Phase II Study of Combined Chemotherapy With Arsenic Trioxide in Stage 4/M Neuroblastoma

(NCT03503864)

Protocol

Version:2.0

March 17 2020

CONTENTS

1. Background

Neuroblastoma (NB) is the most common extra-cranial solid tumor in children, originating from primitive neuroblasts and can develop in any region of the sympathetic nervous system. It accounts for 8%-10% of the incidence of childhood malignancies and contributes to 15% of cancer-related mortality in children. Children with high-risk NB (HR-NB) exhibit a high degree of malignancy and are characterized by an aggressive progression, a heightened probability of early distant metastasis, and a significantly increased risk of mortality [1-2]. The 5-year survival rate for stage 4/M neuroblastoma using chemotherapy alone is approximately 30%. Despite a multidisciplinary sequential comprehensive treatment regimen involving chemotherapy, radiotherapy, surgery, immunotherapy, and hematopoietic stem cell transplantation (HSCT), the 5-year survival rate for patients with HR-NB remains below 50%[3-5]. Consequently, chemotherapy remains the main method for treating HR-NB, and cost-effective and efficient treatment alternatives for children with HR-NB is urgently needed.

The low survival rate in stage 4/M NB can be attributed to multiple factors, including the difficulty in completely eradicating minimal residual disease (MRD) during treatment [6-7] and the emergence of multidrug resistance (MDR) in NB cells post-chemotherapy [8-10], which leads to a significant proportion of relapsed or refractory NB cases. Acquired MDR is currently a major barrier to the chemotherapeutic eradication of NB cells and a principal reason for the stagnation in improving cure rates. In recent years, several innovative research approaches have been undertaken to address these issues, such as autologous stem cell transplantation (ASCT) following high-dose chemotherapy and combined anti-GD2 antibody targeted therapy, which have improved the 5-year event-free survival (EFS) rate for high-risk NB to approximately 56% [11]. However, the prerequisite medication for NB ASCT, mafosfamide, is not available in China, and most facilities lack the capabilities to perform pre-transplant autologous bone marrow purification. The immunotherapy drug anti-GD2 antibody (Unituxin, dinutuximab), approved by the FDA on March 10,

2015, is extremely costly (at $10,000 per vial, totaling about $200,000 for all five treatment cycles), and is not readily accessible in China in the short term. The prohibitive cost and regulatory constraints of Chinese drug policies significantly limit the implementation of ASCT and anti-GD2 antibody immunotherapy as consolidation treatments for stage 4/M NB in children within the country. Therefore, chemotherapeutic treatment remains the main approach for managing high-risk NB in China at present, and finding effective treatment strategies suitable for the Chinese context is an ongoing challenge for pediatric oncology specialists.

NB is a highly heterogeneous tumor disease with significant variations in biological behavior and clinical outcomes. HR-NB patients may experience early widespread distant metastasis leading to death. However, some patients, such as infants with localized congenital and stage 4s NB, may see tumors spontaneously shrink or even disappear, or transform into benign ganglioneuromas, with minimal or no systemic treatment [12]. This phenomenon of natural regression in NB has captivated many oncologists and biologists. Although the precise nature of this spontaneous regression remains unclear, extensive research suggests that the maturation and differentiation of NB cells may be one of the mechanisms behind this natural regression [13]. Consequently, finding effective treatments that promote the maturation and natural regression of highly malignant NB cells is a current focus under therapeutic challenges. Among the strategies to induce tumor differentiation, retinoids (Retinoic acid, RA) have been proven to induce differentiation of malignant immature NB cells into mature ganglion cells or promote apoptosis of tumor cells. However, after comprehensive treatment using RA to induce differentiation, 40%-50% of high-risk NB patients still relapse, with RA-resistant minimal residual tumor cells being a significant factor in recurrence [14]. Currently, due to the limited availability of suitable drugs for treating RA-resistant HR-NB in children, finding and testing the combined use of effective drugs, especially those approved by the China National Medical Products Administration, is crucial in formulating new treatment plans and may play a key role in finding new therapeutic strategies for RA-resistant HR-NB.

