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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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068112
Article Type:	Protocol
Date Submitted by the Author:	07-Sep-2022
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Keywords:	Dermatopathology < DERMATOLOGY, Clinical trials < THERAPEUTICS, Neuropathology < NEUROLOGY, Neurogenetics < NEUROLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Speech pathology < OTOLARYNGOLOGY

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Manuscripts

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4 **A Clinical Trial on the Efficacy and Safety of NPC-15 for Patients with Xeroderma**
5 **Pigmentosum Exaggerated Sunburn Reaction Type: A Multicenter, Double-Blinded,**
6 **Placebo-Controlled, Two-Group Crossover Study followed by a Long-Term Open**
7 **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.

Word count: 3672 words excluding title page, references, figures and tables

1 INTRODUCTION

2 Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder that is
3 associated with severe photosensitivity, freckle-like pigmented and depigmented maculae,
4 and skin cancers in the sun-exposed areas. Without strict sun protection, numerous skin
5 cancers can develop before the age of 10 years. Furthermore, neurological symptoms, such
6 as sensorineural hearing impairment, and progressive peripheral and central nervous
7 disorders beginning around the age of 6 years, have also been observed in approximately
8 60% of patients [1, 2].

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

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

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

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

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

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

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

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

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

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

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

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

13 As this crossover study was to confirm the short-term efficacy of the drug to obtain
14 a drug indication, the investigators decided to target patients with XP with exaggerated
15 sunburn reaction type for this clinical trial in order to investigate the efficacy and safety of
16 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

1 administration.

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

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

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

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

8 (6) Presence or absence of skin cancer

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

12 Eligibility criteria

13 Inclusion criteria

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

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

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

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

24 Exclusion criteria

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

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

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4 1 (2) Patients receiving other investigational drugs (including placebo) within the 4 months
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6 2 prior to obtaining consent.

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8 3 (3) Patients who have been using N-acetyl-5methoxytryptamine (including health foods
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10 4 containing melatonin as the principal component) and Fluvoxamine maleate (Lubox,
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12 5 Depmerol, etc.) in the 4 weeks prior to the start of drug administration

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14 6 (4) Pregnant, lactating women, women who wish to become pregnant during the study
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16 7 period, or women who are fertile and cannot accept an effective contraceptive method.

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18 8 (5) Patients deemed inappropriate by the investigators in participation of this clinical study
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21 10 **Randomization**

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23 11 All subjects who provide consent to participate and fulfill the sampling criteria will be
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25 12 randomized. Subjects will be randomly assigned to either the NP group or the PN group with
26
27 13 a 1:1 allocation using the permutation random block method stratified by category (whether
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29 14 XP genotype is XP-A or not). The block size will not be disclosed to ensure that blinding is
30
31 15 maintained. The allocation sequence for the randomization method will be generated by a
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33 16 person in charge from DOT world company, CRO. The trial participants, care providers, and
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35 17 endpoint assessors will be blinded. Either the principal or sub-investigator will send a subject
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37 18 enrollment form by EDC to the data center. The staff at the data center will confirm the
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39 19 subject's eligibility and issue the subject enrollment confirmation form that contains the
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41 20 eligibility judgment, the randomization assignment result from the generated random
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43 21 sequence, and the enrollment number. The form will then be sent to the principal investigator
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45 22 or sub-investigator.

46 23 47 48 24 **Data collection and management**

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50 25 Either the principal investigator or sub-investigator will enter the case report form (CRF)
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52 26 data for each subject into the electronic data capture (EDC) system. The principal
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54 27 investigator will confirm that the entered CRF data are complete and correct, electronically
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1 sign the CRF in Viedoc™, and then make a printout of the signed CRF for record keeping.
2 The CRF printout will be retained for audit trail purposes. If there are any queries about the
3 CRF data, the principal investigator or sub-investigator will promptly respond. Only the
4 biostatistician will have access to the final dataset.

5 6 **Monitoring and Audit**

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

17 18 **Intervention and treatment protocol**

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

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

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

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

2

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

	Study period															
	Screening period		Crossover period I							Crossover period II						
			Treatment period					Washout	Treatment period					Washout		
			NPC-15 or Placebo						Placebo or NPC-15							
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	X		
Concomitantly administered medications ¹⁰																		

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1 ¹ In the case of five or more consecutive holidays, including weekends and national holidays such as
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3 New Year's Eve and Golden Week, the allowable range for the treatment period is ± 3 days, and the

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4 1 allowable range for the washout period after the crossover study period I and II is +22/-4, and ± 4
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7 2 days, respectively.
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10 3 ² Allowance (-3~ +21) is based on the point Day 35.
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13 4 ³ Consent should be obtained within the 12 weeks prior to drug allocation.
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16 5 ⁴ Visit tolerance on the UV test day is ± 2 , but evaluation should be made at 24, 48, 72, and 96 hours
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18 ± 6 hours after the test day. Re-evaluation is prohibited.
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22 7 ⁵ Neurologic severity scale scores will be evaluated in subjects 3 years of age or older.
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25 8 ⁶ Methods of the hearing test (pure-tone audiometry or conditioned play audiometry) will be recorded
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27 in the medical records.
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30 10 ⁷ 5-meter walk test will be conducted including the subjects who wear braces when the
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32 principal/participating investigator deems the subject can tolerate the test. Whether with or without
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34 the brace and what kinds of brace they wear the brace will be recorded in the medical records. When
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36 the subject wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as
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38 far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace
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40 will be described in the medical records.
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47 16 ⁸ Data will be collected within 62 weeks prior to administration of the study drug.
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50 17 ⁹ Laboratory urine test: oxidative stress markers and N-acetyl 5-methoxytryptamine metabolites.
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53 18 ¹⁰ Four weeks prior to the initiation of the study drug.
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1 **Table 2.** Summary of study assessments and procedures in the open period
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	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

3 ^a The study will be conducted only for subjects who are deemed by the principal/sub-investigator at
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4 1 Visit 305 (after 52 weeks of the open study), to require a visit to the hospital for evaluation of
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7 2 adverse events, etc.

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10 3 ^bNeurologic severity scale score will be evaluated in subjects of 3 years of age or older.

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13 4 ^c Methods of the hearing test (pure-tone audiometry or conditioned play audiometry, etc) will be
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16 5 recorded in the medical records.

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19 6 ^d 5-meter walk test will be conducted including the subjects who wear the brace when the
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22 7 principal/participating investigator deems the subject can tolerate the test. Whether with or without
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25 8 the brace and what kinds of brace they wear the brace will be recorded in the medical records. When
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28 9 the subject wears the brace, the test at visit 1 and Visit 305 will be conducted using the same brace as
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31 10 far as possible, and if the brace is changed, the kinds of brace and the reason for the change of brace
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34 11 will be described in the medical records.

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36 12 ^e Urine examination for research use: Oxidative stress marker and N-acetyl 5-methoxytryptamine
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39 13 metabolites.

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43 44 15 **Statistical analysis**

45 46 16 **Analysis set**

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48 17 A summary of the planned statistical analysis for this study is provided below. The final
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50 18 analysis will be performed after data from the subjects have been obtained and fixed at the
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52 19 end of the follow-up period.

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54 20 The full analysis set is the set of randomized subjects who receive at least one dose of the
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1 study drug. The per-protocol set is the subset of subjects in the full analysis set who
2 sufficiently comply with all aspects of the clinical trial protocol, including the drug
3 administration methods and schedule. The safety analysis set is the set of subjects who
4 receive at least one dose of the study drug. In this study, the safety analysis set is the same as
5 the full analysis set.

6 7 **Statistical analysis**

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

15 For the crossover study, we will analyze under the assumption that there is no carryover
16 effect due to a short half-life, and therefore a 2-week wash-out period will take place after
17 the period 1. The analysis will be based on the difference in endpoints between period 1 and
18 2 for each subject. The difference between the mean endpoints (MED in each time point,
19 melanin index, and concentrations of oxidative stress markers) of the NPC-15 and placebo
20 and its 95% confidence interval will be estimated. Statistical significance will be assessed
21 using the Student's t-test, with a significance level for hypothesis testing of 2.5%
22 (one-sided).

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

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

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

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

12

13 **Sample size calculation**

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

15 We estimated the difference in MED between the placebo and actual drug to be
16 approximately 4.4 mJ/cm², which was 1/5th of the difference between the mean MED at 24
17 hours after irradiation in 7 healthy subjects (54.3 mJ/cm²) and 28 XP patients (31.9 mJ/cm²).

18 We believe that a difference of 4.4 mJ/cm² is sufficient to reduce the biological effects of
19 UV exposure for XP patients with photosensitivity. The standard deviation of the difference
20 between period 1 and 2 was conservatively assumed to be 6.0 mJ/cm², twice the irradiation
21 unit. In the 2 × 2 crossover design of this study, when we expect a difference of 4.4 mJ/cm²
22 between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0
23 mJ/cm², we would need 8 cases to maintain a significance level of 2.5% (one-sided) and a
24 total of 16 subjects to achieve a power of 80%. Assuming a few dropouts, a total of 20
25 subjects are required.

26

27 **Study period**

1 This manuscript is based on the current version of the study protocol (version 1.2, last
2 updated on 1 June 2022). The study was first approved on 24 January 2022 by the
3 institutional review board of Kobe University, Graduate School of Medicine, and authorized
4 by the Pharmaceutical and Medical Devices Agency. Participant recruitment began on 1
5 April 2022. The expected date of completion (last visit of the last patient) is 15 December
6 2023.

