Phase II study of vincristine, actinomycin-D, cyclophosphamide and irinotecan for patients with newly diagnosed low-risk subset B rhabdomyosarcoma

A study protocol

Mitsuru Miyachi, MD, PhD,a,*, Kunihiro Tsuchiya, MD, PhD,b, Ako Hosono, MD,c, Atsushi Ogawa, MD,d, Katsuyoshi Koh, MD,e, Atsushi Kikuta, MD, PhD,f, Junichi Hara, MD, PhD,g, Satoshi Teramukai, PhD,h, Hajime Hosi, MD, PhDa

Abstract

Background: Approximately 80% to 90% of patients with low-risk rhabdomyosarcoma can be cured. However, cured patients often face long-term complications associated with the treatment. An important factor in the treatment plan is the dose of cyclophosphamide administered because the dose can have both acute and long-term side effects. It is therefore essential to investigate whether the dose can be reduced without a negative effect on treatment outcome. The ARST0331 trial revealed that drastically reducing the cyclophosphamide dose to 4.8g/m² negatively affected treatment outcomes. The current study aims to determine whether reducing the cyclophosphamide dose to 10.8g/m² while introducing a new drug, irinotecan, can prevent the negative effect on treatment outcome. We also aim to investigate whether the reduced cyclophosphamide dose results in a decrease in infertility, one of the long-term complications of this treatment.

Methods: The subjects are patients with stage 1 group III rhabdomyosarcoma (excluding those with orbital group III N0 and NX) or patients with stage 3 group I and II low-risk subset B embryonal rhabdomyosarcoma who will alternately undergo VAC 1.2 treatment (vincristine, actinomycin D, cyclophosphamide 1.2g/m²) and VI treatment (vincristine, irinotecan). The effectiveness and safety of this treatment regimen will be assessed. Data will be presented at international conferences and will be published in peer-reviewed journals.

Discussion: This study is significant because it aims to establish that the use of irinotecan in patients with low-risk subset B embryonal rhabdomyosarcoma (aged 30 or younger) allows the dose of cyclophosphamide to be reduced and is associated with few short-term adverse effects and long-term complications. The open-label and single-arm design of this study may be a limitation.

Trial registration and ethical approval: The trial registration number is jRCTs051180200 (Japan Registry of Clinical Trials). The study protocol was approved by the institutional review board at each of the participating centers and the data will be presented at international conferences and published in peer-reviewed journals.

Abbreviations: COG = Children’s oncology group, CR = complete response, DPE = delayed primary excision, ECOG = Eastern Cooperative Oncology Group, EFS = event free survival, FFS = failure free survival, jRCT = Japan Registry of Clinical Trials, OS = overall survival, PR = partial response, PS = performance status, RT = radiotherapy, SEER = Surveillance, Epidemiology, and End Results, STS = soft tissue sarcoma, VA = vincristine, actinomycin D, VAC = vincristine, actinomycin D, cyclophosphamide, VI = vincristine, irinotecan.

Keywords: cyclophosphamide, irinotecan, low-risk, microRNA, rhabdomyosarcoma, UGT1A1
1. Introduction

Rhabdomyosarcoma is a malignant tumor that originates in the mesoderm or mesenchymal tissue of the fetus and develops during the ensuing formation of skeletal muscles or during skeletal muscle differentiation following malignant transformation. Treatment consists of a combination of chemotherapy and radiotherapy after tumor resection or biopsy.

In the low-risk subset B patients followed in the JRS-I trial (conducted in Japan between 2004 and 2012), an experimental protocol was utilized that consisted of 24 weeks of VAC (vincristine, actinomycin D, cyclophosphamide) therapy and 24 weeks of VA (vincristine, actinomycin D) therapy. Enrollment closed in March 2012 and analysis of the data is on-going, but no clear declines in treatment outcomes were observed. However, the dose of cyclophosphamide used in each cycle of the 24-week VAC therapy + 24-week VA therapy for JRS-I low-risk subset B patients was as high as 2.2g/m^2. The total amount of the drug that was administered during the treatment cycle was 17.6g/m^2, which is high enough to cause known acute toxicities such as veno-occlusive disease and late effects such as infertility.

