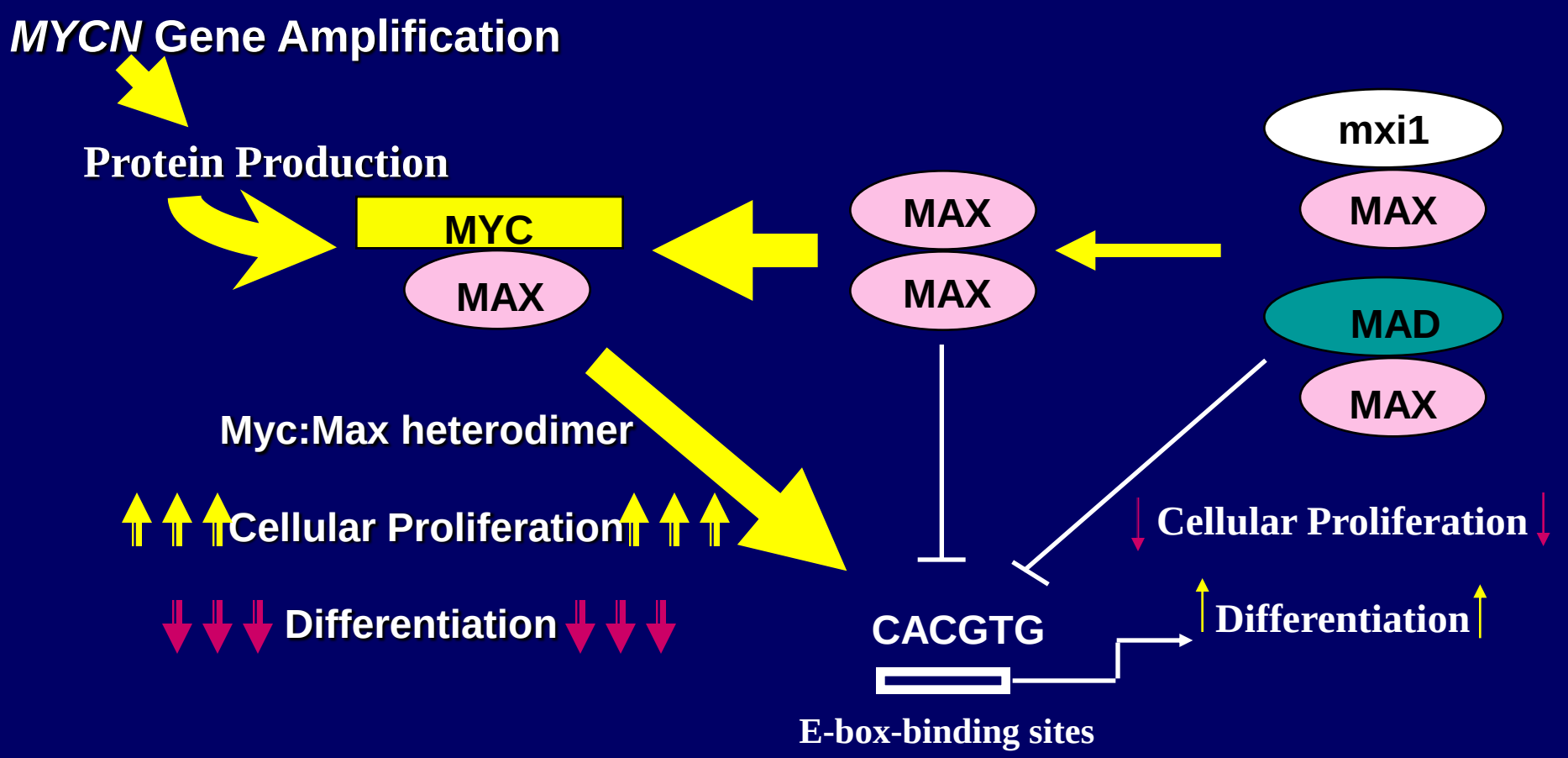
Role of *MYCN* in Organogenesis

MYCN Gene & MAX Transcription Factor Network

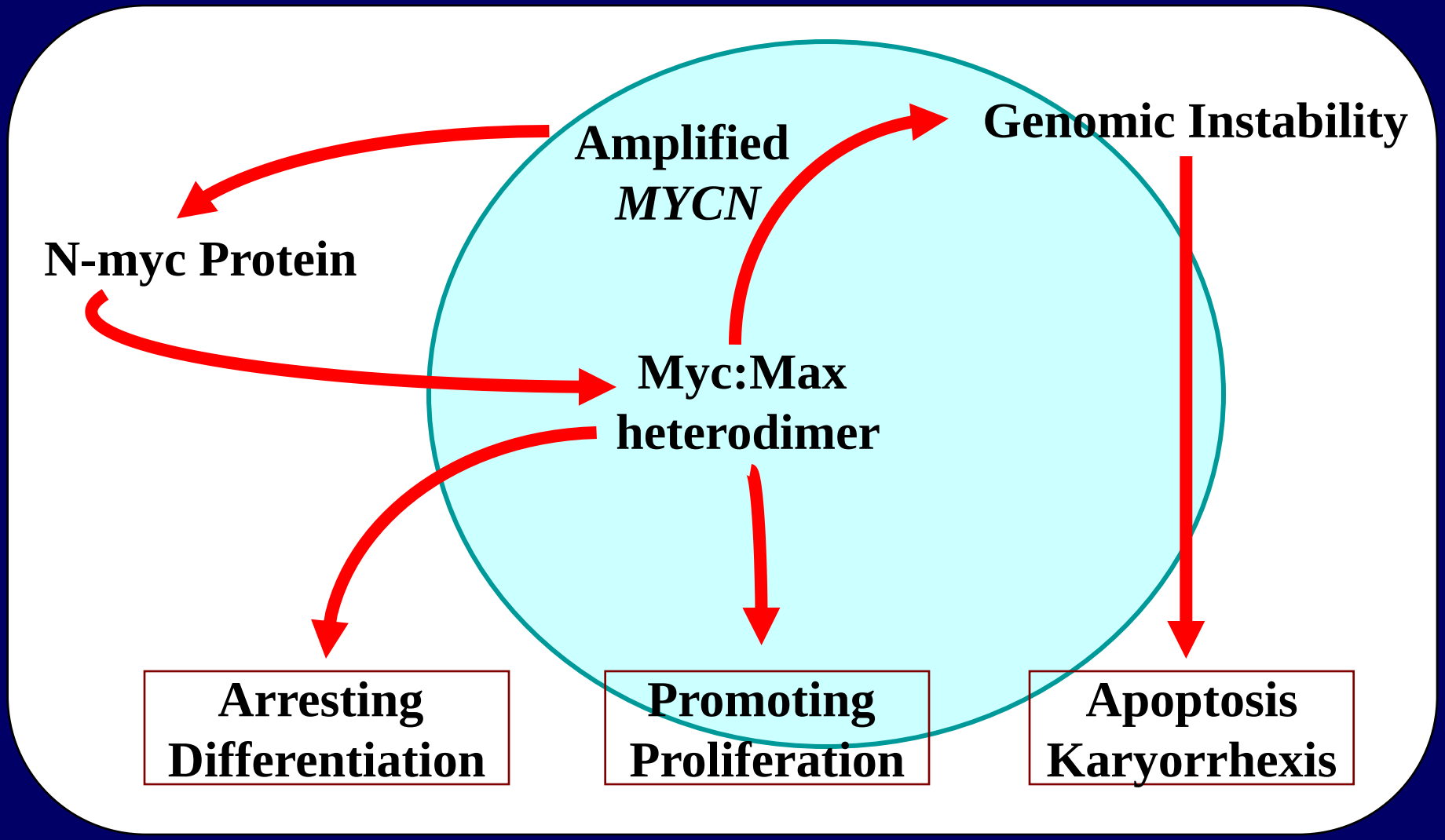


MYCN Amplification in Neuroblastoma

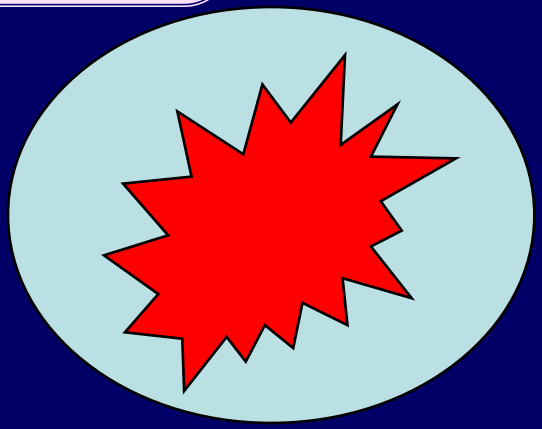
MYCN Gene & MAX Transcription Factor Network