7 8 **Patient and public involvement**

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

15 16 **ETHICS AND DISSEMINATION**

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

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

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

4 **Author affiliations**

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6 Medicine, Kobe University, Kobe, Japan.
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- 14 7) Japanese Red Cross Hyogo Blood Center

16 **Acknowledgments**

17 We would like to thank Editage (<http://www.editage.jp>) for English language editing.

19 **Contributors**

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

Funding

This work is supported by the Japan Agency for Medical Research and Development (AMED) under Grant Number JP15ek0109028h0002(CN, MT, RO), 21ek0109450h0002 (CN, MT, RO, TF), 21ek0109562h0001 (CN, MT, RO, TF, TU), and by Ministry of Health, 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

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4 1 be made available from the author upon reasonable request once the trial has been completed.
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7 2 Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).
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11 4 **Open access**

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15 7 remix, adapt, build upon this work non-commercially, and license their derivative works on
16 8 different terms.
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4 **1 FIGURE CAPTIONS**

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6 2 Table 1. Summary of study assessments and procedures in the crossover period

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8 3 Table 2. Summary of study assessments and procedures in the open period

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10 4 Figure 1. Flow chart of participants

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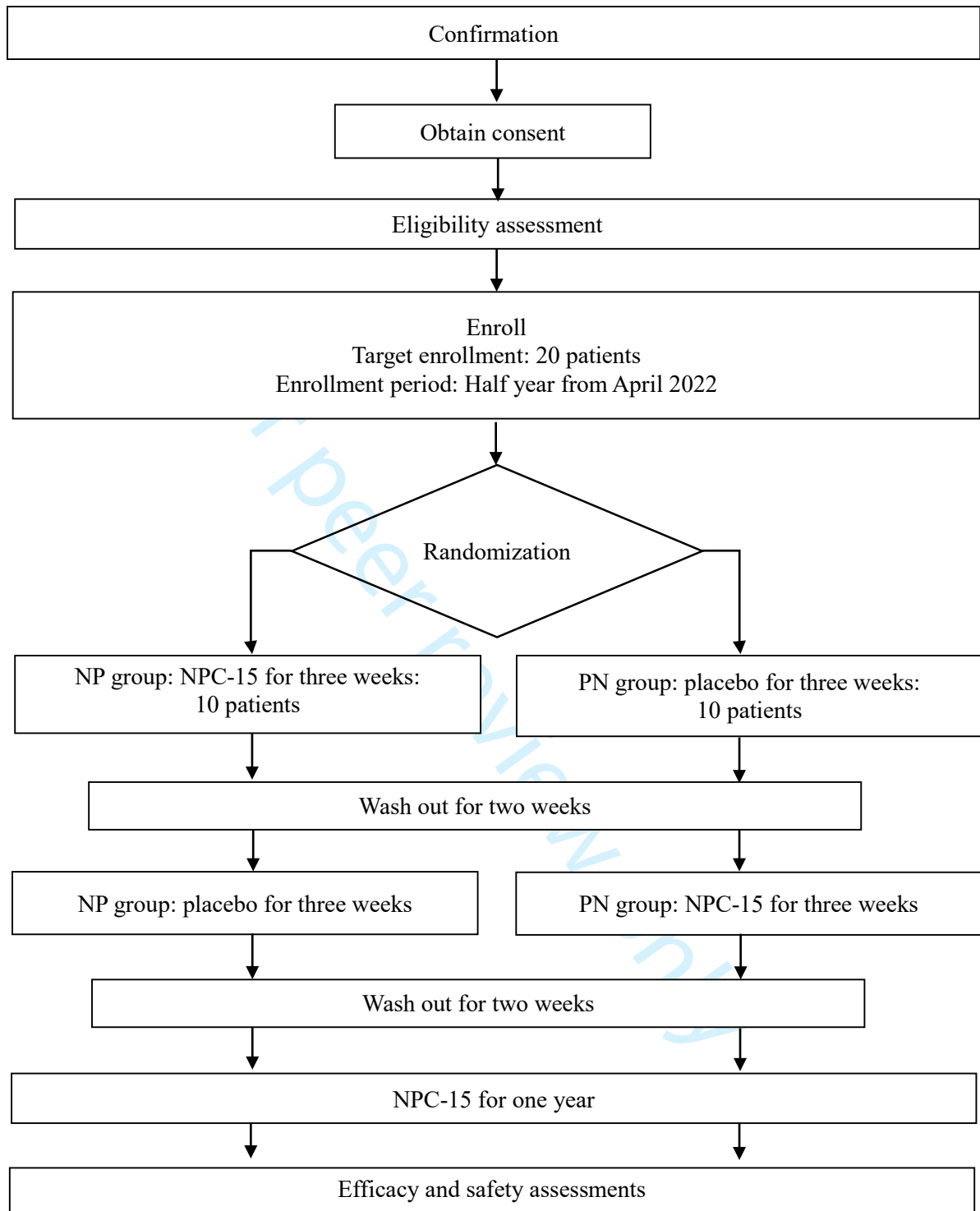


Figure 1. Flowchart of participants.

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



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].

1
2 Roles and
3 responsibilities

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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>

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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.

1			
2		6b	Explanation for choice of comparators
3			The aim of this study is to evaluate the efficacy and safety of the NPC-
4			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
5			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
6			study, 10 weeks; open study, 52 weeks) in patients with XP
7			exaggerated sunburn reaction type.
8			
9			
10	Objectives	7	Specific objectives or hypotheses
11			The aim of this study is to evaluate the efficacy and safety of the NPC-
12			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
13			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
14			study, 10 weeks; open study, 52 weeks) in patients with XP
15			exaggerated sunburn reaction type.
16			We hypothesized that NPC-15 could contribute to mitigate sunburn
17			reactions and slow down the progress of neurological symptoms in
18			patients with XP exaggerated sunburn reaction type.
19			
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21			
22	Trial design	8	Description of trial design including type of trial (eg, parallel group,
23			crossover, factorial, single group), allocation ratio, and framework (eg,
24			superiority, equivalence, noninferiority, exploratory)
25			This is a multicenter, placebo-controlled, double-blinded, randomized
26			two-group crossover study followed by a long-term open study.
27			
28			

Methods: Participants, interventions, and outcomes

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30			
31	Study setting	9	Description of study settings (eg, community clinic, academic hospital)
32			and list of countries where data will be collected. Reference to where
33			list of study sites can be obtained
34			This study will be performed at Kobe University Hospital, Kobe, and
35			Osaka Medical and Pharmaceutical University, Osaka, Japan.
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2 Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility
3 criteria for study centres and individuals who will perform the
4 interventions (eg, surgeons, psychotherapists)
5

6
7 Inclusion Criteria

- 8 ✓ Patients with XP (according to the XP diagnostic criteria
9 by the Japan Dermatology Society, 2015) that have been
10 diagnosed with Exaggerated sunburn-reaction type (XP-
11 A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
12
13 ✓ Patients aged 1 year old or older with a weight of 7.5 kg
14 or more at the time of consent. However, patients under 6
15 years of age will be enrolled after confirming the safety
16 for the first 10 cases of the subjects aged 6 years or
17 older during the crossover study by the Safety Evaluation
18 Committee.
19
20 ✓ Patients (or their caregivers/guardians) who have
21 provided written informed consent to participate in this
22 study.
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30 Exclusion Criteria

- 31 ✓ Patients with a history of allergies to N-acetyl 5-
32 methoxytryptamine or ramelteon.
33
34 ✓ Patients receiving other investigational drugs (including
35 placebo) within the 4 months prior to obtaining consent.
36
37 ✓ Patients who have been using N-acetyl-
38 5methoxytryptamine (including health foods containing
39 melatonin as the principal component) and Fluvoxamine
40 maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to
41 the start of drug administration.
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43 ✓ Patients who are pregnant or may become pregnant.
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45 ✓ Patients judged by the investigator to be ineligible for this
46 study.
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- 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 administered 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.

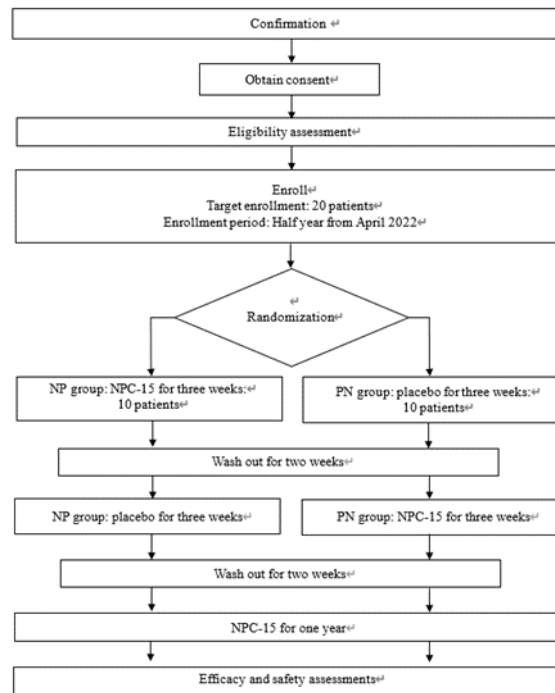
Outcomes

- 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 XP¹⁶, 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.

view only

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/cm², which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm²) and 28 XP patients (31.9 mJ/cm²). We believe that a difference of 4.4 mJ/cm² 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/cm² 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/cm² between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm², 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%.

1
2 Recruitment 15 Strategies for achieving adequate participant enrolment to reach
3 target sample size
4 All subjects who provide consent to participate and who fulfil the
5 inclusion criteria and who do not meet any of the exclusion criteria will
6 be randomized.
7

8
9 **Methods: Assignment of interventions (for controlled trials)**

10 Allocation:

11
12 Sequence 16a Method of generating the allocation sequence (eg, computer-
13 generation generated random numbers), and list of any factors for stratification.
14 To reduce predictability of a random sequence, details of any planned
15 restriction (eg, blocking) should be provided in a separate document
16 that is unavailable to those who enrol participants or assign
17 interventions
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19 All subjects who provide consent to participate and fulfill the sampling
20 criteria will be randomized. Subjects will be randomly assigned to
21 either the NP group or the PN group with a 1:1 allocation using the
22 permutation random block method stratified by category (whether XP
23 genotype is XP-A or not). The block size will not be disclosed to
24 ensure that blinding is maintained. The allocation sequence for the
25 randomization method will be generated by a person in charge from
26 DOT world company, CRO. The trial participants, care providers, and
27 outcome assessors will be blinded.
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32 Allocation 16b Mechanism of implementing the allocation sequence (eg, central
33 concealment telephone; sequentially numbered, opaque, sealed envelopes),
34 mechanism describing any steps to conceal the sequence until interventions are
35 assigned
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37 Either the principal or sub-investigator will send a subject enrollment
38 form by EDC to the data center. The staff at the data center will
39 confirm the subject's eligibility and issue the subject enrollment
40 confirmation form that contains the eligibility judgment, the
41 randomization assignment result from the generated random
42 sequence, and the enrollment number. The form will then be sent to
43 the principal investigator or sub-investigator.
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46 Implementation 16c Who will generate the allocation sequence, who will enrol participants,
47 and who will assign participants to interventions
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49 The allocation sequence for the randomization method will be
50 generated by a person in charge from DOT world company, CRO.
51 Either the principal or sub-investigator will send a subject enrollment
52 form by EDC to the data center. The staff at the data center will
53 confirm the subject's eligibility and issue the subject enrollment
54 confirmation form that contains the eligibility judgment, the
55 randomization assignment result from the generated random
56 sequence, and the enrollment number. The form will then be sent to
57 the principal investigator or sub-investigator.
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- 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.

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Methods: Data collection, management, and analysis

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- 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.

For peer review only

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.

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- 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.

17 Methods: Monitoring

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- 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.