Arsenic trioxide (ATO) is the major ingredient of a longstanding traditional Chinese

herbal preparation for thousands of years. Clinical studies have demonstrated that low doses of ATO can induce complete remission in patients with relapsed acute promyelocytic leukemia (APL) with favorable safety profile, and are now widely adopted in the clinical management of APL [15]. The utilization of ATO for the treatment of APL was officially sanctioned by the US Food and Drug Administration (FDA) in 2000. Furthermore, numerous investigations and clinical trials have provided substantial evidence supporting the effectiveness of conventional doses of ATO in eliminating various types of tumor cells, including NB, multiple myeloma, breast cancer, ovarian cancer, hepatocellular carcinoma, and osteosarcoma, etc[16]. Numerous Phase I and II clinical trials are currently underway to determine the efficacy and safety of ATO in the clinical treatment of tumors [17-22]. The Memorial Sloan-Kettering Cancer Center in the United States has preliminarily completed a Phase II clinical trial on ATO for the treatment of refractory/recurrent solid tumors in children, including NB (detailed information can be found at https://clinicaltrials.gov/ct2/show/study/NCT00024258?term=neuroblastoma+arsenic+trioxide&rank=1). Although specific methods and efficacy details have not been published, these studies suggest that ATO holds potential for treating MDR NB. The mechanisms of ATO's cytotoxic effects on tumor cells are complex, involving the induction of tumor cell differentiation, inhibition of growth, and promotion of apoptosis[23]. In APL cells, ATO primarily targets the PML/RARA oncogene for its anti-tumor effects; in other tumor cells, it acts mainly through Caspase-dependent apoptosis pathways and down-regulation of the Bcl-2 gene expression. ATO induces apoptosis independently of P53 gene expression; it works primarily by down-regulating Bcl-2, activating Caspases, generating reactive oxygen species, disrupting mitochondrial membrane potential, and blocking the cell cycle at G0 or G2/M phase, thereby promoting tumor cell apoptosis [16]. ATO acts on multiple targets in NB cells to promote their apoptosis and reduce the likelihood of developing drug resistance. Amplification of the MYCN gene is a prognostically adverse factor closely associated with neuroblastoma. The MYCN gene primarily promotes the proliferation of NB cells through mechanisms such as the transcriptional activation of target genes

like ODC, MCM7, MDM2, MDR1, and PAX3, and by promoting the transition of NB cells from G1 to S phase. Kim DW et al. [24] found that the anti-tumor effects of STI-571 (Imatinib Mesylate) and As2O3 are not diminished in NB with MYCN gene amplification. Yin Hua et al. [25] found that different concentrations of ATO could reduce the expression of MYCN mRNA in LA-N-5 NB cells, with the most significant reduction at a concentration of 3.0 μmol/L. The numerous mechanisms of ATO in treating NB avoid inducing MDR, such as its cytotoxic effects on NB cells being independent of the P53 gene, and its undiminished anti-tumor effects in MYCN-positive NB cells, as well as its ability to down-regulate MYCN gene expression. The most significant advantage of ATO in treating NB is its efficacy in treating MDR NB and its synergistic effects with various anti-tumor drugs; unlike traditional chemotherapy, the cytotoxicity of ATO against resistant NB cells does not significantly decrease under both normoxic and hypoxic conditions, making it a potential effective drug for future treatment of MDR NB. Currently, there are no reported clinical studies on the combined use of ATO with chemotherapy in treating HR-NB internationally or domestically.

The findings from our prior investigations [26-28] have established that the administration of ATO exhibits a notable capacity to augment the cytotoxic effects on NB cells. ATO significantly blocked SK-N-SH cells in G2/M phase; the expression of drug-resistant proteins (e.g. P-gp) in SK-N-SH cells did not increase with the increase of ATO concentration and time; and the expression of TrkA and Trk C, which have the effect of promoting NB differentiation and apoptosis, increased significantly with the increase of ATO concentration. Our in vitro experiments showed that the IC_{50} of ATO in SK-N-SH cell line was 3 umol/L, which is very close to its effective serum concentration in the clinical treatment of APL, suggesting that the use of ATO in combination with chemotherapy, which is similar to that used in the treatment of APL, can be effectively used in the treatment of NB. In addition, further studies showed that the combination of ATO and chemotherapeutic agents (vincristine, docetaxel, etoposide or cisplatin) enhanced their cytotoxic effect on SK-N-SH cells; and when As2O3 was used in combination with M-phase specific chemotherapy (vincristine,

