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Excellent Early Outcomes of Combined Chemotherapy With Arsenic Trioxide for Stage 4/M Neuroblastoma in Children: A Multicenter Nonrandomized Controlled Trial

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This nonrandomized, multicenter cohort, open-label clinical trial evaluated the efficacy and safety of combined chemotherapy with arsenic trioxide (ATO) in children with stage 4/M neuroblastoma (NB). We enrolled patients who were newly diagnosed with NB and assessed as stage 4/M and received either traditional chemotherapy or ATO combined with chemotherapy according to their own wishes. Twenty-two patients were enrolled in the trial group (ATO combined with chemotherapy), and 13 patients were enrolled in the control group (traditional chemotherapy). Objective response rate (ORR) at 4 weeks after completing induction chemotherapy was defined as the main outcome, and adverse events were monitored and graded in the meantime. Data cutoff date was December 31, 2019. Finally, we found that patients who received ATO combined with chemotherapy had a significantly higher response rate than those who were treated with traditional chemotherapy (ORR: 86.36% vs. 46.16%, $p = 0.020$). Reversible cardiotoxicity was just observed in three patients who were treated with ATO, and no other differential adverse events were observed between the two groups. ATO combined with chemotherapy can significantly improve end-induction response in high-risk NB, and our novel regimen is well tolerated in pediatric patients. These results highlight the superiority of chemotherapy with ATO, which creates new opportunity for prolonging survival. In addition, this treatment protocol minimizes therapeutic costs compared with anti-GD2 therapy, MIBG, and proton therapy and can decrease the burden to families and society. However, we also need to evaluate more cases to consolidate our conclusion.

Key words: Chemotherapy; Arsenic trioxide (ATO); Neuroblastoma (NB); Children; Clinical Trial

INTRODUCTION

Neuroblastoma (NB), originating from the nerve crest of the sympathetic nervous system, is one of the most common malignant extracranial solid tumors in children, accounting for approximately 10% of pediatric malignancies and contributing to 15% of all pediatric cancer mortality^{1,2}. About 40% of patients are defined as high-risk NB (HRNB) with distant metastasis diseases at diagnosis. Although new strategies have been developed for treatment in recent years, the outcome of patients with

HRNB remains poor, with long-term survival less than 50%³. Currently, chemotherapy still plays a key role in the treatment of NB, and it is reported that improved end-induction response in HRNB is associated with longer survival⁴. Therefore, searching for innovative regimens of induction chemotherapy is strategic to improve prognosis of patients with HRNB.

Arsenic trioxide (ATO) is an ancient drug used in traditional Chinese medicine for more than 2000 years, and currently, it is a Food and Drug Administration (FDA)-approved drug to treat acute promyelocytic

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leukemia (APL)^{5,6}. In recent years, ATO has been reported to exert potent cytotoxic activity against a large variety of cancer cells of solid tumor including NB⁷. Particularly, our previous studies^{8,9} have confirmed that ATO could inhibit proliferation of NB cells by retarding cell cycle in the G₀ or G₂/M phase and has synergetic cytotoxic effects to NB cells when used in combination with other chemotherapy drug like etoposide, cisplatin, vinorelbine, and docetaxel. Recently, ATO is demonstrated as a Hedgehog (HH) pathway inhibitor acting at the level of GLI. The HH signaling pathway plays an important role in the development of neural crest stem cells¹⁰. It is reported that the signaling molecules of this pathway such as SHH, PTCH1, SMO, and GLI are highly expressed in NB patients, and activated HH pathway accounts for the poor prognosis of NB patients¹¹. Evidence has shown that ATO could suppress tumor growth of NB by blocking HH/GLI both in vitro and in vivo^{12,13}. Therefore, we conducted an innovative clinical study to evaluate the efficacy and safety of ATO combined with chemotherapy for patients with newly diagnosed stage 4/M NB. Here we report the preliminary results.

MATERIALS AND METHODS

Ethics Statement

All patients and/or guardians gave written informed consent approved by the Ethics Committee of Sun Yet-Sen Memorial Hospital, and the study was approved by both the protocol review committee and the institutional review board of each institution. This trial was also registered with ClinicalTrial.gov (NCT03503864) and Chinese Clinical Trial Registry (ChiCTR1800014748).