Since most rhabdomyosarcoma patients in the low-risk group can expect long-term survival due to curative treatment of the disease, late effects are not desired. To this end, studies on lower doses of cyclophosphamide have been conducted in the United States. The ARST0331 trial, conducted by the Soft Tissue Adrioncctine side effects are minimal; however, lower doses are more likely to lead to unsatisfactory outcomes. Another study further explored this difference and revealed that blood serum test results for rhabdomyosarcoma showed elevated expression of muscle-specific miR-206. However, that study utilized a small sample size. Validation of their results in studies with larger numbers of subjects is therefore required.

This study aims to verify the validity of the use of serum miR-206 as a biomarker to estimate therapeutic response and prognosis.

2. Methods

2.1. Study aims

The objective of this clinical trial is to assess the efficacy and safety of alternating use of VAC 1.2 treatment (vincristine, actinomycin D, cyclophosphamide) and VI treatment (vincristine, irinotecan) on low-risk subset B embryonal rhabdomyosarcoma patients classified as Stage 1, Group III (excluding orbital Group III NO and NX) or Stage 3, Group I and II.

2.2. Study design

This study is an open-label, single-arm, multicenter phase II trial that will be performed at 100 centers in Japan. The primary endpoint is event-free survival (EFS). Secondary endpoints include overall survival, time to treatment failure, overall response rate, frequency of adverse events, and grade assessment using CTCAE ver. 4.0. Exploratory endpoints include the correlation between serum miR-206 expression level at diagnosis and EFS, changes in miR-206 expression level during and after treatment, and the correlation between the expression level of miR-206 expression in cerebrospinal fluid at the time of diagnosis of parameningeal primary tumor and central nervous system recurrence. Other exploratory endpoints are the frequency of adverse effects of irinotecan and the quality control of radiotherapy.

2.3. Patient registration and data collection

Patient registration started on February 1, 2016 and the target enrolment is 18 patients. A log with the patients’ names and dates of birth will be kept along with their unique study numbers at
each participating center. All the data generated from the study will be stored in an anonymized form in a database, which will also be password protected. Only anonymized information will be stored on this, and participants will only be identifiable by their study number. All paperwork will be kept in a locked filing cabinet in a locked office. All data will be stored on a password-protected computer with limited access to the research team.

2.4. Eligibility criteria

Inclusion criteria

1) Diagnosis of low-risk subset B rhabdomyosarcoma. Patients switched from other risk groups according to the results of central pathology review are acceptable.
2) Age: Under 30 years of age at the time consent was obtained.
3) Start of treatment within 42 days after the date of initial surgery.
4) Initial onset of malignant tumor.
5) Patients determined to have the following performance status (PS) according to the Eastern Cooperative Oncology Group (ECOG) Performance Status Score:
   - PS 0–2: In cases of were aged 15 or younger at the time consent was obtained, the standard is a Lansky performance status score of at least 50 points.
6) Patients whose major organ functions have been preserved. Maintenance of the following: WBC of at least 2000/μl, neutrophil count of at least 500/μl, hemoglobin of at least 7.0 g/dl, platelet count of at least 50,000/μl.
7) Patients for whom a consent form was obtained from the patient him/herself or from a legally authorized representative (in the case of patients aged between 16 and 20 years who have been informed of their diagnosis, the consent must be obtained from the patient and his/her parents).

