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Neuroblastoma

Current Drug Therapy Recommendations as Part of the Total Treatment Approach

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Abstract

Neuroblastoma represents one of the most challenging malignancies for treatment decisions because of its unusual biological behaviour. The features include spontaneous regression (regressive type), maturation to ganglioneuroma (maturative type) and largely treatment-resistant progression (progressive type). Current

knowledge allows only partial prediction of type. For practical reasons, patients may be categorised as an 'observation', a 'standard risk' or a 'high risk' treatment arm.

During the last 2 decades, 5-year survival rates for children with neuroblastoma have increased from 48 to 67%. The main achievements were the reduction of chemotherapy in patients with localised disease and the increased efficacy of chemotherapy in metastatic neuroblastoma stage 4 (5-year survival increased from 8 to 33%). Different goals for chemotherapy (e.g. stopping rapid progression, improvement of symptoms, induction and maintenance of remission) require different dosages and durations of treatment (range 1 week to 9 months). The main risks of chemotherapy are toxic death (rate up to 15%) predominantly during the periods of bone marrow depression and the development of secondary leukaemias (up to 7% cumulative risk after 4 years).

In conclusion, the use of cytotoxic drugs can be completely omitted in a substantial proportion of low risk patients with neuroblastoma. On the other hand, for high risk patients with the disease, intensive polychemotherapy represents the basis and the backbone of treatment among other modalities.

1. Biology of Neuroblastoma

1.1 Definition

Neuroblastoma is defined histologically.[1] It is characterised by neuroblastic tumour cells resembling embryonal sympathetic neuroblasts of the neural crest. Almost all neuroblastoma cells express the gangliosid GD2 and the neural cell adhesion molecule (NCAM) on their surface. The grade of differentiation may vary considerably from the undifferentiated small blue round cell with only discrete differentiating features (neuron specific enolase, chromogramin A) to differentiated patterns (ganglioneuroblastoma). Schwann cells are present in most neuroblastomas. These cells are considered reactive and part of the maturation process.[2]

The diagnosis of neuroblastoma may also be established by the presence of typical tumour cell clumps in the bone marrow and by the simultaneous detection of elevated levels of catecholamine metabolites, vanilmandelic acid (VMA) and/or homovanillyl acid (HVA), in the urine or serum.[1]

Utilising noninvasive measures, neuroblastoma can be diagnosed if a radiologically typical adrenal or midline tumour shows distinct metaiodobenzylguanidine (mIBG) uptake and produces abnormal VMA/HVA excretion.

Before drug therapy modalities can be discussed it is necessary to outline the different presentations and biological courses which make neuroblastoma such an unusual and interesting paediatric tumour.

1.2 Incidences

After acute lymphoblastic leukaemia, neuroblastoma represents the second most frequent malignancy in childhood (relative frequency 8.3%). The age-standardised incidence rate in Germany (1989-1998) was 1.2 cases per 100 000 children aged less than 15 years and per year; the cumulative incidence during childhood was 16.9 cases per 100 000 children.[3] The sex ratio is nearly equal (girls : boys = 1 : 1.1). Neuroblastoma typically presents during infancy (6.8 cases per 100 000 infants) or toddler years (2.1 cases per 100 000 children aged 1 to 4 years).[3] 90% of children with the disease are diagnosed within the first 5 years of life.

1.3 Staging

Table I demonstrates the internationally accepted definition of neuroblastoma stages according to the International Neuroblastoma Staging System (INSS).[1] The minimum diagnostic workup includes radiological imaging [sonography/magnetic resonance imaging (MRI)], the neuroblastoma spe-

Table I. Main characteristics of the International Neuroblastoma Staging System (INSS) and relative frequency of stages in 1161 patients

Stage	Characteristics	Percentage of children at various stages of neuroblastoma		
		all patients (n = 1161)	<1 year (n = 432)	≥1 year (n = 729)
1	Complete gross resection ± microscopic residuals (removed adherent lymph nodes may be positive)	19.6	26.3	15.6
2	Unilateral tumour			
	a) Incomplete gross resection	5.0	6.5	4.2
	b) Ipsilateral nonadherent lymph nodes positive for tumour ± incomplete gross resection	7.0	6.0	7.5
3	Bilateral tumour Tumour and/or lymph nodes infiltrating across the midline (opposite site of vertebral column)	18.0	17.7	18.2
4	Distant metastases (bone marrow, bone, lymph nodes, skin, CNS)	39.9	15.1	54.5
4S	Primary tumour as in stage 1 or 2 + dissemination limited to skin, liver, bone marrow (bone marrow <10% of nucleated cells); age <1 year	10.5	28.4.	0.0

cific mIBG scintigraphy, determination of catecholamine metabolites VMA and HVA and four site bone marrow investigation. Incidence figures show that advanced stage (3 and 4) disease occurs more frequently in older children. The median age at diagnosis increased from 13 months in stage 1 to 15 months in stage 2, 17 months in stage 3 and 33 months in stage 4 (n = 820; trial NB 90) [unpublished data].

1.4 Prognosis

The 10-year survival was 61 ± 1% for 2151 unselected patients with neuroblastoma (unpublished data). Figure 1 shows a quite stable plateau of the Kaplan-Meier survival plot between 5 and 15 years. Death due to tumour progression is a very rare event more than 15 years from diagnosis. Survival and neuroblastoma mortality rates were similar in Western countries, including France, Austria and Germany. A somewhat higher annual mortality rate in the UK (1987 to 1991: 5.45 per 10^6 in the UK *vs* 4.16 per 10^6 in Germany; $p < 0.01$) is believed to be related to a higher incidence of advanced stages of neuroblastoma in the UK and not to treatment differences.[4] During the last 2 decades, an approximate 20% gain in the rate of survival was achieved [German Society of Pediatric Oncology and Hematology (GPOH) trial NB 79: 48%; trial NB 90: 67% survival at 5 years; log-rank test $p < 0.001$] (unpublished data). The prognosis of the individual patient is heavily dependent on the presence or absence of adverse prognostic factors. Commonly accepted factors are 'age over 1 year' (fig. 1), 'stage 4' (fig. 2) and *MYCN* amplification (fig. 3), although these[5,6] and other[7] factors are at least partially associated with each other. While the correlation of clinical factors (e.g. stage and age, serum lactate dihydrogenase and ferritin, mIBG and tumour marker response) has been elucidated multivariately, the prognostic impact of the many molecular characteristics needs to be evaluated in a homogeneously treated population of patients. Table II lists some of the most interesting factors.

