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A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: A Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study: XP-1 study

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A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: A Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study: XP-1 study

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ABSTRACT

Introduction: Xeroderma pigmentosum (XP) is a rare intractable disease without a fundamental treatment, presenting with severe photosensitivity, freckle-like pigmented and depigmented maculae, and numerous skin cancers before the age of 10 years without strict sun protection. About 70% of the patients exhibit extremely severe sunburn reactions and most of them develop neurological symptoms, including sensorineural hearing impairment and progressive peripheral and central nervous disorders beginning from childhood ages. In the preclinical study, we found that N-acetyl-5-methoxytryptamine showed effective to suppress skin tumor development in addition to improvement of auditory brainstem response in chronically UV irradiated XP-A model mice.

Methods and analysis: On the bases of the preclinical study, we conduct a clinical trial on the efficacy of NPC-15 for patients with XP with Exaggerated sunburn-reaction type by a multicenter, double-blinded placebo-controlled, two-group crossover study followed by a 52- weeks open study.

Ethics and dissemination: Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board, and the study is conducted in accordance with the approved protocol. All participants will be required to provide written informed consent. Findings will be disseminated through scientific and professional conferences and peer-reviewed journal publications. The datasets generated during the study will be available from the corresponding author upon reasonable request.

Trial registration number: Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered on February 23, 2022.

Strengths and limitations of this study

- ✓ This is the first clinical trial that has been conducted for patients with Xeroderma Pigmentosum, an extremely rare genetic disease.

- ✓ The clinical study is designed as a double-blinded cross-over trial, followed by open study because the number of study patients is limited even in Japan, where its frequency is higher than in other countries and NPC has been already approved as a medicine for other disorders.
- ✓ The randomization and evaluation of the minimum erythema dose, the primary endpoint, and the Efficacy and Safety Evaluation Committee are organized independently of the investigators.
- ✓ The limitation of the study is the duration of the open study is not enough for evaluating the new onset of skin cancer and several genotypes are considered together.

Keywords: Xeroderma Pigmentosum, Minimum Erythema Dose, randomized controlled trial, double-blind, crossover, UV irradiation test, NPC-15

A short title: The trial of NPC-15 in patients with Xeroderma Pigmentosum sunburn enhancement.

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INTRODUCTION

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers in the sun-exposed areas. Without strict sun protection, numerous skin cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such as sensorineural hearing impairment, and progressive peripheral and central nervous disorders beginning around the age of 6 years, have also been observed in approximately 60% of patients [1, 2].

The number of patients medically recorded to have XP in Japan is 300–600 patients. However, the estimated frequency of XP in the Japanese population, which was calculated by the number of patients diagnosed with the eight clinical subtypes of XP (XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-G, and XP-V), is approximately 1:22,000 persons. To elaborate, the percentage of patients with XP-A accounts for about 50% of all XP in Japan, and 90 % of them harbor the homozygous founder mutation of IVS3-1G>C in the *XPA* gene (responsible gene for XP-A), and its carrier ratio was 1: 113 in the general population (heterozygous for the founder mutation in the *XPA* gene [3]. This discrepancy in the estimated and actual number of patients was assumed to be because some patients have not yet been diagnosed or are in facilities that limit their access to regular check-ups. Further, as the carrier data are based on archived pathological sections (from 1957–2011) stored at medical facilities in Hiroshima, it is possible that the frequency of carriers may have been higher in the previous era. Regardless, the frequency of XP is higher in Japan than in both Europe and the U.S.A, whereby it is only observed at a rate of 1:1,000,000 people [1, 2].

XP is represented by 8 clinical subtypes, seven of which (A-G) are caused by mutations in genes of the Nucleotide Excision Repair (NER) pathway and one (V) is NER proficient but deficient in Translesion Synthesis (TLS) DNA polymerase η. In Japanese XP patients, XP-A accounts for 52.7% of patients, followed by XP-V for 30.8%, XP-D for 7.3%, XP-F for 4.0%, XP-C for 2.7%, and XP-G for 2.0% of patients [2]. Patients with XP-A

exhibit the lowest ability of DNA repair and the most severe cutaneous and neurological symptoms. The ratio of male to female patient incidence of XP is almost 1:1.

NER is the process of repairing DNA lesions caused by ultraviolet radiation (UV), and NER defects, as in XP, result in the accumulation of DNA lesions that lead to carcinogenesis. Whilst the pathogenesis of severe sunburn, pigmentary abnormalities, and concomitant neurological symptoms is still unclear [2], several hypotheses have been postulated that relate to the fact that NER is involved in oxidative DNA lesions such as cyclopurine that accumulate in the neuronal cells of humans [4, 5], and that XP patients show an impaired mitophagy in their neuronal mitochondria due to oxidative stress [6].

Symptoms vary with each group; XP-A, -B, -D, -F, and -G are categorized as exaggerated sunburn reaction types and patients with these groups manifest with severe sunburn with minimum sun exposure, and a prolonged reaction, with the peak at 48-72 hours after UV exposure. In addition, these groups exhibit neurological symptoms to varying degrees. Patients with XP-A show severe photosensitivity from birth, freckle-like pigmented and depigmented maculae after UV exposure to the sun-exposed skin, and the development of skin cancer before the age of 10 if strict sun protection is not enforced. XP is associated with almost normal development in early childhood, with mild delays in speech and walking usually observed. However, neurological symptoms generally start with sensorineural hearing impairment at the age of 5–6 years, gait imbalance from 6 years, and severe gait disturbance by 20 years. Breathing problems because of laryngeal dystonia, dysphagia, and aspiration occur frequently, and sensorineural hearing impairment and dysarthria cause severe communication challenges in their late teens. Any patients with XP may develop skin cancer in childhood and adolescence if not protected from the sun [7, 8, 9].

Progressive central and peripheral neurological symptoms occur in almost 100% of Japanese patients with XP-A, about 10% of XP-D patients, and some -F and -G patients. The rate of neurological symptoms varies with age, but they appear in almost all XP-A patients in their mid-teens. There is no fundamental treatment for XP yet, although cutaneous

conditions have improved over the last 30 years due to improved education for strict sun protection, and early diagnosis and treatment for skin cancers with regular check-ups [2]. However, there is no way to treat or prevent the progression of neurological symptoms, although symptomatic treatments by an interdisciplinary team, such as doctors from dermatology, pediatrics, neurology, otolaryngology, orthopedics, ophthalmology, urology, and dentistry, can assist with education for sun-protection, skin cancer checks, hearing aids, and rehabilitation modalities to prevent secondary complications. The UV-blocking film is strongly recommended to be applied to windows in everyday living spaces at home and school.

The Anti-inflammatory drug has shown to reduce UV-induced inflammation as well as UV- induced skin tumor development in XP animal models [10], XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppressed inflammatory response and oxidative stress after UV irradiation. In addition, it suppressed skin tumor formation, and improved auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiated XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

Although N-Acetyl-5-methoxytyptamine was first identified as a hormone in the pineal gland by Lerner [11], it is believed to be derived from an antioxidant synthesized in photosynthetic cyanobacteria 3 billion years ago that has been conserved in almost all living species, since then, with little change in chemical structure [12]. N-Acetyl-5-methoxytyptamine is responsible for regulating circadian rhythms, and endogenous synthesis and its secretion are regulated by the light/dark cycle, with the highest plasma concentrations at night. In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytyptamine as a free radical scavenger and antioxidant [13]. It is particularly believed to act as an antioxidant within mitochondria [12]. Pharmacologically, N-Acetyl-5-methoxytyptamine reduces the severity of injury in several disease models due

to its effect on acute and chronic inflammation and CNS protection.

In Japan, Nobelpharma Co. Ltd. submitted a manufacturing and marketing authorization application in April 2019, and a 0.2% granule of N-Acetyl-5-methoxytyptamine was approved in March 2020 for the treatment of sleep difficulties associated with childhood neurodevelopmental disorders. However, since no drugs for XP have been approved and marketed in Japan and overseas, the development of an additional indication was promoted.

Considering that anti-inflammatory drugs reduced the UV-induced inflammation as well as UV- induced skin tumor development in the XP animal model [10]. Furthermore, it has been shown that level of N-Acetyl-5-methoxytyptamine metabolites is reduced in patients with XP [14], and there is a strong need to establish an early treatment for XP in actual clinical practice.

As this crossover study was to confirm the short-term efficacy of the drug to obtain a drug indication, the investigators decided to target patients with XP with exaggerated sunburn reaction type for this clinical trial in order to investigate the efficacy and safety of this drug in Japanese patients with an exaggerated sunburn reaction type of XP.

MATERIALS & METHODS

Study design

This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study. The patient flowchart is shown in Figure 1.

Study setting

This study will be performed at Kobe University Hospital and Osaka Medical and Pharmaceutical University Hospital. All study data will be stored and archived in the data center of DOT World using Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), an electronic data system (EDC) for clinical research, to manage the data and protect confidentiality before, during, and after the trial.

Purpose

The purpose of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type.

Endpoints

Primary endpoint

The Minimum Erythema Dose (MED) 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration.

Secondary endpoint

The secondary efficacy endpoints are the following:

(1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (stage I and stage II) of investigational drug

administration.

(2) Evaluation of Melanin index regarding the pigmented area in MED judgment area.

(3) Pigmented maculae inspection (number, area, and color tone) [15].

(4) Neurological symptoms (neurologic severity scale score on XP [16], hearing test, and 5 m gait test).

(5) Presence or absence of onset of acute skin symptoms

(6) Presence or absence of skin cancer

The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Eligibility criteria

Inclusion criteria

Patients will be included in the study when they satisfy all the following criteria:

(1) Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) that have been diagnosed with Exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.

(2) Patients aged 1 year old or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the subjects aged 6 years or older during the crossover study by the Safety Evaluation Committee.

(3) Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

Exclusion criteria

Patients will be excluded from the study when any of the following criteria apply:

(1) Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon

(2) Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.

(3) Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration

(4) Pregnant, lactating women, women who wish to become pregnant during the study period, or women who are fertile and cannot accept an effective contraceptive method.

(5) Patients deemed inappropriate by the investigators in participation of this clinical study

Randomization

All subjects who provide consent to participate and fulfill the sampling criteria will be randomized. Subjects will be randomly assigned to either the NP group or the PN group with a 1:1 allocation using the permutation random block method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. The trial participants, care providers, and endpoint assessors will be blinded. Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Data collection and management

Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each subject into the electronic data capture (EDC) system. The principal investigator will confirm that the entered CRF data are complete and correct, electronically

sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping. The CRF printout will be retained for audit trail purposes. If there are any queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Monitoring and Audit

Monitoring of the study will be performed to ensure that the human rights and welfare of the subjects are being protected, the study is conducted safely in accordance with the protocol and applicable regulatory requirements under the Good Clinical Practice, and data are being collected properly. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring. For quality assurance, the study will be examined 4 times, before the initiation of the clinical trial, after the first patient in, before the last patient in, and before the completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

Intervention and treatment protocol

The NPC-15 0.2% granules sold by Melatobel™ for pediatrics are manufactured at Nobelpharma Co., Ltd. The placebo formulation is the same except that it does not contain N-acetyl 5-methoxytryptamine.

NPC-15-Placebo (NP) group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. Placebo-NPC-15 (PN) group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.

The relationships between the interventions, endpoints, other assessments, and visits for the

subjects in this study are shown in Table 1 and Table 2.

Table 1. Summary of study assessments and procedures in the crossover period

	Study period														
	Screening period		Crossover period I							Crossover period II						
			Treatment period NPC-15 or Placebo						Washout	Treatment period Placebo or NPC-15						Washout
	Enrolment	Allocation	Day 1	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22–35	Day 1	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22–35
Informed Consent[3]	X															
Baseline data	X															
Enrollment	X															
Prescription			←	—	—	—	—	→			←	—	—	—	→	
UV irradiation[4]				X							X					
MED[4]					X	X	X	X				X	X	X	X	
Melanin index										X						
neurological severity scale score[5]	X															

hearing test[6]	X															
5-meter walk test[7]		X														
Acute skin symptom	X [8]															
Skin cancer	X [8]															
urine test for oxidative stress marker[9]		X						X							X	
Laboratory test	X							X							X	
Adverse events			←	—	—	—	—	—	—	—	—	—	—	—	→	
drowsiness and dizziness		X			X							X				
Body weight		X														
Medication status				X	X	X	X	X		X	X	X	X	X	X	
Concomitantly administered medications[10]		←	—	—	—	—	—	—	—	—	—	—	—	—	—	→

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2 [1] In the case of five or more consecutive holidays, including weekends and national holidays such as

3 New Year's Eve and Golden Week, the allowable range for the treatment period is ± 3 days, and the

allowable range for the washout period after the crossover study period I and II is +22/-4, and ± 4 days, respectively.

[2] Allowance (-3～＋21) is based on the point Day 35.

[3] Consent should be obtained within the 12 weeks prior to drug allocation.

[4] Visit tolerance on the UV test day is ± 2, but evaluation should be made at 24, 48, 72, and 96 hours ± 6 hours after the test day. Re-evaluation is prohibited.

[5] Neurologic severity scale scores will be evaluated in subjects 3 years of age or older.

[6] Methods of the hearing test (pure-tone audiometry or conditioned play audiometry) will be recorded in the medical records.

[7] 5-meter walk test will be conducted including the subjects who wear braces when the principal/participating investigator deems the subject can tolerate the test. Whether with or without the brace and what kinds of brace they wear the brace will be recorded in the medical records. When the subject wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[8] Data will be collected within 62 weeks prior to administration of the study drug.

[9] Laboratory urine test: oxidative stress markers and N-acetyl 5-methoxytryptamine metabolites.

[10] Four weeks prior to the initiation of the study drug.

Table 2. Summary of study assessments and procedures in the open period

	Open trial				
	Day1	Week 13	Week 26	Week39	Week52
Prescription (NPC-15)	←	—	—	—	→
Melanin index	X				
Facial pigmentation	X				X
Neurologic severity scale score [b]					X
Hearing test [c]					X
5-meter walk test [d]					X
Acute skin symptoms					X
Skin cancer					X
Laboratory test for Research (urine)[e]	X		X		X
Laboratory tests (blood and urine)					X
Adverse events	←	—	—	—	→
drowsiness and dizziness	X	X	X	X	X
Body weight	X	X	X	X	
Medication status		X	X	X	X

[a] The study will be conducted only for subjects who are deemed by the principal/sub-investigator at

Visit 305 (after 52 weeks of the open study), to require a visit to the hospital for evaluation of adverse events, etc.

[b] Neurologic severity scale score will be evaluated in subjects of 3 years of age or older.

[c] Methods of the hearing test (pure-tone audiometry or conditioned play audiometry, etc) will be recorded in the medical records.

