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Improved Outcomes with Induction Chemotherapy Combined with Arsenic Trioxide in Stage 4 Neuroblastoma: A Case Series

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Abstract

Objective: The apoptotic and cytotoxic effects of arsenic trioxide (ATO) makes it a potentially suitable agent for the treatment of patients with neuroblastoma with poor prognosis; therefore, we try to evaluate the effectiveness and safety of ATO combined with reinduction/induction chemotherapy in children with recurrent/refractory or newly diagnosed stage 4 neuroblastoma. **Methods:** Retrospective analysis was performed on seven pediatric patients with recurrent /refractory or newly diagnosed stage 4 neuroblastoma treated with traditional reinduction/induction chemotherapy combined with ATO. **Results:** A total of 7 patients were treated synchronously with ATO and chemotherapy for up to nine courses; all patients received conventional chemotherapy plus a 0.16 mg/kg/day dose of intravenous ATO during reinduction/induction chemotherapy. Treatment was effective in five patients and ineffective in the other two patients. The overall response rate was 71.43% (5 of 7). The side effects of the ATO combination were minor, whereby only treatment in one patient was terminated at the sixth course due to a prolonged QT interval (0.51 s), which returned to normal after symptomatic treatment. **Conclusions:** ATO can be safely and effectively combined with chemotherapy drugs as a potential alternative means of treatment for high-risk stage 4 neuroblastoma, and we have observed that ATO can restore the sensitivity of chemotherapy in some patients who were resistant to previous chemotherapy. Further investigations and clinical data are required to confirm these observations.

Keywords

arsenic trioxide, neuroblastoma, relapse, metastasis, chemotherapy

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Introduction

Neuroblastoma (NB) is the most common type of extra cranial solid pediatric malignancy, originating from neural crest cells that were destined to form the sympathetic nervous system. NB accounts for 8 to 10% of all childhood malignant cancer cases.[1] NB is a heterogeneous disease, and for low-risk or intermediate-risk NB, the prognosis is favorable following surgical resection alone or minimal chemotherapy.[2] However, more than 50% of patients with high-risk NB succumb to recurrence despite responding well to aggressive chemotherapy at first.[3] Recently, treatments to improve the prognosis, including autologous stem cell transplantation (ASCT), GD2 antigen-

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targeted therapy and immunobiological therapy have been developed for relapsed and refractory NB[4,5]; however, the use of such treatments is limited due to various restrictions in China. For example, ASCT therapy cannot be used due to unachievable autologous bone marrow purging and limited access to drugs used prior to bone marrow transplantation (eg melphalan is still a non-registered drug in China) in the majority of hospitals in China. The use of different chemotherapy regimens for recurrent/refractory stage 4 NB has been extensively studied, but rescue treatment for these patients is often difficult, therefore making the identification of an effective chemotherapy regimen for patients with high-risk NB is still a direction worthy of our efforts.

In the process of thinking and searching for the possibility of new therapeutic drugs for high-risk NB, we noticed that a traditional anti-cancer drug with a long history from China, arsenic trioxide (ATO), may be a candidate. Arsenic-containing substances occur naturally and despite the fact that different arsenical compounds have been used as medical agents for more than 2000 years, concerns regarding toxicity and carcinogenesis has contributed to the reduction in its use for therapeutic purposes.[6] It was not until in the 1990s, when Chinese studies demonstrated that ATO is highly effective for the treatment of acute promyelocytic leukemia (APL), the clinical use of ATO was revived.[1,7] The results of ours and other previous studies have reported that ATO can inhibit cellular proliferation and induce apoptosis in a large variety of tumor cells, such as liver cancer cells.[8]