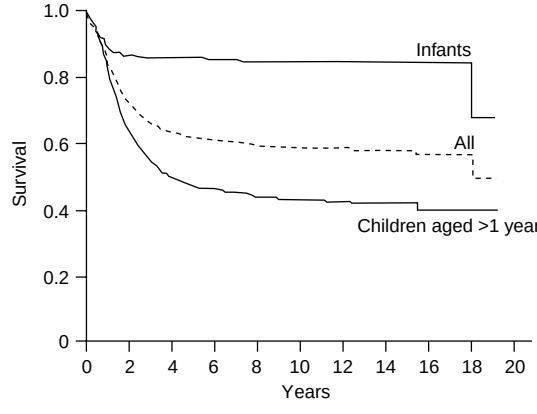

Fig. 1. Survival in 2151 unselected neuroblastoma patients by age, $p < 0.0001$.

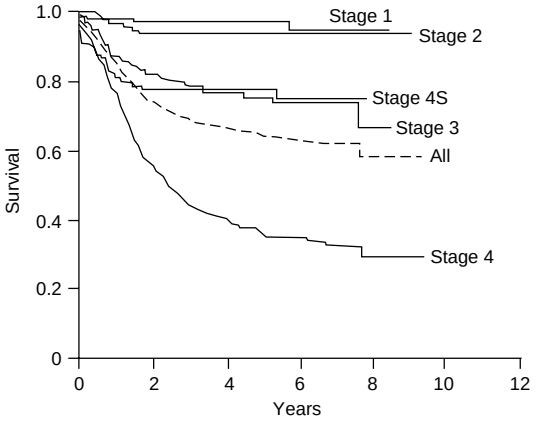

Fig. 2. Survival in 923 unselected neuroblastoma patients by stage, trial NB 90.

1.5 Neuroblastoma Features

The biological behaviour of morphologically identical neuroblastomas is extremely variable and in some respects unique. Although the basic clinical types have been known for almost a century (regressive refers to Peppers's type, progressive to Hutchinson's type) the detection of associated molecular characteristics is currently underway. The growing body of evidence will enable us not only to characterise the extremes (e.g. 4S stage neuroblastoma in infants versus stage 4 in toddlers) but also to define intermediate subtypes, to predict clinical behaviour and thus to tailor therapy. Table III describes the current understanding of biologically different subtypes.

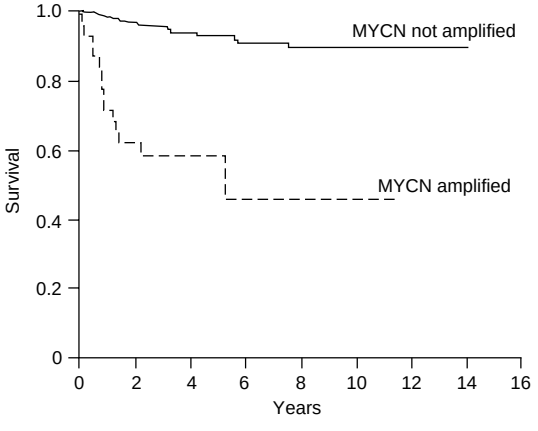

Fig. 3. Survival in 550 neuroblastoma patients with localised disease by *MYCN* amplification, $p < 0.0001$.

1.5.1 Spontaneous Regression

Infants with a primary tumour of limited size and tumour manifestation confined to liver, skin and bone marrow may present with a huge tumour load (particularly in the liver) resulting in death from tumour progression in 10 to 15% of affected patients. If the progressive phase does not result in a life-threatening condition or can be stopped by chemotherapy or radiotherapy, a spontaneous regression phase always follows with disappearance (apoptosis) of the tumour lesions. In some patients, the disappearance of lesions remains incomplete (in particular, the primary tumour) or lesions show signs of maturation (in particular, skin nodules). Approximately 6% (10 of 161) of children with stage 4S neuroblastoma convert to true progressive stage 4 tumours within 6 months (range 2.4 to 15.9 months).[24] It is unclear whether the nature of dissemination in stage 4S is metastatic or a multilocular disease.

A neuroblastoma screening programme in Canada performed at 3 weeks and 6 months after birth resulted in the detection of 2.2-fold higher rates of neuroblastoma compared with the neighbouring non-screened area.[25] It can be concluded that the surplus cases detected by screening correspond to children in the non-screening area whose tumour regressed undetected spontaneously. Thus, regression seems not to be confined to stage 4S. Our observations with 8 untreated stage 3 patients surviving more than 4 to 14 years[24] support this view. It must be emphasised that the fraction of regressive stage 2 and 3 tumours is totally unclear, since those patients have usually received chemotherapy or radiotherapy in the past. The current 'observation trials' for stage 2 Localised Neuroblastoma European Study Group (LNESG) or stage 2 and 3 (Germany) are aimed to define that subset better.

From a clinical point of view, it is very clear that chemotherapy may play an important role in stopping the progression of disease and in inducing the

Table II. Molecular characteristics of neuroblastoma tumours with potential prognostic information

Characteristic	Prognostically adverse expression	References
MYCN	Amplification	8, 9
DNA ploidy	Euploid	10, 11
Deletion 1p 36.2 → ter	Present	12-14
17q gain	Present	15, 16
MRP expression	High	17
Telomerase	High activity	18, 19
trkA expression	Absent	20, 21
CD44 expression	Absent	22, 23

MRP = multidrug resistance-associated protein.

regressive phase in molecular favourable neuroblastomas. However, it remains unclear how long chemotherapy needs to be continued because of the lack of markers indicating when the regression process becomes irreversible.

1.5.2 Progression

Patients with stage 4 neuroblastoma or with localised disease and *MYCN* gene amplification (± other adverse genetic factors) show typically progressive disease which proves to be highly resistant to treatment. The use of maximum chemotherapy, including megatherapy with autologous stem cell support, repeated surgical tumour resections, radiotherapy and immunotherapy, resulted in a 30% 5-year event-free survival and 15 to 20% event-free long term survival only. Approximately 40% of all patients with neuroblastoma belong to that high risk group (36 to 38% stage 4; 3 to 4% stage 1, 2, 3, 4S with *MYCN* amplification) [unpublished data].

