NEUROBLASTOMA: BIOLOGICAL INSIGHTS INTO A CLINICAL ENIGMA

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Neuroblastoma is a tumour derived from primitive cells of the sympathetic nervous system and is the most common solid tumour in childhood. Interestingly, most infants experience complete regression of their disease with minimal therapy, even with metastatic disease. However, older patients frequently have metastatic disease that grows relentlessly, despite even the most intensive multimodality therapy. Recent advances in understanding the biology and genetics of neuroblastomas have allowed classification into low-, intermediate- and high-risk groups. This allows the most appropriate intensity of therapy to be selected — from observation alone to aggressive, multimodality therapy. Future therapies will focus increasingly on the genes and biological pathways that contribute to malignant transformation or progression.

PLOIDY
A general term that is used to describe the overall chromosome number of a cell. A normal diploid cell has a karyotype with 46 chromosomes and a DNA content of 1.0. A triploid cell with 69 chromosomes has a DNA content of 1.5.

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Few tumours have engendered as much fascination and frustration for clinical and laboratory investigators as neuroblastoma, the most common and deadly solid tumour of childhood (see BOX 1). These tumours either regress spontaneously, particularly in infants, or they mature into a benign ganglioneuroma. However, most children of more than 1 year of age have extensive or metastatic disease at the time of diagnosis, and their overall prognosis has been poor. This incredible heterogeneity defied explanation until molecular genetic and biochemical analysis of tumour tissue began to shed light on these disparate clinical behaviours.

Many genetic features of neuroblastomas, such as the PLOIDY status, oncogene amplification or ALLELIC LOSS, have now been identified that correlate with clinical outcome. For instance, near-triploidy is associated with favourable outcome, whereas *MYCN* oncogene amplification or allelic loss at sites such as chromosome 1p are linked with more aggressive tumours and poor prognosis. High expression of the NEUROTROPHIN receptor TrkA (also known as NTRK1) is a favourable indicator — perhaps mediating either apoptosis or differentiation in these tumours. Conversely, high expression of TrkB (also known as NTRK2) with its ligand might provide an AUTOCRINE survival pathway in unfavourable tumours, particularly those with *MYCN* amplification. These and other observations have given us insight into mechanisms of malignant transformation and progression, as well as spontaneous differentiation and regression.

The specific genetic changes that have been identified allow tumours to be classified into subsets with distinct biological features and clinical behaviour. Indeed, certain genetic abnormalities are very powerful predictors of response to therapy and outcome, and, as such, they have become essential components of tumour characterization at diagnosis. So, neuroblastoma serves as a model solid tumour in which the genetic and biological analysis of tumour cells provides important information that guides optimal patient management. The challenge of the next decade will be to translate this information into more effective and less toxic therapy for these patients.

So, what is the current understanding about the biological and genetic features of neuroblastomas? Are all neuroblastomas derived from a common precursor, or do favourable and unfavourable neuroblastomas arise independently? What is the biological explanation for spontaneous regression of neuroblastomas in infants? And how can our knowledge of the biological and genetic features of the tumour be used for screening, predicting patient outcome, selecting therapies and developing new therapies? These and other questions are addressed in this review.

ALLELIC LOSS
(or loss of heterozygosity (LOH)). If the DNA is polymorphic in the normal constitutional DNA (two alleles identified) of a patient and only one allele is present in the tumour, then there is presumptive loss of DNA at that locus. Regions with high frequency of LOH are believed to harbour tumour-suppressor genes.

NEUROTROPHIN
A protein that binds to a receptor on a nerve cell, which, in turn, activates signalling pathways that support cell survival.

AUTOCRINE
A mechanism of self-activation through a ligand–receptor pathway. Autocrine activation results from a ligand that is produced by a cell binding to and activating a receptor on the same cell.

Summary

- Neuroblastoma is the most common extracranial tumour of childhood. This tumour originates from precursor cells of the peripheral (sympathetic) nervous system and usually arises in a paraspinal location in the abdomen or chest.
- The aetiology of neuroblastoma is unknown, but it seems unlikely that environmental exposures are important. A subset of patients inherits a genetic predisposition to neuroblastoma, and these patients usually have multifocal primary tumours that arise at an early age. A predisposition locus has been mapped to the short arm of chromosome 16.
- Neuroblastomas can be classified into subtypes that are predictive of clinical behaviour based on the patterns of genetic change. This information can be useful in the selection of therapy.
- Favourable tumours are characterized by near-triploid karyotypes with whole chromosome gains. These tumours rarely have structural rearrangements, and they usually express the TrkA neurotrophin receptor. Patients with these tumours are more likely to be less than 1 year of age, have localized tumours and a good prognosis.
- Unfavourable tumours are characterized by structural changes, including deletions of 1p or 11q, unbalanced gain of 17q and/or amplification of the *MYCN* protooncogene. They might also express the TrkB neurotrophin receptor and its ligand, brain-derived neurotrophic factor (BDNF). These patients are usually older than 1 year of age, have more advanced stages of disease and a much worse prognosis, even with aggressive treatment.
- Mass screening for neuroblastoma at 6–12 months of age led to an increased prevalence of neuroblastoma detected in the screened populations, but no decrease in mortality from this disease. The tumours detected have overwhelmingly been of the favourable genetic subtype.
- Novel, biologically based therapies are being developed that would specifically target the genes, proteins and pathways that are responsible for malignant transformation and progression in neuroblastomas. These approaches are likely to be more effective and less toxic than conventional therapy.
- In the future, it is likely that more extensive molecular profiling of the genetic changes and expression patterns of neuroblastoma will lead to an even more precise subclassification system that will be predictive of outcome, as well as therapies to which the tumour is most likely to be responsive.

Genetics of neuroblastoma predisposition

A subset of patients with neuroblastoma shows a predisposition to develop this disease, and this predisposition follows an AUTOSOMAL-DOMINANT pattern of inheritance. Knudson and Strong estimated that up to 22% of all neuroblastomas could be the result of a germinal mutation[1]. Regression analysis of neuroblastoma data was consistent with KNUDSON'S TWO-HIT HYPOTHESIS for the origin of childhood cancer. The median age at diagnosis of patients with familial neuroblastoma is 9 months, which contrasts with a median age of ~18 months for neuroblastoma in the general population. At least 20% of patients with familial neuroblastoma have bilateral adrenal or multifocal primary tumours[2,3]. The concordance for neuroblastoma in twins during infancy indicates that hereditary factors might be predominant, whereas the discordance in older twins indicates that random mutations or other factors might also be important[4].

A constitutional predisposition syndrome or associated congenital anomalies have not yet been identified in human neuroblastoma. Patients with both neuroblastoma and NEUROFIBROMATOSIS TYPE I (von Recklinghausen disease) have been reported. As both disorders result from abnormalities in development of the neural-crest cells in the embryo, a relationship has been suggested. However, an analysis of the reported coincidence of neuroblastoma and neurofibromatosis indicates that most of these cases can probably be accounted for by chance[5]. Lack of ganglia in the colon (HIRSCHSPRUNG DISEASE) is also a disorder of neural-crest origin that has been associated with neuroblastoma, but linkage to genes associated with this disorder has not been seen[6,7]. Various other congenital anomalies and genetic syndromes have been reported in association with neuroblastoma, but no specific abnormality has been identified with increased frequency.

Several cases of constitutional chromosome abnormalities have been reported in individuals with neuroblastoma, but no consistent pattern has emerged as yet[8]. There have been three reports of constitutional abnormalities involving the short arm of chromosome 1 (REFS 9–11). Considering the frequent deletion of 1p36 in sporadic neuroblastomas (see below), these cases indicated that constitutional deletions or rearrangements involving a gene or genes on 1p36 might have a role in malignant transformation or predisposition to neuroblastoma in some cases. However, a report that familial neuroblastoma is not linked to 1p36 indicates that the predisposition locus lies elsewhere[6]. Indeed, there has been a recent report of a genome-wide genetic-linkage analysis of neuroblastoma predisposition indicating that a locus at 16p12-13 is responsible[12]. At the present time, it is unclear if this is the only predisposition locus, or if there are multiple loci, but this locus could account for most high-risk families.

Somatic genetic changes

Although some patients with neuroblastoma have a predisposition to the disease, most neuroblastomas occur spontaneously. Somatic changes, such as gain of alleles and activation of oncogenes, loss of alleles or changes in tumour-cell ploidy have been shown to be important in the development of sporadic neuroblastomas.

DNA content: near-diploidy versus near-triploidy. Although most tumours have KARYOTYPES in the diploid range, tumours from patients with lower stages of disease are often hyperdiploid or near-triploid[13,14]. The karyotype of tumour cells can have prognostic value, but karyotype analysis is frequently unsuccessful. Flow cytometric analysis of DNA content is a simple, semi-automated way of measuring total cell DNA, and it correlates well with modal chromosome number, but it provides no information about specific chromosomal changes or rearrangements. Studies by Look and colleagues have shown that determination of the ploidy status content of neuroblastomas from infants can be predictive of outcome[15,16]. Unfortunately, ploidy loses its prognostic significance for patients who are older than 1–2 years of age[16]. This is probably because hyperdiploid and near-triploid tumours from infants generally have whole chromosome gains without structural rearrangements, whereas hyperdiploid/near-triploid tumours in older patients also have several structural rearrangements.

AUTOSOMAL DOMINANT
A pattern of inheritance through the non-sex chromosomes, in which a gene (allele) on one chromosome in a pair results in a phenotype and is dominant over the phenotype conferred by the other allele.

KNUDSON'S TWO-HIT HYPOTHESIS
Alfred Knudson proposed that familial cancers result from two rate-limiting mutations. One mutation is inherited in the constitutional DNA, and a single somatically acquired mutation in any cell of the target tissue could result in a tumour. In sporadic cases, both mutations are somatically acquired.

NEUROFIBROMATOSIS TYPE I
(Or von Recklinghausen disease). An autosomal-dominant disorder that is characterized by pigmented patches of skin and by the formation of neurofibromas (tumours involving nerve tissue) in the skin, subcutaneous tissue, cranial nerves and spinal root nerves.

HIRSCHSPRUNG DISEASE
A congenital condition that results from a failure to completely enervate the distal colon. This leads to obstruction of the large intestine from inadequate motility and collapse of this distal segment.

Box 1 | Basic facts about neuroblastoma

Occurrence

Neuroblastoma probably derives from primitive SYMPATHETIC NEURAL PRECURSORS. About half of all neuroblastomas arise in the ADRENAL MEDULLA, and the rest originate in PARASPINAL sympathetic ganglia in the chest or abdomen, or in pelvic ganglia. Neuroblastomas account for 7–10% of all childhood cancers, and it is the most common cancer diagnosed during infancy[181]. The prevalence is about one case in 7,000 live births, and there are about 700 new cases per year in the United States[29]. This incidence is fairly uniform throughout the world, at least for industrialized nations. The median age at diagnosis for neuroblastoma patients is about 18 months; so about 40% are diagnosed by 1 year of age, 75% by 4 years of age and 98% by 10 years of age[29]. The aetiology of neuroblastoma is unknown, but it seems unlikely that environment exposure has a significant role.

Progression and prognosis

Neuroblastomas typically spread to regional lymph nodes, bone and bone marrow. However, in infants there is sometimes a unique pattern of metastatic spread (stage 4S) that is primarily to the liver and skin. Despite the presence of metastatic disease at diagnosis, these children generally have an excellent prognosis. Indeed, some might be 'cured' by observation alone, as these and other tumours can undergo spontaneous regression. Unfortunately, older children frequently have metastatic disease, but they have a much worse prognosis. Recent research has provided possible explanations for these very different behaviours.

Histology

Most neuroblastomas are undifferentiated tumours, consisting of small, round cells called NEUROBLASTS that have little, if any, evidence of neural differentiation. However, some tumours show partial histological differentiation and are called ganglioneuroblastomas. The most differentiated end of the spectrum is a ganglioneuroma, which consists of clusters of mature neurons surrounded by a dense stroma of SCHWANN CELLS. The differentiation state of the tumour has some prognostic significance, but a more sophisticated histopathological classification has been developed to help predict outcome and select therapy.

Recently, Kaneko and Knudson proposed a hypothesis to explain how the ploidy state of the tumour has a fundamental role in the heterogeneity of clinical behaviour of neuroblastomas[17]. They proposed that both near-diploid and near-triploid tumours might arise from near-triploid cells with tripolar mitoses (see below).

