Original Article

Efficacy and safety of sirolimus treatment for intractable lymphatic anomalies: A study protocol for an open-label, single-arm, multicenter, prospective study (SILA)

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ARTICLE INFO

Article history:
Received 20 September 2018
Received in revised form 22 November 2018
Accepted 6 December 2018

Keywords:
Lymphatic abnormalities
Lymphatic malformation

ABSTRACT

Introduction: Lymphatic anomalies (LAs) refer to a group of diseases involving systemic dysplasia of lymphatic vessels. These lesions are classified as cystic lymphatic malformation (macrocytic, microcytic or mixed), generalized lymphatic anomaly, and Gorham–Stout disease. LAs occur mainly in childhood, and present with various symptoms including chronic airway problems, recurrent infection, and organ disorders. Individuals with LAs often experience progressively worsening symptoms with a deteriorating quality of life. Although limited treatment options are available, their efficacy has not been validated in prospective clinical trials, and are usually based on case reports. Thus, there are no validated standards of care for these patients because of the lack of prospective clinical trials.

Abbreviations: LAs, lymphatic anomalies; LM, lymphatic malformation; GLA, generalized lymphatic anomaly; GSD, Gorham–Stout Disease; mTOR, mammalian target of rapamycin; QOL, quality of life; ADL, activities of daily living; BSA, body surface area; MRM, magnetic resonance imaging; DICOM, Digital Imaging and Communications in Medicine; ROL, region of interest; FACT-G, Functional Assessment of Cancer Therapy-General.

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Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2018.12.001

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1. Introduction

Lymphatic anomalies (LAs) represent a group of diseases involving systemic dysplasia of the lymphatic vessels. Refractory and fatal diseases, termed intractable LAs, can also occur. Cystic lymphatic malformation (LM) also called as lymphangioma, generalized lymphatic anomaly (GLA) also called as lymphangiomatosis, and Gorham–Stout Disease (GSD) are representative of these disorders [1]. Classification of LAs was performed by the International Society for the Study of Vascular Anomalies (Table 1) [2].

Cystic LMs present at birth in up to 60% of LA cases, and by 2 years of age in approximately 90% [3]. They can be characterized as macrocystic, microcystic, or mixed cystic lesion. The pathology of cystic LMs is quite variable, ranging from a focal area with minimal swelling to large areas of diffusely infiltrating aberrant lymphatic channels. Depending on their location and extent of the lesion, LMs can affect vital physiological functions. For example, up to 75% of LMs are found in the cervicofacial region, which can cause chronic airway problems, recurrent infection, and functional issues related to speech, oral hygiene, and malocclusion. Treatment of LMs includes observation, surgical excision, sclerotherapy, and pharmacotherapy. Surgical resection and sclerotherapy are usually effective for treating and resolving macrocystic LMs. However, microcystic LMs are more infiltrative and difficult to treat. Interferon, corticosteroids, and propranolol are also used for treatment of inoperable LM, although their effects are limited as they are not indicated for this disease [3].

GLA is characterized by diffuse or multicentric proliferation of dilated lymphatic vessels resembling common LM [4]. GLA has a variable presentation and can affect several different sites including bone, liver, spleen, mediastinum, lungs, and soft tissues. Thoracic involvement may be associated with poor prognosis compared with cases with soft tissue or bone involvement [4]. GSD is a rare disease characterized by osteolysis in bony segments, with localized proliferation of lymphatic or vascular channels in areas adjacent to the affected bone [5]. While GSD mainly involves the skeletal system, it can also involve the viscera, and clinical findings of GSD and GLA closely overlap. In patients with multifocal lesions, therapeutic options are palliative, and therapy is often aimed at reducing symptoms associated with bone lesions and chylosous effusions. For inoperable and intractable cases, corticosteroids, interferon, propranolol, and chemotherapy agents (vincristine) are used, although these treatment approaches are associated with limited responses and significant side effects [4].

Sirolimus is an immunosuppressive agent that inhibits the action of mammalian target of rapamycin (mTOR), which regulates cell division, multiplication, and survival [6]. Sirolimus was approved for use to ‘prevent organ rejection reaction in kidney transplant patients’ in the United States in September 1999 and in the European Union in March 2001. As of September 2011, it had been used in 89 countries (although it is not approved for use in Japan) under the product name ‘Rapamune’. In Japan, sirolimus was developed to treat lymphangioleiomyomatosis, and in October 2013, Nobelpharma applied for manufacturing and marketing approval for use of sirolimus in lymphangioleiomyomatosis, which was approved in July 2014.