MYCN Amplification in Neuroblastoma

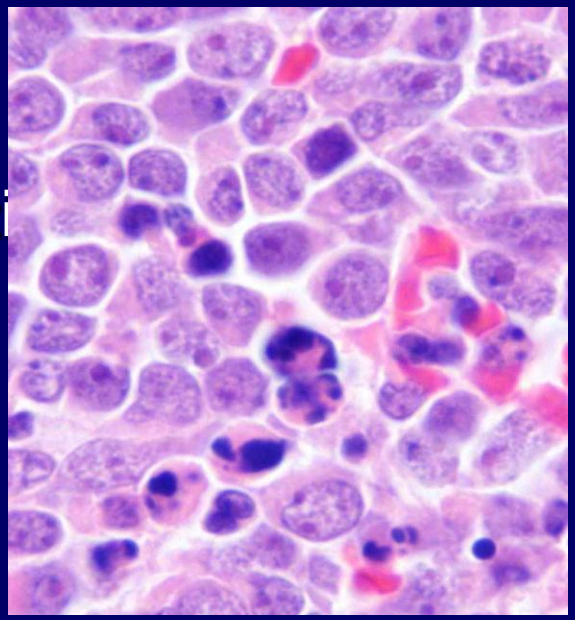


MYCN Amplification in Neuroblastoma

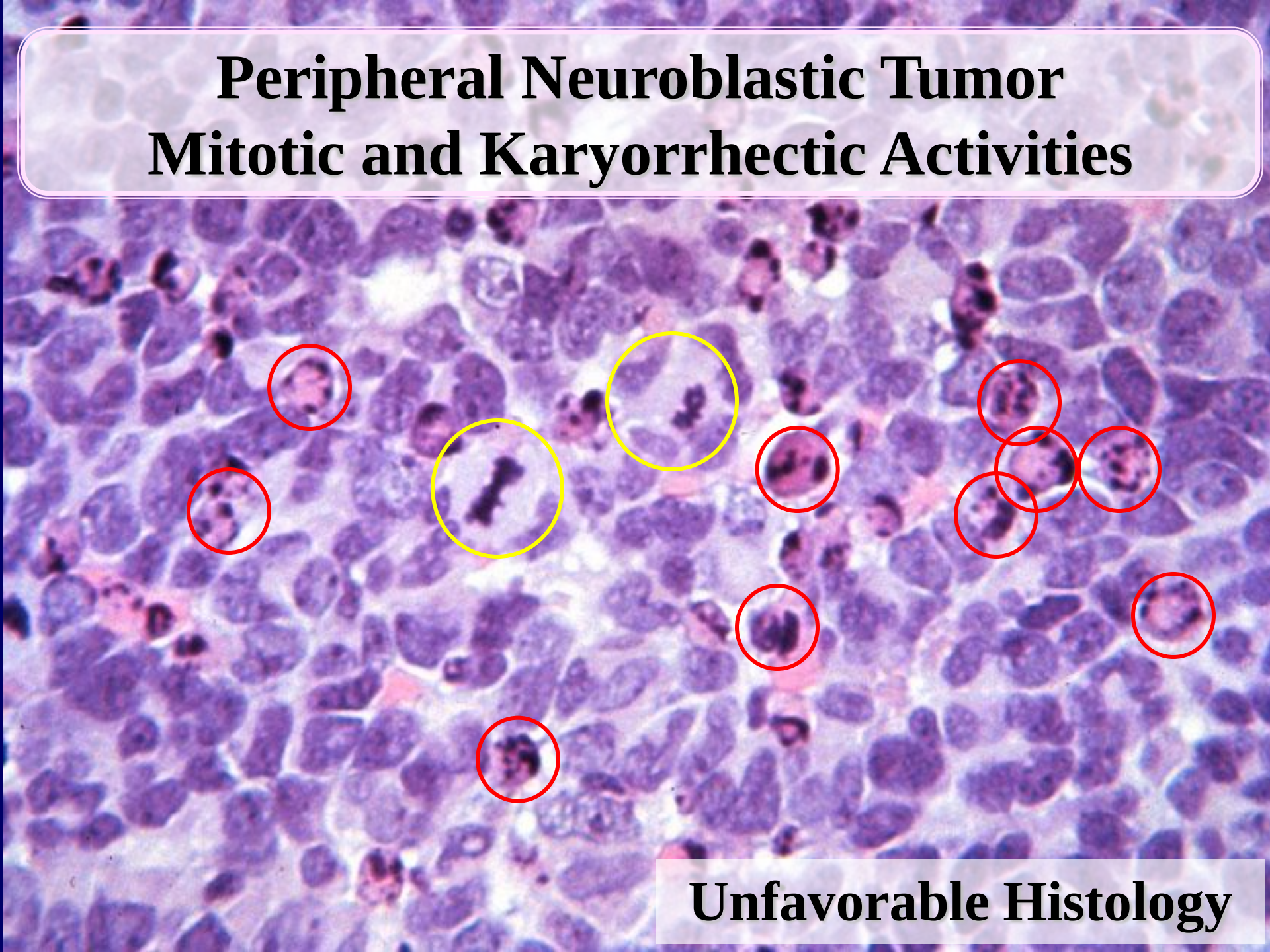


Powerful Driving Force for:

- **Preventing Cellular Differentiation**
- **Increasing Mitotic and Karyorrhecti Activities**

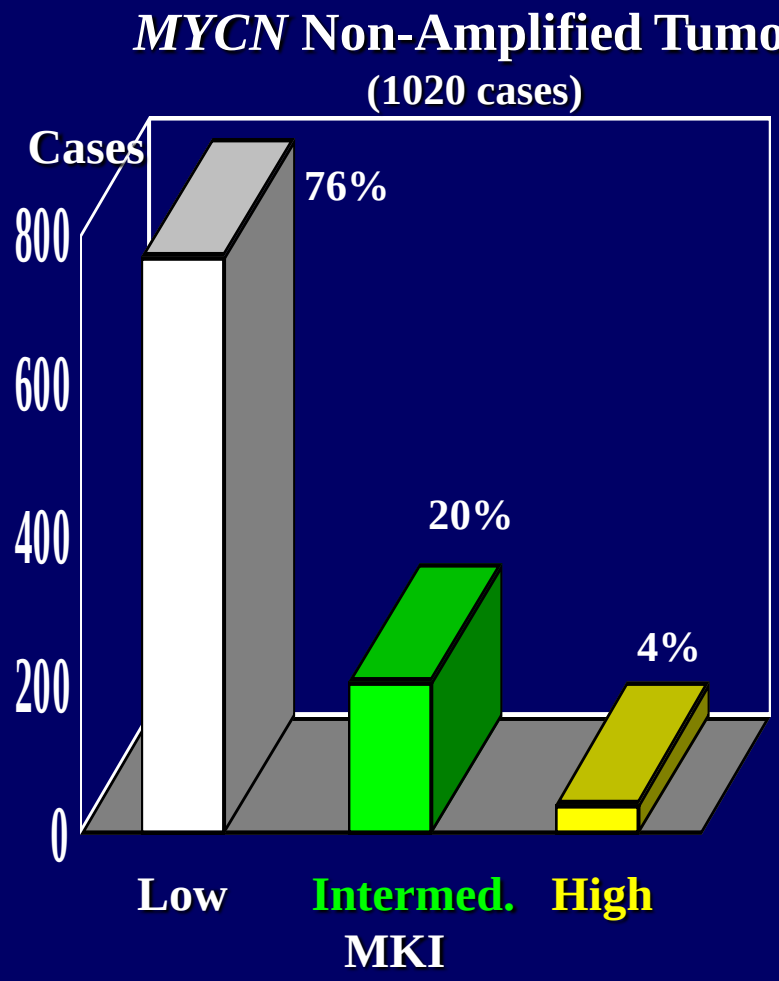


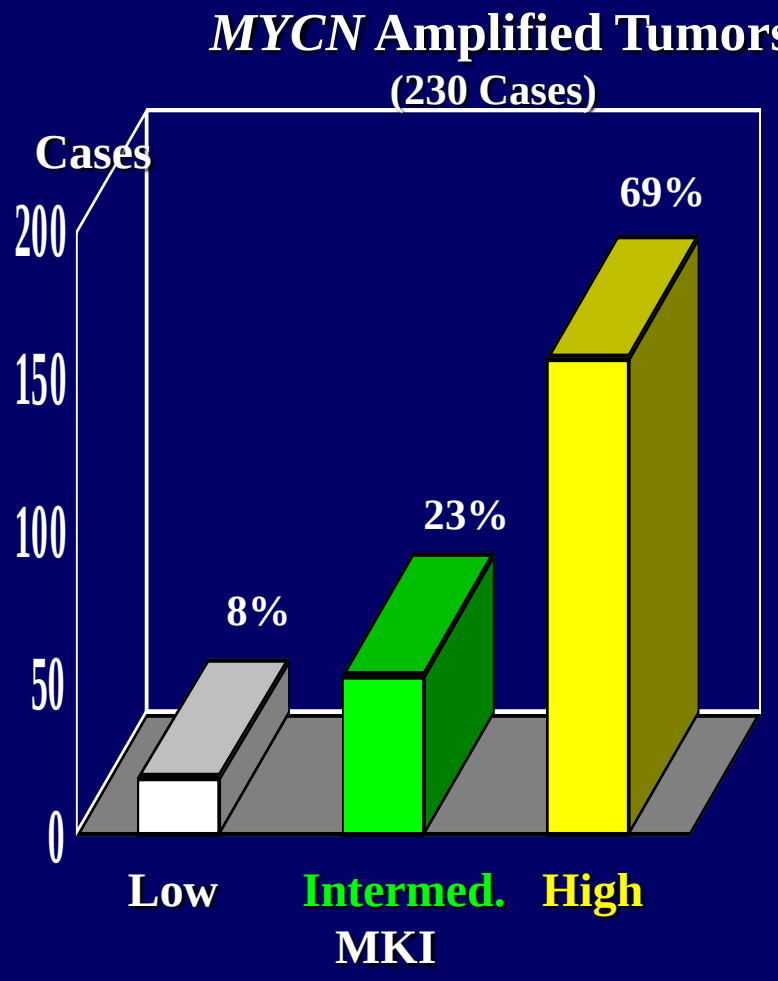
Peripheral Neuroblastic Tumor
Mitotic and Karyorrhectic Activities