[d] 5-meter walk test will be conducted including the subjects who wear the brace when the principal/participating investigator deems the subject can tolerate the test. Whether with or without the brace and what kinds of brace they wear the brace will be recorded in the medical records. When the subject wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[e] Urine examination for research use: Oxidative stress marker and N-acetyl 5-methoxytryptamine metabolites.

Statistical analysis

Analysis set

A summary of the planned statistical analysis for this study is provided below. The final analysis will be performed after data from the subjects have been obtained and fixed at the end of the follow-up period.

The full analysis set is the set of randomized subjects who receive at least one dose of the

study drug. The per-protocol set is the subset of subjects in the full analysis set who sufficiently comply with all aspects of the clinical trial protocol, including the drug administration methods and schedule. The safety analysis set is the set of subjects who receive at least one dose of the study drug. In this study, the safety analysis set is the same as the full analysis set.

Statistical analysis

Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The subject baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints.

For the crossover study, we will analyze under the assumption that there is no carryover effect due to a short half-life, and therefore a 2-week wash-out period will take place after the period 1. The analysis will be based on the difference in endpoints between period 1 and 2 for each subject. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided).

For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's test, one-sample t-test, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker concentrations before and after the open study will be assessed using a one-sample t-test.

The change of facial pigmented maculae at the start and end of the study which will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum test, one-sample t-test, and McNemar's test, respectively. The difference between acute skin symptoms and the development of skin cancer during the 62 weeks before the start of treatment (Visit 101) and after the start of treatment (Visit 101 – Visit305) will be assessed using McNemar's test.

The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall currency, and by treatment.

For evaluation of delayed sexual maturation or development, no statistical test will be performed and secondary sexual characteristics and blood hormone (prolactin) levels in each subject will be individually described.

Sample size calculation

The total target number of subjects is 20, with 10 subjects in both the NP and PN groups.

We estimated the difference in MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation of the difference between period 1 and 2 was conservatively assumed to be 6.0 mJ/cm^2, twice the irradiation unit. In the 2 × 2 crossover design of this study, when we expect a difference of 4.4 mJ/cm^2 between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm^2, we would need 8 cases to maintain a significance level of 2.5% (one-sided) and a total of 16 subjects to achieve a power of 80%. Assuming a few dropouts, a total of 20 subjects are required.

Study period

This manuscript is based on the current version of the study protocol (version 1.2, last updated on 1 June 2022). The study was first approved on 24 January 2022 by the institutional review board of Kobe University, Graduate School of Medicine, and authorized by the Pharmaceutical and Medical Devices Agency. Participant recruitment began on 1 April 2022. The expected date of completion (last visit of the last patient) is 15 December 2023.

Patient and public involvement

Patients and the public were not involved in the development of the research questions, selection of endpoint measures, study design, patient recruitment, or conduction of the study. The burden of intervention was assessed by representatives of patient associations participating in the ethical review committee. As mentioned in the individual consent form, participants may obtain access to the final results of the study through the principal investigator.

ETHICS AND DISSEMINATION

The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol.

Written informed consent is obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is

obtained from the substitute. The protocol was submitted to the Japan registry of clinical trial (jRCT)

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Contributors

MT obtained the grant funding and drafted the manuscript. RO, TF, and TU obtained the grant funding and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

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The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. The funding agency can be contacted at the following e-mail address: rare-koubo@amed.go.jp.

Competing interests

Nobelpharma Co., Ltd. provided the NPC-15 and placebo during the study period; however, the company had no role in this clinical trial. All authors have no conflicts of interest to declare.

Patient consent for publication

Not applicable.

Provenance and peer review

Not commissioned; externally peer-reviewed.

Data availability statement

The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol as no datasets were generated. However, the data will

be made available from the author upon reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).

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FIGURE CAPTIONS

Table 1. Summary of study assessments and procedures in the crossover period

Table 2. Summary of study assessments and procedures in the open period

Figure 1. Flow chart of participants

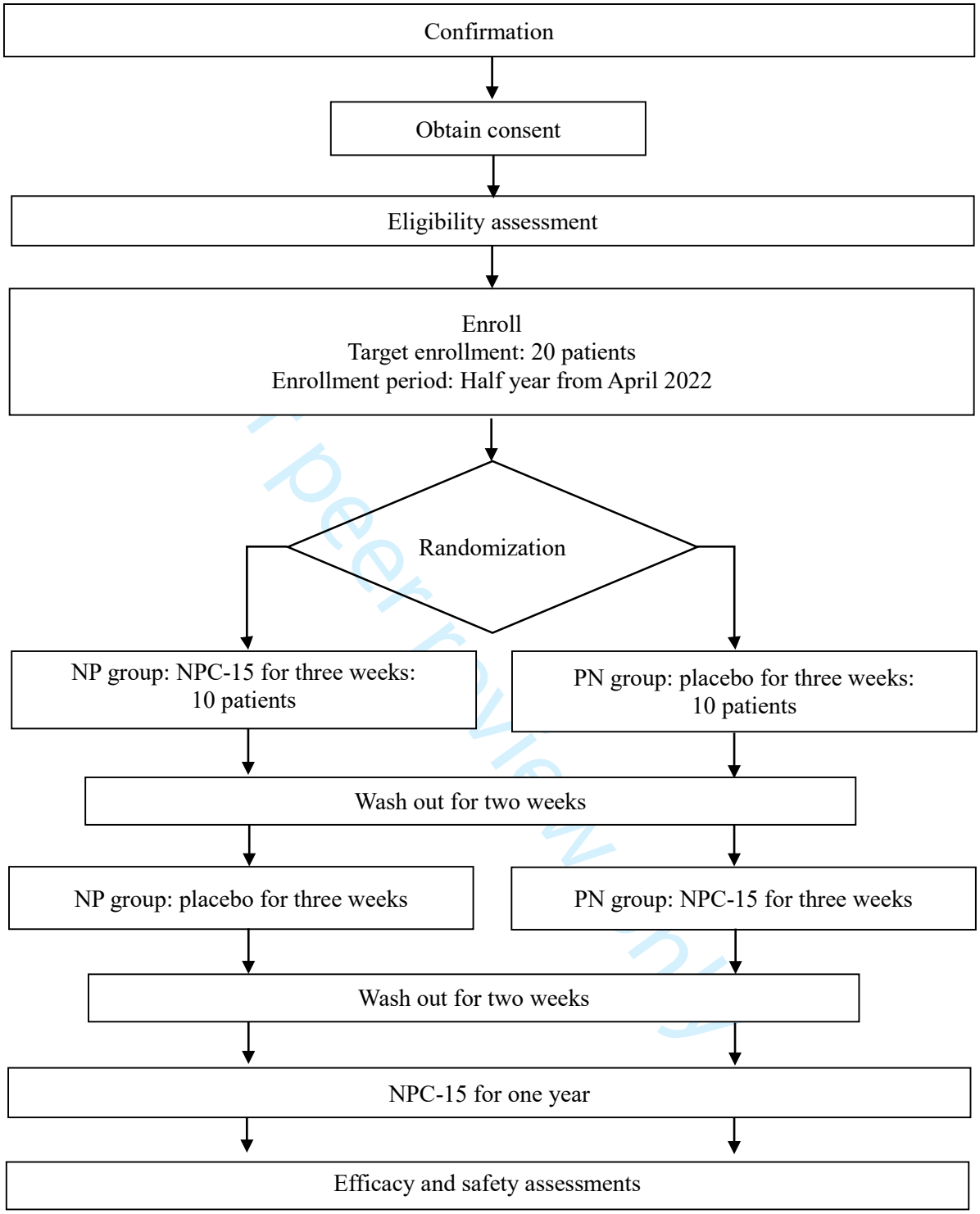

Figure 1. Flowchart of participants.

NP: NPC-15 to placebo, PN: placebo to NPC-15.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym A multicenter, double-blind, placebo-controlled, two-group crossover study and a long-term open study evaluating the efficacy and safety of NPC-15 in patients with xeroderma pigmentosum (XP) sunburn enhancement. (XP-1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinical Trials: Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 23 Feb 2022.
	2b	All items from the World Health Organization Trial Registration Data Set This information is available at the Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 17 Feb 2022. (https://jrct.niph.go.jp/en-latest-detail/jRCT2051210181)
Protocol version	3	Date and version identifier Version 1.2, last updated on 1 June 2022
Funding	4	Sources and types of financial, material, and other support This work is supported by the Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

MT, Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe, Japan.

YK, Clinical and Translational Research Center, Kobe University Hospital, and Department of Oral and Maxillofacial Surgery, Kobe University Graduate School of Medicine, Kobe, Japan

NY, Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe, Japan.

TF, Department of Otolaryngology-Head and Neck Surgery, Kobe University Graduate School of Medicine, Kobe, Japan

TU, Division of Neurology, Kobe University Graduate School of Medicine, Kobe, Japan

RO, Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe, Japan.

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SM, Department of Dermatology, Osaka Medical and Pharmaceutical University, Osaka, Japan

CN, Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, and Japanese Red Cross Hyogo Blood Center, Japan

MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

	5b	Name and contact information for the trial sponsor

The Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Contact information:

The funding agency can be contacted at the following web address: https://www.amed.go.jp/en/aboutus/index.html

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
A coordinating centre, steering committee, endpoint adjudication committee, and other individuals and groups are not participating in the composition of the trial and have no roles or responsibilities in the trial.

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers at sun-exposed areas. Without strict sun protection, numerous skin cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such as sensorineural hearing impairment, and progressive peripheral and central nervous disorders beginning around the age of 6 years, have also been observed in approximately 60% of patients.

The anti-inflammatory drug has shown to reduce the UV induced inflammation as well as UV-induced skin tumor development in XP animal models, XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppressed inflammatory response and oxidative stress after UV irradiation. In addition, it suppressed skin tumor formation, and improvement of auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiated XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytyptamine as a free radical scavenger and antioxidant. It is particularly believed to act as an antioxidant within mitochondria. Pharmacologically, N-Acetyl-5-methoxytyptamine reduces the severity of injury in several disease models due to its effect on acute and chronic inflammation and CNS protection.

Considering that anti-inflammatory drug reduced the UV-induced inflammation as well as UV-induced skin tumor development in the XP animal model. Furthermore, it has been shown that level of N-Acetyl-5-methoxytyptamine metabolites is reduced in patients with XP, and there is a strong need to establish an early treatment for XP in actual clinical practice.

	6b	Explanation for choice of comparators The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type.
Objectives	7	Specific objectives or hypotheses The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type. We hypothesized that NPC-15 could contribute to mitigate sunburn reactions and slow down the progress of neurological symptoms in patients with XP exaggerated sunburn reaction type.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study.
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained This study will be performed at Kobe University Hospital, Kobe, and Osaka Medical and Pharmaceutical University, Osaka, Japan.

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)

Inclusion Criteria

- ✓ Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) that have been diagnosed with Exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
- ✓ Patients aged 1 year old or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the subjects aged 6 years or older during the crossover study by the Safety Evaluation Committee.
- ✓ Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

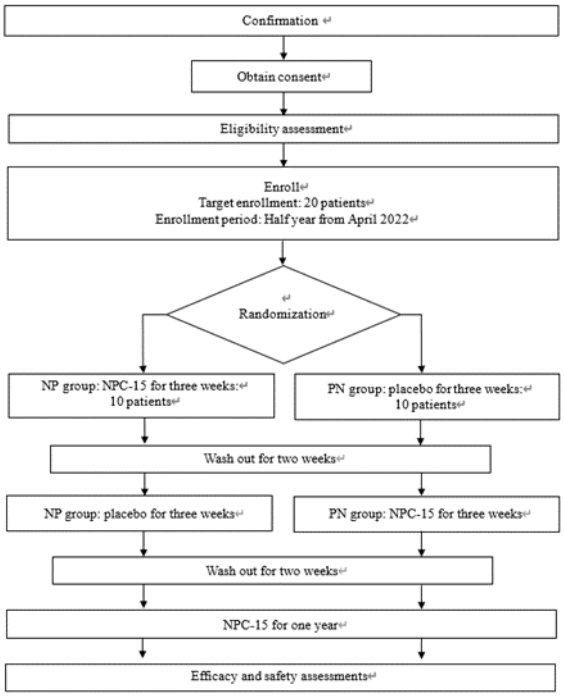
Exclusion Criteria

- ✓ Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon.
- ✓ Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.
- ✓ Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration.
- ✓ Patients who are pregnant or may become pregnant.
- ✓ Patients judged by the investigator to be ineligible for this study.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (1) NPC-15-Placebo (NP) group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. (2) Placebo-NPC-15 (PN) group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) The patients will return the empty medicine pouches at the end of the treatment period.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Concomitant administration of N-acetyl-5-methoxytryptamine (including health foods containing it as a major ingredient) and Fluvoxamine maleate (Luvox, Depmerol, etc.) is prohibited from the date of initiation to the date of termination of administration of the investigational drug.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended The primary endpoint is the Minimum Erythema Dose (MED) 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration. The secondary efficacy endpoints are the following: (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (stage I and stage II) of investigational drug administration. (2) Evaluation of Melanin index regarding the pigmented area in MED judgment area. (3) Pigmented maculae inspection (number, area, and color tone)[15]. (4) Neurological symptoms (neurologic severity scale score on XP[16], hearing test, and 5 m gait test). (5) Presence or absence of onset of acute skin symptoms (6) Presence or absence of skin cancer The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations The total target number of subjects is 20, with 10 subjects in both the NP and PN groups. We estimated the difference in MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation of the difference between period 1 and 2 was conservatively assumed to be 7.0 mJ/cm^2 because the estimated time point (24 hours) and the time of the main endpoint (72 hours) are different. In the 2 × 2 crossover design of this study, when we expect a difference of 4.4 mJ/cm^2 between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm^2, we would need 8 cases to maintain a significance level of 2.5% (one-sided) and a total of 16 cases to achieve a power of 80%.

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size All subjects who provide consent to participate and who fulfil the inclusion criteria and who do not meet any of the exclusion criteria will be randomized.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions All subjects who provide consent to participate and fulfill the sampling criteria will be randomized. Subjects will be randomly assigned to either the NP group or the PN group with a 1:1 allocation using the permutation random block method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. The trial participants, care providers, and outcome assessors will be blinded.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how The trial participants, care providers, and endpoint assessors will be blinded.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Because this is a crossover study in which both groups receive the actual drug, an unblinding procedure will not be incorporated into the study.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol. Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Data from participants who discontinue their participation in the study or who deviate from the protocol will be included in the FAS analysis. All data acquired during the study period will be analyzed.