docetaxel), the cytotoxic effect on SK-N-SH cells was enhanced, and when ATO was used in combination with M-phase specific chemotherapy (vincristine, docetaxel), it was not effective in treating APL. When ATO was combined with M-phase-specific chemotherapeutic agents (vincristine, docetaxel), the cytotoxic effect on SK-N-SH cells was significantly enhanced if ATO was administered first to block the SK-N-SH cell cycle at the G2/M phase and then M-phase-specific chemotherapeutic agents were added. When ATO was combined with non-M phase-specific chemotherapeutic drugs (etoposide and cisplatin), the administration of ATO first did not further increase the cytotoxic effect on NB cells, but it was still significantly better than that of the single drug administration.

In summary, to further improve the chemotherapy response rate, event-free survival, and long-term survival rates for children with HR-NB, there is an urgent need to identify new therapeutic drugs. Inducing maturation, differentiation, and apoptosis in NB cells is a key strategy for treating relapsed or refractory NB. ATO has a broad spectrum of antitumor activities, and its efficacy and safety in treating malignant tumors have been clinically validated. In vitro studies suggest that ATO can act on NB cells through multiple mechanisms, and its combined use with traditional chemotherapy drugs can enhance chemotherapeutic effects. This study primarily investigates the clinical efficacy and safety of ATO combined with chemotherapy in treating stage 4/M NB, offering a new treatment approach and option for relapsed or refractory NB.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

By observing the objective response rate (ORR) of 4/M stage NB treated with ATO combined chemotherapy at the fourth week after the completion of induction chemotherapy, and using the current ORR of 4/M stage NB as an external control, this study aims to evaluate the clinical efficacy of ATO combined chemotherapy in treating 4/M stage NB.

2.2 SECONDARY OBJECTIVES

2.2.1 To investigate the overall survival rate (OS) of 4/M stage NB treated with ATO combined chemotherapy.

2.2.2 To investigate the progression-free survival (PFS) of 4/M stage NB treated with ATO combined chemotherapy.

2.2.3 To investigate the disease control rate (DCR) of 4/M stage NB treated with ATO combined chemotherapy.

2.2.4 To evaluate the safety of ATO combined chemotherapy in treating 4/M stage NB.

3. STUDY DESIGN

3.1. DESCRIPTION OF STUDY

This study is a prospective, single-arm, open-lable, multi-center clinical trial. This study employs the Simon two-stage optimal design, planning to enroll 65 patients (19 patients in the first stage and 40 patients in the second stage) who are ≤ 14 years old, have evaluable lesions, and are newly diagnosed with NB classified as stage 4 according to INSS and/or stage M according to INRG. The enrollment is expected to be completed within 36 months, with follow-up for OS continuing for 36 months after the last patient is enrolled. The entire study is projected to last 72 months.

The enrolled patients will receive ATO combined with the traditional induction chemotherapy regimen for stage 4/M NB. The study will calculate the objective response rate and overall survival rate following ATO combined induction chemotherapy, and analyze chemotherapy-related adverse reactions. The overall treatment level of 4/M stage NB reported in the literature will serve as an external control to evaluate the efficacy and safety of ATO combined chemotherapy for 4/M stage NB.

Patients will continue the specified treatment until objective disease progression, symptom deterioration, unacceptable toxicity, death, or withdrawal of consent

(whichever occurs first). Chemotherapy-related adverse reactions will be closely monitored and recorded throughout the treatment. Comprehensive disease assessments will be conducted every 2-3 cycles, at the fourth week after the end of induction chemotherapy, and at the fourth week after the completion of all treatments. Following the completion of all treatments, patients will be followed up every 3-6 months to monitor disease and health status until death or the end of the study.

3.2. SELECTION OF STUDY POPULATION

3.2.1 Inclusion Criteria

(1) Untreated Stage 4/M neuroblastoma patients according to the INSS or the INRG staging system.

(2) Patients not more than 14 years old.

(3) There are measurable lesions.

(4) Guardians agreed and signed informed consent.