Patients

Children 14 years of age were eligible for this study if they were newly diagnosed with NB and assessed as stage 4 according to the International Neuroblastoma Staging System (INSS) or stage M according to the International Neuroblastoma Risk Group (INRG), respectively^{14,15}. Patients were divided into two groups: (a) trial group: patients received traditional chemotherapy combined with ATO; (b) control group: patients received traditional chemotherapy alone.

Treatment

Patients in the control group received traditional comprehensive treatment following SMHPO-N-2012 NB protocol, a protocol based on N7 and NB2004 protocols (Table 1). Traditional chemotherapy was a nine-cycle treatment and consisted of three regimens: CAV (cycles 1, 2, 4, and 6), PVP (cycles 3, 5, and 7), and CT (cycles 8 and 9). The CAV regiment was composed of vincristine 0.022 mg/kg or 0.67 mg/m² daily for 2 h on days 1 and 2, doxorubicin 25 mg/m² over 3 h, and cyclophosphamide 1.2 g/m² for 3 h daily on days 1 to 3. The PVP regimen consisted of cisplatin 50 mg/m² daily for 6 h over days 1 to 4 and etoposide (light resistant) 200 mg/m² daily for more than 4 h over days 1 to 3. The CT regimen consisted of cyclophosphamide 1.2 g/m² for 3 h daily on days 1 and 2 and topotecan (light resistant) 2 mg/m² daily as a continuous infusion over days 1 to 3 (72 h in total).

In the trial group, patients received ATO combined with chemotherapy for nine courses in total (Fig. 1). ATO was administered 2 days in advance at a dose of 0.16 mg/kg per day for 10 days (Table 1). The ATO injection was administered at a constant rate over 8 h in 250–500 ml of normal saline or a 5% glucose solution through a central

Table 1. Dose and Usage of Chemotherapeutics

Regimen/Drug	Dose and Usage	Time
ATO		
Arsenic trioxide	0.16 mg/kg/day, IV drip, PI > 4 h	d (-2)–d7
CAV		
CTX	1.2 g/m ² /day, IV drip, PI = 3 h	d1–d2
THP	25 mg/m ² /day, IV drip, PI > 3 h	d1–d3
VCR*	0.022 mg/kg/day or 0.67 mg/m ² /day, IV drip, PI = 2 h	d1–d3
PVP		
DDP	50 mg/m ² /day, IV drip, PI = 6 h (light resistant)	d1–d4
VP-16	200 mg/m ² /day, IV drip, PI > 4 h	d1–d3
CT		
CTX	1.2 g/m ² /day, IV drip, PI = 3 h	d1–d2
Topotecan	2 mg/m ² /day, IV drip, PI = 24 h (light resistant)	d1–d3

CTX, cyclophosphamide; THP, pyranodomyacin; VCR, vincristine; DDP, carboplatin; VP-16, etoposide.

*Take the lower value of the two calculation methods; maximum dose = 0.67 mg/day and total dose of 3 days 2 mg.

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Regimens	▲			▲			●			▲			⊗		●			▲			●				■					■
	↑			↑			↑			↑					↑			↑			↑				↑				↑	

Figure 1. Arsenic trioxide (ATO) combined with induction chemotherapy of SMHPO-N-2012 neuroblastoma (NB) protocol.

venous catheter. Simultaneously, patients received 0.5–1.0 g ascorbic acid along with 5% 100- to 250-ml glucose injections in another vein channel. Surgery could be performed at one of the two different time points if necessary: before treatment or after four courses of our protocols. As for surgery, we consulted the HR-NBL1/SIOPEN study¹⁶ and strongly encouraged complete macroscopic excision (CME) of the primary tumor, including all visible and palpable tumor and related involved lymph nodes. If surgery was performed, evaluation of patient's status should be conducted before surgery and at least 2 weeks after it.

General imaging examinations such as computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET) CT were conducted every two or four courses of chemotherapy. In our assessment, if patients presented for surveillance PET CT, high standard uptake value (SUV) of tumors (>2.5) was interpreted as “a suspicious malignant process,” and further examinations should be required to fully understand this distinction from other malignant tumors.