Amplification of MYCN and the 2p24 locus. Some neuroblastomas are characterized cytogenetically by double-minute chromatin bodies (DMs) or homogeneously staining regions (HSRs), which are both cytogenetic manifestations of gene amplification. The gene or genetic region amplified was not known initially, but Schwab and colleagues identified a novel *MYC*-related oncogene — *MYCN* — that was amplified in a panel of neuroblastoma cell lines[18]. MYC oncoproteins are transcription factors that can lead to deregulated growth and proliferation when overexpressed. *MYCN* is normally located on the distal short arm of chromosome 2, but in cells with *MYCN* amplification it also maps to the DMs or HSRs[19,20]. A large region from chromosome 2p24 (including the *MYCN* locus) becomes amplified, presumably because it provides some selective advantage to the cells. The mechanism of amplification is unknown, but the *MYCN* locus might be copied to form an extrachromosomal circular element or DM, with retention of the normal copies of *MYCN* at 2p24 (REFS 20,21). DMs might accumulate by uneven segregation during mitosis; however, in some cases, the amplified DNA might integrate into a chromosomal locus to form an HSR[22]. Other genes might be co-amplified with *MYCN* in a subset of cases, but *MYCN* is the only gene that is consistently amplified from this locus[23,24].

We have shown that *MYCN* amplification occurs in many primary neuroblastomas in untreated patients[25] (FIG. 1). Amplification of *MYCN* is associated predominantly with advanced stages of disease and a poor outcome, but it is also associated with rapid tumour progression and a poor prognosis, even in infants and patients with lower stages of disease[22,26] (FIG. 2). These studies have been extended to 3,000 patients participating in cooperative group protocols in the United States (TABLE 1), and the overall prevalence of *MYCN* amplification in neuroblastomas is about 22% (REFS 27–30).

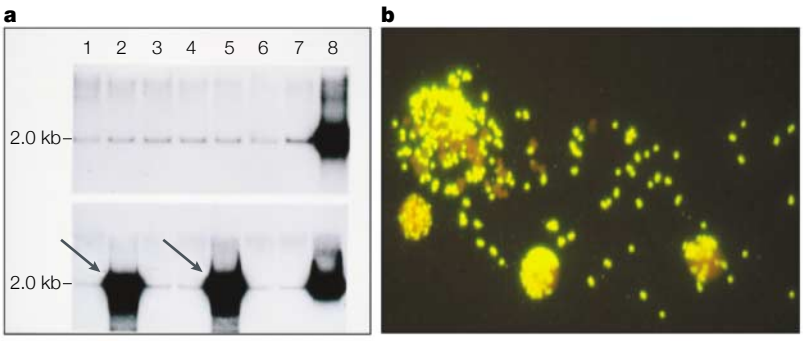

Figure 1 | ***MYCN* amplification in neuroblastomas. a** | DNA was extracted from cell lines and neuroblastoma samples and hybridized with a labelled *MYCN* probe in a Southern blot assay. In both rows, lane 1 shows DNA from a normal lymphoblastoid cell line as a single-copy control, and lane 8 shows DNA from a cell line that has 150 copies of *MYCN* per haploid genome. Lanes 2–7 in the top row show DNA taken from neuroblastomas with a single copy of *MYCN* per haploid genome. Lanes 2 and 5 (highlighted with arrows) in the bottom row show examples of tumours with *MYCN* amplification, whereas the DNA in the other lanes is taken from tumours that have the normal single-copy signal. **b** | Fluorescence *in situ* hybridization of a labelled *MYCN* probe to interphase and metaphase neuroblastoma cells. Interphase nuclei have heterogeneity of copy distribution, and multiple double-minute chromatin bodies (DMs) can be seen in the metaphase nucleus.

KARYOTYPE
A presentation of the chromosomes of a cell organized in pairs and by size. Normal human cells have a karyotype of 46 chromosomes (23 pairs).

SYMPATHETIC NERVOUS SYSTEM
The peripheral nervous system that is characterized by the neurotransmitter noradrenaline.

ADRENAL MEDULLA
The centre of the adrenal gland, where ganglion cells produce chemicals such as noradrenaline and adrenaline. This is a common site from which neuroblastomas originate.

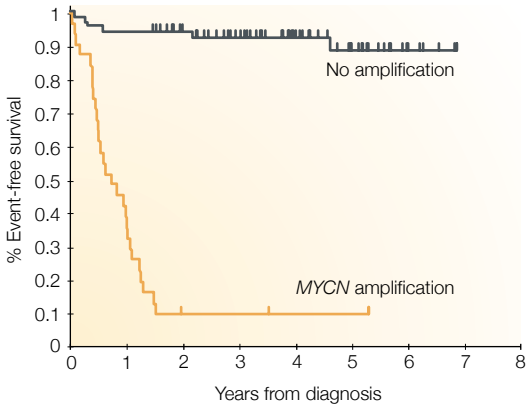

Figure 2 | **Survival of infants with metastatic neuroblastoma based on *MYCN* status.** A Kaplan–Meier survival curve of infants less than 1 year of age with metastatic neuroblastoma who were treated in a recent study[182]. The 3-year event-free survival (EFS) of infants whose tumours lacked *MYCN* amplification was 93%, whereas those with tumours that had *MYCN* amplification had only a 10% EFS.

Interestingly, we found a consistent pattern of *MYCN* copy number (either amplified or unamplified) in different tumour samples taken from an individual patient either simultaneously or consecutively[31], indicating that *MYCN* amplification is an intrinsic biological property of a subset of aggressive neuroblastomas, and that tumours without amplification at diagnosis rarely, if ever, develop this abnormality. Neuroblastomas from patients on clinical trials in the United States, Europe and Japan are currently routinely assessed for the presence of *MYCN* amplification, because it is a powerful predictor of a poor prognosis.

The reason why *MYCN* amplification is associated with a more aggressive phenotype is still uncertain. However, there is corresponding overexpression of the MYCN protein[32]. MYCN forms a heterodimer with MAX, and this protein complex functions as a transcriptional activator. In the absence of MYCN (or MYC), MAX forms a homodimer that is transcriptionally repressive. Only a few targets of MYCN are known — for example, *ODC*, *MCM7* and *MRP1* (REFS 33,34) — but activation of these genes leads to progression through the G1 phase of the cell cycle. Even though MYCN has a short half-life, the extremely high steady-state levels (~100 times normal) in amplified tumour cells probably ensures that cells stay in cycle and do not enter G0 (REF. 35).

Table 1 | **Correlation of *MYCN* amplification and stage in neuroblastomas**

Stage at diagnosis	*MYCN* amplification	3-year survival
Benign ganglioneuromas	0/64 (0%)	100%
Low stages (1,2)	31/772 (4%)	90%
Stage 4S	15/190 (8%)	80%
Advanced stages (3,4)	612/1,974 (31%)	30%
TOTAL	658/3,000 (22%)	50%

See REFS 27–30.

In general, there is a correlation between *MYCN* copy number and expression. Tumours with amplification usually express *MYCN* at much higher levels than are seen in tumours without amplification[35], and this subset of neuroblastomas is highly malignant. However, it is controversial whether or not overexpression of *MYCN* mRNA or MYCN protein has prognostic significance in tumours lacking *MYCN* amplification. Some neuroblastoma cell lines express high levels of *MYCN* mRNA or MYCN protein without gene amplification[32,36], perhaps due to alterations in normal protein degradative pathways rather than loss of *MYCN* transcriptional autoregulation[37,38]. One study indicated that MYCN expression correlates inversely with survival probability[39], whereas others either found no such correlation, or the correlation was confined to older children[40,41]. Further studies using standardized methods in a larger cohort of consistently treated patients will be necessary to determine if quantitative assessment of MYCN expression in tumours lacking *MYCN* amplification provides further prognostic information.

Our studies have also shown a strong correlation between *MYCN* amplification and 1p loss of heterozygosity (LOH)[42]. Both *MYCN* amplification and deletion of chromosome 1p are strongly correlated with a poor outcome and with each other, but it is controversial whether they are independent prognostic variables[43–45]. Nevertheless, they seem to characterize a genetically distinct subset of highly aggressive neuroblastomas. Most cases with *MYCN* amplification also have 1p LOH, but not all cases with 1p LOH have *MYCN* amplification, indicating that 1p deletion might precede the development of amplification. Indeed, it might be necessary to delete a gene that regulates *MYCN* expression, or one that mediates programmed cell death in the presence of high *MYCN* gene expression, for amplification to occur. Alternatively, there might be an underlying genetic abnormality that leads to genomic instability that predisposes to both 1p LOH and *MYCN* amplification.

Amplification of other loci. Amplification of at least six regions that are nonsyntenic with the *MYCN* locus at 2p24 has been shown in neuroblastoma cell lines or primary tumours. These include amplification of DNA from chromosome 2p22 and 2p13, the *MDM2* gene on 12q13 and the *MYCL* gene at 1p32 (REFS 27,46–48). However, no neuroblastoma has been shown to amplify another gene that did not also amplify *MYCN*. Allelic gain or amplification of other loci, including 4q, 6p, 7q, 11q and 18q, and other sites, have been identified using COMPARATIVE GENOMIC HYBRIDIZATION (CGH) approaches[49–51], but they mainly occur concurrently with *MYCN* amplification, so their prevalence, as well as biological and clinical significance, is unclear.

Trisomy for 17q. The only other specific karyotypic abnormality that has been detected with increased frequency so far is trisomy for the long arm of chromosome 17 (17q). Allelotyping and CGH studies have indicated that this abnormality might occur in more than half of all neuroblastomas[52,53]. Even accounting for

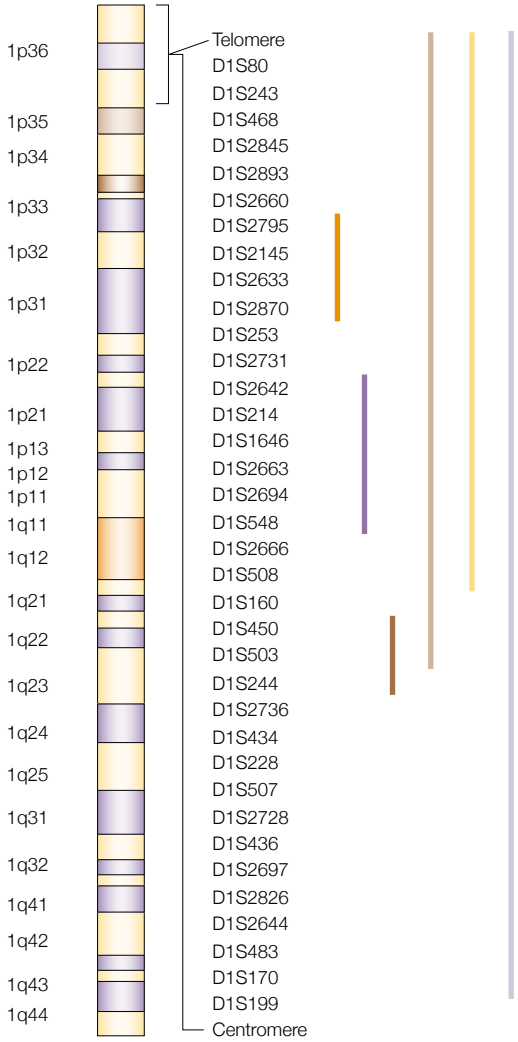

Figure 3 | **Loss of heterozygosity of chromosome 1p in neuroblastomas.** Studies from several laboratories have identified the approximate location of the smallest region of overlap (SRO) on the short arm of chromosome 1 (1p), at 1p36. This genetic map of 1p36 shows the regions identified by Brodeur[66,67], Schwab[65], Hyashi[69], Nakagawara[68], Martinsson[62,63] and Versteeg[64], shown in this order from left to right by the coloured lines.

PARASPINAL
Adjacent to the spine. This is a common location of sympathetic nerve cells, from which neuroblastomas arise.

NEUROBLASTS
Immature nerve cells.

SCHWANN CELLS
Cells that are derived from a group of embryonic cells called the neural crest, which are associated with and supportive of nerve cells. Schwann cells are the stromal cells in mature ganglioneuromas.