3.2.2 Exclusion Criteria

(1) Patients who had suffered from other tumors and received chemotherapy or abdominal radiotherapy.

(2) Patients with one or more critical organs failure such as heart, brain, kidney failure and so on.

3.2.3 Discontinuation Criteria

(1) Premature termination of treatment

(2) Non-compliance with treatment regimen, not treating according to staging and tissue type

(3) Disease progression after completing four cycles of induction chemotherapy

(4) Occurrence of severe adverse events, complications, or special physiological changes that make continued treatment inadvisable

3.2.4 Case Numbers

Based on the large-sample case analysis reports from multiple medical centers domestically and internationally, the ORR (including complete and partial responses) after standard induction chemotherapy for stage 4/M NB is approximately 70%. We hypothesize that the induction remission rate of ATO combined with chemotherapy reaching 85% would be considered clinically valuable, using a power of 80% and a two-sided significance level of 0.05.

According to the Simon two-stage optimal design for single-group clinical trials, calculated using the Power and Sample Size Program, this study expects to enroll 19 patients ≤ 14 years old with stage 4/M NB in the first phase. If no more than 14 patients benefit, the trial will be terminated. Otherwise, the second phase will continue to enroll 40 patients. If the total number of effective cases in both phases exceeds 46, it will be considered that ATO combined with chemotherapy can benefit patients. A total of 59 cases need to be enrolled in both phases. Considering the 10% dropout rate, 65 cases are planned to be enrolled in this study.

3.3. Clinical Pathological Diagnosis and Staging

All cases must have a confirmed pathological histological classification and staging before starting treatment.

3.3.1 Histological Classification

(1) Neuroblastoma: Undifferentiated; Poorly Differentiated; Differentiated

(2) Ganglioneuroblastoma: intermixed; Nodular

(3) Ganglioneuroma: Maturing subtype; Mature subtype

3.3.2 International Neuroblastoma Staging System (INSS)

Stage	Description
1	Localized tumor with complete gross excision, with / without microscopic residual disease Ipsilateral lymph nodes negative for tumor microscopically Lymph nodes attached to and removed with primary tumor may be positive
2A	Localized tumor with incomplete gross excision Ipsilateral lymph nodes negative for tumor microscopically

2B	Localized tumor with / without complete gross excision Ipsilateral, nonadherent lymph nodes positive for tumor Enlarged contralateral lymph nodes must be negative for tumor microscopically
3	Unresectable unilateral tumor infiltrating across the midline (midline is defined as the vertebral column) with / without regional lymph node involvement **OR** Localized unilateral tumor With contralateral regional lymph node involvement **OR** Midline tumor With bilateral extension by infiltration (unresectable) or by lymph node involvement
4	Disseminated tumor to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for stage 4S)
4S	oLocalized primary tumor (as defined for stage 1, 2A, or 2B) with dissemination limited to skin, liver and/or bone marrow (<10% of nucleated cells) Only in infants <1 year in age

3.4. Pre-treatment Examination

3.4.1 Medical History Collection

In addition to the current medical history, it is necessary to include past health status, family history of cancerous diseases, and social and environmental exposure to harmful physical and chemical factors. Check for any congenital malformations.

3.4.2 Physical Examination

Height, weight, body surface area. Blood pressure, examination of the head, face, limbs, chest, cardio-pulmonary, superficial lymph nodes, abdominal masses, reproductive organs, etc.

3.4.3 Laboratory Tests

(1) Routine tests: complete blood count, blood type, routine urine analysis (including occult blood), stool routine.

(2) Biochemical tests: liver and kidney functions, electrolytes, coagulation function, blood NSE, and 24-hour urine VMA (for young children who have difficulty retaining urine, a single 20ml urine VMA/Cr ratio may be performed).

(3) Cardiac function tests: ECG, UCG, and cardiac enzyme tests.

(4) Imaging studies of the lesion include: X-ray, CT or MRI scans of the lesion site, head, chest, and abdomen (plain and enhanced), whole-body bone scan, and if necessary, PET-CT to determine the extent and location of tumor invasion.

(5) Routine human PPD skin test, bone marrow examination (single or double iliac crest).