Chemotherapy plays the key role in the treatment of high risk patients in respect of improving quality of life (the majority of patients), extending the duration of survival (an increasing proportion of patients) and survival ratio (the minority).

1.5.3 Maturation

Ganglioneuroma, the end-point of maturation, is a completely benign tumour and therefore not systematically recorded in any of the worldwide tumour registries. Primary ganglioneuroma is rarely observed in clinical settings (<5% of neuroblastomas), but secondary to chemotherapy a, mainly incomplete, maturation process is well known. Whether all ganglioneuromas evolve by a maturation process from a neuroblastoma is unclear. Histogenetic and cell culture studies suggest an important functional role of Schwann cells invading from extratumorous sites into neuroblastoma tissue.[2] Chemotherapeutic agents appear to exert their activity by producing apoptosis of immature neuroblastic cells (preferentially); they also seem to have a potential for inducing maturation (e.g. alkylating agents).[26] However, whether maturation

Table III. Biological types of neuroblastoma

Type	Presentation	Adverse molecular markers	Course of the disease	Treatment approach	Current outcome
Regressive	Multilocular (stage 4S)	Absent	Progression (may be fast!) → regression	Minimal therapy: inhibition of rapid tumour growth by "mild" chemotherapy, biopsy (resection) + observation only	80-85% survival
	Unilocular (stages 1-3)	Absent			
Progressive	Metastatic (stage 4) Unilocular (stages 1-3)	Present or absent present	Progression	Maximum therapy: polychemotherapy, megatherapy with stem cell support, surgery, radiotherapy, immunotherapy	20-30% survival
Maturative	Unilocular (stages 1-3)	Absent	Maturation	No approach because this subtype can be identified only retrospectively	100% (?)

Table IV. Comparison of ED_{50}a drug concentrations as determined by monolayer proliferation assay with peak plasma concentrations (reproduced from Fulda et al.,[27] with permission)

Range of effectiveness	Drugs	Mean ED_{50} (μmol/L)	Plasma concentrations (μmol/L)	ED_{50}/plasma concentrations
Most effective[b]	Mitoxantrone	0.06	1.2-5.42	0.01-0.05
	Doxorubicin	0.02	0.4-1	0.02-0.05
	Hydroxyurea (hydroxycarbamide)	34	300-2000	0.02-0.11
	Bleomycin	0.196	1-1-	0.02-0.2
	Dactinomycin	0.00023	0.000597	0.04
	Cisplatin	0.52	6-12	0.04-0.08
	Thiotepa	2.93	53-63	0.05-0.06
	Melphalan	2.25	32-49	0.05-0.07
	Carboplatin	9.25	54-200	0.05-0.17
	Etoposide	0.167	3.57-11.9	0.06-0.19
	Vincristine	0.04	0.4	0.1
	Cytarabine	1.63	10	0.16
	Thioguanine	3.37	6-10	0.3-0.6
	Cyclophosphamide	2.19	1.4-7	0.3-1.6
	Ifosfamide	5.58	1-12	0.5-5.6
	Zilascorb	320	100-400	0.8-3.2
	Fluorouracil	2.4	0.5-2.5	1-4.8
	Mercaptopurine	18	4-8	2.25-4.5
	Lomustine	26-83	4.3-8.5	3.2-6.2
Less effective[c]	Procarbazine	190	0.3-3	63-633

a *In vitro* concentration for 50% growth inhibition.

b Drugs where mean ED_{50} <human plasma concentrations.

c Drugs where mean ED_{50} ≥human plasma concentrations.

or regression is more important for survival from immature neuroblastic tumours is unknown. The majority of investigators feel that chemotherapy has only a marginal role in inducing the process of maturation.

2. Drug Therapy

2.1 Sensitivity and Resistance

The cytostatic effects of a large number of drugs on various neuroblastoma cell lines (± *MYCN* amplification, ± P glycoprotein expression) have been investigated *in vitro*.[27] Using the ratio EC_{50} (dose effective at inhibiting 50% growth of cell population) *in vitro* **:** peak plasma concentration *in vivo*, the drugs evaluated as 'more effective' included all those which were empirically selected for clinical use. These included anthracyclines (mitoxantrone, doxorubicin), platinum compounds (cisplatin, carboplatin), etoposide, vincristine and alkylating agents (thiotepa, melphalan, cyclophosphamide, ifosfamide) [compare table IV with table V]. Other drugs, like hydroxyurea (hydroxycarbamide) and bleomycin, showing *in vitro* activity lack evidence of clinical usefulness so far. In interpreting *in vitro* data, one has to consider that these data provide only a rough and sometimes an incorrect estimate of the *in vivo* situation as a result of marked differences in bioavailability (dose, route of administration, tumour vascularisation) and catabolic pathways.

Neuroblastoma may acquire a substantial drug-resistant phenotype from exposure to chemotherapeutic agents. The resistance progressively increased with the intensity of the therapy delivered *in vivo*.[35] This was highest in cells obtained at relapse after bone marrow transplantation (BMT). They exhibited IC_{90} (concentration for 90% growth inhibition) values distinctly higher than clinically achievable

drug concentrations by 1 to 37 times for melphalan, 1 to 9 times for carboplatin, 25 to 78 times for cisplatin, 6 to 719 times for doxorubicin and 3 to 52 times for etoposide.