Recent studies have found a role of the phosphoinositide 3-kinase/AKT/mTOR pathway in the development of blood vessels and lymphatic vascular tissues, and new therapeutic agents that target this pathway are under development [6,7]. The mTOR inhibitor sirolimus has also been shown to inhibit lymphangiogenesis, and is thought to act on lymphatic tissues within lesions to regulate the production and leakage of lymph by decreasing lymphatic endothelial cell activity. Thus, we hypothesized that treatment with sirolimus will reduce volume of lymphatic tissues and improve the clinical symptoms of patients with LAs. Thus, we have proposed a multicenter study to investigate the efficacy and safety of sirolimus for intractable lymphatic anomalies (SILA study). Herein, we present the SILA study protocol.

Methods: This open-label, single-arm, multicenter, prospective study will assess the efficacy and safety of a mammalian target of the rapamycin inhibitor sirolimus in the treatment of intractable LAs. Participants will receive oral sirolimus once a day for 52 weeks. The dose is adjusted so that the nadir concentration remains within 5–15 ng/ml. The primary endpoint is the response rate of radiological volumetric change of the target lesion confirmed by central review at 52 weeks after treatment. The secondary endpoints are the response rates at 12 and 24 weeks, respiratory function, pleural effusion, ascites, blood coagulation parameters, bleeding, pain, quality of life, activities of daily living, adverse events, side effects, laboratory examinations, vital signs, and pharmacokinetic data.

Results: This is among the first multicenter studies to evaluate sirolimus treatment for intractable LAs, and few studies to date have focused on the standard assessment of the efficacy for LAs treatment. Our protocol uses novel, uncomplicated methods for radiological assessment, with reference to the results of our previous retrospective survey and historical control data from the literature.

Conclusions: We propose a multicenter study to investigate the efficacy and safety of sirolimus for intractable LAs (SILA study; trial registration UMIN000028905). Our results will provide pivotal data to support the approval of sirolimus for the treatment of intractable LAs.

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2.2. Study outline and enrollment

This is an open-label, single-arm, multicenter, prospective trial at 5 institutions in Japan (Gifu University Hospital, Kyoto Prefectural University of Medicine, Kyushu University Hospital, National Center for Child Health and Development, and Keio University School of Medicine). Patients who meet the eligibility criteria and who provide informed consent will be enrolled within 28 days of obtaining consent. The first sirolimus dose is administered within 7 days of enrollment (Fig. 1).

2.3. Eligibility criteria

2.3.1. Inclusion criteria

1) Over 0.6 m² of body surface area (BSA) at enrollment, and judged by the investigator to be able to take tablets. The trial is open to patients of either sex and of any age.
2) Definitely diagnosed with cystic LM (head, neck, thoracic, peritoneal cavity, or retroperitoneum), GLA, or GSD according to diagnostic criteria, and excluding other lymphatic diseases (channel type LM, primary lymphedema, and others).
3) Having at least one target lesion (e.g., cystic LM, lymphedema) that is measurable by magnetic resonance imaging (MRI).
4) With intractable and severe disorders and symptoms because of the target disease (bleeding, chronic pain, recurrent cellulitis >3 episodes/year, ulceration, visceral and/or bone involvement, potential effects on organ function including the eye, airway, and ear).
5) Written consent to participate in this trial was provided by the subjects in person or by a legal representative guardian (when the subject is younger than 20 years at consent).