COMPARATIVE GENOMIC HYBRIDIZATION
(CGH). A technique that is used to detect chromosome gain or loss by hybridizing DNA from a target cell and a normal cell that are differentially labelled with unique fluors to a normal karyotype.

near-triploid cases with gain of the entire chromosome, 17q trisomy might be the most prevalent genetic abnormality that has been identified so far in neuroblastomas. Although unbalanced gain of 17q can occur independently, it frequently occurs as part of an unbalanced translocation between chromosomes 1 and 17 (REF. 54). The 17q breakpoints vary, but preferential gain of a region from 17q22-qter indicates a dosage effect that provides a selective advantage rather than interruption of a gene[55]. The gene (or genes) responsible for the selective advantage is unknown, but overexpression of survivin — a member of the inhibitor of apoptosis proteins — has been proposed[56]. Gain of 17q is associated with more aggressive neuroblastomas, although its prognostic significance relative to other genetic and biological markers awaits a large prospective trial and multivariate analysis.

HRAS and oncogene activation. Although *NRAS* was first identified as the transforming gene of a human neuroblastoma cell line, subsequent studies of primary neuroblastomas by us and others indicate that activating mutations of *RAS* proto-oncogenes are rare[57,58]. However, there is evidence that high expression of *HRAS* in neuroblastomas is associated with a lower stage of disease and a better outcome[59]. RAS protein activation is a frequent consequence of activating tyrosine kinase receptors (such as TrkA, see below), which, in turn, are associated with neural differentiation. So, RAS activation or overexpression could mimic activation of this aspect of the signal-transduction pathway. However, the ultimate clinical use of the analysis of oncogene expression in neuroblastomas remains to be determined. Activation of other oncogenes by amplification, mutation or other mechanisms have not been found except for a few rare examples seen primarily in established cell lines. So, other than *MYCN* amplification, which occurs in only a subset of tumours, there is no consistent evidence for activation of any other oncogene in human neuroblastomas.

Chromosome deletion or allelic loss at 1p. Deletion of the short arm of chromosome 1 (1p) is a common abnormality that has been identified in 70–80% of the near-diploid tumours that have been karyotyped[22,60]. However, DNA polymorphism approaches are more accurate and the actual prevalence is probably closer to 35% (REFS 11,43,61,62). Deletions of chromosome 1 are found more commonly in patients with advanced stages of disease, and 1p allelic loss is highly associated with *MYCN* amplification. The independent prognostic significance of 1p LOH has been controversial, but current evidence indicates that allelic loss at 1p36 predicts for disease progression but not overall survival in neuroblastoma patients[43–45].

Most studies indicate that there is a single site of deletion on distal 1p36 in neuroblastomas, but there might be more than one. Indeed, there is not agreement as to the exact site, as studies by different groups have identified at least three discrete regions (FIG. 3). These regions are being mapped intensively to identify potential candidate genes for the putative tumour-suppressor gene that has been deleted from this region[63–69]. Furthermore, some other studies identify larger regions that overlap two or more of the smaller regions, so it remains unclear if there is more than one tumour suppressor on distal 1p36 that is involved in the pathogenesis of neuroblastomas.

Chromosome deletion or allelic loss at other sites. Allelic loss of 11q has been detected by analysis of DNA polymorphisms and by CGH techniques[49–51,70,71]. In a recent study of 267 cases, 11q allelic loss was found in 43% patients, making it the most common deletion detected so far in neuroblastomas[72]. Deletion of 11q was directly associated with 14q deletion, but it was inversely correlated with 1p deletion and *MYCN* amplification. Interestingly, 11q LOH was associated with decreased event-free survival, but only in patients lacking *MYCN* amplification. This is presumably

because few tumours with 11q loss have *MYCN* amplification, and when the two abnormalities occur together, the prognostic impact of *MYCN* amplification is dominant. Nevertheless, loss of 11q might prove to be a useful predictor of outcome in clinically high-risk patients without *MYCN* amplification.

There is also evidence that LOH for the long arm of chromosome 14 occurs with increased frequency in neuroblastomas[70,73–75]. A recent study of 280 neuroblastomas found allelic loss in 23%, and a consensus region of deletion was found in 14q23-32 (REF. 75). There was a strong correlation with 11q allelic loss and an inverse relationship with 1p deletion and *MYCN* amplification. However, no correlation was found with other biological or clinical features or outcome. Deletion or allelic loss has been shown at various other sites by genome-wide allelotyping or by CGH, but their biological or clinical significance is unclear.

Specific tumour-suppressor genes. The *TP53* gene, which encodes the p53 protein, is one of the most commonly mutated genes in human neoplasia. p53 is a key regulator of cell-cycle control, and so inactivation of p53 function can contribute to malignant transformation. However, mutations are rarely found in primary neuroblastomas[76,77]. There is recent evidence that the *TP53* gene might be mutated more commonly in cell lines that are derived from patients at relapse[78,79], but there is still controversy about the involvement of this gene in neuroblastomas. Some reports have shown cytoplasmic sequestration in undifferentiated neuroblastomas, so impairing the normal G1 checkpoint after DNA damage[80,81]. Others have shown that, although p53 is primarily located in the cytoplasm, ionizing radiation induces normal translocation of p53 to the nucleus, where it can then induce G1 arrest[82].

The *CDKN2A* gene (which encodes INK4A, also known as p16) is deleted or mutated in many types of adult cancer. INK4A is important in cell-cycle control, and is frequently inactivated in various cancers. Nevertheless, three studies have found no evidence of inactivation in neuroblastomas or the related genes of *CDKN2A* — *CDKN2B* (which encodes KIP1, also known as p27) and *CDKN2C* (which encodes INK4C, also known as p18)[83–85]. Another study found homozygous deletion of *CDKN2A* at 9p21 in 4 out of 46 neuroblastoma cell lines, but it seems to be uncommon in primary tumours[86]. These results indicate that, for neuroblastoma, biallelic inactivation of *CDKN2A* might contribute to tumorigenicity, but in a minority of cases. The only other example of suppressor-gene inactivation is deletions or mutations in the *NF1* gene, which has been reported in two neuroblastoma cell lines[87,88], but there are no reports of this in primary tumours.

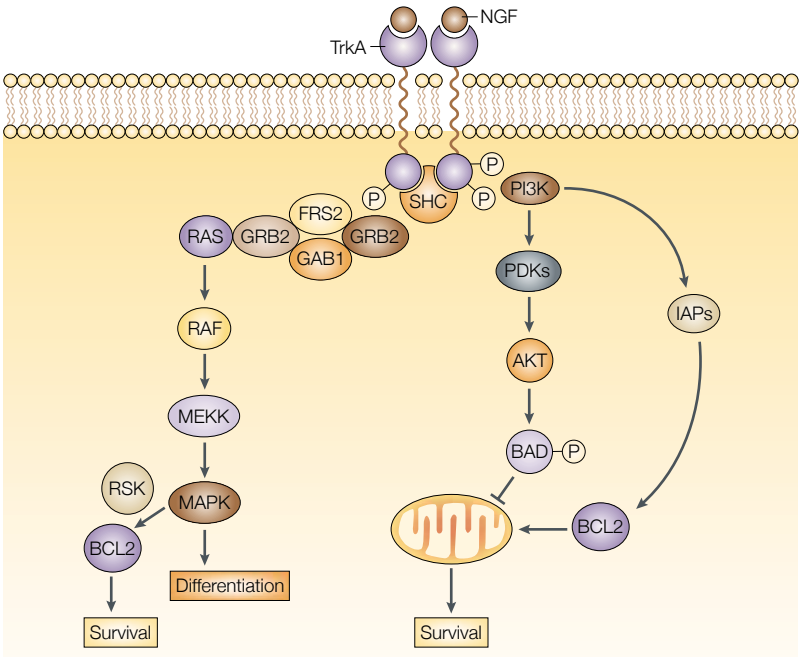

Figure 4 | **Signal-transduction pathway of the TrkA tyrosine kinase receptor.** Binding of nerve growth factor (NGF) to the ligand-binding domain of TrkA leads to TrkA autophosphorylation and activation of various signalling cascades. Proteins that are thought to interact directly with the Trk intracellular domain are SHC, PLCγ1, SH2B and IAPs, some of which are shown here. Binding of a ligand to TrkA can also trigger the RAS signalling pathway, leading to survival and differentiation, and an alternative survival signalling pathway through phosphatylinositol 3-kinase (PI3K).

Abnormal patterns of gene expression

Expression of neurotrophin receptors. The factors that are responsible for regulating the malignant transformation of sympathetic neuroblasts to neuroblastoma cells are not well understood, but they probably involve one or more neurotrophin-receptor pathways that signal the cell to differentiate. Recently, three tyrosine kinase receptors for a homologous family of neurotrophin factors have been cloned. The main ligands for the TrkA, TrkB and TrkC (also known as NTRK3) receptors are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3), respectively. Neurotrophin-4 (NT4, also known as NT5) also seems to function through TrkB[89,90].

TrkA is a transmembrane receptor that probably functions as a homodimer. Binding of TrkA to a homodimer of NGF activates autophosphorylation of the receptor, docking of signalling proteins, signal transduction and induction of gene transcription (FIG. 4). Activation of specific signalling pathways has been linked to survival or to differentiation, whereas inhibition of TrkA activation can lead to programmed cell death, depending in part on the state of differentiation of the cell. So, the presence or absence of NGF can have a profound effect on cellular behaviour.

We studied the relationship between TrkA mRNA expression and patient survival in a series of neuroblastomas and ganglioneuromas to determine its clinical significance[91] (TABLE 2). High levels of *TrkA* expression were correlated with younger age, lower stage and absence of *MYCN* amplification. Furthermore, TrkA expression was highly correlated with favourable outcome, and the combination of TrkA expression and *MYCN* amplification provided even greater prognostic power. Similar results have been

Table 2 | **Role of TrkA and B in neuroblastomas**

	TrkA	TrkB
Clinical group	Favourable	Unfavourable
Ligand	NGF	BDNF
Ligand expression	No	Yes
Cell survival	Yes	Yes
Differentiation	Yes	No
Angiogenesis	Inhibits	Promotes
Drug resistance	Inhibits?	Promotes
Tumorigenicity	Inhibits	Promotes

BDNF, brain-derived nerve factor; NGF, nerve growth factor.

obtained independently by other groups[92,93]. These data indicate that the TrkA/NGF pathway might have an important role in the propensity of some neuroblastomas to differentiate (or regress) in selected patients. Activation of the receptor by NGF would lead to survival and differentiation of neuroblasts into GANGLION CELLS. Selected neuroblasts induce the invasion and proliferation of Schwann cells, and these stromal cells produce neurotrophic factors that lead to neuroblast differentiation[94,95]. Conversely, neurotrophin deprivation might lead to programmed cell death in normal sympathetic neurons and TrkA-expressing neuroblastoma cells in culture[91,96]. So, the regression that is seen, particularly in TrkA-expressing neuroblastomas in infants, might be due in part to delayed activation of developmentally programmed cell death, resulting from the absence of NGF in the microenvironment.

Recently, we have examined the expression and function of TrkB and TrkC in neuroblastomas. Both of these neurotrophin receptors can be expressed in a truncated form (lacking the tyrosine kinase) and a full-length form. Interestingly, expression of full-length TrkB was strongly associated with *MYCN*-amplified tumours[97]. Because these tumours also expressed the cognate TrkB ligand (BDNF), this might represent an autocrine or PARACRINE loop, thereby providing some survival or growth advantage[98,99]. Maturing tumours were more likely to express the truncated TrkB, whereas most immature, non-amplified tumours expressed neither[100,101]. The TrkB/BDNF autocrine pathway seems to contribute to both enhanced angiogenesis and to drug resistance[100,101] (TABLE 2). By contrast, the expression of TrkC was found predominantly in lower-stage tumours, and, like TrkA, TrkC was not expressed in *MYCN*-amplified tumours[102,103]. This indicates that favourable neuroblastomas are characterized by the expression of TrkA, with or without TrkC, but unfavourable tumours express full-length TrkB plus its ligand BDNF.

