Table 4 Multivariate analyses of PFS and OS in localized rhabdomyosarcoma ($n = 73$)

	No. of patients	PFS			OS		
		HR	95 % CI	*p* value	HR	95 % CI	*p* value
Age							
<21	51						
≥21	22	2.60	1.18–5.70	0.018	1.67	0.62–4.52	0.311
Stage							
1	25						
2	13	2.73	0.90–8.29	0.076	7.36	1.38–39.2	0.019
3	35	2.42	0.94–6.24	0.069	5.66	1.19–26.9	0.029
Radiotherapy							
No	21						
Yes	52	0.69	0.29–1.63	0.394	0.95	0.32–2.85	0.924
Surgery							
No	30						
Yes	43	0.60	0.29–1.24	0.167	0.63	0.26–1.54	0.312
Presence of CNS invasion							
No	66						
Yes	7	1.68	0.52–5.36	0.384	1.75	0.52–5.84	0.363

CNS central nervous system, *PFS* progression-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval

Table 5 Multivariate analyses of PFS and OS in metastatic rhabdomyosarcoma ($n = 25$)

	No. of patients	PFS			OS		
		HR	95 % CI	*p* value	HR	95 % CI	*p* value
Age							
<21	11	1.00			1.00		
≥21	14	0.97	0.37–2.55	0.947	1.03	0.35–3.06	0.960
Radiotherapy							
No	7						
Yes	18	0.14	0.04–0.51	<0.001	0.24	0.07–0.82	0.023
Surgery							
No	20						
Yes	5	1.15	1.15–3.95	0.394	0.30	0.06–1.47	0.137
Presence of CNS invasion							
No	24						
Yes	1	1.85	0.20–16.8	0.585	0.99	0.12–8.45	0.995

CNS central nervous system, *PFS* progression-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval

PFS and OS. Radiotherapy was the only significant factor in improved PFS or OS in metastatic disease.

Local therapy for metastatic disease

Of 25 patients with metastatic disease, 21 patients (84.0 %) received local therapy as part of their primary treatment that included radiotherapy ($n = 16$), surgery and radiotherapy ($n = 3$), and surgery ($n = 2$). Sixteen of these 21 patients (76.2 %) experienced relapse at the following sites: distant metastatic site ($n = 11$) and primary site progression ($n = 5$). Among four patients with metastatic disease who did not receive local therapy, three patients relapsed: two experienced local relapse and one relapsed at a distant metastatic site. In the 25 patients with metastatic disease, median PFS times in patients with or without local therapy (surgery and/or radiotherapy) were 13.4 versus 7.0 months ($p < 0.001$) (Fig. 2a), respectively, and median OS times were 36.1 versus 7.6 months ($p < 0.001$), respectively (Fig. 2b).

Timing of local therapy

We further sought information about the optimal timing of local therapy in 53 patients who received local therapy (both radiotherapy and surgery) during their course of treatment (localized disease, $n = 38$; metastatic disease, $n = 15$). PFS and OS were not significantly different

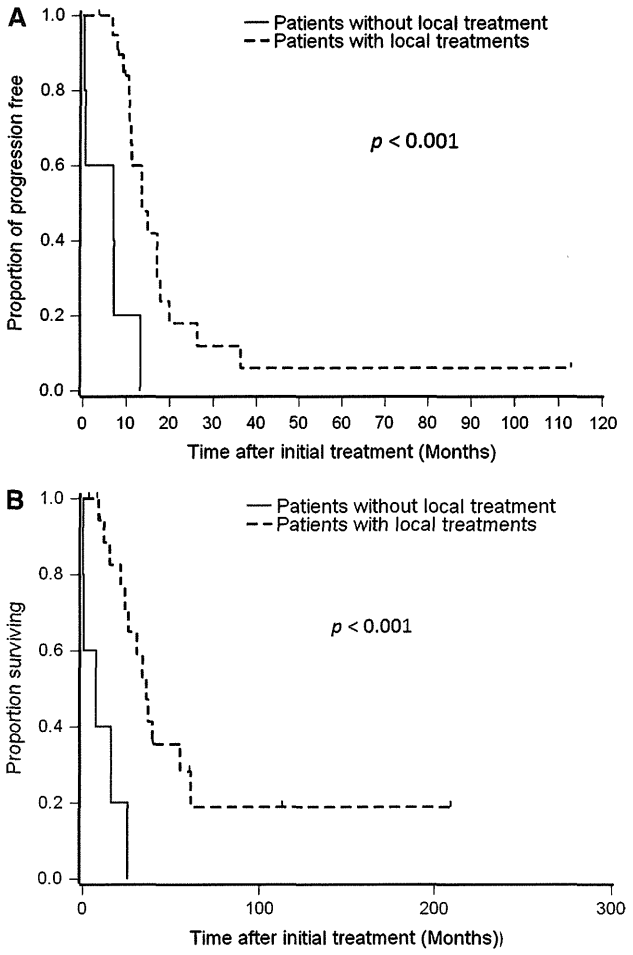

Fig. 2 Kaplan–Meier curve of progression-free survival (**a**) and overall survival (**b**) in patients with metastatic disease with local therapy (*dashed line*) and without local therapy (*solid line*)

between patients who received local therapy within or after 18 weeks of starting initial treatment (see Table 6 for our multivariance analysis of OS). In the 38 patients with localized disease, median PFS was 134.7 months in patients who received radiotherapy in the induction phase and 101.6 months in patients who received it during the maintenance phase ($p = 0.921$); median OS was similar for patients during both phases ($p = 0.277$). Median PFS and OS times were also similar in patients who received surgery either in the induction phase or in the maintenance phase ($p = 0.304$ for PFS and $p = 0.502$ for OS).

In the 15 patients with metastatic disease, the median PFS in patients who received radiotherapy was similar for both phases (induction, 18.4 months; maintenance, 13.3 months, $p = 0.177$); however, median OS was significantly longer for the patients receiving radiotherapy in the induction phase than for those receiving radiotherapy in the maintenance phase (60.7 and 25.7 months, respectively, $p = 0.048$). Median PFS and OS were similar in patients who received surgery in either the induction or maintenance phase ($p = 0.304$ for PFS and $p = 0.214$ for OS).

Discussion

In this study, we evaluated the clinical outcomes of adults and children with RMS who received VAC/VAC-like chemotherapy as their initial treatment. This study resulted in two main findings. First, we showed that age was an independent negative prognostic factor for PFS in RMS with localized disease, but it was not associated with survival in metastatic disease. Second, local therapy to the

Table 6 Multivariate analysis of OS to determine the significance of timing for local therapy in localized and metastatic rhabdomyosarcoma

	Localized disease ($n = 38$)			Metastatic disease ($n = 15$)		
	HR	95 % CI	p value	HR	95 % CI	p value
Age						
<21	1			1		
≥21	1.04	0.24–4.49	0.961	0.63	0.11–3.66	0.610
Stage						
1	1			–	–	–
2	30.74	0.68–1,390.3	0.078	–	–	–
3	12.98	0.50–339.5	0.124	–	–	–
Timing of radiotherapy						
≥18 weeks	1			1		
<18 weeks	0.89	0.23–3.36	0.857	0.30	0.04–2.31	0.246
Timing of surgery						
≥18 weeks	1			1		
<18 weeks	0.45	0.04–3.31	0.429	3.11	0.07–138.2	0.558
Presence of CNS invasion						
No	1			1		
Yes	1.45	0.38–5.56	0.587	2.81	0.18–45.0	0.465

CNS central nervous system, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval

primary tumor site during the treatment course may be necessary for metastatic RMS, as the patients who received local therapies showed significantly longer survival than those who did not. Although our findings suggest that patients with metastatic RMS should be treated at an early stage with local radiotherapy to improve OS, this aspect of our results requires more research; thus, the timing of local therapy should be individually determined depending on patient conditions.