Drug resistance is a complex phenomenon that occurs as a result of altered expression of drug resistance genes, tumour cells in sanctuary sites of low drug penetration, tumour hypoxia and cells resting out of the cell cycle. Although *MDR-1*/P glycoprotein is detectable[36] and functional[37] in neuroblastoma cells, its presence seems not to play a significant role for the patient.[37] In contrast, the expression of another gene encoding the multidrug-resistance-associated protein was strongly associated with increased numbers of tumour recurrences and reduced survival, and showed prognostic impact in addition to the *MYCN* status.[17] More recently, drug induced apoptosis mediated by the CD95 receptor, CD95 ligand and the caspase system has been described. Neuroblastoma cells that were resistant to CD95-triggered apoptosis displayed increased resistance to cisplatin and doxorubicin.[38]

Twelve to 15 cytostatic drugs are commonly used for the treatment of patients with neuroblastoma (table V).[28-30] Nevertheless, the specific impact of the various drugs has not yet been clearly defined. Clinical phase II trials with single agents were conducted between 1962 and 1977. Cyclophosphamide, cisplatin, doxorubicin and teniposide consistently yielded significant complete and partial response rates ranging from 34 to 45% and have become the backbone of many multidrug regimens.[39] Based on cell cycle kinetics, phase-nonspecific and phase-specific drugs were combined (e.g. cyclophosphamide followed by doxorubicin, cisplatin followed by teniposide) and produced response rates up to 80 to 90%.[39] Table V represents an overview and is not intended for the use of constructing a chemotherapy programme, since doses and schedules are highly dependent on the particular overall regimen in which they are included. For further information, the reader is referred to the original articles.[28-34]

2.2 The Dose Intensity Concept

Cheung and Heller[40] analysed 44 different clinical trials published over a period of 25 years for the impact of dose intensity (mg/m^2 per week for the entire treatment period) on the outcome. They reported that response, median survival and median progression-free survival was better with higher doses of teniposide and cisplatin and somewhat

Table V. Cytostatic drugs commonly used for the treatment of neuroblastoma

	Preferred dose range (mg/m^2 per day)[28-34]								
	Low (stopping progression, e.g. in 4S stage neuroblastoma)			Standard (intensive polychemotherapy)			High (megatherapy with ASCR)		
Drug	dose	IV time (h)/day	days	dose	IV time (h)/day	days	dose	IV time (h)/day	days
Busulfan	-	-	-	-	-	-	150	Oral	4
Carboplatin	200	1	3	200-500	-	1	500-1000	1	1-3
Cisplatin	90	1	1	25-90	0.5-24	1-5	-	-	-
Cyclophosphamide	150-300	Oral or 1h	7	70-1200	1-6 or oral	1-8	2200	3	2
Dacarbazine	-	-	-	200	0.5-1	5	-	-	-
Doxorubicin	15-35	0.5	1-3	30-60	0.5-4	1-3	-	-	-
Etoposide	150	2	3	90-200	1-24	1-5	250-1200	3-4	1-5
Ifosfamide	-	-	-	1500-3000	1-24	3-5	-	-	-
Melphalan	-	-	-	-	-	-	45-200	Bolus-0.5	1-4
Teniposide	100	1	1	125-150	1-24	3	-	-	-
Thiotepa	-	-	-	10-50	1-2	2-3	300	2	3
Vinblastine	-	-	-	3	Bolus-1	1	-	-	-
Vincristine	0.75	Push	3	1-1.5	Bolus-1	1-2	0.5 (+1.5 push)	24	5

ASCR = autologous stem cell rescue; **IV** = intravenous.

better with higher cyclophosphamide and doxorubicin dose intensity, while no correlation was found with the use of vincristine. Criticism of this study includes the considerable bias inherent in the retrospective nature of the analysis, lack of consideration of the different structure of the cytostatic cycles (infusion time and days of administration, other cytostatic agents given, realisation of the recommended schedule), the potential selection of patients in the reported series and the effect of supportive therapy. In our experience, increasing cisplatin doses from 100 to 200 mg/m^2 and teniposide doses from 100 to 500 mg/m^2 per cycle (5 cycles, trial NB 85, 135 patients) did not result in improved event-free survival ($p = 0.37$) or survival ($p = 0.21$); all other treatment modalities were constant in this trial.

Nonetheless, the event-free survival and survival curves of unselected patients with stage 4 disease have improved during recent years (fig. 4) [5-year survival NB 79: 8%, NB 90: 33%; $p < 0.001$]. Increasing the number and dosages of drugs resulted in better survival of patients in NB 82 compared with those in NB 79, but not of NB 85 compared with NB 82 patients, although 22% of the NB 85 trial patients underwent BMT. Furthermore, the increase in survival of patients in trial NB 90 compared with NB 85 was not the result of higher doses (and not of the higher proportion of BMT participants in the NB 90 study; see section 2.4), since the number of drugs was decreased, the doses kept the same or decreased but the infusion time in 6 out of 7 cytostatic drugs increased. The improvement could have been even greater if chemotherapy-related deaths had been avoided. Thus, even in a single country with 97% participation in the national trial and no selection of patients, it appears very difficult to sort out the many factors, in addition to dose intensity, which seem to influence the outcome of patients with neuroblastoma. Similarly, the improvements in the French trial results over the last 10 years in patients with with stage 4 neuroblastoma (LMCE3: 29% *vs* LMCE1: 8% progression free survival 7 and more years after diagnosis; $p = 0.004$) have been attributed to better supportive care as well as to more effective components during induction, postinduction and consolidation chemotherapy.[41] Although higher doses or enlarged areas under the concentration-time curves of some selected drugs may have a favourable impact on the outcome of these patients, compelling data for a broader validity of the dose intensity concept in neuroblastoma have yet to be presented.

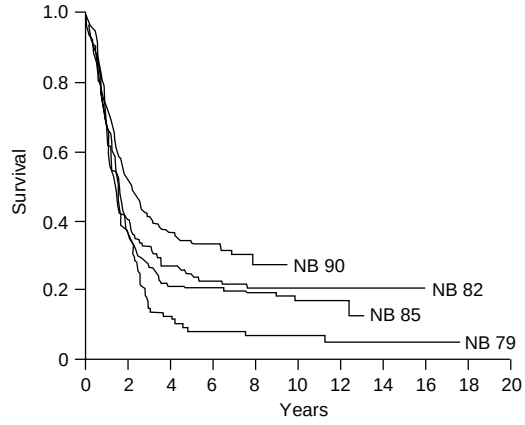

Fig. 4. Survival by trial in 631 unselected neuroblastoma patients stage 4.

2.3 Induction Chemotherapy

With the exception of phase I/II trials, current chemotherapy for patients with neuroblastoma consists of a combination of cytostatic drugs (polychemotherapy). The upper range of the standard doses given in table V are commonly utilised. The aim is to achieve a tumour remission or at least a better resectability of the primary tumour. Furthermore, chemotherapy should be able to stop rapid and dangerous tumour progression, and to improve the general condition and quality of life of the patient. The main problem with induction chemotherapy is making the decision as to who needs it and, if indicated, when should it be given.

