Molecular Genetics of Paediatric Solid Tumours – aetiology, diagnosis, prognostic & therapeutic implications

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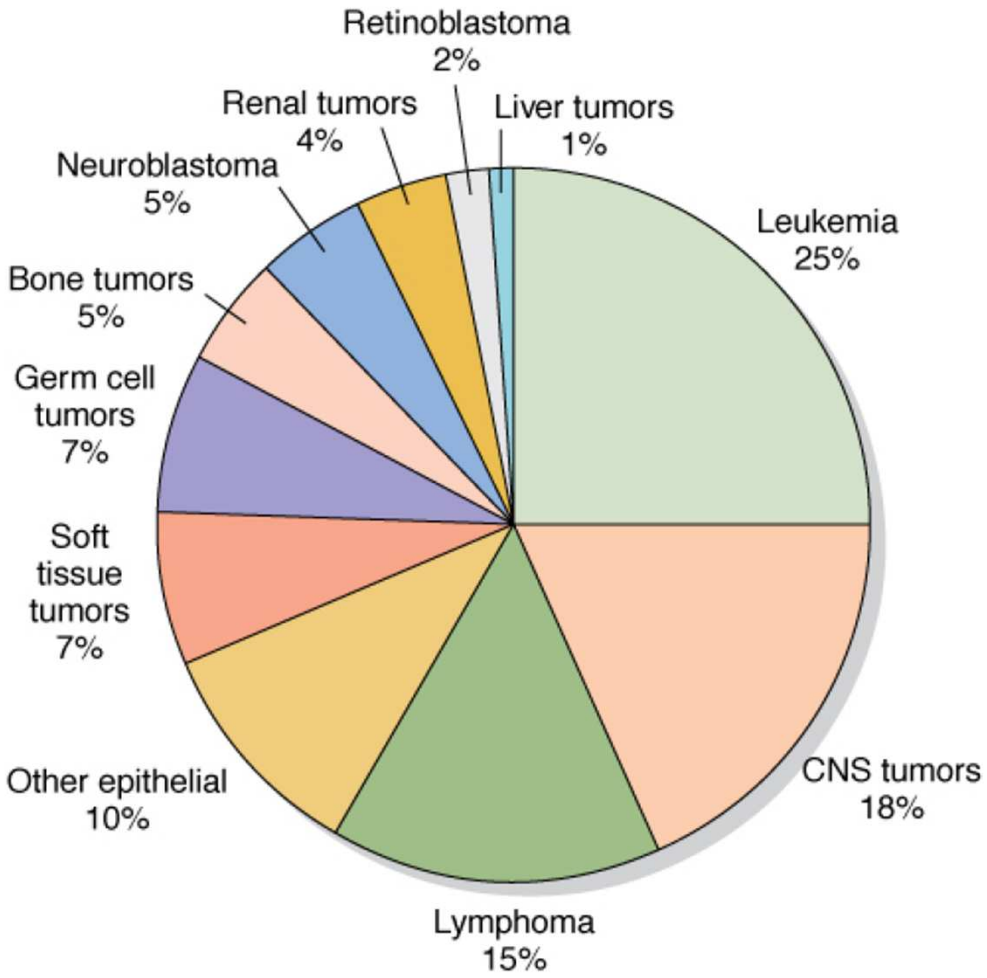
Distribution of childhood tumours



Developmental origins

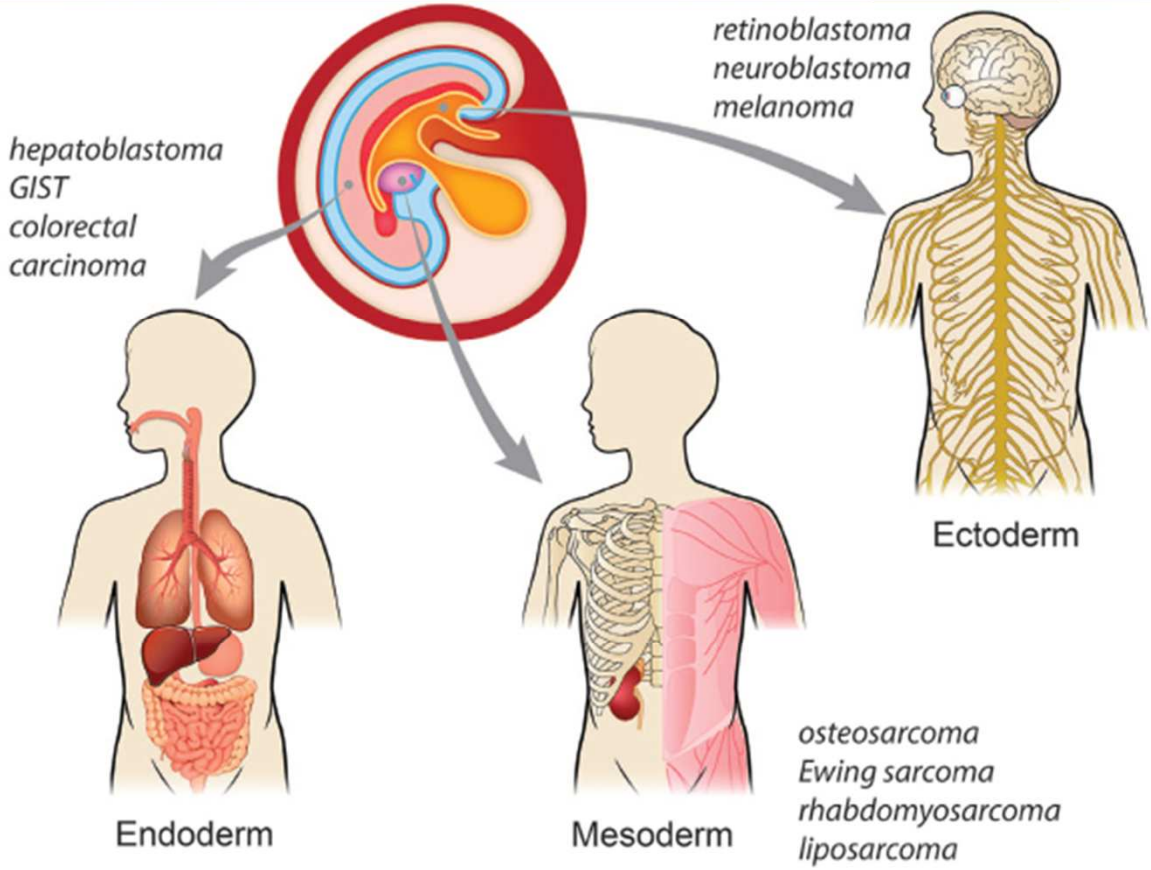
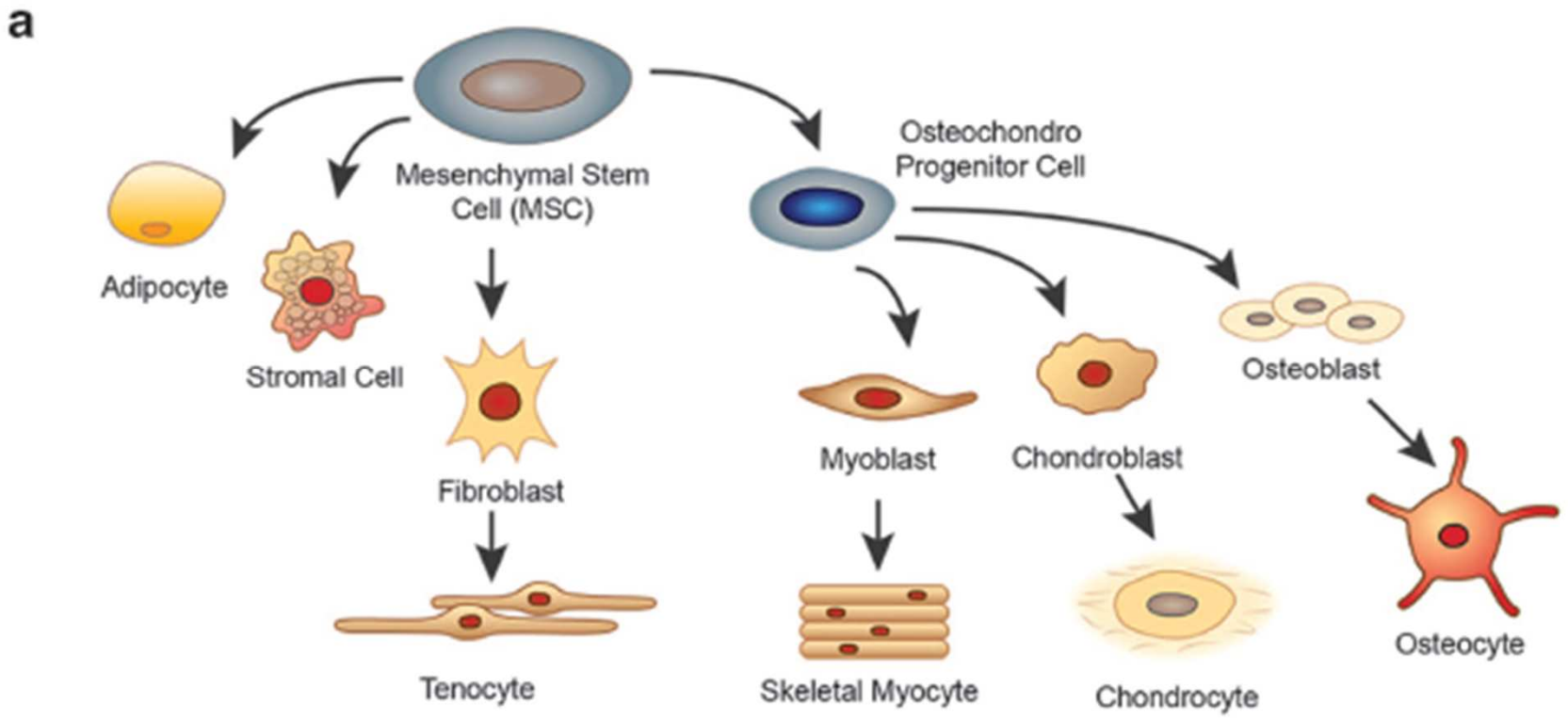
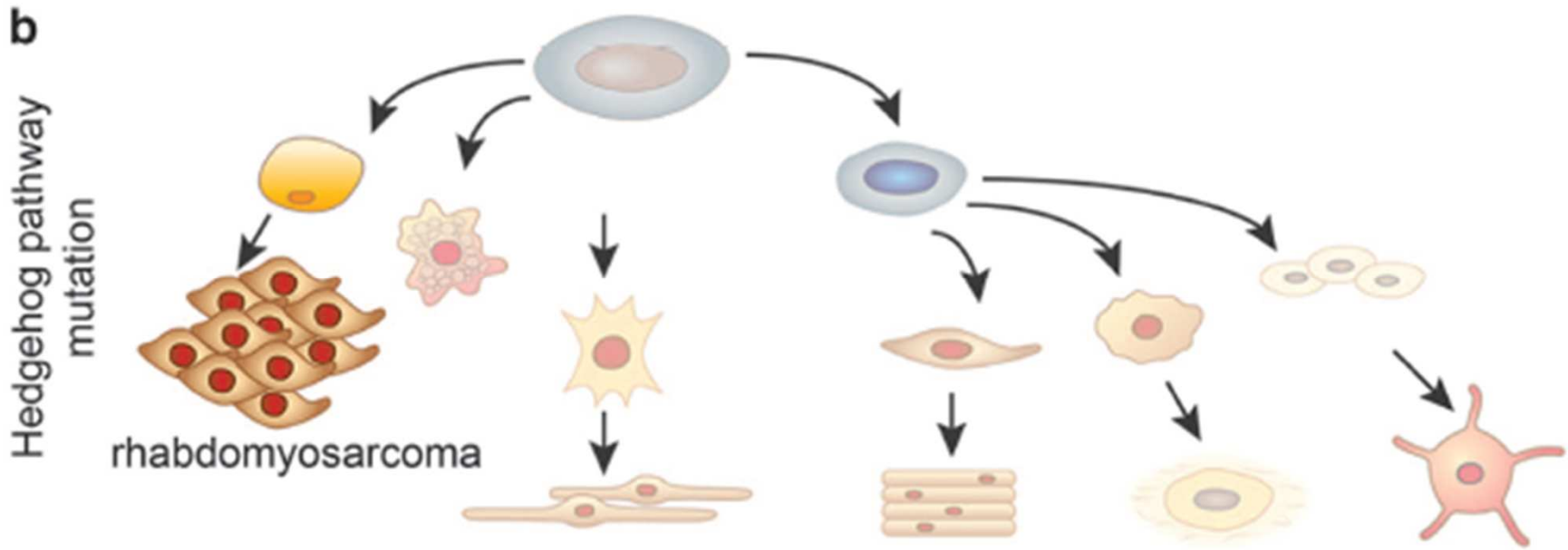


Figure 1. Pediatric solid tumors have diverse developmental origins. Unlike hematologic malignancies and brain tumors, pediatric solid tumors can arise from any of the three germ layers in the embryo. Representative examples are highlighted for each lineage.