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

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17 Research ethics 24 Plans for seeking research ethics committee/institutional review board
18 approval (REC/IRB) approval
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21 The study is being conducted in compliance with the principles of the
22 Declaration of Helsinki (1996), the principles of Good Clinical Practice,
23 and all applicable regulatory requirements. Ethics approval is
24 overseen by the Kobe University Institutional Review Board and
25 Osaka Medical and Pharmaceutical University Institutional Review
26 Board and the study is conducted in accordance with the approved
27 protocol.
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30 Protocol 25 Plans for communicating important protocol modifications (eg,
31 amendments changes to eligibility criteria, outcomes, analyses) to relevant parties
32 (eg, investigators, REC/IRBs, trial participants, trial registries, journals,
33 regulators)
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35 Any changes required by the ethics committee will be communicated
36 to the participants by the investigators.
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38 Consent or assent 26a Who will obtain informed consent or assent from potential trial
39 participants or authorised surrogates, and how (see Item 32)

40 Written informed consents are being obtained from all participants
41 before any study procedure is performed. The participants will have
42 the opportunity to review the participant consent form and agree that
43 they fully understand the details of the study procedures. Informed
44 consent will be administered by a suitably qualified and experienced
45 individual who has been delegated this duty by the principal
46 investigator. If a patient aged 20 years or older is not able to consent
47 to participate in this study in writing due to intellectual disability,
48 consent is obtained from the substitute. The protocol was submitted in
49 Japan registry of clinical trial (jRCT).
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54 26b Additional consent provisions for collection and use of participant data
55 and biological specimens in ancillary studies, if applicable

56 Secondary use of the data will occur only if the patients provide
57 written informed consent for additional use of their data.
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2	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
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5			We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden),
6			which is an electronic data system for clinical research, to manage the
7			data and protect confidentiality before, during, and after the trial.
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
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12			The authors declare that they have no competing interests.
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14	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
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16			Only the biostatistician will have access to the final dataset.
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20	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
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22			This study is insured for clinical trials, with up to 100 million yen
23			guaranteed for death cases, for example.
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26	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
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28			The results of the study will be published in a paper.
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33		31b	Authorship eligibility guidelines and any intended use of professional writers
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35			MT drafted the manuscript and obtained the grant. RO, TF, and TU
36			obtained the grant and reviewed the manuscript. YK managed the
37			study and drafted the manuscript. CN is the chief investigator who
38			conceived and designed the study and obtained the grant funding and
39			drafted the manuscript. SM designed the statistical analysis plan and
40			reviewed the manuscript. NY and SM reviewed the manuscript. All
41			authors provided final approval of the manuscript.
42			Editage (http://www.editage.jp) provided editing of the draft of this
43			manuscript.
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48		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
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50			The full study protocol is available in the supplementary materials and
51			at the Japan Registry of Clinical Trials (jRCT):
52			https://jrct.niph.go.jp/latest-detail/jRCTs2051210181 . Data sharing is
53			not applicable to this study protocol as no datasets were generated.
54			However, the data will be made available from the author on
55			reasonable request once the trial has been completed. Please contact
56			the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).
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Appendices

Informed consent 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 (高校生以上者用、保護者用) 作成日：2022 年 6 月 1 日

同意書

医師印

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 NPO-15 の有効性及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 群ランダム化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、覚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、本同意書に当たり、説明文書および同意書を受領しました。

<ul style="list-style-type: none"> 試験の目的について 試験の方法について 参加者の数について 参加者の選定について 参加者の利益について 参加者の負担について 参加者のリスクについて 参加者の権利について 参加者のプライバシーの保護について 参加者の個人情報について 参加者のデータについて 参加者のデータの利用について 参加者のデータの開示について 参加者のデータの保存について 参加者のデータの廃棄について 参加者のデータのバックアップについて 参加者のデータのセキュリティについて 参加者のデータの監査について 参加者のデータの検証について 参加者のデータの報告について 参加者のデータの公表について 参加者のデータの撤回について 参加者のデータの削除について 参加者のデータの復元について 参加者のデータのバックアップの頻度について 参加者のデータのバックアップの場所について 参加者のデータのバックアップの方法について 参加者のデータのバックアップの検証について 参加者のデータのバックアップの記録について 参加者のデータのバックアップの承認について 参加者のデータのバックアップの監査について 参加者のデータのバックアップの検証の頻度について 参加者のデータのバックアップの検証の方法について 参加者のデータのバックアップの検証の記録について 参加者のデータのバックアップの検証の承認について 参加者のデータのバックアップの検証の監査について 参加者のデータのバックアップの検証の記録の保存について 参加者のデータのバックアップの検証の記録の廃棄について 参加者のデータのバックアップの検証の記録のバックアップについて 参加者のデータのバックアップの検証の記録のセキュリティについて 参加者のデータのバックアップの検証の記録の監査について 参加者のデータのバックアップの検証の記録の報告について 参加者のデータのバックアップの検証の記録の公表について 参加者のデータのバックアップの検証の記録の撤回について 参加者のデータのバックアップの検証の記録の削除について 参加者のデータのバックアップの検証の記録の復元について 参加者のデータのバックアップの検証の記録のバックアップの頻度について 参加者のデータのバックアップの検証の記録のバックアップの場所について 参加者のデータのバックアップの検証の記録のバックアップの方法について 参加者のデータのバックアップの検証の記録のバックアップの検証について 参加者のデータのバックアップの検証の記録のバックアップの記録について 参加者のデータのバックアップの検証の記録のバックアップの承認について 参加者のデータのバックアップの検証の記録のバックアップの監査について 参加者のデータのバックアップの検証の記録のバックアップの記録の保存について 参加者のデータのバックアップの検証の記録のバックアップの記録の廃棄について 参加者のデータのバックアップの検証の記録のバックアップの記録のバックアップについて 参加者のデータのバックアップの検証の記録のバックアップの記録のセキュリティについて 参加者のデータのバックアップの検証の記録のバックアップの記録の監査について 参加者のデータのバックアップの検証の記録のバックアップの記録の報告について 参加者のデータのバックアップの検証の記録のバックアップの記録の公表について 参加者のデータのバックアップの検証の記録のバックアップの記録の撤回について 参加者のデータのバックアップの検証の記録のバックアップの記録の削除について 参加者のデータのバックアップの検証の記録のバックアップの記録の復元について 	<ul style="list-style-type: none"> 試験の中止について 試験による健康被害に対する治療および補償 試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 この試験に関する第三者からの問い合わせについて 試験に賛同する費用を負担している企業会社と利益相反について 参加者に与えていた利益について 試験に関する苦情
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ご本人氏名（署名または記名・捺印）
 同意日 年 月 日
 住所 〒 丁目 番 号
 代読者（必要時）
 氏名（署名）
 同意日 年 月 日

【関係機関記載欄】
 ●同意説明医師（治験責任医師・治験分指医師）
 同意説明日 年 月 日
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 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（立会人がある場合）） 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
 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068112.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Dec-2022
Complete List of Authors:	Tsujimoto, Mariko; Kobe University Graduate School of Medicine School of Medicine, Dermatology Kakei, Yasumasa; Kobe University Hospital, Clinical & Translational Research Center; Kobe University Graduate School of Medicine School of Medicine, Oral and Maxillofacial Surgery Yamano, Nozomi; Kobe University Graduate School of Medicine School of Medicine, Dermatology Fujita, Takeshi; Kobe University Graduate School of Medicine School of Medicine, Otolaryngology-Head and Neck Surgery Ueyama, Takehiro; Kobe University Graduate School of Medicine School of Medicine, Neurology Ono, Ryusuke; Kobe University Hospital, Dermatology Murakami, Sae; Kobe University Hospital, Clinical and Translational Research Center Moriwaki, Shinichi; Osaka Medical and Pharmaceutical University, Dermatology Nishigori, Chikako; Kobe University Hospital, Dermatology; Hyogo Red Cross Blood Center
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Neurology
Keywords:	Dermatopathology < DERMATOLOGY, Clinical trials < THERAPEUTICS, Neuropathology < NEUROLOGY, Neurogenetics < NEUROLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Speech pathology < OTOLARYNGOLOGY

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5 Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter,
6 Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a
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8 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.

Word count: 3578 words excluding title page, references, figures and tables

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%,

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

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

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

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

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

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

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

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4 1 N-Acetyl-5-methoxytryptamine reduces the severity of injury in several disease models due
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6 2 to its effect on acute and chronic inflammation and central nervous system (CNS) protection.

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8 3 In Japan, Nobelpharma Co. Ltd. submitted a manufacturing and marketing
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10 4 authorization application in April 2019, and a 0.2% granule of
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12 5 N-Acetyl-5-methoxytryptamine was approved in March 2020 for the treatment of sleep
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14 6 difficulties associated with childhood neurodevelopmental disorders. However, since no
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16 7 drugs for XP have been approved and marketed in Japan and overseas, the development of
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18 8 an additional indication was promoted.

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20 9 Furthermore, it has been shown that level of N-Acetyl-5-methoxytryptamine
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22 10 metabolites is reduced in patients with XP [14], and there is a strong need to establish an
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24 11 early treatment for XP in actual clinical practice.

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26 12 As this crossover study was to confirm the short-term efficacy of the drug to obtain
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28 13 a drug indication, the investigators decided to target patients with XP with exaggerated
29
30 14 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 cm² on the participant's back are

1 irradiated with UVB from 3 mJ/cm² to 30 or 60 mJ/cm², which are serially dosed up by 3
2 mJ/cm² each session. The upper limit of the irradiation dose is set between 30 and 60 mJ/cm²,
3 considering the degree of photosensitivity based on the patient's age (skin thickness) and
4 genetic diagnosis. The irradiated area is set so as not to overlap each other for both the left
5 and right back, during crossover period I and II, respectively.

6 7 **Secondary endpoint**

8 The secondary efficacy endpoints are the following:

- 9 (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after
10 UV irradiation on the 15th day (period I and period II) of investigational drug
11 administration.
- 12 (2) Evaluation of Melanin index regarding the pigmented area in MED judgment area.
- 13 (3) Pigmented maculae inspection (number, area, and color tone) [15].
- 14 (4) Neurological symptoms (neurologic severity scale score on XP [16], hearing test, and 5
15 m gait test).
- 16 (5) Presence or absence of onset of acute skin symptoms
- 17 (6) Presence or absence of skin cancer

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

21 22 **Eligibility criteria**

23 **Inclusion criteria**

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

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

1 (2) Patients aged 1 year or older with a weight of 7.5 kg or more at the time of consent.

2 However, patients under 6 years of age will be enrolled after confirming the safety for
3 the first 10 cases of the patients aged 6 years or older during the crossover study by the
4 Safety Evaluation Committee.