Another transmembrane receptor called p75 (p75^{NTR}, also known as TNFRSF16) binds all the NGF family of neurotrophins with low affinity. This receptor is a member of the tumour necrosis factor receptor (TNFR) death-receptor family. Theoretically, p75 could lead to either cell death or differentiation in response to ligand, depending on whether or not Trk receptors were co-expressed[104,105]. p75 expression in neuroblastomas has generally been associated with a favourable outcome[91–93]. However, its biological and prognostic significance independent of Trk expression is unclear.

Expression of other important genes. Some tumour cells become resistant to several chemotherapeutic agents simultaneously by overexpressing genes that confer this resistance, probably by enhanced drug efflux. The genes associated with this phenomenon are the multidrug resistance gene 1 (*MDR1*), the gene for multidrug resistance-related protein (*MRP*) and other members of these families. Most of the investigation of these genes and their encoded proteins has been done *in vitro*, but their expression and potential clinical significance in neuroblastomas has been addressed recently[106–108].

Telomerase is a reverse transcriptase that is important in maintaining the length of telomeres — structures that protect the ends of chromosomes — in normal cells. Cancer cells often have increased telomerase activity, which prevents telomere loss and so maintains cell viability. Hiyama and colleagues[109] studied 79 neuroblastomas from untreated patients for telomerase activity. Their results show an inverse correlation between telomerase activity and outcome of neuroblastoma patients, and a direct correlation between high expression and *MYCN* amplification. So, the extremes of telomerase activity might provide some prognostic information, but telomerase activity is correlated more with malignancy *per se* than with specific types or behaviour[110]. Brodeur has also shown a correlation of high expression of the RNA component of telomerase with stage 4 disease and a poor outcome[110].

Neuroblastoma has the highest rate of spontaneous regression observed in human cancers, so delayed activation of normal apoptotic pathways might be important in this phenomenon. Activation of programmed cell death can originate from various stimuli, such as the presence or absence of exogenous ligand or from DNA damage. However, other cell-surface proteins, such as members of the TNFR family (for example, p75 and CD95) might be involved in initiating apoptosis in neuronal cells and neuroblastomas[111–113]. Intracellular molecules that are responsible for relaying the apoptotic signal include the BCL2 family of proteins. *BCL2* is highly expressed in most neuroblastomas, and the level of expression is inversely related to the proportion of cells undergoing apoptosis and the degree of cellular differentiation[114,115]. The BCL2 proteins might also be important in acquired resistance to chemotherapy[116,117]. Finally, there is evidence that increased expression of caspases — the proteolytic enzymes that are responsible for the execution of the apoptotic signal — in neuroblastomas is associated with favourable biological features and improved disease outcome[118]. So, neuroblastomas that are prone to undergoing apoptosis are more likely to spontaneously regress and/or respond well to chemotherapy.

GANGLION CELLS
Mature, post-mitotic, fully differentiated nerve cells.

PARACRINE
Paracrine activation results from a ligand produced by one cell binding to and activating a receptor on an adjacent cell.

Genetic model of neuroblastoma development

So, the accumulated research on the genetics of neuroblastoma indicates that there are at least two genetic subsets of neuroblastomas that are highly predictive of clinical behaviour. One popular classification takes into account abnormalities of 1p, *MYCN* copy number and assessment of DNA content, and distinct genetic types and subsets of neuroblastomas can be identified[27,28,30] (FIG. 5; TABLE 3). Indeed, it is possible that all neuroblastomas have a single mutation in common. However, a commitment is made shortly after initiation of tumour formation to develop into one of two main types.

The first type is characterized by mitotic dysfunction leading to a hyperdiploid or near-triploid modal karyotype, with few if any cytogenetic rearrangements. These tumours lack specific genetic changes such as *MYCN* amplification, 1p LOH or 17q gain. They generally express high levels of TrkA, and these tumours are prone to differentiation or programmed cell death, which might depend on the presence or absence of NGF. The patients are generally less than 1 year of age with localized disease and a very good prognosis.

The second is characterized by gross chromosomal aberrations and these tumours generally have a near-diploid karyotype. No consistent abnormality has been identified so far, but 17q gain is common, and high TrkA expression is rare. Within this type, two subsets can be distinguished. One subset is characterized by 11q deletion, 14q deletion or other changes, but they lack *MYCN* amplification and generally lack 1p LOH. Patients with these tumours are generally older, with more advanced stages of disease that is slowly progressive and often fatal. The most aggressive subset has *MYCN* amplification, usually with 1p LOH. These tumours frequently express *TrkB* plus *BDNF*, which presumably represents an autocrine survival pathway that confers a selective advantage. These patients are generally between 1 and 5 years of age with advanced-stage, rapidly progressive disease that is frequently fatal.

Proposals to explain the genetic and clinical heterogeneity based on patterns of genetic change have been made recently by others[17,119]. These proposals relate the tumour-cell ploidy with the presence or absence of structural abnormalities in the tumour, particularly involving chromosome 1. Indeed, they indicate how both diploid and triploid tumours could arise from divisions of tetraploid cells. However, once established, there is little evidence to indicate that genetically and biologically favourable tumours ever evolve into unfavourable tumours. In any case, neuroblastomas have very different clinical behaviours that can generally be predicted by the patterns of genetic change and gene expression. This information, in turn, can be used to predict outcome and select the most appropriate intensity of therapy.

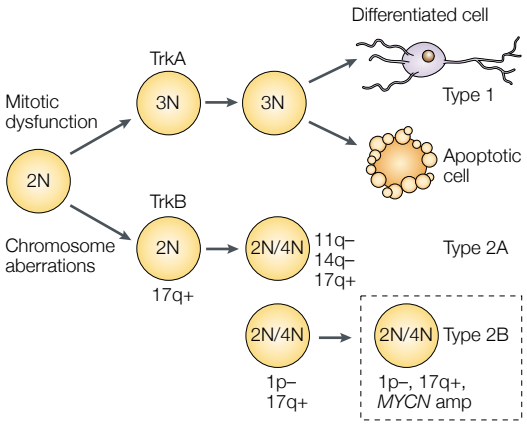

Figure 5 | **Genetic model of neuroblastoma development.** According to this model, all neuroblastomas have a common precursor and might have a common mutation (for example, the one responsible for familial neuroblastoma). However, a commitment is made to develop into one of two main types. The first type is characterized by mitotic dysfunction leading to a hyperdiploid or near-triploid karyotype (3N) with whole chromosome gains, but few, if any, structural cytogenetic rearrangements. These tumours usually express high levels of TrkA, so they are prone to either differentiation or apoptosis, depending on the presence or absence of nerve growth factor (NGF) in their microenvironment. The second type generally has a near-diploid (2N) or near-tetraploid karyotype, but is characterized by gross chromosomal aberrations. No consistent abnormality has been identified, but 17q gain is common. Within this type, two subsets can be distinguished. One subset is characterized by 11q deletion and/or 14q deletion, whereas the second subset is characterized by 1p loss of heterozygosity (LOH), with or without *MYCN* amplification. The tumours with LOH at 1p frequently express TrkB plus brain-derived neurotrophic factor (BDNF), probably representing an autocrine survival pathway. Modified with permission from REF. 28 © (2000) Elsevier Science B. V.

Prognostic considerations

Clinical features. The most important clinical variables in predicting patient outcome are the stage of disease (as defined by the International Neuroblastoma Staging System[120] (INSS; see TABLE 4), the age of the patient at diagnosis and the site of the primary tumour[3,29]. The 2-year disease-free survival of patients with stage 1, 2 and 4S is 80–90%, whereas those with stages 3 and 4 have a range of 40–50%. The outcome of infants who are less than 1 year of age is substantially better than older patients with the same stage of disease, particularly those with more advanced stages of disease. Patients with primary tumours in the adrenal gland seem to do worse than patients with tumours originating at other sites. However, these clinical features are imperfect predictors of tumour behaviour, so further prognostic markers are needed.

Serum markers. Various serum markers have been proposed, either to predict outcome or to follow disease activity. For example, serum ferritin levels are increased in some patients with advanced-stage tumours[121], but increased levels might simply be a marker of rapid tumour growth and/or large tumour burden. Neuron-specific enolase (NSE) is a cytoplasmic protein that is associated with neural cells, and survival is substantially worse for patients with advanced disease and high NSE[122]. The disialoganglioside G_{D2} is found on the

Table 3 | **Biological/clinical types of neuroblastoma**

Feature	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
TrkA expression	High	Low or absent	Low or absent
TrkB expression	Truncated	Low or absent	High (full length)
TrkC expression	High	Low or absent	Low or absent
Age	Usually <1 year	Usually >1 year	Usually 1–5 years
INSS stage	Usually 1,2,4S	Usually 3,4	Usually 3,4
5-year survival	95%	40–50%	25%

See REFS 27–30. LOH, loss of heterozygosity. INSS, International Neuroblastoma Staging System.

surface of most neuroblastomas. Increased levels have been found in the plasma of neuroblastoma patients, and gangliosides that are shed by tumour cells might be important in accelerating tumour progression[123]. Although not specific to neuroblastoma, serum lactate dehydrogenase (LDH) levels have been used as a prognostic marker for neuroblastoma, and they might reflect rapid cellular turnover or large tumour burden[124,125]. Nevertheless, none of these markers are used at present to predict outcome or to select therapy.

Tumour pathology. Differentiated histology, such as ganglioneuroblastoma, is generally associated with localized tumours, but this type of histological classification does not have prognostic value that adds substantially to age and stage. More detailed analysis of histology, such as the classification devised by Shimada (called the Shimada classification), takes into consideration the amount of Schwann cells in the stroma, mitotic figures and degeneration of nuclei[126]. This classification seems to be a more powerful predictor of outcome. There is now an International Neuroblastoma Pathology Classification (INPC) — based on the Shimada Classification — that will supersede the above-mentioned systems and become the international standard for histopathological classification, particularly as a prognostic variable[127,128]. Expression of the cell-surface glycoprotein CD44 has also been shown to have prognostic significance, with high expression being associated with more differentiated tumours and a better outcome[129].

Genetic markers. Various genetic changes in neuroblastoma cells (discussed above) have been proposed as useful prognostic markers. These include changes in the DNA content or modal karyptype, gains of genetic material (for example, *MYCN* amplification and unbalanced 17q gain), loss of genetic material (for example, deletions of 1p, 11q or other sites), as well as changes in gene expression (for example, *TRK* genes, *RAS* genes, *MRP1*, telomerase and others). Although all of these markers have predictive value in univariate analyses, only a few remain predictive after multivariate analysis and correction for clinical variables such as age, stage and tumour histology. Indeed, it will take a large clinical trial with analysis of many variables to determine if any of these can substantially improve the current risk stratification.

Biologically based risk groups. Most current studies for the treatment of neuroblastoma patients are based on risk groups that take into account various biological features (*MYCN* copy number, histopathology and tumour ploidy in infants) in addition to patient age and INSS stage (TABLE 4). Preliminary data — adjusted for age and stage — indicate that analysis of DNA content in infants and *MYCN* copy number in all patients allow more precise determination of risk[130]. Tumour histopathology — determined by the Shimada classification and the newer INPC classification — also seems to be an important independent prognostic marker, at least for certain subsets of patients[127,128]. Judging the independent prognostic impact of other biological variables, such as 1p allelic loss, 11q allelic loss, TrkA expression or others, must await large prospective therapeutic and biological studies.

Table 4 | **Neuroblastoma risk groups based on clinical and biological features**

Stage*	Low risk	Intermediate risk	High risk
1	All	None	None
2A, 2B	Age <1 year, or age 1–21 years and *MYCN* non-AMP, or age 1–21 years and *MYCN* AMP + FH	None	Age 1–21 years and *MYCN* AMP + UH
3	None	Age <1 year and *MYCN* non-AMP, or age 1–21 years and *MYCN* non-AMP + FH	Age 0–21 years and *MYCN* AMP, or age 1–21 years and *MYCN* non-AMP + UH
4	None	Age <1 year and *MYCN* non-AMP	Age <1 year and *MYCN* AMP, or age 1–21 years
4S	*MYCN* non-AMP; FH; DI >1	*MYCN* non-AMP; UH; DI=1	*MYCN* AMP

See REFS 143,144,146. *International Neuroblastoma Staging System[136]. AMP, amplified; DI, DNA index (ploidy); FH, favourable histology; non-AMP, not amplified; UH, unfavourable histology.