(6) Lesion biopsy and pathological type report; if surgical biopsy is not possible at initial diagnosis, one of the following two diagnostic criteria is needed: a. Presence of metastatic NB cells in bone marrow with corresponding immunohistochemistry and morphological features; b. Imaging supports NB diagnosis along with significantly elevated 24-hour urine VMA (or single urine VMA/Cr ratio).

(7) N-myc gene testing: send bone marrow sample affected by the tumor or excised tumor pathological sample.

(8) Arsenic baseline value: Collect any urine sample before arsenic treatment and send the same urine sample for arsenic testing (conducted by KingMed Diagnostics) and urine creatinine (tested by Sun Yat-sen University Sun Yat-sen Memorial Hospital), reported as: μg arsenic/g creatinine

3.5. Treatment Protocol

The induction treatment for stage 4/M neuroblastoma (NB) includes chemotherapy, surgery, and radiation therapy. The induction protocol consists of 9 chemotherapy cycles: Cycles 1, 2, 4, and 6 follow the CAV regimen (Cyclophosphamide + Pirarubicin + Vincristine); Cycles 3, 5, and 7 follow the PVP regimen (Cisplatin + Etoposide); Cycles 8 and 9 follow the CT regimen (Cyclophosphamide + Topotecan). The chemotherapy intervals for the CAV and PVP regimens are 2-3 weeks; for the CT regimen, the interval is 3-4 weeks. Surgical operations are generally scheduled between cycles 4 and 5 (as shown in Table 1). Radiation therapy, for children aged ≥1 year, is generally administered within one month after surgery, and chemotherapy is continued at the corresponding intervals after the completion of radiation therapy; for children who do not require radiation therapy (aged < 1 year), chemotherapy is administered according to the diagram. Some NB patients, after completing 4 cycles

of chemotherapy (3 CAV regimens + 1 PVP regimen) and undergoing a PET-CT scan that shows no solid tumor lesions, do not require surgery and will continue the remaining chemotherapy cycles at the corresponding intervals.

Table 1. Protocol of the Induction Chemotherapy

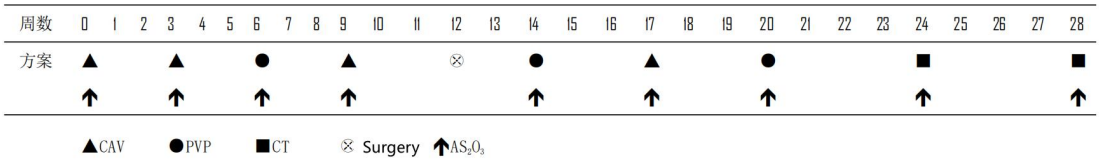

Table 2.Composition of Chemotherapy Regimen

ATO	ATO	0.16 mg/kg/day, iv drip, PI > 4h	d1 to d10
CAV Regimen	Cyclophosphamide (CTX)	1.2 g/m²/day, iv drip, PI = 3h	d3-d4
	Pirarubicin (THP)	25 mg/m²/day, iv drip, PI > 3h	d3-d5
		0.022 mg/kg/day or 0.67 mg/m²/day, iv drip, PI = 2h	d3-d5
		Use the lower of the two calculation methods; maximum daily dose ≤ 0.67 mg, total dose over 3 days > 2 mg	d3-d5
	Vincristine (VCR)	0.022 mg/kg/day or 0.67 mg/m²/day, iv drip, PI = 2h	d3-d5
PVP Regimen	Cisplatin (DDP)	50 mg/m²/day, iv drip, PI = 6h (light-protected)	d3-d6
	Etoposide (VP-16)	200 mg/m²/day, iv drip, PI > 4h	d3-d5
CT Regimen	Cyclophosphamide (CTX)	1.2 g/m²/day, iv drip, PI = 3h	d3-d4
	Topotecan	2 mg/m²/day, iv drip, PI = 24h (light-protected)	d3-d5

Precautions:

(1) Entry criteria for starting chemotherapy: Neutrophils > 0.5 × 10^9/L, Platelets > 50 × 10^9/L.

(2) Entry requirements for treatment with ATO: a. Serum potassium: 3.5-5.3 mmol/L, serum magnesium: 0.74-1.03 mmol/L, serum calcium: 2.02-2.60 mmol/L; b. Total serum bilirubin ≤ 1.5 mg/dL, serum creatinine ≤ 1.5 mg/dL; c. 12-lead ECG showing: QT interval < 0.5 s.