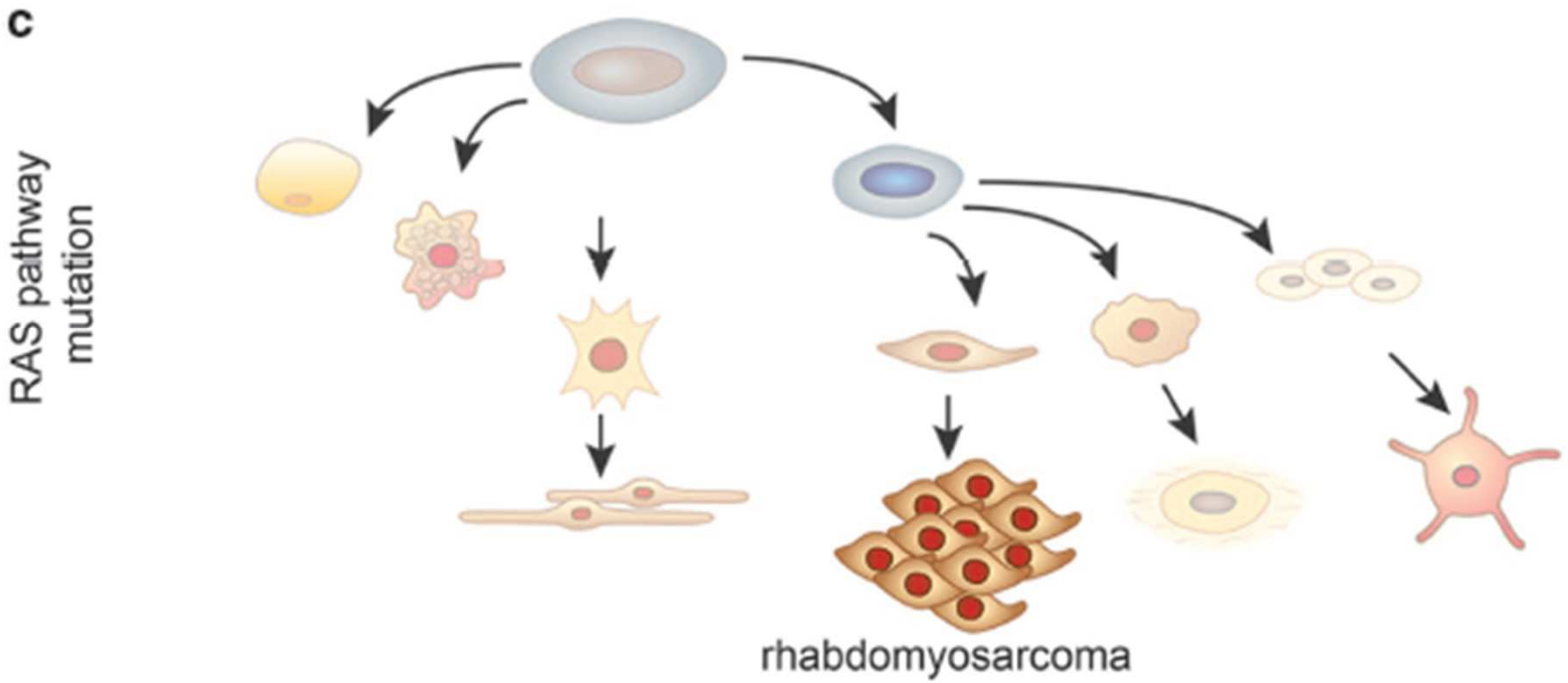
Simplified lineage diagram for several mesodermally derived lineages



Hedgehog pathway mutations



Ras Pathway mutations



Hereditary Syndromes Associated with Childhood Cancer Predisposition

Syndrome	OMIM Entry[a]	Major Tumor Types	Mode of Inheritance	Genes
Hereditary Gastrointestinal Malignancies				
Adenomatous polyposis of the colon	175100	Colon, thyroid, stomach, intestine, hepatoblastoma	Dominant	*APC*
Juvenile polyposis	174900	Gastrointestinal	Dominant	*SMAD4/DPC4*
Peutz-Jeghers syndrome	175200	Intestinal, ovarian, pancreatic	Dominant	*STK11*

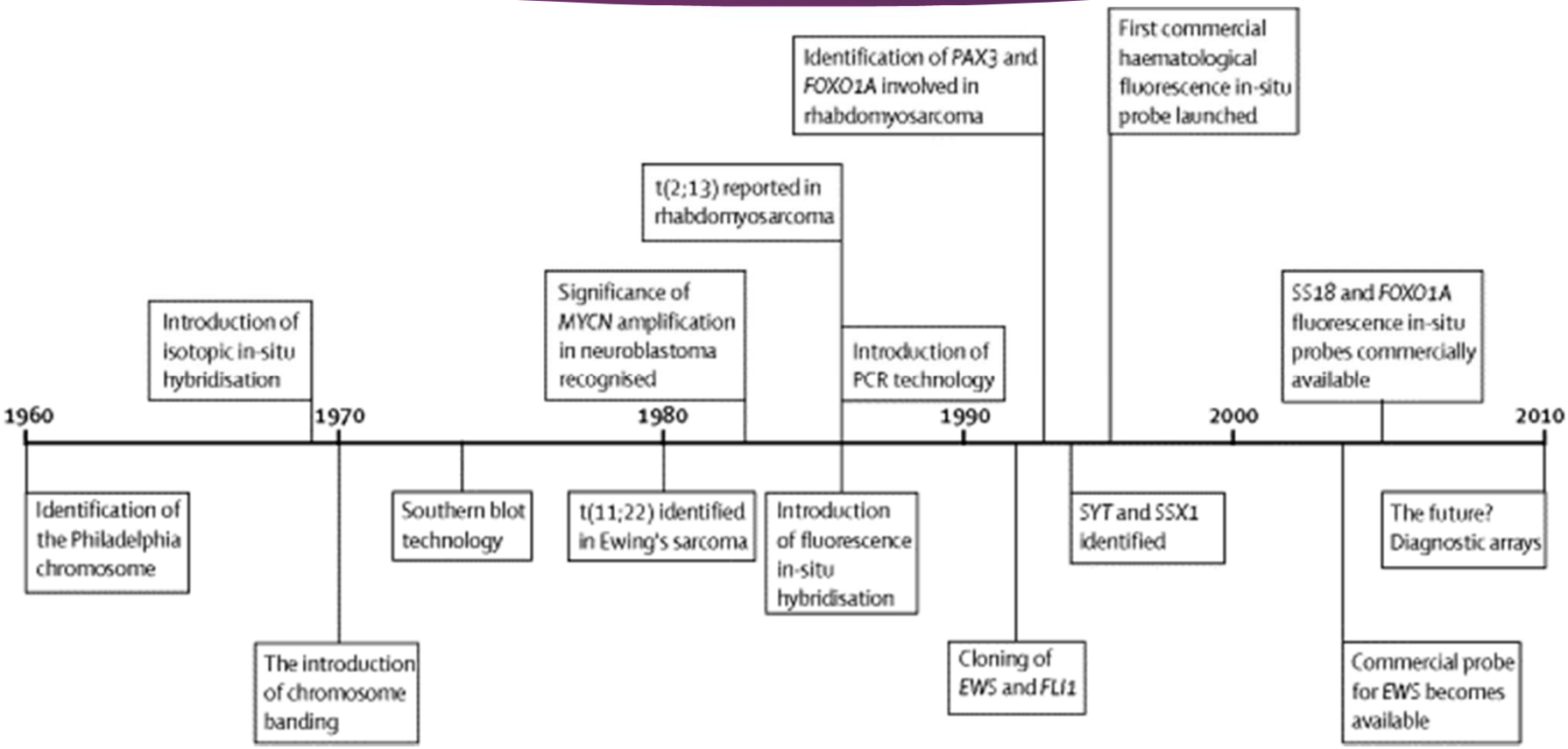
Genitourinary Predispositions…

Simpson-Golabi-Behmel syndrome	312870	Embryonal tumors, Wilms tumor	X-linked	*GPC3*
Von Hippel-Lindau syndrome	193300	Retinal and central nervous hemangioblastoma, pheochromocytoma, renal cell carcinoma	Dominant	*VHL*
Beckwith-Wiedemann syndrome	130650	Wilms tumor, hepatoblastoma, adrenal carcinoma, rhabdomyosarcoma	Dominant	*CDKN1C/NSD1*
Wilms tumor syndrome	194070	Wilms tumor	Dominant	*WT1*
WAGR syndrome	194072	Wilms tumor, gonadoblastoma	Dominant	*WT1*
Costello syndrome	218040	Neuroblastoma, rhabdomyosarcoma, bladder carcinoma	Dominant	*H-Ras*

Central Nervous System Predisposition syndromes

Retinoblastoma	180200	Retinoblastoma, osteosarcoma	Dominant	*RB1*
Rhabdoid predisposition syndrome	601607	Rhabdoid tumor, medulloblastoma, choroid plexus tumor		*SNF5/INI1*
Medulloblastoma predisposition	607035	Medulloblastoma	Dominant	*SUFU*

Timeline of technologies for identification of gene rearrangements



Boo Messahel , Ruth Nash , Iona Jeffrey , Kathy Pritchard-Jones , Sandra Hing

Common Cytogenetic Rearrangements in Solid Tumors of Childhood

Solid Tumor	Cytogenetic Rearrangement	Genes[a]
Ewing sarcoma	t(11;22) (q24;q12), +8	*EWS(22) FLi-1(11)*
Neuroblastoma	del1p32–36, DMs, HSRs, +17q21-qter	*N-MYC*
Retinoblastoma	del13q14	*Rb*
Wilms tumor	del11p13, t(3;17)	*WT1*
Synovial sarcoma	t(X;11) (p11;q11)	*SSX(X) SYT(18)*
Osteogenic sarcoma	del13q14	*?*
Rhabdomyosarcoma	t(2;13) (q37;q14), t(2;11),3p-,11p-	*PAX3(2) FOXO1(13)*
Peripheral neuroepithelioma	t(11;22) (q24;q12), +8	*EWS(22) FLi-1(11)*
Astrocytoma	i(17q)	*?*
Meningioma	delq22, -22	*MN1, NF2, ?*
Atypical teratoid/rhabdoid tumor	delq22.11	*SNF 5*
Germ cell tumor	i(12p)	

Table 1. Chromosomal Regions and Genes Proven or Speculated Involved in Neuroblastoma Tumorigenesis