Recently, the combination of ATO with certain cytotoxic drugs has been identified to exhibit a synergistic effect on various tumor types, such as NB.[6] Our previous study along with others[1] demonstrated that treatment with ATO alone or combined with chemotherapeutic drugs exhibits a satisfactory cytotoxic effect on chemoresistant NB cells. TrkA, TrkB, and TrkC gene expression levels in an NB cell line are upregulated with increasing concentrations of ATO,[9] and treatment with ATO also reduces the expression of the resistance-associated protein, P-glycoprotein (P-gp).[7,8] We also found that preincubation with ATO followed by a mitosis-phase specific agent (vinorelbine or docetaxel) may have a higher anticancer ability compared with single drug treatment or followed by a non-mitosis-phase-specific agent (etoposide or cisplatin),[13] suggested that the combination strategy of ATO and different chemotherapeutics can lead to different tumor killing effects, and it might have clinical implications. Especially important, accumulated recent evidence has shown that ATO can effectively inhibit hedgehog (HH) signaling at the level of GLI,[14] induce apoptosis and block proliferation in all major types of NB cells, and abrogate the tumorigenicity of NB cells.[17,18] Thus, ATO may be a promising therapeutic option for the treatment of NB through inhibition of multiple targets closely related to tumorigenesis.

Although there are more than 100 trials investigating the use of ATO for the treatment of leukemia or solid tumors in the US National Library of Medicine Clinical Trials database to date, to the best of our knowledge, there is only one phase II study that has investigated the therapeutic use of ATO in pediatric patients with advanced NB or other childhood solid tumors at the Memorial Sloan Kettering Cancer Center (NCT00024258); in this study, patients received ATO alone intravenously over 1 to 4 h on days 1 to 5 and 8 to 12, and the treatment was repeated every 28 days for a maximum of six courses in the absence of disease progression or unacceptable toxicity. The results were not ideal in this clinical trial as among the 21 patients investigated, only five patients achieved stable disease (SD), with the other 16 patients exhibiting progressive disease (PD). As far as we know, there have been no other clinical reports on the effects of ATO combined with conventional chemotherapeutic agents for the treatment of high-risk NB.

Based on the results of our previous preclinical studies, whereby ATO combined with chemotherapy drugs was demonstrated to effectively kill NB cells *in vitro,* and its clinical efficacy remains to be determined, the present study aimed to determine whether ATO can be safely and effectively combined with chemotherapy for the treatment of recurrent/ refractory or newly diagnosed stage 4 NB.

Patient Characteristics

Patients were enrolled from September 2009 to June 2016. A coordinating pathologist confirmed the diagnosis of NB in all patients. Seven eligible patients were enrolled and assigned up to nine cycles chemotherapy including ATO, of which four patients (Patient 1-4) were relapsed/refractory, and the other three patients (Patient 5-7) were newly diagnosed patients.

Patient #1 had an intermittent fever that lasted more than 20 days, patients #5 and #6 had pain in the lower limbs, and the other 4 patients presented with repeated abdominal pain and distension. The primary tumor lesions of all patients were located in the abdominal cavity, and the extensive metastatic sites before reinduction chemotherapy combined with ATO were located in the liver, kidney, muscle, lymph nodes, bone and bone marrow or surrounding large blood vessels (Table 1).

All patients exhibited increased serum neuron-specific enolase (NSE) levels. A total of 5 cases were vanillylmandelic acid/creatinine (VMA/Cr)-positive, whereas the levels in patients #2 and #7 were not examined. Patients #3 and #5 exhibited MYCN amplification, whereas patients #4 and #6 did not. MYCN amplification was not assessed in the other three patients.

Because there is no clear consensus on optimal therapy for relapse and a lack of randomized clinical trial, before entering reinduction therapy with ATO in this study, four relapsed patients had previously received a series of >2 lines chemotherapy. If the response to the previous one or two courses of chemotherapy was poor, other protocols would be given : Patient #1 received the regimen COPE (cyclophosphamide, vincristine, cisplatin, and etoposide) and regimen IPP (ifosfamide, carboplatin and pirarubicin [THP]) interchangeably for a total of 6 sessions and followed by surgery. The review of the positron emission tomography/computed tomography (PET/CT) scan showed no tumor recurrence in the operated area; however,

Table 1. Patient characteristics.