Serum neuron-specific enolase (NSE) and urinary vanillylmandelic acid/creatinine (VMA/Cr) ratio was measured at each course. Bone marrow examination was performed at each course until bone marrow metastasis converted negative.

Assessments

The primary analytic end point was objective response rate (ORR). Time to event was defined as time from diagnosis until time of 4 weeks after completing nine cycles of chemotherapies or the time of first occurrence of relapse, progression. Effective evaluation was performed using the International NB Staging System criteria¹⁴ at the end point. (1) A complete response (CR) was defined as the complete resolution of all clinical evidence of disease for at least 4 weeks. (2) A partial response (PR) was defined as a 50%–90% reduction in the sum of the products of the perpendicular diameters of all measurable lesions for at least 4 weeks and no appearance of new lesions. (3) Stable disease (SD) was defined as a decrease <50% in tumor size less than a PR, but no disease progression or any lesion enlarged by <25%. (4) Progressive disease (PD) was defined as the appearance of new lesions or a 25% increase in the product of the two longest perpendicular diameters in any previously measurable lesion (excluding bone).

The toxicities associated with ATO include cardiotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity,

metabolic disturbance, fluid retention, skin discoloration, xeroderma, conjunctivitis, etc¹⁷. Adverse events (AEs) were monitored and graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5.0 (<https://ctep.cancer.gov>).

Statistical Analyses

Categorical variables (efficiency of induction chemotherapy and determination of cytotoxic activity) were compared by using Fisher's exact test, and count data were analyzed by using nonparametric test as appropriate. Statistical significance was defined as a value of $p < 0.05$. All analyses were performed with SPSS 25.0.

RESULTS

Patient Characteristics

During January 1, 2018, and December 31, 2019, a total of 22 patients were enrolled in the trial group, while 13 patients were included in the control group during January 1, 2011 and December 31, 2019. All of these 35 patients were evaluated as high risk, and patient demographics and baseline characteristics were comparable between groups (Tables 2 and 3).

Primary NB sites were adrenal gland ($n = 17$, 77.27%), mediastinum ($n = 2$, 9.09%), bone ($n = 2$, 9.09%), and cervical region ($n = 1$, 4.55%) in the trial group, and adrenal gland ($n = 10$, 76.92%) and bone ($n = 3$, 23.08%) in the control group. The most common metastasis was bone marrow in both groups (trial group: 15/22; control group: 9/13). MYCN amplification detection was performed in all patients, and positive rates were 59.09% (13/22) and 61.54% (8/13) in the trial group and control group, respectively.

In the trial group, 22 patients received ATO combined with chemotherapy, and 13 patients in the control group were treated with traditional chemotherapy.

Efficacy

Among patients treated with combined chemotherapy, 12 patients achieved CR, 7 were PR, and 3 were PD after induction therapy. In the control group, 3 patients achieved CR and 3 were PR, and 7 patients were assessed as PD. Patients who received ATO combined with chemotherapy had a higher response rate than those who were treated with traditional chemotherapy (ORR: 86.36% vs. 46.16%, $p = 0.020$) (Table 3, Figs. 2 and 3).

Table 2. Patient Demographic and Baseline Clinical Characteristics

	Trial Group (N = 22)	Control Group (N = 13)	Total (N = 35)	p Value*
Age				0.384
Median (years)	3.00	4.00	4.90	
Range (IQR) (years)	0.7–7.0 (3.49)	0.1–8.0 (3.41)	0.1–8.0 (2.85)	
<18 months	3 (13.64%)	1 (7.69%)	4 (11.43%)	
18 months	19 (86.36%)	12 (92.31%)	31 (88.57%)	
Gender				0.488
Male	12 (54.55%)	9 (69.23%)	21 (60.00%)	
Female	10 (45.45%)	4 (30.77%)	14 (40.00%)	
Stage of disease				1.000
INSS stage 4	20 (90.91%)	12 (92.31%)	32 (91.43%)	
INRG stage M	2 (9.09%)	1 (7.69%)	3 (8.57%)	
Primary site				1.000
Adrenal gland	17 (77.27%)	10 (76.92%)	27 (77.14%)	
Mediastinum	2 (9.09%)	0 (0%)	2 (5.71%)	
Bone	2 (9.09%)	3 (23.08%)	5 (14.29%)	
Cervical region	1 (4.55%)	0 (0%)	1 (2.86%)	
BM metastasis				1.000
Yes	15 (68.18%)	9 (69.23%)	24 (68.57%)	
No	7 (31.82%)	4 (30.77%)	11 (31.43%)	
VMA/Cr				0.432
Median	42.60	44.70	44.70	
Range (IQR)	6.18–108.6 (43.92)	17.8–388 (66.03)	6.18–388 (51.00)	
MYCN amplification				1.000
Positive	13 (59.09%)	8 (61.54%)	21 (60.00%)	
Negative	9 (40.91%)	5 (38.46%)	14 (40.00%)	