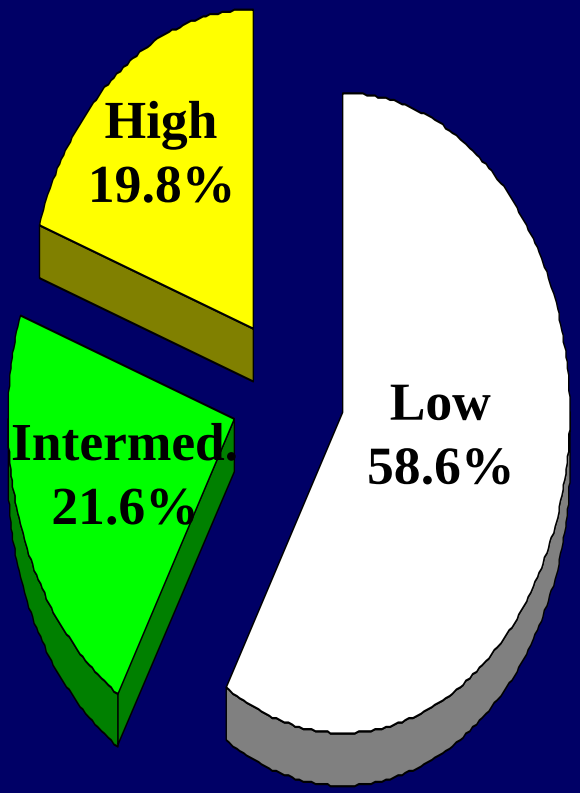
Unfavorable Histology

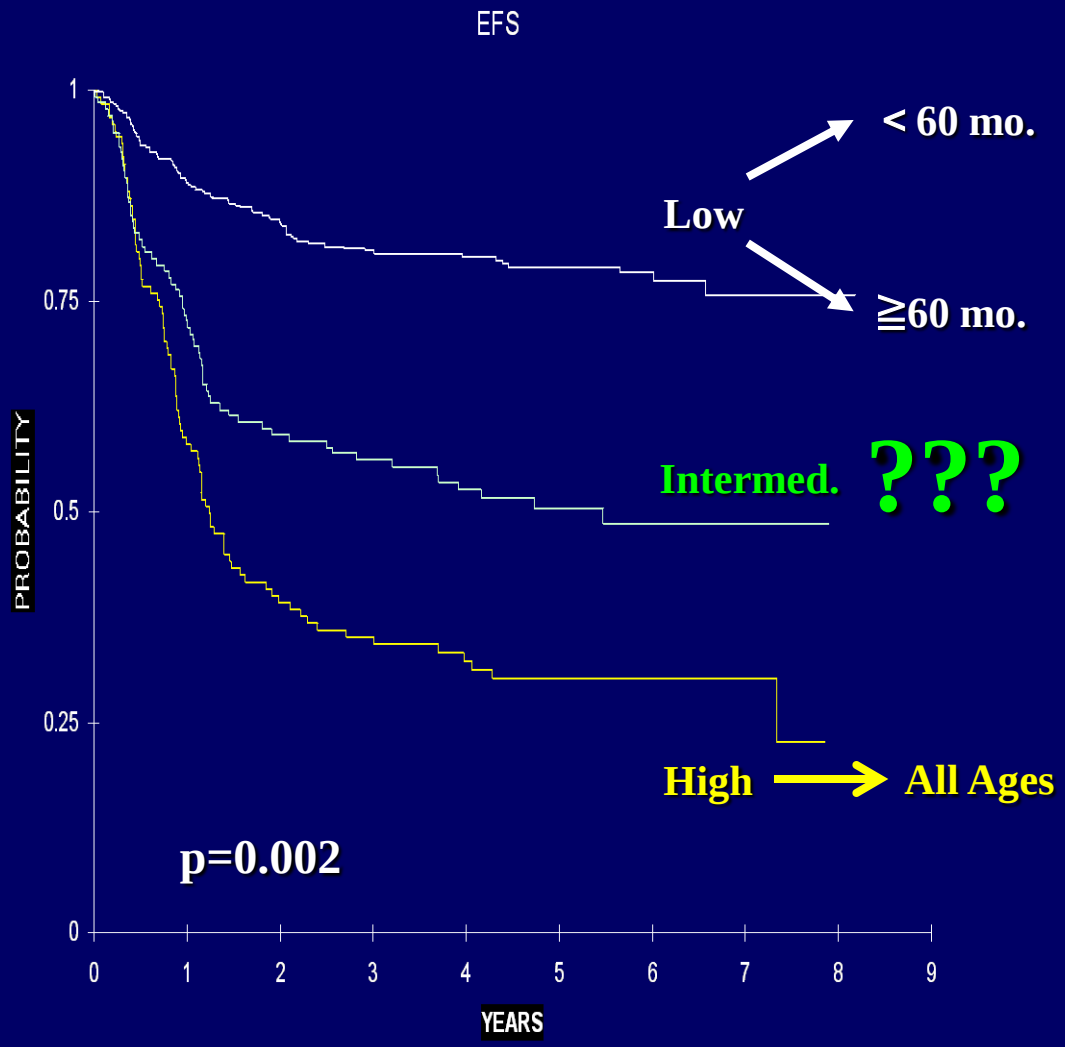
Neuroblastoma: *MYCN* Status and MKI





Neuroblastoma: Prognosis by MKI





Neuroblastoma: Prognostic Effects of Intermediate MKI

Non-Amplified *MYCN*

80%

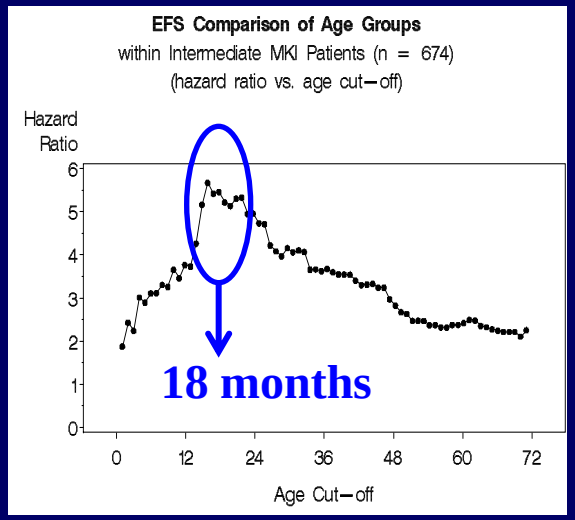
Amplified *MYCN*

20%

Intermed. MKI
N-myc Protein
Expression

To be terminated

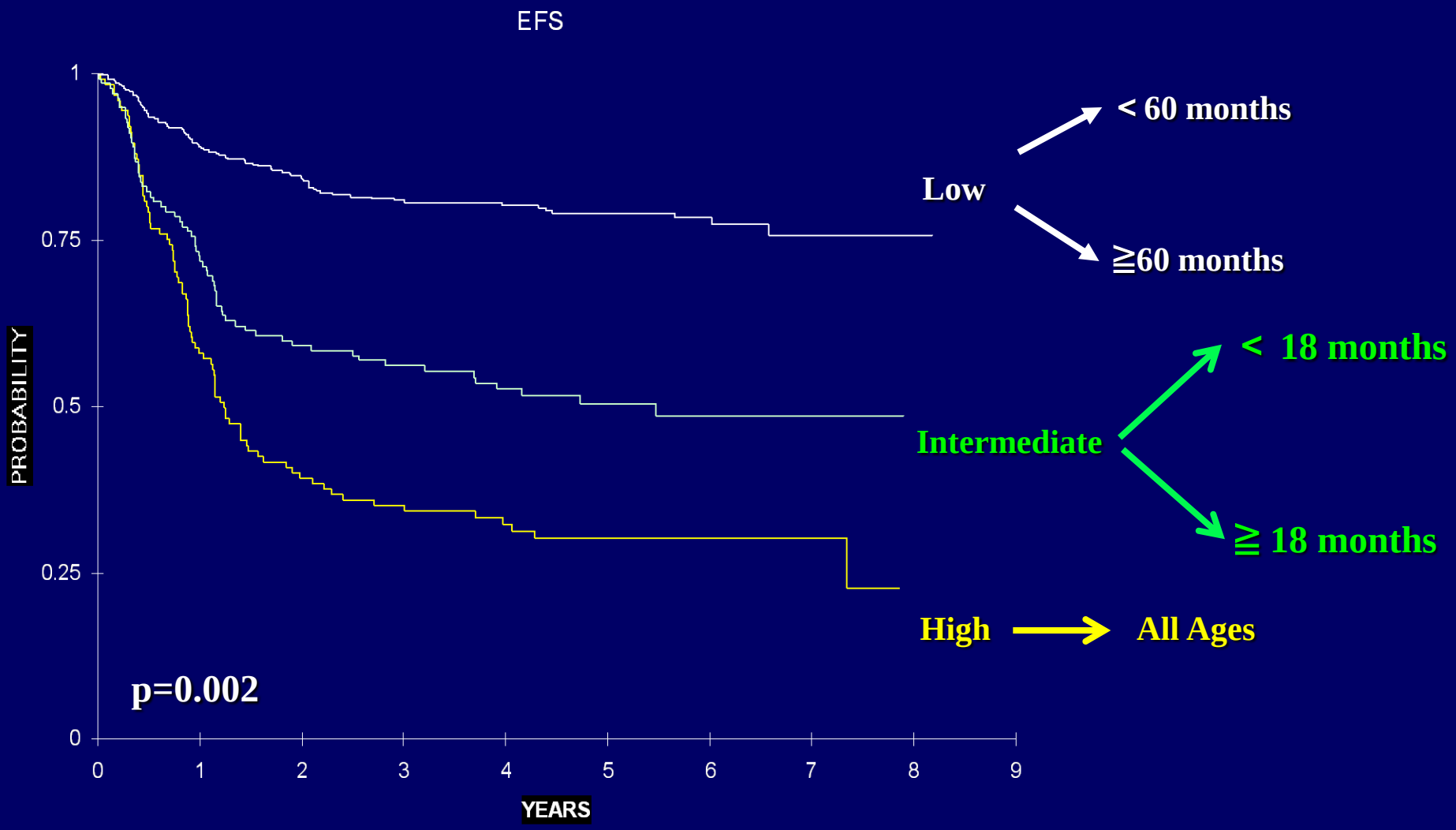
Good Prognosis



Continuous

Poor Prognosis

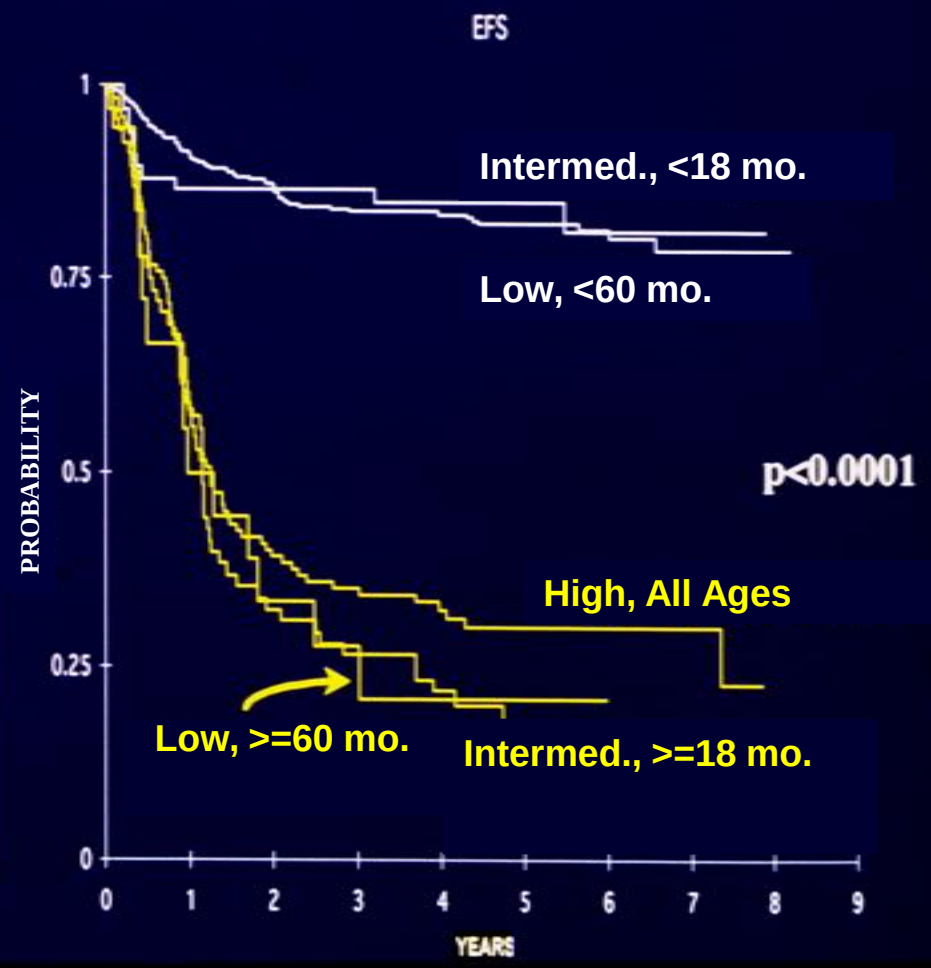
Neuroblastoma: Prognosis by MKI



MKI in Neuroblastoma
Age-linked Prognostic Effects (CCG-3881/-3891 Study)

Prognostic Grouping

	<18 mo.	18-60 mo.	≥60 mo.
Low			
Inter-med.			
High			

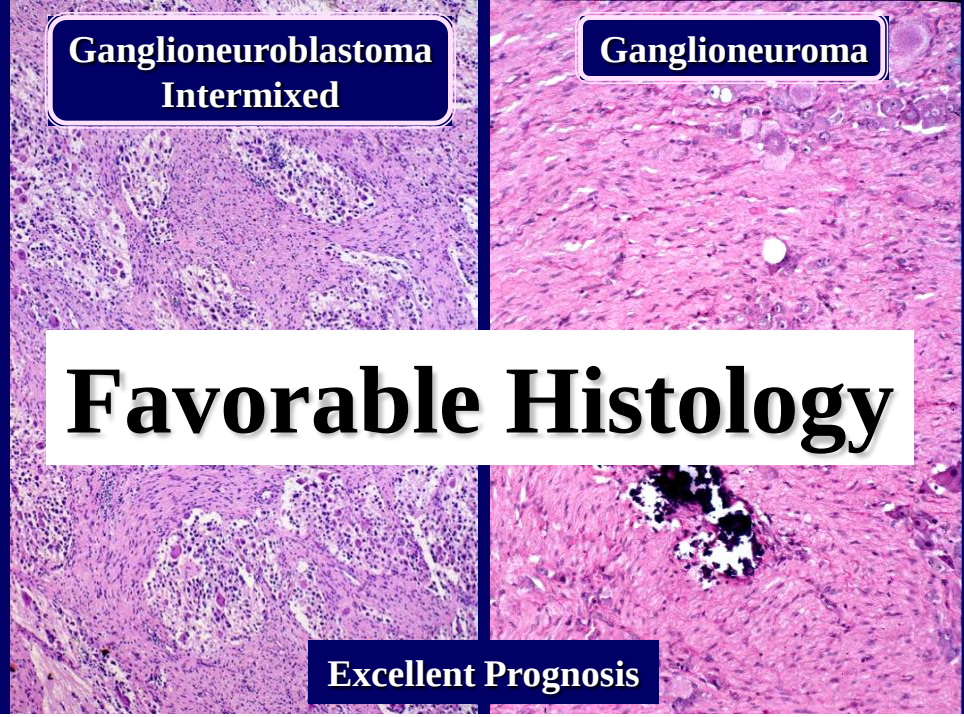


Neuroblastoma (Schwannian stroma-poor)

Grade of Differentiation	MKI	< 18 mo.	18-60mo.	> 60 mo.
Undifferentiated	Any	Unfavorable Histology		
Poorly Differentiated	Low	Favorable Histology	Unfavorable Histology	
	Intermed.	Favorable Histology	Unfavorable Histology	
	High	Unfavorable Histology		
Differentiating	Low	Favorable Histology	Favorable Histology	Unfavorable Histology
	Intermed.	Favorable Histology	Unfavorable Histology	
	High	Unfavorable Histology		

