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Neuroblastoma is the most frequently diagnosed extracranial solid tumour in childhood. While a subset of tumours show spontaneous regression or complete remission following conventional treatment, a substantial number remain resistant to intensive multimodal therapies. Survival rates approaching 40% place high-risk neuroblastoma as one of the greatest challenges in paediatric oncology. This contemporary review provides an update on the diagnosis, risk stratification and management for this enigmatic tumour.

Neuroblastoma is the most common extracranial solid tumour in childhood and the most frequently diagnosed neoplasm during infancy.[1] This malignant tumour consists of undifferentiated and/or differentiating cells originating from neural crest-derived sympathoadrenal precursors. Neuroblastoma is often described as "enigmatic" and "unpredictable" because of the broad spectrum of clinical behaviour ranging from life-threatening progression despite intensive treatment to complete spontaneous regression. Although outcome for certain subsets of patients has improved over the past few decades, children with high-risk disease continue to have less than 40% long-term survival.[2] New therapeutic options are being sought through advances in basic science and translational clinical research.

EPIDEMIOLOGY, GENETIC PREDISPOSITION AND RISK FACTORS

Neuroblastoma accounts for more than 7% of malignancies in patients younger than 15 years and around 15% of all paediatric oncology deaths.[3] The incidence of neuroblastoma in predominantly Caucasian populations is 9–12 per million children.[4] A family history of neuroblastoma has been reported in 1% to 2% of patients[5] and follows an autosomal dominant pattern of inheritance. Supporting the Knudson two-mutation hypothesis, the median age at diagnosis for familial neuroblastoma cases is 9 months compared to 18 months in sporadic cases.[3] Associations with Hirschsprung disease and congenital central hypoventilation syndrome[6] with a shared PHOX2b mutation proposed to explain this link[7] have been reported, as has neurofibromatosis type 1.[8] At present, genetic predisposition to neuroblastoma appears to be heterogeneous and hence tumourigenesis is postulated to need multiple genetic alterations.[3]

Although several factors including maternal gestational weight gain, type of delivery, birth weight, gestational age, maternal smoking and prenatal hormone exposure have been investigated as potential risk factors for development of neuroblastoma, little evidence exists to prove definitively the role of any of these in the aetiology of this condition.

PATHOGENESIS: CELL OF ORIGIN AND CANCER STEM CELL HYPOTHESIS

Neuroblastoma originates from neural crest-derived cells and most commonly arises from the adrenal medulla or the abdominal sympathetic ganglia. The cancer stem cell hypothesis suggests that many if not all tumours contain a small number of cancer stem cells (CSCs) which express early developmental markers and may act as a reservoir of cancer cells.[9] CSCs are thought to be responsible for tumourigenesis, progression and metastasis, as well as relapse, and should be regarded as critical therapeutic targets in eradicating cancer. Cytotoxic agents preferentially kill tumour cells with a rapid turnover. However, CSCs can escape this process by a slower proliferation rate and the presence of cell membrane-associated drug transporters, thus allowing subsequent relapse. The search for CSCs in neuroblastoma remains elusive because specific reliable markers for their identification are not available. Of the three cell types that have been well characterised in neuroblastoma cell lines, namely, stromal (S type), neuroblastic (N type) and intermediate (I type), the I-type cell most closely resembles a CSC by its unique morphological, biochemical, differentiative and tumourigenic properties.[10] To what extent these findings can be translated to neuroblastoma tumours is still under investigation.