Exclusion criteria

1) Primary tumor in the central nervous system, central nervous system metastasis, or positive cerebrospinal fluid cytology.
2) Active multiple cancer (synchronous multiple cancer and metachronous multiple cancer with a disease-free period of 5 or fewer years).
3) Demyelinating Charcot-Marie-Tooth disease or varicella zoster.
4) History of chemotherapy and radiotherapy.
5) The following severe concurrent diseases:
   - Intersitial pneumonia, pulmonary fibrosis, or advanced emphysema;
   - Poorly controlled diabetes;
   - Poorly controlled hypertension;
   - Disease with marked ECG abnormality or clinical problems (cardiac failure, myocardiak infarction, ischemic disease);
   - Cirrhosis of the liver, liver failure;
   - Renal failure.
6) Presence of contraindications for the treatment drugs.
7) Any of the following:
   - Women who are or may be pregnant or women hoping to become pregnant;
   - Women who are breastfeeding;
   - Men whose partners hope to become pregnant during the treatment period;
8) Any other reason determined by the principal investigator or secondary investigators not eligible for the study.

2.5. Treatment methods (Figure 1)

Subgroup B patients will receive 9 cycles (27 weeks) of vincristine, dactinomycin, and cyclophosphamide at 1.2 g/m²/cycle (VAC1.2) and 5 cycles (15 weeks) of vincristine and irinotecan (VI) (total cumulative doses: V = 54 mg/m², A = 0.405 mg/kg, C = 10.8 g/m², I = 1250 mg/m²) (Fig. 1).

Radiotherapy (RT) will begin at week 13 for most patients with residual microscopic or gross embryonal rhabdomyosarcoma. Patients in group II/III will receive RT as follows: 36 Gy for stage 1 group IIA patients, 41.4 Gy for group IIB/IIIC patients, and 50.4 Gy for group III patients.

Two exceptions will be made. Subgroup B patients with tumors in the vulva, uterus, biliary tract, and superficial non-parameningal head/neck sites can undergo a delayed primary excision (DPE) at week 12 to remove gross residual tumor. RT will be administered after the DPE. Subgroup B patients with vaginal tumors will begin RT at week 12 if N1 or at week 28 if N0, in an attempt to preserve vaginal tissue and to avoid RT; N0 patients in whom repeated biopsies showed no residual tumor before or by week 28 will receive no RT.

2.6. Follow-up

The planned blood tests and imaging studies will be performed at enrollment, at 12 weeks, at 24 weeks, and at the conclusion of the treatment. Imaging studies will additionally be performed every three months following the conclusion of treatment.

2.7. Study endpoints and statistical methods

The primary endpoint is event-free survival (EFS). EFS is defined as the period from the enrollment date until disease worsening/recurrence or death from any cause. The EFS curve will be estimated using the Kaplan-Meier estimator, and the null hypothesis will be tested using a test based on the maximum likelihood estimator of hazard. When one sided P value for this test is below .05 (equivalent to a 3-year EFS rate higher than 78%), it will be determined that the EFS in the treatment group was superior to that reported by ARST0331. Sub-group analysis will be performed by estimating the EFS curve by age, postoperative group, tissue type, presence of fusion gene, and number of copies of serum miR-206 at diagnosis (high/low).
The target sample size is 18 patients. Assuming that the enrollment rate will remain constant during the enrollment period, all cases will be completely followed, and the distribution of EFS will be the exponential distribution. The 3-year EFS rate of the ARST0331 (63%) is used as a threshold. When the expected 3-year EFS rate for this treatment is 85% (event hazard = 0.054/year), the number of subjects required in order to reject the null hypothesis of 3-year EFS rate (63%; event hazard = 0.154/year) at a one-sided significance level of 5% and a power of 80%, using a test based on the maximum likelihood estimator for hazard is 18 patients.

Analysis of secondary endpoints:
1) Overall survival (OS)
   The OS curve will be estimated using the Kaplan–Meier estimator.
2) Tumor response
   The response rate will be estimated by the proportion for complete response (CR) or partial response (PR).
3) Adverse events
   Data on adverse events will be tabulated by type.