Patient no.	Age	MYCN staus	Primary site	Metastatic site	Stage	Induction Chemotherapy	Reinduction chemotherapy combined with ATO	Status after reinduction[a] chemotherapy with ATO	Prognosis
1	9	NE	Abdominal	Gluteus medius, bone, bone marrow	4	COPE/IPP × 6, CPV × 2, CT × 2	CPV × 3,CT × 3	CR	DOD
2	7	NE	Abdominal	Aorta, renal hilum, diaphragm angle	4	CPV × 4, PVP × 2	CPP × 2, PVP × 2, CD × 2,CT × 2,	PD	DOD
3	7	(+)	Abdominal	Major vessels, lymph nodes	4	CPV × 3, PVP × 1	CPV × 1, PVP × 2, CT × 2	CR	CR
4	9	(−)	Abdominal	Lymph nodes, bone marrow	4	CPP × 18, CPV × 2, CT × 2, GO × 2, IVT × 4	IVT × 3, CD × 1[b], decitabine × 5[b]	NR	DOD
5	7	(+)	Abdominal	Lymph nodes, bone, bone marrow, liver	4	CPV × 4, PVP × 3, CT × 2 (combined with ATO)		CR	CR
6	5	(−)	Abdominal	Lymph nodes, bone, bone marrow	4	CPV × 4, PVP × 3, CT × 2 (combined with ATO)		CR	CR
7	6	NE	Abdominal	Thoracoabdominal aorta, bone marrow	4	CPV × 3, PVP × 2 (combined with ATO)		CR	CR

Abbreviations: CD, cyclophosphamide/dacarbazine; COPE, cyclophosphamide/vincristine/cisplatin/etoposide; CPP, cyclophosphamide/pirarubicin/cisplatin; CPV, cyclophosphamide/pirarubicin/vincristine; CR, complete response; CT, cyclophosphamide/topotecan; DOD, dead of disease; GO, gemcitabine/oxaliplatin; IPA, ifosfamide/carboplatin/pirarubicin; ITV, irinotecan/vincristine/temozolomide; NE, not evaluated; NR, no response; PD, progressive disease; PR, partial response; PVP, cisplatin/etoposide; SD, stable disease.
[a]Initial induction chemotherapy in newly diagnosed patients.
[b]Without ATO.

both right and left thigh root stocks of the middle intermediate muscle nodule exhibited increased metabolic activity, indicative of muscle transfer. The mineral density of multiple vertebrae, primarily those of the lumbar spine and the pelvis bone, was uneven with increased metabolic activity, indicative of bone marrow infiltration. The patient received 2 CPV (cyclophosphamide, pirarubicin and vincristine) and 2 CT (cyclophosphamide and topotecan) chemotherapy courses in our hospital, and the PET/CT scan revealed that the original muscle metastases had increased with increased metabolic activity observed. We found increased metabolic activity in the spinal cord of the spinal canal at T12-L1, indicative of intramedullary infringement. The vertebrae and pelvis exhibited high focal metabolism.

Patient #2 received 4 CPV and 2 PVP (cisplatin and etoposide) chemotherapy courses, and the PET/CT scan review showed high metabolic activity in the retroperitoneal focal nodules, indicative of lymph node metastasis.

Patient #3 received 3 CPV and 1 PVP chemotherapy courses, and the primary tumor was then resected. A 70 mm × 60 mm × 40 mm tumor located in the pancreas was identified intraoperatively behind the edge near the aortic hiatus, the right edge of the right adrenal area and the left edge of the pancreatic tail, and was wrapped around the abdominal aorta, celiac axis, hepatic artery and proximal splenic artery with poor mobility. As the retroperitoneal primary tumors could not be completely resected, the patient only received palliative resection.