PATHOLOGY AND GENETIC FEATURES

Tumour pathology

The currently accepted International Neuroblastoma Pathology Classification (INPC) system (table 1)[10] was modified from the Shimada classification of neuroblastoma, and is based on age at diagnosis, mitosis–karyorrhexis index (MKI), neuroblastic differentiation and stromal content. INPC has a proven role in predicting outcome.[11]

Genetic features

MYC oncoproteins are transcription factors that may cause deregulated growth on overexpression. In neuroblastoma, MYCN amplification is strongly associated with rapid disease progression and poor

Table 1 Prognostic evaluation of neuroblastoma tumours according to the International Neuroblastoma Pathology Classification (INPC/Shimada) system

Age	Pathology	Prognostic group
<1.5 years	Poorly differentiated or differentiating and low or intermediate MKI	Favourable
<1.5 years	Undifferentiated tumour or high MKI	Unfavourable
1.5–5 years	Undifferentiated or poorly differentiated and intermediate or high MKI	Unfavourable
>5 years	All tumours	Unfavourable

MKI, mitosis–karyorrhexis index.

outcome in patients at all ages, infants included, and at all stages.[12] However, about 80% of neuroblastomas harbour non-amplified MYCN. Several other somatically acquired chromosomal aberrations associated with DNA copy number alterations (CNAs), together with tumour cell DNA content, have been shown to predict neuroblastoma behaviour. In particular, aggressive tumour behaviour and poor outcome are associated with deletions at the chromosomal region 1p36.3[13] or 11q23,[14] and with unbalanced gain of the long arm of chromosome 17 (17q21 to 17qter).[15] Since 11q23 deletion is inversely associated with MYCN amplification,[14] this feature is now regarded as a powerful marker of poor outcome in patients with no MYCN amplification. Tumour cell DNA content in neuroblastoma falls into two main categories, near-diploidy or hyperdiploidy (more often triploidy); in patients younger than 18 months with metastatic disease near diploid DNA content is a predictor of poor outcome.[16 17] Recently, the Biology Committee of the International Neuroblastoma Risk Group (INRG) has recommended that for accurate and reproducible risk class stratification, neuroblastoma tumours should at least be assessed for MYCN, tumour cell DNA content and 11q23, while the meaning of several other genetic aberrations deserves further prospective validation.[18] Furthermore, genome-wide assessment of DNA aberrations using comprehensive genomic hybridisation arrays has increasingly been utilised as a powerful tool to detect complex patterns of CNAs and identify distinct genetic subgroups.[19–21] Improved approaches integrating genome-wide analysis of DNA alterations with gene expression profiles are likely to replace region-specific methods in the analysis of DNA CNAs to be employed in risk group stratification.

CLINICAL PRESENTATION, DIAGNOSIS AND STAGING

Clinical features

Neuroblastoma presents in a myriad of different ways depending on the site of the primary, extent of metastatic disease and associated paraneoplastic syndromes. In addition, neuroblastoma may present as an incidental finding or as elevated urinary catecholamine metabolites identified during screening in otherwise healthy infants or children. Non-specific symptoms such as pain and malaise may accompany early stage disease. Thoracic tumours may present as incidental masses on chest radiography, associated with Horner syndrome in cervicothoracic tumours or with symptoms related to cord compression in intraspinal tumours. Abdominal neuroblastomas can present with abdominal distension and/or symptoms due to compression of abdominal viscera such as constipation or urinary retention. Children with extensive metastatic disease tend to be quite unwell at presentation. Characteristic periorbital ecchymosis (''raccoon eyes'') and proptosis are frequently seen in children with metastatic neuroblastoma. A minority of cases present with diarrhoea secondary to vasoactive intestinal peptide secreting tumours (''VIPomas''), flushing and excessive sweating in catecholamine secreting tumours or immune-mediated cerebellar ''opsoclonus–myoclonus'' syndrome.

