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# Safety and efficacy of oral cancer vaccine B440 in patients with PD-1/PD-L1 inhibitor-resistant advanced urothelial cancer: a study protocol for a phase 1 multicenter, open-label, single-arm clinical trial

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## Abstract

**Background** This is a multicenter, open-label, single-arm clinical trial to evaluate the safety and efficacy of oral cancer vaccine B440 in patients with PD-1/PD-L1 inhibitor-resistant advanced urothelial cancer.

**Methods** The trial will be performed at three university hospitals in Japan. The target number of patients will be 12. The patients will be treated orally with B440 once daily for 5 days followed by 2 days for four consecutive courses (4 weeks, 20 treatments). The low-dose group will receive 800 mg (4 capsules) per dose and the high-dose group will receive 1,600 mg (8 capsules) per dose. The primary outcome will be the number and incidence of DLT cases the start of treatment and Day 28. Secondary outcomes are the presence or absence of a response, the best overall response and PFS.

**Discussion** If this trial shows B440 to be safe and effective, it may lead to a late phase randomized controlled trial in advanced urothelial cancer. Ultimately, we hope to provide a new treatment option for such patients.

**Trial registration** Japan Registry of Clinical Trials (jRCT) identifier: jRCT2051220143. Registered on December 27, 2022.

**Keywords** B440, WT-1 protein, Clinical trial, advanced urothelial cancer, ICI, Phase 1

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## Introduction

More than 90% of cancers of the renal pelvis, ureter, and bladder are urothelial carcinomas arising from the urothelial mucosa. Squamous cell carcinoma, adenocarcinoma, and small cell carcinoma are less common. In 2018, the number of patients with bladder cancer in Japan was estimated to be 20,800 [1] and 549,393 worldwide [2]. For patients with metastatic or recurrent advanced unresectable urothelial carcinoma, platinum-based M-VAC (methotrexate, vinblastine, doxorubicin, cisplatin) and GC (gemcitabine, cisplatin) are generally used to prolong life [3], but the response rate is about 50%, and the number of long-term survivors is low [4]. Also, the toxicity of these systemic chemotherapies is high and they cannot be administered to patients with reduced renal function or ureteral cancer after total nephroureterectomy due to their nephrotoxicity [5]. Cancer immunotherapy (IO: Immuno-Oncology) has been used for urothelial carcinoma since the 1970s to prevent recurrence of intermediate- to high-risk bladder cancer, which frequently recurs, and to treat carcinoma in situ (CIS). Again, the side effects, including symptomatic bladder irritation, are strong and the effect is limited to the bladder area [6]. Recently, the efficacy of an anti-PD-1 antibody (pembrolizumab) for advanced urothelial carcinoma after platinum-based chemotherapy was confirmed in a phase III clinical trial, and pembrolizumab was approved for urothelial carcinoma in Japan. However, the response rate to pembrolizumab in that trial was 21.1%, and no correlation between PD-L1 expression in tumor tissue and drug sensitivity was observed [7]. Currently, PD-1/PD-L1 inhibitors such as anti-PD-1 antibody (pembrolizumab) and anti-PD-L1 antibody (avelumab) [8] are approved in Japan for the treatment of metastatic or recurrent advanced urothelial cancer. Enfortumab vedotin [9] has been approved for patients who have become resistant to these immune checkpoint inhibitors. However, there remains an unmet need for combination agents that improve the response rate to PD-1/PD-L1 inhibitors and for the development of novel therapies for PD-1/PD-L1 inhibitor-refractory patients.

In recent years, cancer immunotherapy has rapidly gained popularity in clinical practice due to the commercialization of such immune checkpoint inhibitors (ICIs) as anti-PD-1 antibodies [10]. On the other hand, the fundamental principle of cancer immunotherapy is the induction of cancer-specific tumor immunity, and the development of "cancer vaccines" that can artificially induce tumor immunity remains highly important [11]. The response rate when ICIs are used alone is about 30% [12–14], and complex cancer immunotherapies combining therapies with different points of action are expected to become the mainstream treatment in the future. Theoretically, combination therapy, such as inactivation of tumor-associated antigen (TAA)-specific cytotoxic T lymphocytes (CTLs) induced by cancer vaccines and inhibition of TAA-specific CTLs by ICIs, can be expected to have a significant synergistic effect [14], and there is an increasing need for the development of cancer vaccines as combination drugs to improve the response rate and therapeutic outcome of ICIs.