IQR, Interquartile range; INSS, International Neuroblastoma Staging System; INRG, International Neuroblastoma Risk Group; BM, bone marrow; VMA/Cr, urinary vanillylmandelic acid/creatinine ratio.

*Categorical variables were compared using Fisher's exact test, and count data were analyzed using nonparametric test.

Toxicity

There were no treatment-related deaths. Table 4 lists all grade 3 or 4 AEs that occurred in 35 patients (22 in the trial group and 13 in the control group) who were enrolled in this trial.

All patients had grade 4 leukopenia and neutropenia during chemotherapy, with no significant difference observed between groups. The nonhematologic grade 4 toxicities were not found in our study. The nonhematologic grade 3 toxicities included infection, alanine aminotransferase (ALT) elevation, and aspartate aminotransferase (AST) elevation. The most common grade 3 nonhematologic AE was infection, and there were 59.09% (13/22) and 53.85% (7/13) in the trial group and

control group, respectively. In addition, AST and ALT elevation simultaneously occurred in six patients (each three in the trial group and control group) but could be reversed by using hepatinica. Reversible cardiotoxicity was observed in three patients who received ATO combined with chemotherapy, presenting as increased heart rates in one case and asymptomatic serum increased heart type creative arouses enzyme (CK-MB) in another two cases (the CK-MB levels of two patients were less than 2.5 times the upper limit of normal and evaluated as grade 1 according to NCI-CTCAE). Initial cardiac workup of these two patients with elevated CK-MB, including electrocardiogram (ECG), echocardiography, and Holter monitoring, yielded normal results. We

Table 3. Treatment Responses of End-Induction Chemotherapy

Group	Enrolled Cases (N)	Response			ORR (%)
		CR (N)	PR (N)	NR/PD (N)	
ATO combined with chemotherapy	22	12	7	3	86.36
Conventional chemotherapy	13	3	3	7	46.16

CR, complete response; PR, partial response; NR, no response; PD, progressive disease; ATO, arsenic trioxide. Using Fisher's exact probabilities: $p = 0.020$.