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

7 8 **Exclusion criteria**

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

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

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

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

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

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

19 20 **Randomization**

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

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

7 8 **Data collection and management**

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

16 17 **Monitoring and Audit**

18 Monitoring of the study will be performed to ensure that the human rights and welfare of the
19 patients are being protected, the study is conducted safely in accordance with the protocol
20 and applicable regulatory requirements under the Good Clinical Practice, and data are being
21 collected properly. The principal investigator will appoint someone to responsibly monitor
22 the study. The items to be checked at monitoring are specified in the written procedure for
23 the implementation of study monitoring. For quality assurance, the study will be examined
24 four times, before the initiation of the clinical trial, after the first patient in, before the last
25 patient in, and before the completion of the integrated study report, to determine that it is
26 being conducted in accordance with the protocol and written procedures, independently and
27 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 administered 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							Washout	Treatment period					Washout
				NPC-15 or Placebo								Placebo or NPC-15					
Enrollment	All locations	Day 1 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	

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4 1 3 days, and the allowable range for the washout period after the crossover study period I and
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7 2 II is +22/-4, and ± 4 days, respectively.
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10 3 ² Allowance (-3~+21) is based on the point Day 35.
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13 4 ³ Consent should be obtained within the 12 weeks before drug allocation.
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16 5 ⁴ Visit tolerance on the UV test day is ± 2 , but evaluation should be made at 24, 48, 72, and
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19 6 96 hours ± 6 hours after the test day. Re-evaluation is prohibited.
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22 7 ⁵ Neurologic severity scale scores will be evaluated in patients 3 years of age or older.
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25 8 ⁶ Methods of the hearing test (pure-tone audiometry or conditioned play audiometry) will be
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28 9 recorded in the medical records.
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30 10 ⁷ 5-meter walk test will be conducted including the patients who wear braces when the
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33 11 principal/participating investigator deems the patient can tolerate the test. Whether with or
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36 12 without the brace and what kinds of brace they wear will be recorded in the medical records.
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39 13 When the patient wears the brace, the test at visit 1 and Visit 305 will be conducted using the
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42 14 same brace as far as possible, and if the brace is changed, the kinds of brace and the reason
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45 15 for the change of brace will be described in the medical records.
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48 16 ⁸ Data will be collected within 62 weeks before administration of the study drug.
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51 17 ⁹ Laboratory urine test: oxidative stress markers (Malondialdehyde,
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54 18 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine
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1 metabolites (6-sulfatoxymelatonin).

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

4 ¹¹ Four weeks prior to the initiation of the study drug.

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

6

	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

1

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

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

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

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

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4 1 When the patient wears the brace, the test at visit 1 and Visit 305 will be conducted using the
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7 2 same brace as far as possible, and if the brace is changed, the kinds of brace and the reason
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10 3 for the change of brace will be described in the medical records.

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13 4 ^e Urine examination for research use: Oxidative stress markers (Malondialdehyde,
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16 5 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine
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19 6 metabolites (6-sulfatoxymelatonin).

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22 7 ^f Urine examination: Urinary protein and urinary urobilinogen.

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25 8 ^g Confirmation of secondary sexual characteristics status and measurement of prolactin
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28 9 levels in blood. To be performed on patients between 10 and 17 years of age.

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12 **Statistical analysis**

13 **Analysis set**

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

17 The full analysis set is the set of randomized patients who receive at least one dose of the
18 study drug. The per-protocol set is the subset of patients in the full analysis set who
19 sufficiently comply with all aspects of the clinical trial protocol, including the drug
20 administration methods and schedule. The safety analysis set is the set of patients who
21 receive at least one dose of the study drug. In this study, the safety analysis set is the same as
22 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.

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

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

6 7 **Sample size calculation**

8 The primary outcome will be to compare the changes of MED in crossover period I and II
9 between the two groups. MED of XP patients is approximately 5 to 10 times smaller than
10 that of healthy controls. For healthy controls, a difference of 10 mJ/cm² in MED is
11 apparently sufficient in the clinical setting. Therefore, we thought the difference of 1-2
12 mJ/cm² should be clinically sufficient for XP patients. If we compare irradiation areas on
13 one side of a child's back, avoiding apparently curved areas, we postulate that 10 areas are
14 the limit. Trying to evaluate all participants with the same irradiation dose difference and
15 considering that 10-areas are limit for children, a 3 mJ/cm² difference per area was
16 considered most appropriate. The minimum effect of improved MED should be larger than
17 this irradiation dose difference. It is understandable to assume that the difference in MED
18 between the placebo and actual drug is not as great as the difference in MED between
19 healthy controls and XP patients, in fact, it is possibly much lower. From the above, we
20 estimated the difference in change of MED between the placebo and actual drug to be
21 approximately 4.4 mJ/cm², which was 1/5th of the difference between the mean MED at 24
22 hours after irradiation in 7 healthy controls (54.3 mJ/cm²) and 28 XP patients (31.9 mJ/cm²).
23 We believe that a difference of 4.4 mJ/cm² is sufficient to reduce the biological effects of
24 UV exposure for XP patients with photosensitivity. The standard deviation was assumed to
25 be 6.0 mJ/cm², twice the irradiation unit dose for MED examination. Differences in NPC-15
26 and placebo in a crossover design can be substituted for differences in period between
27 groups. The difference in period in NP group is denoted by [Period I (NPC-15) – Period II

1 (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/cm² and a standard deviation of 6.0 mJ/cm² 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.

9 **Study period**

10 This study is based on the current version of the study protocol (version 1.2, last updated on 11 1 June 2022). The study was first approved on 24 January 2022 by the institutional review 12 board of Kobe University, Graduate School of Medicine, and authorized by the 13 Pharmaceutical and Medical Devices Agency. Participant recruitment began on 1 April 2022. 14 The expected date of completion (last visit of the last patient) is 15 December 2023.

16 **Patient and public involvement**

17 Patients and the public were not involved in the development of the research questions, 18 selection of endpoint measures, study design, patient recruitment, or conduction of the study. 19 The burden of intervention was assessed by representatives of patient associations 20 participating in the ethical review committee. As mentioned in the individual consent form, 21 participants may obtain access to the final results of the study through the principal 22 investigator.

24 **ETHICS AND DISSEMINATION**

25 The study is being conducted in compliance with the principles of the Declaration of 26 Helsinki (1996), the principles of Good Clinical Practice, and all applicable regulatory 27 requirements. Ethics approval is overseen by the Kobe University Institutional Review

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

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

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24 25 **Acknowledgments**

26 We would like to thank Editage (<http://www.editage.jp>) for English language editing.

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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.

Funding

This work is supported by the Japan Agency for Medical Research and Development (AMED) under Grant Number JP15ek0109028h0002(CN, MT, RO), 21ek0109450h0002 (CN, MT, RO, TF), 21ek0109562h0001 (CN, MT, RO, TF, TU), and by Ministry of Health, 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

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4 1 Not applicable.
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8 **3 Provenance and peer review**

9 4 Not commissioned; externally peer-reviewed.
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13 **6 Data availability statement**

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16 7 The full study protocol is available in the supplementary materials and at the Japan Registry
17
18 8 of Clinical Trials (jRCT): <https://jrct.niph.go.jp/latest-detail/jRCTs2051210181>. Data sharing
19
20 9 is not applicable to this study protocol, as no datasets were generated. However, the data will
21
22 10 be made available from the author upon reasonable request once the trial has been completed.
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25 11 Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).
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32 **13 Open access**

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1 **FIGURE CAPTIONS**

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

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

4 Figure 1. Flow chart of participants

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For peer review only

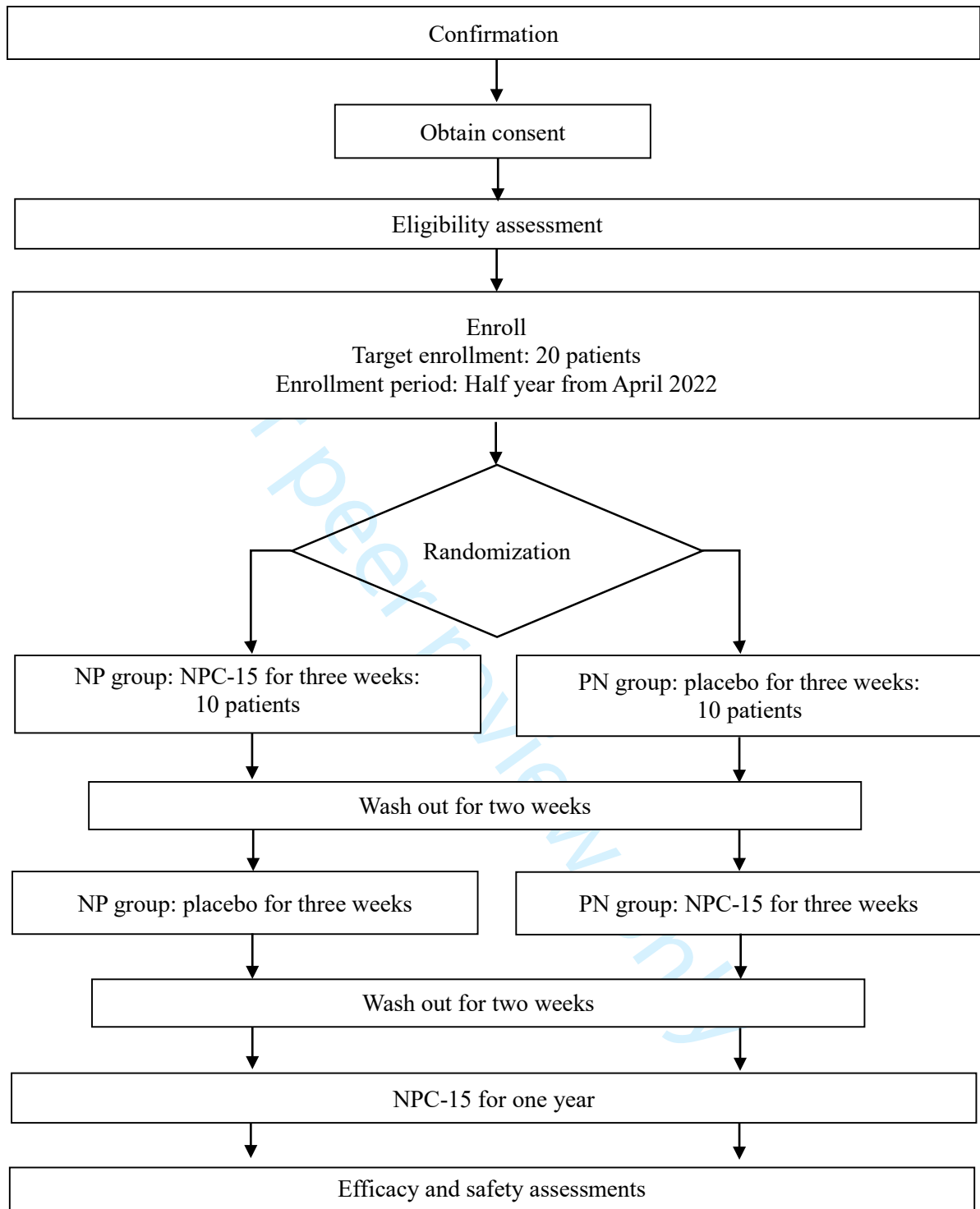


Figure 1. Flowchart of participants.