Interaction between bifidobacteria and the intestinal immune system has been studied increasingly in recent years. An experiment in which bifidobacteria were orally administered to mice confirmed bacteria in Peyer's patches within 1 hour and migration with dendritic cells to the mesenteric lymph nodes (MLN) within 22 hours [15]. To date, bacterial vectors that have been used as vaccines and cancer drugs include Salmonella, Listeria, and Cholera as attenuated pathogens, and Lactobacillus probiotics [16]. In particular, a vaccine that expresses the E7 protein of human papillomavirus using lactic acid bacteria as a platform has successfully induced antigen-specific immune responses in a clinical study of cervical intraepithelial neoplasms (CIN) [17]. We have developed an oral vaccine platform using Bifidobacterium longum as an antigen delivery system to the intestinal immune system. In this platform, antigen proteins are expressed on the surface layer of Bifidobacterium longum by the anchor protein gltA (GL-BP protein) derived from Bifidobacterium longum. Bifidobacteria expressing antigen protein on the surface layer are specifically taken up by M cells in the intestinal epithelium and can deliver antigen protein to intestinal-associated lymphoid tissues including Peyer's patches with high efficiency, making them an ideal platform for oral cancer vaccines in terms of safety. To date, we have produced several oral vaccines and succeeded in inducing strong cellular immunity in animal experiments [18, 19].

In this study, we produced a WT1 oral cancer vaccine using this oral vaccine platform, obtained promising data in animal experiments [20], and started clinical development. We have produced a variety of genetically engineered bifidobacteria expressing approximately 70% of the WT1 antigen protein (B. longum 420; mouse WT1 antigen protein expression, B. longum 440; human WT1 antigen protein expression, B440; lyophilized powder of B. longum 440 inactivated by the present experimental drug) as a WT1 oral cancer vaccine [20–22], and developed B. longum 2012, which expresses only GL-BP protein on the surface layer, as a control group for these drugs. We confirmed the high antitumor efficacy of this oral cancer vaccine in combination with anti-PD-1 antibody and its very high antitumor efficacy compared to WT1 peptide vaccine in a mouse prostate cancer model [20, 22]. These results provide a strong rationale for the

clinical development of a safe oral cancer vaccine with efficacy in PD-1/PD-L1 inhibitor-resistant patients, as a novel cancer immunotherapeutic agent for advanced urothelial carcinoma.

## Methods and analysis

### Study design/setting

This study is a non-randomized, prospective, open-label, multicenter clinical trial for patients with PD-1/PD-L1 inhibitor-resistant advanced urothelial cancer that commenced on January 1, 2023. The expected date of completion (final visit of the last patient) is the end of December 2024. A summary of the study is presented in Fig. 1. This study will be performed at three university hospitals in Japan, Kobe University Hospital, Hamamatsu University Hospital, and Hiroshima University Hospital. The follow-up period will continue for half a year after the date of registration. This study protocol follows the SPIRIT statement. All study data will be stored and archived in the data center of DOT World Co., Ltd. using Viedoc (Viedoc Technologies AB, Uppsala, Sweden) to manage the data and protect confidentiality before, during and after the trial.

### Sample size calculation

A total of 12 patients, 6 in each dose group, are planned. The sample size was set based on Target Toxicity Level of 33% for the development of anti-neoplastic agents. This sample size allows us to detect if the incidence rate of DLT exceeds 33%, at which point the study can be safely discontinued. If the incidence rate of DLT is 33%, the probability of observing adverse events in at least 2 out of 6 patients (discontinuation criteria) is calculated as $1-((6\times(1-0.33)^5)\times(0.33)+(1-0.33)^6)=0.649$. Therefore, we considered that the study could be safely discontinued if the incidence rate of DLT exceeds 33%.