Spontaneous regression. Microscopic neuroblastic nodules are found with increased frequency in infants less than 3 months of age who died of other causes[131]. This finding was initially interpreted to mean that neuroblastoma *in situ* develops considerably more often than it is detected clinically, but that these microscopic tumours regress spontaneously in most cases. However, others have shown that these neuroblastic nodules occur uniformly in all fetuses studied, peaking between 17 and 20 weeks of gestation, and gradually regressing by the time of birth[132,133]. So, these microscopic neuroblastic nodules are likely to be remnants of fetal adrenal development. Nevertheless, these remnants might be the cells from which neuroblastomas develop, at least in the adrenal medulla. These nodules would never be detected clinically, and would not be detected by urinary mass screening of infants for neuroblastoma (see below). However, neuroblastoma apparently has a high rate of spontaneous regression. Indeed, there are several well-documented cases of infants with neuroblastoma who have had complete regression of their tumour[134]. The actual frequency of neuroblastomas that are detected clinically and subsequently regress without treatment is 5–10%. However, on the basis of estimates from the mass-screening studies (see below), the frequency of true asymptomatic neuroblastomas that regress spontaneously is probably much higher, and might be equal to the number detected clinically.

Current status of mass screening

Infants with neuroblastoma have a better outcome than children who are more than 1 year of age at diagnosis. Therefore, a potential approach to improve the long-term outcome of neuroblastoma patients would be to identify patients earlier in the course of their disease. Because neuroblastomas frequently produce increased levels of CATECHOLAMINES, the metabolites of which are readily detectable in the urine, mass urinary screening of infants for neuroblastoma was undertaken initially in Japan[135,136]. Similar efforts were undertaken subsequently in North America and in Europe to answer questions concerning the feasibility and utility of screening for neuroblastoma[137,138].

The rationale for mass screening assumes that aggressive, biologically unfavourable disease seen in patients has evolved over time from more localized, biologically favourable tumours in infants, and early detection would improve their outcome. Indeed, the clinical and cytogenetic features of tumours that have been identified as a result of mass screening of infants for neuroblastomas in Japan indicate that most patients identified have lower stages of disease, and virtually all of the tumours are in the near-triploid range with whole chromosome gains[139–142]. Previous studies have shown that such findings are generally associated with a favourable outcome. Therefore, the results of this study indicated at least two possibilities — either all neuroblastomas originate with a favourable genotype and phenotype, and some evolve into more aggressive tumours with adverse genetic features, or there are at least two different subsets of neuroblastoma, and the more favourable group presents earlier and is therefore the predominant group detected by screening. The accumulating body of genetic information is more consistent with the latter explanation.

Evidence also indicates that the prevalence of neuroblastoma in screened populations is increased by at least 50–100% compared with unscreened populations, and that the prevalence of neuroblastoma in patients over the age of 1 year has not changed appreciably[137,138,143,144]. Taken together, this indicates that screening detects tumours in a substantial number of patients who would probably never develop symptomatic disease because their tumours would have regressed or matured without therapy. Many of the tumours detected by screening at 6–12 months of age have favourable biological features and could be cured easily with relatively mild therapy[142]. A few patients with unfavourable biological features have presented clinically during the first 6–12 months of age in the screened population, and they have had an unfavourable outcome[14,142,145,146]. Finally, there has been no improvement in the mortality from neuroblastoma in patients over the age of 1 year as a consequence of mass screening[143,144].

So, mass screening for neuroblastoma rarely detects biologically unfavourable tumours early, and it has not improved the overall mortality from neuroblastoma. Indeed, it has resulted in substantial 'overdetection' of tumours in the first year of life, probably leading to unnecessary testing, surgery and even chemotherapy for patients with a high likelihood of spontaneous regression. However, mass screening has taught us a great deal about the natural history of neuroblastoma, and it has given us a better sense of the frequency of spontaneous regression in these patients. Also, it indicates that there are at least two distinct types of neuroblastoma — a biologically favourable type that develops in infants, and a biologically unfavourable type that develops in older patients. The former type rarely, if ever, evolves into the latter, so mass screening at 6–12 months of age is unlikely to be successful.

Future directions: biology and therapy

Molecular profiling. MYCN amplification is the only example of oncogene activation known to occur with substantial frequency in neuroblastomas. However, it seems likely that other examples of oncogene activation affecting cell-cycle control or DNA repair will be identified as these pathways become better understood. Several sites of recurring allelic loss have been identified in neuroblastomas, but so far no specific tumour-suppressor genes have been identified at these sites. Finally, a neuroblastoma predisposition gene has been linked to 16p, but there might be other genes responsible for familial neuroblastomas.

Microarray analysis of DNA or RNA, as well as other technologies (such as the serial analysis of gene expression or SAGE analysis)[147,148], are emerging that might allow the identification of patterns of genetic change, as well as gene-expression profiles, that would provide a more complete picture of each individual tumour. For example, Khan and colleagues have used microarray

CATECHOLAMINES
Catecholamines are small molecules such as DOPA, dopamine and norepinephrine that function as neurotransmitters in the central and peripheral nervous systems. These compounds are broken down into urinary metabolites that can be measured in the urine.

expression profiling to distinguish neuroblastomas from other small, round, blue-cell tumours of childhood[149]. Microarray analysis has been used to identify novel targets of the MYCN transcription factor in neuroblastomas with *MYCN* amplification[34], as well as genes that are involved in the early stages of retinoid-induced differentiation[150]. Global patterns of gene expression can be used to distinguish particular types of tumours, or to identify subsets. Expression analysis can also be used to determine if genes involved in particular pathways — such as survival, differentiation and apoptosis — are expressed. This, in turn, might be used to predict whether an individual tumour is likely to be sensitive (or resistant) to conventional or biologically based therapies.

Animal models. In the past, the only models available to study neuroblastoma biology and therapy *in vivo* were either human neuroblastoma xenografts growing in immunosuppressed animals, or a few syngeneic rodent models — for example, C1300 and its derivatives in the mouse, and B104 in the rat. However, Weiss and colleagues have genetically engineered a transgenic mouse that overexpresses the *MYCN* proto-oncogene under the control of the tyrosine kinase promoter, and these animals have a high prevalence of neuroblastoma[151]. The neuroblastomas that develop in these animals have genetic and biological features that are similar to those seen in primary human neuroblastomas, indicating that this is a useful model for studying the genetic evolution of these tumours[152,153]. Furthermore, this model might be particularly tractable to the study of novel, biologically based therapies that are aimed at both treating and preventing neuroblastomas.

Biologically based therapies. Most neuroblastomas are treated with conventional therapeutic approaches, including surgery, external beam radiation therapy and cytotoxic chemotherapy. However, as the genes, proteins and pathways that are important for the pathogenesis of neuroblastomas are identified and characterized, it is anticipated that these will provide insights into the development of more biologically based therapies. These therapies will probably target pathways that are common to other forms of cancer, as well as some that are relatively unique to neuroblastoma. These approaches promise greater specificity and/or less toxicity than standard modalities.

Induction of differentiation is an approach that would seem to be particularly promising for neuroblastomas. Retinoic-acid derivatives have been shown to induce differentiation and slow the growth of neuroblastoma cells in culture[154–156]. Subsequently, treatment of high-risk neuroblastoma patients with 13-*cis* retinoic acid after bone-marrow transplantation was carried out in a randomized clinical trial, and showed a significant survival advantage with minimal extra toxicity[157]. Indeed, this approach has now become standard practice in the management of high-risk neuroblastoma patients after marrow or stem-cell transplantation. There might be other approaches to induce neuronal differentiation in neuroblastoma cells that target the TrkA or other neurotrophin-receptor pathways.

Induction of apoptosis is an increasingly popular approach to the treatment of human cancer. Indeed, a novel synthetic retinoid — *N*-(4-hydroxyphenyl)retinamide (fenretinide) — induces apoptosis rather than differentiation, and this agent is, now, undergoing clinical trials in neuroblastoma patients[158–160]. Antisense oligonucleotides for the *BCL2* gene (Genasense) can block a crucial survival pathway and induce cancer cells to undergo apoptosis, or to become more sensitive to chemotherapy[161]. Finally, the expression of TrkB and BDNF in high-risk neuroblastomas, particularly those with *MYCN* amplification, might represent an important survival pathway that confers resistance to treatment[97–99]. Blocking this pathway with Trk-specific tyrosine kinase inhibitors might reduce this resistance and promote apoptosis when used alone or in combination with conventional agents[101,162].

Inhibition of angiogenesis is a promising approach for the treatment of neuroblastomas because of the high degree of vascularity of these tumours[163]. The agent TNP-470 has proved effective in treating neuroblastomas growing in animal models[164–166], but it might be too toxic to be a useful therapeutic agent. Various other agents are under development that target selected aspects of the angiogenic process[167–170]. In addition, the chronic administration of low-dose chemotherapy — known as metronomic therapy — might be another effective way to inhibit angiogenesis[171].

Immunotherapy of neuroblastoma is another approach that is gaining in popularity. Neuroblastomas are not highly immunogenic, in part because of the low levels of expression of antigens that are required for proper recognition by or presentation to the immune system. However, several antibodies have been raised against neuroblastoma surface antigens that target the disialoganglioside G_{D2} and have been used as therapeutic agents[172–174]. These antibodies might also provide a means of targeting other therapeutic molecules selectively to neuroblastoma cells. A better understanding of the interface between neuroblastoma cells and the immune system will allow the development of effective immunotherapy approaches.

Targeted radiation therapy might also be useful in the treatment of both localized and disseminated neuroblastomas and obviate some of the limitations and toxicities of conventional radiation therapy. Meta-iodobenzylguanidine (MIBG) is a compound that is actively taken up by neuroblastoma cells and concentrated in secretory granules. Radioactive MIBG has proven to be useful for diagnostic imaging, and for treatment of *de novo* and recurrent neuroblastomas[175–177]. Targeting with radiolabelled anti-G_{D2} antibodies might also be a useful approach. PROTON-BEAM THERAPY should become more widely available in the next few years to treat children with neuroblastoma and other tumours[178–180]. Although not biologically based, proton therapy approaches can deliver radiation with much greater precision, thereby increasing local control and decreasing toxicity to the surrounding normal tissues.

PROTON-BEAM THERAPY
Radiation therapy for local tumour control using a proton beam, as opposed to an electron or photon beam (used in more conventional radiation therapy).

Conclusions

Genetic and molecular profiling of neuroblastomas using microarray, SAGE or other techniques are likely to be used increasingly to identify genetic signatures of subsets of patients that are predictive of outcome. These approaches will also help to identify genes, proteins and pathways that are responsible for malignant transformation and progression in neuroblastomas and this will aid development of novel approaches that target these specific biological pathways. Although the conventional modalities of surgery, chemotherapy and radiation therapy will continue to be important in the treatment of neuroblastomas, biologically based therapies will be used increasingly in the management of these patients. This, in turn, should allow more effective and less toxic treatment, improving the cure rate and reducing the development of late effects.