Patient #4 achieved complete remission following 18 courses of CPP (cyclophosphamide, pirarubicin, and cisplatin) chemotherapy. The levels of neuron-specific enolase (NSE) and VMA/Cr increased 2 years later, and the review of the PET/CT identified new multiple hypermetabolic foci in the left supraclavicular area, mediastinum, behind the diaphragm, on the right foot, and the retroperitoneal and right iliac blood vessels, indicative of lymph node metastasis. At our department, he was treated with 2 CPV, 2 CT, 2 GO (gemcitabine and oxaliplatin) and 4 IVT (irinotecan, vincristine, and temozolomide) chemotherapy courses, and the PET/CT scan showed shrinkage of the original metabolic foci with reduced metabolic activity.

The remaining three patients (#5, #6, and #7) were newly diagnosed and treated with a combination of ATO induction chemotherapy from the beginning (Table 1).

Treatment Plan

All patients received a chemotherapy protocol (SMHPO-N-2012 NB, modified from the N7 protocol[19,20]) combined with ATO, including up to 9 cycle treatments and consisted of 3 regimens: CPV (cycle 1, 2, 4, and 6), PVP (cycle 3, 5, and 7) and CT (cycle 8 and 9). The CPV regiment was composed of vincristine (0.022 mg/kg or 0.67 mg/m^2 daily for 2 h on days 1 and 2), pirarubicin (25 mg/m^2 over3 h) and cyclophosphamide (1.2 g/m^2 for 3 h daily on days 1-3). The PVP regimen consisted of cisplatin (50 mg/m^2 daily for 6 h

over days 1-4) and etoposide (light-resistant, 200 mg/m^2 daily for more than 4 h over days 1-3). The CT regimen consisted of a cyclophosphamide dose (1.2 g/m^2) for 3 h daily on days 1 to 2 and of a topotecan dose (light-resistant, 2 g/m^2) daily as a continuous infusion over days 1 to 3 (72 h duration of administration in total). In the trial group, patients were treated with ATO-combined chemotherapy for 9 courses in total (Figure 1). ATO was administered 2 days in advance at the 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 to 500 mL of normal saline or in a 5% glucose solution through a central venous catheter. Simultaneously, patients received 0.5 to 1.0 g ascorbic acid with an injection containing 5% glucose solution (100-250 mL) in another vein channel. The general imaging examinations, such as CT, magnetic resonance imaging (MRI) or PET/CT, were conducted every 2 or 4 courses of chemotherapy, while serum neuron-specific enolase and urinary vanillyl mandelic acid/creatinine ratio assessment were performed in each course of treatment. Bone marrow examination was performed in each course of treatment until bone marrow metastasis was absent.

Administration

A total of 7 patients in our study signed informed consent forms following chemotherapy using intravenous ATO. The ATO injection was produced by Beijing SLPharmaceutical Company (Beijing, China) and Harbin Medical University Pharmaceutical Company (Harbin, China). The drug was administered at a constant rate over 8 h in 250 to 500 mL of normal saline or a 5% glucose solution through a central venous catheter at a dose of 0.16 mg/kg/day for 10 days. Simultaneously, patients received 0.5 to 1.0 g ascorbic acid along with 5% 100 to 250 mL glucose injection in another vein channel.

Treatment Assessment

During treatment, anti-tumor efficacy was assessed according to the International NB Staging System criteria at the end of treatment[21]: (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 to 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) A stable disease (SD) was defined as a decrease <50% in tumor size less than a partial response, but with no disease progression or any lesion enlarged by <25%. (4) A 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). We detected tumor markers (NSE, VMA, or VMA/Cr) and performed imaging (B ultrasonic, CT, MRI, and PET/CT) during chemotherapy. Common adverse reactions, such as nervous system damage, electrolyte disorder, and cardiac toxicity, following ATO administration were monitored. The follow-up period was 3 years.