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each subject into the electronic data capture (EDC) system. The principal investigator will confirm that the entered CRF data are complete and correct, electronically sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping. The CRF printout will be retained for audit trail purposes. If there are any queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The subject baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints. For the crossover study, we will analyze under the assumption that there is no carryover effect due to a short half-life, and therefore a 2-week wash-out period will take place after the period 1. The analysis will be based on the difference of endpoints between period 1 and 2 for each subject. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided). For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's test, one-sample t-test, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker concentrations before and after the open study will be assessed using one-sample t-test. The change of facial pigmented maculae at the start and end of the study that will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum test, one-sample t-test, and McNemar's test, respectively. The difference between acute skin symptoms and the development of skin cancer during the 62 weeks before the start of treatment (Visit 101) and after the start of treatment (Visit 101 – Visit305) will be assessed using the McNemar's test. The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall occurrence, and by treatment. For evaluation of delayed sexual maturation or development, no statistical analysis will be performed and secondary sexual characteristics and blood hormone (prolactin) level in each subject will be individually described.

	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) No additional analyses (e.g., subgroup and adjusted analyses) will be performed.
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) If there is any doubt about the data summarization or analysis, the biostatistician and the study representative will discuss the issue and decide how it will be handled. When missing data are identified, the researcher will contact the subject.
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Monitoring of the study will be performed once three months to ensure that the human rights and welfare of the subjects are being protected, study is conducted safely in accordance with the protocol and applicable regulatory requirements under the Good Clinical Practice, and data are being collected properly. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial An evaluation of the interim results is not planned.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct In our study, an adverse event is defined as any disease, disability, death, or infection that occurs during this study. Adverse event monitoring will begin on first day of period and continue to the last day of the open study. The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor For quality assurance, the study will be examined at 4 times, before the initiation of clinical trial, after the first patient in, before the last patient in and before the completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

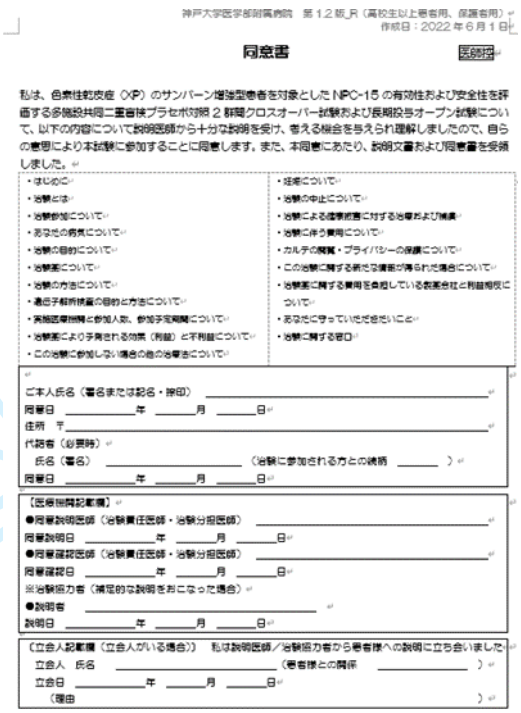
Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any changes required by the ethics committee will be communicated to the participants by the investigators.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute. The protocol was submitted in Japan registry of clinical trial (jRCT).
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Secondary use of the data will occur only if the patients provide written informed consent for additional use of their data.

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), which is an electronic data system for clinical research, to manage the data and protect confidentiality before, during, and after the trial.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site The authors declare that they have no competing interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Only the biostatistician will have access to the final dataset.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation This study is insured for clinical trials, with up to 100 million yen guaranteed for death cases, for example.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions The results of the study will be published in a paper.
	31b	Authorship eligibility guidelines and any intended use of professional writers MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript. Editage (http://www.editage.jp) provided editing of the draft of this manuscript.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol as no datasets were generated. However, the data will be made available from the author on reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates



神戸大学医学部附属病院　第1.2版_R（高校生以上患者用、保護者用）
作成日：2022年6月1日

同意書　　医師用

私は、色素性乾皮症（XP）のサンバーン増強型患者を対象としたNPC-15の有効性および安全性を評価する多施設共同二重盲検プラセボ対照2群間クロスオーバー試験および長期投与オープン試験について、以下の内容について説明医師から十分な説明を受け、考える機会を与えられ理解しましたので、自らの意思により本試験に参加することに同意します。また、本同意にあたり、説明文書および同意書を受領しました。

- はじめに
- 治験とは
- 治験参加について
- あなたの病気について
- 治験の目的について
- 治験薬について
- 治験の方法について
- 遺伝子解析検査の目的と方法について
- 実施医療機関と参加人数、参加予定期間について
- 治験薬により予測される効果（利益）と不利益について
- この治験に参加しない場合の他の治療法について
- 症状について
- 治験の中止について
- 治験による健康被害に対する治療および補償
- 治験に伴う費用について
- カルテの閲覧・プライバシーの保護について
- この治験に関する新たな情報が得られた場合について
- 治験薬に関する費用を負担している製薬会社と利益相反について
- あなたに守っていただきたいこと
- 治験に関する窓口

ご本人氏名（署名または記名・捺印）
同意日　　年　　月　　日
住所　〒
代諾者（必要時）
氏名（署名）　（治験に参加される方との続柄　　）
同意日　　年　　月　　日

【医療機関記載欄】
●同意説明医師（治験責任医師・治験分担医師）
同意説明日　　年　　月　　日
●同意確認医師（治験責任医師・治験分担医師）
同意確認日　　年　　月　　日
※治験協力者（補足的な説明をおこなった場合）
●説明者
説明日　　年　　月　　日

（立会人記載欄（立会人がいる場合））　私は説明医師／治験協力者から患者様への説明に立ち会いました。
立会人　氏名　（患者様との関係　　）
立会日　　年　　月　　日
（理由　　）

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable There are plans for the collection, laboratory evaluation, and storage of biological specimens for molecular analysis in the current trial and for future use in ancillary studies.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study in Japan

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A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study in Japan

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ABSTRACT

Introduction: Xeroderma pigmentosum (XP) is a rare intractable disease without a fundamental treatment, presenting with severe photosensitivity, freckle-like pigmented and depigmented maculae, and numerous skin cancers before the age of 10 years without strict sun protection. About 70% of the patients exhibit extremely severe sunburn reactions and most of them develop neurological symptoms, including sensorineural hearing impairment and progressive peripheral and central nervous disorders beginning from childhood ages. In the preclinical study, we found that N-acetyl-5-methoxytryptamine showed effective to suppress skin tumor development in addition to improvement of auditory brainstem response in chronically UV irradiated XP-A model mice.

Methods and analysis: On the bases of the preclinical study, we conduct a clinical trial on the efficacy of NPC-15 for patients with XP with exaggerated sunburn-reaction type by a multicenter, double-blinded placebo-controlled, two-group crossover study followed by a 52-weeks open study.

Ethics and dissemination: Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board, and the study is conducted in accordance with the approved protocol. All participants will be required to provide written informed consent. Findings will be disseminated through scientific and professional conferences and peer-reviewed journal publications. The datasets generated during the study will be available from the corresponding author upon reasonable request.

Trial registration number: Japan Registry of Clinical Trials (jRCT) identifier: jRCTs051210181. Registered on February 23, 2022.

Strengths and limitations of this study

- ✓ The clinical study is designed as a double-blinded cross-over trial, followed by open study because the number of study patients is limited even in Japan.

- ✓ The randomization and evaluation of the minimum erythema dose, the primary endpoint, and the Efficacy and Safety Evaluation Committee are organized independently of the investigators.
- ✓ The limitations of the study is the duration of the open study, which is not enough for evaluating the new onset of skin cancer, and several genotypes are considered together.

Keywords: Xeroderma Pigmentosum, Minimum Erythema Dose, randomized controlled trial, double-blind, crossover, UV irradiation test, NPC-15

Short title: Trial of NPC-15 in patients with Xeroderma Pigmentosum sunburn enhancement.

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INTRODUCTION

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers in the sun-exposed areas. Without strict sun protection, numerous skin cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such as sensorineural hearing impairment, and progressive peripheral and central nervous disorders beginning around the age of 6 years, have also been observed in approximately 60% of patients [1, 2].

The number of patients medically recorded to have XP in Japan is 300–600. However, the estimated frequency of XP in the Japanese population, which was calculated by the number of patients diagnosed with the eight clinical subtypes of XP (XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-G, and XP-V), is approximately 1:22,000 persons. To elaborate, the percentage of patients with XP-A accounts for about 50% of all XP in Japan, and 90 % of them harbor the homozygous founder mutation of IVS3-1G>C in the *XPA* gene (responsible gene for XP-A), and its carrier ratio was 1:113 in the general population (heterozygous for the founder mutation in the *XPA* gene [3]. This discrepancy in the number of patients estimated (approximately 5600 patients) and those with officially recorded diagnoses was assumed to be because some patients have not yet been diagnosed or are in facilities that limit their access to regular check-ups. Further, as the carrier data are based on archived pathological sections (from 1957-2011) stored at medical facilities in Hiroshima, it is possible that the frequency of carriers may have been higher in the previous era. Regardless, the frequency of XP is higher in Japan than in both Europe and the U.S.A, whereby it is only observed at a rate of 1:1,000,000 people [1, 2].

XP is represented by eight clinical subtypes, seven of which (A-G) are caused by mutations in genes of the Nucleotide Excision Repair (NER) pathway and one (V) is NER proficient but deficient in Translesion Synthesis (TLS) DNA polymerase η. In Japanese XP patients, XP-A accounts for 52.7% of patients, followed by XP-V for 30.8%, XP-D for 7.3%,

XP-F for 4.0%, XP-C for 2.7%, and XP-G for 2.0% of patients [2]. Patients with XP-A exhibit the lowest ability of DNA repair and the most severe cutaneous and neurological symptoms. The ratio of male to female patient incidence of XP is almost 1:1.

NER is the process of repairing DNA lesions caused by ultraviolet radiation (UV), and NER defects, as in XP, result in the accumulation of DNA lesions that lead to carcinogenesis. Whilst the pathogenesis of severe sunburn, pigmentary abnormalities, and concomitant neurological symptoms is still unclear [2], several hypotheses have been postulated that relate to the fact that NER is involved in oxidative DNA lesions such as cyclopurine that accumulate in the neuronal cells of humans [4, 5], and that XP patients show an impaired mitophagy in their neuronal mitochondria due to oxidative stress [6].

Symptoms vary with each group; XP-A, -B, -D, -F, and -G are categorized as exaggerated sunburn reaction types and patients with these groups manifest with severe sunburn with minimum sun exposure, and a prolonged reaction, with the peak at 48-72 hours after UV exposure. In addition, these groups exhibit neurological symptoms to varying degrees. Patients with XP-A show severe photosensitivity from birth, freckle-like pigmented and depigmented maculae after UV exposure to the sun-exposed skin, and the development of skin cancer before the age of 10 years if strict sun protection is not enforced. XP is associated with almost normal development in early childhood, with mild delays in speech and walking usually observed. However, neurological symptoms generally start with sensorineural hearing impairment at the age of 5–6 years, gait imbalance from 6 years, and severe gait disturbance by 20 years. Breathing problems because of laryngeal dystonia, dysphagia, and aspiration occur frequently, and sensorineural hearing impairment and dysarthria cause severe communication challenges in their late teens. Any patients with XP may develop skin cancer in childhood and adolescence if not protected from the sun [7, 8, 9].

Progressive central and peripheral neurological symptoms occur in almost 100% of Japanese patients with XP-A, about 10% of XP-D patients, and some -F and -G patients. The rate of neurological symptoms varies with age, but they appear in almost all XP-A patients in

their mid-teens. There is no fundamental treatment for XP yet, although cutaneous conditions have improved over the last 30 years due to improved education for strict sun protection and early diagnosis and treatment for skin cancers with regular check-ups [2]. However, there is no way to treat or prevent the progression of neurological symptoms, although symptomatic treatments by an interdisciplinary team, such as doctors from dermatology, pediatrics, neurology, otolaryngology, orthopedics, ophthalmology, urology, and dentistry, can assist with education for sun-protection, skin cancer checks, hearing aids, and rehabilitation modalities to prevent secondary complications. The UV-blocking film is strongly recommended to be applied to windows in everyday living spaces at home and school.

The anti-inflammatory drug has shown to reduce UV-induced inflammation as well as UV- induced skin tumor development in XP animal models [10]; XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppressed inflammatory response and oxidative stress after UV irradiation. In addition, it suppressed skin tumor formation, and improved auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiation in XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

Although N-Acetyl-5-methoxytryptamine was first identified as a hormone in the pineal gland by Lerner [11], it is believed to be derived from an antioxidant synthesized in photosynthetic cyanobacteria 3 billion years ago that has been conserved in almost all living species since then, with little change in chemical structure [12]. N-Acetyl-5-methoxytryptamine is responsible for regulating circadian rhythms, and endogenous synthesis and its secretion are regulated by the light/dark cycle, with the highest plasma concentrations at night. In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytryptamine as a free radical scavenger and antioxidant [13]. It is particularly believed to act as an antioxidant within mitochondria [12]. Pharmacologically,

N-Acetyl-5-methoxytryptamine reduces the severity of injury in several disease models due to its effect on acute and chronic inflammation and central nervous system (CNS) protection.

In Japan, Nobelpharma Co. Ltd. submitted a manufacturing and marketing authorization application in April 2019, and a 0.2% granule of N-Acetyl-5-methoxytryptamine was approved in March 2020 for the treatment of sleep difficulties associated with childhood neurodevelopmental disorders. However, since no drugs for XP have been approved and marketed in Japan and overseas, the development of an additional indication was promoted.

Furthermore, it has been shown that level of N-Acetyl-5-methoxytryptamine metabolites is reduced in patients with XP [14], and there is a strong need to establish an early treatment for XP in actual clinical practice.

As this crossover study was to confirm the short-term efficacy of the drug to obtain a drug indication, the investigators decided to target patients with XP with exaggerated sunburn reaction type.

MATERIALS & METHODS

Study design

This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study. The patient flowchart is shown in Figure 1. Since the number of patients with XP is limited and the half-life of NPC-15 is about 3 hours, a 2 × 2 crossover design was adopted. We ensured that the carry over effect would not affect the research results by setting the washout period to 14 days.

Study setting

This study will be performed at Kobe University Hospital and Osaka Medical and Pharmaceutical University Hospital. All study data will be stored and archived in the data center of DOT World using Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), an electronic data system (EDC) for clinical research, to manage the data and protect confidentiality before, during, and after the trial.

Purpose

The purpose of this study is to evaluate the efficacy and safety of orally administered NPC-15 (Nobelpharma, Tokyo, Japan) before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) *per* day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with exaggerated sunburn reaction type XP.