## Study population

### Inclusion criteria

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

(1) Patients with histologically confirmed urothelial carcinoma.
(2) Patients with urothelial carcinoma resistant or intolerant to PD-1/PD-L1 inhibitors.
(3) Patients with unresectable urothelial carcinoma refractory or intolerant to standard treatment (including Enfortumab vedotin).
(4) Patients diagnosed with at least 1 measurable lesion (including lymph node lesion) based on RECIST (Response Evaluation Criteria in Solid Tumors) ver1.1 that has not been irradiated.
(5) Adverse events associated with prior treatment have resolved to Grade 1 or less in CTCAE (Common Terminology Criteria for Adverse Events)

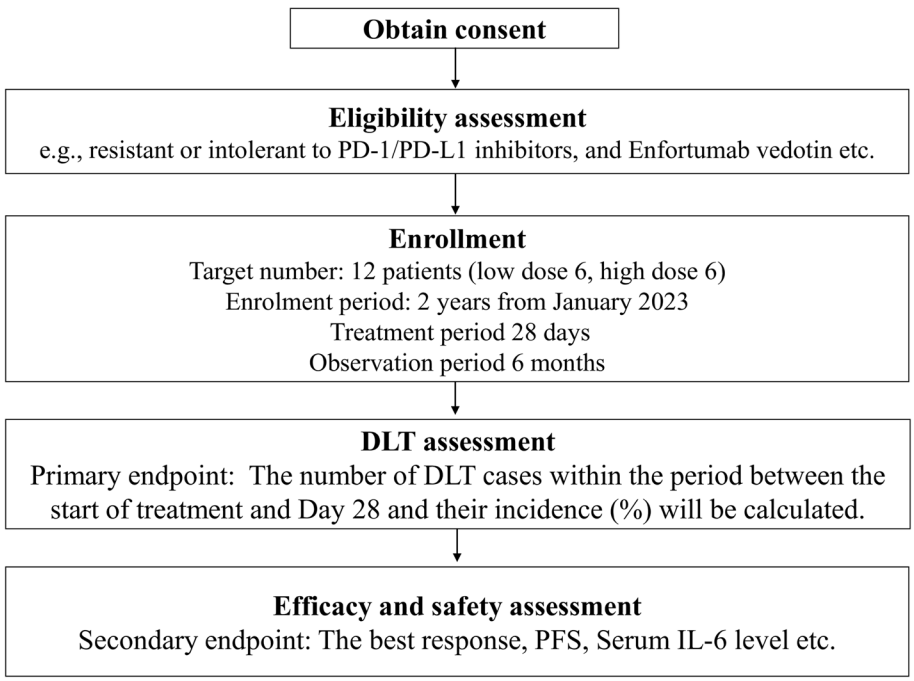


**Fig. 1** Summary of the study protocol

v5.0-JCOG (Japan Clinical Oncology Group); or Grade 2 or higher symptoms (including hair loss, peripheral sensory neuropathy, skin hyperpigmentation, dysgeusia) are stable.

(6) Eastern Cooperative Oncology Group performance status 0–1.
(7) Age between 18 and 85 at the time of informed consent.
(8) Patients must provide written informed consent for participation in this study.
(9) The test value within 14 days before registration satisfies all the following test criteria, and no blood transfusion has been performed within 14 days inclusive before the examination.

a. Neutrophil count 1.5 x10^3/μL or more
b. Hemoglobin 8.0g/dL or more
c. Platelet count 75 x10^3/μL or more
d. Total bilirubin 1.5 mg/dL or less
e. AST (GOT) 100 U/L or less, ALT (GPT) 100 U/L or less
f. Serum creatinine 1.5mg/dL or less, or calculated creatinine clearance (Cockcroft-Gault formula) 50mL/min or more
g. $SpO_2$ 93 percent or more