Chromosomal Locus	Gene Name	Gene Type/Function*
1p36.2-p36.3		Tumor suppressor
1p13	*NGF*	Neurotrophin; ligand for *NTRK1*
1q23-q31	*NTRK1 (TRK-A)*	Receptor tyrosine kinase
2p12-13	*MAD*	May regulate *MYCN*
2p24.1	*MYCN*	Proto-oncogene
2p24	*DDX1*	RNA helicase/oncogene
3p		Tumor suppressor
4p		Tumor suppressor
7q21	*PGY1 (MDR1)*	Multidrug resistance
9q22.1	*NTRK2 (TRK-B)*	Receptor tyrosine kinase
11p13	*CD44*	Integrin/metastasis suppression
11p13	*BDNF*	Neurotrophin; ligand for *NTRK1*
11q23		Tumor suppressor
12p13	*NTF3 (NT-3)*	Neurotrophin; ligand for *NTRK3*
14q23	*MAX*	Regulates *MYCN*
14q23-qter		Tumor suppressor
15q24-q25	*NTRK3 (TRK-C)*	Receptor tyrosine kinase
16p13.1	*MRP*	Multidrug resistance
17q22	*NME1*	Nucleoside kinase/metastasis suppression
17q23-qter		Oncogene
18q21.1	*DCC*	Tumor suppressor
18q21.3	*BCL2*	Apoptosis suppression
19	*NTF4 (NT-4)*	Neurotrophin; ligand for *NTRK2*

Nmyc interactions

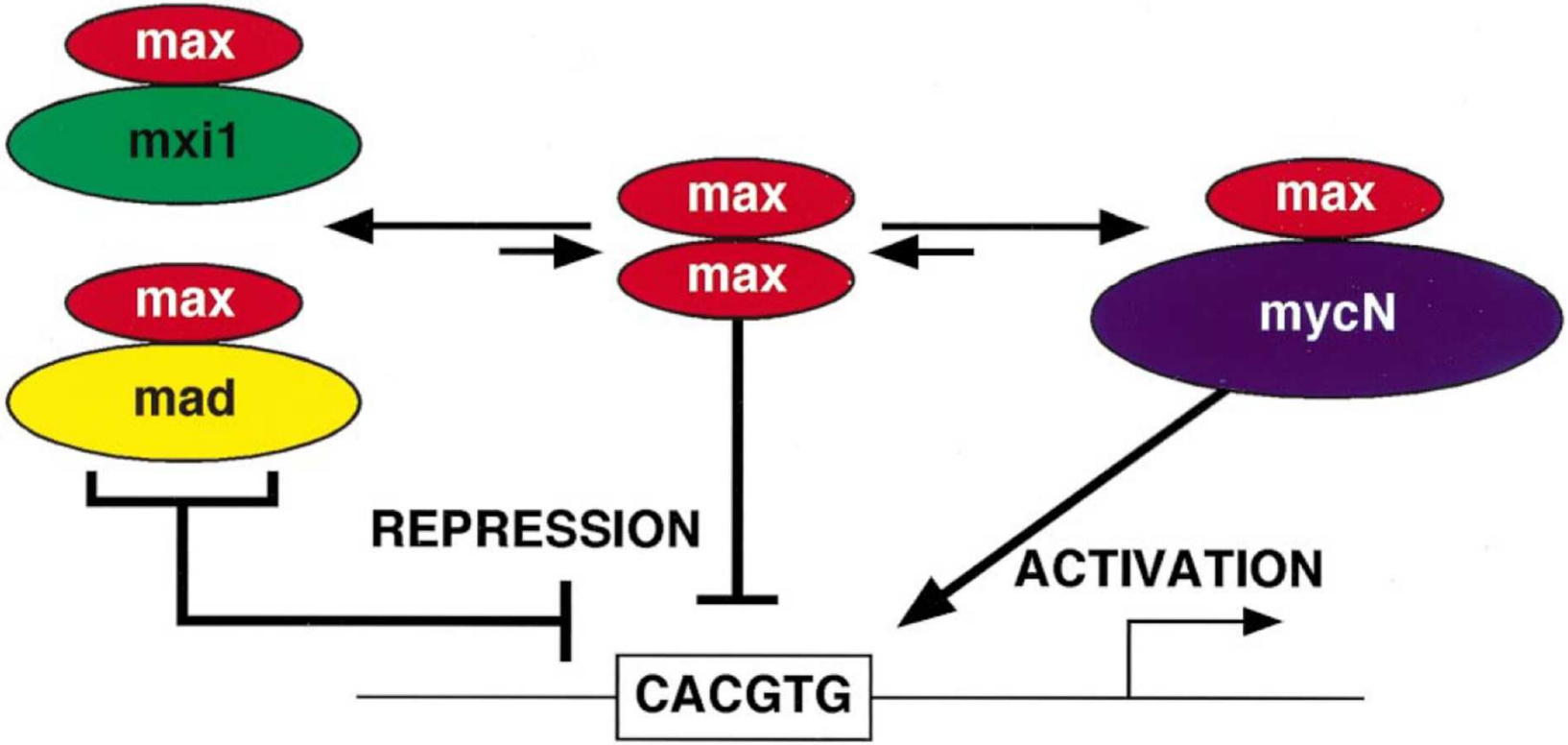


Fig 1. Model of MycN interactions with other proteins. Genomic amplification of *MYCN* leads to increased MycN nuclear protein. Heterodimerization of MycN and Max is favored in the presence of excess MycN (arrows). Transcriptional activation of target genes occurs after interaction of the MycN/Max complex with the promoter region through an E-box motif (CACGTG). In the absence of MycN, it is postulated that the transcriptional repressing protein complexes Max/Max, Max/Mad, and Max/Mxi1 predominate and function to repress transcription.

CGH in neuroblastoma

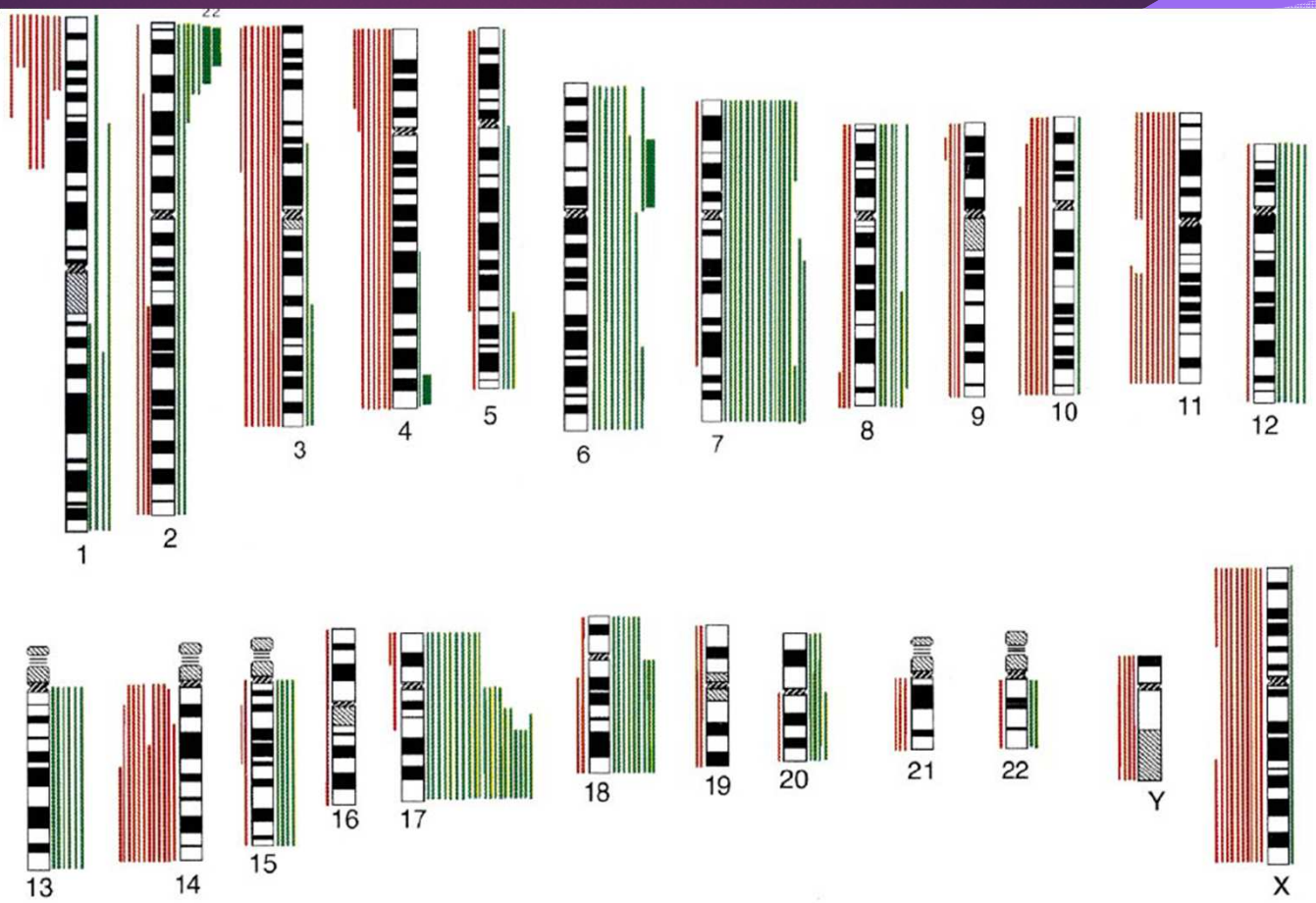
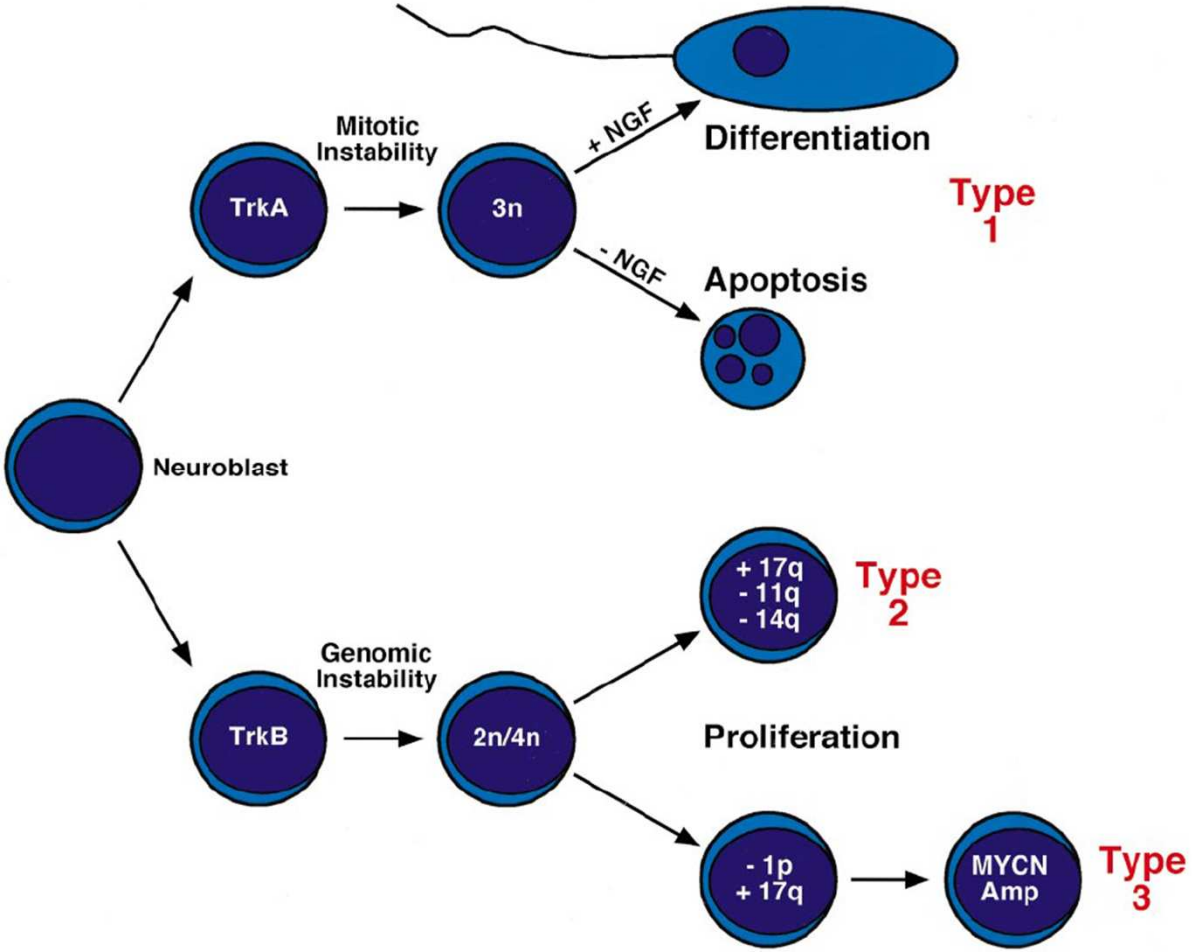
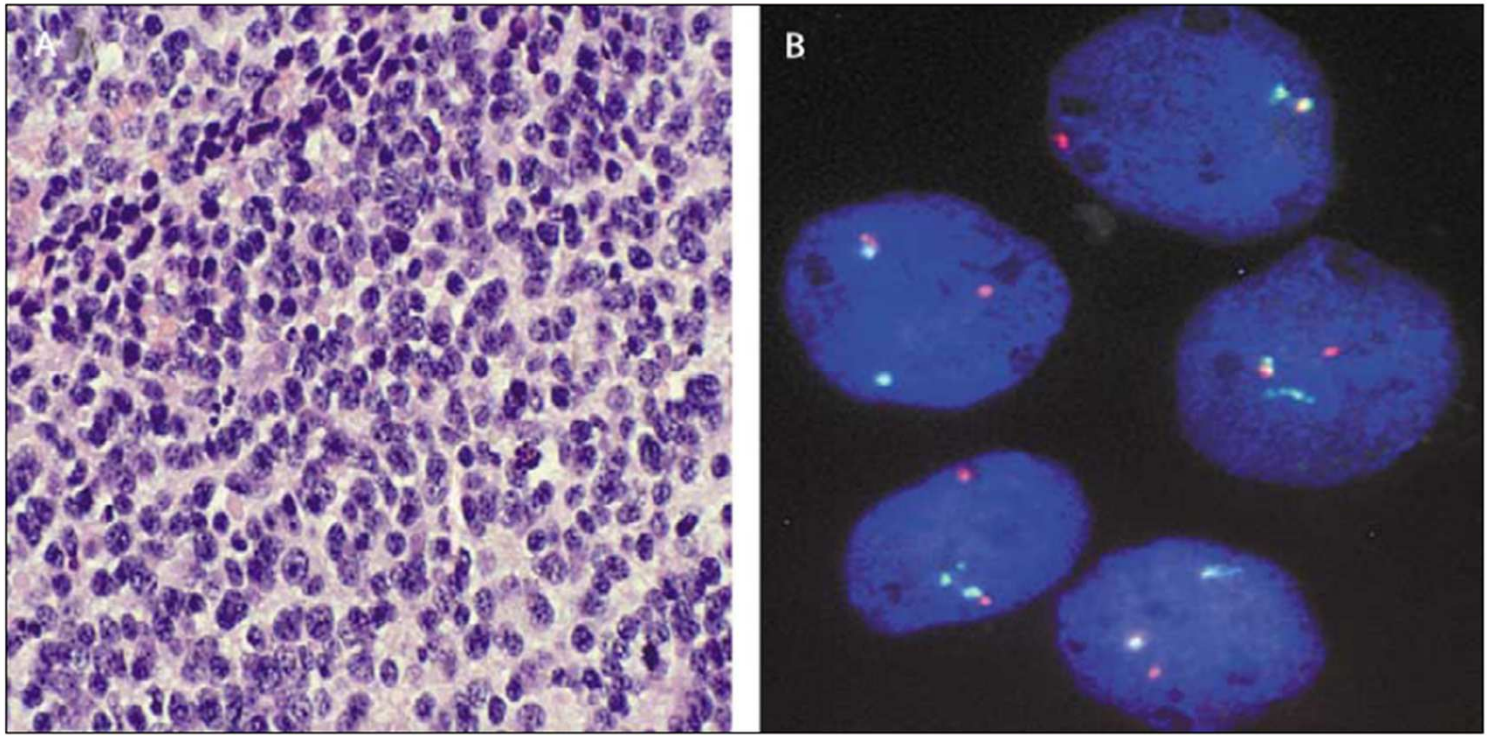