2.3.1 No Indication

All investigators agree that patients with stage 1 and infants with asymptomatic stage 4S neuroblastoma do not need chemotherapy at all. Microscopic residuals from stage 1 tumours as well as the disseminated tumour lesions of infants with stage

4S appear to regress spontaneously. There is no uniform recommendation for patients with stage 2 or with stage 3 neuroblastomas. Children's Cancer Group (CCG) studies demonstrated an excellent outcome by surgery alone in Evans stage II patients (89% 6-year progression free survival, n = 75). The addition of radiotherapy did not further improve the result (94%; n = 66).[42] Evans et al.[43] reported an 86% 5-year event-free survival in 21 stage 2b patients with surgery alone. The current European trial (LNESG) follows the 'wait and see strategy' for patients with stage 2 neuroblastoma with macroscopic residuals or spread to regional lymph nodes. The German protocol includes stage 3 neuroblastoma of infants with bilateral, unresectable tumours in this 'wait and see strategy', unless patients have serious symptoms or high speed progression which require an immediate therapeutic approach.

2.3.2 Standard Risk Patients

Standard risk patients include children over 1 year of age with unresectable tumours that are not *MYCN* amplified and infants with significant tumour-related symptoms from stage 2, 3 and 4S neuroblastoma. Three indications for induction chemotherapy are accepted.

Stopping Rapid Tumour Progression in Infants with Stage 4S Neuroblastoma

The huge tumourous liver of infants with stage 4S neuroblastoma may rapidly enlarge within a few days, compressing bowel, kidneys and lungs. This results in serious complications in approximately half of the patients and in an early fatal outcome in 20% of the patients. The best indicators for the necessity of chemotherapy were critical general condition at diagnosis and thrombocytopenia, according to a multivariate analysis.[44] Molecular parameters are still lacking. Although only induction of tumour regression has to be achieved, the intensity of chemotherapy needs to be balanced between the high speed of liver enlargement and the high susceptibility of young infants to chemotherapy. Proposed blocks of chemotherapy are carboplatin (6.6 mg/kg per day on days 1 to 3, 1h infusion) plus etoposide (5 mg/kg per day on days 1 to 3, 1h infusion) or block 4 containing doxorubicin, vincristine, cyclophosphomide (see fig. 5). Cyclophosphamide (10 mg/kg per day) only was a popular treatment approach, but was too low in efficacy. Low dose radiotherapy to the liver also appeared to be inadequately effective and was difficult to use in critically ill patients in the intensive care unit. A surgical artificial abdominal hernia may be beneficial, but should be reserved for extreme situations.

Treatment of Serious Symptoms

Immanent or present transverse myelopathy, dangerous bowel obstruction, hypoventilation or kidney dysfunction and other serious symptoms require immediate chemotherapy (e.g. N4 for infants <6 months or N5 + N6 for older children; see fig. 5). In patients with intraspinal neuroblastoma with partial neurological deficits, recovery was seen in most after chemotherapy or surgical laminectomy,[44] but laminectomy produced a higher incidence of spinal deformities. Long standing severe cord compression resulted in a low incidence of recovery regardless of the treatment modality.[46]

Relief from symptoms is usually quickly observed (during or shortly after the first chemotherapy block) while better resectability of the tumour needs more time and is largely achieved after 2 to 4 cycles.

Improvement of the Resectability of Stage 2 and 3 Tumours

Chemotherapy may convert unresectable neuroblastoma into resectable tumours. Of 109 patients with stage 3 neuroblastoma, at the time of diagnosis half the tumours could only be biopsied (biopsy 50%, partial resection 22%, complete removal 28%). Following preoperative chemotherapy, 3 of 4 tumours were completely excised (biopsy 6%, partial resection 20%, complete removal 74%) [GPOH NB 90 trial]. The surgical complication rate decreased with the use of chemotherapy (first look at diagnosis: 11.3%, delayed first look after preoperative chemotherapy: 4.8%, second look following first look and chemotherapy: 5.7%). The favourable influence of chemotherapy on the resectability on tumours must not prevent a – rather defensive – surgical approach at diagnosis, since obtaining tumour tis-

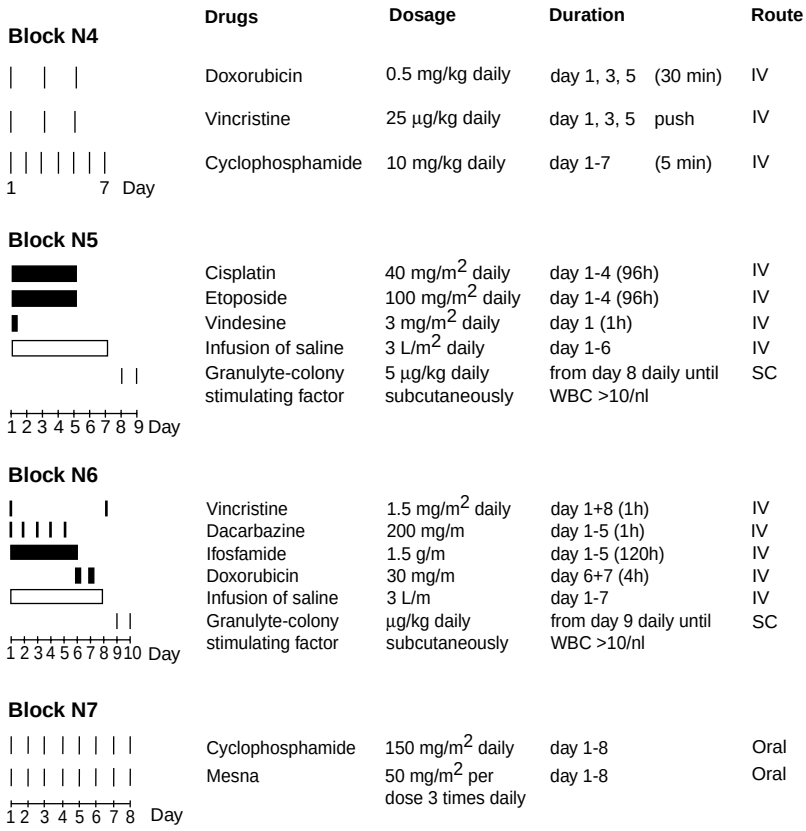

Fig. 5. Structure of chemotherapy cycles [German Society of Pediatric Oncology and Hematology (GPOH) study NB 97].[45] IV = intravenously; **SC** = subcutaneously.

sue for histological and biological studies is mandatory in any case. Furthermore, preoperative estimation of resectability and the intraoperative situation may differ. In the case of unexpected complete removal of the tumour in stage 1 disease, the need for chemotherapy may be abandoned.