#### Exclusion criteria

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

(1) Patients who have received anticancer drugs and other therapeutic drugs within 28 days before registration.
(2) Patients who have an infection requiring systemic treatment.
(3) Patients who have serious complications (intestinal paralysis, intestinal obstruction, pulmonary fibrosis, uncontrolled diabetes, heart failure, myocardial infarction, angina pectoris, renal failure, liver failure, psychiatric disease, cerebrovascular disease, ulcer required transfusion).
(4) Patients with a history or complication of interstitial pneumonia requiring treatment.
(5) Patients with severe mental disorders.
(6) Patients who are pregnant or possibly pregnant diagnosed by doctor interview, or breast-feeding. Breast-feeding women who discontinue breast-feeding are not allowed to be enrolled in this study.
(7) Patients with no intention of conception with his/her partner from the start of administration of B440 to 180 days after the last dose of the drug in males, or 154 days after the last dose of the drug in females who may become pregnant.
(8) Patients of childbearing potential who are unable to perform a pregnancy test at screening.
(9) Patients with history of serious drug allergy.
(10) Patients who are positive for either HBs antigen or HCV antibody in a test within 6 months before the enrollment.
(11) Patients who are positive for HIV antibody in past tests.
(12) Patients who are receiving continuous systemic administration (oral or intravenous) of steroids or other immunosuppressive agents (except for immune-related adverse events caused by immune checkpoint inhibitors).
(13) Patients who have complications of autoimmune disease or a history of chronic or recurrent autoimmune disease (except immune-related adverse events caused by immune checkpoint inhibitors).
(14) Patients who have a history of hypersensitivity to the ingredients of this drug or bifidobacteria protein.
(15) Patients who are inappropriate to participate in this study at physician's discretion.

#### Intervention

This study is a dose-escalation study using a modified 3 + 3 design. Six cases will constitute one cohort, and the dose will be increased to next stage according to the number of cases with the onset of DLT. After screening for eligibility registration, the patients will be treated orally with B440 once daily for 5 days with 2 days of rest for four consecutive courses (4 weeks, 20 times). The low-dose group will be 800 mg (4 capsules) per dose and the high-dose group will be 1,600 mg (8 capsules) per dose. The administration should be on an empty stomach, defined as at least 2 hours after a meal and at least 1 hour before the next meal. The administration interval should be at least 20 hours. A summary of other study outcomes, assessments, and procedures is presented in Table 1. Anticancer drugs, radiation therapy, hyperthermia, immunosuppression therapy, and granulocyte colony-stimulating factor are prohibited as concomitant medications and therapies.

## Outcomes

#### Primary outcome

DLT: The number of DLT cases within the period between the start of treatment and Day 28 and their incidence (%) will be calculated.

**Table 1** Data collection schedule

| | **Before medication** | | | **Treatment Period[e] (Day1 ~28)** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Screening[g]** | **Baseline** | | | | | | | | | | | | | | |
| Day | | | -1 | 1 | 2 | 3 | 4 | 5 | 8 | 9 | 10 | 11 | 12 | 15 | 16 | 17 |
| Visit window (Day): | -28~ | -14~ | -3 | - | - | - | - | - | +2 | - | - | - | - | ±2 | - | - |
| Visit No. | 100-1 | 100-2 | | 101 | 102 | 103 | 104 | 105 | 201 | | | | | 301 | | |
| Obtain consent | • | | | | | | | | | | | | | | | |
| Registration | | • | | | | | | | | | | | | | | |
| Medication[a] | | | | • | • | • | • | • | • | • | • | • | • | • | • | • |
| Patient characteristics | • | | | | | | | | | | | | | | | |
| Body weight Vital Sign[b] | • | | • | • | • | • | • | • | • | | | | | • | | |
| PS [b] | • | | • | • | • | • | • | • | • | | | | | • | | |
| Adverse event | | | | → | → | → | → | → | → | → | → | → | → | → | → | → |
| Prohibited concomitant medications and therapies | • | | • | → | → | → | → | → | → | → | → | → | → | → | → | → |
| Laboratory test | • | | • | | • | • | | • | • | | | | | • | | |
| Thyroid function test | • | | | | | | | | • | | | | | • | | |
| Urine test[b] | • | | • | | • | • | | • | • | | | | | • | | |
| Electrocardiogram[b] | • | | • | | • | | | • | • | | | | | • | | |
| Examination for infectious diseases[c] | • | | | | | | | | | | | | | | | |
| Chest X-ray[d] | • | | | | | | | | | | | | | | | |
| Contrast CT or MRI[d] | • | | | | | | | | | | | | | | | |
| Pregnancy test[f] | • | | | | | | | | | | | | | | | |
| Blood test for immune analysis | | | • | | | | | | | | | | | • | | |