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



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].

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2 Roles and
3 responsibilities

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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>

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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.

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2		6b	Explanation for choice of comparators
3			The aim of this study is to evaluate the efficacy and safety of the NPC-
4			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
5			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
6			study, 10 weeks; open study, 52 weeks) in patients with XP
7			exaggerated sunburn reaction type.
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10	Objectives	7	Specific objectives or hypotheses
11			The aim of this study is to evaluate the efficacy and safety of the NPC-
12			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
13			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
14			study, 10 weeks; open study, 52 weeks) in patients with XP
15			exaggerated sunburn reaction type.
16			We hypothesized that NPC-15 could contribute to mitigate sunburn
17			reactions and slow down the progress of neurological symptoms in
18			patients with XP exaggerated sunburn reaction type.
19			
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22	Trial design	8	Description of trial design including type of trial (eg, parallel group,
23			crossover, factorial, single group), allocation ratio, and framework (eg,
24			superiority, equivalence, noninferiority, exploratory)
25			This is a multicenter, placebo-controlled, double-blinded, randomized
26			two-group crossover study followed by a long-term open study.
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Methods: Participants, interventions, and outcomes

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31	Study setting	9	Description of study settings (eg, community clinic, academic hospital)
32			and list of countries where data will be collected. Reference to where
33			list of study sites can be obtained
34			This study will be performed at Kobe University Hospital, Kobe, and
35			Osaka Medical and Pharmaceutical University, Osaka, Japan.
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2 Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility
3 criteria for study centres and individuals who will perform the
4 interventions (eg, surgeons, psychotherapists)
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7 Inclusion Criteria

- 8 ✓ Patients with XP (according to the XP diagnostic criteria
9 by the Japan Dermatology Society, 2015) that have been
10 diagnosed with Exaggerated sunburn-reaction type (XP-
11 A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
12
13 ✓ Patients aged 1 year old or older with a weight of 7.5 kg
14 or more at the time of consent. However, patients under 6
15 years of age will be enrolled after confirming the safety
16 for the first 10 cases of the subjects aged 6 years or
17 older during the crossover study by the Safety Evaluation
18 Committee.
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20 ✓ Patients (or their caregivers/guardians) who have
21 provided written informed consent to participate in this
22 study.
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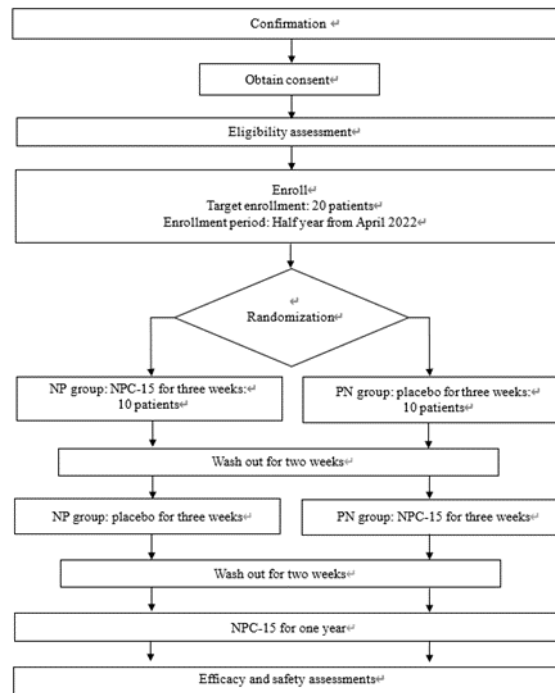
30 Exclusion Criteria

- 31 ✓ Patients with a history of allergies to N-acetyl 5-
32 methoxytryptamine or ramelteon.
33
34 ✓ Patients receiving other investigational drugs (including
35 placebo) within the 4 months prior to obtaining consent.
36
37 ✓ Patients who have been using N-acetyl-
38 5methoxytryptamine (including health foods containing
39 melatonin as the principal component) and Fluvoxamine
40 maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to
41 the start of drug administration.
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43 ✓ Patients who are pregnant or may become pregnant.
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45 ✓ Patients judged by the investigator to be ineligible for this
46 study.
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- 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 administered 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.

1
2 Outcomes 12 Primary, secondary, and other outcomes, including the specific
3 measurement variable (eg, systolic blood pressure), analysis metric
4 (eg, change from baseline, final value, time to event), method of
5 aggregation (eg, median, proportion), and time point for each
6 outcome. Explanation of the clinical relevance of chosen efficacy and
7 harm outcomes is strongly recommended
8
9 The primary endpoint is the Minimum Erythema Dose (MED) 72 hours
10 (+/- 6 hours) after UV irradiation on the 15th day (Crossover period I
11 and Crossover period II) of investigational drug administration.
12
13 The secondary efficacy endpoints are the following:
14 (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96
15 hours (+/- 6 hours) after UV irradiation on the 15th day (stage I and
16 stage II) of investigational drug administration.
17
18 (2) Evaluation of Melanin index regarding the pigmented area in MED
19 judgment area.
20
21 (3) Pigmented maculae inspection (number, area, and color tone)¹⁵.
22
23 (4) Neurological symptoms (neurologic severity scale score on XP¹⁶,
24 hearing test, and 5 m gait test).
25
26 (5) Presence or absence of onset of acute skin symptoms
27
28 (6) Presence or absence of skin cancer
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30 The secondary endpoint for safety is the presence or absence of any
31 adverse events and the intensity of drowsiness and dizziness (visual
32 analog scale score; VAS) associated with the conduct of this clinical
33 study.
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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/cm², which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm²) and 28 XP patients (31.9 mJ/cm²). We believe that a difference of 4.4 mJ/cm² 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/cm² 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/cm² between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm², 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%.

1
2 Recruitment 15 Strategies for achieving adequate participant enrolment to reach
3 target sample size
4 All subjects who provide consent to participate and who fulfil the
5 inclusion criteria and who do not meet any of the exclusion criteria will
6 be randomized.
7

8
9 **Methods: Assignment of interventions (for controlled trials)**

10 Allocation:

11
12 Sequence 16a Method of generating the allocation sequence (eg, computer-
13 generation generated random numbers), and list of any factors for stratification.
14 To reduce predictability of a random sequence, details of any planned
15 restriction (eg, blocking) should be provided in a separate document
16 that is unavailable to those who enrol participants or assign
17 interventions
18

19
20 All subjects who provide consent to participate and fulfill the sampling
21 criteria will be randomized. Subjects will be randomly assigned to
22 either the NP group or the PN group with a 1:1 allocation using the
23 permutation random block method stratified by category (whether XP
24 genotype is XP-A or not). The block size will not be disclosed to
25 ensure that blinding is maintained. The allocation sequence for the
26 randomization method will be generated by a person in charge from
27 DOT world company, CRO. The trial participants, care providers, and
28 outcome assessors will be blinded.
29
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32 Allocation 16b Mechanism of implementing the allocation sequence (eg, central
33 concealment telephone; sequentially numbered, opaque, sealed envelopes),
34 mechanism describing any steps to conceal the sequence until interventions are
35 assigned
36

37 Either the principal or sub-investigator will send a subject enrollment
38 form by EDC to the data center. The staff at the data center will
39 confirm the subject's eligibility and issue the subject enrollment
40 confirmation form that contains the eligibility judgment, the
41 randomization assignment result from the generated random
42 sequence, and the enrollment number. The form will then be sent to
43 the principal investigator or sub-investigator.
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47 Implementation 16c Who will generate the allocation sequence, who will enrol participants,
48 and who will assign participants to interventions

49 The allocation sequence for the randomization method will be
50 generated by a person in charge from DOT world company, CRO.
51 Either the principal or sub-investigator will send a subject enrollment
52 form by EDC to the data center. The staff at the data center will
53 confirm the subject's eligibility and issue the subject enrollment
54 confirmation form that contains the eligibility judgment, the
55 randomization assignment result from the generated random
56 sequence, and the enrollment number. The form will then be sent to
57 the principal investigator or sub-investigator.
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- 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.

17 Methods: Data collection, management, and analysis

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- 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.

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- 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.

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- 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.

17 Methods: Monitoring

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- 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.

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2 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and
3 whether the process will be independent from investigators and the
4 sponsor

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7 For quality assurance, the study will be examined at 4 times, before
8 the initiation of clinical trial, after the first patient in, before the last
9 patient in and before the completion of the integrated study report, to
10 determine that it is being conducted in accordance with the protocol
11 and written procedures, independently and separately from the routine
12 activities of monitoring.
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15 Ethics and dissemination

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17 Research ethics 24 Plans for seeking research ethics committee/institutional review board
18 approval (REC/IRB) approval

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21 The study is being conducted in compliance with the principles of the
22 Declaration of Helsinki (1996), the principles of Good Clinical Practice,
23 and all applicable regulatory requirements. Ethics approval is
24 overseen by the Kobe University Institutional Review Board and
25 Osaka Medical and Pharmaceutical University Institutional Review
26 Board and the study is conducted in accordance with the approved
27 protocol.
28
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30 Protocol 25 Plans for communicating important protocol modifications (eg,
31 amendments changes to eligibility criteria, outcomes, analyses) to relevant parties
32 (eg, investigators, REC/IRBs, trial participants, trial registries, journals,
33 regulators)

34
35 Any changes required by the ethics committee will be communicated
36 to the participants by the investigators.
37