Fig 2. Comparative genomic hybridization of primary neuroblastomas. Summary of the chromosomal gains (green) and losses (red) present in a representative panel of 29 primary tumors.[37] Thick bars represent high level genomic amplification and each thick bar at the *MYCN* locus (2p24) represents the pattern of gain in two distinct cases.

Hypothetical models of neuroblastomas



The small round blue-cell tumours of childhood



Dual-colour fluorescent in-situ hybridisation using two probes spanning the *EWS* gene locus on chromosome 22 . A split in the green and red signal indicates rearrangement of *EWS*. This shows high-level amplification

Schematic diagram of normal *EWS* on chromosome 22 (green exons), normal *FLI1* on chromosome 11 (blue)

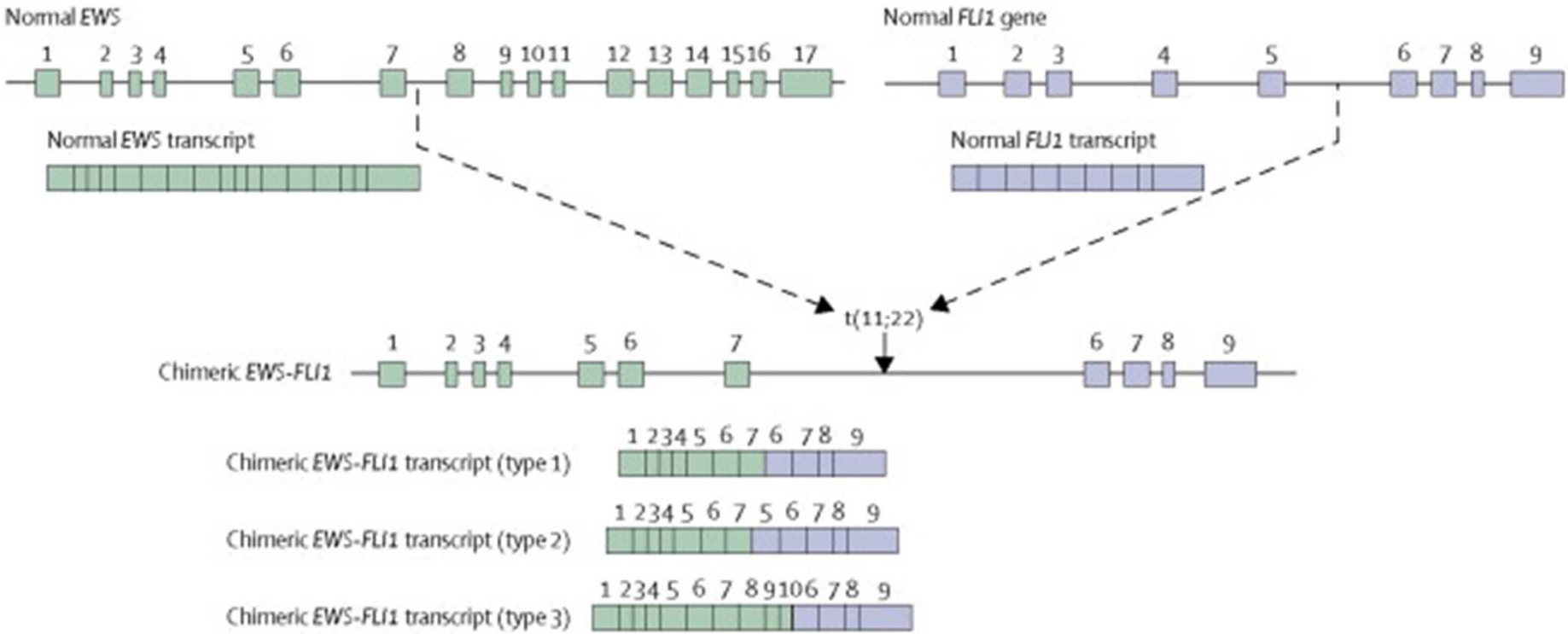


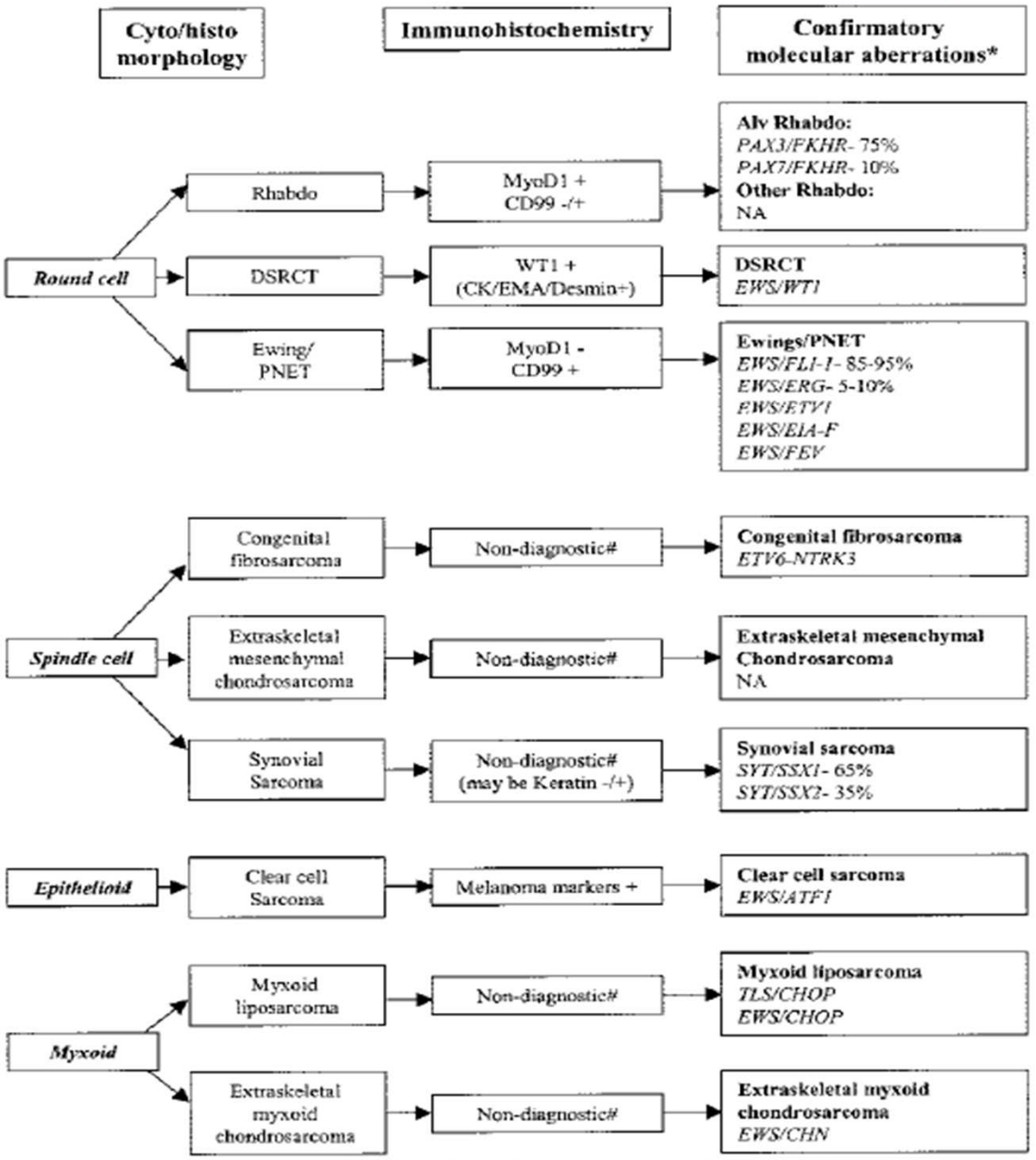
Table 1. Summary of Genetic/Molecular Lesions in Pediatric Soft Tissue Tumors