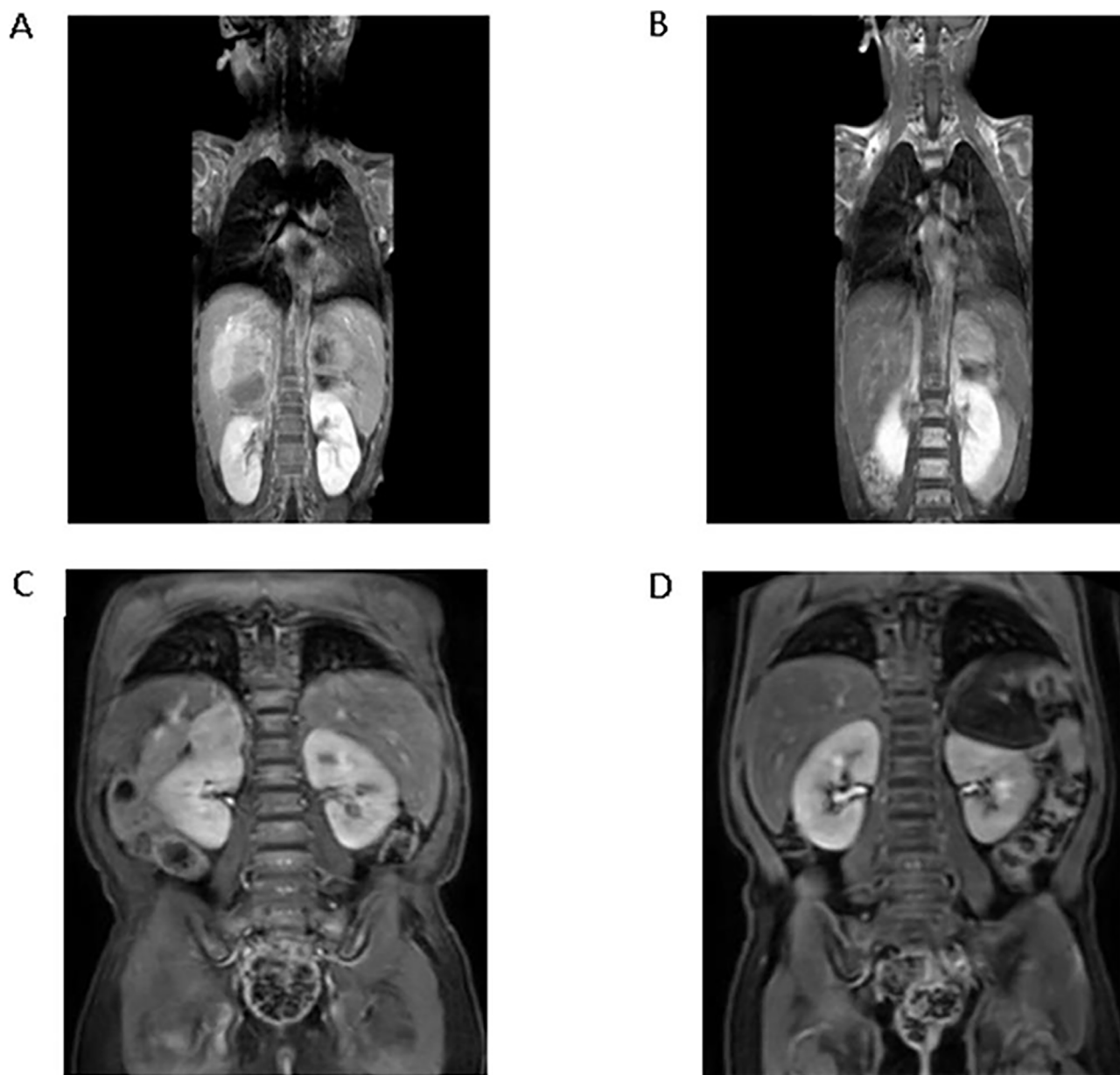


Figure 2. Magnetic resonance imaging (MRI) of a 3-year-old girl in the trial group (A: before treatment; B: after ATO combined with chemotherapy and surgery). MRI of a 2-year-old girl in the trial group (C: before treatment; D: after ATO combined with chemotherapy and surgery).

found no significant difference in systolic ejection fraction (range: 59%–64%) with baseline prior to treatment (range: 60%–66%) either. These cardiac side reactions disappeared after suspension of ATO, and no abnormality was observed in careful reexamination.

DISCUSSION

NB is the most common extracranial solid tumors in children that can occur anywhere in the sympathetic nervous system, accounting for about 8% to 10% of

childhood malignancies and 15% of childhood tumor-related mortality². HRNB is a tumor with a high degree of malignancy with 5 years overall survival (OS) rate of less than 40% before the multimodality therapy, such as chemotherapy, surgery, radiotherapy, and hematopoietic stem cell transplantation². Worse still, the recurrence rate of HRNB within 2 years (median time: 4.5–18.7 months) after the initial diagnosis has exceeded 80%, and only 7% to 12.7% of relapsed cases could survive more than 5 years^{18,19}. In an effort to enhance the survival of HRNB,