Endpoints

Primary endpoint

The primary endpoint is the minimum erythema dose (MED) 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration. UV irradiation in MED measurement requires special caution in XP, and the following points were considered. Square areas of 1 cm^2 on the participant's back are

irradiated with UVB from 3 mJ/cm^2 to 30 or 60 mJ/cm^2, which are serially dosed up by 3 mJ/cm^2 each session. The upper limit of the irradiation dose is set between 30 and 60 mJ/cm^2, considering the degree of photosensitivity based on the patient's age (skin thickness) and genetic diagnosis. The irradiated area is set so as not to overlap each other for both the left and right back, during crossover period I and II, respectively.

Secondary endpoint

The secondary efficacy endpoints are the following:

(1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (period I and period II) of investigational drug administration.

(2) Evaluation of Melanin index regarding the pigmented area in MED judgment area.

(3) Pigmented maculae inspection (number, area, and color tone) [15].

(4) Neurological symptoms (neurologic severity scale score on XP [16], hearing test, and 5 m gait test).

(5) Presence or absence of onset of acute skin symptoms

(6) Presence or absence of skin cancer

The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Eligibility criteria

Inclusion criteria

Patients will be included in the study when they satisfy all the following criteria:

(1) Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) that have been diagnosed with exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.

(2) Patients aged 1 year or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the patients aged 6 years or older during the crossover study by the Safety Evaluation Committee.

(3) Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

Exclusion criteria

Patients will be excluded from the study when any of the following criteria apply:

(1) Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon

(2) Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.

(3) Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration

(4) Pregnant, lactating women, women who wish to become pregnant during the study period, or women who are fertile and cannot accept an effective contraceptive method.

(5) Patients deemed inappropriate by the investigators for participation in this clinical study

Randomization

All patients who provide consent to participate and fulfill the sampling criteria will be randomized. Patients will be randomly assigned to either the NPC-15-placebo (NP) or Placebo-NPC-15 (PN) group with a 1:1 allocation using the permutation random block method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, Contract Research Organization. The trial participants, care providers, and endpoint

assessors will be blinded. Either the principal or sub-investigator will send a patient enrollment form by EDC to the data center. The staff at the data center will confirm the patient's eligibility and issue the patient enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Data collection and management

Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each patient into the electronic data capture (EDC) system. The principal investigator will confirm that the entered CRF data are complete and correct, electronically sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping. The CRF printout will be retained for audit trail purposes. If there are any queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Monitoring and Audit

Monitoring of the study will be performed to ensure that the human rights and welfare of the patients are being protected, the study is conducted safely in accordance with the protocol and applicable regulatory requirements under the Good Clinical Practice, and data are being collected properly. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring. For quality assurance, the study will be examined four times, before the initiation of the clinical trial, after the first patient in, before the last patient in, and before the completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

Intervention and treatment protocol

The NPC-15 0.2% granules sold by Melatobel™ for pediatrics are manufactured at Nobelpharma Co., Ltd. The placebo formulation is the same except that it does not contain N-acetyl 5-methoxytryptamine.

NP group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. PN group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.

The relationships between the interventions, endpoints, other assessments, and visits for the patients in this study are shown in Table 1 and Table 2.

Table 1. Summary of study assessments and procedures in the crossover period

	Study period														
	Screening period		Crossover period I							Crossover period II					
			Treatment period NPC-15 or Placebo						Washout	Treatment period Placebo or NPC-15					Washout
	Enrollment	Allocation	Day1[1]	Day 15[1]	Day 16[1]	Day 17[1]	Day 18[1]	Day 19[1]	Day 22–35[2]	Day 1[1]	Day 15[1]	Day 16[1]	Day 17[1]	Day 18[1]	Day 19[1]	Day 22–35[2]
Informed	X														

Consent[3]																
Baseline data	X															
Enrollment	X															
Prescription			←	—	—	—	→			←	—	—	—	→		
UV irradiation[4]				X							X					
MED[4]					X	X	X	X				X	X	X	X	
Melanin index										X						
neurological severity scale score[5]	X															
hearing test[6]	X															
5-meter walk test[7]		X														
Acute skin symptom	X [8]															
Skin cancer	X [8]															
urine test for oxidative stress		X						X							X	

marker[9]																
Laboratory test	X							X							X	
Secondary sexual characteristics status[10]	X															
Adverse events			←	—	—	—	—	—	—	—	—	—	—	—	→	
drowsiness and dizziness		X			X							X				
Body weight		X														
Medication status				X	X	X	X	X		X	X	X	X	X	X	
Concomitantly administered medications[11]		←	—	—	—	—	—	—	—	—	—	—	—	—	—	→

1

2 [1] In the case of five or more consecutive holidays, including weekends and national holidays

3 such as New Year's Eve and Golden Week, the allowable range for the treatment period is ±

3 days, and the allowable range for the washout period after the crossover study period I and II is +22/-4, and ± 4 days, respectively.

[2] Allowance (-3~＋21) is based on the point Day 35.

[3] Consent should be obtained within the 12 weeks before drug allocation.

[4] Visit tolerance on the UV test day is ± 2, but evaluation should be made at 24, 48, 72, and 96 hours ± 6 hours after the test day. Re-evaluation is prohibited.

[5] Neurologic severity scale scores will be evaluated in patients 3 years of age or older.

[6] Methods of the hearing test (pure-tone audiometry or conditioned play audiometry) will be recorded in the medical records.

[7] 5-meter walk test will be conducted including the patients who wear braces when the principal/participating investigator deems the patient can tolerate the test. Whether with or without the brace and what kinds of brace they wear will be recorded in the medical records. When the patient wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[8] Data will be collected within 62 weeks before administration of the study drug.

[9] Laboratory urine test: oxidative stress markers (Malondialdehyde, 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine

metabolites (6-sulfatoxymelatonin).

[10] Confirmation of secondary sexual characteristics status and measurement of prolactin levels in blood. To be performed on patients between 10 and 17 years of age.

[11] Four weeks prior to the initiation of the study drug.

Table 2. Summary of study assessments and procedures in the open period

	Open trial				
	Day1	Week 13	Week 26	Week39	Week52
Prescription (NPC-15)	←				→
Melanin index	X				
Facial pigmentation	X				X
Neurologic severity scale score [b]					X
Hearing test [c]					X
5-meter walk test [d]					X
Acute skin symptoms					X
Skin cancer					X
Laboratory test for Research	X		X		X

(urine)[e]					
Laboratory tests (blood and urine) [f]					X
Secondary sexual characteristics status [g]					X
Adverse events	←				→
drowsiness and dizziness	X	X	X	X	X
Body weight	X	X	X	X	
Medication status		X	X	X	X

[a] The study will be conducted only for patients who are deemed by the principal/sub-investigator at Visit 305 (after 52 weeks of the open study), to require a visit to the hospital for evaluation of adverse events, etc.

[b] Neurologic severity scale score will be evaluated in patients of 3 years of age or older.

[c] Methods of the hearing test (pure-tone audiometry or conditioned play audiometry, etc) will be recorded in the medical records.

[d] 5-meter walk test will be conducted including the patients who wear the brace when the principal/participating investigator deems the patient can tolerate the test. Whether with or without the brace and what kinds of brace they wear will be recorded in the medical records.

When the patient wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[e] Urine examination for research use: Oxidative stress markers (Malondialdehyde, 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine metabolites (6-sulfatoxymelatonin).

[f] Urine examination: Urinary protein and urinary urobilinogen.

[g] Confirmation of secondary sexual characteristics status and measurement of prolactin levels in blood. To be performed on patients between 10 and 17 years of age.

Statistical analysis

Analysis set

A summary of the planned statistical analysis for this study is provided below. The final analysis will be performed after data from the patients have been obtained and fixed at the end of the follow-up period.

The full analysis set is the set of randomized patients who receive at least one dose of the study drug. The per-protocol set is the subset of patients in the full analysis set who sufficiently comply with all aspects of the clinical trial protocol, including the drug administration methods and schedule. The safety analysis set is the set of patients who receive at least one dose of the study drug. In this study, the safety analysis set is the same as the full analysis set.

Statistical analysis

Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The patient baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of patients, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints.

For the crossover study, we will analyze under the assumption that there is no carryover effect due to a short half-life, and, therefore, a 2-week wash-out period will take place after the period Ⅰ. The analysis will be based on the difference in endpoints between period Ⅰ and Ⅱ for each patient. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided).

For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's test, one-sample t-test, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker (Malondialdehyde, 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) concentrations before and after the open study will be assessed using a one-sample t-test.

The change of facial pigmented maculae at the start and end of the study, which will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum test, one-sample t-test, and McNemar's test, respectively. McNemar's test will be used to compare the incidence of acute cutaneous symptoms and development of skin cancer during the 62 weeks study period and the 62 weeks before the study.

The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall currency, and by treatment.

For evaluation of delayed sexual maturation or development, no statistical test will be performed and secondary sexual characteristics and blood hormone (prolactin) levels in each patient will be individually described.

Sample size calculation

The primary outcome will be to compare the changes of MED in crossover period I and II between the two groups. MED of XP patients is approximately 5 to 10 times smaller than that of healthy controls. For healthy controls, a difference of 10 mJ/cm^2 in MED is apparently sufficient in the clinical setting. Therefore, we thought the difference of 1-2 mJ/cm^2 should be clinically sufficient for XP patients. If we compare irradiation areas on one side of a child's back, avoiding apparently curved areas, we postulate that 10 areas are the limit. Trying to evaluate all participants with the same irradiation dose difference and considering that 10-areas are limit for children, a 3 mJ/cm^2 difference per area was considered most appropriate. The minimum effect of improved MED should be larger than this irradiation dose difference. It is understandable to assume that the difference in MED between the placebo and actual drug is not as great as the difference in MED between healthy controls and XP patients, in fact, it is possibly much lower. From the above, we estimated the difference in change of MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy controls (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation was assumed to be 6.0 mJ/cm^2, twice the irradiation unit dose for MED examination. Differences in NPC-15 and placebo in a crossover design can be substituted for differences in period between groups. The difference in period in NP group is denoted by [Period I (NPC-15) – Period II

(Placebo)] and the difference in period in PN group by [Period I (Placebo) – Period II (NPC-15)], thus the difference between two groups can be written as {[Period I (NPC-15) – Period II (Placebo)] – [Period I (Placebo) – Period II (NPC-15)] = 2*[(NPC-15)-(Placebo)}. We expected a difference of 4.4 mJ/cm^2 and a standard deviation of 6.0 mJ/cm^2 between the two groups-in MED. Using a one-sided t-test with a significance level of 0.025, we need 8 patients in each group to have at least 80% power. Assuming a few dropouts, a total of 10 patients in each group are required.

Study period

This study is based on the current version of the study protocol (version 1.2, last updated on 1 June 2022). The study was first approved on 24 January 2022 by the institutional review board of Kobe University, Graduate School of Medicine, and authorized by the Pharmaceutical and Medical Devices Agency. Participant recruitment began on 1 April 2022. The expected date of completion (last visit of the last patient) is 15 December 2023.

Patient and public involvement

Patients and the public were not involved in the development of the research questions, selection of endpoint measures, study design, patient recruitment, or conduction of the study. The burden of intervention was assessed by representatives of patient associations participating in the ethical review committee. As mentioned in the individual consent form, participants may obtain access to the final results of the study through the principal investigator.

ETHICS AND DISSEMINATION

The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review

Board and Osaka Medical and Pharmaceutical University Institutional Review Board, and the study is conducted in accordance with the approved protocol.

Written informed consent is obtained from all participants before any study procedure is performed. The participants (or their caregivers/guardians) will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. For participants under 18 years of age, or individuals over 20 years of age who are unable to consent due to intellectual disability, consent is obtained from the substitute/caregivers/guardians. The protocol was submitted to the Japan registry of clinical trial (jRCT).

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Contributors

MT obtained the grant funding and drafted the manuscript. RO, TF, and TU obtained the grant funding and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

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The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. The funding agency can be contacted at the following e-mail address: rare-koubo@amed.go.jp.

Competing interests

Nobelpharma Co., Ltd. provided the NPC-15 and placebo during the study period; however, the company had no role in this clinical trial. All authors have no conflicts of interest to declare.

Patient consent for publication

Not applicable.

Provenance and peer review

Not commissioned; externally peer-reviewed.

Data availability statement

The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol, as no datasets were generated. However, the data will be made available from the author upon reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).

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FIGURE CAPTIONS

Table 1. Summary of study assessments and procedures in the crossover period

Table 2. Summary of study assessments and procedures in the open period

Figure 1. Flow chart of participants

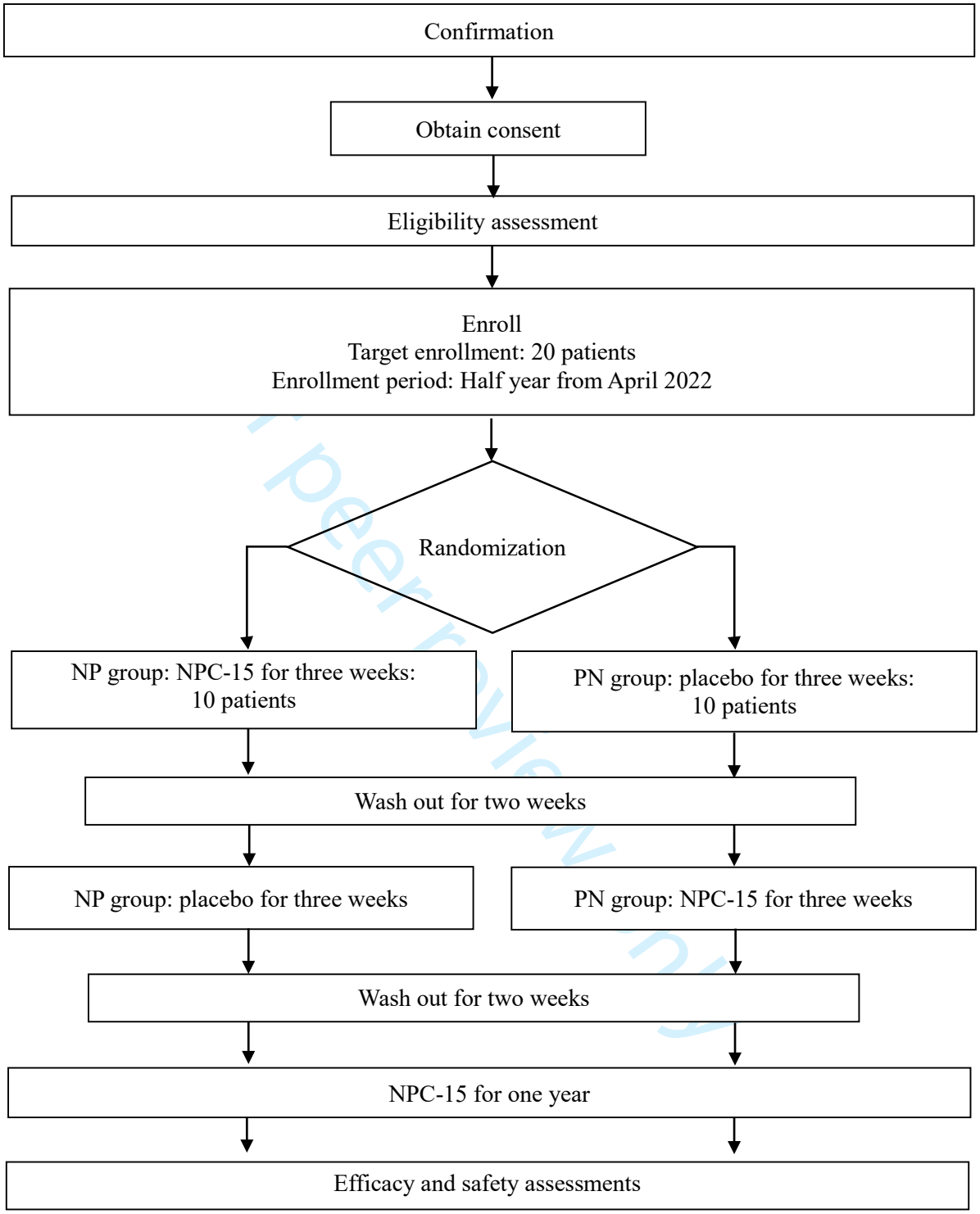

Figure 1. Flowchart of participants.