2.3.2. Exclusion criteria

1) Patients with inadequate liver, renal, and cardiac function: all of the following criteria must be met. Cases in whom liver, renal, or cardiac function has deteriorated because of the disease will be not excluded
   • Serum total bilirubin: < three times the upper limit of normal for the age [8].
   • Serum creatinine: < three times the upper limit of normal for the age [8].
2) Having received molecular target drugs associated with the mTOR pathway, such as sirolimus, other mTOR inhibitors (e.g., everolimus), or a tyrosine kinase inhibitor (e.g., bevacizumab, sorafenib) within 8 weeks before the first administration of the trial drug.
3) With active infections that require systemic treatment
4) Performance status (PS): Karnofsky PS score <30 (>10 years of age) or Lansky play-PS < 30 (<10 years) because of permanent sequelae from brain injury.
5) With uncontrolled diabetes, hypertension and hyperlipidemia, chronic liver, and kidney disease.
6) Having been administered an immunosuppressant drugs (e.g., cyclosporine, tacrolimus) or corticosteroids over 4 weeks, except for local injection, inhaled, topical, or physiological maintenance dose administration.
7) With a history of an allergic reaction to sirolimus or any of its additives.
8) Having been administered a drug that inhibits/induces CYP3A4 enzyme activity within 1 week of test drug initiation.
9) With immunodeficiency conditions such as human immunodeficiency virus infection or primary immunodeficiency diseases.
10) Carriers of hepatitis B and/or hepatitis C virus
11) Patients who may suffer from malabsorption of sirolimus.
12) Having received surgery (resection, sclerotherapy, or endovascular treatment) for the target lesion within at least 2 weeks prior to the date of obtaining consent for participation in this trial or not be able to deny the possibility of remaining effects induced by surgery.
13) Having been administered therapeutic drugs for the target disease (including prapranolol, epipikapakusato, auigikenchuto, interferon, octreotide, bisphosphonate, or denosumab) within 2 weeks prior to the date of obtaining consent for participation in this trial.
14) Having been administered chemotherapy agents that lead to myelosuppression, or biological products or treatment not covered by insurance, within 4 weeks prior to the date of obtaining consent for participation in this trial.
15) Having received radiation therapy for the target lesion within 24 weeks prior to the date of obtaining consent for participation in this trial.
16) Patients who participated in another clinical trial within 4 weeks prior to the date of obtaining consent for participation in this trial.
17) Patients with orthodontic appliances, cochlear implants, or other devices that may affect image assessment of MRI.
18) Pregnant, breast-feeding, or may be pregnant, or without consent to contraception during the clinical trial period.
19) Judged by the principal investigator/sub-investigator to be inappropriate for participation in this clinical trial for other reasons.

2.4. Treatment

Sirolimus (Rapamune tablet, 1 mg) will be administered orally once daily after meals or on an empty stomach. Patient’s with a BSA ≥1.0 m² will be administered 2 mg (2 tablets) once a day, while those with a BSA of <1.0 m² will be administered 1 mg (1 tablet) once a day. The dose is adjusted so that the nadir concentration remains within 5—15 ng/ml. The first sirolimus level for adjustment will be measured during week 2 (day 12—16) to allow for loading to occur and to approach steady state concentrations. If the trough level of sirolimus is under 5 ng/ml, the dose will be increased by 1 mg steps. The maximum dose per day is 4 mg. If the trough level of sirolimus is over 15 ng/ml within administration of 1 mg once daily, it will be reduced each 48 h. Subsequent trough sirolimus levels will be obtained every 4 weeks.

During sirolimus treatment, patients will not be allowed the following therapies: drugs that may cause adverse effects combined with sirolimus (attenuated live vaccine, chemotherapy
agents that lead to myelosuppression), drugs that inhibit/induce CYP3A4 enzyme activity, and therapies for the target disease such as surgery, sclerotherapy, and pharmacotherapies (e.g., prednisone or interferon). However, supportive therapies (antibiotics, blood transfusion, antipyretics, and analgesics) and other drugs that may not affect assessment of the lymphatic lesion will be allowed.

2.5. Evaluation, laboratory tests and follow

The study assessment schedules are summarized in Table 2. For baseline, all patients require an assessment of the affected area by MRI. All MR examinations are performed on a 1.5 T scanner with patients in the supine position. Routine T1-weighted, T2-weighted, T2-weighted fat saturated, and three-dimensional fat sat T2-weighted sequences are obtained. The axial T2-weighted fat saturated sequence and the effects of slice thickness between 5 and 8 mm without gap will be used as definitive evaluation. The areas of the lymphatic cyst, lymphedema, and lymph fluids will be measured using T2 fat-saturated images, as these lesions show clear high-signal intensities. If the lesions are diffuse or large, we will base the measuring range on the normal organ position and landmarks (e.g., location of spine). After 12, 24, and 52 weeks, the patient will be assessed for clinical response by MRI. If the patients show disease progression (>20% increase of target lesion evident on radiographic imaging from baseline) after 24 weeks, the patient will be removed from the study protocol. Other patients will continue sirolimus treatment for up to a further 28 weeks (total 52 weeks). Tests for QOL, respiration, pain, and bleeding will be performed at weeks 12, 24, and 52. Other physical and laboratory examinations will be performed monthly. There will also be a post-observation period at 4 weeks after final administration.