Type of tumor	Chromosomal abnormality	Gene involved or fusion gene	Prevalence	FISH	RT-PCR*	Prognosis†
Rhabdomyosarcoma						
Botryoid	NA	NA	NA	NA	NA	Good
Spindle cell	NA	NA	NA	NA	NA	Good
Embryonal	Gains of 2, 7, 8, 12, 13; losses of 1, 6, 9, 14, and 17[50]	*IGF2, GOK, PTCH TP53*	NA	NA	NA	Good
Alveolar	t(2;13)(q35;q14);	*PAX3-FKHR*	75%	Ref.67	Ref.13	Poor‡
	t(1;13)(p36;q14)	*PAX7-FKHR*	10%			
Undifferentiated	NA	NA	NA	NA	NA	Poor
Non-rhabdomyosarcoma-EWS family						
EWS/PNET	t(11;22)(q24;q12)	*EWS-FLI-1*	85–95%	Ref.108	Ref.109	Good with type I fusion transcripts‡
	t(21;22)(q22;q12)	*EWS-ERG*	5–10%			
	t(7;22)(p22;q12)	*EWS-ETV1*	<1%			
	t(17;22)(q21;q12)	*EWS-E1AF*	<1%			
	t(2;22)(q33;q12)	*EWS-FEV*	<1%			
	Inversion of 22q	*EWS-ZSG*	<1%			
DSRCT	t(11;22)(p13;q12)	*EWS-WT1*	>95%	NA	Ref.24	Poor
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS-ATF1*	90%	Ref.110	Ref.37	Poor
Extraskeletal myxoid chondrosarcoma§	t(9;22)(q22–23;q11–12)	*EWS-TEC (CHN)*	75%	Ref.111	Ref.43, 96	Good
	t(9;15)(q22;q21)	*TCF12-TEC*	NA		Ref.43, 112	
	t(9;17)(q22;q11)	*TAF2N-TEC*	25%		Ref.113	
Extraskeletal mesenchymal chondrosarcoma	der(13;21)(q10;q10)	NA	NA	Ref.114	NA	Poor

Algorithm highlighting diagnostic contribution by molecular genetic study

Chang and Shidham

JMD August 2003, Vol. 5, No. 3



*Requires proper clinical and morphological correlation. Performed with molecular diagnostic techniques using frozen tissue (preferred), cytology smears, or formalin-fixed paraffin-embedded tissue.

#No diagnostic immunohistochemistry markers available for aiding in diagnosis.

The phenotype of the tumour varies with the fusion partner

Chang and Shidham

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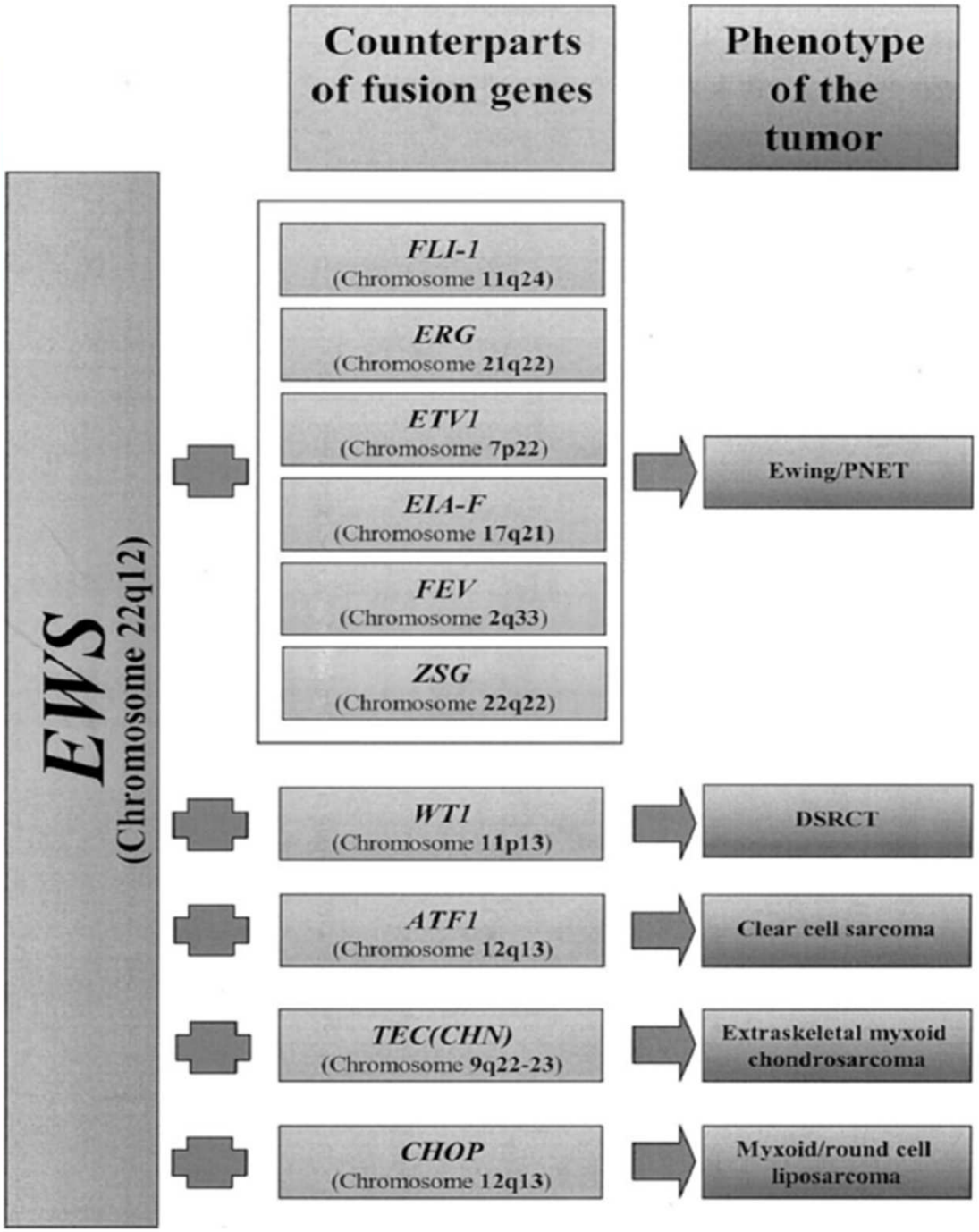
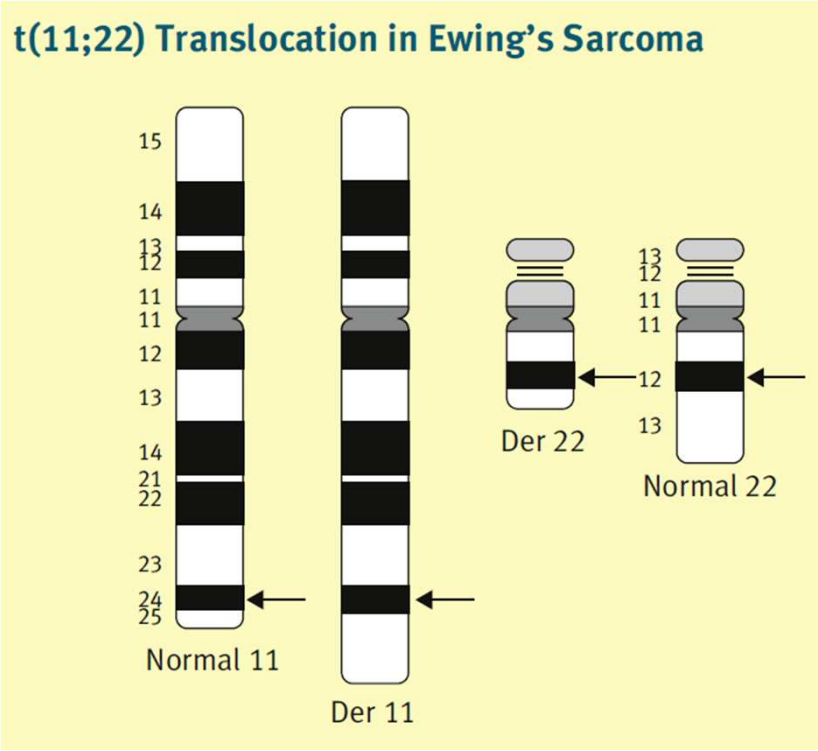
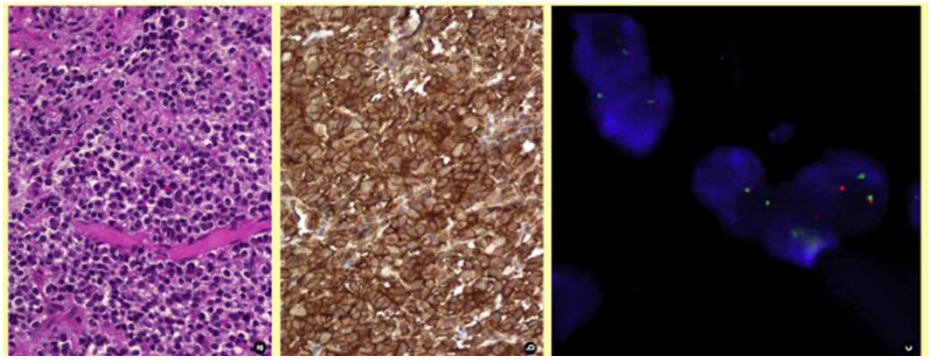