Several studies have reported that age is associated with poor survival in patients with RMS. Sultan et al. reported on the prognosis of pediatric (age $\leqq$ 19 years) and adult (age $>$ 19 years) RMS patients, and their findings suggested that the 5-year survival rate was significantly poorer in adults compared to that in children (5-year OS, 27 and 61 %, respectively; $p < 0.0001$) (Sultan et al. 2009). Another study clarified that the outcomes of patients with intermediate-risk RMS varied depending on age (Meza et al. 2006). Oberlin et al. (2008) also reported on the prognosis of metastatic RMS, and their data suggested that the 3-year event-free survival rate was significantly poorer in RMS patients <1 year and >9 years of age compared to that in RMS patients aged 1–9 years ($p < 0.001$). In our study, age was a negative prognostic factor of PFS in RMS with localized disease, but outcomes for metastatic disease were not different between adults and children. Previous studies have mostly focused on age in children, but our study reported different prognoses for adults and children in both localized and metastatic disease. Therefore, our results would be expected to be different than those of Oberlin et al. (2008). The poor prognosis in adult metastatic RMS may depend on the tumor biology and drug delivery.

Histopathological classification of adult RMS is somewhat difficult to categorize conventional subtypes. Although our data include alveolar subtype most, pleomorphic and spindle subtypes in part may be included in the heterogeneous tumor and these subtypes are suggested poor prognosis (Mentzel, 2000 #3204). For the drug delivery, unpublished data in our institute suggest that the dose intensity of vincristine and cyclosporine is lower in adult when compared to children as hematological toxicities and neurotoxicity are severe. These data suggest that categorizing adult RMS and its treatment may be necessary to be developed independently to that of child RMS.

Radiation therapy and surgery are important for local tumor control and survival in the treatment of RMS. However, the optimal timing of radiation therapy is unclear. IRSG and COG protocols incorporate radiation therapy scheduled at weeks 9 or 12 after the induction of initial chemotherapy (Crist et al. 2001; Arndt et al. 2009). Minn et al. (2010) analyzed the risk of early treatment failure in intermediate-risk RMS, and the majority of patients with early progression experienced local failure. Earlier radiation therapy may improve outcomes by the prevention of early local progression, and the current COG study (ARST0531, http://www.clinicaltrials.gov) plans to perform radiation therapy at week 4 for intermediate-risk RMS. Although there has been no randomized trial to compare the timing of local therapy in RMS, early initiation of local treatment would seem to be preferable. In our study, local therapy was effective in improving survival even in metastatic disease. We could find the efficacy of radiotherapy in metastatic patients but not for surgery probably because of the shortage of patients number included. However, except for local radiotherapy in patients with metastatic disease, the timing of local therapy had no significantly different effect on outcomes in patients who received local therapy during the induction phase versus the maintenance phase. The threshold we used for dichotomization (within 18 weeks or later than 18 weeks after initial treatment onset) may have been a factor in our inability to detect a significant difference in outcomes. This result implies that the timing of local therapy for metastatic disease, whether radiotherapy or surgery, may be varied depending on the individual patient's characteristics, that is, the radiotherapeutic field or the operability of the patient's local site.

Several limitations to our study should be mentioned. Our analysis was limited by its retrospective design and small sample size. Patients receiving VAC-like chemotherapy had undergone chemotherapy during the period prior to 2000, and the dose intensity of chemotherapy varied by protocol. The dose of irradiation and radiation methods also varied. Further, the patients who received local therapy might have been in better general condition or had a smaller primary tumor, which could be included in one radiation field, compared with those who did not. To reduce these biases, we compared the baseline characteristics of each group and demonstrated their similarity. However, adult RMS is a rare cancer; thus, our results should contribute to further advances in this field of oncology.

In conclusion, we showed that age was a negative prognostic factor for PFS and OS in RMS patients with localized disease, but age was not associated with survival in metastatic disease. For metastatic disease, local therapy may have a beneficial effect on survival, but the optimal timing of local therapy is unclear and should be determined individually. Future clinical trials for metastatic RMS should focus on the timing of local therapy, and evaluation of treatment strategies limited to adult RMS patients is warranted.

Conflict of interest None.

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REVIEW ARTICLE

Development of treatment strategies for advanced neuroblastoma

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Abstract Neuroblastoma is the most common cancer in childhood. The majority of patients with neuroblastoma are assigned to the high-risk group based on age at diagnosis, stage, histology, MYCN status, and DNA ploidy. Their prognosis remains unsatisfactory; the 5-year event-free survival (EFS) rate is generally 40 %. During the past 20 years, much effort has been made to reinforce chemotherapy, including the introduction of high-dose chemotherapy with autologous stem cell rescue, resulting in a 5-year EFS rate of around 30 %. Subsequently, maintenance therapy aimed at eradicating residual tumors after induction and consolidation therapies was introduced, consisting of differentiation-inducing agents, retinoids, and immunotherapy using anti-GD2 antibodies combined with cytokines. However, such additional treatment provided benefit to only 10–20 % of patients, while the prognosis of about half the patients remains poor. Currently, novel targeted agents are under development. Among them, anaplastic lymphoma kinase (ALK) inhibitors and aurora kinase A inhibitors are promising. ALK somatic mutation or gene amplification predisposing neuroblastoma development occurs in up to 15 % of neuroblastomas. Crizotinib is a dual-specific inhibitor of ALK/Met and inhibits proliferation of neuroblastoma cells harboring R1275Q-mutated ALK or amplified wild-type ALK, but not cells harboring F1174L. Instead, cells with F1174L are sensitive to another small molecule ALK inhibitor, TAE684. Aurora kinase A plays a pivotal role in centrosome maturation and spindle formation during mitosis. MLN8237 (alisertib) is a small molecule inhibitor of aurora kinase A that is currently in early-phase clinical testing. Future treatment will be individually planned, adapting targeted agents based on personal biological tumor characteristics.

Keywords Neuroblastoma · High-risk · Treatment · Review

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Introduction

Neuroblastoma is the most common extracranial cancer in childhood and generally occurs in very young children, with a median age at diagnosis of 17 months [1]. The tumors arise in tissues of the sympathetic nervous system, the adrenal medulla, or paraspinal ganglia. Patients with neuroblastoma are stratified into very low-, low-, intermediate-, and high-risk groups based on age at diagnosis, stage, histology, MYCN status, and DNA ploidy [2]. Neuroblastomas have unique characteristics, with age at diagnosis being a powerful prognostic factor. Patients with hyperdiploidy and no MYCN amplification are assigned to the low-risk group, if younger than 18 months even if stage 4 disease, while in very low-risk patients, a subset of tumors shows spontaneous regression or complete remission with short-term chemotherapy [3, 4]. The prognosis of patients assigned to the high-risk group remains poor; the 5-year event-free survival (EFS) rate is around 40 %. The high-risk group is currently defined by MYCN amplification or age over 18 months. Neuroblastomas comprise several subsets of diseases currently characterized by surrogates. Molecular characterization for identifying underlying tumor

biology is in progress using modern molecular technologies. In this article, recent developments in the treatment of high-risk neuroblastoma are described.