2.6. Endpoints

The primary endpoint is response rate, defined as the proportion of patients who achieved complete response or partial response at 52 weeks after initiating treatment with the trial drug. The radiological volumetric change will be assessed by MR Imaging of the target lesion. The area dimensions of lymphatic tissue or cysts demonstrated by MR imaging with T2 fat saturated sequences will be measured with the Digital Imaging and Communications in Medicine (DICOM) viewer (OsiriX © v.9.0; Pixmeo, Bernex, Switzerland). MR images are evaluated by 2 independent central radiologists blinded to patient identity. Quantitative analysis will be automatically performed to measure the area dimensions of the lesion using the region of interest (ROI) tool (Fig. 2). If ROIs cannot be calculated because of intricate shapes of the lesion, measurement is performed with use of a manual computing tool (closed polygon ROIs). Other pathologic lesions, namely inflammation, bleeding, and hematoma, will be removed. The volume of the target lesion is calculated by multiplying these ROI areas by the slice width. The evaluation criteria are defined as follows: Complete response: disappearance of all target lesions, partial response: at least a 20% decrease of the volume of the target lesion, progressive disease: a 20% or greater increase in volume of the target lesion, and stable disease: insufficient shrinkage to qualify for partial response and insufficient growth to qualify for progressive disease.

The secondary endpoints are the response rate at 12 weeks and 24 weeks, respiratory function, pleural effusion, ascites, blood coagulation parameters, bleeding, pain, QOL, ADL, adverse events, side effects (aphthous ulcers, leukopenia, hypercholesterolemia and opportunistic infections are common), laboratory examinations (complete blood count, liver and lipid profile), vital signs, and pharmacokinetic data. Bleeding, pain, QOL, ADL, and adverse events will be measured using the World Health Organization-Bleeding...
2.7. Primary-endpoint analysis

Primary analysis will be performed on the full analysis set consisting of all enrolled patients who will receive at least one dose of sirolimus, will be measured for the efficacy variable at least once after enrollment, and will have no critical good clinical practice violation. The response rate at 52 weeks and its exact 95% confidence interval will be calculated. If the lower limit of the 95% confidence interval regarding the response rate is greater than 5%, we would conclude that the efficacy of sirolimus is confirmed. Furthermore, the exact binomial test for null hypothesis that the response rate is less than 5% will be performed.

2.8. Sample size

Because there is little evidence regarding the natural history of intractable LAs and the efficacy of traditional therapies for these conditions, we used retrospective data from a national survey on intractable LAs (unpublished data) to determine sample size. We examined data from 40 patients (22 with GLA, 18 with GSD) who had not received surgery, radiation therapy, or drug therapies. Volumetric changes on radiologic examination were measured and response rates were calculated retrospectively. None of the 40 patients showed radiological improvement (more than 20% decrease in volume of the target lesion) for at least 1 year. Among 19 patients with intractable cystic LM, only one (5.3%) showed radiological improvement. Thus, of the 59 cases evaluated, the lesion shrank in only one (1.7%), with a threshold response rate of 5%.

In a clinical trial conducted in the United States in 2009 that included 53 cases, the response rate (shrinkage exceeding 20% of the target lesion size) determined by MRI after 12 months of sirolimus administration (12 courses) was 52% (95% confidence interval: 36–69%) [8]. Furthermore, in a clinical trial in Japan (unpublished data), the response rate (shrinkage exceeding 20% of the target lesion size) for cystic LM, GLA, and GSD was 66.7% (10/15 cases) at 12 months after administration of sirolimus, while the response rate was 50.0% (4/8 cases) when patients aged 6 years or younger (who were unable to take tablets) were excluded. Thus, the expected response rate was determined as 50%. The sample size was calculated based on the probability that the lower limit of the exact 95% confidence interval for the primary endpoint would be greater than the threshold of 5%. To maintain a probability of at least 90%, nine patients were needed. In consideration of drop-out, the target sample size was 10.

2.9. Ethics and dissemination

Approval for this clinical trial was obtained from the institutional review boards of each participating facility (Gifu University Hospital, Kyoto Prefectural University of Medicine, Kyushu University Hospital, National Center for Child Health and Development, and Keio University School of Medicine). The study protocol, informed consent form, and other documents were reviewed and approved. This study has been registered at the Clinical Trial Registry (UMIN-CTR) (UMIN000028905). The principal investigator (sub-investigator) will provide sufficient explanation to subjects using consent information sheets before their
participation in the trial, and will obtain written consent to participate in the present clinical trial based on each subject's free will. Final data will be publicly disseminated regardless of the results. A report releasing the study results will be submitted in an appropriate journal.