Serum markers

Although not specific markers for neuroblastoma itself, high levels at diagnosis of serum markers including ferritin (>142 ng/ml), neuron specific enolase (NSE, >100 ng/ml) and lactate dehydrogenase (LDH, >1500 IU/litre) have been shown to be predictive of poor outcome.[22–24] Whether the measurement at diagnosis of serum levels of chromogranin A (CGA), a protein coreleased with cathecolamines from neurosecretory granules, can provide additional diagnostic and prognostic information is not yet clear.[25]

Urinary markers

Urinary analysis for catecholamines and catecholamine metabolites including dopamine (DA), vanillylmandelic acid (VMA) and homovanillic acid (HVA), are used in the initial diagnostic investigation. VMA/HVA ratio and DA/VMA ratio have been shown to provide useful additional information.[26]

Imaging

The adrenal gland is the most frequent site for the primary tumour, which typically presents as an abdominal mass. Once such a mass is suspected, ultrasonography is usually the initial imaging tool. Contrast-enhanced CT scans can demonstrate the extent of primary disease and may delineate some metastatic deposits. CT (with or without 3D reconstructions to display the vascular anatomy) is also important in presurgical assessment of tumour resectability.[27] In adrenal primary tumours, the ipsilateral kidney is usually displaced by the primary tumour; identification of the kidney separate from the tumour and the presence of microcalcification tend to favour the diagnosis of neuroblastoma over Wilms tumour.[28] MRI can be helpful in tumours with suspected spinal involvement. Metaiodobenzylguanidine (MIBG) structurally resembles norepinephrine and, as such, enters neuroendocrine cells by an active reuptake mechanism and is stored in the neurosecretory granules, thus resulting in a specific concentration

compared to non-neuroendocrine cells.[29] In patients with neuroblastoma, MIBG scintigraphy is a sensitive and specific tool to demonstrate bone, bone marrow and soft tissue metastatic involvement.[29] Since about 10% of neuroblastomas do not show MIBG uptake, ^{99m}Technetium methylene diphosphonate (MDP) bone scintigraphy is used in conjunction with ^{131}I or ^{123}I MIBG to accurately stage disease.[30] Positron emission tomography (PET) scanning uses ^{18}F-fluorodeoxyglucose (^{18}FDG), which is avidly taken up by rapidly proliferating cells such as tumour cells. ^{18}FDG is therefore also useful for monitoring tumours that fail, either at diagnosis or following treatment, to concentrate MIBG.[31 32]

Tissue diagnosis

Adequate tumour tissue sampling at diagnosis is necessary for accurate risk stratification and treatment assignment. Open surgical biopsy is preferred to image-guided needle biopsy in many UK centres. A recent study comparing the two techniques reported increased incidence of insufficient tissue for biological studies for the latter, with no difference in complication rates.[33] Although laparoscopic biopsy has been proposed for neuroblastoma, the extent of its usefulness is still unclear.[34] Multiple bone marrow biopsies and aspirates are also performed at diagnosis to assess metastatic extent.

Staging

Stage according to the International Neuroblastoma Staging System (INSS) is one of the main clinical variables that affect outcome in neuroblastoma (table 2).[35] As current approaches to risk stratification and treatment assignment vary considerably across the world, it can be difficult to directly compare risk-based clinical trials. The INRG task force has recently developed a new staging system (INRGSS) (table 3) to establish a consensus approach for pretreatment risk stratification.[36] This latest scoring system uses histology and MYCN status in addition to INSS, to stratify risk categories for treatment. The risk categories in INRGSS are defined according to event free survival (EFS).

To improve the outcome and safety of surgical treatment, the European International Society of Paediatric Oncology Neuroblastoma Group activated the Localised Neuroblastoma European Study Group 1 (LNESG1) study on localised neuroblastoma aimed at identifying surgical risk factors (SRFs) or image-defined risk factors (IDRFs) based on the radiological characteristics of the tumour (table 4).[37] The IDRFs have been reported to predict the outcome of surgery and in particular the risk of postoperative complications and residual disease, but have not been shown to predict overall survival (OS).[38]

SPONTANEOUS REGRESSION AND STAGE 4S

A small proportion of neuroblastomas frequently undergo complete spontaneous regression (ie, disappearance or terminal differentiation to ganglioneuroma) or complete remission after minimal treatment.[39] The incidence of spontaneous regression in neuroblastoma is between 10 and 100 times greater than that for any other human cancer and the underlying biological mechanisms are yet not fully understood.[40] This outcome is generally associated with a clinically recognisable syndrome in infants called stage 4S (S for special), which is defined as a small primary tumour associated with widespread involvement of liver, bone marrow (but not of cortical bone) and/or skin.[35 41]