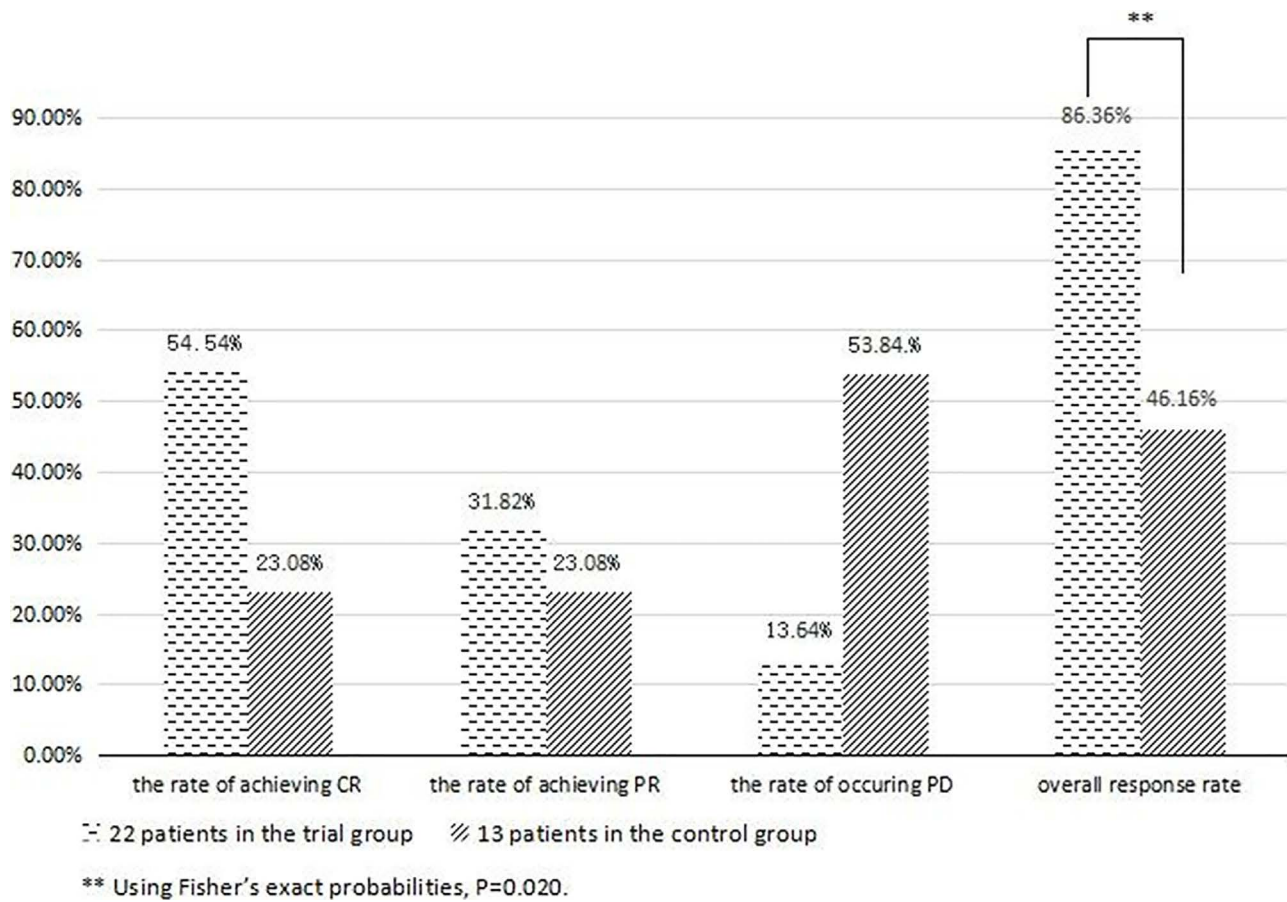


Figure 3. Different results between the trial group and control group in evaluation. **Patients who received ATO combined with chemotherapy had a higher overall response rate (ORR) than those who were treated with traditional chemotherapy.

scholars have focused on immunotherapy and targeted therapy. Dinutuximab, an anti-GD2 monoclonal antibody, was reported to raise 2-year event-free survival (EFS) and OS rates to $66 \pm 5\%$ and $86 \pm 4\%$, respectively, compared to the standard treatment in a phase III study²⁰. It is important to note that anti-GD2 therapy may enhance the incidence of low GD2-expressing tumor, decreasing the effectiveness of maintenance therapy²¹. A study in 2015 (NCT01355679) in which therapy is assigned based on results of molecular profiling for patients with relapsed disease, and only 1 of 14 subjects achieved partial remission (the median time of EFS was 59 days)²². Association studies indicated that some patients had responses to other molecularly guided approaches for MYCN, ALK, and PI3K/AKT/mTOR pathways in the short term; however, the efficacy of these treatments showed a gradual decline due to drug resistance and reduction in target cells²³. With this situation, the molecularly guided approaches are not as satisfied until now in clinical practices.