Principles of therapy

Since high-risk neuroblastoma including localized disease is a systemic disease, the role of modalities for local treatment is limited and the significance of total resection or local radiation has not been proven. Chemotherapy plays a major role in the treatment of high-risk neuroblastoma. Historically, the probability of long-term survival for high-risk neuroblastoma patients was <15 %. The survival rate has increased in proportion to the intensity of chemotherapy [5]. The development of supportive therapy has made it possible to increase chemotherapy intensity. Treatment consists of induction, consolidation, and maintenance phases. Tumors are usually resected during or after the induction phase, and irradiation is delivered to the primary site and residual metastatic sites after completion of the induction phase. The role of induction and consolidation therapies is to reduce tumor burden as much as possible and rapidly, before tumor cells acquire drug resistance. In the 1970s, even vincristine plus cyclophosphamide showed a considerable effect at an early phase of treatment, but almost all tumors recurred after 3–4 months, indicating that neuroblastoma cells become resistant faster than other pediatric tumors. Therefore, it is important to accomplish treatment without delay according to a well-scheduled plan. The aim of maintenance therapy is to eradicate minimal residual disease after high-dose myeloablative chemotherapy. Since residual neuroblastoma cells are highly resistant to conventional chemotherapy, alternative strategies are desirable. In this context, tumor differentiation therapy and immunotherapy are currently under development, using retinoids and anti-GD2 monoclonal antibodies combined with cytokines, respectively.

Induction chemotherapy

In the last 30 years, phase II clinical trials have established active agents against neuroblastoma, such as platinum analogs, anthracyclines, alkylating agents, and epipodophyllotoxins. More recently, the topoisomerase I inhibitors, topotecan and irinotecan, were shown to be effective. These agents demonstrated a 30–50 % response rate in newly diagnosed patients. Current first-line chemotherapy regimens generally consist of combinations of cisplatin, doxorubicin, vincristine, cyclophosphamide, and etoposide. Table 1 shows the induction chemotherapy regimens used by major cooperative groups. Regimens using drugs at higher doses achieved higher response rates; POG-8742 Regimen 1 containing higher doses of cisplatin and etoposide achieved better results than Regimen 2 [7]. The Japanese Cooperative Group uses higher doses of cisplatin and pirarubicin (THP-adriamycin) for induction therapy and reported a 92 % response rate [9]. Older studies may seemingly have shown better results but they lacked a sensitive method for assessment of tumor response at the time, ^{123}I-meta-iodobenzylguanidine (MIBG) scintigraphy. There is virtual consensus about the reinforcement of the dose intensity to raise response rates. To strengthen treatment intensity, one method is to shorten the treatment interval. Recently, the European Neuroblastoma Study Group (ENSG-5) compared the standard schedule (OPEC/OJEC) using a 21-day interval with a rapid COJEC schedule using a 10-day interval. The same total drug doses were administered in 11 and 21 weeks in the rapid and standard schedules, respectively [10]. There was no significant difference in overall survival (OS) between the rapid and standard regimens at 5 and 10 years, while there

Table 1 Induction therapies and their response rates

Study [reference]	Period	Dose per course (mg/m^2)				Course duration	No. of courses	Response rate (CR + PR) (%)
		Cisplatin	Doxorubicin	Etoposide	Cyclophosphamide			
CCG-321P2 [6]	1985–1989	60	30	100 × 2	900 × 2	Day 1–6	5–7	76
POG-8742 (1) [7]	1987–1991 A[a]	40 × 5		100 × 3		Day 1–5	3	77
	1987–1991 B[a]		35		150 × 7 (po)	Day 1–8	2	
POG-8742 (2) [7]	1987–1991	90	35	100	150 × 8	Day 1–11	5	68
CCG-3891 [8]	1991–1996	60	30	100 × 2	900 × 2	Day 1–6	5	78
Japanese Cooperative Group [9]	1985–1990	90	THP-ADM 40	VCR 1.5	1200	Day 1–5	6	92

CR complete response, *po* orally, *PR* partial response, *THP-ADM* pirarubicin (THP-adriamycin), *VCR* vincristine

[a] Alternative administration

was a significant difference in the 5-year EFS rate (30.2 vs. 18.2 %; $P = 0.022$). Myeloablative consolidation therapy was given a median of 55 days earlier in patients assigned to rapid treatment than in those given standard treatment. Although this study showed that shortening of the chemotherapy interval might be a promising method, the significance of the results should be carefully considered as there was no difference in OS and, furthermore, the survival rates for both regimens were lower than current regimens. The Japanese Cooperative Group (the Japan Neuroblastoma Study Group) is currently conducting a phase II trial under the hypothesis that the interruption of chemotherapy with local therapy might contribute to acquisition of chemoresistance. In this study, local treatment including tumor resection and radiotherapy are postponed till the end of myeloablative consolidation therapy. However, it is certain that the strategy of strengthening chemointensity for the improvement of survival probability is approaching a limit and incorporation of new drugs is required. Topoisomerase I inhibitors are good candidates in this respect as their toxicity is limited and their myelotoxicity is less than for classic drugs [11–13]. The Children's Oncology Group has shown the efficacy of a combination of cyclophosphamide and topotecan in a phase I study and has followed this with an ongoing phase III study incorporating this combination in induction chemotherapy [14–17].

Myeloablative consolidation therapy

An increase in antineoplastic drug dosages has been considered as a means of overcoming tumor cell chemoresistance. Neuroblastoma is a unique tumor in that the advantage of myeloablative chemotherapy has been demonstrated in phase III studies (Table 2) [8, 18, 19]. In consolidation therapy, it is required to eradicate surviving tumor cells that have acquired chemoresistance after induction chemotherapy. The first confirmed evidence was obtained with the CCG-3891 randomized study, in that myeloablative therapy including total body irradiation, carboplatin, etoposide, and melphalan (CEM) followed by purged autologous bone marrow stem cell rescue significantly improved 5-year EFS (myeloablative therapy 34 ± 4 % vs. intensive chemotherapy 22 ± 4 %; $P = 0.034$) [8]. This observation was confirmed during longer follow-up (5-year EFS: 30 ± 4 vs. 19 ± 3 %, respectively; $P = 0.04$) [20]. The German Cooperative Group compared a non-total body irradiation myeloablative regimen including CEM with oral maintenance chemotherapy [18]. They demonstrated that patients allocated megatherapy had increased 3-year EFS compared with those allocated maintenance therapy [47 % (95 % confidence interval (CI) 38–55) vs. 31 % (95 % CI 23–39); hazard ratio (HR) 1.404 (95 % CI 1.048–1.881); $P = 0.0221$], but did not significantly increase 3-year OS [62 % (95 % CI 54–70) vs. 53 % (95 % CI 45–62); HR 1.329 (95 % CI 0.958–1.843); $P = 0.0875$]. More recently, the European Cooperative Group (SIOPEN) compared CEM and busulfan plus melphalan (BuMel) myeloablative regimens [19]. A significant difference in EFS in favor of BuMel (3-year EFS: 49 vs. 33 %, $P < 0.001$) was observed as well as in OS (3-year OS: 60 vs. 48 %, $P = 0.004$). Trials incorporating ^{131}I-MIBG as a component of myeloablative regimens have been performed and showed their feasibility [21].