This type of chemotherapy does not need a high dose intensity regimen, i.e. spontaneous haematological recovery may be awaited. Granulocyte-colony stimulating factor (G-CSF) support is not required. Most protocols recommend a short treatment duration with a maximum of 1 to 3 cycles for inducing regression and 4 to 6 cycles for achieving symptom relief and better resectability.

2.3.3 High Risk Patients

High risk neuroblastomas (stage 4, and other stages *MYCN* amplified) frequently carry chemotherapy-resistant clones. Thus, higher dose intensity is intended. This may be achieved by shortening the intervals between the blocks of chemotherapy by using G-CSF or by the use of more drugs, higher doses and longer infusion times (see fig. 5 for example). The treatment duration is considerably longer (6 to 8 blocks). Two aims of treatment are pursued.

Improvement of Subjective Condition

Only 5% of patients with high risk neuroblastoma are asymptomatic. The most prominent symp-

toms in GPOH NB 90 were reduced general condition (54%), pain (52%), fever (39%), tumour swelling (36%) and bodyweight loss (22%). Normal activity was seen in 13% at diagnosis, 43% after 3 to 5 cycles and 57% at the end of chemotherapy.[47] Thus, although chemotherapy imposes a burden on young children, most patients experience improved activity and quality of life even during the treatment period.

Achievement of Remission

Complete remission requires disappearance of all detectable tumour lesions at primary and metastatic sites. In neuroblastoma, bone marrow clearance (cytology, mIBG) usually precedes the normalisation of lymph node and bone (mIBG + technetium scintigraphy) metastasis. Disappearance of the primary tumour solely by chemotherapy is rarely observed, but improvement of the surgical condition is an achievable goal. While only 19% of 188 stage 4 patients had complete tumour excision at tumour diagnosis, the figure increased to two-thirds after preoperative chemotherapy (67 to 71% delayed first- and second-look surgery). The complete remission rate increased in most published series with the duration of chemotherapy (GPOH NB 90 trial: 13.8% complete remission after 4 cycles, 48.6% after 8 cycles, 63.1% at the end of therapy). This is in contrast with the experience in patients with leukaemia who achieve the highest response rates early during remission induction. Furthermore, in patients with neuroblastoma, the achieved response status is only a weak predictor for the long term outcome. Complete and very good partial response rates of more than 70% have been reported[48] but could not be duplicated by other institutions.

2.4 Consolidation Chemotherapy

2.4.1 Megatherapy with Autologous Stem Cell Rescue

Two randomised trials for consolidation treatment in patients with stage 4 neuroblastoma have demonstrated that myeloablative chemotherapy with autologous stem cell rescue (ASCR) is superior compared with none[31] or to continuing the intensive induction chemotherapy.[32] The estimated gain is in the range of 10 to 20% 5-year survival, not more. Because of the early data of the European Neuroblastoma Study Group (ENSG)1 trial,[31] all of the myeloablative regimens contain high dose melphalan. Particularly poor responders in bone marrow or bone lesions appeared to benefit from the addition of busulfan (and cyclophosphamide).[33] Double or triple megatherapy regimens did not further improve the outcome.

Total body irradiation as part of the regimen has been abandoned in most studies because of a lack of convincing data showing its efficacy and the high toxicity potential of this approach in young patients (2 to 5 years at graft). No advantage was demonstrated for allogenic compared with autologous stem cells in a case-control analysis of the European Bone Marrow Transplantation Registry.[34] Removal of residual tumour cells from the graft by purging procedures has been increasingly utilised.

Although chemical *in vitro*-purging with mafosfamide is still adopted in several studies, the immunological positive selection of CD34 cells has advantages of 80 to 99% purity and avoidance of further stem cell impairment.[49] Reports of the presence of CD34 receptors on neuroblastoma cell surfaces[50] require cautious evaluation and need reinvestigation of the collected cell suspension e.g. by GD2 staining or by TH-rt polymerase chain reaction (PCR) analysis in order to avoid a selection of tumour cells. Currently, immunomagnetic depletion of stem from tumour cells as developed by Kemshead et al.[51] remains the standard method.

2.5 Maintenance Chemotherapy

The role of maintenance chemotherapy in high risk neuroblastoma is not established. Results of 1-year maintenance chemotherapy using cyclophosphamide suggested a 10 to 15% better survival in 31 patients compared with matched controls who did not receive maintenance chemotherapy.[47] In a consecutive trial, the 5-year survival using megatherapy for consolidation was 36 ± 11% (n = 40) and equal to 37 ± 7% with maintenance chemotherapy for 1-year consisting of oral cyclophosphamide, etoposide, melphalan and intravenous vincristine

(n = 92).[52] The maintenance arm could have been superior were it not for a substantial number of patients in this arm developing secondary leukaemia. Thus, continuing a chemotherapy regimen different from the induction regimen may well prove to be an efficient consolidation modality. This is currently being studied in a randomised fashion (GPOH NB 97, fig. 6).

2.6 The Risks of Chemotherapy

The increased survival rates achieved in patients with neuroblastoma who received chemotherapy (5-year survival NB 79: 48 ± 4%, NB 82: 60 ± 4%, NB 85: 60 ± 3%, NB 90: 64 ± 2%) were hampered by a significant therapy-related death rate (NB 79: 15%, NB 82: 4%, NB 85: 9%, NB 90: 15% of all 511 deaths). In localised neuroblastoma, tumour progression (n = 17) and toxic death (n = 10 by treatment, n = 3 by relapse treatment) were of similar magnitude (trial NB 90) and death by chemotherapy and surgery equally balanced. In patients with stage 4 disease, chemotherapy contributed more (15.5% of all deaths) than surgery to therapy-related deaths. Censoring the toxic deaths, the 5-year survival rate would improve from 30 to 35 ± 2% (NB 90, n = 206). Since the majority of chemotherapy-related deaths were caused by the development of sepsis during neutropenia, the reduction of fatal events may be possible. In principle, the use of growth factors (e.g. G-CSF) and some reduction of dose intensity of the blocks of chemotherapy is being currently investigated. While G-CSF appears to be generally well tolerated and beneficial,[53] stem cell factor is produced by some neuroblastoma cells themselves and able to protect the tumour cells via an autocrine loop from apoptosis.[54] In patients with stage 1 to