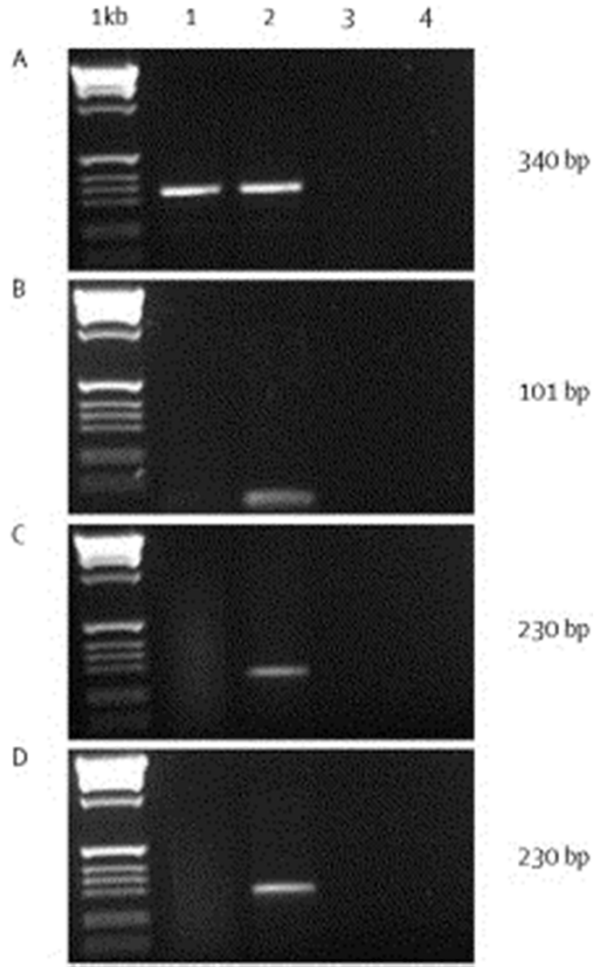
Ewing sarcoma



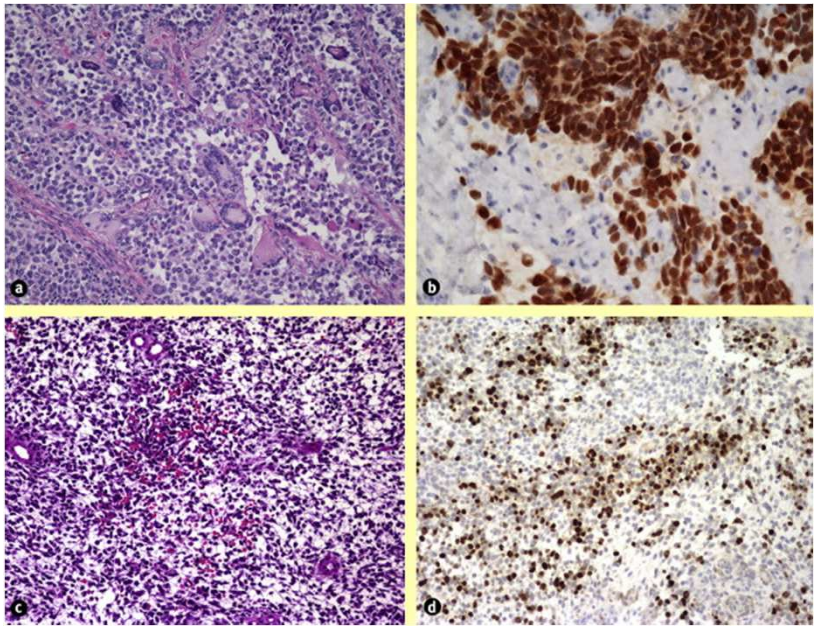


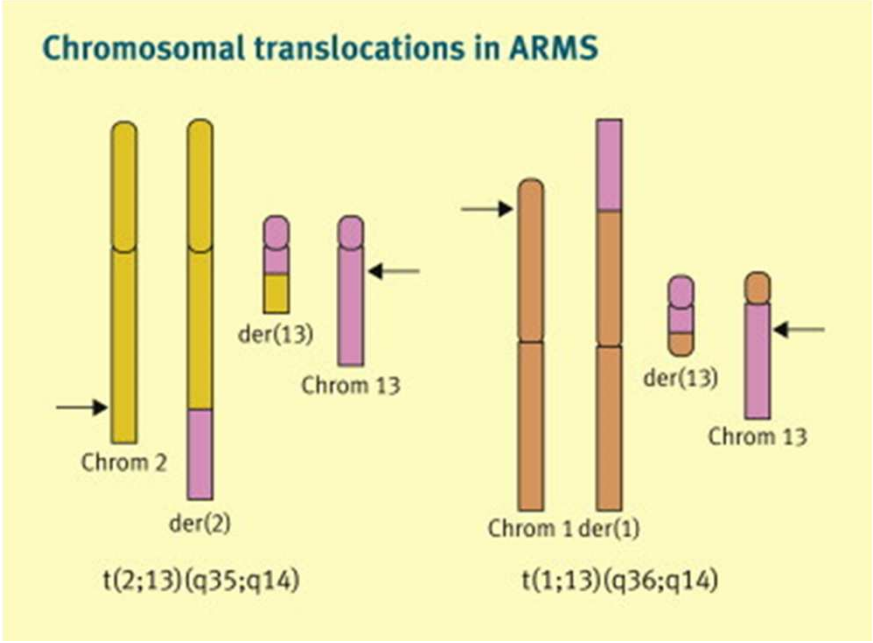
a Primitive cells arranged in formless sheets
b CD99 strong perimembranous staining
c Dual colour FISH with break-apart EWS probe

Figure 4 Differential diagnosis of a soft-tissue sarcoma by reverse transcriptase-PCR. Amplification of the expected size transcript for *EWS-FLI1* (A), *SYT-SSX* (B). *PAX3-FKHR* (C), a...



Alveolar rhabdomyosarcoma. Note the discohesive nature of the primitive rhabdomyoblasts lining fibrovascular septae. When present, giant cells are a helpful feature in distinguishing ARMS from embryonal rhabdomyosarcoma





Bruce R. Pawel **Recent advances in the molecular diagnosis of paediatric soft tissue sarcomas** Diagnostic Histopathology, Volume 17, Issue 1, 2011, 25 - 35

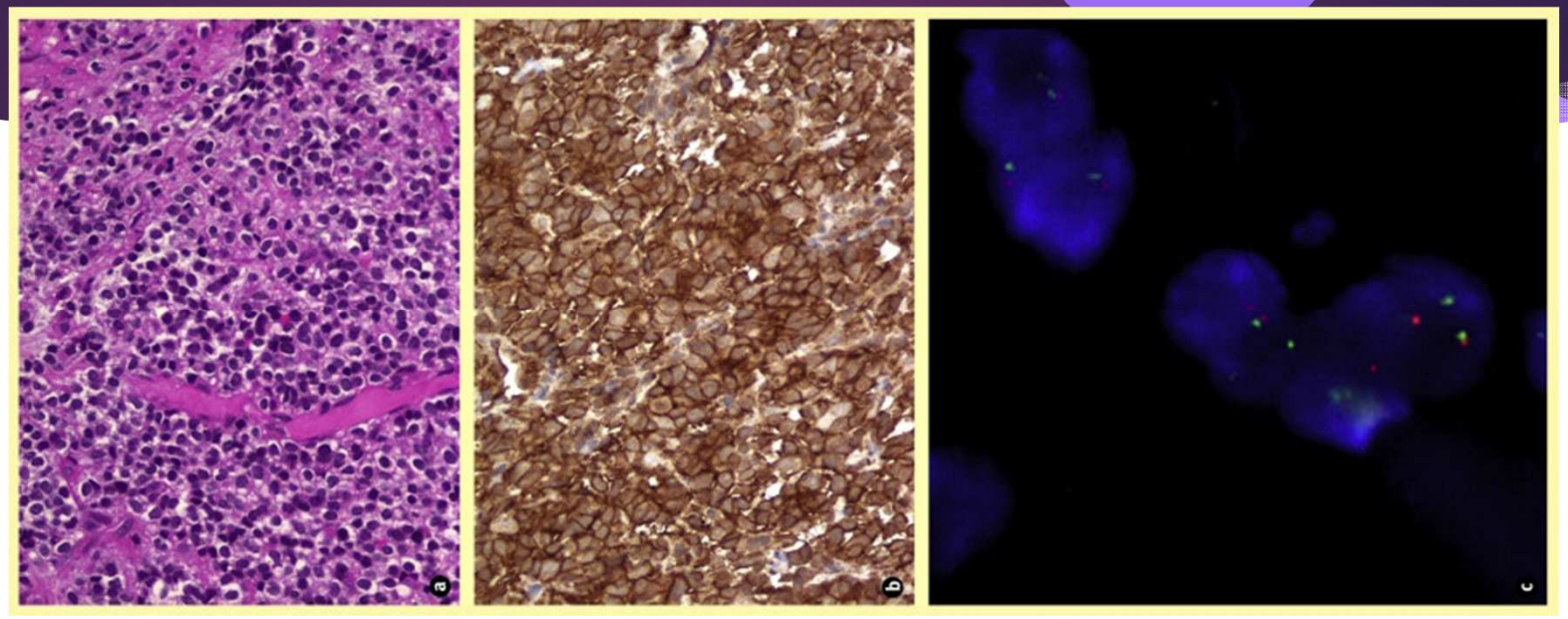


Figure 3 a Ewing sarcoma. Primitive cells arranged in formless sheets (haematoxylin & eosin). b CD99 strong perimembranous staining in Ewing sarcoma. c Dual colour FISH with break-apart EWS probe, performed on interphase nuclei in a case of Ewing...

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Recent advances in the molecular diagnosis of paediatric soft tissue sarcomas

Diagnostic Histopathology, Volume 17, Issue 1, 2011, 25 - 35

http://dx.doi.org/10.1016/j.mpdhp.2010.10.003

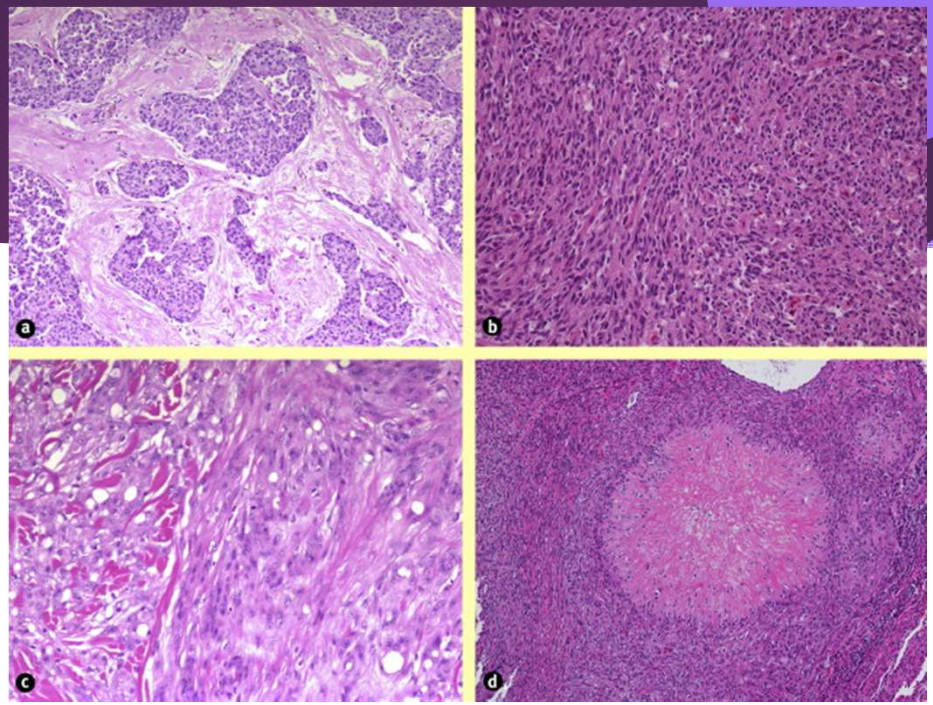

Figure 5 a Desmoplastic small round cell tumour. b Infantile fibrosarcoma. c Clear cell sarcoma. d Low-grade fibromyxoid sarcoma, with large fibrous rosette (all haematoxylin & eosin).

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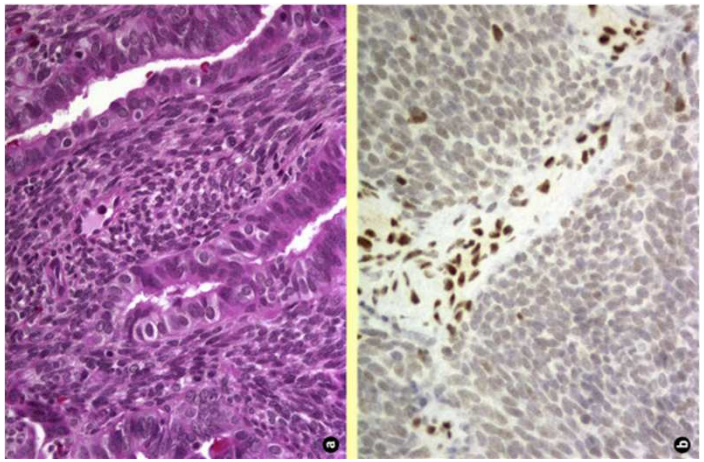

Figure 6 Synovial sarcoma. a Biphasic pattern with glands and spindled cells (haematoxylin & eosin). b Immunohistochemistry for INI1 in synovial sarcoma, with endothelial cells serving as positive internal controls. Tumour cells demonstrate weak to...