Another approach to consolidation is the administration of two or three consecutive courses of myeloablative therapy with peripheral blood stem cell (PBSC) rescue. Extensive pilot studies have shown its feasibility and have suggested its efficacy [18, 22–24]. The Cooperative Oncology Group (COG) is currently comparing tandem myeloablative consolidation with a thiotepa and cyclophosphamide regimen followed by an attenuated CEM regimen to a single CEM regimen (COG-ANBL0532).

In autologous stem cell transplantation, contaminating tumor cells in autografts play a role in spreading disease after myeloablative therapy. Since the number of tumor cells in peripheral blood is small after several courses of induction chemotherapy and PBSC rescue provides rapid

Table 2 Phase III clinical trials of myeloablative consolidation therapy

Study [reference]	No. of patients	Regimen	EFS (%)	EFS observation period (years)	*P* value
CCG-3891 [8]	379	Myeloablative carboplatin/etoposide/melphalan + rescue vs. intensive chemotherapy	34 vs. 22	5	0.034
German Cooperative Group [18]	295	Myeloablative carboplatin/etoposide/melphalan + rescue vs. oral maintenance chemotherapy with cyclophosphamide	47 vs. 31	3	0.0221
European Cooperative Group (SIOPEN) [19]	598	Myeloablative carboplatin/etoposide/melphalan + rescue vs. myeloablative busulfan/melphalan + rescue	33 vs. 49	3	<0.001

EFS event-free survival

hematopoietic recovery, PBSC is preferential to bone marrow. The COG confirmed no benefit of immunomagnetic bead-based purging of pheresates on EFS or OS [25].

Maintenance therapy

Retinoid compounds

Retinoids are natural and synthetic derivatives of vitamin A that have been shown to induce terminal differentiation of neuroblastoma cells [26]. Among the retinoids, 13-*cis*-retinoic acid has been shown to have high bioavailability in a phase I study [27]. In the CCG-3891 phase II study, patients who achieved a complete or very good partial response after induction therapy were randomly assigned to 6-month treatment with 13-*cis*-retinoic acid or no further treatment following consolidation therapy [8]. This study showed a significant benefit of 13-*cis*-retinoic acid on outcome. Oral administration of 13-*cis*-retinoic acid following consolidation therapy has since become the standard for treating minimal residual disease in high-risk patients. Currently, clinical studies are focused on exploring more effective and less toxic retinoids with high bioavailability and a capacity for maximum tumor terminal differentiation. Fenretinide, a synthetic retinoid, is under development. In a phase II clinical trial of fenretinide in patients with recurrent or refractory diseases conducted by the COG, 14 of 59 evaluable patients (24 %) experienced response (1 partial response and 13 prolonged stable disease). Low bioavailability may have limited the activity of fenretinide [28]. Novel fenretinide formulations with improved bioavailability are currently being evaluated in pediatric phase I studies.

Anti-GD2

GD2 is a surface disialoganglioside that is almost uniformly expressed on the surface of neuroblastoma cells, making it an optimal target for an immunotherapeutic approach. Since GD2 expression in normal tissues is restricted to the central nervous system, peripheral sensory nerves, and skin melanocytes, monoclonal antibodies against GD2 have been expected to be suitable candidates for tumor-specific therapy [29]. Their function is not fully understood; antitumor effects can be either dependent or independent of the immune system. Immune-mediated mechanisms include antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity. Murine, chimeric, and humanized antibodies have been developed and their antitumor activities have been demonstrated in preclinical models and in phase I and II studies [30–37]. However, since their activity has been minimal, development of anti-GD2 antibodies has been aimed at eliminating minimal residual disease. Murine anti-GD2 antibody, 3F8, showed modest activity in clearing residual neuroblastoma cells contaminating bone marrow [38]. A major adverse event is neuropathic pain, which is universal among all antibodies and dose limiting. The human–mouse chimeric antibody ch14.18 has been extensively tested in clinical trials. It is 50–100 times more efficient at mediating tumor ADCC in vitro than murine antibody 14G2a [39]. In German trials (NB90 and 97) for patients with newly diagnosed high-risk neuroblastoma, ch14.18 was administered to 166 patients every 2 months over a period of 1 year in the maintenance phase. A total of 99 patients received a 12-month course of maintenance chemotherapy and 65 had no further treatment. There was no significant difference in EFS or OS [40]. To strengthen immunocytotoxicity, coadministration of interleukin-2 (IL-2) and GM-CSF has been attempted. The COG evaluated the toxicities and efficacy of a combination of ch14.18, IL-2 alternating with granulocyte–macrophage colony stimulating factor (GM-CSF) in a phase I trial followed by a phase III randomized clinical trial [36, 41]. In the phase III trial of newly diagnosed patients with high-risk neuroblastoma, patients who achieved a complete or partial response to induction therapy were randomized after myeloablative consolidation therapy to receive maintenance therapy with *cis*-retinoic acid versus *cis*-retinoic acid plus ch14.18 in combination with IL-2 and GM-CSF. Randomization was stopped early because interim monitoring revealed significantly improved 2-year OS and EFS rates. Immunotherapy was superior to standard therapy with respect to 2-year EFS rate (66 ± 5 vs. 46 ± 5 %, $P = 0.01$) and 2-year OS rate (86 ± 4 vs. 75 ± 5 %, $P = 0.02$). Major toxicities were neuropathic pain, capillary leak syndrome, and hypersensitivity reaction [36]. To reduce systemic toxicities associated with the addition of cytokines, fusion antibodies in which the cytokine is linked to the Fc end of the monoclonal antibody are currently under development. Fusion antibodies provide high cytokine concentrations to the tumor microenvironment. The COG has conducted a phase I followed by a phase II trial of the humanized hu14.18 linked to IL-2 [34, 35]. In the phase II trial, while no objective response was observed in patients with disease measurable by standard radiographic criteria, in patients with disease evaluated only by MIBG scintigraphy and/or bone marrow histology, five patients (21.7 %) achieved a complete response.

New drug development

The Pediatric Preclinical Testing Program (PPTP) was established with National Cancer Institute support in the

US for new drug development. It is a comprehensive program to systematically evaluate new agents against molecularly characterized childhood solid tumor and leukemia models. The primary goal of the PPTP is to identify new agents that have the potential for significant activity when evaluated clinically against selected childhood cancers. The PPTP seeks to test these agents near the time that they are entering phase I evaluation in adults with cancer. So far, an NEDD8-activating enzyme inhibitor (MLN4942) [42], a CENP-E inhibitor (GSK923295A) [43], a polyamine analog (PG11047) [44], insulin-like growth factor-1 receptor inhibitors (BMS-754807, IMC-A12, SCH717454) [45–47], an aurora kinase A inhibitor (MLN8237) [48], a multikinase inhibitor (sunitinib) [49], an HSP90 inhibitor alvespimycin (17-DMAG, KOS-1022) [50], and a vascular endothelial growth factor inhibitor (AZD2171) have been tested [51]. Of them, the aurora kinase A inhibitor is the most encouraging. MLN8237 is a small molecule inhibitor of aurora kinase A that is currently in early-phase clinical testing. Aurora kinase A plays a pivotal role in centrosome maturation and spindle formation during mitosis [52]. A phase III trial of adult peripheral T-cell lymphoma has just started.