1. Knudson, A. G. J. & Strong, L. C. Mutation and cancer: neuroblastoma and pheochromocytoma. *Am. J. Hum. Genet.* **24**, 514–522 (1972).
2. Kushner, B. H., Gilbert, F. & Helson, L. Familial neuroblastoma: case reports, literature review, and etiologic considerations. *Cancer* **57**, 1887–1893 (1986).
3. Maris, J. M. & Matthay, K. K. Molecular biology of neuroblastoma. *J. Clin. Oncol.* **17**, 2264–2279 (1999).
4. Kushner, B. H. & Helson, L. Monozygotic siblings discordant for neuroblastoma: etiologic implications. *J. Pediatr.* **107**, 405–409 (1985).
5. Kushner, B. H., Hajdu, S. I. & Helson, L. Synchronous neuroblastoma and von Recklinghausen's disease: a review of the literature. *J. Clin. Oncol.* **3**, 117–120 (1985).
6. Maris, J. M. *et al.* Familial predisposition to neuroblastoma does not map to chromosome band 1p36. *Cancer Res.* **56**, 3421–3425 (1996).
7. Weiss, M. J. *et al.* Localization of a hereditary neuroblastoma predisposition gene to 16p12-p13. *Med. Pediatr. Oncol.* **35**, 526–530 (2000).
8. Bown, N. P., Pearson, A. D. J. & Reid, M. M. High incidence of constitutional balanced translocations in neuroblastoma. *Cancer Genet. Cytogenet.* **69**, 166–167 (1993).
9. Biegel, J. A. *et al.* Constitutional 1p36 deletion in a child with neuroblastoma. *Am. J. Hum. Genet.* **52**, 176–182 (1993).
10. Laureys, G. *et al.* Constitutional translocation t(1;17)(p36.31-p36.13;q11.2-q12.1) in a neuroblastoma patient. Establishment of somatic cell hybrids and identification of PND/A12M2 on chromosome 1 and NF1/SCYA7 on chromosome 17 as breakpoint flanking single copy markers. *Oncogene* **10**, 1087–1093 (1995).
11. White, P. S. *et al.* Detailed molecular analysis of 1p36 in neuroblastoma. *Med. Pediatr. Oncol.* **36**, 37–41 (2001).
12. Maris, J. M. *et al.* Evidence for a hereditary neuroblastoma predisposition locus at chromosome 16p12-13. *Cancer Res.* **62**, 6651–6658 (2002).
The first report of linkage analysis, identifying a candidate locus on 16p12-13.
13. Kaneko, Y. *et al.* Different karyotypic patterns in early and advanced stage neuroblastomas. *Cancer Res.* **47**, 311–318 (1987).
The first report to associate karyotypic pattern with stage and prognosis, and the first to show the association of near-triploid tumours in infants with whole chromosome gains.
14. Kaneko, Y. *et al.* Current urinary mass screening or catecholamine metabolites at 6 months of age may be detecting only a small portion of high-risk neuroblastomas: a chromosome and N-myc amplification study. *J. Clin. Oncol.* **8**, 2005–2013 (1990).
15. Look, A. T., Hayes, F. A., Nitschke, R., McWilliams, N. B. & Green, A. A. Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. *N. Engl. J. Med.* **311**, 231–235 (1984).
The first report to show the prognostic significance of tumour-cell DNA content in infants with neuroblastoma.
16. Look, A. T. *et al.* Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma. A Pediatric Oncology Group Study. *J. Clin. Oncol.* **9**, 581–591 (1991).
17. Kaneko, Y. & Knudson, A. G. Mechanism and relevance of ploidy in neuroblastoma. *Genes Chromosom. Cancer* **29**, 89–95 (2000).
18. Schwab, M. *et al.* Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* **305**, 245–248 (1983).
Reports the cloning of the *MYCN* proto-oncogene as the gene amplified in neuroblastoma cell lines and a primary tumour.
19. Schwab, M. *et al.* Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc. *Nature* **308**, 288–291 (1984).
20. Corvi, R., Amler, L. C., Savelyeva, L., Gehring, M. & Schwab, M. *MYCN* is retained in single copy at chromosome 2 band p23-24 during amplification in human neuroblastoma cells. *Proc. Natl Acad. Sci. USA* **91**, 5523–5527 (1994).
21. Schneider, S. S. *et al.* Isolation and structural analysis of a 1.2-megabase N-myc amplicon from a human neuroblastoma. *Mol. Cell. Biol.* **12**, 5563–5570 (1992).
22. Brodeur, G. M. & Fong, C. T. Molecular biology and genetics of human neuroblastoma. *Cancer Genet. Cytogenet.* **41**, 153–174 (1989).
23. Reiter, J. L. & Brodeur, G. M. High-resolution mapping of a 130-kb core region of the *MYCN* amplicon in neuroblastomas. *Genomics* **32**, 97–103 (1996).
24. Reiter, J. L. & Brodeur, G. M. *MYCN* is the only highly expressed gene from the core amplified domain in human neuroblastomas. *Genes Chromosom. Cancer* **23**, 134–140 (1998).
25. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* **224**, 1121–1124 (1984).
Amplification of the *MYCN* oncogene is strongly associated with advanced stages of disease in neuroblastoma.
26. Seeger, R. C. *et al.* Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N. Engl. J. Med.* **313**, 1111–1116 (1985).
The first report to show the adverse prognostic significance of *MYCN* amplification in neuroblastoma patients.
27. Brodeur, G. M., Maris, J. M., Yamashiro, D. J., Hogarty, M. D. & White, P. S. Biology and genetics of human neuroblastomas. *J. Pediatr. Hematol. Oncol.* **19**, 93–101 (1997).
28. Brodeur, G. M. & Ambros, P. F. in *Neuroblastoma* (eds Brodeur, G. M., Sawada, T., Tsuchida, Y. & Voûte, P. A.) 355–369 (Elsevier Science B. V., Amsterdam, 2000).
29. Brodeur, G. M. & Maris, J. M. in *Principles and Practice of Pediatric Oncology* (eds Pizzo, P. & Poplack, D.) 895–937 (2002).
30. Brodeur, G. M. in *The Genetic Basis of Human Cancer* (eds Vogelstein, B. & Kinzler, K. W.) 751–772 (McGraw–Hill, Inc., New York, 2002).
31. Brodeur, G. M. *et al.* Consistent N-myc copy number in simultaneous or consecutive neuroblastoma samples from sixty individual patients. *Cancer Res.* **47**, 4248–4253 (1987).
32. Seeger, R. C. *et al.* Expression of N-myc by neuroblastomas with one or multiple copies of the oncogene. *Prog. Clin. Biol. Res.* **271**, 41–49 (1988).
33. Norris, M. D. *et al.* Evidence that the *MYCN* oncogene regulates MRP gene expression in neuroblastoma. *Eur. J. Cancer* **33**, 1911–1916 (1997).
34. Shohet, J. M. *et al.* Minichromosome maintenance protein MCM7 is a direct target of the MYCN transcription factor in neuroblastoma. *Cancer Res.* **62**, 1123–1128 (2002).
35. Nakagawara, A., Arima, M., Azar, C. G., Scavarda, N. J. & Brodeur, G. M. Inverse relationship between TRK expression and N-MYC amplification in human neuroblastomas. *Cancer Res.* **52**, 1364–1368 (1992).
36. Wada, R. K. *et al.* Human neuroblastoma cell lines that express N-myc without gene amplification. *Cancer* **72**, 3346–3354 (1993).
37. Cohn, S. L. *et al.* High levels of N-myc protein in a neuroblastoma cell line lacking N-myc amplification. *Prog. Clin. Biol. Res.* **366**, 21–27 (1991).
38. Sivak, L. E. *et al.* Autoregulation of the human N-myc oncogene is disrupted in amplified but not single-copy neuroblastoma cell lines. *Oncogene* **15**, 1937–1946 (1997).
39. Chan, H. S. *et al.* MYCN protein expression as a predictor of neuroblastoma prognosis. *Clin. Cancer Res.* **3**, 1699–1706 (1997).
40. Bordow, S. B., Norris, M. D., Haber, P. S., Marshall, G. M. & Haber, M. Prognostic significance of *MYCN* oncogene expression in childhood neuroblastoma. *J. Clin. Oncol.* **16**, 3286–3294 (1998).
41. Cohn, S. L. *et al. MYCN* expression is not prognostic of adverse outcome in advanced-stage neuroblastoma with nonamplified MYCN. *J. Clin. Oncol.* **18**, 3604–3613 (2000).
42. Fong, C. T. *et al.* Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastomas: correlation with N-myc amplification. *Proc. Natl Acad. Sci. USA* **86**, 3753–3757 (1989).
43. Gehring, M., Berthold, F., Edler, L., Schwab, M. & Amler, L. C. The 1p deletion is not a reliable marker for the prognosis of patients with neuroblastoma. *Cancer Res.* **55**, 5366–5369 (1995).
44. Caron, H. *et al.* Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. *N. Engl. J. Med.* **334**, 225–230 (1996).
45. Maris, J. M. *et al.* Loss of heterozygosity at 1p36 independently predicts for disease progression but not decreased overall survival probability in neuroblastoma patients: a Children's Cancer Group study. *J. Clin. Oncol.* **18**, 1888–1899 (2000).
46. Jinbo, T., Iwamura, Y., Kaneko, M. & Sawaguchi, S. Coamplification of the L-myc and N-myc oncogenes in a neuroblastoma cell line. *Jpn. J. Cancer Res.* **80**, 299–301 (1989).
47. Corvi, R. *et al.* Non-syntenic amplification of *MDM2* and *MYCN* in human neuroblastoma. *Oncogene* **10**, 1081–1086 (1995).
48. Van Roy, N. *et al.* Identification of two distinct chromosome 12-derived amplification units in neuroblastoma cell line NGP. *Cancer Genet. Cytogenet.* **82**, 151–154 (1995).
49. Brinkschmidt, C. *et al.* Comparative genomic hybridization (CGH) analysis of neuroblastomas — an important methodological approach in paediatric tumour pathology. *J. Pathol.* **181**, 394–400 (1997).
50. Lastowska, M. *et al.* Comparative genomic hybridization study of primary neuroblastoma tumors. United Kingdom Children's Cancer Study Group. *Genes Chromosom. Cancer* **18**, 162–169 (1997).
51. Vandesompele, J. *et al.* Genetic heterogeneity of neuroblastoma studied by comparative genomic hybridization. *Genes Chromosom. Cancer* **23**, 141–152 (1998).
52. Caron, H. Allelic loss of chromosome 1 and additional chromosome 17 material are both unfavourable prognostic markers in neuroblastoma. *Med. Pediatr. Oncol.* **24**, 215–221 (1995).
53. Bown, N. *et al.* Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. *N. Engl. J. Med.* **340**, 1954–1961 (1999).
Definitive report of the prevalence and clinical significance of unbalanced 17q gain in neuroblastomas.
54. Van Roy, N. *et al.* Analysis of 1;17 translocation breakpoints in neuroblastoma: implications for mapping of neuroblastoma genes. *Eur. J. Cancer* **33**, 1974–1978 (1997).
55. Lastowska, M. *et al.* Breakpoint position on 17q identifies the most aggressive neuroblastoma tumors. *Genes Chromosom. Cancer* **34**, 428–436 (2002).
56. Islam, A. *et al.* High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. *Oncogene* **19**, 617–623 (2000).
57. Ireland, C. M. Activated N-ras oncogenes in human neuroblastoma. *Cancer Res.* **49**, 5530–5533 (1989).
58. Moley, J. F. *et al.* Low frequency of *ras* gene mutations in neuroblastomas, pheochromocytomas and medullary thyroid cancers. *Cancer Res.* **51**, 1596–1599 (1991).