**Table 1** (continued)

| | Before medication | | Treatment Period[e] (Day1 ~ 28) | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screening[g] | Baseline | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Stool collection for intestinal bacteria and physicochemical analysis | | | [j] | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Immunohistological examination of WT1 antigen protein[k] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Confirmation of medication status | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Table 1** (continued)

| | Treatment Period[e](Day1～28) | | | | | | | | Observation period (Day 29～169) | | | | | | Cancel protocol treatment | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | 18 | 19 | 22 | 23 | 24 | 25 | 26 | Visiting day | 29 | 57 | 85 | 113 | 141 | 169 | Time of discontinuance [h] | 28 days after the date of discontinuation[h] |
| Visit window (Day): | - | - | ±2 | - | - | - | - | Visit window (day): | +3 | ±5 | ±14 | ±14 | ±14 | ±14 | +5 | ±7 |
| Visit No. | | | 401 | | | | | Visit no. | 501 | 502 | 503 | 504 | 505 | 506 | 901 | 902 |
| Obtain consent | | | | | | | | Body weight Vital sign[b] | • | • | • | • | • | • | • | • |
| Registration | | | | | | | | PS[b] | • | • | • | • | • | • | • | • |
| Medication [a] | • | • | • | • | • | • | • | Adverse event | → | → | → | → | → | → | → | → |
| Patient characteristics | | | | | | | | Prohibited concomitant medications and therapies | → | → | → | → | → | → | → | → |
| Body weight Vital Sign [b] | | | • | | | | | Laboratory test [b] | • | • | • | • | • | • | • | • |
| PS [b] | | | • | | | | | Thyroid function test | • | | • | | • | • | | • |
| Adverse event | → | → | → | → | → | → | → | Urine test [b] | • | • | • | • | • | • | • | • |
| Prohibited concomitant medications and therapies | → | → | → | → | → | → | → | Electrocardiogram [b] | • | | • | | • | • | | • |
| Laboratory test | | | • | | | | | Examination for infectious disease [c] | | | | | | | | |
| Thyroid function test | | | | | | | | Chest X-ray [d] | (•) | (•) | | (•) | | (•) | | |
| Urine test [b] | | | • | | | | | Contrast CT or MRI [d] | (•) | (•) | | (•) | | (•) | | (•) |
| Electrocardiogram [b] | | | | | | | | Pregnancy test [f] | | | | | | | | |
| Examination for infectious diseases[c] | | | | | | | | Blood test for immune analysis | • | • | | • | | • | | • |

**Table 1** (continued)

| Treatment Period[e](Day1～28) | | Observation period (Day 29～169) | | Cancel protocol treatment |
|---|---|---|---|---|
| Chest X-ray[d] | | Stool collection for intestinal bacteria and physicochemical analysis | ↵ ↵ | |
| Contrast CT or MRI[d] | | Immunohistological examination of WT1 antigen protein [k] | | |
| Pregnancy test[f] | | Confirmation of medication status | • | • |
| Blood test for immune analysis | | | | |
| Stool collection for intestinal bacteria and physicochemical analysis | | | | |
| Immunohistological examination of WT1 antigen protein[k] | | | | |
| Confirmation of medication status | • | | | |

[a] If the subject's visit to the hospital is delayed, the medication should be staggered. On the day of the visit, take the medication after the [a]If the subject's visit to the hospital is delayed, the medication should be staggered. On the day of the visit, take the medication after the consultation.

[b] If discharged after the 9th day of administration, confirm 2 days prior to discharge and on the day of discharge.

[c] Allow test results within 6 months prior to registration.

[d] Conduct as needed.

[e] Evaluate up to 169 days after the start of the medication of B440, even if the drug is discontinued.

[f] Performed only on subjects of childbearing potential.

[g] Screening laboratory data can be substituted as baseline data if they are within 4 days prior to the start of drug administration.

[h] At the time of discontinuation of the clinical trial, if Visit 505 is completed, the subject will be tested for Visit 506.

[i] If a stool sample cannot be collected during the baseline examination, it will still be collected during Visit 501 and Visit 502.

[j] If stool samples could not be collected at Visit 501 and Visit 502 after the start of study drug administration, if possible, the most recent fecal sample after each Visit should be collected and stored in the freezer and submitted at the next clinic visit.

[k] If the data have already been obtained prior to the start of this study, the data will be used and no additional tests will be performed.

### Secondary outcomes

(1) Presence or absence of a complete response (CR) or partial response (PR) as best overall response recorded from the start of treatment until the end of the trial. In this study, the 4-week confirmation period for determining the best overall response is not required.
(2) Progression-Free Survival (PFS): The period from the registration date to the earlier of the date of imaging that indicates progression or the date of death by any cause. 'Progression' is determined based on Progressive Disease according to the RECIST.
(3) Serum IL-6 level.