ROLE OF SCREENING

In view of the significantly better outcome for younger children and those with localised disease, extensive screening studies were conducted in Japan, Europe and North America using urinary HVA and VMA analysis. Reports from these studies demonstrated that the detection rate of neuroblastoma in a screened infant population substantially increases compared to the unscreened population.[42–44] However, the vast majority of tumours detected by screening have favourable clinical and biological features, and therefore screening does not reduce either the prevalence of advanced disease over 1 year of age or the overall death rate.[42–44]

UP-FRONT TREATMENT

Principles of therapy

The various modalities used in the treatment of newly diagnosed neuroblastoma include surgery, chemotherapy, radiotherapy, differentiation therapy, immunotherapy and in selected cases careful

Table 2 International Neuroblastoma Staging System (INSS)

Stage	Description
1	Localised tumour with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive)
2a	Localised tumour with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically
2b	Localised tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. Enlarged contralateral lymph nodes must be negative microscopically.
3	Unresectable unilateral tumour infiltrating across the midline (vertebral column) with or without regional lymph node involvement; or localised unilateral tumour with contralateral regional lymph node involvement; or midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement
4	Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for stage 4S)
4s	Localised primary tumour (as defined for stage 1, 2A or 2B), with dissemination limited to skin, liver and/or bone marrow (limited to infants <1 year of age)

Table 3 International Neuroblastoma Risk Group (INRG) consensus pretreatment classification schema

INRG stage	Age, months	Histological category	Grade of tumour differentiation	MYCN	11q aberration	Ploidy	Pretreatment risk group
L1/L2		GN maturing; GNB intermixed					A: very low
L1		Any except GN maturing or GNB intermixed		NA			B: very low
				Amp			K: high
L2	<18	Any except GN maturing or GNB intermixed		NA	No		D: low
					Yes		G: intermediate
	⩾18	GNB nodular; neuroblastoma	Differentiating	NA	No		E: low
					Yes		H: intermediate
			Poorly differentiated or undifferentiated	NA			
M	<18			NA		Hyperdiploid	F: low
	<12			NA		Diploid	I: intermediate
	12 to<18			NA		Diploid	J: intermediate
	<18			Amp			O: high
	⩾18						P: high
MS				NA	No		C: very low
					Yes		Q: high
				Amp			R: high

Very low risk = 5-year EFS>85%; low risk = 5-year EFS 75 to 85%; high risk = 5-year EFS <50%.
Amp, amplified; EFS, event free survival; GN, ganglioneuroma; GNB, ganglioneuroblastoma; L1, localised tumour confined to one body compartment and with the absence of image-defined risk factors; L2, locoregional tumour with presence of one or more image-defined risk factors (IDRF); M, distant metastatic disease (except stage MS); MS, metastatic disease confined to skin, liver or bone marrow in children <18 months of age; NA, not amplified.

observation only. Most clinical trials now stratify patients into low, intermediate or high-risk groups based on age of the patient, INSS stage, histology according to the INPC, MYCN status and DNA ploidy.[40]

Most localised tumours with favourable biological characteristics respond well to surgical resection. Local recurrences may be treated with surgery, whereas rare metastatic recurrences need chemotherapy and in selected cases radiotherapy. More than half of all children with neuroblastoma have unresectable or metastatic disease at presentation, and treatment of children with stage 4 disease remains one of the greatest challenges in paediatric oncology. Children with favourable biology/histology INSS stage 3 or infants with favourable biology INSS stage 4 (intermediate-risk group) require moderately intensive chemotherapy followed by surgical resection where possible. Children with high-risk neuroblastoma need intensive multimodal treatment including induction chemotherapy, surgical resection of primary tumour, radiotherapy, consolidation therapy usually including myeloablative chemotherapy and haematopoietic rescue, followed by further therapy for eradicating minimal residual disease.