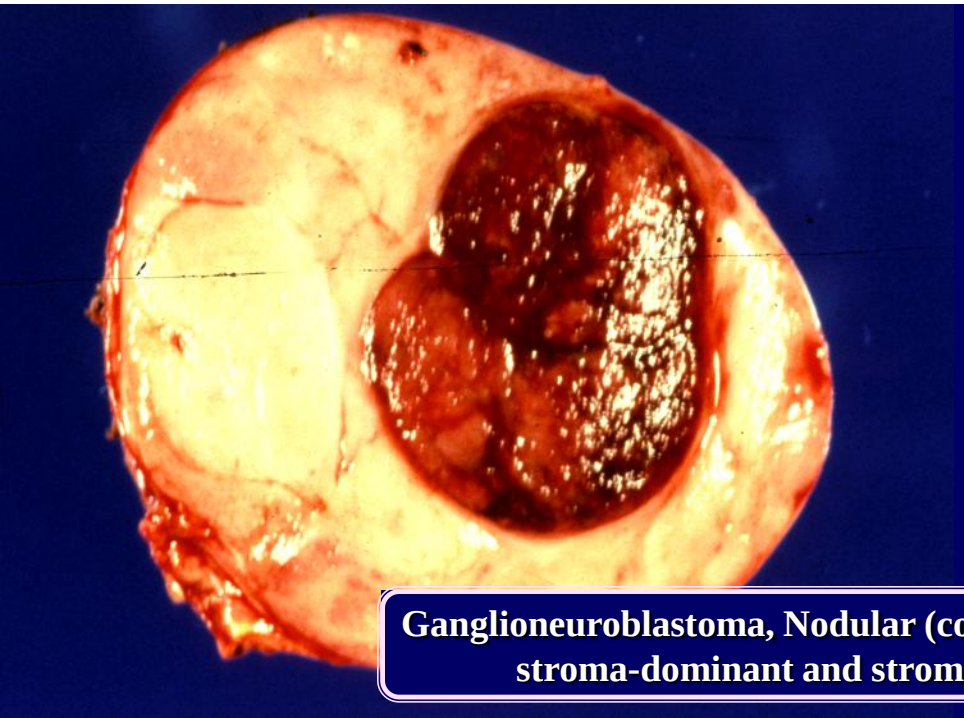
Favorable Histology

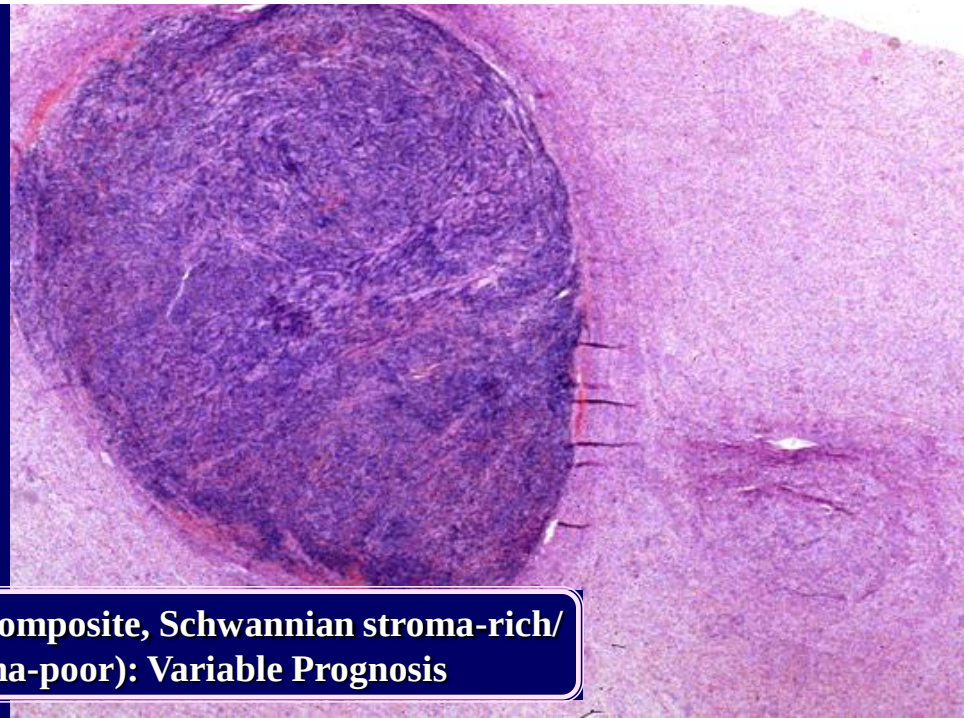
Unfavorable Histology



Favorable Histology

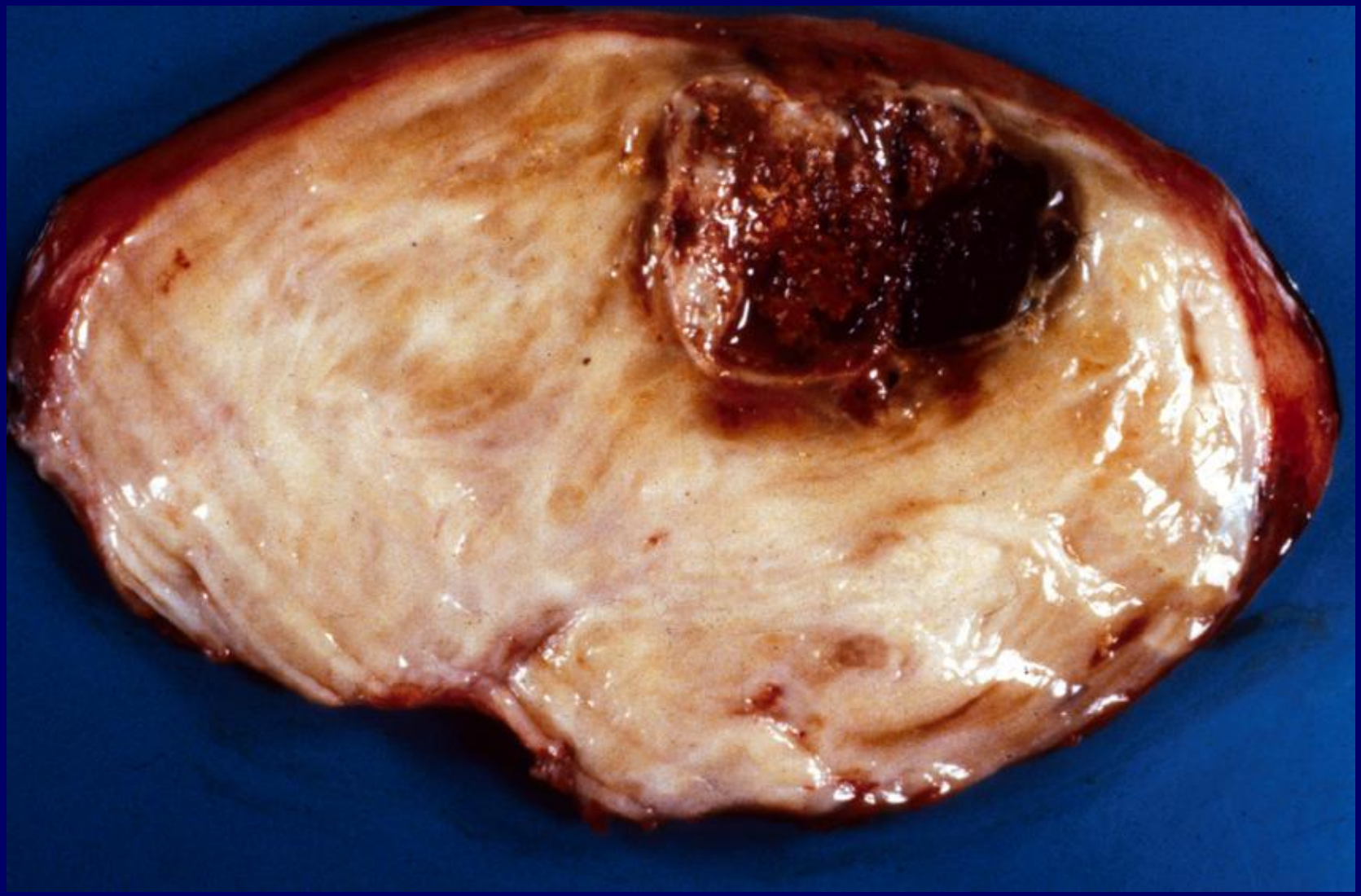
Excellent Prognosis

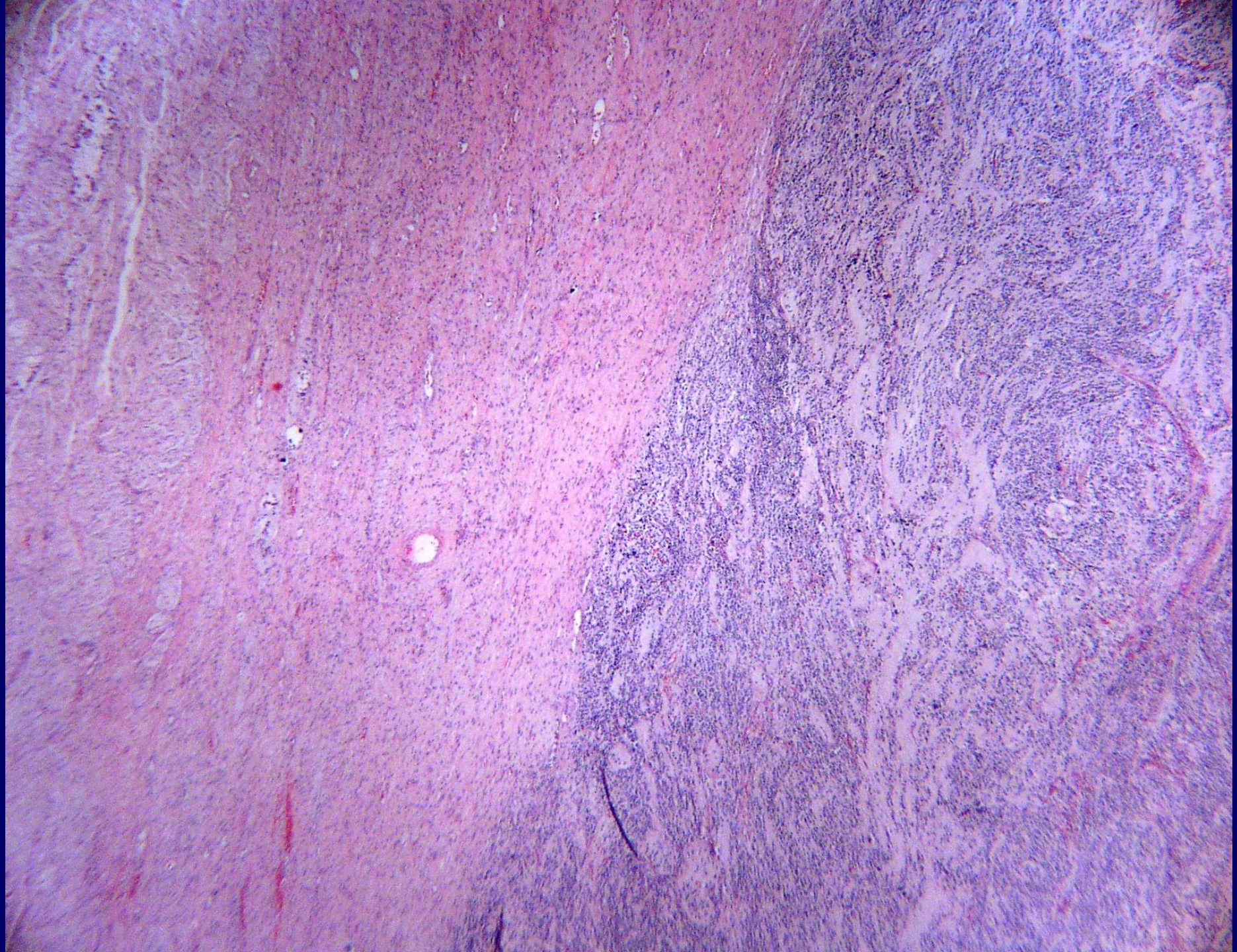




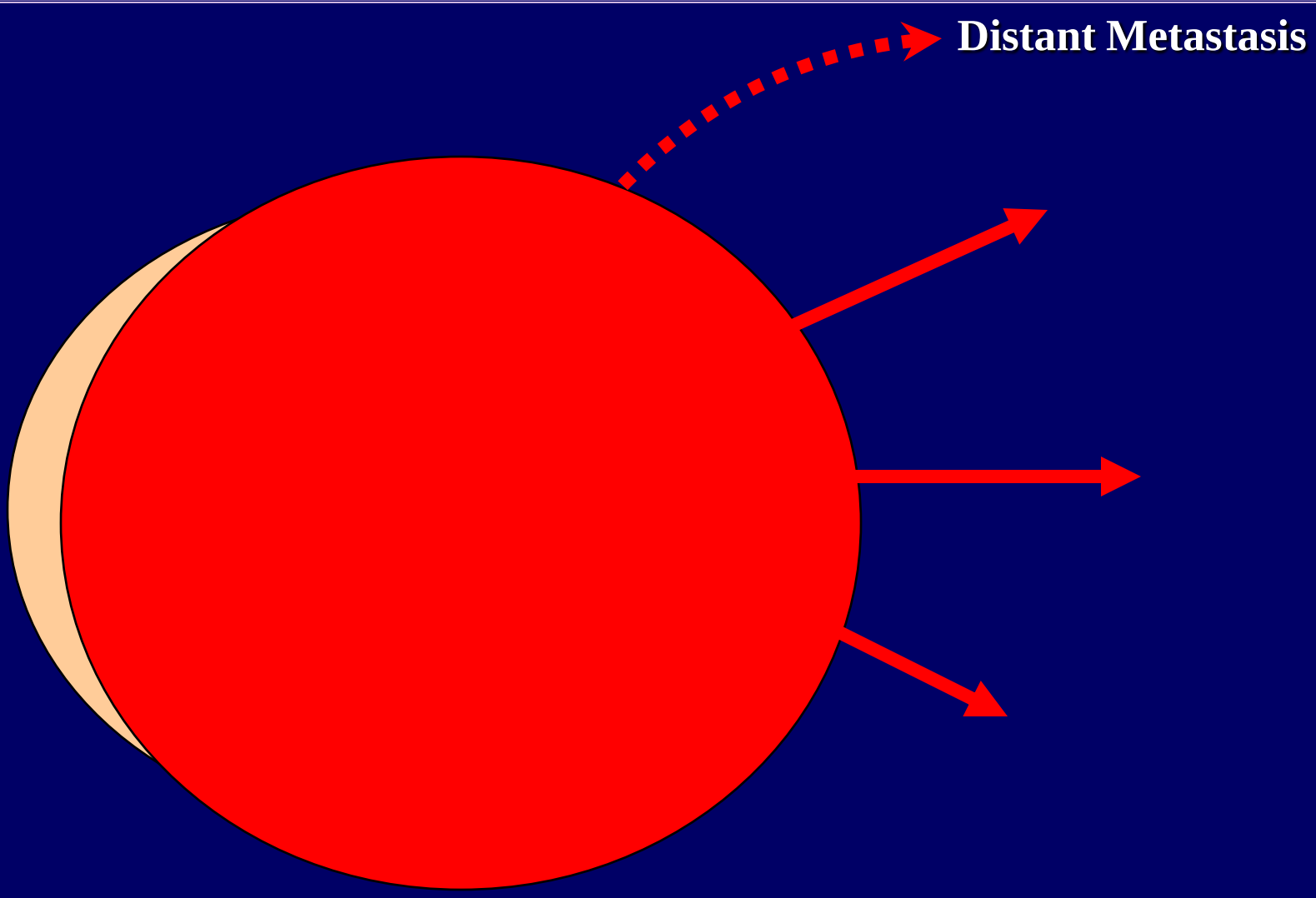
Ganglioneuroblastoma, Nodular (composite, Schwannian stroma-rich/ stroma-dominant and stroma-poor): Variable Prognosis

Ganglioneuroblastoma, Nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)



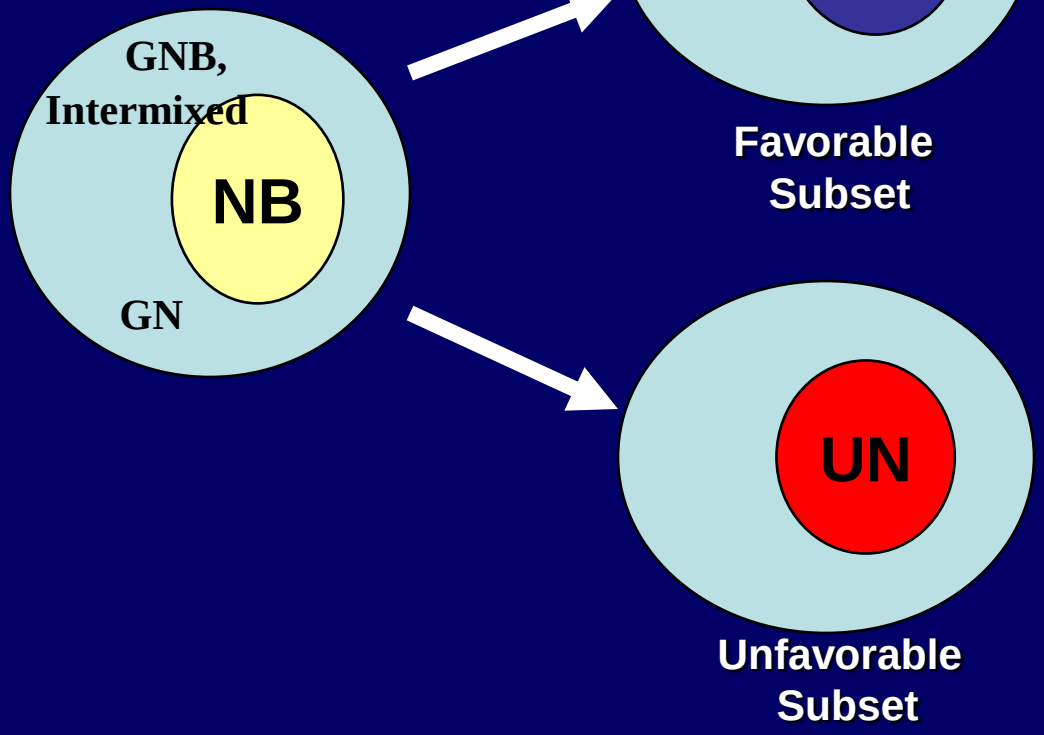


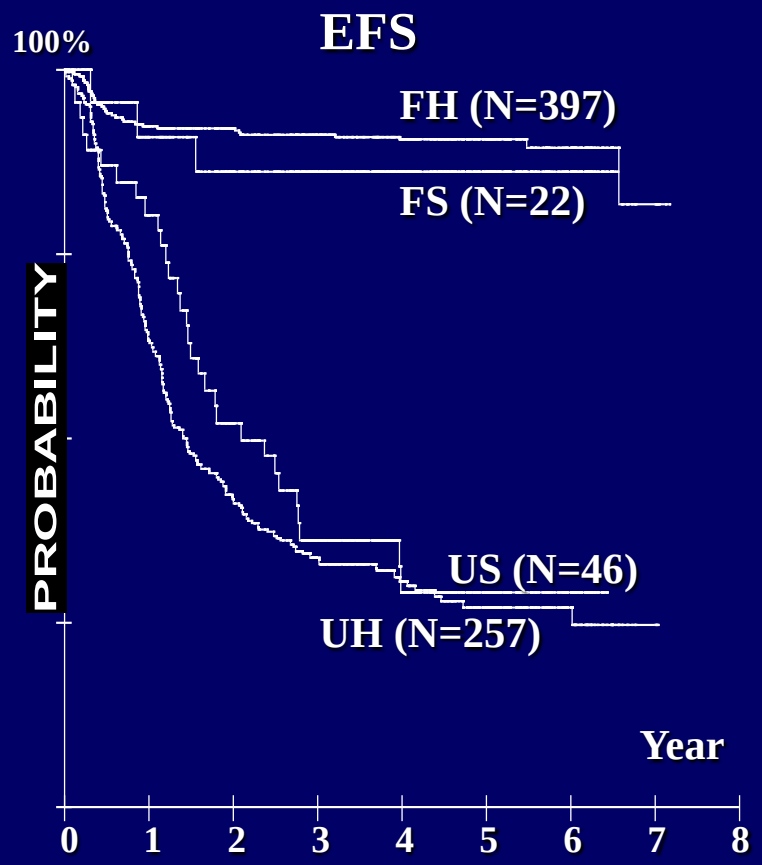
Ganglioneuroblastoma, Nodular