### Exploratory outcomes

(1) WT-1 protein-specific T-cell response (ELISPOT assay, intracellular cytokine immunostaining, Simultaneous multiple cytokine quantification).
(2) CD8 T cell induction specific to WT-1 epitopes in peripheral blood (Tetramaer assay HLA-A02:01 RMFPNAPYL or HLA-A 24:02 CYTWNQMNL, but not performed if HLA is neither type).
(3) Bacterial composition of the intestinal flora (Microbiome metagenomic analysis using next-generation sequencing).
(4) Fecal organic acids (Fecal physiochemical analysis).
(5) WT-1 protein expression in tumor cells (immunohistological examination of WT-1 antigen protein).

### Patient enrolment and data management

Patients will be recruited from January 1, 2023, to June 30, 2024, at three university hospitals in Japan, namely, Kobe University Hospital, Hamamatsu University Hospital, and Hiroshima University Hospital. All patients who provide consent to participate, fulfill the inclusion criteria, and do not meet any of the exclusion criteria will be enrolled. The data center will issue the patient enrolment confirmation form that contains the eligibility judgment after the data center confirms the patient's eligibility. The primary investigator or sub-investigator will enter the case report form (CRF) data for each patient. The principal investigator will confirm that the entered CRF data are complete and correct, sign the CRF, and copy the signed CRF for filing. The primary investigator will retain the CRF printout. If there are any queries about the CRF data that are entered by the staff at the data center, the primary investigator or sub-investigator should promptly respond to the queries.

### Analysis population

The analysis populations for efficacy are the full analysis set (FAS). The FAS will also serve as the analysis for safety population (SP). The FAS is defined as all patients enrolled in this study and administered at least one dose of B440. The population evaluated for DLT will be defined by excluding certain patients from the FAS. The specific exclusions were as follows: 1) patients in which the total amount administered was less than 80% of the prescribed total amount due to reasons other than the occurrence of adverse events. 2) Patients in which the prescribed tests and observations were not performed at all. 3) Patients in which prohibited concomitant drugs or therapies were administered.

All analyses for efficacy will be carried out with the FAS, and analyses for safety, except for DLT, will be performed in SP.

### Data handling and analysis procedures

The handling of the enrolled patients for analysis will be determined by discussion among the coordinating investigators, committee, and chief of statistical analysis before data lock. If data are missing, they will not be inputted for analysis of the primary outcome. All cases will be analyzed after data are fixed.

### Primary analysis of primary outcome

We will make a list of the DLT by patients by the name of DLT. Additionally, we will calculate the proportion and its 95% confidence interval of the patients with DLT in the population evaluated for DLT in each dose group. As a reference, we will calculate the one-sided p-value under the null hypothesis that the "DLT occurrence proportion is over 33%." The 33% is set as the Target Toxicity Level in phase I trials of antineoplastic drug development. The p-value is calculated based on an exact calculation using the binomial distribution.

### Analysis of secondary outcomes

We will calculate objective response rate (ORR) as a proportion of the CR or PR as the best overall response along with its 95% confidence interval and 80% confidence interval. The interval estimation of ORR will use Wilson's confidence interval based on the binomial distribution. PFS will be estimated using the Kaplan-Meier method, and 95% confidence intervals will be calculated using Greenwood's method. Descriptive statistics will be performed for Serum IL-6 level.

### Analysis for safety outcomes

For safety analysis, all adverse events will be listed by term, drug dosage, and grade.

### Exploratory outcomes

Descriptive statistics will be conducted for the exploratory outcomes and interval estimation will be performed.

### Monitoring and auditing

Periodic monitoring of the study will be performed to ensure that the human rights and welfare of patients are protected and that the reported trial data are accurate, complete, and verifiable from source documents. The study will be safely conducted in accordance with the protocol and the applicable regulatory requirements under the Good Clinical Practice. The coordinating investigator will appoint monitors for the study. The items to be checked at monitoring are specified in the "Written procedure for implementation of study monitoring". For quality assurance, an audit will be performed in the study.

### 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. As mentioned in the individual consent form, participants may obtain access to the final results of the study through the principal investigator.