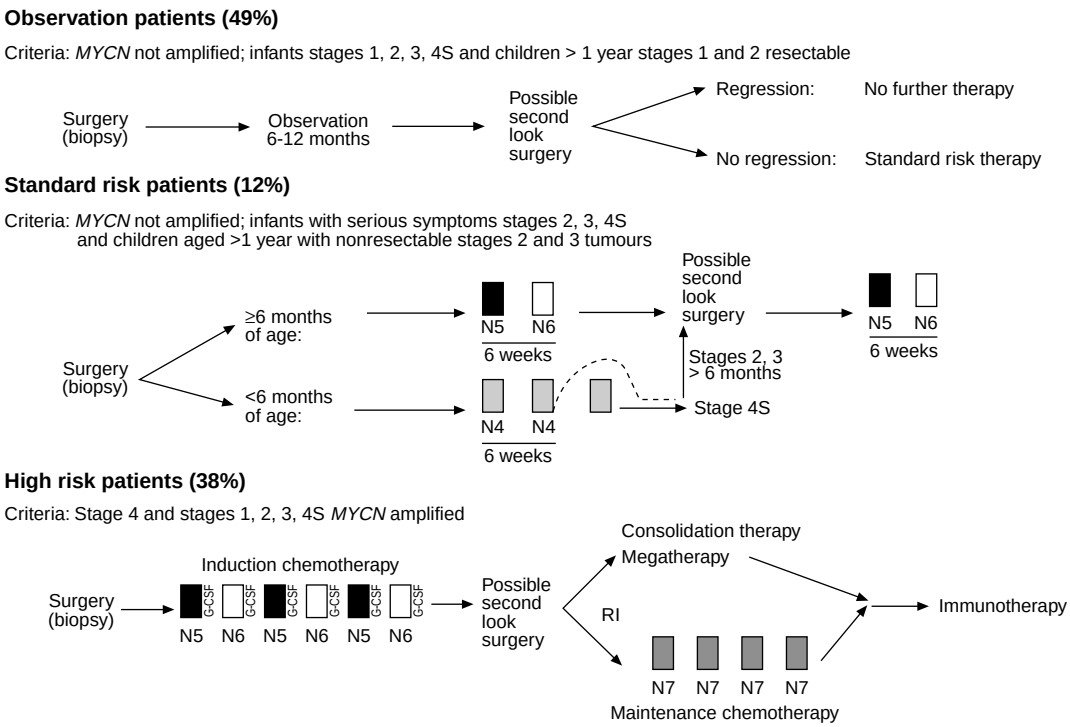

Fig. 6. Flow sheet for the treatment of neuroblastoma according to the German Society of Pediatric Oncology and Hematology (GPOH) study NB 97.[45]

3 disease, the avoidance of therapy-related death would further reduce the few fatal events in this patient group.

In the literature, we found no thorough analysis of all treatment-related deaths (including those resulting from chemotherapy + surgery + stem cell transplantation + late deaths), which makes it difficult to put our quite stringent definitions in perspective with those of other groups. Generally, a toxic death rate of between 2 and 3% is considered acceptable.

Another disturbing feature of chemotherapy in patients with neuroblastoma is the development of secondary myelodysplastic syndromes and transformed acute myeloid leukaemias (t-AML). Many series show that high risk neuroblastoma itself is not associated with a higher frequency of secondary malignancies (cumulative <1% after 10 years). Kushner et al.[48] reported the development of t-AML in 3 out of 53 patients in whom the most intensive Memorial Sloan-Kettering Cancer Center (MSKCC)-N6 regimen was applied (3-year cumulative incidence 7%). Three other patients developed t-AML after treatment for relapsed or refractory neuroblastoma. In our experience,[52] 8 of 92 patients who started and completed the 1-year maintenance chemotherapy regimen in NB 90 (which included oral etoposide) developed t-AML, compared with none of the 40 patients who received megatherapy (including bioequivalent doses of intravenous etoposide) for consolidation. All t-AML cases presented within 0.6 and 4 years (median 2 years) after the start of maintenance chemotherapy (4-year cumulative incidence 7%). After omitting oral etoposide, melphalan and intravenous vincristine from the maintenance chemotherapy arm, no other case of secondary leukaemia was observed.

2.7 New Approaches in Drug Therapy

2.7.1 Topoisomerase I Inhibitors

DNA topoisomerase I is expressed in neuroblastoma and neuroblastoma xenografts, while lower activity has been observed in ganglioneuroblastoma and normal adrenal gland.[55] Preclinical studies using topotecan revealed complete or partial remissions in 8 of 10 different xenografts. Three phase I/II studies that included 61 patients with neuroblastoma reported 3 complete remissions, 3 partial remissions and 11 minor responses or disease stabilisations. Other investigators found a 4% response rate in patients with relapsed or refractory neuroblastoma.[55] In a phase I study combining topotecan with cyclophosphamide, 1 of 7 patients achieved a complete remission, 1 of 7 a partial remission and 2 of 7 stable disease.[56] The contrast between preclinical and the thus far reported clinical activity is even more pronounced with use of irinotecan, which may be administered orally. Taken together, the optimal schedule for the use and the issue of combining with other drugs (alkylating agents) has still to be addressed.

2.7.2 Differentiating Agents

In vitro, 13 cis retinoic acid rapidly reduces *MYCN* overexpression, induces differentiation and promotes apoptosis. The randomised CCG trial demonstrated a 46 ± 6% 3-year event free survival rate with and 29 ± 5% without retinoic acid (160 mg/m^2 per day on days 1 to 15 each month for 6 months[32]) for patients with poor risk stage 3 and with stage 4 neuroblastoma ($p = 0.027$). A similar European approach with lower doses (0.75 mg/kg per day) and longer exposure (2 years) did not find retinoic acid effective for children with stage 4 disease [J. Kohler, personal communication, 1999]. *In vitro*, 9 cis retinoic acid and fenretinide also showed a promising capacity to influence maturation and apoptosis. Their antitumour activity in the clinical setting is yet to be determined.