Sorafenib, a multikinase inhibitor, has demonstrated inhibition of neuroblastoma growth in a xenograft mouse model [53]. Sorafenib treatment also decreases neuroblastoma cell proliferation, attenuates ERK signaling, and enhances G1/G0 cell cycle arrest in vitro. Sorafenib inhibits phosphorylation of signal transducer and activator of transcription 3 (STAT3), which is associated with inhibition of phosphorylated Janus kinase 2 (JAK2), an upstream kinase that mediates STAT3 phosphorylation. Sorafenib also inhibits the phosphorylation of STAT3 induced by IL-6 and sphingosine-1-phosphate (S1P), a recently identified regulator for STAT3, in tumor cells. Moreover, sorafenib downregulates phosphorylation of MAP kinase (p44/42) in neuroblastoma cells, consistent with inhibition of their upstream regulators MEK1/2. Sorafenib inhibited expression of cyclin E, cyclin D1/D2/D3, key regulators for cell cycling, and the antiapoptotic proteins Mcl-1 and survivin [54].

Recently, polo-like kinase 1 (PLK1) was identified as a key player in oncogenesis in neuroblastoma-initiating cells [55]. Polo-like kinases are recognized as key regulators of mitosis, meiosis, and cytokinesis [56]. PLK1 is being studied as a target for cancer drugs. Many colon and lung cancers caused by K-RAS mutations are dependent on PLK1. When PLK1 expression is silenced with RNA interference in cell culture, K-RAS cells are selectively killed, without harming normal cells [57, 58]. Treatment with PLK1 inhibitors in clinical trials of adult malignancies has shown that BI2536 or BI6727 (volasertib) are cytotoxic to neuroblastoma-initiating cells. Furthermore, BI2536 significantly inhibited tumor growth in a xenograft model [55].

The discovery of anaplastic lymphoma kinase (ALK) as the major neuroblastoma predisposition gene was immediately extended to show that ALK somatic mutation or gene amplification occurs in up to 15 % of neuroblastomas [59, 60]. The ALK gene is located at 2p23, near the MYCN locus (2p24). ALK mutations frequently occurred within the kinase domain, in which three highly conserved amino acid positions were predominantly affected. The constitutive activation induced by mutations or amplification transmits signals through activation of a variety of signal transducers, including PLCγ, PI3K/AKT, STAT3 and RAS [61–64]. ALK mutations are distributed evenly across different clinical stages, although the most frequent somatic mutation, F1174L, is associated with MYCN amplification. The combination appears to confer a worse prognosis than MYCN amplification alone. ALK encodes an orphan receptor tyrosine kinase with an extracellular domain, belonging to the insulin family of proteins [65]. Expression of ALK is largely restricted to neural tissues [66–69] and is observed at high frequencies in primary neuroblastoma specimens [70]. Since several ALK inhibitors have been shown to be effective for non-small-cell lung cancers (NSCLCs) and ALK-deficient mice seem to show apparently normal development, these inhibitors are expected to play a substantial role in the treatment of neuroblastoma. Currently, the sole commercially available ALK inhibitor, crizotinib, is a dual-specific inhibitor of the ALK and Met tyrosine kinases. It shows substantial activity against NSCLCs and also inhibits proliferation of neuroblastoma cells expressing R1275Q-mutated ALK or amplified wild-type ALK. In contrast, cell lines harboring F1174L-mutated ALK were relatively resistant to crizotinib [71]. Another small molecule ALK inhibitor, TAE684, inhibited neuroblastoma cells harboring F1174L-mutated ALK [72]. Recently, an antagonistic ALK antibody has been reported, which inhibits cell growth and induces in-vitro ADCC [73]. This strategy may overcome intrinsic insensitivity against small molecule inhibitors.

Conclusion

During the past 20 years, much effort has been directed towards the improvement of treatment results in advanced neuroblastoma. Most effort has been to reinforce chemotherapy, including the introduction of high-dose chemotherapy with autologous stem cell rescue. As a result, improvement of treatment results was achieved little by little. More recently, the introduction of maintenance therapy including administration of differentiation agents and immunotherapy has contributed to further improvement.

However, such treatment provided benefit only to 10–20 % of patients, while the prognosis of about half the patients remains poor. Thus, it is difficult to expect further improvement of treatment results using past treatment strategies. It is obvious that novel strategies are required to develop further improvement. Fortunately, a large number of novel targeted agents are under development. Comprehensive genome-wide characterization is now being increasingly used to extensively profile individual tumors. Future treatment would appear to be heading towards individualization of therapy by adapting targeted agents based on personal biological tumor characteristics.

Conflict of interest The author has no conflict of interest to declare.

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ORIGINAL ARTICLE

Implications of surgical intervention in the treatment of neuroblastomas: 20-year experience of a single institution

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Abstract
Purpose The implications of surgical intervention for neuroblastomas were assessed in one institution.
Methods We analyzed the clinical characteristics and extension of resection in 123 pediatric patients with neuroblastoma diagnosed between 1985 and 2004.
Results The 5-year survival rate of the 82 patients under 12 months of age, 59 of whom were treated with complete resection of the primary tumor, was 97%. The 5-year survival rate of the 41 patients over 12 months of age did not differ significantly according to whether complete ($n = 19$) or incomplete resection ($n = 22$) was performed (46 vs. 38%, respectively). No local recurrence was observed in ten patients over 12 months of age with stage 4 disease who underwent complete resection of the primary tumor; however, four of these ten patients died of metastatic recurrence.
Conclusion Considering that the majority of infantile neuroblastomas in this study had favorable biology, complete resection might be unnecessary for patients under 12 years of age. For advanced neuroblastomas in patients over 12 months of age, the main treatment for metastasis is systemic chemotherapy, although extirpation of the primary tumor without extensive surgery might prevent local recurrence when combined with radiation therapy.

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Keywords Neuroblastoma · Surgical intervention · Biology

Introduction

Neuroblastoma is the most common solid tumor in children, and its development is still uncharacterized [1]. The prognosis varies greatly, based on the clinical prognostic and biological prognostic factors [2]; thus, it is important to select the optimal therapy according to the properties of these tumors [3]. There are three types of surgical intervention for neuroblastoma: initial tumor extirpation, biopsy of the tumor at initial diagnosis; and radical surgery as a second-look operation after biopsy and induction chemotherapy. The role of surgical resection in the treatment of neuroblastomas is still controversial [4]. We conducted the present study to evaluate the implications of surgical intervention for neuroblastomas in patients under 12 months of age versus those over 12 months of age, based on an analysis of patients treated at one institution.

Patients and methods

A total of 123 patients had neuroblastoma diagnosed and treated at the Department of Pediatric Surgery, Kyushu University, between 1985 and 2004. This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan on July 30, 2003. Consent for tumor preservation and biological analysis was obtained from the parents of each pediatric patient before surgery. Of the 123 patients, 82 were less than 12 months of age and 41 were 12 months of age or older. Of the 82 neuroblastomas in

patients under 12 months of age, 73 were detected through mass screening at 6 months of age. In all cases, the status of MYCN amplification was determined by Southern blotting, quantitative polymerase chain reaction (PCR), and fluorescent in situ hybridization (FISH) [5]. According to the International Neuroblastoma Staging System (INSS) [6], there were 79 (64%) patients with stages 1, 2, and 4S; 16 (13%) with stage 3; and 28 (23%) with stage 4. Seventeen (14%) patients had MYCN amplification. The 5-year survival rate was 76%.