(3) Each chemotherapy cycle starts with two days of ATO chemotherapy alone, followed by combination with the standard induction chemotherapy regimens (CAV,

PVP, CT) starting from the third day. ATO is used for a total of 10 days per cycle (d1–d10).

(4) Dosage and administration of ATO: 0.16 mg/kg.d, mixed with 5% glucose solution or 0.9% normal saline 250-500ml, intravenous drip over more than 4 hours; concurrently administer vitamin C to reduce adverse reactions and enhance its effectiveness in the body: vitamin C 0.5-1.0g, mixed in 5% glucose solution 100-250 ml, administered simultaneously with ATO by intravenous drip.

(5) In the CAV regimen, Cyclophosphamide (CTX) requires pre-hydration and alkalinization half a day in advance, along with Mesna (administered concurrently with CTX, daily dose of 1200 mg/m^2, divided into three doses, continued for 2 days, total dose equal to 100% of the CTX dose), alkalinization, hydration, diuresis, antiemetic etc., starting from the first day of CTX, continuous hydration and alkalinization for at least 5 days; Pirarubicin (THP) dissolved in 5% glucose solution 250-500 ml IV drip over more than 3 hours, with antiemetics; Vincristine (VCR) dissolved in normal saline 100ml IV drip over 2 hours, maximum daily dose ≤ 0.67 mg, total dose over 3 days ≤ 2mg.

(6) In the PVP regimen, starting from the day of Cisplatin (DDP) administration, continue hydration and alkalinization for at least 7 days, fluid replacement 3000 ml/m^2.d, 20% mannitol for diuresis, monitor urine volume, check routine urine; before administering DDP, give mannitol (20% mannitol 1.25-5.0 ml/kg + normal saline 100-250 ml, rapid IV drip), start hydration treatment 2 hours before, when urine output reaches 250 ml/h, start DDP administration (in normal saline, concentration not exceeding 1mg/ml, IV drip = 6h, protect IV bottle from light); if cardiovascular function is compromised (output < 45%), appropriately reduce hydration volume and limit the single dose of DDP; antiemetics; monitor for nephrotoxicity and severe, persistent electrolyte disturbances caused by DDP, and manage accordingly if significant hyponatremia, hypomagnesemia, hypocalcemia, or hypokalemia occur, which can happen days after DDP treatment; for allergic reactions caused by DDP, treatment with epinephrine, antihistamines, and corticosteroids is effective; Etoposide (VP16) dissolved in normal saline 250-500 ml (ensure drug preparation concentration

limits, not exceeding 0.25mg/ml) IV drip over more than 4 hours, used for 3 consecutive days, avoid using glucose solution for preparation, slow drip required.

(7) In the CT regimen, Cyclophosphamide (CTX) requires pre-hydration and alkalinization half a day in advance, along with Mesna (administered concurrently with CTX, daily dose of 1200 mg/m^2, divided into three doses, continued for 2 days, total dose equal to 100% of the CTX dose), alkalinization, hydration, diuresis, antiemetic etc., starting from the first day of CTX, continuous hydration and alkalinization for at least 5 days; Topotecan dissolved in normal saline 250ml–500ml IV drip over 24 hours (continuous IV drip, best protected from light), used for 3 consecutive days, with antiemetics.

(8) Audiometric testing (ENT examination items: a. Pure tone audiometry b. Acoustic impedance + stapedius muscle reflex attenuation c. Acoustic reflex)

3.6. Adverse Reactions and Management

(1) Leukocytosis syndrome: A few patients may experience an increase in white blood cells, appearing 2-3 weeks after medication initiation. There is no need to stop the treatment; white cell counts can decrease on their own or can be reduced with oral hydroxyurea within a week.

(2) Digestive system: Nausea, vomiting, anorexia, abdominal pain, and diarrhea are common adverse reactions. Symptomatic treatment is effective, and these symptoms usually resolve after stopping the medication. Some patients may experience liver damage, including elevated transaminases and jaundice; liver-protective medication can be used, and liver function typically returns to normal after stopping the medication.