GNBn : Determination of Prognostic Subsets

Applying Histopathology Evaluation to the Neuroblastomatous Compon

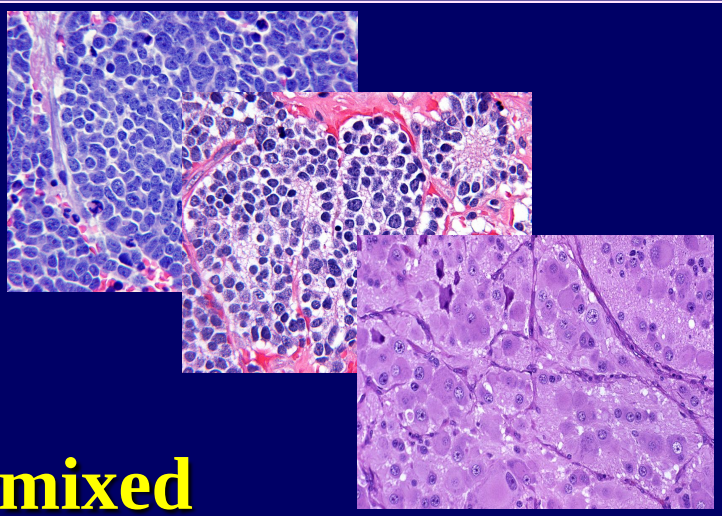




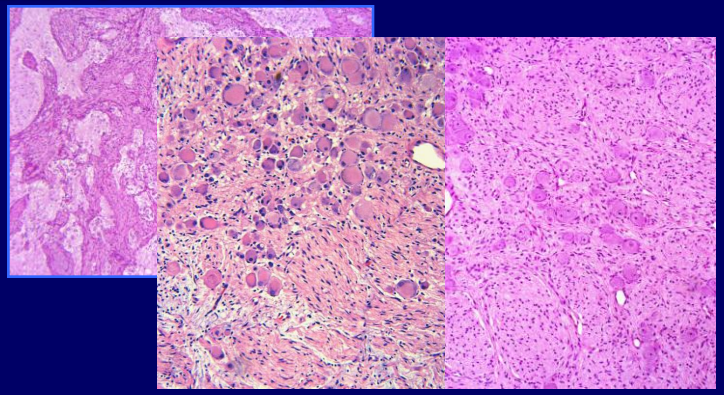
FS: GNBn, Favorable Subset
US: GNBn, Unfavorable Subset
FH: non-GNBn, Favorable Subset
UH: non-GNBn, Unfavorable Subset

Peripheral Neuroblastic Tumors

- **Neuroblastoma**
  （Schwannian stroma-poor）
  - Undifferentiated Subtype
  - Poorly Differentiated Subtype
  - Differentiating Subtype

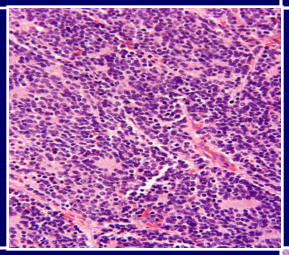
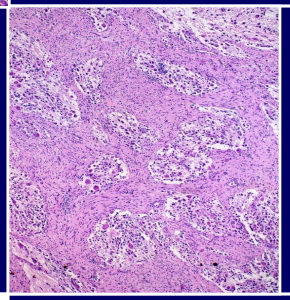
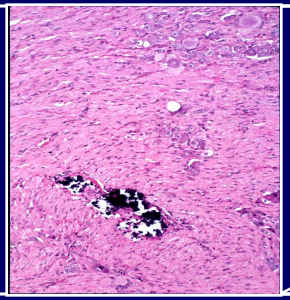
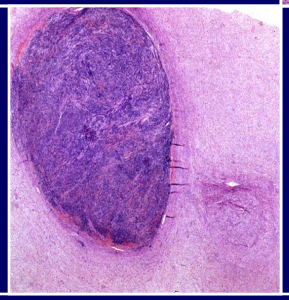