In our study, 13 patients in the control group received traditional comprehensive treatment, a protocol based on

N7 and NB2004 protocols. Of these patients, the value of the response rate (6/13, 46.16%) was significantly smaller compared to literature-reported results. N7 protocol was tested by Cheung et al.²⁴, and 24 patients more than 1 year of age with newly diagnosed HRNB were enrolled in their study. Among these patients, the overall response after induction chemotherapy consisted of 21 CR/very good partial response (VGPR) (87.50%), 2 PR (8.33%), and 1 PD (4.17%). According to Kholer et al.²⁵, of the 30 patients with positive mIBG scans, there were 16 in CR giving a response rate of 53.3% [95% confidence interval (CI): 36.1%–69.8%]. This difference might be explained as follows. Firstly, this might be related to the small sample size, resulting in greater fluctuation of the predicted value. Secondly, in the control group of our study, the positive rate of MYCN amplification (61.54%) was higher than the other two studies (45.83% reported by Cheung et al.²⁴ and 28.13% reported by Kholer et al.²⁵), and it indicated that patients in our group might have a higher degree of malignancy with poor therapeutic effects. The overall response rate of the trial group was

Table 4. Incidence and Severity of Adverse Events

Adverse Event	Trial Group (N = 22)	Control Group (N = 13)	p Value*
Hematologic			
Leukopenia			1.000
Grade 3	0 (0.00%)	0 (0.00%)	
Grade 4	22 (100.00%)	13 (100.00%)	
Neutropenia			1.000
Grade 3	0 (0.00%)	0 (0.00%)	
Grade 4	22 (100.00%)	13 (100.00%)	
Anemia			0.259
Grade 3	14 (63.64%)	11 (84.62%)	
Grade 4	8 (36.36%)	2 (15.38%)	
Thrombocytopenia			1.000
Grade 3	18 (81.82%)	10 (76.92%)	
Grade 4	4 (18.18%)	3 (23.08%)	
Nonhematologic			
AST elevation			1.000
Grade 3	3 (13.64%)	3 (23.08%)	
Grade 4	0 (0.00%)	0 (0.00%)	
Others	19 (86.36%)	10 (76.92%)	
ALT elevation			1.000
Grade 3	3 (13.64%)	3 (23.08%)	
Grade 4	0 (0.00%)	0 (0.00%)	
Others	19 (86.36%)	10 (76.92%)	
Infection			0.734
Grade 3	13 (59.09%)	7 (53.85%)	
Grade 4	0 (0.00%)	0 (0.00%)	
Others	9 (40.91%)	6 (46.15%)	

*Categorical variables were compared using Fisher's exact test, and count data were analyzed using nonparametric test.

higher than might be expected that 86.36% of patients achieved PR or better, which significantly surpassed the control group without any obvious difference in the toxicities. Compared with published data of the Children's Oncology Group (COG)⁴, the ORR of our induction effect was higher than COG (86.36% vs. 78.4%). Our investigation defined the antitumor efficiency and safety of this regimen, suggesting a potential role for ATO in the management of patients with HRNB. In our study, all patients had grade 4 leukopenia and neutropenia. Infection occurred about 59.09% (13/22) and 53.85% (7/13) in the trial group and control group, respectively. AST and ALT elevation simultaneously went up in six patients (each three in the trial group and control group). Three patients who received ATO combined with chemotherapy were found with cardiotoxicity. After corresponding symptomatic treatment and suspension chemotherapy, all these AEs could be reversed.