38 Consent or assent 26a Who will obtain informed consent or assent from potential trial
39 participants or authorised surrogates, and how (see Item 32)
40
41 Written informed consents are being obtained from all participants
42 before any study procedure is performed. The participants will have
43 the opportunity to review the participant consent form and agree that
44 they fully understand the details of the study procedures. Informed
45 consent will be administered by a suitably qualified and experienced
46 individual who has been delegated this duty by the principal
47 investigator. If a patient aged 20 years or older is not able to consent
48 to participate in this study in writing due to intellectual disability,
49 consent is obtained from the substitute. The protocol was submitted in
50 Japan registry of clinical trial (jRCT).
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54 26b Additional consent provisions for collection and use of participant data
55 and biological specimens in ancillary studies, if applicable
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57 Secondary use of the data will occur only if the patients provide
58 written informed consent for additional use of their data.
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2	Confidentiality	27	How personal information about potential and enrolled participants will
3			be collected, shared, and maintained in order to protect confidentiality
4			before, during, and after the trial
5			We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden),
6			which is an electronic data system for clinical research, to manage the
7			data and protect confidentiality before, during, and after the trial.
8			
9			
10	Declaration of	28	Financial and other competing interests for principal investigators for
11	interests		the overall trial and each study site
12			The authors declare that they have no competing interests.
13			
14	Access to data	29	Statement of who will have access to the final trial dataset, and
15			disclosure of contractual agreements that limit such access for
16			investigators
17			Only the biostatistician will have access to the final dataset.
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20	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
21	post-trial care		compensation to those who suffer harm from trial participation
22			This study is insured for clinical trials, with up to 100 million yen
23			guaranteed for death cases, for example.
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26	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
27	policy		participants, healthcare professionals, the public, and other relevant
28			groups (eg, via publication, reporting in results databases, or other
29			data sharing arrangements), including any publication restrictions
30			The results of the study will be published in a paper.
31			
32			
33		31b	Authorship eligibility guidelines and any intended use of professional
34			writers
35			MT drafted the manuscript and obtained the grant. RO, TF, and TU
36			obtained the grant and reviewed the manuscript. YK managed the
37			study and drafted the manuscript. CN is the chief investigator who
38			conceived and designed the study and obtained the grant funding and
39			drafted the manuscript. SM designed the statistical analysis plan and
40			reviewed the manuscript. NY and SM reviewed the manuscript. All
41			authors provided final approval of the manuscript.
42			Editage (http://www.editage.jp) provided editing of the draft of this
43			manuscript.
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46			
47		31c	Plans, if any, for granting public access to the full protocol, participant-
48			level dataset, and statistical code
49			The full study protocol is available in the supplementary materials and
50			at the Japan Registry of Clinical Trials (jRCT):
51			https://jrct.niph.go.jp/latest-detail/jRCTs2051210181 . Data sharing is
52			not applicable to this study protocol as no datasets were generated.
53			However, the data will be made available from the author on
54			reasonable request once the trial has been completed. Please contact
55			the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).
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Appendices

Informed consent 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 (高校生以上者専用、保護者用) 作成日: 2022 年 6 月 1 日

同意書 医師印

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 NPO-15 の有効性及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 群ランダム化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、覚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、本同意書に当たり、説明文書および同意書を受理しました。

<ul style="list-style-type: none"> ・試験の目的について ・試験の利益について ・試験のリスクについて ・試験の利益とリスクのバランスについて ・試験の利益とリスクのバランスが不明な場合について ・試験の利益とリスクのバランスが不明な場合の他の治療法について 	<ul style="list-style-type: none"> ・試験の中止について ・試験による健康被害に対する治療および補償 ・試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 ・この試験に関する第三者が得られた場合について ・試験に関する費用を負担している保険会社と利益相反について ・その他に守っていただきたいこと ・試験に関する窓口
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ご本人氏名 (署名または記名・捺印) _____

同意日 _____ 年 _____ 月 _____ 日

住所 〒 _____

代読者 (必要時)

氏名 (署名) _____ (試験に参加される方との続柄 _____)

同意日 _____ 年 _____ 月 _____ 日

【関係機関記載欄】

●同意説明医師 (試験責任医師・試験分担医師) _____

同意説明日 _____ 年 _____ 月 _____ 日

●同意確認医師 (試験責任医師・試験分担医師) _____

同意確認日 _____ 年 _____ 月 _____ 日

※試験協力者 (補足的な説明をおこなった場合)

●説明者 _____

説明日 _____ 年 _____ 月 _____ 日

【立代人記載欄 (立代人がいる場合)】 私は説明医師/試験協力者から患者様への説明に立ち会いました。

立代人 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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-068112.R2
Article Type:	Protocol
Date Submitted by the Author:	13-Feb-2023
Complete List of Authors:	Tsujimoto, Mariko; Kobe University Graduate School of Medicine School of Medicine, Dermatology Kakei, Yasumasa; Kobe University Hospital, Clinical & Translational Research Center; Kobe University Graduate School of Medicine School of Medicine, Oral and Maxillofacial Surgery Yamano, Nozomi; Kobe University Graduate School of Medicine School of Medicine, Dermatology Fujita, Takeshi; Kobe University Graduate School of Medicine School of Medicine, Otolaryngology-Head and Neck Surgery Ueda, Takehiro; Hyogo Prefectural Amagasaki General Medical Center, Neurology Ono, Ryusuke; Kobe University Hospital, Dermatology Murakami, Sae; Kobe University Hospital, Clinical and Translational Research Center Moriwaki, Shinichi; Osaka Medical and Pharmaceutical University, Dermatology Nishigori, Chikako; Kobe University Hospital, Dermatology; Hyogo Red Cross Blood Center
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Neurology
Keywords:	Dermatopathology < DERMATOLOGY, Clinical trials < THERAPEUTICS, Neuropathology < NEUROLOGY, Neurogenetics < NEUROLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Speech pathology < OTOLARYNGOLOGY

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5 Pigmentosum Exaggerated Sunburn Reaction Type: XP-1 Study Protocol for a Multicenter,
6 Double-Blinded, Placebo-Controlled, Two-Group Crossover Study followed by a
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8 Long-Term Open Study in Japan
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For peer review only

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.

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4 ✓ The randomization and evaluation of the minimum erythema dose, and the primary
5 endpoint, along with the Efficacy and Safety Evaluation Committee are organized as
6 independent of the investigators.
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10 ✓ The limitations of the study include the duration of the open study, which is not enough
11 for evaluating the new onset of skin cancer, and several genotypes are considered
12 together.
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18 **Keywords:** Xeroderma Pigmentosum, Minimum Erythema Dose, randomized controlled
19 trial, double-blind, crossover, UV irradiation test, NPC-15
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24 **Short title:** Trial of NPC-15 in patients with Xeroderma Pigmentosum sunburn
25 enhancement.
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30 Word count: 3998 words excluding title page, abstract, references, figures, and tables
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1 INTRODUCTION

2 Xeroderma Pigmentosum (XP) is an autosomal recessive genetic disorder
3 associated with severe photosensitivity, freckle-like pigmented and depigmented maculae,
4 and skin cancers in sun-exposed areas. Numerous skin cancers can develop before the age of
5 10 years without strict sun protection. Furthermore, neurological symptoms, such as
6 sensorineural hearing impairment and progressive peripheral and central nervous disorders
7 beginning around 6 years of age, have also been observed in approximately 60% patients [1,
8 2].

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4

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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

1 mentioned in the Introduction section. The “MED” is defined as the minimum dose that
2 elicits very faint, but discernible erythema (NOT USUAL SUNBURN). Usually, MED is
3 measured 24 hours after UV irradiation. However, delay of the erythematous peak is a
4 characteristic of exaggerated sunburn type XP. In this clinical trial, MED at 72 hours will be
5 evaluated; therefore, the irradiation dose can be minimized. The upper limit of the irradiation
6 dose will be set between 30 and 60 mJ/cm², considering the degree of photosensitivity based
7 on the patient’s age (skin thickness) and genetic diagnosis. The irradiated area will be set in
8 a way such that it does not overlap for both the left and right back, during crossover period I
9 and II, respectively.

11 **Secondary endpoint**

12 The secondary efficacy endpoints will be the following:

- 13 (1) Minimum Erythema Dose (MED) at 24 hours, 48 hours, and 96 hours (+/- 6 hours) after
14 UV irradiation on the 15th day (period I and period II) of investigational drug
15 administration.
- 16 (2) Evaluation of melanin index regarding the pigmented area in MED judgment area.
- 17 (3) Pigmented maculae inspection (number, area, and color tone) [15].
- 18 (4) Neurological symptoms (neurologic severity scale score on XP [16], hearing test, and 5
19 m gait test).
- 20 (5) Presence or absence of onset of acute skin symptoms.
- 21 (6) Presence or absence of skin cancer.

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

26 **Eligibility criteria**

27 **Inclusion criteria**

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

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

11 12 **Exclusion criteria**

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

- 14 (1) Patients with a history of allergies to N-acetyl 5-methoxytryptamine or ramelteon.
- 15 (2) Patients receiving other investigational drugs (including placebo) within the 4 months
16 prior to obtaining consent.
- 17 (3) Patients who have been using N-acetyl-5methoxytryptamine (including health foods
18 containing melatonin as the principal component) and Fluvoxamine maleate (Lubox,
19 Depmerol, etc.) in the 4 weeks prior to the start of drug administration.
- 20 (4) Pregnant, lactating women, women who wish to become pregnant during the study
21 period, or women who are fertile and cannot accept an effective contraceptive method.
- 22 (5) Patients deemed inappropriate by the investigators for participation in this clinical study

23 24 **Randomization**

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

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

11 12 **Data collection and management**

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

20 21 **Monitoring and Audit**

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

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

6 **Intervention and treatment protocol**

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

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

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

17 **Statistical analysis**

18 **Analysis set**

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

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

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2 **Statistical analysis**

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

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

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

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

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4 1 The number of adverse events and their frequency and percentage of occurrence will be
5
6 2 summarized according to time, overall currency, and by treatment.

7
8 3 For evaluation of delayed sexual maturation or development, no statistical test will be
9
10 4 performed and secondary sexual characteristics and blood hormone (prolactin) levels in each
11
12 5 patient will be individually described.