59. Tanaka, T. *et al.* Expression of Ha-ras oncogene products in human neuroblastomas and the significant correlation with a patient's prognosis. *Cancer Res.* **48**, 1030–1034 (1988).
60. Brodeur, G. M. *et al.* Cytogenetic features of human neuroblastomas and cell lines. *Cancer Res.* **41**, 4678–4686 (1981).
Definitive report of distal 1p deletions as a genetic change characteristic of neuroblastomas. The clinical significance of deletion of 1p was shown subsequently in large studies.
61. White, P. S. *et al.* A region of consistent deletion in neuroblastoma maps within 1p36.2-3. *Proc. Natl Acad. Sci. USA* **92**, 5520–5524 (1995).
62. Martinsson, T., Shoberg, P.-M., Hedborg, F. & Kogner, P. Deletion of chromosome 1p loci and microsatellite instability in neuroblastomas analyzed with short-tandem repeat polymorphisms. *Cancer Res.* **55**, 5681–5686 (1995).
63. Ejeskar, K. *et al.* Fine mapping of a tumour suppressor candidate gene region in 1p36.2-3, commonly deleted in neuroblastomas and germ cell tumours. *Med. Pediatr. Oncol.* **36**, 61–66 (2001).
64. Caron, H. *et al.* Chromosome bands 1p35-36 contain two distinct neuroblastoma tumor suppressor loci, one of which is imprinted. *Genes Chromosom. Cancer* **30**, 168–174 (2001).
65. Bauer, A. *et al.* Smallest region of overlapping deletion in 1p36 in human neuroblastoma: a 1 Mbp cosmid and PAC contig. *Genes Chromosom. Cancer* **31**, 228–239 (2001).
66. Hogarty, M. D. *et al.* Identification of a 1-megabase consensus region of deletion at 1p36.3 in primary neuroblastomas. *Med. Pediatr. Oncol.* **35**, 512–515 (2000).
67. Maris, J. M. *et al.* Comprehensive analysis of chromosome 1p deletions in neuroblastoma. *Med. Pediatr. Oncol.* **36**, 32–36 (2001).
68. Ohira, M. *et al.* Identification and characterization of a 500-kb homozygously deleted region at 1p36.2-p36.3 in a neuroblastoma cell line. *Oncogene* **19**, 4302–4307 (2000).
69. Chen, Y. Z. *et al.* Homozygous deletion in a neuroblastoma cell line defined by a high-density STS map spanning human chromosome band 1p36. *Genes Chromosom. Cancer* **31**, 326–332 (2001).
70. Srivatsan, E. S., Ying, K. L. & Seeger, R. C. Deletion of chromosome 11 and of 14q sequences in neuroblastoma. *Genes Chromosom. Cancer* **7**, 32–37 (1993).
71. Plantaz, D. *et al.* Comparative genomic hybridization (CGH) analysis of stage 4 neuroblastoma reveals high frequency of 11q deletion in tumors lacking MYCN amplification. *Int. J. Cancer* **91**, 680–686 (2001).
72. Guo, C. *et al.* Allelic deletion at 11q23 is common in MYCN single copy neuroblastomas. *Oncogene* **18**, 4948–4957 (1999).
73. Suzuki, T. *et al.* Frequent loss of heterozygosity on chromosome 14q in neuroblastoma. *Cancer Res.* **49**, 1095–1098 (1989).
74. Hoshi, M. *et al.* Detailed deletion mapping of chromosome band 14q32 in human neuroblastoma defines a 1.1-Mb region of common allelic loss. *Br. J. Cancer* **82**, 1801–1807 (2000).
75. Thompson, P. M. *et al.* Loss of heterozygosity for chromosome 14q in neuroblastoma. *Med. Pediatr. Oncol.* **36**, 28–31 (2001).
76. Vogan, K. *et al.* Absence of p53 gene mutations in primary neuroblastomas. *Cancer Res.* **53**, 5269–5273 (1993).
77. Hosoi, G. *et al.* Low frequency of the p53 gene mutations in neuroblastoma. *Cancer* **73**, 3087–3093 (1994).
78. Keshelava, N. *et al.* Loss of p53 function confers high-level multidrug resistance in neuroblastoma cell lines. *Cancer Res.* **61**, 6185–6193 (2001).
79. Tweddle, D. A., Malcolm, A. J., Bown, N., Pearson, A. D. & Lunec, J. Evidence for the development of p53 mutations after cytotoxic therapy in a neuroblastoma cell line. *Cancer Res.* **61**, 8–13 (2001).
80. Moll, U. M., LaQuaglia, M., Benard, J. & Riou, G. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc. Natl Acad. Sci. USA* **92**, 4407–4411 (1995).
81. Moll, U. M. *et al.* Cytoplasmic sequestration of wild-type p53 protein impairs the G1 checkpoint after DNA damage. *Mol. Cell. Biol.* **16**, 1126–1137 (1996).
82. Goldman, S. C., Chen, C. Y., Lansing, T. J., Gilmer, T. M. & Kastan, M. B. The p53 signal transduction pathway is intact in human neuroblastoma despite cytoplasmic localization. *Am. J. Pathol.* **148**, 1381–1385 (1996).
83. Beltinger, C. P., White, P. S., Sulman, E. P., Maris, J. M. & Brodeur, G. M. No CDKN2 mutations in neuroblastomas. *Cancer Res.* **55**, 2053–2055 (1995).
84. Iolascon, A. *et al.* Structural and functional analysis of cyclin-dependent kinase inhibitor genes (*CDKN2A*, *CDKN2B*, and *CDKN2C*) in neuroblastoma. *Pediatr. Res.* **43**, 139–144 (1998).
85. Kawamata, N., Seriu, T., Koeffler, H. P. & Bartram, C. R. Molecular analysis of the cyclin-dependent kinase inhibitor family: p16(CDKN2/MTS1/INK4A), p18(INK4C) and p27(Kip1) genes in neuroblastomas. *Cancer* **77**, 570–575 (1996).
86. Thompson, P. M. *et al.* Homozygous deletion of *CDKN2A* (p16INK4a/p14ARF) but not within 1p36 or at other tumor suppressor loci in neuroblastoma. *Cancer Res.* **61**, 679–686 (2001).
87. Johnson, M. R., Look, A. T., DeClue, J. E., Valentine, M. B. & Lowy, D. R. Inactivation of the NF1 gene in human melanoma and neuroblastoma cell lines without impaired regulation of GTP Ras. *Proc. Natl Acad. Sci. USA* **90**, 5539–5543 (1993).
88. The, I. *et al.* Neurofibromatosis type 1 gene mutations in neuroblastoma. *Nature Genet.* **3**, 62–66 (1993).
89. Yano, H. & Chao, M. V. Neurotrophin receptor structure and interactions. *Pharm. Acta Helv.* **74**, 253–260 (2000).
90. Patapoutian, A. & Reichardt, L. F. Trk receptors: mediators of neurotrophin action. *Curr. Opin. Neurobiol.* **11**, 272–280 (2001).
91. Nakagawara, A. *et al.* Association between high levels of expression of the TRK gene and favorable outcome in human neuroblastoma. *N. Engl. J. Med.* **328**, 847–854 (1993).
The first report to show the favourable prognostic value of TrkA expression in neuroblastomas. Other reports were reported independently with similar results.
92. Suzuki, T., Bogenmann, E., Shimada, H., Stram, D. & Seeger, R. C. Lack of high-affinity nerve growth factor receptors in aggressive neuroblastomas. *J. Natl Cancer Instit.* **85**, 377–384 (1993).
93. Kogner, P. *et al.* Coexpression of messenger RNA for TRK protooncogene and low affinity nerve growth factor receptor in neuroblastoma with favorable prognosis. *Cancer Res.* **53**, 2044–2050 (1993).
94. Ambros, I. M. *et al.* Role of ploidy, chromosome 1p, and Schwann cells in the maturation of neuroblastoma. *N. Engl. J. Med.* **334**, 1505–1511 (1996).
95. Ambros, I. M. *et al.* Neuroblastoma cells provoke Schwann cell proliferation *in vitro*. *Med. Pediatr. Oncol.* **36**, 163–168 (2001).
96. Nakagawara, A. & Brodeur, G. M. Role of neurotrophins and their receptors in human neuroblastomas: a primary culture study. *Eur. J. Cancer* **33**, 2050–2053 (1997).
97. Nakagawara, A., Azar, C. G., Scavarda, N. J. & Brodeur, G. M. Expression and function of TRK-B and BDNF in human neuroblastomas. *Mol. Cell. Biol.* **14**, 759–767 (1994).
The first report to associate TrkB and BDNF expression with high-risk neuroblastomas that have *MYCN* amplification.
98. Acheson, A. *et al.* A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature* **374**, 450–453 (1995).
99. Matsumoto, K., Wada, R. K., Yamashiro, J. M., Kaplan, D. R. & Thiele, C. J. Expression of brain-derived neurotrophic factor and $p145^{TrkB}$ affects survival, differentiation, and invasiveness of human neuroblastoma cells. *Cancer Res.* **55**, 1798–1806 (1995).
100. Eggert, A. *et al.* Expression of neurotrophin receptor TrkA inhibits angiogenesis in neuroblastoma. *Med. Pediatr. Oncol.* **35**, 569–572 (2000).
101. Ho, R. *et al.* Resistance to chemotherapy mediated by TrkB in neuroblastomas. *Cancer Res.* **62**, 6462–6466 (2002).
102. Yamashiro, D. J., Nakagawara, A., Ikegaki, N., Liu, X.-G. & Brodeur, G. M. Expression of TrkC in favorable human neuroblastomas. *Oncogene* **12**, 37–41 (1996).
103. Ryden, M. *et al.* Expression of mRNA for the neurotrophin receptor TrkC in neuroblastomas with favourable tumour stage and good prognosis. *Br. J. Cancer* **74**, 773–779 (1996).
104. Casaccia-Bonnefil, P., Gu, C. & Chao, M. V. Neurotrophins in cell survival/death decisions. *Adv. Exp. Med. Biol.* **468**, 275–282 (1999).
105. Hempstead, B. L. The many faces of p75NTR. *Curr. Opin. Neurobiol.* **12**, 260–267 (2002).
106. Goldstein, L. J. *et al.* Expression of the multidrug resistance, MDR1, gene in neuroblastomas. *J. Clin. Oncol.* **8**, 128–136 (1990).
107. Chan, H. S. *et al.* P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N. Engl. J. Med.* **325**, 1608–1614 (1991).
108. Norris, M. D. *et al.* Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. *N. Engl. J. Med.* **334**, 231–238 (1996).
109. Hiyama, E. *et al.* Correlating telomerase activity levels with human neuroblastoma outcomes. *Nature Med.* **1**, 249–255 (1995).
110. Brodeur, G. M. Do the ends justify the means? *Nature Med.* **1**, 203–205 (1995).
111. Brodeur, G. M. & Castle, V. P. in *Apoptosis and Cancer Chemotherapy* (eds Hickman, J. A. & Dive, C.) 305–318 (Humana, New Jersey, 1999).
112. Bunone, G., Mariotti, A., Compagni, A., Morandi, E. & Della Valle, G. Induction of apoptosis by p75 neurotrophin receptor in human neuroblastoma cells. *Oncogene* **14**, 1463–1470 (1997).
113. Fulda, S., Sieverts, H., Friesen, C., Herr, I. & Debatin, K. M. The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. *Cancer Res.* **57**, 3823–3829 (1997).
114. Castle, V. P. *et al.* Expression of the apoptosis-suppressing protein bcl-2, in neuroblastoma is associated with unfavorable histology and N-myc amplification. *Am. J. Pathol.* **143**, 1543–1550 (1993).
115. Oue, T. *et al. In situ* detection of DNA fragmentation and expression of bcl-2 in human neuroblastoma: relation to apoptosis and spontaneous regression. *J. Pediatr. Surg.* **31**, 251–257 (1996).
116. Dole, M. *et al.* Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res.* **54**, 3253–3259 (1994).
117. Dole, M. G. *et al.* Bcl-x_L is expressed in neuroblastoma cells and modulates chemotherapy-induced apoptosis. *Cancer Res.* **55**, 2576–2582 (1995).
118. Nakagawara, A. *et al.* High levels of expression and nuclear localization of interleukin-1β converting enzyme (ICE) and CPP32 in favorable human neuroblastomas. *Cancer Res.* **57**, 4578–4584 (1997).
119. Westermann, F. & Schwab, M. Genetic parameters of neuroblastomas. *Cancer Lett.* **184**, 127–147 (2002).
120. Brodeur, G. M. *et al.* Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. *J. Clin. Oncol.* **11**, 1466–1477 (1993).
A description of the International Neuroblastoma Staging System currently used throughout the world.
121. Hann, H. W. L. *et al.* Prognostic importance of serum ferritin in patients with stages III and IV neuroblastoma. The Children's Cancer Study Group Experience. *Cancer Res.* **45**, 2843–2848 (1985).
122. Zeltzer, P. M., Marangos, P. J., Evans, A. E. & Schneider, S. L. Serum neuron-specific enolase in children with neuroblastoma. Relationship to stage and disease course. *Cancer* **57**, 1230–1234 (1986).
123. Ladisch, S. & Wu, Z. L. Detection of a tumour-associated ganglioside in plasma of patients with neuroblastoma. *Lancet* **1**, 136–138 (1985).
124. Quinn, J. J., Altman, A. J. & Frantz, C. N. Serum lactic dehydrogenase, an indicator of tumor activity in neuroblastoma. *J. Pediatr.* **97**, 89–91 (1980).
125. Shuster, J. J. *et al.* Serum lactate dehydrogenase in childhood neuroblastoma. A Pediatric Oncology Group recursive partitioning study. *Am. J. Clin. Oncol.* **15**, 295–303 (1992).
126. Shimada, H. *et al.* Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. *J. Natl Cancer Inst.* **73**, 405–413 (1984).
The original report of the popular histopathological classification for predicting outcome of neuroblastoma patients. This was subsequently revised into the International Neuroblastoma Pathology Classification.
127. Shimada, H. *et al.* Terminology and morphologic criteria of neuroblastic tumors: recommendations by the International Neuroblastoma Pathology Committee. *Cancer* **86**, 349–363 (1999).
128. Shimada, H. *et al.* The International Neuroblastoma Pathology Classification (the Shimada system). *Cancer* **86**, 364–372 (1999).
129. Combaret, V. *et al.* Clinical relevance of CD44 cell-surface expression and N-myc gene amplification in a multicentric analysis of 121 pediatric neuroblastomas. *J. Clin. Oncol.* **14**, 25–34 (1996).
130. Castleberry, R. P. *et al.* The International Neuroblastoma Risk Groups (INRG): a preliminary report. *Eur. J. Cancer* **33**, 2113–2116 (1997).
First report of an international consensus on neuroblastoma risk groups using a combination of clinical and biological variables.
131. Beckwith, J. & Perrin, E. *In situ* neuroblastomas: a contribution to the natural history of neural crest tumors. *Am. J. Pathol.* **43**, 1089–1104 (1963).
132. Turkel, S. B. & Itabashi, H. H. The natural history of neuroblastic cells in the fetal adrenal gland. *Am. J. Pathol.* **76**, 225–243 (1975).
133. Ikeda, Y., Lister, J., Bouton, J. M. & Buyukpamukcu, M. Congenital neuroblastoma, neuroblastoma *in situ*, and the