NP: NPC-15 to placebo, PN: placebo to NPC-15.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym A multicenter, double-blind, placebo-controlled, two-group crossover study and a long-term open study evaluating the efficacy and safety of NPC-15 in patients with xeroderma pigmentosum (XP) sunburn enhancement. (XP-1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinical Trials: Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 23 Feb 2022.
	2b	All items from the World Health Organization Trial Registration Data Set This information is available at the Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 17 Feb 2022. (https://jrct.niph.go.jp/en-latest-detail/jRCT2051210181)
Protocol version	3	Date and version identifier Version 1.2, last updated on 1 June 2022
Funding	4	Sources and types of financial, material, and other support This work is supported by the Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

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MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

	5b	Name and contact information for the trial sponsor

The Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Contact information:

The funding agency can be contacted at the following web address: https://www.amed.go.jp/en/aboutus/index.html

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
A coordinating centre, steering committee, endpoint adjudication committee, and other individuals and groups are not participating in the composition of the trial and have no roles or responsibilities in the trial.

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers at sun-exposed areas. Without strict sun protection, numerous skin cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such as sensorineural hearing impairment, and progressive peripheral and central nervous disorders beginning around the age of 6 years, have also been observed in approximately 60% of patients.

The anti-inflammatory drug has shown to reduce the UV induced inflammation as well as UV-induced skin tumor development in XP animal models, XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppressed inflammatory response and oxidative stress after UV irradiation. In addition, it suppressed skin tumor formation, and improvement of auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiated XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytyptamine as a free radical scavenger and antioxidant. It is particularly believed to act as an antioxidant within mitochondria. Pharmacologically, N-Acetyl-5-methoxytyptamine reduces the severity of injury in several disease models due to its effect on acute and chronic inflammation and CNS protection.

Considering that anti-inflammatory drug reduced the UV-induced inflammation as well as UV-induced skin tumor development in the XP animal model. Furthermore, it has been shown that level of N-Acetyl-5-methoxytyptamine metabolites is reduced in patients with XP, and there is a strong need to establish an early treatment for XP in actual clinical practice.

	6b	Explanation for choice of comparators The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type.
Objectives	7	Specific objectives or hypotheses The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type. We hypothesized that NPC-15 could contribute to mitigate sunburn reactions and slow down the progress of neurological symptoms in patients with XP exaggerated sunburn reaction type.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study.
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained This study will be performed at Kobe University Hospital, Kobe, and Osaka Medical and Pharmaceutical University, Osaka, Japan.

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)

Inclusion Criteria

- ✓ Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) that have been diagnosed with Exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
- ✓ Patients aged 1 year old or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the subjects aged 6 years or older during the crossover study by the Safety Evaluation Committee.
- ✓ Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

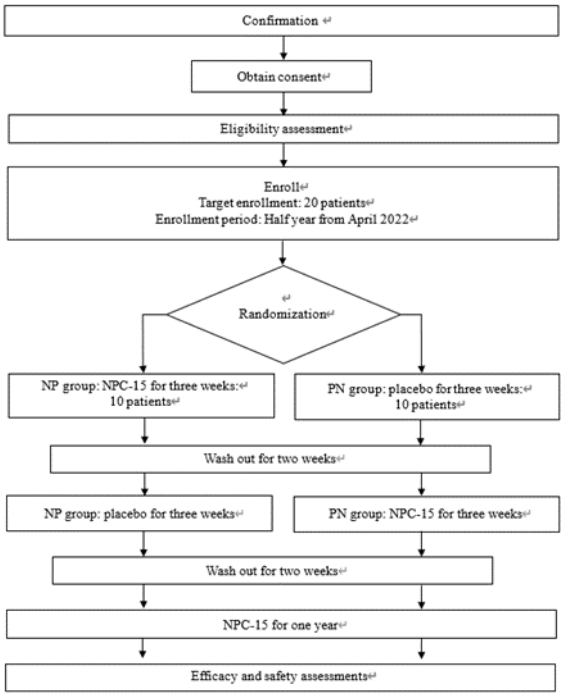
Exclusion Criteria

- ✓ Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon.
- ✓ Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.
- ✓ Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration.
- ✓ Patients who are pregnant or may become pregnant.
- ✓ Patients judged by the investigator to be ineligible for this study.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (1) NPC-15-Placebo (NP) group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. (2) Placebo-NPC-15 (PN) group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) The patients will return the empty medicine pouches at the end of the treatment period.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Concomitant administration of N-acetyl-5-methoxytryptamine (including health foods containing it as a major ingredient) and Fluvoxamine maleate (Luvox, Depmerol, etc.) is prohibited from the date of initiation to the date of termination of administration of the investigational drug.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended The primary endpoint is the Minimum Erythema Dose (MED) 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration. The secondary efficacy endpoints are the following: (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (stage I and stage II) of investigational drug administration. (2) Evaluation of Melanin index regarding the pigmented area in MED judgment area. (3) Pigmented maculae inspection (number, area, and color tone)[15]. (4) Neurological symptoms (neurologic severity scale score on XP[16], hearing test, and 5 m gait test). (5) Presence or absence of onset of acute skin symptoms (6) Presence or absence of skin cancer The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations The total target number of subjects is 20, with 10 subjects in both the NP and PN groups. We estimated the difference in MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation of the difference between period 1 and 2 was conservatively assumed to be 7.0 mJ/cm^2 because the estimated time point (24 hours) and the time of the main endpoint (72 hours) are different. In the 2 × 2 crossover design of this study, when we expect a difference of 4.4 mJ/cm^2 between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm^2, we would need 8 cases to maintain a significance level of 2.5% (one-sided) and a total of 16 cases to achieve a power of 80%.

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size All subjects who provide consent to participate and who fulfil the inclusion criteria and who do not meet any of the exclusion criteria will be randomized.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions All subjects who provide consent to participate and fulfill the sampling criteria will be randomized. Subjects will be randomly assigned to either the NP group or the PN group with a 1:1 allocation using the permutation random block method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. The trial participants, care providers, and outcome assessors will be blinded.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how The trial participants, care providers, and endpoint assessors will be blinded.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Because this is a crossover study in which both groups receive the actual drug, an unblinding procedure will not be incorporated into the study.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol. Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Data from participants who discontinue their participation in the study or who deviate from the protocol will be included in the FAS analysis. All data acquired during the study period will be analyzed.

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each subject into the electronic data capture (EDC) system. The principal investigator will confirm that the entered CRF data are complete and correct, electronically sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping. The CRF printout will be retained for audit trail purposes. If there are any queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The subject baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints. For the crossover study, we will analyze under the assumption that there is no carryover effect due to a short half-life, and therefore a 2-week wash-out period will take place after the period 1. The analysis will be based on the difference of endpoints between period 1 and 2 for each subject. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided). For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's test, one-sample t-test, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker concentrations before and after the open study will be assessed using one-sample t-test. The change of facial pigmented maculae at the start and end of the study that will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum test, one-sample t-test, and McNemar's test, respectively. The difference between acute skin symptoms and the development of skin cancer during the 62 weeks before the start of treatment (Visit 101) and after the start of treatment (Visit 101 – Visit305) will be assessed using the McNemar's test. The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall occurrence, and by treatment. For evaluation of delayed sexual maturation or development, no statistical analysis will be performed and secondary sexual characteristics and blood hormone (prolactin) level in each subject will be individually described.

	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) No additional analyses (e.g., subgroup and adjusted analyses) will be performed.
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) If there is any doubt about the data summarization or analysis, the biostatistician and the study representative will discuss the issue and decide how it will be handled. When missing data are identified, the researcher will contact the subject.
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Monitoring of the study will be performed once three months to ensure that the human rights and welfare of the subjects are being protected, study is conducted safely in accordance with the protocol and applicable regulatory requirements under the Good Clinical Practice, and data are being collected properly. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial An evaluation of the interim results is not planned.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct In our study, an adverse event is defined as any disease, disability, death, or infection that occurs during this study. Adverse event monitoring will begin on first day of period and continue to the last day of the open study. The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor For quality assurance, the study will be examined at 4 times, before the initiation of clinical trial, after the first patient in, before the last patient in and before the completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

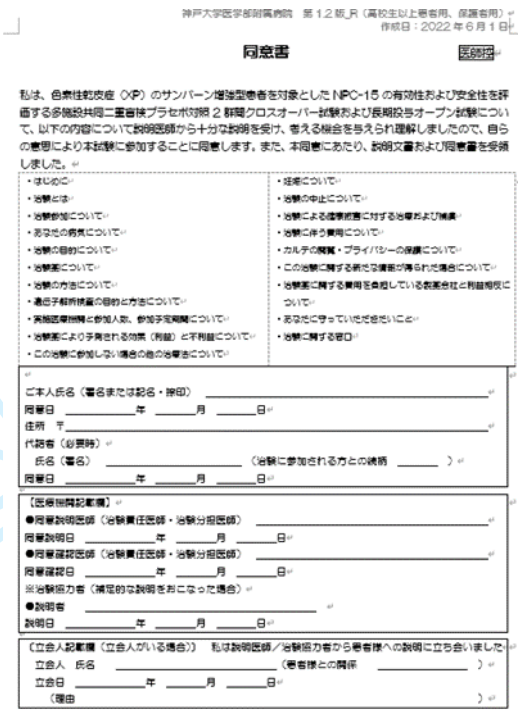
Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any changes required by the ethics committee will be communicated to the participants by the investigators.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute. The protocol was submitted in Japan registry of clinical trial (jRCT).
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Secondary use of the data will occur only if the patients provide written informed consent for additional use of their data.

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), which is an electronic data system for clinical research, to manage the data and protect confidentiality before, during, and after the trial.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site The authors declare that they have no competing interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Only the biostatistician will have access to the final dataset.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation This study is insured for clinical trials, with up to 100 million yen guaranteed for death cases, for example.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions The results of the study will be published in a paper.
	31b	Authorship eligibility guidelines and any intended use of professional writers MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript. Editage (http://www.editage.jp) provided editing of the draft of this manuscript.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol as no datasets were generated. However, the data will be made available from the author on reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates



神戸大学医学部附属病院　第1.2版_R（高校生以上患者用、保護者用）
作成日：2022年6月1日

同意書　　医師用

私は、色素性乾皮症（XP）のサンバーン増強型患者を対象としたNPC-15の有効性および安全性を評価する多施設共同二重盲検プラセボ対照2群間クロスオーバー試験および長期投与オープン試験について、以下の内容について説明医師から十分な説明を受け、考える機会を与えられ理解しましたので、自らの意思により本試験に参加することに同意します。また、本同意にあたり、説明文書および同意書を受領しました。

- はじめに
- 治験とは
- 治験参加について
- あなたの病気について
- 治験の目的について
- 治験薬について
- 治験の方法について
- 遺伝子解析検査の目的と方法について
- 実施医療機関と参加人数、参加予定期間について
- 治験薬により予測される効果（利益）と不利益について
- この治験に参加しない場合の他の治療法について
- 症状について
- 治験の中止について
- 治験による健康被害に対する治療および補償
- 治験に伴う費用について
- カルテの閲覧・プライバシーの保護について
- この治験に関する新たな情報が得られた場合について
- 治験薬に関する費用を負担している製薬会社と利益相反について
- あなたに守っていただきたいこと
- 治験に関する窓口

ご本人氏名（署名または記名・捺印）＿＿＿＿＿＿＿＿＿＿
同意日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日
住所 〒＿＿＿＿＿＿＿＿＿＿
代諾者（必要時）
氏名（署名） ＿＿＿＿＿＿＿＿（治験に参加される方との続柄 ＿＿＿ ）
同意日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日

【医療機関記載欄】
●同意説明医師（治験責任医師・治験分担医師） ＿＿＿＿＿＿＿＿＿＿
同意説明日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日
●同意確認医師（治験責任医師・治験分担医師） ＿＿＿＿＿＿＿＿＿＿
同意確認日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日
※治験協力者（補足的な説明をおこなった場合）
●説明者 ＿＿＿＿＿＿＿＿＿＿
説明日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日

（立会人記載欄（立会人がいる場合）） 私は説明医師／治験協力者から患者様への説明に立ち会いました。
立会人 氏名 ＿＿＿＿＿＿＿＿＿＿（患者様との関係 ＿＿＿＿＿＿ ）
立会日 ＿＿＿＿年 ＿＿＿月 ＿＿＿日
（理由 ）

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable There are plans for the collection, laboratory evaluation, and storage of biological specimens for molecular analysis in the current trial and for future use in ancillary studies.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study in Japan

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A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter, Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open Study in Japan

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ABSTRACT

Introduction: Xeroderma pigmentosum (XP) is a rare intractable disease without a fundamental treatment, presenting with severe photosensitivity, freckle-like pigmented and depigmented maculae, and numerous skin cancers before the age of 10 years without strict sun protection. About 70% of the patients exhibit extremely severe sunburn reactions and most of them develop neurological symptoms, including sensorineural hearing impairment and progressive peripheral and central nervous disorders beginning from childhood ages. In the preclinical study, we found that N-acetyl-5-methoxytryptamine was effective in suppressing skin tumor development in addition to improvement of auditory brainstem response in chronically UV irradiated XP-A model mice.

Methods and analysis: On the bases of the preclinical study, we conduct a clinical trial on the efficacy of NPC-15 for patients with XP with exaggerated sunburn-reaction type by a multicenter, double-blinded placebo-controlled, two-group crossover study followed by a 52-weeks open study.