Table 4 Surgical risk factors (SRF) or image-defined risk factors (IDRF)

Site of tumour	Risk factors
All site	Tumour size
	Tumour fragility
Neck	Encasement of vertebral artery
	Involvement of other major vessels
	Encasement of brachial plexus
	Crossing of midline
	Thoracic extension
	Dumbbell tumour
Thorax	Encasement of subclavian vessels
	Encasement of other thoracic vessels
	Lower mediastinal tumour
	Abdominal extension
	Encasement of trachea and/or principal bronchii
	Dumbbell tumour
Abdomen	Encasement of celiac axis
	Encasement of superior mesenteric artery
	Encasement of aorta
	Encasement of inferior vena cava
	Encasement of iliac or hypogastric vessels
	Infiltration of renal pedicle(s)
	Infiltration of portal hepatis
	Compromise of kidney or ureter
	Dumbbell tumour
	Pelvis tumour crossing the sciatic notch
	Muscular infiltration

Surgery

Surgical resection and external beam radiotherapy to the primary and/or metastatic sites are given with the goal of minimising residual viable tumour tissue. The basic principles of surgical resection, namely, adequate vascular control and optimal exposure of the tumour to permit complete resection holds good for neuroblastoma. The aim of surgery in low-risk and intermediate-risk groups is complete resection wherever possible, with minimal damage to the adjacent neurovascular structures that are frequently adherent to, if not encased by the tumour. Neuroblastoma being an infiltrative tumour, it is not usually possible to get microscopically negative resection margins, and hence the aim is "gross total resection".

Japanese experience has shown that aggressive surgery for abdominal neuroblastoma can result in renal infarction and atrophy in up to 15% of patients.[45] Kiely proposed a "subadventitial" approach to facilitate resection of neuroblastoma

with vascular involvement, but associated morbidity related to this technique is diarrhoea secondary to intestinal sympathetic denervation.[46] Moreover, a significant survival advantage for radical surgical clearance in stage 4 disease has never been clearly demonstrated.[47 48] Abnormalities in imaging studies may not necessarily reflect the extent of neuroblastoma, resectability being difficult to determine by CT or MRI, and being subjectively influenced by factors such as the surgeon's experience.[49] The major surgical complications for neuroblastoma vary with the site and extent of primary tumour, and include haemorrhage, neurovascular injury, gut and renal infarction. A retrospective multicentre European study has shown that laparoscopic surgery, in experienced hands, may be feasible to achieve effective local control even in advanced stages of neuroblastoma.[50]

In most European centres, surgery for high-risk neuroblastoma is performed after three to five courses of intensive induction chemotherapy following the initial biopsy. Induction chemotherapy facilitates the likelihood of complete resection and may minimise morbidity. However, a Children's Cancer Group study (CCG-3381) showed no effect on EFS, OS and extent of surgical complications in high-risk neuroblastoma for timing of surgery, whether at diagnosis or following induction chemotherapy.[51]

The role of surgical resection in infants with stage 4S disease is unclear and there is no compelling evidence that surgical resection of primary tumour is beneficial on survival. Recent European trials also suggest that a considerable number of localised non-MYCN-amplified neuroblastomas in infants can regress spontaneously, and question the role of chemotherapy and surgery in patients who are asymptomatic.[52] The ongoing trial, NB2004, aims to assess if a similar "wait and see" policy can be justified for children over 1 year of age.