2.7.3 Other Compounds

In vitro, several other agents have demonstrated interesting effects on neuroblastoma cell lines and xenografts.[55] These agents include modulators of chemoresistance [e.g. verapamil, valspodar (PSC 833)], apoptosis (e.g. botulinic acid), and glutathione depletion (e.g. buthionine sulphoximide), and inhibitors of angiogenesis [e.g. AGM-1470 (TNP 470)]. Thus far, no or only limited clinical studies have been performed and their value in the clinical setting remains to be elucidated. Other new approaches, like hyperbaric oxygen, interleukin-2,

anti-GD2 monoclonal antibodies and targeted radiotherapy with mIBG are beyond the scope of this article and therefore not discussed.

3. Recommendations

3.1 General Considerations

The complex biology of neuroblastoma and the use of all currently available treatment modalities demand that each patient must be treated according to the guidelines of a modern protocol. Exemptions for 'not participating' are unacceptable even for single patients because of the known inferior prognosis for nontrial patients. Several well conducted trials are available in all continents and can be recommended. Special attention has to be paid to the correct categorisation of patients. The increasing number of protocols, dealing with sometimes quite small subgroups, only makes it often difficult to select the correct trial for a given patient. Since the German trial is one of the few covering all the different aspects within one protocol, we are following those lines. This does not mean in any respect that other trials might be less recommendable. The principles of treatment are internationally accepted by all study groups.

3.2 Specific Recommendations

The current GPOH NB 97 approach stratifies patients into 3 groups: observation, standard risk and high risk patients. The criteria for the categorisation and the flow sheet is shown in figure 6.

3.2.1 Observation Patients

The observation group comprises approximately half of the patients enrolled. Initial surgery is performed to obtain tissue for histological and molecular studies (*MYCN*) and with the intention of removing as much of the tumour as possible without increased risk for life and organs (e.g. no kidney removal *a priori* accepted). Following surgery, patients are observed on an outpatient basis by consecutive clinical checkups, urinary VMA and HVA determinations, and ultrasound investigations. The goal is to follow closely the natural regression process. If regression is observed, no further therapy is needed. If regression remains insufficient (residual >10% of the initial volume or >2 to 5ml) after 6 to 12 months, a second look should be performed to see whether the tumour may be removed now more easily and to study potentially initiated regression or maturation in the tissue. If more than 50% of vital neuroblastic tumour cells are found or more than 50% of neuroblastic cells remain immature (mature = ganglioneuroma giant cells) then 4 blocks of induction chemotherapy are indicated. Distinct tumour progression during the observation period should also lead to utilising standard risk chemotherapy. It is estimated that chemotherapy may be avoided in approximately half of the stage 2 and 3 patients. So far, all patients with progression have responded well to delayed chemotherapy compared with other protocols.

3.2.2 Standard Risk Patients

The standard risk group may disappear in the future with better understanding of the biology and more accurate discrimination of the patients belonging to different prognostic groups. Today, roughly one-tenth of patients are categorised as standard risk. The main goal for chemotherapy is to stop progression, to treat serious symptoms and to induce regression in large residual tumours. In some cases (particularly in stage 4S patients with huge liver tumours) the start has to be very quick and even under intensive care unit conditions. A regimen that can easily be administered intravenously without the need for large bypass volumes of fluid appears preferable (e.g. block N4). We prefer N4 blocks for all infants less than 6 months of age (fig. 5) and continue with the cisplatin-containing N5 and the ifosfamide containing N6 block if the patient reaches the age of ≥6 months. Since high dose intensity is not considered a key issue, the use of G-CSF between chemotherapy blocks is not routinely recommended. Second-look surgery to remove the residual tumour may be performed after 2 or 4 blocks of chemotherapy, depending on the speed of the response. If after 4 cycles an unresectable, mIBG and contrast medium uptaking residual tumour remains, local irradiation (36 to 40 Gy) is recommended. Unsatisfactory results after radio-

therapy qualify the patients to experimental approaches or to transfer to the high risk arm.

3.2.3 High Risk Patients

This is the best defined therapeutic group in neuroblastoma and comprises approximately 40% of patients. In the future, a small increase in this group is expected as poor risk patients from the observation and standard risk groups will be identified molecularly. After obtaining tissue for histological and biological studies from primary or metastatic lesions, 6 cycles of chemotherapy are administered for remission induction. By aiming for short intervals between the cycles (3 weeks) the use of prophylactic G-CSF is recommended. The main adverse effects are bone marrow depression, nephrotoxicity and ototoxicity. If the extension of time period to substantially more than 4 weeks [day 1 N5 (6) → day 1 N6 (5)] a dose reduction of etoposide (N5) or ifosfamide (N6) may be considered. Ototoxicity of >grade 2 (WHO definition) should result in the substitution of cisplatin by carboplatin.

The necessity of consolidation chemotherapy is accepted today. Most protocols recommend megatherapy with autologous stem cell rescue. The German trial currently asks the question of equality of a maintenance chemotherapy programme (4 blocks of N7 = 4.2 g/m^2 cyclophosphamide), which is different from induction chemotherapy and may be performed on an outpatient basis.

The long-lasting cascade of relapses (fig. 2) prompted most investigators to add more recent treatment modalities including immunotherapy with monoclonal antibodies (e.g. anti-GD2) and retinoic acid after the end of chemotherapy. Their attraction results from different therapeutic principles and the assumption that they work best at the minimal residual disease status. However, the efficacy remains to be confirmed.

4. Outlook

The refined exploitation of chemotherapy has produced significantly better survival rates in poor risk patients with neuroblastoma, but the level is still unsatisfactory. It appears unlikely that chemotherapy has much more potential, i.e. the capacity for curing those patients cannot be greatly expanded on the basis of current knowledge. Thus, newer technical developments like immunotherapy, vaccine or gene therapy look more promising but are still at an early stage.

The reduction of chemotherapy without compromising the already good results in standard and low risk patients represents one of the major, somewhat overlooked achievements of the last decade. For a substantial fraction of patients, the non-necessity of chemotherapy has become clear. A better understanding of regression and maturation processes and their early detection with the use of molecular markers is necessary to identify those patients at diagnosis (without the need for an observation period) and to tailor therapy.

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