Analysis of exploratory endpoints:
1) We will assess the correlation between miR-206 expression level at diagnosis and EFS, changes in serum miR-206 expression during and after treatment, and miR-206 expression levels in cerebrospinal fluid in cases of parameningeal primary tumor and central nervous system recurrence. The Kaplan–Meier estimator will be used to estimate the EFS curve by serum number of miR-206 copies (high/low) at diagnosis. The frequencies of subsequent central nervous system recurrence by number of miR-206 copies (high/low) at diagnosis will be compared.
2) In order to assess the correlation between the frequency of UGT1A1 gene polymorphisms and the adverse effects of irinotecan, data will be compiled on the incidence of diarrhea and neutropenia associated with the presence vs absence of UGT1A1 gene polymorphisms (*6, *28).

3. Discussion
Rhabdomyosarcoma is the most common type of soft-tissue tumor in childhood. This study will determine whether a cyclophosphamide dose of 10.8g/m², administered alongside irinotecan, is an effective and less toxic treatment for low-risk subset B rhabdomyosarcoma patients. The data from this study will also elucidate whether circulating miR-206 can serve as a novel prognostic biomarker for rhabdomyosarcoma.

Author contributions
All authors were members of the protocol planning working group and contributed to the design of the study. MM is a principal investigator of the study. MM drafted the manuscript. All authors critically reviewed and approved the final version of the manuscript.

References
[1] Walterhouse D, Pappo AS, Meza JL, et al. Vincristine (V), Dactinomycin (A), and lower doses of cyclophosphamide (C) with or without radiation therapy for patients with newly diagnosed low-risk embryonal rhabdomyosarcoma (ERMS): a report from the Children’s Oncology Group (COG). J Clin Oncol 2012;30: abstr. 9309.
[2] Sultan I, Qaldoumi I, Yaser S, et al. Comparing adult and pediatric rhabdomyosarcoma in the surveillance, epidemiology and end results program, 1973 to 2005: an analysis of 2600 patients. J Clin Oncol 2009;27:3391–7.
[3] Pappo AS, Lyden E, Breitfeld P, et al. Two consecutive phase II window trials of irinotecan alone or in combination with vincristine for the treatment of metastatic rhabdomyosarcoma: the Children’s Oncology Group. J Clin Oncol 2007;25:362–9.
[4] Toffoli G, Cucchin E, Corona G, et al. The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 2006;24:3061–8.
[5] Liu CY, Chen PM, Chiou TJ, et al. UGT1A1*28 polymorphism predicts irinotecan-induced severe toxicities without affecting treatment outcome and survival in patients with metastatic colorectal carcinoma. Cancer 2008;112:1932–40.
[6] Hirasa wa A, Zama T, Akahane T, et al. Polymorphisms in the UGT1A1 gene predict adverse effects of irinotecan in the treatment of gynecologic cancer in Japanese patients. J Hum Genet 2013;58:794–8.
[7] Moriya H, Saito K, Helsby N, et al. Association between the low-dose irinotecan regimen-induced occurrence of grade 4 neutropenia and genetic variants of UGT1A1 in patients with gynecological cancers. Oncol Lett 2014;7:2035–40.
[8] Bomgaars LR, Bernstein M, Kraito M, et al. Phase II trial of irinotecan in children with refractory solid tumors: a Children’s Oncology Group Study. J Clin Oncol 2007;25:4622–7.
[9] Stewart CF, Panetta JC, O’Shaughnessy MA, et al. UGT1A1 promoter genotype correlates with SN-38 pharmacokinetics, but not severe toxicity in patients receiving low-dose irinotecan. J Clin Oncol 2007;25:2594–600.
[10] Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:837–46.
[11] Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
[12] Wei JS, Johansson P, Chen QR, et al. MicroRNA profiling identifies cancer-specific and prognostic signatures in pediatric malignancies. Clin Cancer Res 2009;15:5560–8.
[13] Miyachi M, Tsuchiya K, Yoshida H, et al. Circulating muscle-specific microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma. Biochem Biophys Res Commun 2010;400:89–93.