Survival curves for each category were constructed using the Kaplan–Meier method and then statistically evaluated by the log-rank test.

Results

Treatment and outcomes of the patients less than 12 months of age

In principle, since 1994, the protocol of the Japanese Infantile Neuroblastoma Study Group has been applied to patients under 12 months of age [7, 8]. This protocol consists of the following: An initial tumor extirpation is performed for localized resectable neuroblastomas; then, if no MYCN amplification is detected, postoperative chemotherapy is not administered. A biopsy is taken of an unresectable neuroblastoma, followed by mild chemotherapy if no MYCN amplification is detected in the tumor. Usually, no second-look operation is performed for residual tumors that decrease in size with chemotherapy after a biopsy, if the biology, including the MYCN gene status, is favorable. If MYCN amplification is detected in the tumor, intensive chemotherapy with a decrease in dose according to age is given, with or without tumor extirpation.

Of our 82 patients under 12 months of age, 70 (85%) had stage 1, 2, or 4S and 2 (2%) had MYCN amplification; the 5-year survival rate was 97% (Table 1). There were no significant differences in the survival rates between the 59 patients who underwent complete resection and the 23 who underwent incomplete resection (Fig. 1). Moreover, of the 59 patients who underwent complete resection, there were no significant differences in the survival rates of the 38 who received chemotherapy and the 21 who did not. All 23 patients who underwent incomplete resection of the tumor received postoperative chemotherapy, and all these patients survived (Table 2).

Of the nine patients with neuroblastoma detected clinically, and not through mass screening, four had stage 4S, three had stage 1, and two had stage 4 disease. One patient with stage 4S disease had MYCN amplification. Seven of these nine patients underwent complete resection of the primary tumor. Six patients with stage 4 or 4S received mild chemotherapy after operation. Three patients with stage 1 received no postoperative chemotherapy after complete resection of primary tumor. The patient who had stage 4S disease with MYCN amplification died of the disease.

Surgical complications developed in five patients: postoperative bleeding in one, renal atrophy in three, and adhesive intestinal obstruction in one. One patient with partial resection of a stage 2 tumor suffered postoperative bleeding; two patients with complete resection of a stage 1 tumor and one patient with partial resection of a stage 3 tumor suffered renal atrophy; and one patient with complete resection of a stage 1 tumor suffered adhesive intestinal obstruction. All five patients with a surgical complication were alive without disease at the time of writing.

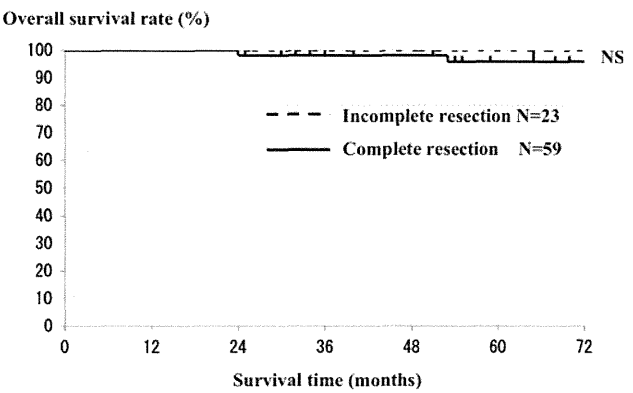

Fig. 1 Correlation between tumor resection and prognosis in 82 neuroblastoma patients less than 1 year of age. *NS* not significant

Table 1 Characteristics of 82 patients less than 12 months old with neuroblastoma

	No. of patients
Stages 1, 2, 4S	70 (85%)
Stages 3, 4	12
No MYCN amplification	80 (98%)
With MYCN amplification	2
5-year survival rate	97%

Table 2 Outcome of the 82 patients less than 12 months old with neuroblastoma based on chemotherapy and type of resection

Chemotherapy	Tumor resection	Survival
Received ($n = 61$)	CR ($n = 38$)	35 (92%)
	ICR ($n = 23$)	23 (100%)
Not received ($n = 21$)	CR ($n = 21$)	21 (100%)
	ICR ($n = 0$)	

CR complete resection, *ICR* incomplete resection

Treatment and outcomes of the patients aged 12 months or older with advanced neuroblastoma

In principle, since 1985, the protocol of the Japan Study Group for Advanced Neuroblastoma (JANB) has been applied to patients aged 12 months or older with advanced neuroblastomas [9, 10]. This protocol consists of the following: First, a biopsy is taken of the tumor, followed by intensive induction chemotherapy based on the MYCN gene status. A second-look operation is performed for residual tumors that decreased in size following the induction chemotherapy after biopsy. The second-look operation usually involves complete resection of the primary tumor, avoiding injury to the surrounding organs and major vessels. Enlarged lymph nodes are usually resected with sampling the surrounding lymph nodes, but systematic lymphadenectomy is not performed. If the second-look operation involves the complete resection of the tumor, then postoperative localized external-beam radiation (2–30 Gy) is given, followed by high-dose chemotherapy with stem cell transplantation. If the second-look operation involves incomplete resection of the tumor, postoperative localized external-beam radiation (20–30 Gy) is given, followed by consolidation chemotherapy.

Regarding the degree of resection of the primary tumor, macroscopic complete resection is defined as complete resection without systematic lymphadenectomy at the initial diagnosis or at the second-look operation after induction chemotherapy, and surgical intervention for the primary tumor, such as a biopsy only, partial resection, and subtotal resection, is defined as incomplete resection.

Of our 41 patients more than 1 year old with neuroblastomas, 32 (78%) had stage 3 and 4 disease and 15 (36%) showed MYCN amplification. The 5-year survival rate of these 41 patients was 42% (Table 3). The 86% 5-year survival rate of the 9 patients with stage 1 and 2 disease was significantly better than the 30% 5-year survival rate of the 32 patients with stage 3 and 4 disease ($P < 0.05$). Moreover, the 25% 5-year survival rate of the 15 patients with MYCN amplification was significantly worse than the 53% 5-year survival rate of the 26 patients without MYCN amplification ($P < 0.05$) (Fig. 2). Of 9 patients with early (stage 1 or 2) disease and no MYCN amplification, 5 with stage 1 underwent initial complete resection of the tumor, and 4 with stage 2 underwent initial incomplete resection of the tumor. All except 1 of the 8 patients with stage 1 disease underwent postoperative mild chemotherapy. One of these patients died of another disease.

Table 3 Clinical characteristics of the 41 patients aged 12 months or older with neuroblastoma

Clinical characteristics	No. of patients
Stage	
Stage 1, 2, and 4S	9
Stage 3 and 4	32 (78%)
MYCN amplification	
No amplification	26
Amplification	15 (36%)
5-year survival rate	42%

There was no significant difference in 5-year survival rate between the 19 (46%) patients who underwent complete resection and the 22 (38%) who underwent incomplete resection (Fig. 3). Furthermore, the survival rate of the 32 patients with stage 3 and 4 disease did not differ significantly between the 11 patients who underwent complete resection and the 21 who underwent incomplete resection.

No local recurrence was observed in the ten patients over 1 year old with stage 4 disease who underwent complete resection of the primary tumor without systematic lymphadenectomy and local irradiation after 1994, although four of these patients died of metastatic recurrence (Table 4). Seven of these ten patients underwent stem cell transplantation (SCT); however, SCT was not associated with their outcome. No major surgical complications occurred.