Chemotherapy

Most induction regimens use different combinations of cisplatin or carboplatin, etoposide, cyclophosphamide, vincristine and doxorubicin. These drugs have substantial dose–response activity, and hence are generally used at high dose and according to very intensive schedules, especially cisplatin and cyclophosphamide. However, a review of 17 different regimens for induction chemotherapy in patients with metastatic neuroblastoma found no evidence of positive association between marrow response rate and either dose of individual drugs or schedule utilised.[53]

The results of the 10-year randomised controlled trial by the European Neuroblastoma Study Group (ENSG-5) have been recently published, comparing the standard OPEC/OJEC schedule with a 21-day interval (depending on bone marrow recovery) with a dose intensive rapid COJEC schedule with a 10-day interval (irrespective of haematological recovery).[54] No significant difference in OS between the standard and rapid schedules was demonstrated at 5 and 10 years, while EFS at 5 years (but not at 10 years) was increased in patients treated with the rapid schedule.[54]

More recently, the combination of topotecan and cyclophosphamide was shown to be active in patients with either resistant[55] or newly diagnosed[56] neuroblastoma, and a pilot study suggested the opportunity of incorporating this combination into an intensive induction regimen.[57] Accordingly, the Children's Oncology Group (COG), COG-ANBL02P1 phase 1 pilot study is presently evaluating the toxicity and feasibility of including two up-front courses of topotecan and cyclophosphamide into the standard induction regimen in patients with newly diagnosed or resistant high-risk neuroblastoma.

Consolidation therapy aims to eradicate the most, if not all, remaining tumour cells, and usually involves cytotoxic agents at myeloablative doses, followed by haemopoietic stem cell rescue. The CCG-3891 study was the first randomised study to demonstrate that myeloablative therapy followed by autologous bone marrow transplantation significantly improves 3-year EFS in children with high-risk neuroblastoma as compared with intensive chemotherapy alone.[58] A recent update on the long-term outcome for these patients has confirmed the benefit of myeloablative therapy in terms of 5-year EFS and OS.[59] In Europe, similar randomised studies substantially supported the better outcome associated with myeloablative therapy.[60 61] Advances in haemopoietic stem cell transplant protocols, including infection prophylaxis, supportive care and more recently the advent of peripheral blood stem cells (PBSCs), have resulted in significant improvements in treatment-related death rate.[62] Despite challenges in the collection, processing and administration of PBSCs, this tool provides faster haematopoetic recovery in comparison to the conventional marrow transplantation.[62] Encouraging results have also been reported following the administration of two consecutive courses of myeloablative therapy with PBSC rescue, which allows an even greater dose intensification.[63]

An interesting alternative approach to the induction and consolidation therapy of children with high-risk neuroblastoma has been undertaken at Memorial Sloan–Kettering Cancer Center (MSKCC). Firstly, immunotherapy using anti-ganglioside D2 (G_{D2}) 3F8 monoclonal antibody (mAb) has been integrated into the induction therapy.[64 65] Secondly, radioimmunotherapy with ^{131}I-3F8 mAb (with haemopoietic stem cells rescue) followed by adjuvant immunotherapy with cold 3F8 mAb has replaced conventional myeloablative chemoradiotherapy.[64 65] Results reported by the MSKCC group in terms of toxicity and response rate are extremely promising, but to what extent these monoinstitutional results can be reproduced in a multi-institutional setting is questionable.[65]

Radiotherapy

Myeloablation using high-dose chemotherapy and total body irradiation (TBI) followed by haematopoietic stem cells rescue is widely utilised with

success.[58 59] TBI has been shown to decrease relapse and increase EFS in high-risk neuroblastoma, although its value in young children is controversial in view of the long-term side effects. Currently COG recommends that patients with high-risk disease receive radiation to the primary site regardless of the extent of surgical resection, and to the metastatic sites that display persistent MIBG avidity on pretransplantation scans. Fractionated doses are generally kept below 21Gy for local control in any stage of the disease. Radiotherapy is not advised for low-risk tumours even with local residual disease, as risks outweigh potential benefits. In low-risk and intermediate-risk groups, radiation therapy is reserved for patients with progressive clinical deterioration despite chemotherapy and surgery. Infants with stage 4S disease are also excluded from radiotherapy except those with respiratory or abdominal compartment syndrome following massive liver enlargement. Acute and long-term side effects as well as tumour response may be enhanced by concurrent use of radiosensitising agents. The most highly radiosensitising drugs are doxorubicin and actinomycin D, which are contraindicated with radiotherapy, while mild radiosensitisers including cisplatin, topotecan and irinotecan are usually safe to give alongside radiation therapy.