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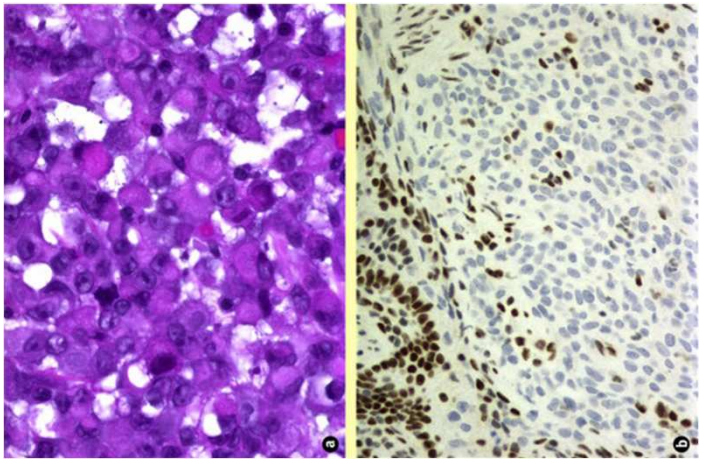

Figure 7 Malignant rhabdoid tumour. a Typical rhabdoid cells with eccentric eosinophilic globular cytoplasmic inclusions, vesicular nuclei and prominent nucleoli (haematoxylin & eosin). b Immunohistochemistry for INI1, showing absence of nuclear st...

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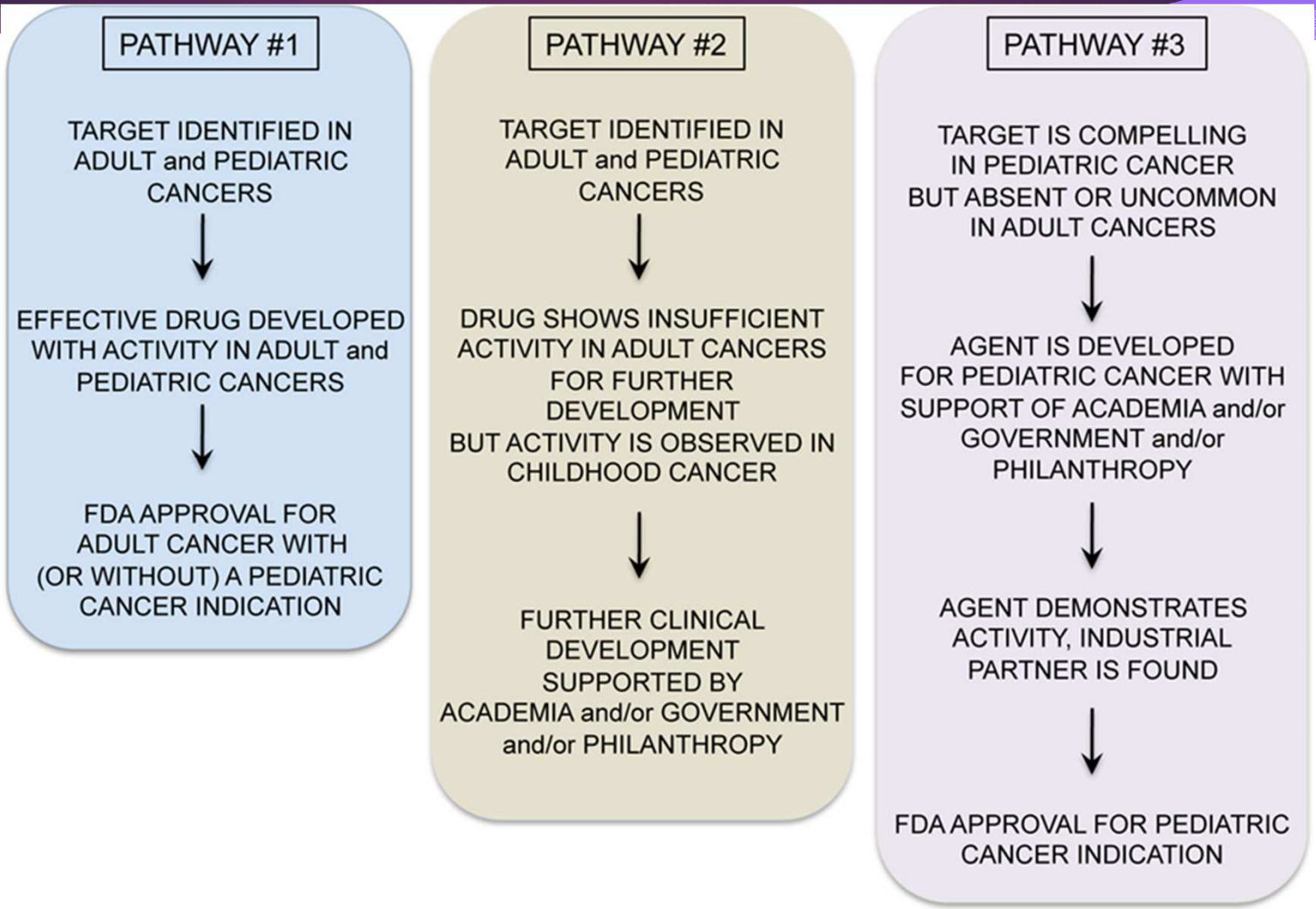
Using molecular tools to prognosticate

	Type 1	Type 2A	Type 2B
MYCN	Normal	Normal	Amplified
DNA ploidy	Hyperdiploid or near triploid	Near diploid or near tetraploid	Near diploid or near tetraploid
17q gain	Rare	Common	Common
11q, 14q LOH	Rare	Common	Rare
1p LOH	Rare	Rare	Common
NTRK1 expression	High	Low or absent	Low or absent
NTRK2 expression	Truncated	Low or absent	High (full length)
NTRK3 expression	High	Low or absent	Low or absent
Age	<1 year*	>1 year*	1–5 years*
INSS	1, 2–4S*	3, 4*	3, 4
5-year survival (%)	95	40–50	25

LOH=loss of heterozygosity; INSS=international neuroblastoma staging system; *In most cases.