Ethics and dissemination: Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board, and the study is conducted in accordance with the approved protocol. All participants will be required to provide written informed consent. Findings will be disseminated through scientific and professional conferences and peer-reviewed journal publications. The datasets generated during the study will be available from the corresponding author upon reasonable request.

Trial registration number: Japan Registry of Clinical Trials (jRCT) identifier: jRCTs051210181. Registered on February 23, 2022.

Strengths and limitations of this study

- ✓ The clinical study is designed as a double-blinded cross-over trial, followed by open study because the number of study patients is limited even in Japan.

- ✓ The randomization and evaluation of the minimum erythema dose, and the primary endpoint, along with the Efficacy and Safety Evaluation Committee are organized as independent of the investigators.
- ✓ The limitations of the study include the duration of the open study, which is not enough for evaluating the new onset of skin cancer, and several genotypes are considered together.

Keywords: Xeroderma Pigmentosum, Minimum Erythema Dose, randomized controlled trial, double-blind, crossover, UV irradiation test, NPC-15

Short title: Trial of NPC-15 in patients with Xeroderma Pigmentosum sunburn enhancement.

Word count: 3998 words excluding title page, abstract, references, figures, and tables

INTRODUCTION

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers in sun-exposed areas. Numerous skin cancers can develop before the age of 10 years without strict sun protection. Furthermore, neurological symptoms, such as sensorineural hearing impairment and progressive peripheral and central nervous disorders beginning around 6 years of age, have also been observed in approximately 60% patients [1, 2].

The number of patients medically recorded to have XP in Japan is 300–600. However, estimated frequency of XP in the Japanese population, calculated by the number of patients diagnosed with the eight clinical subtypes of XP (XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-G, and XP-V), is approximately 1:22,000 persons. To elaborate, the percentage of patients with XP-A accounts for about 50% of all XP in Japan, and 90% of them harbor the homozygous founder mutation of IVS3-1G>C in the *XPA* gene (responsible gene for XP-A); its carrier (heterozygote) ratio was 1:113 in the general population (heterozygous for the founder mutation in the *XPA* gene [3]. This discrepancy in the estimated number of patients (approximately 5600 patients) and those with officially recorded diagnoses was assumed to be because some patients were undiagnosed or were in facilities that limited their access to regular check-ups. Further, as the carrier data are based on archived pathological sections (from 1957-2011) stored at medical facilities in Hiroshima, it is possible that the frequency of carriers may have been higher in the previous era. Regardless, the frequency of XP is higher in Japan than in both Europe and the U.S.A, whereby it is only observed at a rate of 1:1,000,000 people [1, 2].

XP is represented by eight clinical subtypes, seven of which (A-G) are caused by mutations in genes of the Nucleotide Excision Repair (NER) pathway and one subtype (V) is NER proficient but deficient in Translesion Synthesis (TLS) DNA polymerase η. Among Japanese XP patients, XP-A accounts for 52.7% of the patients, followed by XP-V for 30.8%,

XP-D for 7.3%, XP-F for 4.0%, XP-C for 2.7%, and XP-G for 2.0% of patients [2]. Symptoms vary among groups. Patients with XP-A exhibit the lowest ability of DNA repair and the most severe cutaneous and neurological symptoms. The ratio of male to female patient incidence of XP is almost 1:1.

NER is the process of repairing DNA lesions caused by ultraviolet radiation (UV), and NER defects, as in XP, result in the accumulation of DNA lesions that lead to carcinogenesis. Whilst the pathogenesis of severe sunburn, pigmentary abnormalities, and concomitant neurological symptoms is still unclear [2], several hypotheses have been postulated; NER is involved in oxidative DNA lesions such as cyclopurine that accumulate in the neuronal cells of humans [4, 5], and XP patients show an impaired mitophagy in their neuronal mitochondria due to oxidative stress [6].

XP-A, -B, -D, -F, and -G are categorized as exaggerated sunburn reaction types and patients with these subtypes manifest severe sunburn with minimum sun exposure and a prolonged reaction, with the erythema peak at 48-72 hours after UV exposure. In addition, these subtypes exhibit neurological symptoms to varying degrees. Patients with XP-A show severe photosensitivity from birth, freckle-like pigmented and depigmented maculae after UV exposure to the sun-exposed skin, and development of skin cancer before the age of 10 years, if strict sun protection is not enforced. XP is associated with almost normal development in early childhood, with a usual observation of mild delays in speech and walking. However, neurological symptoms generally start with sensorineural hearing impairment at the age of 5–6 years, gait imbalance from 6 years, and severe gait disturbance by 20 years. Breathing problems due to laryngeal dystonia, dysphagia, and aspiration occur frequently, and sensorineural hearing impairment and dysarthria cause severe communication challenges in the late teens. Patients with XP may develop skin cancer in childhood and adolescence, if not protected from the sun [7, 8, 9].

Progressive central and peripheral neurological symptoms occur in almost all Japanese patients with XP-A, about 10% of XP-D patients, and some -F and -G patients. The

rate of neurological symptoms varies with age; however, they appear in almost all XP-A patients in their mid-teens. There is no fundamental treatment for XP yet; although cutaneous conditions have improved over the last 30 years due to improved education for strict sun protection and early diagnosis and treatment for skin cancers with regular check-ups [2]. However, treatment or prevention of the progression of neurological symptoms is not possible. Symptomatic treatments by an interdisciplinary team, such as doctors from dermatology, pediatrics, neurology, otolaryngology, orthopedics, ophthalmology, urology, and dentistry, can assist with education for sun-protection, skin cancer checks, hearing aids, and rehabilitation modalities to prevent secondary complications. Application of a UV-blocking film on windows in living spaces at home and schools is strongly recommended.

Anti-inflammatory drugs reduce UV-induced inflammation and skin tumor development in XP animal models [10]; XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppresses inflammatory response and oxidative stress after UV irradiation. In addition, it suppresses skin tumor formation and improves auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiation in XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

Although N-Acetyl-5-methoxytryptamine was first identified as a hormone in the pineal gland by Lerner [11], it is believed to have been derived from an antioxidant synthesized in photosynthetic cyanobacteria, 3 billion years ago, and have been conserved in almost all living species since then, with little change in its chemical structure [12]. N-Acetyl-5-methoxytryptamine is responsible for regulating circadian rhythms and endogenous synthesis, and its secretion is regulated by the light/dark cycle, with the highest plasma concentrations at night. In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytryptamine as a free radical scavenger and antioxidant [13]. It is

particularly believed to act as an antioxidant within mitochondria [12]. Pharmacologically, N-Acetyl-5-methoxytryptamine reduces the severity of injury in several disease models due to its effect on acute and chronic inflammation and central nervous system (CNS) protection.

In Japan, Nobelpharma Co. Ltd. submitted a manufacturing and marketing authorization application in April 2019, and a 0.2% granule of N-Acetyl-5-methoxytryptamine was approved in March 2020 for the treatment of sleep difficulties associated with childhood neurodevelopmental disorders. However, as no drugs for XP have been approved and marketed in Japan and overseas, the development of an additional indication was promoted.

Furthermore, it has been shown that the level of N-Acetyl-5-methoxytryptamine metabolites is reduced in patients with XP [14], and there is a strong need to establish an early treatment for XP in actual clinical practice.

As this crossover study is to confirm the short-term efficacy of the drug to obtain a drug indication, the investigators have decided to target patients with XP with exaggerated sunburn reaction type.

In this trial, setting the primary endpoint will be difficult. An evaluation of the efficacy of the trial drug based on the progression level of neurological symptoms is desired. As the progression of neurological symptoms is gradual over the years, it is practically difficult to conduct a 10-year-clinical trial on this disease. Furthermore, neurological symptoms differ with age, making the evaluation difficult. On the other hand, exaggerated and prolonged sunburn response is a lifelong symptom that is always and exclusively observed in patients with exaggerated sunburn type XP. Additionally, neurological symptoms are also observed in this type of XP. Furthermore, in our preclinical study, both cutaneous and neurologic symptoms, such as ABR were ameliorated. Therefore, we theoretically considered that setting the primary endpoint as the improvement of minimum erythema dose (MED) is the best and most reliable (can be quantitatively evaluated) method for evaluation of drug efficacy in XP. However, irradiation of XP patients with UV

significantly increases the risk of skin cancers; therefore, patients with XP are educated to strictly avoid UV exposure. It has an ethical issue. After careful deliberation, we decided to set the primary endpoint as the MED at 72 hours to minimize UV exposure dose.

MATERIALS & METHODS

Study design

This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study. The patient flowchart is shown in Figure 1. As the number of patients with XP is limited and the half-life of NPC-15 is about 3 hours, a 2 × 2 crossover design was adopted. We ensured that the carry over effect would not affect the research results by setting the washout period to 14 days.

Study setting

This study will be performed at Kobe University Hospital and Osaka Medical and Pharmaceutical University Hospital. All study data will be stored and archived in the data center of DOT World using Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), an electronic data capture (EDC) system for clinical research, to manage the data and protect confidentiality before, during, and after the trial.

Purpose

The purpose of this study is to evaluate the efficacy and safety of orally administered NPC-15 (Nobelpharma, Tokyo, Japan) before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) *per* day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with exaggerated sunburn reaction type XP.

Endpoints

Primary endpoint

The primary endpoint is the MED, 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration. A UV-irradiated examination is a burdensome examination for patients with XP; therefore, determination of the study's primary endpoint was done after sufficient discussion, as

mentioned in the Introduction section. The "MED" is defined as the minimum dose that elicits very faint, but discernible erythema (NOT USUAL SUNBURN). Usually, MED is measured 24 hours after UV irradiation. However, delay of the erythematous peak is a characteristic of exaggerated sunburn type XP. In this clinical trial, MED at 72 hours will be evaluated; therefore, the irradiation dose can be minimized. The upper limit of the irradiation dose will be set between 30 and 60 mJ/cm^2, considering the degree of photosensitivity based on the patient's age (skin thickness) and genetic diagnosis. The irradiated area will be set in a way such that it does not overlap for both the left and right back, during crossover period I and II, respectively.

Secondary endpoint

The secondary efficacy endpoints will be the following:

(1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (period I and period II) of investigational drug administration.

(2) Evaluation of melanin index regarding the pigmented area in MED judgment area.

(3) Pigmented maculae inspection (number, area, and color tone) [15].

(4) Neurological symptoms (neurologic severity scale score on XP [16], hearing test, and 5 m gait test).

(5) Presence or absence of onset of acute skin symptoms.

(6) Presence or absence of skin cancer.

The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Eligibility criteria

Inclusion criteria

Patients who meet the following criteria will be included in the study:

(1) Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) who have been diagnosed with exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.

(2) Patients aged 1 year or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the patients aged 6 years or older during the crossover study by the Safety Evaluation Committee.

(3) Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

Exclusion criteria

Patients with the following criteria will be excluded from the study:

(1) Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon.

(2) Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.

(3) Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration.

(4) Pregnant, lactating women, women who wish to become pregnant during the study period, or women who are fertile and cannot accept an effective contraceptive method.

(5) Patients deemed inappropriate by the investigators for participation in this clinical study

Randomization

All patients who provide consent to participate and fulfill the sampling criteria will be randomized. Patients will be randomly assigned to either the NPC-15-placebo (NP) or Placebo-NPC-15 (PN) group with a 1:1 allocation using the permutation random block

method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, Contract Research Organization. The trial participants, care providers, and endpoint assessors will be blinded. Either the principal or sub-investigator will send a patient enrollment form by EDC to the data center. The staff at the data center will confirm the patient's eligibility and issue the patient enrollment confirmation form containing the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Data collection and management

Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each patient into the EDC system. The principal investigator will confirm the completion and correctness of entered CRF data, electronically sign the CRF in Viedoc™, and make a printout of the signed CRF for maintaining records. The CRF printout will be retained for audit trail purposes. In case of queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Monitoring and Audit

The study will be monitored to ensure protection of the human rights and welfare of the patients, safe progression of study in accordance with the protocol, and proper collection of applicable regulatory requirements under Good Clinical Practice, and data. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring. For quality assurance, the study will be examined four times, i.e., before

initiation of the clinical trial, after the first patient in, before the last patient in, and before completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

Intervention and treatment protocol

The NPC-15 0.2% granules sold by Melatobel™ for pediatrics are manufactured at Nobelpharma Co., Ltd. The placebo formulation is the same except that it does not contain N-acetyl 5-methoxytryptamine.

NP group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. PN group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.

The relationships between the interventions, endpoints, other assessments, and visits for the patients in this study are shown in Supplementary Table 1 and Supplementary Table 2.

Statistical analysis

Analysis set

A summary of the planned statistical analysis for this study is provided below. The final analysis will be performed after data from the patients have been obtained and fixed at the end of the follow-up period.

The full analysis set is the set of randomized patients who receive at least one dose of the study drug. The per-protocol set is the subset of patients in the full analysis set who sufficiently comply with all aspects of the clinical trial protocol, including the drug administration methods and schedule. The safety analysis set is the set of patients who receive at least one dose of the study drug. In this study, the safety analysis set is the same as the full analysis set.

Statistical analysis

Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The patient baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of patients, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints.

For the crossover study, we will conduct the analysis under the assumption that there is no carryover effect due to a short half-life; therefore, a two-week wash-out period will take place after period Ⅰ. The analysis will be based on the difference in endpoints between period Ⅰ and Ⅱ for each patient. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided).

For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's, one-sample t-, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker (malondialdehyde, 8-hydroxy-2-deoxyguanosine, hexanoyl-Lys) concentrations before and after the open study will be assessed using a one-sample t-test.

The change of facial pigmented maculae at the start and end of the study, which will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum, one-sample t-, and McNemar's test, respectively. McNemar's test will be used to compare the incidence of acute cutaneous symptoms and development of skin cancer during the 62 weeks study period and 62 weeks before the study.

The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall currency, and by treatment.

For evaluation of delayed sexual maturation or development, no statistical test will be performed and secondary sexual characteristics and blood hormone (prolactin) levels in each patient will be individually described.