normal fetal development of the adrenal. *J. Pediatr. Surg.* **16**, 636–644 (1981).
134. Evans, A. E., Gerson, J. & Schnaufer, L. Spontaneous regression of neuroblastoma. *Natl Cancer Inst. Monogr.* **44**, 49–54 (1976).
135. Sawada, T. *et al.* Neuroblastoma. Mass screening for early detection and its prognosis. *Cancer* **53**, 2731–2735 (1984).
A seminal paper that indicates the potential value of mass screening for early detection of disease to improve the prognosis of neuroblastoma. Subsequent reports from mass screening programmes in Quebec and Germany indicate that there is no impact on mortality.
136. Takeda, T. *et al.* Japanese experience of screening. *Med. Pediatr. Oncol.* **17**, 368–372 (1989).
137. Schilling, F. H. *et al.* Screening for neuroblastoma. *Lancet* **344**, 1157–1158 (1994).
138. Woods, W. G. *et al.* A population-based study of the usefulness of screening for neuroblastoma. *Lancet* **348**, 1682–1687 (1996).
139. Kaneko, Y. *et al.* Chromosomes and screening for neuroblastoma. *Lancet* **1**, 174–175 (1988).
140. Hayashi, Y., Inaba, T., Hanada, R. & Yamamoto, K. Chromosome findings and prognosis in 15 patients with neuroblastoma found by VMA mass screening. *J. Pediatr.* **112**, 567–571 (1988).
141. Hayashi, Y., Hanada, R. & Yamamoto, K. Biology of neuroblastomas in Japan found by screening. *Am. J. Pediatr. Hematol. Oncol.* **14**, 342–347 (1992).
142. Brodeur, G. M. *et al.* Biological aspects of neuroblastomas identified by mass screening in Quebec. *Med. Pediatr. Oncol.* **36**, 157–159 (2001).
143. Woods, W. G. *et al.* Screening of infants and mortality due to neuroblastoma. *N. Engl. J. Med.* **346**, 1041–1046 (2002).
144. Schilling, F. H. *et al.* Neuroblastoma screening at one year of age. *N. Engl. J. Med.* **346**, 1047–1053 (2002).
145. Kaneko, Y., Kobayashi, H., Maseki, N., Nakagawara, A. & Sakurai, M. Disomy 1 with terminal 1p deletion is frequent in mass-screening-negative/late-presenting neuroblastomas in young children, but not in mass-screening-positive neuroblastomas in infants. *Int. J. Cancer* **80**, 54–59 (1999).
146. Tajiri, T. *et al.* Clinical and biologic characteristics for recurring neuroblastoma at mass screening cases in Japan. *Cancer* **92**, 349–353 (2001).
147. van Limpt, V. *et al.* SAGE analysis of neuroblastoma reveals a high expression of the human homologue of the *Drosophila* Delta gene. *Med. Pediatr. Oncol.* **35**, 554–558 (2000).
148. Spieker, N. *et al.* The MEIS1 oncogene is highly expressed in neuroblastoma and amplified in cell line IMR32. *Genomics* **71**, 214–221 (2001).
149. Khan, J. *et al.* Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nature Med.* **7**, 673–679 (2001).
150. Truckenmiller, M. E. *et al.* Gene expression profile in early stage of retinoic acid-induced differentiation of human SH-SY5Y neuroblastoma cells. *Restor. Neurol. Neurosci.* **18**, 67–80 (2001).
151. Weiss, W. A., Aldape, K., Mohapatra, G., Feuerstein, B. G. & Bishop, J. M. Targeted expression of *MYCN* causes neuroblastoma in transgenic mice. *EMBO J.* **16**, 2985–2995 (1997).
First report of a transgenic mouse that overexpresses the *MYCN* proto-oncogene under the control of a tyrosine kinase promoter and develops neuroblastoma with high frequency.
152. Weiss, W. A., Godfrey, T., Francisco, C. & Bishop, J. M. Genome-wide screen for allelic imbalance in a mouse model for neuroblastoma. *Cancer Res.* **60**, 2483–2487 (2000).
153. Norris, M. D., Burkhart, C. A., Marshall, G. M., Weiss, W. A. & Haber, M. Expression of N-myc and MRP genes and their relationship to N-myc gene dosage and tumor formation in a murine neuroblastoma model. *Med. Pediatr. Oncol.* **35**, 585–589 (2000).
154. Sidell, N., Altman, A., Haussler, M. R. & Seeger, R. C. Effects of retinoic acid (RA) on the growth and phenotypic expression of several human neuroblastoma cell lines. *Exp. Cell Res.* **148**, 21–30 (1983).
155. Thiele, C. J., Reynolds, C. P. & Israel, M. A. Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* **313**, 404–406 (1985).
156. Reynolds, C. P. *et al.* Comparison of 13-*cis*-retinoic acid to *trans*-retinoic acid using human neuroblastoma cell lines. *Prog. Clin. Biol. Res.* **385**, 237–244 (1994).
157. Matthay, K. K. *et al.* Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-*cis*-retinoic acid. Children's Cancer Group. *N. Engl. J. Med.* **341**, 1165–1173 (1999).
First clinical trial indicating that a survival advantage exists for the treatment of neuroblastoma patients with retinoic acid following bone-marrow transplantation.
158. Lovat, P. E. *et al.* Effector mechanisms of fenretinide-induced apoptosis in neuroblastoma. *Exp. Cell Res.* **260**, 50–60 (2000).
159. Ponzoni, M. *et al.* Differential effects of *N*-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: apoptosis versus differentiation. *Cancer Res.* **55**, 853–861 (1995).
160. Reynolds, C. P. Differentiating agents in pediatric malignancies: retinoids in neuroblastoma. *Curr. Oncol. Rep.* **2**, 511–518 (2000).
161. Galderisi, U., Cascino, A. & Giordano, A. Antisense oligonucleotides as therapeutic agents. *J. Cell Physiol.* **181**, 251–257 (1999).
162. Evans, A. E. *et al.* Antitumor activity of CEP-751 (KT-6587) on human neuroblastoma and medulloblastoma xenografts. *Clin. Cancer Res.* **5**, 3594–3602 (1999).
First report of a tyrosine kinase inhibitor that is selective for Trk receptors with potential use in treating neuroblastomas.
163. Meitar, D., Crawford, S. E., Rademaker, A. W. & Cohn, S. L. Tumor angiogenesis correlates with metastatic disease, N-myc amplification, and poor outcome in human neuroblastoma. *J. Clin. Oncol.* **14**, 405–414 (1996).
Definitive report correlating tumour angiogenesis with high-risk features and outcome in neuroblastomas. This report serves as the rationale for anti-angiogenesis therapy in high-risk neuroblastomas.
164. Wassberg, E., Pahlman, S., Westlin, J. E. & Christofferson, R. The angiogenesis inhibitor TNP-470 reduces the growth rate of human neuroblastoma in nude rats. *Pediatr. Res.* **41**, 327–333 (1997).
165. Katzenstein, H. M. *et al.* Effectiveness of the angiogenesis inhibitor TNP-470 in reducing the growth of human neuroblastoma in nude mice inversely correlates with tumor burden. *Clin. Cancer Res.* **5**, 4273–4278 (1999).
166. Shusterman, S., Grupp, S. A. & Maris, J. M. Inhibition of tumor growth in a human neuroblastoma xenograft model with TNP-470. *Med. Pediatr. Oncol.* **35**, 673–676 (2000).
167. Erdreich-Epstein, A. *et al.* Integrins $\alpha(v)\beta3$ and $\alpha(v)\beta5$ are expressed by endothelium of high-risk neuroblastoma and their inhibition is associated with increased endogenous ceramide. *Cancer Res.* **60**, 712–721 (2000).
168. Jouanneau, E. *et al.* Lack of antitumor activity of recombinant endostatin in a human neuroblastoma xenograft model. *J. Neurooncol.* **51**, 11–18 (2001).
169. Kim, E. S. *et al.* Distinct response of experimental neuroblastoma to combination antiangiogenic strategies. *J. Pediatr. Surg.* **37**, 518–522 (2002).
170. Davidoff, A. M., Leary, M. A., Ng, C. Y. & Vanin, E. F. Gene therapy-mediated expression by tumor cells of the angiogenesis inhibitor flk-1 results in inhibition of neuroblastoma growth *in vivo*. *J. Pediatr. Surg.* **36**, 30–36 (2001).
171. Hanahan, D., Bergers, G. & Bergsland, E. Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. *J. Clin. Invest.* **105**, 1045–1047 (2000).
172. Frost, J. D. *et al.* A phase I/IB trial of murine monoclonal anti-GD2 antibody 14. G2a plus interleukin-2 in children with refractory neuroblastoma: a report of the Children's Cancer Group. *Cancer* **80**, 317–333 (1997).
173. Yu, A. L. *et al.* Phase I trial of a human-mouse chimeric anti-disialoganglioside monoclonal antibody ch14.18 in patients with refractory neuroblastoma and osteosarcoma. *J. Clin. Oncol.* **16**, 2169–2180 (1998).
174. Cheung, N. K., Kushner, B. H., Yeh, S. D. & Larson, S. M. 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma: a phase II study. *Int. J. Oncol.* **12**, 1299–1306 (1998).
155. De Kraker, J. *et al.* First line targeted radiotherapy, a new concept in the treatment of advanced stage neuroblastoma. *Eur. J. Cancer* **31A**, 600–602 (1995).
176. Matthay, K. K. *et al.* Phase I dose escalation of 131I-metaiodobenzylguanidine with autologous bone marrow support in refractory neuroblastoma. *J. Clin. Oncol.* **16**, 229–236 (1998).
177. Yanik, G. A. *et al.* Pilot study of iodine-131-metaiodobenzylguanidine in combination with myeloablative chemotherapy and autologous stem-cell support for the treatment of neuroblastoma. *J. Clin. Oncol.* **20**, 2142–2149 (2002).
178. Fitzek, M. M. *et al.* Neuroendocrine tumors of the sinonasal tract. Results of a prospective study incorporating chemotherapy, surgery, and combined proton-photon radiotherapy. *Cancer* **94**, 2623–2634 (2002).
179. Luttikhuis, M. E. *et al.* Neuroblastomas with chromosome 11q loss and single copy MYCN comprise a biologically distinct group of tumours with adverse prognosis. *Br. J. Cancer* **85**, 531–537 (2001).
180. Bhattacharyya, N., Thornton, A. F., Joseph, M. P., Goodman, M. L. & Amrein, P. C. Successful treatment of esthesioneuroblastoma and neuroendocrine carcinoma with combined chemotherapy and proton radiation. Results in 9 cases. *Arch. Otolaryngol. Head Neck Surg.* **123**, 34–40 (1997).
181. Gurney, J. G. *et al.* Infant cancer in the US: histology-specific incidence and trends, 1973 to 1992. *J. Pediatr. Hematol. Oncol.* **19**, 428–432 (1997).
182. Schmidt, M. L. *et al.* Biologic factors determine prognosis in infants with stage IV neuroblastoma: a prospective Children's Cancer Group study. *J. Clin. Oncol.* **18**, 1260–1268 (2000).

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