Results

Efficacy

A total of 7 patients used ATO in combination with chemotherapy for 4 to 9 courses. Treatment was effective in 5 patients and ineffective in the other 2 patients (Table 1). Patient #1 was treated with 3 CD (cyclophosphamide, dacarbazine) and 3 CPP (cyclophosphamide, pirarubicin, cisplatin) chemotherapy courses, and the review of the PET/CT scan revealed that the original muscle metastases had disappeared. The metabolic activity in the spinal canal at T12-L1, vertebral body and pelvis had returned to normal. However, the patient relapsed and died 11 months after giving up treatment for economic reasons. Patient #2 received 1 PVP and 2 CT chemotherapy courses combined with ATO. The PET/CT scan demonstrated increased lymph node metastasis in the abdominal and retroperitoneal areas, whereby the number and volume of metastatic areas and metabolic activity were increased. Subsequently, the patient continued to receive 2 CD and 2 CPP chemotherapy courses combined with ATO; the PET/CT review showed further deterioration. This child finally succumbed to complications with chemotherapy. Patient #3 received 2 PVP, 1 CPV, and 1 CT courses combining ATO chemotherapy postoperatively, and the PET/CT review showed that the retroperitoneal residual primary tumor foci had disappeared; however, low-density lesions in the liver remained (Figure 2). The patient subsequently received autologous hematopoietic stem cell transplantation and remained recurrence-free during the follow-up period of 32 months. Patient #4 received 3 IVT chemotherapy courses combined with ATO; the PET/CT scan revealed multiple lymph nodes with high metabolic activity and partial volume increases. ATO treatment was stopped, and treatment was switched to 1CD and 5 decitabine chemotherapy courses. The patient finally showed intracranial relapse and treatment was stopped. Patient #5 received 2 CPV courses, then 1 CPV and 1 PVP course combined with ATO; his lesions were then surgically removed completely. The subsequent treatment course continued to combine ATO chemotherapy, and this patient remained recurrence free during the 14 months of follow-up. Patient #6 received 3 CPV courses and 1 PVP course combined with ATO, following treatment, the bone marrow minimal residual disease (MRD) was reduced from 1.3% at early onset to negative, the retroperitoneal lesion size was reduced from 78 mm × 42 mm to 52 mm × 46 mm, and NSE and VMA/Cr levels were reduced to nearly normal. Once the tumors went into remission, this patient underwent operation and received an autologous stem cell transplantation in a hospital in Singapore. At last follow-up, patient #6 survived for more than 4 years. Patient #7 received 2 CPV courses and 1 PVP course combined with ATO. A review of normal bone marrow and MRD indicated complete remission, and NSE and VMA/Cr levels were also reduced. At this point, due to financial reasons, the treatment was temporarily halted for 3 months. Ultimately, this patient suffered from paraplegia and MR image showed that the tumor mass invaded the spinal

Week	0	3	6	9	12	14	17	20	24	28
Cycle	1	2	3	4		5	6	7	8	9
Regiment	▲	▲	●	▲	◎	●	▲	●	■	■
ATO	√	√	√	√	√	√	√	√	√	√

▲CPV ●PVP ■CT ◎Surgery

Figure 1. Schematic of the arsenic trioxide-combined chemotherapy protocol

canal on level T1, 2, 7, 8, 12 and S1. Although his condition gradually improved after radiotherapy plus chemotherapy (1 CPV and 1 PVP), the patient's family avoided the treatment again to avoid the financial costs. The overall response rate after reinduction/induction chemotherapy was 71.43% (5 of 7) and at the time this paper was written, 3 patients (1, 2, and 4) had died and the other 4 patients remained alive with event-free survival (EFS) of 3.5, 3.0, 1.2, and 1.0 years.

Toxicity Monitoring

Only 1 patient who underwent 5 courses of ATO exhibited drug-induced QT prolongation (0.51 s) during the electrocardiogram. Treatment with ATO was suspended and symptomatic treatment was provided until the QT returned to normal. The remaining patients tolerated the combined ATO chemotherapy for 3 to 7 courses well (Table 2).