(3) Fluid retention: Patients may experience weight gain, pleural effusion, pericardial effusion, and facial edema during treatment.

(4) Urinary system: Acute renal failure is rare, but changes in kidney function may occur and generally recover after stopping the medication.

(5) Nervous system damage: Symptoms such as polyneuritis and multiple radiculitis may occur about 10-20 days after medication use. Patients may experience limb pain,

numbness, and a progression from hypersensitivity or abnormal sensations to delayed or absent pain, temperature, and touch sensitivity, even leading to sensory ataxia. Additionally, there may be limb weakness, distal muscle atrophy, and significant autonomic disturbances. Arsenic-induced peripheral neuropathy is indistinguishable from general ataxia. About 34% of patients experience varying degrees of transient cerebrovascular spasm-induced headaches early in the medication regimen.

(6) Cardiovascular system: Symptoms may include palpitations, chest discomfort, and ECG changes, including sinus tachycardia, ST segment depression, T-wave inversion or flattening, prolonged PR interval, or complete atrioventricular block, though these are generally reversible; prolonged QT interval and ventricular arrhythmias based on this may occur.

(7) Skin dryness, erythema, or pigmentation.

(8) Differentiation syndrome: Common in the early stages of arsenic-induced treatment, characterized by elevated white blood cells, fever, weight gain, musculoskeletal pain, respiratory distress, pulmonary interstitial infiltration, pleural effusion, pericardial effusion, skin edema, hypotension, acute renal failure, and even death; severe leukocytosis can also lead to embolisms in the lungs, brain, and other organs. Prevention and management: Monitor blood counts daily during the early induction phase. Whether at the start of induction or during, if WBC > 15 × 10^9/L, administer hydroxyurea (HU) 100 mg/kg.d (based on WBC), divided into 2-3 doses, and use dexamethasone (DXM) 0.3-0.5 mg/Kg.d if symptoms occur, until they disappear. For WBC > 30 × 10^9/L, in addition to HU, it is recommended to use DXM to prevent differentiation syndrome.

Other adverse reactions can be treated symptomatically, and most symptoms disappear after stopping the medication. Some children may experience significant gastrointestinal reactions at the start of each treatment cycle, possibly accompanied by mild headaches, which typically lessen or disappear after 2-3 days. It is recommended to halve the dose for the first 2-3 days, then adjust to the full dose or reduce to 3/4 dose depending on tolerance. To minimize gastrointestinal reactions, doses should be

taken orally at least three times a day, and antiemetic medication such as metoclopramide may be taken half an hour beforehand to alleviate symptoms of nausea and vomiting. For individual cases with severe gastrointestinal reactions that remain intolerable after at least 3 days and additional antiemetics, discontinuation from the study group is recommended.

3.7. Observation Indicators and Follow-up

3.7.1 Observation Indicators During Chemotherapy

Patients need to meet the enrollment criteria. Before each chemotherapy cycle, complete blood count, routine biochemistry, and ECG/echocardiogram are required. During chemotherapy, monitor and record in detail the side effects such as bone marrow suppression, gastrointestinal reactions, liver and kidney damage, cardio-pulmonary toxicity, neurotoxicity, allergic reactions, and local toxicity. After each chemotherapy cycle, perform 24-hour urine VMA and blood NSE tests. Every 2-3 cycles, perform imaging studies of the lesion (abdominal CT and/or chest CT), and assess the tumor status through bone marrow or peripheral blood minimal residual disease.

3.7.2 Follow-up After Treatment Completion

(1) Four weeks after the completion of all ATO combined chemotherapy regimens, conduct a comprehensive re-examination including complete blood count, routine biochemistry, ECG, echocardiogram, chest and abdominal CT, or whole-body PET-CT, and human arsenic content tests (hair, nails, 24-hour urine).

(2) Within the first year after all treatments are completed, perform abdominal ultrasound, chest X-ray, and ECG every three months, and conduct growth and development physical examinations (measuring height and weight); every six months, re-examine abdominal CT and echocardiogram, complete blood count, and routine biochemistry (liver and kidney functions and electrolytes), and conduct growth and development physical examinations.