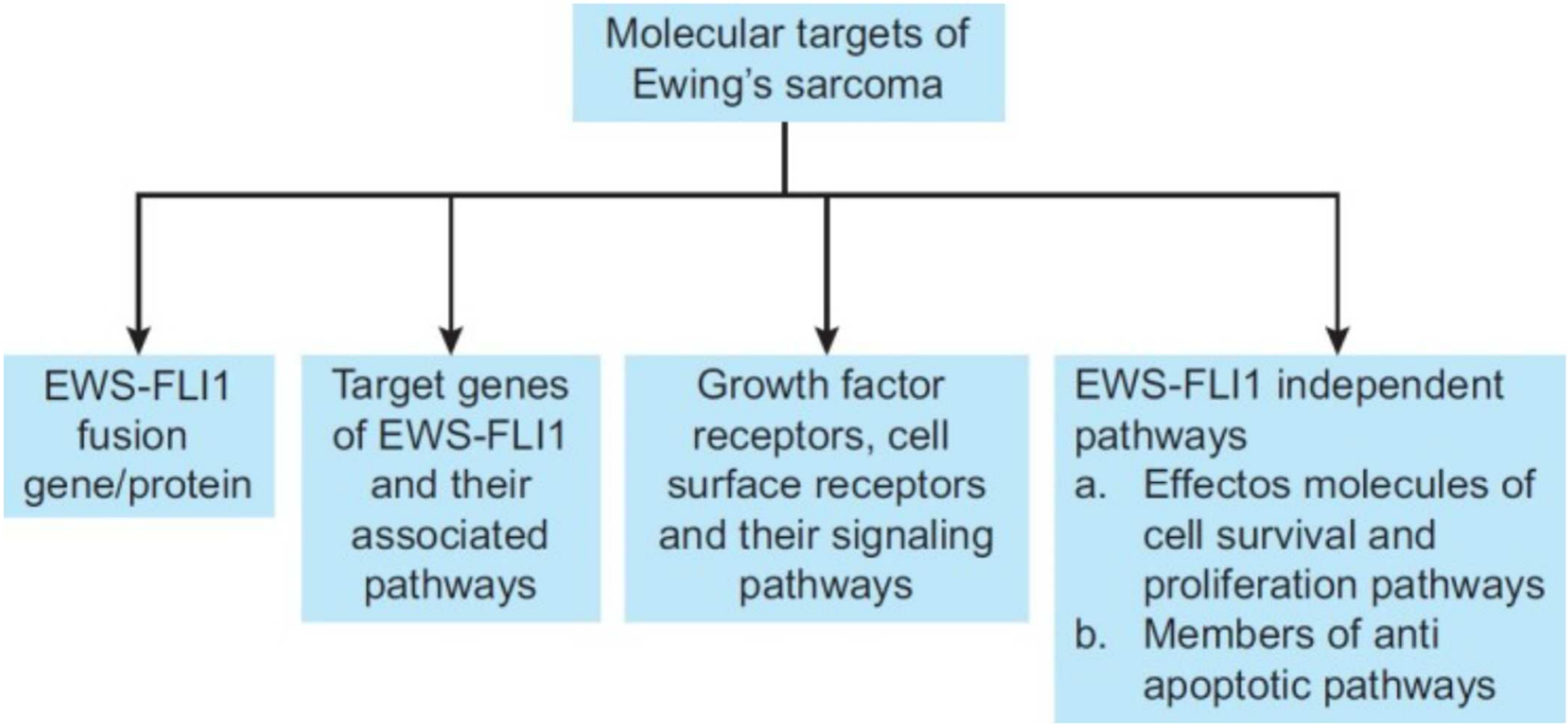
Identifying the target & drug discovery



Dinutuximab

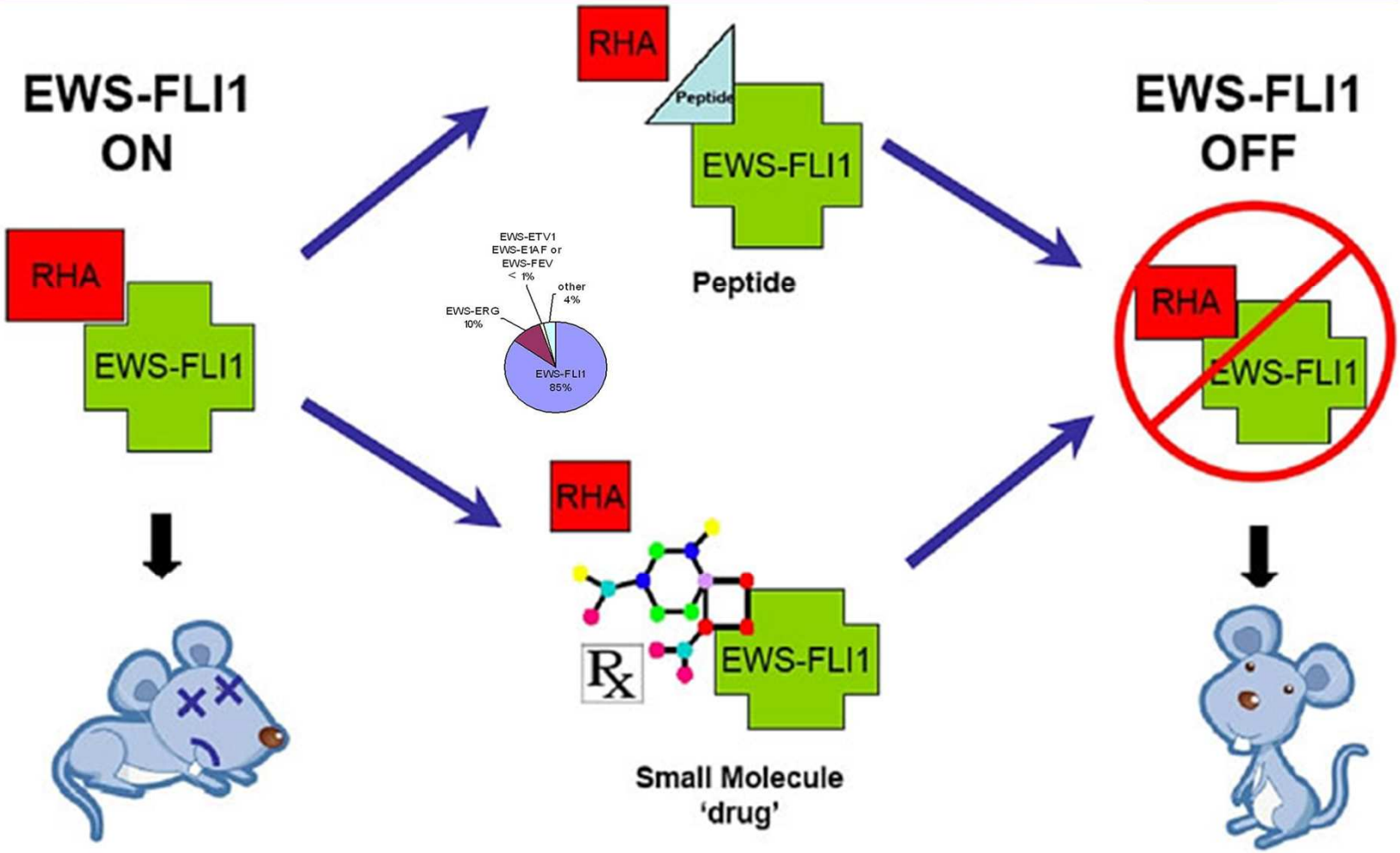
- **226 children with high risk neuroblastoma who had at least achieved a PR to multiple earlier treatments**
- **GD2 surface protein as target:** Dinutuximab binds to cell surface GD2 and induces cell lysis of GD2 expressing cells through antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)
- **Randomised to 13*cis*RA versus Dinutuximab+RA+GM-CSF+IL-2 (combination)**
- **3- years post therapy:**
  - **63% on combination were alive and free of tumor growth or recurrence, compared to 46% of subjects treated with RA alone**
  - **Updated analysis of survival, 73% of subjects who received the combination were alive compared with 58% of those receiving RA alone**

Ewing's sarcoma: approaches to targeting



Jully & Rajkumar Indian J Med Paediatr Oncol. 2012 Oct-Dec; 33(4): 195–202

ES: t(11:22) EWS-FLI1



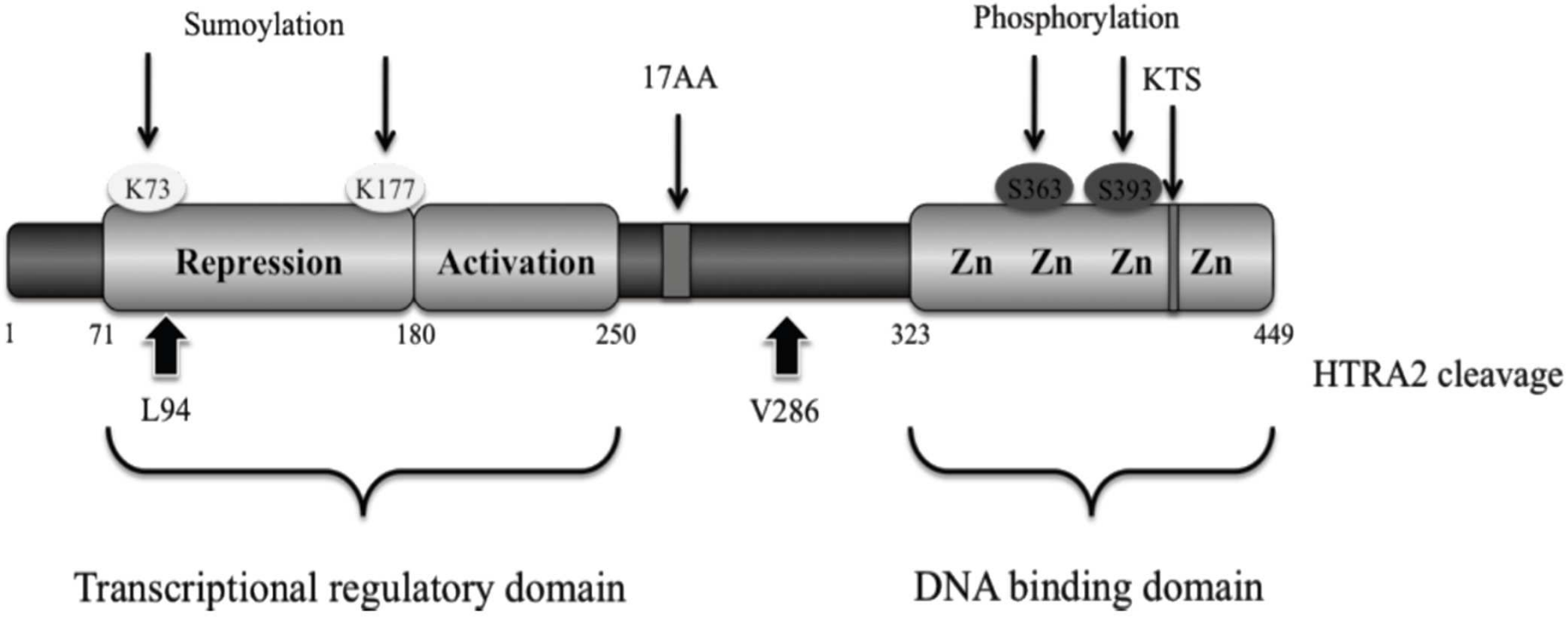
Insulin-like growth factor 1 receptor monoclonal antibodies against Ewing's sarcoma

Drug	Manufacturer	Current status
R1507	Roche	PC
CP-751,871 (figitumumab)	Pfizer	PC
AMG-479	Amgen	C
SCH-717454 (robatumumab)	Schering-Plough	PC*
IMC-A12 (cixutumumab)	Imclone	A+
MK-0646	Merck	A+
BIIB022	Biogen Idec	A
AVE-1642	Sanofi-Aventis	A

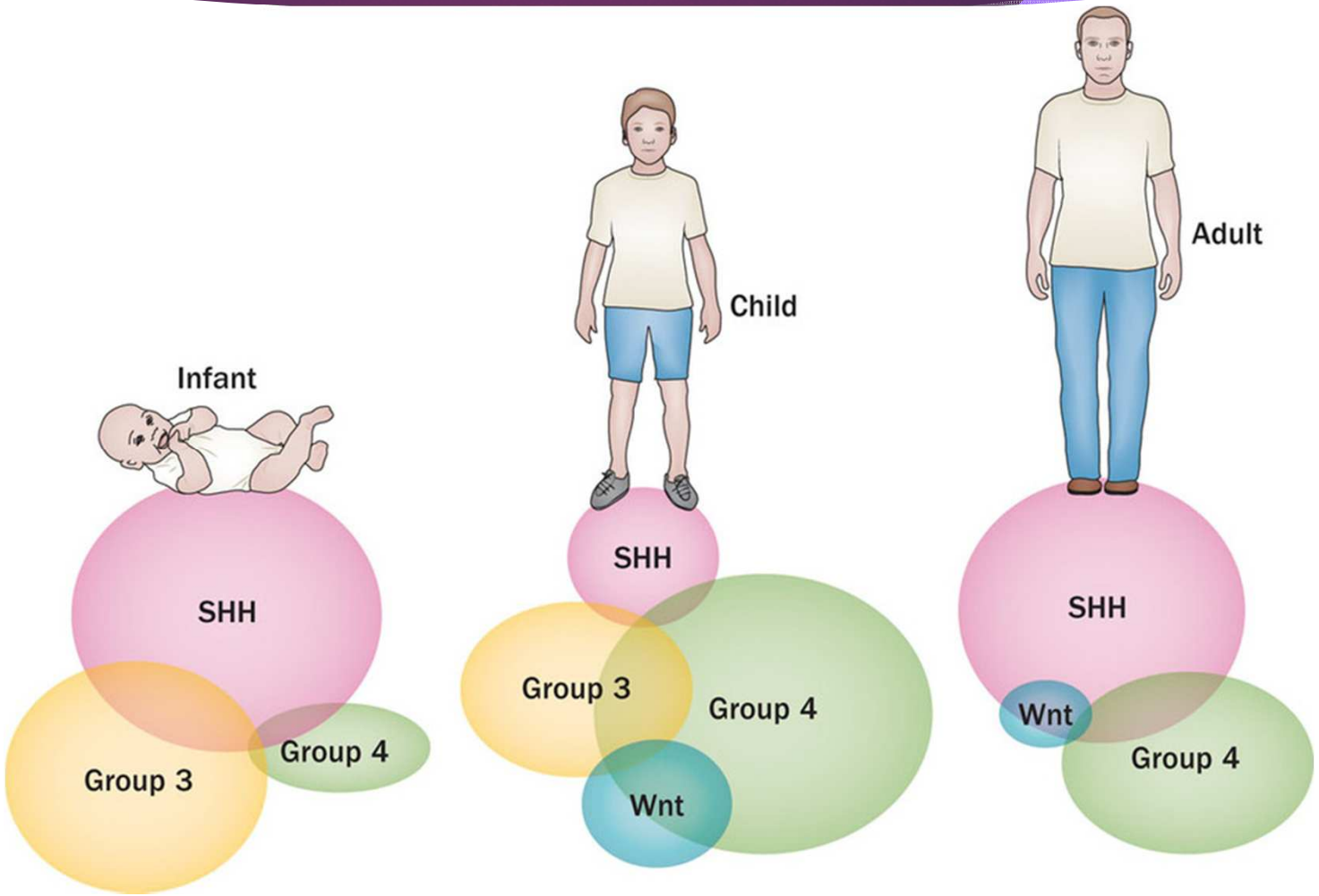
Small-molecule inhibitors of insulin-like growth factor 1 receptor

Drug	Manufacturer	Current status
OSI-906	OSI Pharmaceuticals	In vivo and in vitro activity in EWS, some activity in chondrosarcoma
BMS-554417	Bristol-Myers Squibb	In vitro activity against EWS
XL-228	Exelixis	A multitargeted protein kinase inhibitor targeting IGF1R, FGFR1-3, the Aurora kinases, and the ABL, ALK, and SRC family kinases
INSM-18	Insmed and UCSF	Orally bioavailable small molecule tyrosine kinase inhibitor that has demonstrated selective inhibition of IGF1R and human epidermal growth factor receptor (Her2/Neu).
GSK1904529A and GSK1838705A	GlaxoSmithKline	In vitro activity in EWS cell lines

WT1: Functional motifs and interactions



The 4 molecular subtypes of medulloblastoma



Medulloblastoma

Molecular Subtype	Approximate percent of patients	Typical patient age	Typical Histology	Cytogenetic markers	Molecular markers	Clinical strategy
WNT	10%	Older childhood	Classic	Monosomy 6	Beta-catenin	Reduction in therapy
SHH	25%	Infant and Adult	Desmoplastic or Classic	9q loss	SFRP1[47]or GAB1[50]	SHH pathway inhibitors
Group C	30%	Childhood	Classic or Anaplastic	Isochromosome 17q	MYC activation in 50% of this subtype	Intensified therapy, novel therapeutics
Group D	35%	Childhood	Classic or Anaplastic	Isochromosome 17q	Unknown	Research focus needed

Curr Opin Pediatr. 2012 Feb; 24(1): 33–39

Summary: molecular genetics in childhood solid tumours

- Identifying the molecular pathway helps characterise the tumour
- Allows precision diagnosis, segregates prognostic groups, identifies treatment algorithms
- Helps drive in silico, in vitro identification of druggable targets
- Helps chose agents for targeting childhood solid tumours

Every failure is a stepping stone to success