Discussion

Patients with high-risk NB tend to develop distant metastases early; it has been reported that ~40% of cases are high risk. The 5-year EFS rate is <50%, and >50% of patients succumb to recurrence despite good responses to aggressive chemotherapy at the start of the regimen.[22,23] Patients with recurrent or refractory NB benefit very little from conventional regimens since the tumor cells develop multidrug resistance (MDR) to traditional drugs.[24] It has been reported that the expression of multidrug resistance-related protein (MRP) 1 is significantly increased in MYCN-amplified NB cases, and a high expression level of MRP is closely associated with overall survival (OS) and EFS rates.[25] Furthermore, commonly used chemotherapy drugs, such as etoposide (VP16), cisplatin (DDP) and adriamycin (ADM) have been revealed to increase the expression of MRP in leukemia cells or NB cells. Although higher doses of chemotherapy may reduce the possibility of resistance to some extent, the majority of pediatric patients fail to go through the treatment due to severe side effects; so, there is an urgent need for more effective and available medicine to treat refractory/recurrent NB.

ATO can induce the apoptosis of NB cells and its mechanisms may involve various pathways,[26,27] including the down-regulation of Bcl-2 protein expression, activation of the caspase 3 protein, generation of oxidative damage, reduction in the mitochondrial membrane potential, and induction of cell cycle arrest at the G1 or G2/M phase. Compared with conventional chemotherapeutic agents, ATO has evident advantages for the treatment of NB. Firstly, solid cancer development and progression is usually associated with a hypoxic environment *in vivo*, and the cytotoxic effects of traditional chemotherapy drugs on NB cells are reduced in a hypoxic environment; however, the cytotoxicity of ATO on MDR-NB cells are not significantly affected in hypoxic environments.[1] Secondly, the use of traditional chemotherapy drugs can increase the expression of MDR-associated proteins in NB cells, such as P-gp; thus, the cytotoxicity on MDR-NB cells is decreased.[24,25] However, ATO can significantly kill MDR-NB cells. Our previous studies demonstrated that ATO induces the downregulation of P-gp protein in SK-N-SH cells;[8] in addition, we found that ATO can upregulate the TrkA and TrkC expression in SK-N-BE (2) cells in a dose-dependent manner, to promote NB cell differentiation and apoptosis.[28]

Accumulated evidence suggests a major role for the activation of the HH signaling pathway in the development of neural crest stem cells that give rise to the sympathetic nervous system[29] and HH signaling pathway is highly activated in this class of NB and is closely related to clinical stage and poor prognosis of NB.[30]

Aberrant activation of the HH signaling pathway has been implicated in the pathogenesis of various types of pediatric malignant tumors. In NB, 96%, 100%, and 68% of the specimens stained positive for HH, Patched (PTCH), and Gli1, respectively.[30] We recently performed immunohistochemical detection for HH pathway signaling molecules in 10 stage 4 NB tissues, the positive rates of Sonic hedgehog (SHH), Smoothened (SMO), and Gli1 protein were 58.3%, 81.8%, and 72.7%, respectively (data not shown). It suggested that HH-Gli1 pathways are frequently activated in the specimens of stage 4 NB, which was consistent with the reported literature.[30] The HH signaling pathway may therefore play an important role in the differentiation and malignant potential of NB.[31]

A number of studies have shown that the use of HH pathway antagonists cyclopamine and GANT61 to block SMO and Gli1/2, respectively, can significantly inhibit NB cell proliferation and increase its apoptosis; it is especially important to inhibit Gli1/2 result in down-regulating amplification of the downstream regulatory gene N-myc, which is closely related to the invasiveness and poor prognosis of high-risk NB.[32,33] The above studies suggest that it is feasible to use a targeted drug specific for Gli in the HH signaling pathway closely related to the occurrence and development of NB to treat high-risk NB and now ATO has been proven to be an inhibitor of Gli[34,35] and it may play a