(3) Within the second year after all treatments are completed, every six months,

re-examine abdominal ultrasound or CT, chest X-ray, echocardiogram, complete blood count, and routine biochemistry (liver and kidney functions and electrolytes), and conduct growth and development physical examinations (measuring height and weight).

(4) From the third year after all treatments are completed, perform annual re-examinations including abdominal ultrasound or CT, chest X-ray, ECG, echocardiogram, complete blood count, and routine biochemistry (liver and kidney functions and electrolytes), and conduct growth and development physical examinations (measuring height and weight).

3.8. Criteria for Efficacy Evaluation

WHO criteria for efficacy evaluation are adopted.

3.8.1 Measurable Lesions

(1) Complete Response (CR): All lesions completely disappear, maintained for at least 4 weeks.

(2) Partial Response (PR): For bi-dimensional measurable lesions, the sum of the products of the maximum perpendicular diameters of all lesions (taking the largest diameter and the perpendicular diameter, multiplying them to get the maximum perpendicular product, and then summing these products for all lesions) reduces by more than 50%, maintained for at least 4 weeks; for uni-dimensional measurable lesions, the sum of the maximum diameters of all lesions reduces by more than 50%, maintained for at least 4 weeks.

(3) No Change (NC): For bi-dimensional measurable lesions, the sum of the products of the maximum perpendicular diameters reduces by less than 50% or increases by no more than 25%, maintained for at least 4 weeks; for uni-dimensional measurable lesions, the sum of the maximum diameters reduces by less than 50% or increases by no more than 25%, maintained for at least 4 weeks. NC can only be evaluated after at least two treatment cycles (6 weeks).

(4) Progressive Disease (PD): One or more lesions increase by more than 25%, or

new lesions appear. If new pleural or peritoneal effusions are found with cancer cells in cytology, it should be judged as PD.

3.8.2 Evaluative but Non-Measurable Lesions

(1) CR: All lesions completely disappear, maintained for at least 4 weeks.

(2) PR: Estimated tumor size reduction by more than 50%, maintained for at least 4 weeks.

(3) NC: No significant change in lesions after at least two treatment cycles (6 weeks), estimated tumor reduction by less than 50% or increase by no more than 25%.

(4) PD: New lesions appear, or estimated tumor size increases by more than 25%.

3.8.3 Bone Metastasis Lesions

(1) CR: Osteolytic lesions disappear, bone scans return to normal, maintained for at least 4 weeks.

(2) PR: Partial reduction or calcification of osteolytic lesions, or reduced density of osteoblastic lesions, maintained for at least 4 weeks.

(3) NC: No significant change in lesions, can only be evaluated as NC after at least 8 weeks of treatment.

(4) PD: New lesions appear, or existing bone lesions significantly enlarge. However, bone compression, pathological fractures, or bone healing should not be the sole criteria for efficacy evaluation.

3.8.4 Non-Evaluable Lesions:

(1) CR: All lesions completely disappear, maintained for at least 4 weeks.

(2) NC: No significant change in lesions, estimated tumor reduction by less than 50% or increase by no more than 25%, maintained for at least 4 weeks.

(3) PD: New lesions appear, or estimated tumor size increases by more than 25%. If effusions in cavities increase without other lesion progressions, it should not be evaluated as PD

3.9. Safety Evaluation Criteria

The intensity of adverse events will be graded according to the grading methods recommended by the NCI CTC (version 3.0, 2003-12-12) Common Toxicity Criteria of the National Cancer Institute.

3.10. Statistical Analysis Methods

This study is designed as a single-group clinical trial, with results primarily described using statistical descriptions, and no hypothesis testing will be conducted. The efficacy indicators will be calculated with $\alpha = 0.05$ (two-sided) to compute the 95% CI.

(1) Efficacy Indicators: The remission rate at the fourth week after the end of induction chemotherapy will be the trial evaluation indicator. Additionally, it will serve for further research on the efficacy and safety of arsenic trioxide combined with chemotherapy. The overall survival rate (OS), event-free survival rate (EFS), and progression-free survival time (PFS) will also be calculated and the corresponding survival curves will be plotted.

(2) Safety Data: Detailed descriptions of cases with hematological or non-hematological toxicity reactions will be provided. The incidence rates of different events will be calculated, and the composition ratio of the severity of each event will be computed.

4. Reference

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