- **Ganglioneuroblastoma, Intermixed**
  （Schwannian stroma-rich）
- **Ganglioneuroma**
  （Schwannian stroma-dominant）
  - Maturing Subtype
  - Mature Subtype

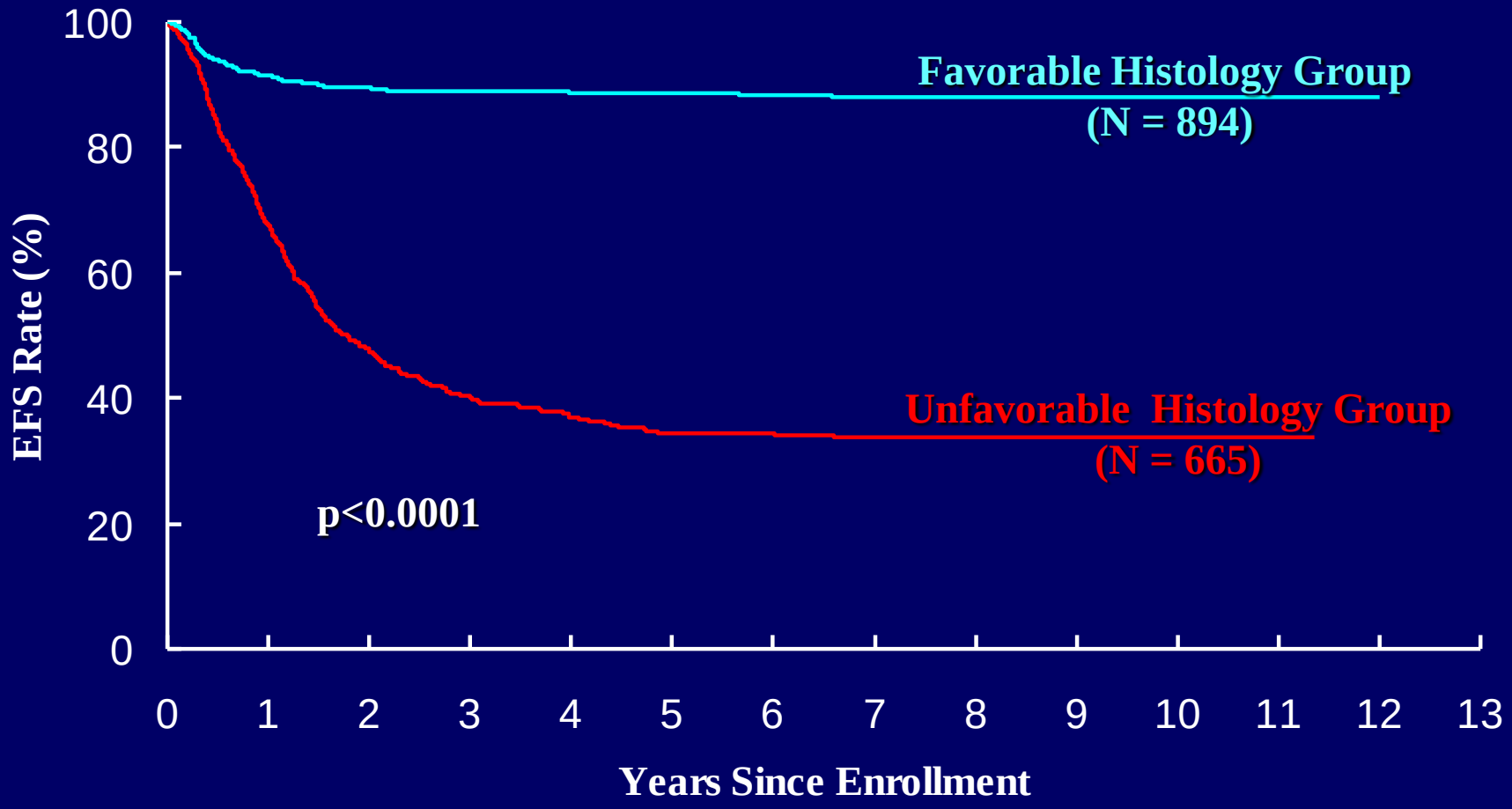


- **Ganglioneurblastoma, Nodular**
  （Composite, Schwannian stroma rich/stroma-dominant and stroma-poor）

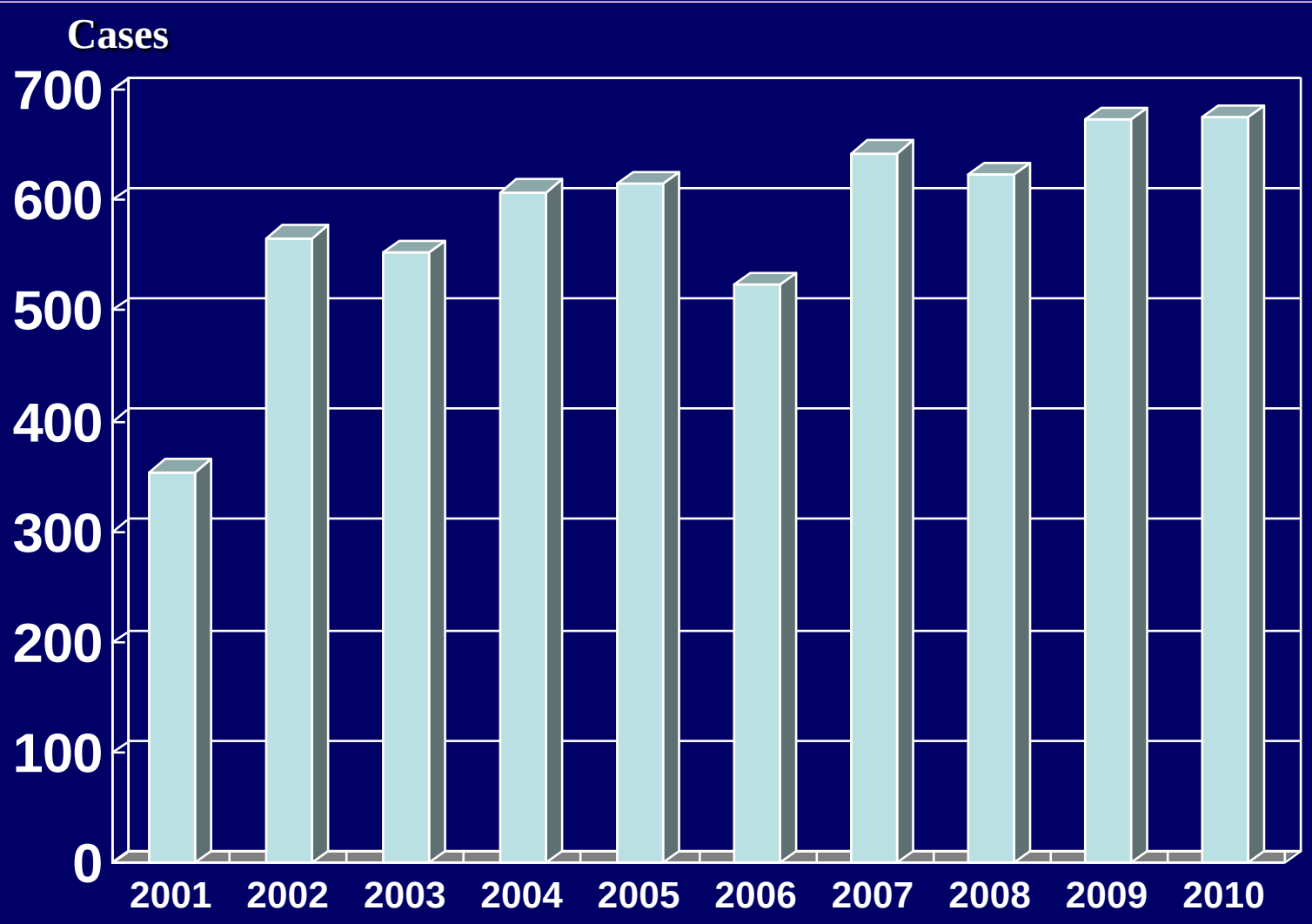
International Neuroblastoma Pathology Classification (INPC)

	Favorable Histology	Unfavorable Histology
Neuroblastoma 	Age-Appropriate Evaluation of: Grade of Differentiation MKI	
Ganglioneuroblastoma, Intermixed Ganglioneuroma  		
Ganglio-neuroblastoma, Nodular 	Neuroblastomatous Nodule Age-Appropriate Evaluation of: Grade of Differentiation MKI	

Event-free Survivals by INPC (n=1559)

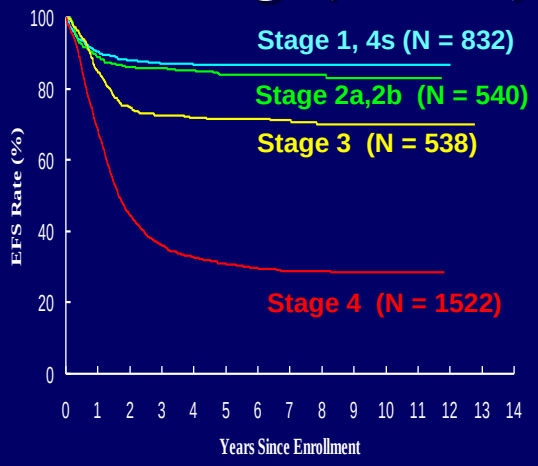


COG Neuroblastoma Cases
Neuroblastoma Pathology Reference Laboratory

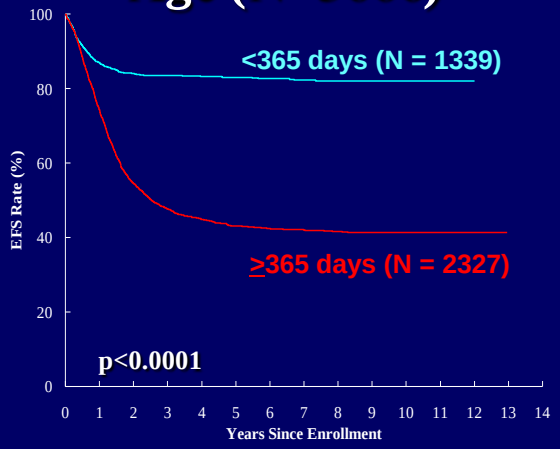


COG Neuroblastoma Biology Study
Prognostic Factors and EFS

INSS Stage (N=3432)

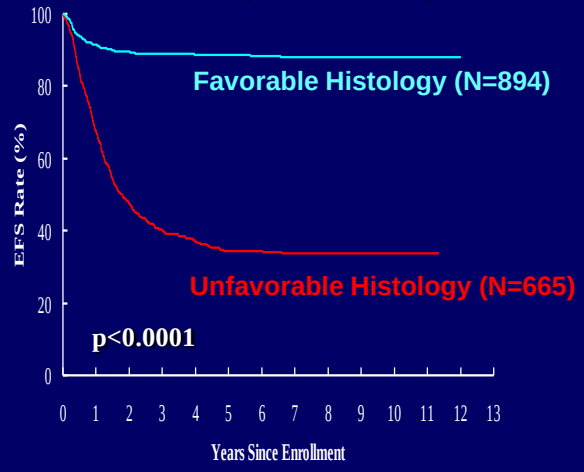


Age (N=3666)

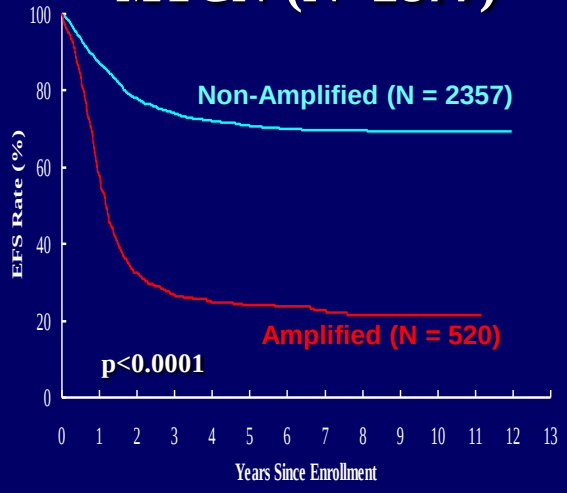


- Clinical Stage (INSS)
- Age at Diagnosis
- Histopathology (INPC)
- *MYCN* Oncogene Status
- DNA Index