Sample size calculation

The primary outcome will be to compare the changes of MED in crossover period I and II between the two groups. MED of XP patients is approximately 5 to 10 times smaller than that of healthy controls. For healthy controls, a difference of 10 mJ/cm^2 in MED is apparently sufficient in the clinical setting. Therefore, we thought the difference of 1-2 mJ/cm^2 should be clinically sufficient for XP patients. If we compare irradiation areas on one side of a child's back, avoiding apparently curved areas, we postulate that 10 areas are the limit. Trying to evaluate all participants with the same irradiation dose difference and considering that 10-areas are limit for children, a 3 mJ/cm^2 difference per area was considered most appropriate. The minimum effect of improved MED should be larger than this irradiation dose difference. It is reasonable to assume that the difference in MED between the placebo and actual drug is not as great as the difference in MED between healthy participants and XP patients; in fact, it is possibly much lower. From the above observation, we estimated the difference in change of MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy participants (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation was assumed to be 6.0 mJ/cm^2, twice the irradiation unit dose for MED examination. Differences in NPC-15 and placebo in a crossover design can be substituted for differences in period between groups. The difference in period in NP group is denoted by

[Period I (NPC-15) – Period II (Placebo)] and the difference in period in PN group by [Period I (Placebo) – Period II (NPC-15)]. Thus, the difference between the two groups can be written as {[Period I (NPC-15) – Period II (Placebo)] – [Period I (Placebo) – Period II (NPC-15)] = 2*[(NPC-15)-(Placebo)}. We expected a difference of 4.4 mJ/cm^2 and a standard deviation of 6.0 mJ/cm^2 between the two groups-in MED. Using a one-sided t-test with a significance level of 0.025, we need 8 patients in each group to have at least 80% power. Assuming a few dropouts, a total of 10 patients in each group are required.

Study period

This study is based on the current version of the study protocol (version 1.2, last updated on 1 June 2022). The study was first approved on 24 January 2022 by the institutional review board of Kobe University, Graduate School of Medicine, and authorized by the Pharmaceutical and Medical Devices Agency. Participant recruitment began on 1 April 2022. The expected date of completion (last visit of the last patient) is 15 December 2023.

Patient and public involvement

Patients and the public were not involved in the development of the research questions, selection of endpoint measures, study design, patient recruitment, or conduction of the study. The burden of intervention was assessed by representatives of patient associations participating in the ethical review committee. As mentioned in the individual consent form, participants may obtain access to the final results of the study through the principal investigator.

ETHICS AND DISSEMINATION

The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review

Board (210040) and Osaka Medical and Pharmaceutical University Institutional Review Board, and the study is conducted in accordance with the approved protocol.

As described in the Introduction section, measurement of MED involves unavoidable ethical issues. We explained this point, along with the following points to the participants (or their caregivers/guardians): (1) difficulties other than the MED measurement in assessing the efficacy of XP therapeutics over a certain period of time; (2) that the dose of UV irradiation in this clinical trial was set lower than that of the UV examination conducted at the time of diagnosis; and (3) that we considered the possibility of developing cancer with MED measurements in this trial to be sufficiently low. Before genetic diagnosis became mainstream, at least 50 XP patients with exaggerated sunburn-reaction type underwent more than 60 mJ/cm^2 UV irradiation on their back for diagnostic purposes in our hospital; however, none of them developed skin cancer on the tested sites, where patients are rarely exposed to the sun.

Written informed consent is obtained from all participants before any study procedure is performed. The participants (or their caregivers/guardians) will have the opportunity to review the participant consent form and provide acknowledgement of complete understanding of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual delegated to this task by the principal investigator. For participants under 18 years of age, or individuals over 20 years of age who are unable to provide consent due to intellectual disability, consent is obtained from the substitute/caregivers/guardians. The protocol was submitted to the Japan registry of clinical trial (jRCT).

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7) Japanese Red Cross Hyogo Blood Center

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Contributors

MT obtained the grant funding and drafted the manuscript. RO, TF, and TU obtained the grant funding and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

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Labor and Welfare under Grant number 20FC1043(CN, TU).

The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. The funding agency can be contacted at the following e-mail address: rare-koubo@amed.go.jp.

Competing interests

Nobelpharma Co., Ltd. provided the NPC-15 and placebo during the study period; however, the company had no role in this clinical trial. All authors have no conflicts of interest to declare.

Patient consent for publication

Not applicable.

Provenance and peer review

Not commissioned; externally peer-reviewed.

Data availability statement

The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol, as no datasets were generated. However, the data will be made available from the author upon reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).

Open access

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FIGURE CAPTIONS

Supplementary Table 1. Summary of study assessments and procedures in the crossover period

Supplementary Table 2. Summary of study assessments and procedures in the open period

Figure 1. Flow chart of participants

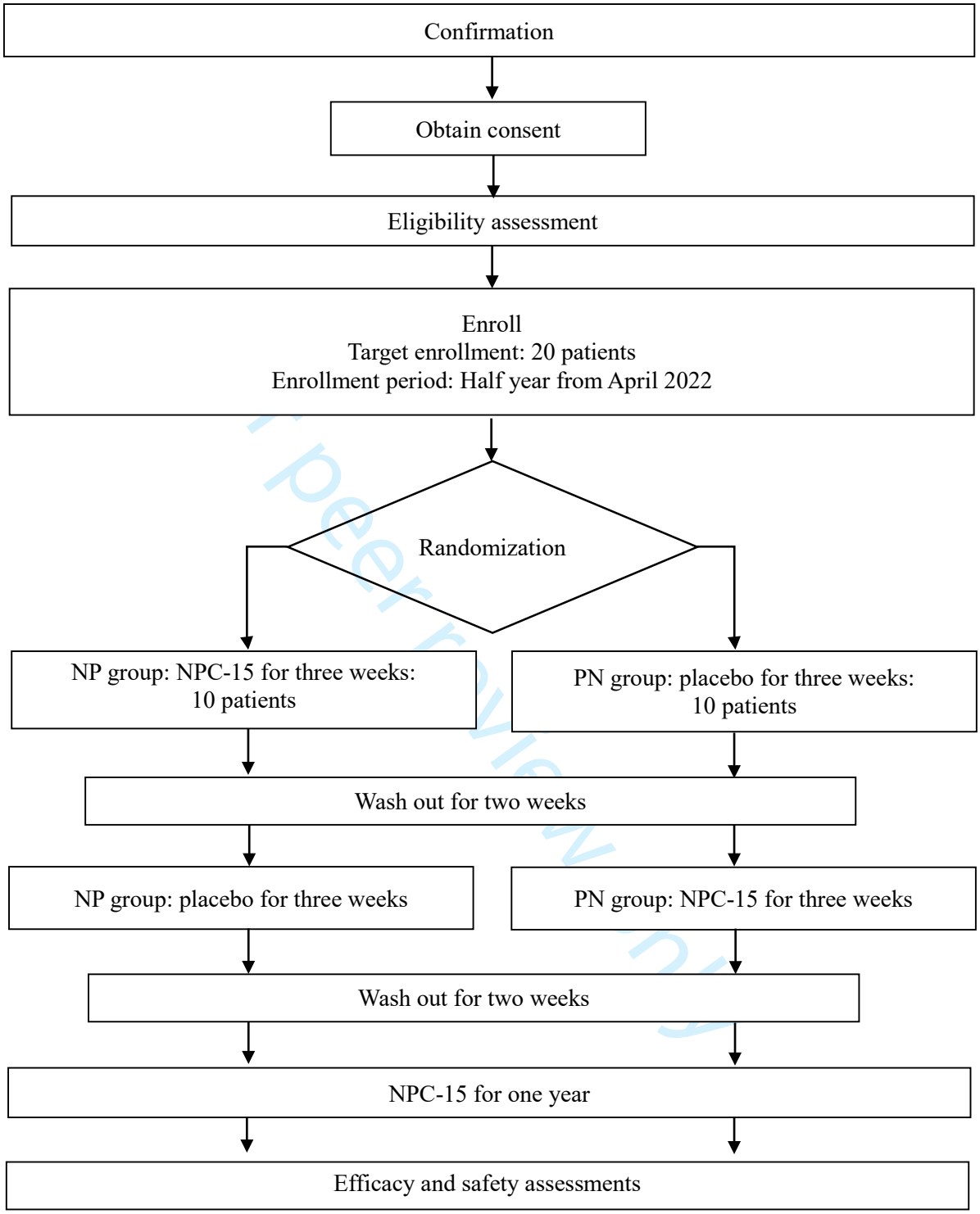

Figure 1. Flowchart of participants.

NP: NPC-15 to placebo, PN: placebo to NPC-15.

Table 1. Summary of study assessments and procedures in the crossover period

	Study period													
	Screening period		Crossover period I							Crossover period II						
			Treatment period NPC-15 or Placebo						Washout	Treatment period Placebo or NPC-15						Washout
	Enrollment	Allocation	Day1[1]	Day 15[1]	Day 16[1]	Day 17[1]	Day 18[1]	Day 19[1]	Day 22–35[2]	Day 1[1]	Day 15[1]	Day 16[1]	Day 17[1]	Day 18[1]	Day 19[1]	Day 22–35[2]
Informed Consent[3]	X															
Baseline data	X															
Enrollment	X															
Prescription			←	—	—	—	—	→			←	—	—	—	→	
UV irradiation[4]				X							X					
MED[4]					X	X	X	X				X	X	X	X	
Melanin index										X						

neurological severity scale score[5]	X															
hearing test[6]	X															
5-meter walk test[7]		X														
Acute skin symptom	X [8]															
Skin cancer	X [8]															
urine test for oxidative stress marker[9]		X						X							X	
Laboratory test	X							X							X	
Secondary sexual characteristics status[10]	X															
Adverse events			←												→	

drowsiness and dizziness		X			X							X				
Body weight		X														
Medication status				X	X	X	X	X		X	X	X	X	X	X	
Concomitantly administered medications[11]		←														→

[1] In the case of five or more consecutive holidays, including weekends and national holidays such as New Year's Eve and Golden Week, the allowable range for the treatment period is ± 3 days, and the allowable range for the washout period after the crossover study period I and II is +22/-4, and ± 4 days, respectively.

[2] Allowance (-3~＋21) is based on the point of day 35.

[3] Consent should be obtained within 12 weeks prior to drug allocation.

[4] Visit tolerance on the UV test day is ± 2; however, evaluation should be made at 24, 48, 72, and 96 hours ± 6 hours after the test day. Re-evaluation is prohibited.

[5] Neurologic severity scale scores will be evaluated in patients aged 3 years or older.

[6] Methods of hearing test (pure-tone audiometry or conditioned play audiometry) will be recorded in the medical records.

[7] The 5-meter walk test will be conducted including the patients who wear braces when the principal/participating investigator deems the patient can tolerate the test. The presence or absence of brace or the type of brace used will be recorded in the medical records. When the patient wears the brace, the tests at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[8] Data will be collected within 62 weeks prior to administration of the study drug.

[9] Laboratory urine test: oxidative stress markers (Malondialdehyde, 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine metabolites (6-sulfatoxymelatonin).

[10] Confirmation of secondary sexual characteristics status and measurement of prolactin levels in blood. To be performed on patients between 10 and 17 years of age.

[11] Four weeks prior to the initiation of the study drug.

Table 2. Summary of study assessments and procedures in the open period

	Open trial				
	Day1	Week 13	Week 26	Week39	Week52
Prescription (NPC-15)	←				→
Melanin index	X				
Facial pigmentation	X				X
Neurologic severity scale score [b]					X
Hearing test [c]					X
5-meter walk test [d]					X
Acute skin symptoms					X
Skin cancer					X
Laboratory test for Research (urine)[e]	X		X		X
Laboratory tests (blood and urine) [f]					X
Secondary sexual					X

characteristics status [g]					
Adverse events	←				→
drowsiness and dizziness	X	X	X	X	X
Body weight	X	X	X	X	
Medication status		X	X	X	X

[a] The study will be conducted only on patients who are deemed by the principal/sub-investigator at Visit 305 (after 52 weeks of the open study), to require a visit to the hospital for evaluation of adverse events, etc.

[b] Neurologic severity scale score will be evaluated in patients aged 3 years or above.

[c] Methods used for testing hearing (pure-tone audiometry or conditioned play audiometry, etc.) will be recorded in the medical records.

[d] The 5-meter walk test will be conducted including the patients who wear the brace when the principal/participating investigator deems the patient can tolerate the test. The presence or absence of brace or the type of brace used will be recorded in the medical records. When the patient wears the brace, the tests at visit 1 and Visit 305 will be conducted using the same brace as far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace will be described in the medical records.

[e] Urine examination for research use: Oxidative stress markers (Malondialdehyde,

8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine metabolites (6-sulfatoxymelatonin).

[f] Urine examination: Urinary protein and urinary urobilinogen.

[g] Confirmation of secondary sexual characteristics status and measurement of prolactin levels in blood. To be performed on patients between 10 and 17 years of age.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym A multicenter, double-blind, placebo-controlled, two-group crossover study and a long-term open study evaluating the efficacy and safety of NPC-15 in patients with xeroderma pigmentosum (XP) sunburn enhancement. (XP-1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinical Trials: Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 23 Feb 2022.
	2b	All items from the World Health Organization Trial Registration Data Set This information is available at the Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051210181. Registered 17 Feb 2022. (https://jrct.niph.go.jp/en-latest-detail/jRCT2051210181)
Protocol version	3	Date and version identifier Version 1.2, last updated on 1 June 2022
Funding	4	Sources and types of financial, material, and other support This work is supported by the Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

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MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript.

	5b	Name and contact information for the trial sponsor

The Japan Agency for Medical Research and Development, AMED, [Grant No. 21ek0109562h0001].

Contact information:

The funding agency can be contacted at the following web address: https://www.amed.go.jp/en/aboutus/index.html

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
The grant funder for this study played no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
A coordinating centre, steering committee, endpoint adjudication committee, and other individuals and groups are not participating in the composition of the trial and have no roles or responsibilities in the trial.

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is associated with severe photosensitivity, freckle-like pigmented and depigmented maculae, and skin cancers at sun-exposed areas. Without strict sun protection, numerous skin cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such as sensorineural hearing impairment, and progressive peripheral and central nervous disorders beginning around the age of 6 years, have also been observed in approximately 60% of patients.

The anti-inflammatory drug has shown to reduce the UV induced inflammation as well as UV-induced skin tumor development in XP animal models, XP animal models and/or patients' cells have shown that N-Acetyl-5-methoxytyptamine suppressed inflammatory response and oxidative stress after UV irradiation. In addition, it suppressed skin tumor formation, and improvement of auditory brainstem response (ABR) threshold, after chronic repetitive UV irradiated XP-A model mice, suggesting that N-Acetyl-5-methoxytyptamine can be a potential new treatment option for XP.

In addition to its pharmacological effects such as circadian rhythm regulation, there are many published references reporting N-Acetyl-5-methoxytyptamine as a free radical scavenger and antioxidant. It is particularly believed to act as an antioxidant within mitochondria. Pharmacologically, N-Acetyl-5-methoxytyptamine reduces the severity of injury in several disease models due to its effect on acute and chronic inflammation and CNS protection.

Considering that anti-inflammatory drug reduced the UV-induced inflammation as well as UV-induced skin tumor development in the XP animal model. Furthermore, it has been shown that level of N-Acetyl-5-methoxytyptamine metabolites is reduced in patients with XP, and there is a strong need to establish an early treatment for XP in actual clinical practice.