Spinal cord compression is treated by chemotherapy, radiotherapy or surgical resection with or without laminectomy depending on individual case. Radiation therapy is contraindicated in intraspinal tumours as it leads to vertebral damage and growth arrest resulting in severe scoliosis, but may be used as an emergency treatment for selected patients with symptomatic spinal cord compression.[66]

Monitoring response to treatment

Following completion of treatment, response is monitored using imaging (CT and MRI for primary site and MIBG and PET for bone and marrow metastases) and urinary catecholamine assessment. The International Neuroblastoma Response Criteria (INRC)[35] (table 5) are used in most centres.

MANAGEMENT OF MINIMAL RESIDUAL DISEASE (MRD) AND RELAPSE

About 50% of children with high-risk neuroblastoma that complete consolidation therapy develop early or late relapse, often from MRD in bone or bone marrow.[67] The utilisation of standardised immunocytology and quantitative reverse transcription (RT)-PCR for tumour specific markers such as G_{D2} and tyrosine hydroxylase has recently been proposed for a reproducible detection and quantification of MRD,[68] and several current therapeutic regimens incorporate biological therapy aimed at eradicating MRD.

Retinoids are natural and synthetic derivatives of vitamin A that include all *trans*-retinoic acid (ATRA), 13-*cis*-retinoic acid (13-*cis*-RA) and fenretinide (4-HPR). Initially, the efficacy of ATRA and 13-*cis*-RA in inducing cell growth arrest and differentiation of neuroblastoma cell lines in vitro suggested that they could both be active in a clinical setting.[69] However, phase I studies showed that higher and more sustained plasma levels were achievable by administering 13-*cis*-RA.[69] Subsequently, the CCG-3891 randomised study demonstrated that 13-*cis*-RA is active against MRD and significantly improves outcome when administered after consolidation chemotherapy.[58 59] Since then, differentiation therapy with 13-*cis*-RA has become the standard for treating MRD in a post-transplantation setting. A synthetic retinoid, 4-HPR, has also been described to have cytotoxic activity on neuroblastoma cell lines in vitro.[69] A favourable toxicity profile and an encouraging response rate were both observed in a phase 1 study that also included children with resistant high-risk neuroblastoma,[70] and a specific phase II study has been completed by COG.

G_{D2} is a surface glycosphingolipid that is abundant in and specific to neuroblastoma cells, making it an optimal target for immunotherapy.[71] Intensive induction and consolidation chemotherapy along with immunotherapy using anti-G_{D2} 3F8 mAb has been shown to significantly improve response rate and outcome in high-risk neuroblastoma.[64 65 71] Granulocyte–monocyte colony stimulating factor (GM-CSF) increases neutrophil and eosinophil production, potentiates antibody-dependent cellular cytotoxicity against neuroblastoma cells in vitro, and is active against MRD when administered in combination with anti-G_{D2} ch14.18 or 3F8 mAbs in children with high-risk neuroblastoma.[72 73] Recently, the addition of immunotherapy with anti-G_{D2} ch14.18 mAb, GM-CSF and interleukin 2 to the standard differentiation therapy with 13-*cis*-RA after myeloablative consolidation therapy has shown to further improve EFS in high-risk neuroblastoma.[74]

Besides differentiation therapy and immunotherapy, a further therapeutic approach for the treatment of MRD in neuroblastoma might be represented by ''metronomic'' chemotherapy. In conventional chemotherapy, closely spaced bolus infusions of cytotoxic agents are administered at or near the maximum tolerated dose (MTD), and are followed by substantial rest periods. In ''metronomic'' chemotherapy, doses of cytotoxic agents well below the MTD are given constantly for an extended period of time, with no prolonged intervals. Studies performed in preclinical models demonstrate that the prolonged utilisation of low doses actively targets tumour stroma and endothelial cells, and suggest that this approach might be useful in optimising the antiangiogenic properties of several cytotoxic drugs when treating slowly growing tumours.[75]