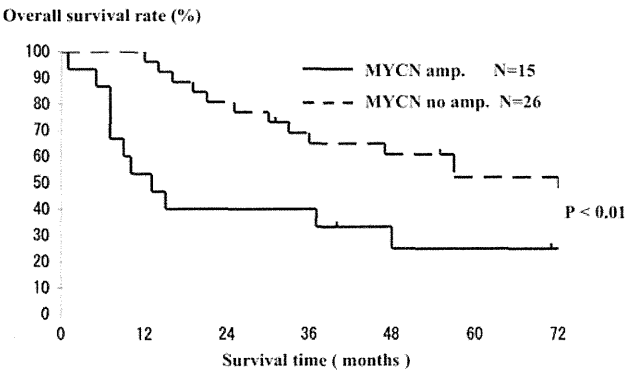

Fig. 2 Correlation between MYCN amplification (amp.) and prognosis in 41 neuroblastoma patients aged 12 months or older

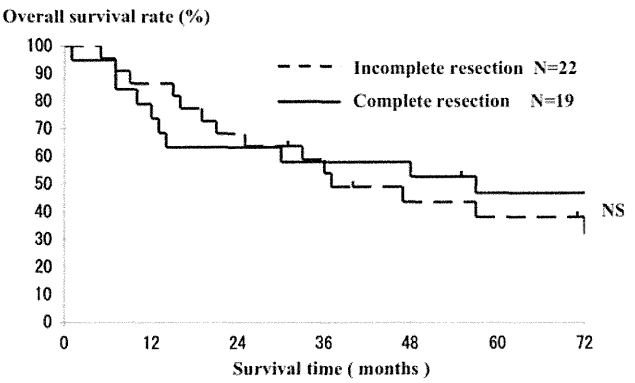

Fig. 3 Correlation between tumor resection and prognosis in 41 neuroblastoma patients aged 12 months or older

Table 4 Clinical course of the ten patients with stage 4 neuroblastoma who underwent complete resection of the primary tumor (1994–2004)

Patient no	Initial metastatic site	MYCN amplification	Local radiation	Local recurrence	Metastatic recurrence	Outcome
1	N, B, E, bm	no amp.	30 Gy	(−)	B	Died
2	N, B, E, bm	amp.	30 Gy	(−)	(−)	Alive 13 years after surgery
3	N, bm	amp.	30 Gy	(−)	(−)	Alive 10 years after surgery
4	N, bm	amp.	30 Gy	(−)	B, bm	Died
5	B	no amp.	30 Gy	(−)	B	Died
6	B, bm	amp.	30 Gy	(−)	N, B, bm	Died
7	B, bm, H	no amp.	30 Gy	(−)	(−)	Alive 6 years after surgery
8	B, bm, H	no amp.	18 Gy	(−)	(−)	Alive 6 years after surgery
9	N, bm	amp.	24 Gy	(−)	(−)	Alive 5 years after surgery
10	B, bm	amp.	18 Gy	(−)	(−)	Alive 5 years after surgery

N lymph node, *B* bone, *E* orbit, *bm* bone marrow, *amp.* amplification, *no amp.* no amplification

Discussion

The prognosis of patients with neuroblastoma tends to vary greatly, based on clinical prognostic factors, such as age and stage, and biological prognostic factors, such as MYCN amplification, DNA ploidy, and chromosome 1p deletion [11]. Thus, it is important to select the optimal therapy, including surgical intervention, according to the characteristics of these tumors [12]. This study is the experience of one institution between 1985 and 2004, during which time the treatment regimen for patients under 12 months of age with neuroblastomas and that for patients aged 12 months or over with advanced neuroblastomas did not change. Other investigators recently reported that 18 months of age is more preferable for risk assessment. In this study, the protocol of the Japanese Infantile Neuroblastoma Study Group was applied for patients less than 12 months of age with neuroblastomas, and the protocol of the Japan Study Group for Advanced Neuroblastoma (JANB) was applied for patients aged 12 months or older with advanced neuroblastomas. Therefore, investigating the borderline of age for risk group classification was not suitable for the analysis in the present study.

The majority of infant neuroblastomas are localized tumors with low-grade malignancy [13]. In particular, neuroblastomas detected by mass screening include spontaneously regressing tumors [14]. In the present series of 82 patients under 12 months of age, the outcome of infant neuroblastomas was excellent, regardless of whether they underwent complete or incomplete resection of the tumor. Furthermore, the outcome of patients who underwent complete resection of the tumor was excellent, regardless of whether they received postoperative chemotherapy. We could not compare patients who underwent initial extirpation with those who did not, because biopsies were performed for all unresectable neuroblastomas, followed by mild chemotherapy. Although complete resection might be unnecessary for infantile neuroblastoma, the majority of which have favorable biology, performing initial tumor extirpation might avoid the need chemotherapy or irradiation.

In the present study, of nine patients whose disease was detected clinically and not through mass screening, only one patient who had stage 4S disease with MYCN amplification died of the disease. Irrespective of mass screening, most patients under 12 months of age had a good prognosis. It is clear that MYCN gene amplification was a powerful prognostic factor, even for infantile neuroblastoma [8].

In the present study, all five major surgical complications occurred in the patients with localized neuroblastoma (stages 1, 2, and 3). Recently, in an effort to establish a new risk-adapted preoperative neuroblastoma staging system, the International Neuroblastoma Risk Group (INRG) task force proposed the use of surgical risk factors (SRFs) to classify localized neuroblastomas [15]. SRFs were defined by objective and subjective radiologic imaging characteristics thought to be associated with an increased risk of surgical complications. These radiologic criteria are known as "image-defined risk factors" (IDRF) [16]. In a retrospective radiologic review of the five patients with surgical complication, all those who suffered postoperative renal atrophy had a positive IDRF. The Japan Neuroblastoma Study Group (JNBSG) proposed guidelines for surgical intervention for localized neuroblastoma based on the IDRF and began observational study of the treatment for low-risk neuroblastomas in 2010.

The role of surgery in the treatment of advanced neuroblastoma in patients 12 months or older remains controversial. La Quaglia et al. [17] reported that gross total resection improved the survival of 39 patients with stage IV neuroblastoma. Conversely, Adkins et al. [18] reported that complete resection was of little benefit for high-risk

neuroblastomas treated by CCG-3891. Kuroda et al. [19] found that intensive surgery with intraoperative radiation therapy dramatically increased local eradication and improved the outcome of patients even if they had advanced neuroblastomas with MYCN amplification. On the other hand, Castel et al. [20] found that delayed surgery after chemotherapy contributes to the good control of stage IV disease, although the final outcome of these patients was determined more by metastatic relapses than by the degree of resection. Kaneko et al. and Kubota et al. reported that systemic extensive surgery for advanced or metastatic neuroblastoma is no longer required if therapy supplemented with intensive pre- and postoperative chemotherapy is given [4, 21]. In the present study, the clinical stage and tumor biology of advanced neuroblastoma in patients aged 12 months or older was associated with the overall survival rate. The degree of tumor resection did not correlate significantly with the overall survival rate. In this study, the second-look operation was conservative tumor resection of the primary tumor, avoiding the injury to the surrounding organs and major vessels, and systematic lymphadenectomy was not performed. Therefore, we could not examine the complications of surgery and the delay in administering intensive chemotherapy resulting from major surgery. No major complications occurred after the second-look operation for the advanced neuroblastomas in patients aged more than 1 year old in this study, and we avoided a delay in intensive chemotherapy after the operation. Furthermore, complete resection of the primary tumor without systematic lymphadenectomy and localized external-beam radiation prevented local recurrence; however, the outcome of patients depended on metastatic recurrence. These results indicate that the main treatment for advanced neuroblastoma in patients aged 12 months or older is systemic chemotherapy, and that extirpation of the primary tumor without major surgery might prevent local recurrence, when combined with irradiation therapy.