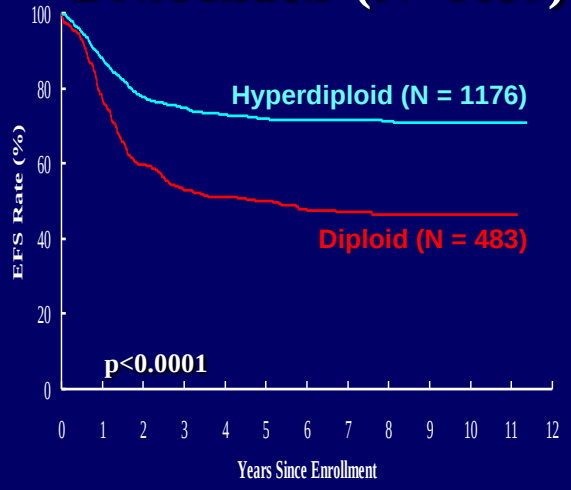
INPC (N=1559)



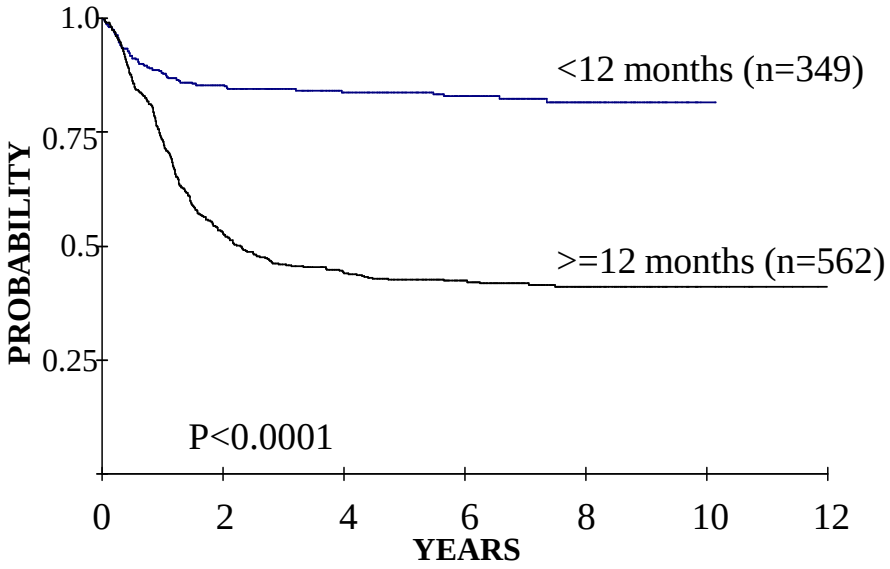
MYCN (N=2877)

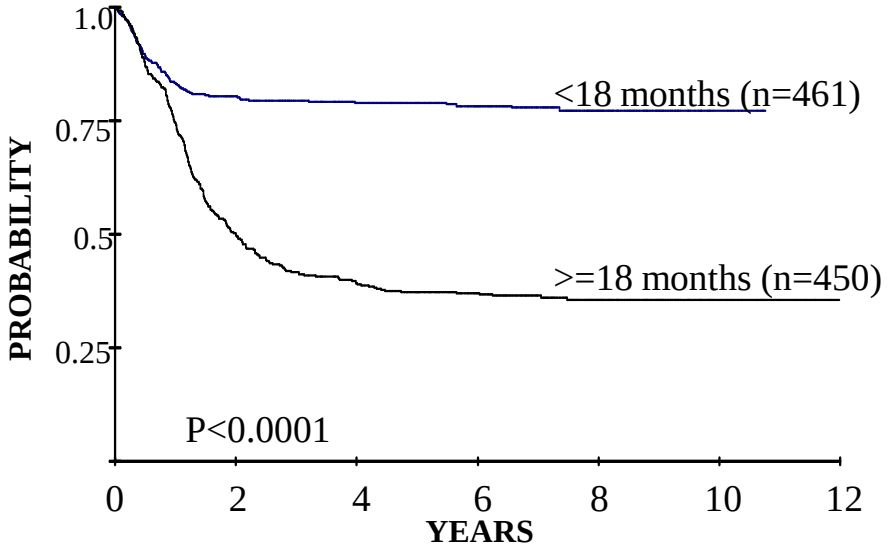


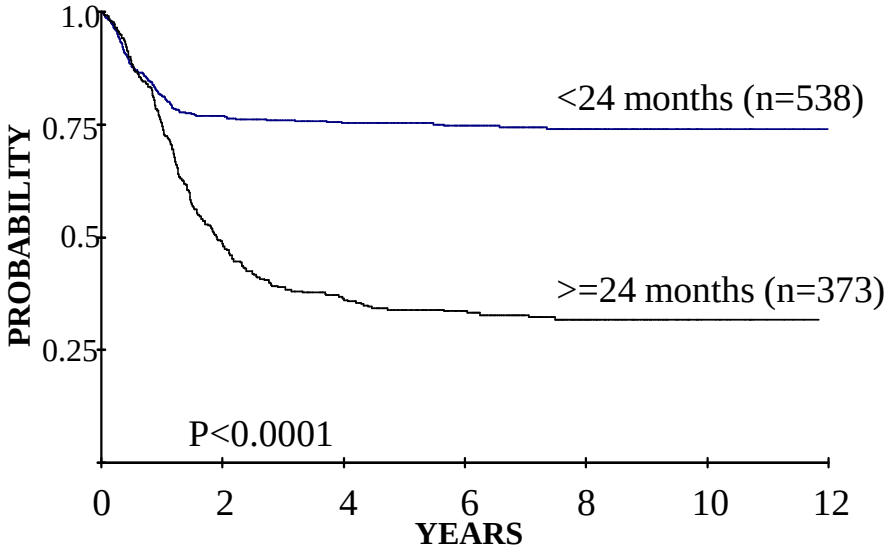
DNA Index (N=1659)



EFS by Different Age Cut-Off's





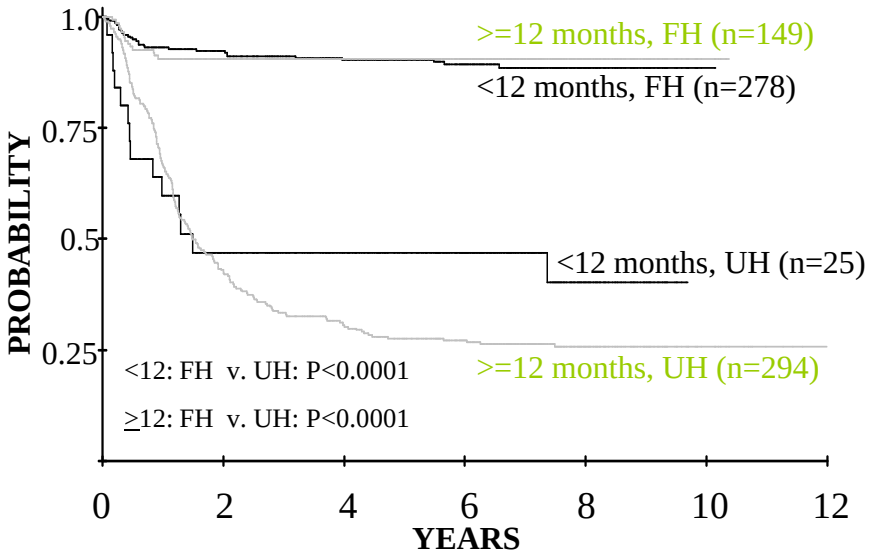


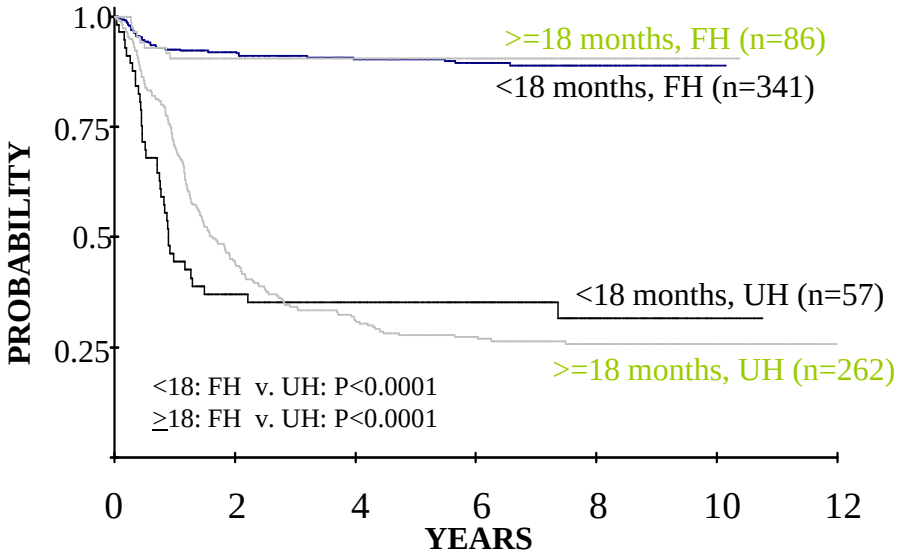
Prognostic effect of Age Factor:

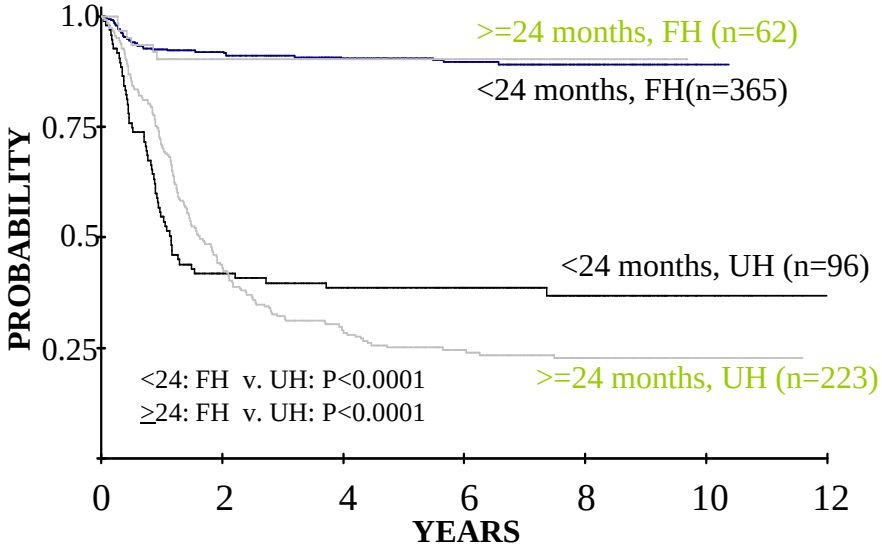
Continuous in Nature

Any choice between 15 and 19 months would work in risk stratification

Prognostic Effects (EFS) by INPC in Different Age Groups



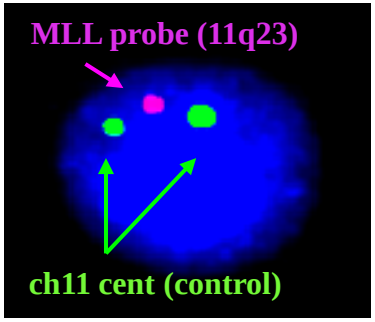




International Neuroblastoma Pathology Classification adds independent prognostic information beyond the prognostic contribution of age