In high-risk neuroblastoma, the persistence of disease or the occurrence of a relapse after induction and consolidation therapy is usually associated with a very poor outcome. The combination of a resistant disease, a decreased bone marrow reserve and an impaired function of critical

Table 5 International Neuroblastoma Response Criteria

Response	Primary tumour	Metastatic sites
CR (complete remission)	No tumour	No tumour, catecholamines normal
VGPR (very good partial response)	Decreased by 90% to 99%	No tumour, cathecholamines normal; residual 99Tc bone changes allowed
PR (partial response)	Decreased by >50%	All measurable sites decreased by>50%. Bones and bone marrow: no. of positive bone sites decreased by >50%, no more than one positive more marrow site is allowed.
MR (mixed response)	No new lesions, >50% reduction of any measurable lesion with 50% reduction in any existing lesion	
NR (no response)	No new lesions, <50% reduction, and <25% reduction in any existing lesion	
PD (progressive disease)	Any new lesion; increase of any measurable lesion by >25%; previous negative marrow positive for tumour	

organs such as kidneys and heart severely limits further therapeutic options, and only incomplete or short-lasting responses are usually obtained. Salvage treatments mainly include: (i) chemotherapy with topotecan and cyclophosphamide,[55] or topotecan, vincristine and doxorubicin,[76] or irinotecan and temozolomide;[77] and (ii) targeted radiotherapy with ^{131}I-MIBG.[78 79]

LATE EFFECTS AND LONG-TERM CONSIDERATIONS

Survivors of advanced stage neuroblastoma often face substantial long-term consequences of aggressive multimodal treatment. In the largest comprehensive study so far, late mortality was determined in a cohort of 1358 5-year neuroblastoma survivors, and incidence of chronic health conditions and second malignancies in 954 survivors from the same cohort.[80] The cumulative 20-year incidence of chronic health conditions was 41%, with an eightfold increased risk when compared with the sibling cohort. The most prevalent chronic conditions affected the neurological, sensory, endocrine and musculoskeletal systems, with cumulative 20-year incidences of 30%, 9%, 8% and 8%, respectively. Survivors that had undergone multimodal treatment were more than twice as likely to develop a chronic health condition compared with those that had only been treated by surgery. Among the 1358 survivors whose data were available, 84 died more than 5 years after diagnosis, with a cumulative 25-year death rate of 6%. The most common causes of late mortality were relapse (51%) and second malignancy (15%). The cumulative incidence of second malignancy was 3.5% at 25 years and 7% at 30 years from diagnosis. The most frequent histotypes were thyroid carcinoma, renal cell carcinoma, soft tissue sarcomas, acute myeloid leukaemia and breast carcinoma. The highest cumulative incidence of chronic health conditions was observed in survivors who were diagnosed and treated more recently, this possibly reflecting the introduction of more intensive treatments.

FUTURE DIRECTIONS

During the last few years, a rapidly expanding portfolio of novel targeted agents in various stages of preclinical research has become available, including agents selectively targeting receptor and non-receptor tyrosine kinases,[81–86] proteasome,[87] MDM2,[88] vascular endothelial growth factor,[89] and mTOR[90] among others. An increasing body of evidence from preclinical models demonstrates that targeted agents in combination with selected cytotoxic drugs can significantly potentiates antitumour activity but not toxicity, thus suggesting that an improvement of therapeutic index can also be achieved in the clinical setting.[91–93] Given the remarkable genotypic and phenotypic heterogeneity of neuroblastoma, comprehensive genome-wide characterisation is being increasingly used to extensively profile individual tumours. Future therapies will be probably based on combined selective strategies targeting multiple molecular pathways and based on unique tumour profiles.

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