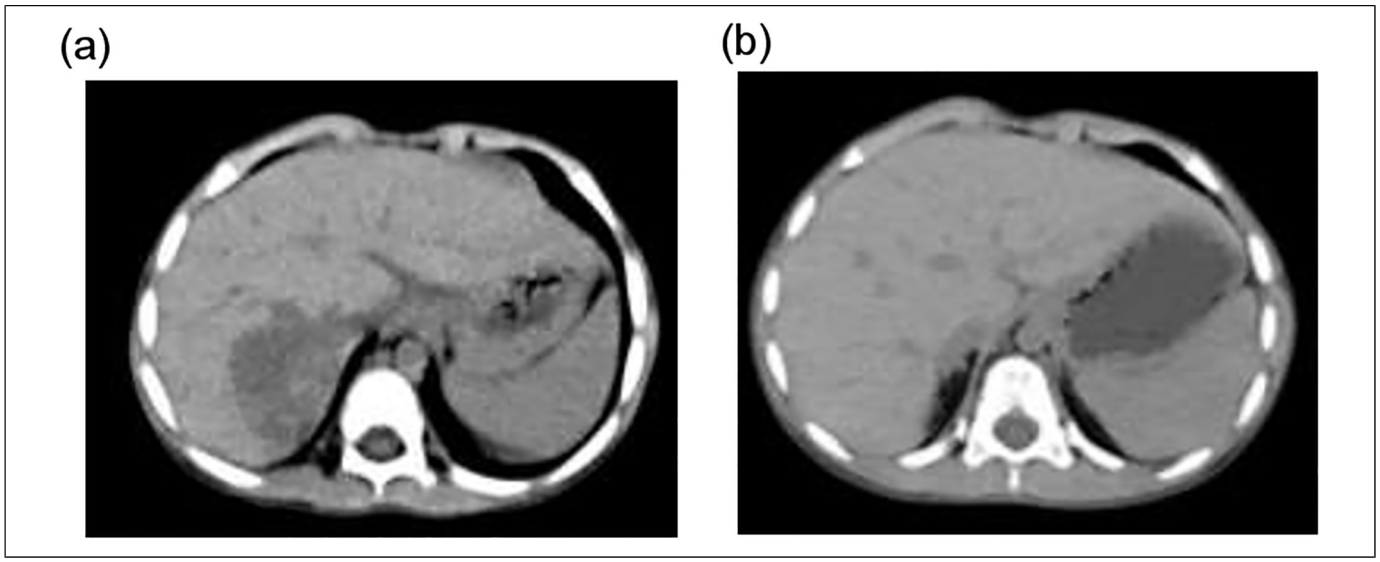

Figure 2. (a) Abdominal MRI scans for Patient 3 at diagnosis, (b) PET/CT scans for Patient 3 following therapy with 2 PVP, 1 CPV and 1 CT courses combining ATO postoperatively.
MRI, magnetic resonance imaging; PET/CT, positron emission tomography/computed tomography; ATO, arsenic trioxide; PVP, cisplatin and etoposide; CPV, cyclophosphamide, pirarubicin and vincristine; CT, cyclophosphamide and topotecan.

role in killing NB cells and implied that ATO may have broad clinical application prospects in such patients.

Large preclinical and clinical studies suggest ATO has therapeutic potential against NB.

These 7 patients enrolled in the present study were all diagnosed with relapsed/refractory or newly diagnosed stage 4 NB, with poor prognosis; therefore, the expected survival time might be short. Our initial result showed that traditional chemotherapy combined with ATO was effective in five patients (patient #1, #3 #5, #6 and #7; the next 3 patients are newly diagnosed cases) and ineffective in the other 2 patients (all were relapsed/refractory cases, patient #2 and #4) after reinduction/induction chemotherapy. The efficacy response in 2relapsed/refractory patients (patient #1 and #3) is interesting. Patient #1 was treated with conventional COPE/IPP, CPV and CT chemotherapeutic regimes at first, and the PET/CT scan suggested extensive metastasis, increased metabolic activity, and spinal cord metastasis in the patient. During the reinduction chemotherapy course, we synchronously combined ATO with almost the same chemotherapy regimens as before; the subsequent PET/CT scan review suggested that the metastases had disappeared and the metabolic activity had returned to normal. We observed the same phenomenon in patient #3; he achieved complete remission after reinduction combined ATO with the same previous chemotherapy regimens. Both patients relapsed following traditional chemotherapy, but the subsequent course using ATO achieved a good therapeutic effect, and we noticed that the majority of chemotherapeutic drugs of both patients in previous induction and reinduction courses are almost the same, suggesting that ATO may improve the efficacy of traditional chemotherapy in relapsed or refractory NB and restore the sensitivity of neuroblastoma cells to chemotherapy.