13 14 6 15 7 **Sample size calculation**

16
17 8 The primary outcome will be to compare the changes of MED in crossover period I and II
18
19 9 between the two groups. MED of XP patients is approximately 5 to 10 times smaller than
20
21 10 that of healthy controls. For healthy controls, a difference of 10 mJ/cm² in MED is
22
23 11 apparently sufficient in the clinical setting. Therefore, we thought the difference of 1-2
24
25 12 mJ/cm² should be clinically sufficient for XP patients. If we compare irradiation areas on
26
27 13 one side of a child's back, avoiding apparently curved areas, we postulate that 10 areas are
28
29 14 the limit. Trying to evaluate all participants with the same irradiation dose difference and
30
31 15 considering that 10-areas are limit for children, a 3 mJ/cm² difference per area was
32
33 16 considered most appropriate. The minimum effect of improved MED should be larger than
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35 17 this irradiation dose difference. It is reasonable to assume that the difference in MED
36
37 18 between the placebo and actual drug is not as great as the difference in MED between
38
39 19 healthy participants and XP patients; in fact, it is possibly much lower. From the above
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41 20 observation, we estimated the difference in change of MED between the placebo and actual
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43 21 drug to be approximately 4.4 mJ/cm², which was 1/5th of the difference between the mean
44
45 22 MED at 24 hours after irradiation in 7 healthy participants (54.3 mJ/cm²) and 28 XP patients
46
47 23 (31.9 mJ/cm²). We believe that a difference of 4.4 mJ/cm² is sufficient to reduce the
48
49 24 biological effects of UV exposure for XP patients with photosensitivity. The standard
50
51 25 deviation was assumed to be 6.0 mJ/cm², twice the irradiation unit dose for MED
52
53 26 examination. Differences in NPC-15 and placebo in a crossover design can be substituted for
54
55 27 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/cm² and a standard deviation of 6.0 mJ/cm² 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

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

3 As described in the Introduction section, measurement of MED involves unavoidable ethical
4 issues. We explained this point, along with the following points to the participants (or their
5 caregivers/guardians): (1) difficulties other than the MED measurement in assessing the
6 efficacy of XP therapeutics over a certain period of time; (2) that the dose of UV irradiation
7 in this clinical trial was set lower than that of the UV examination conducted at the time of
8 diagnosis; and (3) that we considered the possibility of developing cancer with MED
9 measurements in this trial to be sufficiently low. Before genetic diagnosis became
10 mainstream, at least 50 XP patients with exaggerated sunburn-reaction type underwent more
11 than 60 mJ/cm² UV irradiation on their back for diagnostic purposes in our hospital;
12 however, none of them developed skin cancer on the tested sites, where patients are rarely
13 exposed to the sun.

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

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6 Amagasaki, Japan
- 7 6) Department of Dermatology, Osaka Medical and Pharmaceutical University
- 8 7) Japanese Red Cross Hyogo Blood Center

10 **Acknowledgments**

11 We would like to thank Editage (<http://www.editage.jp>) for English language editing.

13 **Contributors**

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

21 **Funding**

22 This work is supported by the Japan Agency for Medical Research and Development
23 (AMED) under Grant Number JP15ek0109028h0002(CN, MT, RO), 21ek0109450h0002
24 (CN, MT, RO, TF), 21ek0109562h0001 (CN, MT, RO, TF, TU), and by Ministry of Health,

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

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

6 **Competing interests**

7 Nobelpharma Co., Ltd. provided the NPC-15 and placebo during the study period; however,
8 the company had no role in this clinical trial. All authors have no conflicts of interest to
9 declare.
10

11 **Patient consent for publication**

12 Not applicable.

13 **Provenance and peer review**

14 Not commissioned; externally peer-reviewed.

15 **Data availability statement**

16 The full study protocol is available in the supplementary materials and at the Japan Registry
17 of Clinical Trials (jRCT): <https://jrct.niph.go.jp/latest-detail/jRCTs2051210181>. Data sharing
18 is not applicable to this study protocol, as no datasets were generated. However, the data will
19 be made available from the author upon reasonable request once the trial has been completed.
20 Please contact the corresponding author, Dr. Yasumasa Kakei (ykakei@med.kobe-u.ac.jp).
21

22 **Open access**

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1 **FIGURE CAPTIONS**

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

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

5 Figure 1. Flow chart of participants

6

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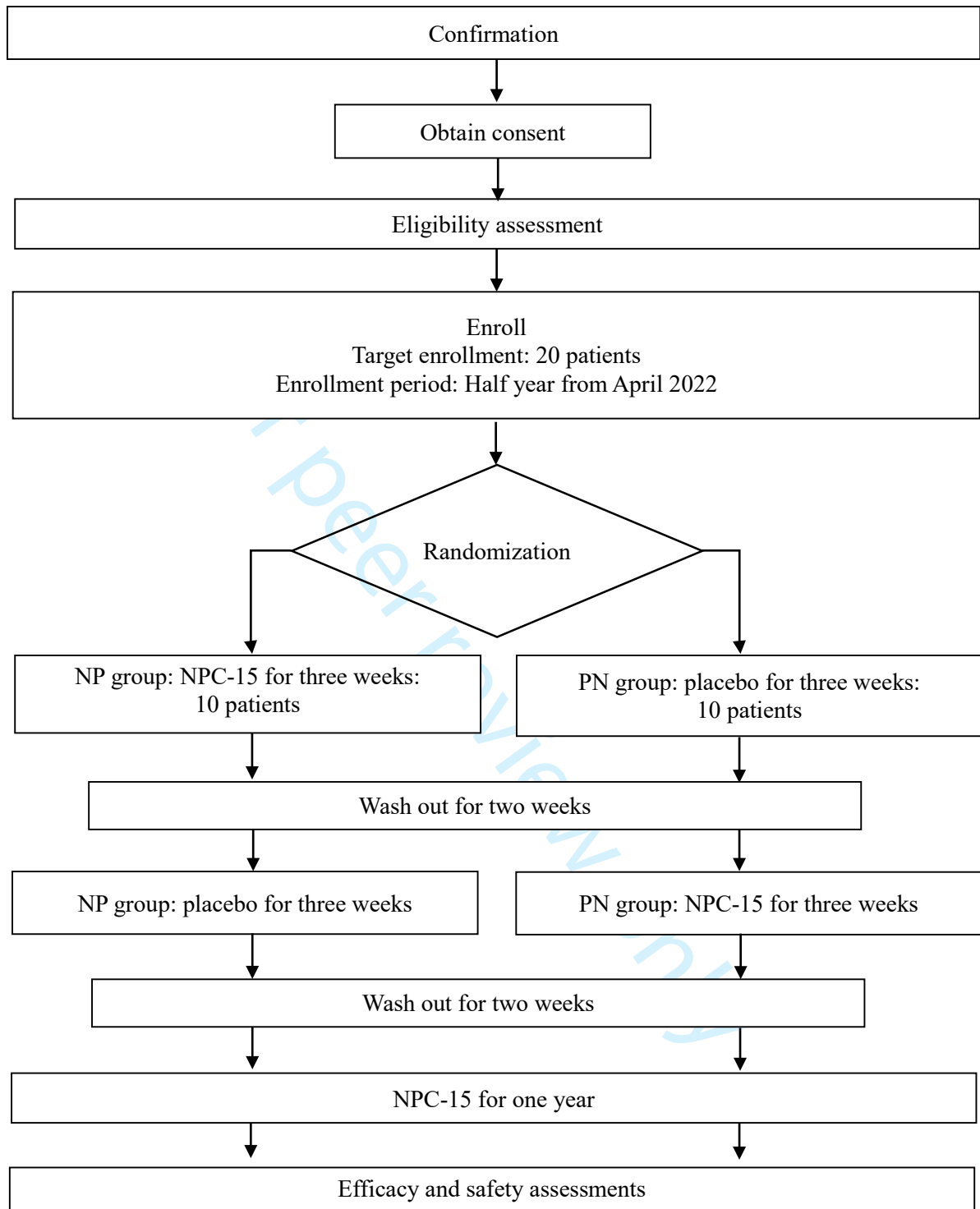


Figure 1. Flowchart of participants.

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

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

	Study period															
	Screening period		Crossover period I						Crossover period II							
			Treatment period				Washout	Treatment period				Washout				
	NPC-15 or Placebo				Placebo or NPC-15											
Enrollment	All	Day 1 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

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

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

4 period is ± 3 days, and the allowable range for the washout period after the crossover study

5 period I and II is $+22/-4$, and ± 4 days, respectively.

6 ² Allowance ($-3\sim+21$) is based on the point of day 35.

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

8 ⁴ Visit tolerance on the UV test day is ± 2 ; however, evaluation should be made at 24, 48,

9 72, and 96 hours ± 6 hours after the test day. Re-evaluation is prohibited.

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

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4 1 ⁶ Methods of hearing test (pure-tone audiometry or conditioned play audiometry) will be
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7 2 recorded in the medical records.
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10 3 ⁷ The 5-meter walk test will be conducted including the patients who wear braces when the
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13 4 principal/participating investigator deems the patient can tolerate the test. The presence or
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16 5 absence of brace or the type of brace used will be recorded in the medical records. When
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19 6 the patient wears the brace, the tests at visit 1 and Visit 305 will be conducted using the
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22 7 same brace as far as possible, and if the brace is changed, the kinds of brace and the reason
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25 8 for the change of brace will be described in the medical records.
26

27 9 ⁸ Data will be collected within 62 weeks prior to administration of the study drug.
28
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30 10 ⁹ Laboratory urine test: oxidative stress markers (Malondialdehyde,
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33 11 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine
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36 12 metabolites (6-sulfatoxymelatonin).
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39 13 ¹⁰ Confirmation of secondary sexual characteristics status and measurement of prolactin
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42 14 levels in blood. To be performed on patients between 10 and 17 years of age.
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45 15 ¹¹ Four weeks prior to the initiation of the study drug.
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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,

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4 8-hydroxy-2-deoxyguanosine, Hexanoyl-Lys) and N-acetyl 5-methoxytryptamine
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7 metabolites (6-sulfatoxymelatonin).
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10 ^f Urine examination: Urinary protein and urinary urobilinogen.
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13 ^g Confirmation of secondary sexual characteristics status and measurement of prolactin
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15 levels in blood. To be performed on patients between 10 and 17 years of age.
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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].

1
2 Roles and
3 responsibilities

5a Names, affiliations, and roles of protocol contributors

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

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

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

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

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29 SM, Clinical and Translational Research Center, Kobe University
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33 SM, Department of Dermatology, Osaka Medical and Pharmaceutical
34 University, Osaka, Japan

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

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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>

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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.