2.10. Maintaining data confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by coded ID numbers only, to maintain participant confidentiality. All local databases will be secured with password-protected access systems. The Data Management Coordinating Center will oversee the study data using electronic data capture.

3. Discussion

There are currently no standard treatments for intractable LAs, and new drugs are urgently required for these refractory and potentially fatal disorders. Both domestic and international reports have indicated the efficacy of sirolimus on intractable LAs, suggesting that further clinical trials are important. Sirolimus has also been shown to improve various clinical symptoms, which may allow subsequent surgery and sclerotherapy. Thus, the results of this trial will provide key new data for approval of sirolimus for treatment of intractable LAs.

The functional mechanism of sirolimus for LM lesions is poorly understood, although several clinical trials showing efficacy of sirolimus have been reported. In 2011, Hammill et al. reported their preliminary experience using sirolimus for treatment of complicated vascular anomalies in children [6]. The prospective trial by Adams et al. also indicated that sirolimus had an excellent effect for LAs, with a good response observed in all GLA and GSD patients [13]. However, it remains unclear how sirolimus acts on the lymphatic endothelial cells and LM lesions in these patients.

The pathophysiology of the mTOR signaling pathway may explain the effectiveness of mTOR inhibitors in vascular anomalies [6]. The mTOR protein is a serine–threonine kinase regulated by phosphoinositide-3-kinase, which has a central role in the complex intracellular signaling pathways involved in important processes such as cell growth, cell proliferation, angiogenesis, cellular metabolism, autophagy, and apoptosis. Sirolimus is thought to reduce phosphoinositide-3-kinase phosphorylation, and thus reduce proliferation and sprouting of LM lymphatic endothelial cells [7]. We assume that sirolimus will help restore the normal structure in the LM lesion and reduce the lesion volume by correcting the lymphatic flow. Thus, we will examine whether a reduction in the target lesion leads to improvement of symptoms in this study, and have designed an objective volumetric analysis of the target lesion.

Fig. 2. Magnetic resonance imaging (MRI) of the regions of interests (ROIs) in a patient with cervical cystic lymphatic malformation (LM) A 1-year-old boy with cystic LM involving the tongue and cervical region (2A, 2C) Axial and coronal view of T2-weighted fat saturated MRI of the neck shows cystic LM. (2B, 2D) The light green area shows the ROIs measured by OsiriX software.
Few studies have assessed the efficacy of pharmacological therapy for LAs. LAs are heterogeneous, and have variable symptoms and lesions. Thus, assessments used in previous studies were not standardized. A previous phase II trial reported 3 distinct assessments involving radiological examination, functional impairment score, and health-related QOL (FACT-G and PedsQL) [13]. In our study, disease response will be established by changes in at least 1 of these parameters. Identifying functional impairment with scores has never been validated for quantification of LAs. Thus, we will also analyze various parameters as secondary endpoints, including respiratory function, pleural effusion, ascites, blood coagulation parameters, bleeding, pain, QOL, ADL, clinical examinations, and vital signs. Nevertheless, it remains difficult to analyze these disparate symptoms unitarily. Development of these assessments should be addressed in future studies.

There are limited studies using radiological assessment for LM lesions. However, we will use MR imaging to assess volumetric changes. MR imaging is the primary noninvasive imaging modality used for evaluation of vascular anomalies. MR imaging provides superior soft tissue contrast and multiplanar imaging capability, and thus is particularly useful for assessment of LM lesions. Furthermore, the use of a central reviewer provides objective radiological assessment. We will measure the area of the lymphatic cyst, lymphedema, and lymph fluid using T2 fat saturated images as these lesions show clear high signal intensities. OsiriX is an easy to use open source software that provides advanced post-processing imaging protocols, thus making it a versatile DICOM viewer. Our study protocol will use novel and simple methods of radiological assessment.