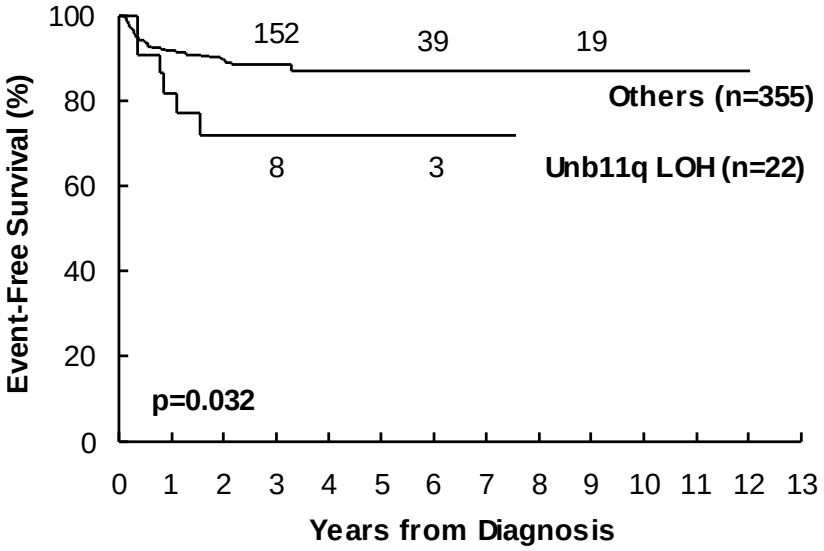
Sano,H et al.
Eur J Cancer 42:1113-9, 2006

1pLOH and 11qLOH Prognostic Effects

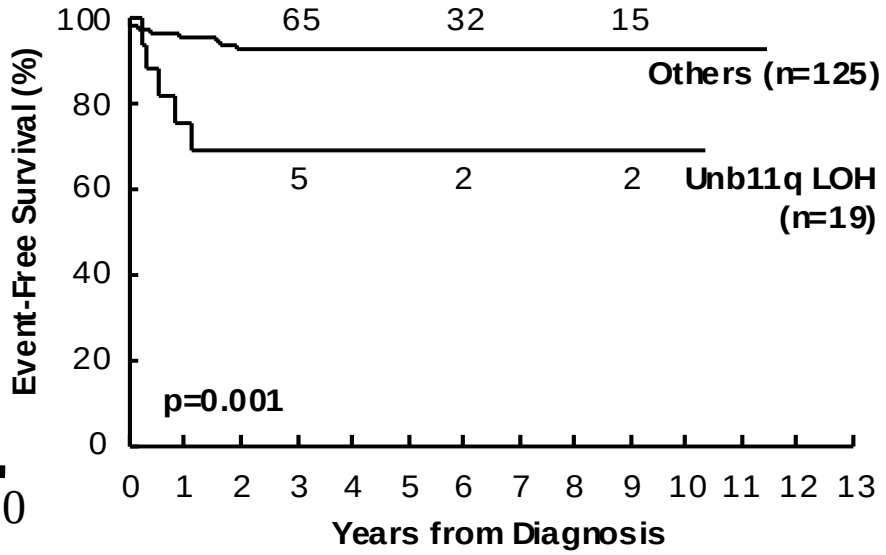


Maris et al: JCO, 2000

Low Risk



Intermediate Risk

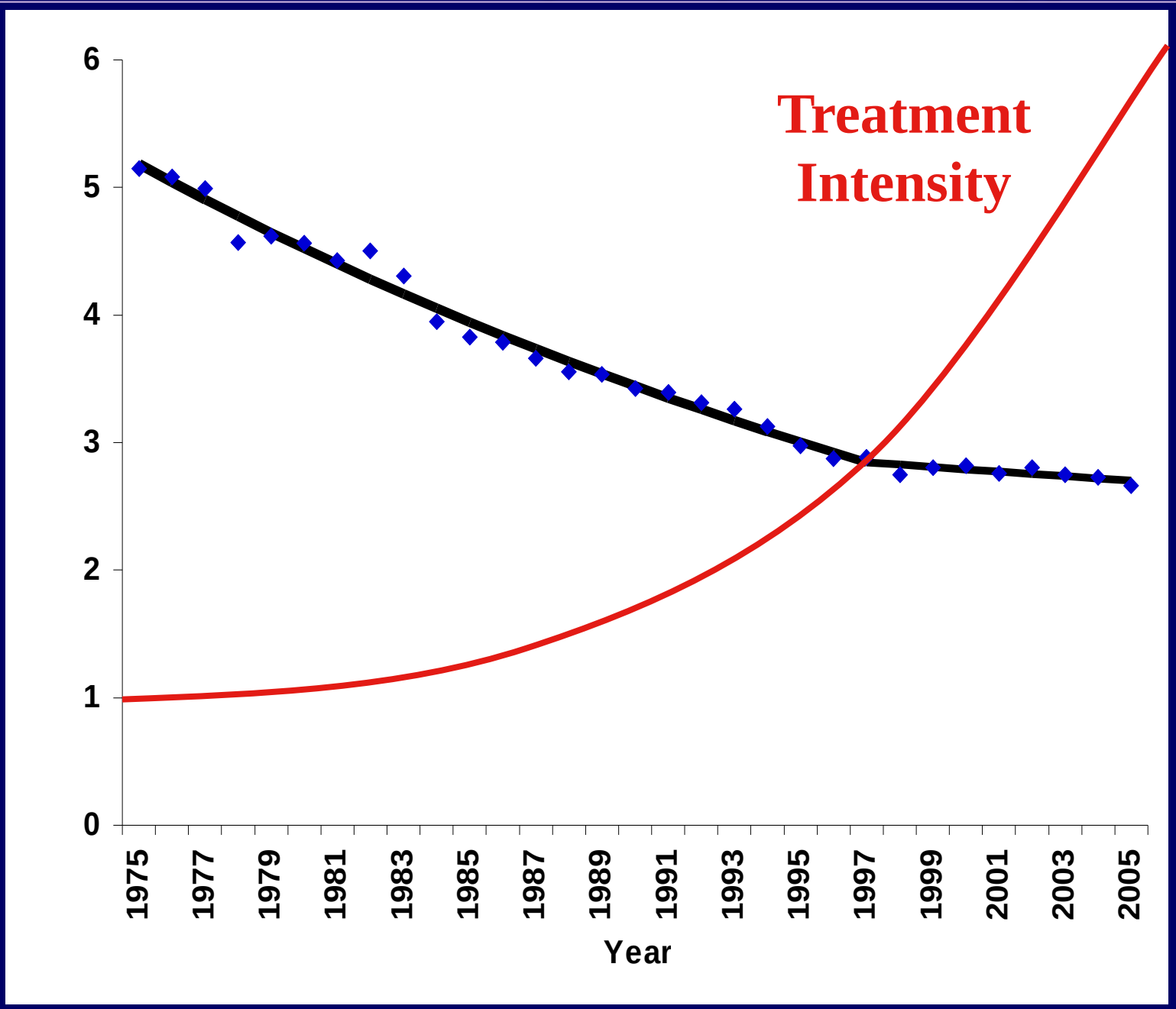


Attiyeh, NEJM: 353:2243, 2005

Peripheral Neuroblastic Tumors
Prognostic factors - COG

	Favorable	Unfavorable
Clinical Stage	Non-Advanced (stages 1,2,4S)	Advanced (stages 3,4)
Age at Diagnosis	<365 days	≥365 days
	<548 days	≥ 548 day
Histopathology Classification	Favorable Histology	Unfavorable Histology
Molecular Genetic Indicators		
MYCN Oncogene	Non-Amplified	Amplified
DNA index	Hyperploid	Diploid
l p LOH	No	Yes
unb11qLOH	No	Yes

Current challenge: Progress has stopped



Peripheral Neuroblastic Tumors
Current Status

Patient Stratification and Protocol Assignment

Low-Risk Group:

>95% Survival by Surgery Alone

Intermediate-Risk Group:

>90% Survival by Surgery/Biopsy plus Non-Intensive Chemotherapy

High-Risk Group：

Therapeutic Modalities, Almost Maximized:

Difficult to Expect Significant Improvement of Survivals

The cost of curing childhood cancer has risen

The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL ARTICLE

Chronic Health Conditions in Adult Survivors of Childhood Cancer

Kevin C. Oeffinger, M.D., Ann C. Mertens, Ph.D., Charles A. Sklar, M.D., Toana Kawashima, M.S., Melissa M. Hudson, M.D., Anna T. Meadows, M.D., Debra L. Friedman, M.D., Neyssa Marina, M.D., Wendy Hobbie, C.P.N.P., Nina S. Kadan-Lottick, M.D., Cindy L. Schwartz, M.D., Wendy Leisenring, Sc.D., and Leslie L. Robison, Ph.D., for the Childhood Cancer Survivor Study*

From Memorial Sloan-Kettering Cancer Center, New York (K.C.O., C.A.S.); the University of Minnesota, Minneapolis (A.C.M.); the Fred Hutchinson Cancer Research Center, Seattle (T.K., D.L.F., W.L.); St. Jude Children's Research Hospital, Memphis, TN (M.M.H., L.L.R.); Children's Hospital of Philadelphia, Philadelphia (A.T.M., W.H.); Stanford University Medical Center, Palo Alto, CA (N.M.); Yale University School of Medicine, New Haven, CT (N.S.K.-L.); and Brown Medical School, Providence, RI (C.L.S.). Address reprint requests to Dr. Oeffinger at the Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021, or at oeffingk@mskcc.org.

*Members of the Childhood Cancer Survivor Study are listed in the Appendix.

ABSTRACT

BACKGROUND

Only a few small studies have assessed the long-term morbidity that follows the treatment of childhood cancer. We determined the incidence and severity of chronic health conditions in adult survivors.

METHODS

The Childhood Cancer Survivor Study is a retrospective cohort study that tracks the health status of adults who received a diagnosis of childhood cancer between 1970 and 1986 and compares the results with those of siblings. We calculated the frequencies of chronic conditions in 10,397 survivors and 3034 siblings. A severity score (grades 1 through 4, ranging from mild to life-threatening or disabling) was assigned to each condition. Cox proportional-hazards models were used to estimate hazard ratios, reported as relative risks and 95% confidence intervals (CIs), for a chronic condition.

RESULTS

Survivors and siblings had mean ages of 26.6 years (range, 18.0 to 48.0) and 29.2

Among 10,397 survivors, 62.3% had at least one chronic condition; 27.5% had a severe or life-threatening condition (grade 3 or 4).

due to a chronic condition.

CONCLUSIONS

Survivors of childhood cancer have a high rate of illness owing to chronic health conditions.

New Challenges
Great Break-Through, Required

More Effective, Less Toxic

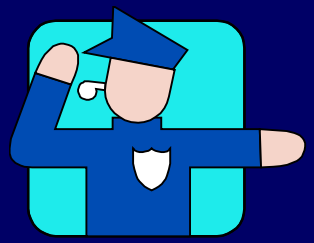
Understanding of Biology

Searching for Genomic/Molecular Targets

New Start!!!

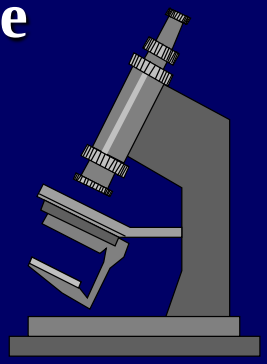
Era of Personalized Medicine

Role of Pathologists in Translational Research of Pediatric Tumors

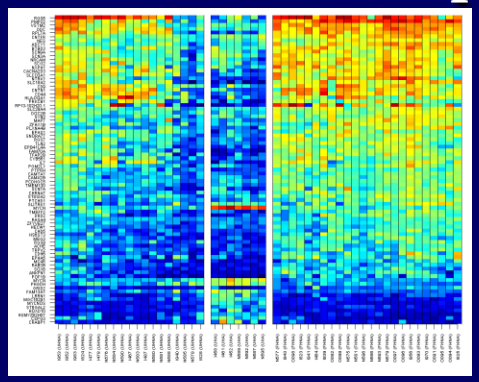


Clinical Bed-Side

Histopathology
Phenotype



Genomic/Molecular Properties



- ✓ Pathology Diagnosis
- ✓ Support – Clinical & Basic Research


Children's Hospital
LOS ANGELES

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International Neuroblastoma Pathology Committee:

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