The common adverse reactions of ATO were electrolyte imbalance, cardiac damage, digestive system damage, and nervous system damage. In the present study, we selected an

Table 2. Times of treatment-emergent grade 2 to 3 adverse events during any phase of therapy including ATO.

Patient no.	Febrile neutropenia	Infection	AST/ALT/GGT	Ileus	Hypokalemia	QTc prolonged	Nausea	Neutrophils	Hemoglobin	Platelets
1	0	0	0	0	0	0	0	3	1	0
2	0	0	0	0	0	0	1	2	0	2
3	0	2	1	2	0	0	0	4	3	2
4	0	0	0	0	2	0	1	1	0	0
5	0	0	0	0	5	0	4	1	0	0
6	0	1	2	0	0	1	1	3	2	0
7	0	1	0	0	0	0	0	2	3	1

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; QTc, QT interval corrected for heart rate; ATO, arsenic trioxide.

ATO dose of 0.16 mg/kg/day, based on the dose of ATO used in the APL chemotherapy regimen. Combined ATO treatment in patient #1 was terminated at the sixth course due to a prolonged QT interval (0.51 s), which returned to normal after symptomatic treatment, while the other 6 patients did not exhibit any ATO-related adverse reactions. The concentration and cumulative dose of ATO in these seven patients did not exceed those in the APL program, confirming that this dose of ATO could be safely used for relapsed or refractory NB.

Our clinical results demonstrated the effectiveness of intravenous ATO combined with chemotherapy in the treatment of relapsed/refractory or newly diagnosed stage 4 neuroblastoma, with little toxicity and few side effects. As far as we know, this is the first clinical study to validate that ATO can be combined with chemotherapy safely and effectively applied to the treatment of relapsed or refractory neuroblastoma; but, it lacked suitable control groups for comparison of clinical efficacy in newly diagnosed patients. Therefore, we carried out a clinical study to confirm the efficacy and safety of combined chemotherapy with ATO in initial stage 4/M NB children. Preliminary results are encouraging.[36] Of the 22 patients in the trial group and 13 in the control group, we found that patients who received ATO combined with chemotherapy had a significantly higher response rate than those who treated with traditional chemotherapy (objective response rate (ORR): 86.36% vs 46.16%, $P = .020$). Reversible cardiotoxicity was just observed in 3 patients who were treated with ATO and no other differential adverse events were observed between 2 groups.

In conclusion, ATO can be safely and effectively combined with chemotherapy drugs as a potential alternative means of treatment for the relapsed/refractory or newly diagnosed stage 4 NB; moreover, further insight into the mechanism of action of ATO on GLI should lead to extended clinical applications of ATO as a HH pathway inhibitor. And, further a multicentered clinical investigation and a larger volume of clinical data are required to validate the clinical effectiveness and safety of this program, and now related multi-center clinical trials (ChiCTR1800014748 and NCT03503864) are ongoing.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its late amendments or comparable ethical standards. Chinese Institutional Ethics Committee approval number is ChiCTR1800014748. Our study was approved by the Ethics Committee of Sun Yet-Sen Memorial Hospital (approval no. 2017-45). All parents of the participants provided written informed consent prior to enrollment in the study.

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Supplemental Material

Supplemental material for this article is available online.

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