	6b	Explanation for choice of comparators The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type.
Objectives	7	Specific objectives or hypotheses The aim of this study is to evaluate the efficacy and safety of the NPC-15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover study, 10 weeks; open study, 52 weeks) in patients with XP exaggerated sunburn reaction type. We hypothesized that NPC-15 could contribute to mitigate sunburn reactions and slow down the progress of neurological symptoms in patients with XP exaggerated sunburn reaction type.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a multicenter, placebo-controlled, double-blinded, randomized two-group crossover study followed by a long-term open study.
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained This study will be performed at Kobe University Hospital, Kobe, and Osaka Medical and Pharmaceutical University, Osaka, Japan.

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)

Inclusion Criteria

- ✓ Patients with XP (according to the XP diagnostic criteria by the Japan Dermatology Society, 2015) that have been diagnosed with Exaggerated sunburn-reaction type (XP-A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
- ✓ Patients aged 1 year old or older with a weight of 7.5 kg or more at the time of consent. However, patients under 6 years of age will be enrolled after confirming the safety for the first 10 cases of the subjects aged 6 years or older during the crossover study by the Safety Evaluation Committee.
- ✓ Patients (or their caregivers/guardians) who have provided written informed consent to participate in this study.

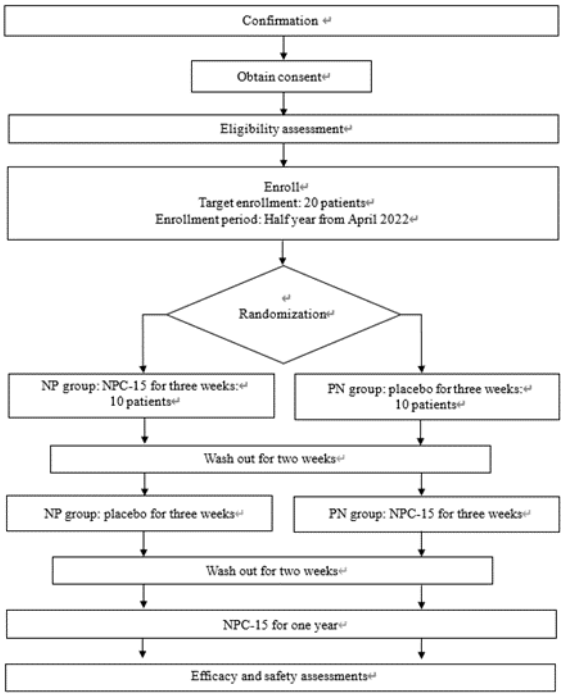
Exclusion Criteria

- ✓ Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon.
- ✓ Patients receiving other investigational drugs (including placebo) within the 4 months prior to obtaining consent.
- ✓ Patients who have been using N-acetyl-5methoxytryptamine (including health foods containing melatonin as the principal component) and Fluvoxamine maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to the start of drug administration.
- ✓ Patients who are pregnant or may become pregnant.
- ✓ Patients judged by the investigator to be ineligible for this study.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (1) NPC-15-Placebo (NP) group will receive NPC-15 for the first 3 weeks, followed by the placebo for 3 weeks after a 2-week drug holiday. (2) Placebo-NPC-15 (PN) group will receive the placebo for the first 3 weeks, followed by NPC-15 for 3 weeks after a 2-week drug holiday. The investigational drug will be administrated orally once daily at a dose of 0.5–4 mg (0.067 mg/kg) before bedtime.
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) The patients will return the empty medicine pouches at the end of the treatment period.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Concomitant administration of N-acetyl-5-methoxytryptamine (including health foods containing it as a major ingredient) and Fluvoxamine maleate (Luvox, Depmerol, etc.) is prohibited from the date of initiation to the date of termination of administration of the investigational drug.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended The primary endpoint is the Minimum Erythema Dose (MED) 72 hours (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I and Crossover period II) of investigational drug administration. The secondary efficacy endpoints are the following: (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after UV irradiation on the 15th day (stage I and stage II) of investigational drug administration. (2) Evaluation of Melanin index regarding the pigmented area in MED judgment area. (3) Pigmented maculae inspection (number, area, and color tone)[15]. (4) Neurological symptoms (neurologic severity scale score on XP[16], hearing test, and 5 m gait test). (5) Presence or absence of onset of acute skin symptoms (6) Presence or absence of skin cancer The secondary endpoint for safety is the presence or absence of any adverse events and the intensity of drowsiness and dizziness (visual analog scale score; VAS) associated with the conduct of this clinical study.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations

The total target number of subjects is 20, with 10 subjects in both the NP and PN groups.

We estimated the difference in MED between the placebo and actual drug to be approximately 4.4 mJ/cm^2, which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm^2) and 28 XP patients (31.9 mJ/cm^2). We believe that a difference of 4.4 mJ/cm^2 is sufficient to reduce the biological effects of UV exposure for XP patients with photosensitivity. The standard deviation of the difference between period 1 and 2 was conservatively assumed to be 7.0 mJ/cm^2 because the estimated time point (24 hours) and the time of the main endpoint (72 hours) are different.

In the 2 × 2 crossover design of this study, when we expect a difference of 4.4 mJ/cm^2 between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm^2, we would need 8 cases to maintain a significance level of 2.5% (one-sided) and a total of 16 cases to achieve a power of 80%.

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size All subjects who provide consent to participate and who fulfil the inclusion criteria and who do not meet any of the exclusion criteria will be randomized.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions All subjects who provide consent to participate and fulfill the sampling criteria will be randomized. Subjects will be randomly assigned to either the NP group or the PN group with a 1:1 allocation using the permutation random block method stratified by category (whether XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is maintained. The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. The trial participants, care providers, and outcome assessors will be blinded.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions The allocation sequence for the randomization method will be generated by a person in charge from DOT world company, CRO. Either the principal or sub-investigator will send a subject enrollment form by EDC to the data center. The staff at the data center will confirm the subject's eligibility and issue the subject enrollment confirmation form that contains the eligibility judgment, the randomization assignment result from the generated random sequence, and the enrollment number. The form will then be sent to the principal investigator or sub-investigator.

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how The trial participants, care providers, and endpoint assessors will be blinded.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Because this is a crossover study in which both groups receive the actual drug, an unblinding procedure will not be incorporated into the study.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol. Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Data from participants who discontinue their participation in the study or who deviate from the protocol will be included in the FAS analysis. All data acquired during the study period will be analyzed.

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Either the principal investigator or sub-investigator will enter the case report form (CRF) data for each subject into the electronic data capture (EDC) system. The principal investigator will confirm that the entered CRF data are complete and correct, electronically sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping. The CRF printout will be retained for audit trail purposes. If there are any queries about the CRF data, the principal investigator or sub-investigator will promptly respond. Only the biostatistician will have access to the final dataset.

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Statistical analyses will be conducted using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). The subject baseline characteristics will be summarized using arms and periods. For continuous variables, the summary statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) will be calculated. For nominal variables, the categorical frequency and proportion will be presented. Missing scores for MED difference will be replaced by zero, and no imputation will be performed for any further endpoints. For the crossover study, we will analyze under the assumption that there is no carryover effect due to a short half-life, and therefore a 2-week wash-out period will take place after the period 1. The analysis will be based on the difference of endpoints between period 1 and 2 for each subject. The difference between the mean endpoints (MED in each time point, melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo and its 95% confidence interval will be estimated. Statistical significance will be assessed using the Student's t-test, with a significance level for hypothesis testing of 2.5% (one-sided). For the open study, neurological symptoms at 52 weeks after the open study will be compared to those before the study using McNemar's test, one-sample t-test, or Wilcoxon rank sum test according to the type of each endpoint. The difference in oxidative stress marker concentrations before and after the open study will be assessed using one-sample t-test. The change of facial pigmented maculae at the start and end of the study that will be examined according to number, area, and color tone, will be analyzed using the Wilcoxon rank sum test, one-sample t-test, and McNemar's test, respectively. The difference between acute skin symptoms and the development of skin cancer during the 62 weeks before the start of treatment (Visit 101) and after the start of treatment (Visit 101 – Visit305) will be assessed using the McNemar's test. The number of adverse events and their frequency and percentage of occurrence will be summarized according to time, overall occurrence, and by treatment. For evaluation of delayed sexual maturation or development, no statistical analysis will be performed and secondary sexual characteristics and blood hormone (prolactin) level in each subject will be individually described.

	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) No additional analyses (e.g., subgroup and adjusted analyses) will be performed.
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) If there is any doubt about the data summarization or analysis, the biostatistician and the study representative will discuss the issue and decide how it will be handled. When missing data are identified, the researcher will contact the subject.
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Monitoring of the study will be performed once three months to ensure that the human rights and welfare of the subjects are being protected, study is conducted safely in accordance with the protocol and applicable regulatory requirements under the Good Clinical Practice, and data are being collected properly. The principal investigator will appoint someone to responsibly monitor the study. The items to be checked at monitoring are specified in the written procedure for the implementation of study monitoring.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial An evaluation of the interim results is not planned.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct In our study, an adverse event is defined as any disease, disability, death, or infection that occurs during this study. Adverse event monitoring will begin on first day of period and continue to the last day of the open study. The principal investigator or sub-investigator will record all the adverse events in the CRF and treat and follow the patient until resolution during the study. If the principal investigator or sub-investigator finds a potentially causal relationship between the adverse event and the study drug, all adverse events will be recorded to report to the review board.

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor For quality assurance, the study will be examined at 4 times, before the initiation of clinical trial, after the first patient in, before the last patient in and before the completion of the integrated study report, to determine that it is being conducted in accordance with the protocol and written procedures, independently and separately from the routine activities of monitoring.

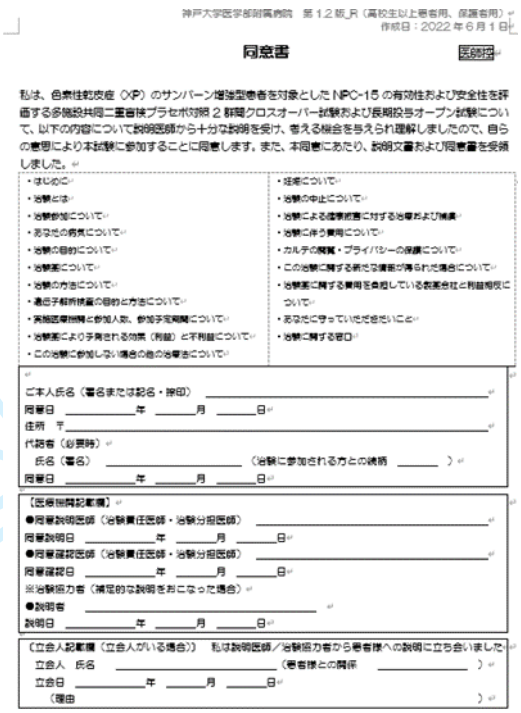
Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval The study is being conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory requirements. Ethics approval is overseen by the Kobe University Institutional Review Board and Osaka Medical and Pharmaceutical University Institutional Review Board and the study is conducted in accordance with the approved protocol.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any changes required by the ethics committee will be communicated to the participants by the investigators.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Written informed consents are being obtained from all participants before any study procedure is performed. The participants will have the opportunity to review the participant consent form and agree that they fully understand the details of the study procedures. Informed consent will be administered by a suitably qualified and experienced individual who has been delegated this duty by the principal investigator. If a patient aged 20 years or older is not able to consent to participate in this study in writing due to intellectual disability, consent is obtained from the substitute. The protocol was submitted in Japan registry of clinical trial (jRCT).
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Secondary use of the data will occur only if the patients provide written informed consent for additional use of their data.

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden), which is an electronic data system for clinical research, to manage the data and protect confidentiality before, during, and after the trial.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site The authors declare that they have no competing interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Only the biostatistician will have access to the final dataset.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation This study is insured for clinical trials, with up to 100 million yen guaranteed for death cases, for example.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions The results of the study will be published in a paper.
	31b	Authorship eligibility guidelines and any intended use of professional writers MT drafted the manuscript and obtained the grant. RO, TF, and TU obtained the grant and reviewed the manuscript. YK managed the study and drafted the manuscript. CN is the chief investigator who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and reviewed the manuscript. NY and SM reviewed the manuscript. All authors provided final approval of the manuscript. Editage (http://www.editage.jp) provided editing of the draft of this manuscript.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code The full study protocol is available in the supplementary materials and at the Japan Registry of Clinical Trials (jRCT): https://jrct.niph.go.jp/latest-detail/jRCTs2051210181. Data sharing is not applicable to this study protocol as no datasets were generated. However, the data will be made available from the author on reasonable request once the trial has been completed. Please contact the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates



神戸大学医学部附属病院　第1.2版_R（高校生以上患者用、保護者用）
作成日：2022年6月1日

同意書　　医師用

私は、色素性乾皮症（XP）のサンバーン増強型患者を対象としたNPC-15の有効性および安全性を評価する多施設共同二重盲検プラセボ対照2群間クロスオーバー試験および長期投与オープン試験について、以下の内容について説明医師から十分な説明を受け、考える機会を与えられ理解しましたので、自らの意思により本試験に参加することに同意します。また、本同意にあたり、説明文書および同意書を受領しました。

- はじめに
- 治験とは
- 治験参加について
- あなたの病気について
- 治験の目的について
- 治験薬について
- 治験の方法について
- 遺伝子解析検査の目的と方法について
- 実施医療機関と参加人数、参加予定期間について
- 治験薬により予測される効果（利益）と不利益について
- この治験に参加しない場合の他の治療法について
- 症状について
- 治験の中止について
- 治験による健康被害に対する治療および補償
- 治験に伴う費用について
- カルテの閲覧・プライバシーの保護について
- この治験に関する新たな情報が得られた場合について
- 治験薬に関する費用を負担している製薬会社と利益相反について
- あなたに守っていただきたいこと
- 治験に関する皆様

ご本人氏名（署名または記名・捺印）
同意日　　年　　月　　日
住所　〒
代諾者（必要時）
氏名（署名）　（治験に参加される方との続柄　　）
同意日　　年　　月　　日

【医療機関記載欄】
●同意説明医師（治験責任医師・治験分担医師）
同意説明日　　年　　月　　日
●同意確認医師（治験責任医師・治験分担医師）
同意確認日　　年　　月　　日
※治験協力者（補足的な説明をおこなった場合）
●説明者
説明日　　年　　月　　日

（立会人記載欄（立会人がいる場合））　私は説明医師／治験協力者から患者様への説明に立ち会いました。
立会人　氏名　（患者様との関係　　）
立会日　　年　　月　　日
（理由　　）

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable There are plans for the collection, laboratory evaluation, and storage of biological specimens for molecular analysis in the current trial and for future use in ancillary studies.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.