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2		6b	Explanation for choice of comparators
3			The aim of this study is to evaluate the efficacy and safety of the NPC-
4			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
5			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
6			study, 10 weeks; open study, 52 weeks) in patients with XP
7			exaggerated sunburn reaction type.
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10	Objectives	7	Specific objectives or hypotheses
11			The aim of this study is to evaluate the efficacy and safety of the NPC-
12			15 (Nobelpharma, Tokyo, Japan) administered orally before bedtime
13			at a dose of 0.5–4 mg (0.067 mg/kg) per day for 62 weeks (crossover
14			study, 10 weeks; open study, 52 weeks) in patients with XP
15			exaggerated sunburn reaction type.
16			We hypothesized that NPC-15 could contribute to mitigate sunburn
17			reactions and slow down the progress of neurological symptoms in
18			patients with XP exaggerated sunburn reaction type.
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22	Trial design	8	Description of trial design including type of trial (eg, parallel group,
23			crossover, factorial, single group), allocation ratio, and framework (eg,
24			superiority, equivalence, noninferiority, exploratory)
25			This is a multicenter, placebo-controlled, double-blinded, randomized
26			two-group crossover study followed by a long-term open study.
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Methods: Participants, interventions, and outcomes

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31	Study setting	9	Description of study settings (eg, community clinic, academic hospital)
32			and list of countries where data will be collected. Reference to where
33			list of study sites can be obtained
34			This study will be performed at Kobe University Hospital, Kobe, and
35			Osaka Medical and Pharmaceutical University, Osaka, Japan.
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2 Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility
3 criteria for study centres and individuals who will perform the
4 interventions (eg, surgeons, psychotherapists)
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7 Inclusion Criteria

- 8 ✓ Patients with XP (according to the XP diagnostic criteria
9 by the Japan Dermatology Society, 2015) that have been
10 diagnosed with Exaggerated sunburn-reaction type (XP-
11 A, XP-B, XP-D, XP-F, XP-G) by genetic testing.
12
13 ✓ Patients aged 1 year old or older with a weight of 7.5 kg
14 or more at the time of consent. However, patients under 6
15 years of age will be enrolled after confirming the safety
16 for the first 10 cases of the subjects aged 6 years or
17 older during the crossover study by the Safety Evaluation
18 Committee.
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20 ✓ Patients (or their caregivers/guardians) who have
21 provided written informed consent to participate in this
22 study.
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30 Exclusion Criteria

- 31 ✓ Patients with a history of allergies to N-acetyl 5-
32 methoxytryptamine or ramelteon.
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34 ✓ Patients receiving other investigational drugs (including
35 placebo) within the 4 months prior to obtaining consent.
36
37 ✓ Patients who have been using N-acetyl-
38 5methoxytryptamine (including health foods containing
39 melatonin as the principal component) and Fluvoxamine
40 maleate (Lubox, Depmerol, etc.) in the 4 weeks prior to
41 the start of drug administration.
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43 ✓ Patients who are pregnant or may become pregnant.
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45 ✓ Patients judged by the investigator to be ineligible for this
46 study.
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- 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 administered 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.

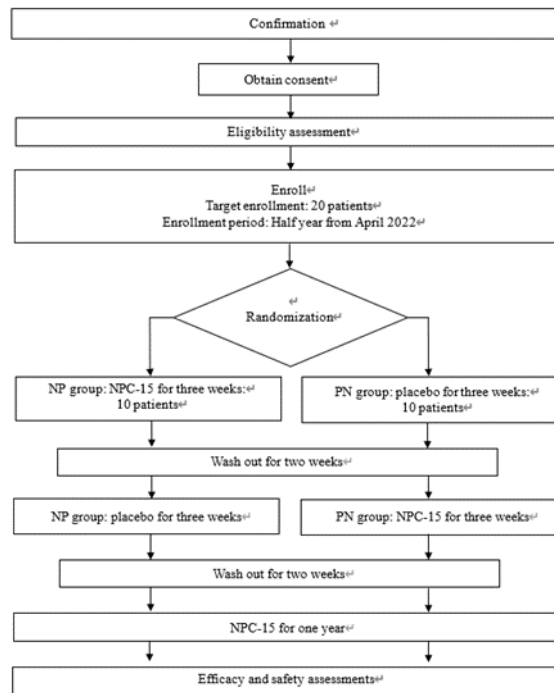
Outcomes

- 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 XP¹⁶, 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.

view only

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/cm², which was 1/5th of the difference between the mean MED at 24 hours after irradiation in 7 healthy subjects (54.3 mJ/cm²) and 28 XP patients (31.9 mJ/cm²). We believe that a difference of 4.4 mJ/cm² 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/cm² 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/cm² between the actual drug and placebo in MED after irradiation and a standard deviation of 6.0 mJ/cm², 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%.

1
2 Recruitment 15 Strategies for achieving adequate participant enrolment to reach
3 target sample size
4 All subjects who provide consent to participate and who fulfil the
5 inclusion criteria and who do not meet any of the exclusion criteria will
6 be randomized.
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9 **Methods: Assignment of interventions (for controlled trials)**

10 Allocation:

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12 Sequence 16a Method of generating the allocation sequence (eg, computer-
13 generation generated random numbers), and list of any factors for stratification.
14 To reduce predictability of a random sequence, details of any planned
15 restriction (eg, blocking) should be provided in a separate document
16 that is unavailable to those who enrol participants or assign
17 interventions
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19 All subjects who provide consent to participate and fulfill the sampling
20 criteria will be randomized. Subjects will be randomly assigned to
21 either the NP group or the PN group with a 1:1 allocation using the
22 permutation random block method stratified by category (whether XP
23 genotype is XP-A or not). The block size will not be disclosed to
24 ensure that blinding is maintained. The allocation sequence for the
25 randomization method will be generated by a person in charge from
26 DOT world company, CRO. The trial participants, care providers, and
27 outcome assessors will be blinded.
28

29 Allocation 16b Mechanism of implementing the allocation sequence (eg, central
30 concealment telephone; sequentially numbered, opaque, sealed envelopes),
31 mechanism describing any steps to conceal the sequence until interventions are
32 assigned
33

34 Either the principal or sub-investigator will send a subject enrollment
35 form by EDC to the data center. The staff at the data center will
36 confirm the subject's eligibility and issue the subject enrollment
37 confirmation form that contains the eligibility judgment, the
38 randomization assignment result from the generated random
39 sequence, and the enrollment number. The form will then be sent to
40 the principal investigator or sub-investigator.
41

42 Implementation 16c Who will generate the allocation sequence, who will enrol participants,
43 and who will assign participants to interventions
44

45 The allocation sequence for the randomization method will be
46 generated by a person in charge from DOT world company, CRO.
47 Either the principal or sub-investigator will send a subject enrollment
48 form by EDC to the data center. The staff at the data center will
49 confirm the subject's eligibility and issue the subject enrollment
50 confirmation form that contains the eligibility judgment, the
51 randomization assignment result from the generated random
52 sequence, and the enrollment number. The form will then be sent to
53 the principal investigator or sub-investigator.
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- 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

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- 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.

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- 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.

For peer review only

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.

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- 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.

17 Methods: Monitoring

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- 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.

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

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17 Research ethics 24 Plans for seeking research ethics committee/institutional review board
18 approval (REC/IRB) approval
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21 The study is being conducted in compliance with the principles of the
22 Declaration of Helsinki (1996), the principles of Good Clinical Practice,
23 and all applicable regulatory requirements. Ethics approval is
24 overseen by the Kobe University Institutional Review Board and
25 Osaka Medical and Pharmaceutical University Institutional Review
26 Board and the study is conducted in accordance with the approved
27 protocol.
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30 Protocol 25 Plans for communicating important protocol modifications (eg,
31 amendments changes to eligibility criteria, outcomes, analyses) to relevant parties
32 (eg, investigators, REC/IRBs, trial participants, trial registries, journals,
33 regulators)
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35 Any changes required by the ethics committee will be communicated
36 to the participants by the investigators.
37

38 Consent or assent 26a Who will obtain informed consent or assent from potential trial
39 participants or authorised surrogates, and how (see Item 32)
40 Written informed consents are being obtained from all participants
41 before any study procedure is performed. The participants will have
42 the opportunity to review the participant consent form and agree that
43 they fully understand the details of the study procedures. Informed
44 consent will be administered by a suitably qualified and experienced
45 individual who has been delegated this duty by the principal
46 investigator. If a patient aged 20 years or older is not able to consent
47 to participate in this study in writing due to intellectual disability,
48 consent is obtained from the substitute. The protocol was submitted in
49 Japan registry of clinical trial (jRCT).
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54 26b Additional consent provisions for collection and use of participant data
55 and biological specimens in ancillary studies, if applicable
56 Secondary use of the data will occur only if the patients provide
57 written informed consent for additional use of their data.
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2	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
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5			We will use Viedoc™ (Viedoc Technologies AB, Uppsala, Sweden),
6			which is an electronic data system for clinical research, to manage the
7			data and protect confidentiality before, during, and after the trial.
8			
9			
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
11			
12			The authors declare that they have no competing interests.
13			
14	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
15			
16			Only the biostatistician will have access to the final dataset.
17			
18			
19			
20	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
21			
22			This study is insured for clinical trials, with up to 100 million yen
23			guaranteed for death cases, for example.
24			
25			
26	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
27			
28			The results of the study will be published in a paper.
29			
30			
31			
32			
33		31b	Authorship eligibility guidelines and any intended use of professional writers
34			
35			MT drafted the manuscript and obtained the grant. RO, TF, and TU
36			obtained the grant and reviewed the manuscript. YK managed the
37			study and drafted the manuscript. CN is the chief investigator who
38			conceived and designed the study and obtained the grant funding and
39			drafted the manuscript. SM designed the statistical analysis plan and
40			reviewed the manuscript. NY and SM reviewed the manuscript. All
41			authors provided final approval of the manuscript.
42			Editage (http://www.editage.jp) provided editing of the draft of this
43			manuscript.
44			
45			
46			
47			
48		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
49			
50			The full study protocol is available in the supplementary materials and
51			at the Japan Registry of Clinical Trials (jRCT):
52			https://jrct.niph.go.jp/latest-detail/jRCTs2051210181 . Data sharing is
53			not applicable to this study protocol as no datasets were generated.
54			However, the data will be made available from the author on
55			reasonable request once the trial has been completed. Please contact
56			the corresponding author, Dr. Y Kakei (ykakei@med.kobe-u.ac.jp).
57			
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Appendices

Informed consent 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

医師印

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 NPO-15 の有効性及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 群ランダム化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、覚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、本同意書にあり、説明文書および同意書を受理しました。

<ul style="list-style-type: none"> ・試験の目的 ・試験の目的について ・追加の負担について ・試験の目的について ・試験の目的について ・試験の方法について ・適応症外観と参加人数、参加予定期間について ・試験により予見される効果（利益）と不利益について ・この試験に参加しない場合の他の治療法について 	<ul style="list-style-type: none"> ・試験について ・試験の中止について ・試験による健康被害に付する治療および補償 ・試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 ・この試験に付する謝辞が得られた場合について ・試験に付する費用を供給している製薬会社と利益相反について ・おなじく守っていただきたいこと ・試験に関する窓口
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ご本人氏名（署名または捺印） _____
 同意日 年 月 日

住所 〒 _____

代読者（必要時）
 氏名（署名） _____（試験に参加される方との続柄 _____）
 同意日 年 月 日

【監修機関監修】

●同意説明医師（試験責任医師・試験分担医師） _____
 同意説明日 年 月 日

●同意確認医師（試験責任医師・試験分担医師） _____
 同意確認日 年 月 日

※試験協力者（補足的な説明をおこなった場合）

●説明者 _____
 説明日 年 月 日

【立代人監修（立大人がいる場合）】 私は説明医師/試験協力者から患者様への説明に立ち会いました

立代人 氏名 _____（患者様との関係 _____）
 立合日 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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.