In the JNBSG, two clinical phase II studies for high-risk neuroblastoma were begun in 2008 and completed in 2010. The guidelines for surgical intervention in these clinical studies recommend complete resection of the primary tumor without systematic lymphadenectomy, and localized irradiation. The long-term outcomes, including late complications, revealed by these clinical studies will be interesting.

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III. 臨床応用の進歩と展望　免疫療法(免疫細胞療法)

Glypican-3などのがん特異的抗原を標的とした免疫療法

澤田　雄　　中面哲也

The cancer specific antigen, glypican-3(GPC3)-targeted immunotherapy

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Abstract

The carcinoembryonic antigen glypican-3(GPC3) is an ideal target of tumor antigen-specific immunotherapy against hepatocellular carcinoma(HCC), because it is overexpressed specifically in HCC. We have reported that a GPC3-derived peptide vaccination was well-tolerated, and immune responses and antitumor efficacy were noted in a phase I trial for HCC patients. We have begun a phase II study of the GPC3-derived peptide vaccine as an adjuvant therapy for HCC patients, and a pilot study of liver biopsies performed before and after GPC3 peptide vaccination for advanced HCC to determine whether tumor-infiltrating lymphocytes are indeed GPC3 peptide-specific CTLs. Furthermore, we are initiating clinical trials of a GPC3-derived peptide vaccine for patients with hepatoblastoma or ovarian clear cell carcinoma.

Key words: glypican-3(GPC3), peptide vaccine, cytotoxic T lymphocyte(CTL)

はじめに

現在までに様々ながん拒絶抗原が同定され，これらの分解産物であるペプチドとHLAクラスI分子を認識するCD8陽性細胞傷害性T細胞(cytotoxic T lymphocyte: CTL)が，がん細胞だけを攻撃するメカニズムを用いたがん抗原特異的免疫療法を目指すことが可能になった．同定されたがん拒絶抗原を用いた臨床試験が世界中で行われており，最近では，前立腺がんに対して樹状細胞療法sipuleucel-T(Provenge®)の第III相臨床試験での免疫療法の有効性も報告され，米国食品医薬品局(Food and Drug Administration: FDA)に承認されている．日本国内でも様々な施設からがんに対するペプチドワクチンの有効例の報告が散見される．著者らは，新規がん胎児性抗原glypican-3(GPC3)を同定し，基礎研究の成果を基に国立がん研究センター東病院でGPC3を標的とするペプチドワクチン療法を行っている．

本稿では，著者らの基礎から臨床応用への一連の研究，完了した肝細胞がんに対するGPC3ペプチドワクチン第I相臨床試験の概略および現在行っている臨床試験について紹介する．

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0047-1852/12/¥60/頁/JCOPY

1. がん特異抗原GPC3の同定と前臨床試験

がん特異抗原の免疫療法への応用を考える場合，がん抗原の発現頻度，腫瘍特異性，免疫原生，消失性(免疫からの逃避が起こりにくい)および自己免疫などの有害事象の誘導の危険性などの特徴をとらえる必要がある．著者らは，東京大学医科学研究所(中村祐輔教授)との共同研究により，cDNAマイクロアレイのデータを基に，肝細胞がんに特異的な新規がん胎児性抗原としてGPC3を同定した[1-4]．がん胎児性抗原GPC3は，580アミノ酸からなる65 kDaの膜タンパク質であり，ヘパラン硫酸プロテオグリカンファミリーに属する．またGPC3遺伝子およびタンパクが，肝細胞がんにおいて特異的に高発現し，正常組織においては胎生期の肝臓あるいは免疫学的に隔離された胎盤でのみ発現していることを確認した．発現の組織特異性が優れていることから，著者らは，この新規がん胎児性抗原GPC3が，理想的な腫瘍拒絶抗原になりうるかどうかを検討し，マウスや肝細胞がん患者の血液中リンパ球を用いて，日本人の約60％が陽性であるHLA-A24拘束性GPC3由来ペプチド(EYILSLEEL)を同定した[5]．同様に日本人の40％が陽性で，欧米白人のメジャータイプであるHLA-A2拘束性GPC3由来ペプチド(FVGEFFTDV)を同定した[6]．またマウスを用いた実験でGPC3抗原の免疫によってペプチド特異的CTLが誘導され[7]，自己免疫現象の誘導なく抗腫瘍効果が認められることを示した．

このようにほとんどのがん患者において高発現しているようながん抗原に関して，HLA-A24あるいはA2拘束性のがん拒絶抗原を同定することにより，多くの患者を対象にペプチドワクチン，樹状細胞ワクチン，更には養子免疫療法などのがん抗原特異的免疫療法が可能になると考えられる．また卵巣明細胞腺がん，肺扁平上皮がん，一部の小児がん(肝芽腫，腎芽腫，卵黄嚢腫)などにも発現があり，卵巣明細胞腺がん，小児がんについては，現在GPC3ペプチドワクチン臨床試験を開始している．

2. 進行肝細胞がんに対するGPC3ペプチドワクチン第I相臨床試験の概要[8-14]

進行肝細胞がん33例を対象にGPC3ペプチドワクチン第I相臨床試験を2007年2月に開始し2009年11月に完了した．主要評価項目は，安全性，副次評価項目として，無増悪期間(TTP)，全生存期間(OS)などの臨床効果のほか，免疫学的反応を設定した．1回の投与量を0.3，1，3，10，30 mgの5段階とし，2週間おきに3回左右の腋窩部，腹部および鼠径部の皮内に不完全フロイントアジュバント(incomplete Freund's adjuvant: IFA)と混ぜたエマルジョン製剤を，安全性を確認しながら用量を増して投与した．

この試験で認められた有害事象として，投与局所の発赤・硬結は，ほぼ全例の患者にみられ，grade 2相当の一過性の発熱，異所性の皮疹が6人にみられるのみであった．grade 3の有害事象として，肝機能障害が4人の患者で観察されたが，効果安全性評価委員会により，いずれもがんの進行によるものと判断された．これらの結果より，GPC3ペプチドワクチン療法は安全性に問題ないと判断された．

臨床効果については，全33症例の無増悪期間中央値は3.4カ月，全生存期間中央値は9.0カ月であった．3回のワクチン投与後1カ月後のCTのRECIST version 1.0での評価では，全33例中，1例部分奏効(PR)と判定され(**図1-a**)，19例で不変(SD)と判定された．2カ月間の病勢コントロール率(PR+SD)は60.6％であった．SDと判定された患者のうち4人で，PRの基準を満たしていないが腫瘍の壊死または腫瘍の部分的な縮小が観察された．腫瘍マーカーであるAFP，PIVKA-IIは，33例中9例(27％)で投与前と比べて少なくとも一度の低下が観察された．

また免疫学的反応の解析のため，全33症例でワクチン投与前後での末梢血単核球細胞中のGPC3ペプチド特異的CTLの検出に，*ex vivo* IFN-γ enzyme-linked immunospot(ELISPOT)