ATO has been used as a drug for the treatment of various diseases in ancient China. As early as the 1970s, Chinese scholars discovered that ATO can induce APL tumor cell differentiation and apoptosis to achieve remarkable results in initial/relapse tumor APL⁶. Dozens

of vitro and vivo studies have shown the broad-spectrum antitumor activities of ATO in multiple tumors²⁶⁻³⁰, suggesting the clinical application value of ATO. Our previous studies have found some mechanisms of ATO cytotoxicity in NB cells. First is the G₂/M cell cycle arrest at 48 h in the SKNSH cell line after ATO administration, which can significantly enhance the cytotoxic efficiency of M phase-specific chemotherapy drugs (vinorelbine, docetaxel, etc)⁹. Second is a dose-dependent upregulation of TrkA and TrkC receptors related to the good prognosis of NB³¹. Furthermore, ATO could induce the downregulation of glycoprotein P (P-gp) in SK-N-SH cells⁹. Relapsing NB has frequently gained multidrug resistance (MDR), so that benefits little from conventional regimens. The P-gp and multidrug resistance-associated protein 1 (MRP1) are the pumps commonly found to confer MDR in cancers. P-gp and MRP1 have been demonstrated to regulate the export of cytotoxic drugs, including vincristine, etoposide, daunorubicin, and others³². It has been reported that MRP1 is a direct transcriptional target of MYCN in NB, which enhanced MRP1-mediated drug resistance³³. In addition to MRP1, an increased expression of other MRP family members MRP4 was confirmed

as predictive of poor clinical outcome in aggressive NB³⁴. Research studies demonstrate upregulation of P-gp, and MDR1 gene expression was strongly upregulated during standard chemotherapy in multifocal HRNB³⁵. Karlsson et al. demonstrated that ATO could efficiently kill NB cells, while the chemotherapeutics of HRNB, i.e., etoposide, doxorubicin, carboplatin, and vincristine, failed to kill multidrug-resistant NB cells³⁶. This result is in agreement with our study demonstrating that ATO has advantages for the treatment of relapsing NB.

Mechanisms underlying the ATO cytotoxic actions are various, such as inducing PML-RAR fusion protein degradation combined with retinoic acid. Retinoic acid targets the RAR portion of the fusion protein, whereas ATO targets the PML part of the protein, which resulted in apoptosis and partial differentiation of the leukemic cells³⁷. Furthermore, ATO can induce ROS production to mediate activation of the downstream caspase-dependent apoptosis pathways³⁸; upregulate tumor apoptosis genes bax, bak, and Fas; inhibit tumor angiogenesis; and so on³⁹. Recently, some considered that the cytotoxic actions of ATO are probably via inhibition of the HH signaling pathway by targeting Gli protein. HH signaling pathway is critical for embryonic patterning and pathologically associated with oncogenesis, maintenance of the tumorigenicity, and prognosis in rhabdomyosarcoma^{40,41}, medulloblastoma⁴², osteosarcoma⁴³, etc. A high proportion of abnormal activation of HH signaling pathway in surgical specimens of embryonic tumors has been confirmed. The positive expression rates of SHH, PTCH, and Gli1 are 96%, 100%, and 68% in NB; 78%, 100%, and 78% in rhabdomyosarcoma; and 71%, 100%, and 43% in Wilms tumor, respectively¹¹, suggesting that the components of the HH pathway may be a target for treatment. Several studies indicated that HH pathway downstream module Gli inhibitors can available inhibit the proliferation of NB cells⁴⁴⁻⁴⁷ by modulating the expression of cell cycle proteins such as cyclin D1 or p21⁴⁸, and decrease the transcription of Gli1 downstream gene MYCN, which is closely related to the invasiveness and poor prognosis of HRNB⁴⁹. Antagonism of ATO to Gli1 was confirmed via replacing zinc finger of Gli1 protein or preventing the accumulation of cilium, resulting in the inactivation of Gli1^{50,51}. All in all, ATO may antagonize NB tumors by means of multiple pathways mentioned above.

CONCLUSION

From our study, we concluded that the ATO combined with chemotherapy has an obvious superiority in end-induction response (ORR: 86.36%) for newly diagnosed stage 4/M NB in children, with mild ATO-related toxicities observed. These results highlight the superiority of chemotherapy with ATO, which creates new opportunity for prolonging survival. Besides, this treatment protocol

minimizes therapeutic costs compared with anti-GD2 therapy, MIBG, and proton therapy and can decrease the burden to families and society. Further exploration is needed to bring in more cases to consolidate our conclusion and enrich the fundamental research of ATO's cytotoxic effect in the NB cell line. We will continue to track the long-term prognosis of patients in this study.

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