## Discussion

This study aims to determine the clinical activity of B440 in patients with incurable advanced urothelial cancer. If this study can demonstrate that B440 is both effective and safe, it may lead to a future randomized trial to create evidence for a systemic treatment option for a patient population that currently lacks options and is often excluded from trial participation. Results of B440 treatment will be disseminated to patients and clinical teams through peer-reviewed journal publications and by engaging with relevant patient organizations.

### Trial status

The study period of this trial began the day that it was released by the Japan Registry of Clinical Trials (jRCT), December 27, 2022; the participant entry period will begin the day it was released by jRCT and continue to October 30, 2024. The study follow-up will be completed by March 31, 2025.

### Abbreviations

| | |
|---|---|
| AE | Adverse Event |
| AMED | Japan Agency for Medical Research and Development |
| B440 | Lyophilized powder of a recombinant Bifidobacterium longum displaying a partial human WT1 protein |
| CD4 | Cluster of Differentiation 4 |
| CD8 | Cluster of Differentiation 8 |
| CIN | Cervical Intraepithelial Neoplasm |
| CIS | Carcinoma in Situ |
| CR | Complete Response |
| CRF | Case Report Form |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTLs | Cytotoxic T Lymphocytes |
| DLT | Dose Limiting Toxicity |
| ELISPOT | Enzyme linked Immunospot |
| FAS | Full Analysis Set |
| GCP | Good Clinical Practice |
| GL-BP | galacto-N-biose-/lacto-N-biose I-binding protein |
| HLA | Human Leukocyte Antigen |
| ICIs | Immune Checkpoint Inhibitors |
| JCOG | Japan Clinical Oncology Group |
| MLN | Mesenteric Lymph Node |
| MRI | Magnetic Resonance Imaging |
| ORR | Objective Response Rate |
| PCR | Polymerase Chain Reaction |
| PD | Progressive Disease |
| PD-1 | Programmed Cell Death-1 |
| PD-L1 | Programmed Death-Ligand 1 |
| PFS | Progression-Free Survival |
| PR | Partial Response |
| SAE | Serious Adverse Event |
| SP | Safety Population |
| TAA | Tumor Associated Antigen |
| WT1 | Wilms' Tumor 1 |

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### Authors' contributions

JF is the chief investigator who designed the study and obtained the grant funding and drafted the manuscript, NH and HM obtained the grant funding and reviewed the manuscript. YK and HK managed the study and drafted the manuscript. TS is the provider of investigational medicinal products who conceived and designed the study and obtained the grant funding and drafted the manuscript. SM designed the statistical analysis plan and drafted the manuscript.HU, TH, JT and MF reviewed the manuscript. All authors provided final approval of the manuscript.

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### Data availability

No datasets were generated or analysed during the current study.

## Declarations

### Ethics approval and consent to participate

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the Clinical Trials Act, and all of the applicable regulatory requirements. This study protocol was first authorized by the institutional review committee on December 21, 2022 at the Kobe University Graduate School of Medicine. Participant recruitment began on January 1, 2023. This study was registered at the jRCT on December 27, 2022 (jRCT2051220143). Ethical approval was obtained for the trial (approved on January 18, 2023, at Hamamatsu University Hospital and February 6, 2023, at Hiroshima University Hospital.). The details of the study are available at the following address: https://jrct.niph.go.jp/latest-detail/jRCT2051220143. Written informed consent will be obtained from all participants before any study procedure is performed. All patients will review the 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 will be delegated this duty by the principal investigator. Any protocol changes that could have an impact on study conduct and/or participant risk-benefit profile, including changes in objectives, design, sample size, participant characteristics, staff changes, or significant administrative aspects, will require approval from the Institutional Review Board. Minor protocol corrections and/or clarifications that could not affect study conduct or the participant risk-benefit profile will be viewed as administrative changes and documented internally. De-identified data will be made available to other interested investigators for additional analyses, on reasonable request, following reports of primary outcomes and with appropriate data use agreement. The findings of this study will be disseminated through scientific and professional conferences and a peer-reviewed journal.

### Consent for publication

Not applicable.

### Competing interests

Toshiro Shirakawa is CEO of Immunorock Co., Ltd., the manufacturer of B440. The remaining authors declare no competing interests.

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