The optimal dose of sirolimus remains controversial. Currently, there are no standardized methods for the optimum dosing of sirolimus. Previous studies utilized trough doses of sirolimus adjusted to 5–15 ng/ml or 10–15 ng/ml. In phase II trials, trough levels were maintained at 10–15 ng/ml [13,14]. A systematic review of sirolimus treatment for vascular anomalies also reported that the expected trough levels of sirolimus for most studies (19/25, 76.0%) were 5–15 ng/ml [15]. Furthermore, in the phase I–II trial of sirolimus treatment for lymphangioleiomyomatosis, the trough levels were maintained at 5–15 ng/ml [16]. In our clinical trial for LAs in Japan, the efficacy and safety of sirolimus treatment was also similar to these studies. Thus, we will measure sirolimus levels at each follow-up visit, and maintain at 5–15 ng/ml.

The standard first dosage of sirolimus is also unclear. In a recent review, Nadal et al. reported heterogeneous dosing, although 1.6 mg/m²/day in 2 doses was most common [15]. Unfortunately, liquid sirolimus is not currently approved in Japan. Thus, our study patients will receive over 0.6 mg m² BSA (as the minimum dose is 1 mg), and be assessed by the investigator to be able to take tablets. We will also divide into 2 patterns according to the patient’s BSA (2 mg: BSA >1.0 m² and 1 mg: BSA of <1.0 m²). Although neonates and infant cannot participate in our study, clinical trials for pediatric patients are required in the future. There are some limitations of this study. First, the natural history of LAs and the expected benefits of other treatments are unknown, and there is no standard treatment for LAs. Thus, there are limited data for statistical analysis. We used several domestic and international studies as a reference. Second, even though the volumetric assessment methods using OsiriX software are standardized, and use experienced assessors, it is difficult to prevent evaluation error between multiple radiologists. To minimize this potential bias, the investigator will measure the area twice, and if the error between each result is >10%, the analysis will be performed again. Third, the sizes of these lesions are sometimes variable because of infection, trauma or spontaneous intrallesional bleeding. We are permitted to move the evaluation date 4 weeks before or after the original evaluation date to prevent incorrect evaluations associated with pseudo-progression, patients with target lesions that are difficult or impossible to evaluate with precision because of obvious infection or bleeding, or because of other factors. Another limitation is the single-arm design of this study, although this was because of ethical considerations for patients with intractable LAs and the rarity of these diseases. Nevertheless, if our study confirms the effectiveness of sirolimus, this will provide important information for clinicians.

4. Conclusions

This is among the first multicenter studies to evaluate sirolimus treatment for intractable LAs. The results of the trial are expected to provide pivotal data to support the approval of sirolimus for the treatment of intractable LAs.

Ethics approval and consent to participate

This study has received approval from the institutional review boards of each participating facility (Gifu University Hospital, Kyoto Prefectural University of Medicine, Kyushu University Hospital, National Center for Child Health and Development, and Keio University School of Medicine). The first institutional review board approval date was 10 July 2017. All procedures are in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and later revisions. Written informed consent will be obtained from all patients prior to inclusion in the study.

Conflict of interests

MO and TF received research funding from Nobelpharma. Sirolimus tablets will be supplied by Nobelpharma. The other authors declare no competing interests.

Funding

This clinical trial will be performed using research funding from the Japan Agency of Medical Research and Development: Clinical research-clinical trial promotion research project.

Authors’ contributions

MO, RA, HH, TM, AF, and FT conceived the study and participated in its design. RA will perform progress management and adjustment of the overall clinical trial. MO, TF, YY, TK, TM, AF, TF, TT, YK, RS, and TF will oversee the operations associated with this clinical trial, including managing and instructing as the leaders of the team comprising sub-investigators and trial collaborators. HH will be responsible for the statistical analysis of this clinical trial. AS will be responsible for case enrollment, data management, and monitoring in this clinical trial. SU, SW, SN, MM, AI, KM, and SH will evaluate the efficacy and safety in this study.

Acknowledgements

We thank Dr. Akifumi Nozawa, Dr. Shiho Yasue, Dr. Saori Endo, Dr. Tomohiro Hori, Dr. Shigemi Suzuki, and Ms. Asuka Ogawa from Gifu University; Dr. Motoi Kato, and Dr. Tadashi Iwanaka from Saitama Children’s Medical Center; Dr. Masatake Takahashi, Dr. Katsuhiro Ogawa, and Dr. Yoko Shioda from National Center for Child Health and Development; Dr. Shigeisha Fumino from Kyoto Prefectural University of Medicine; and Dr. Kyoichi Deie and Miho
Watanabe from The University of Tokyo, for their helpful comments.

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