Phase II trial of treatment based on gene profiling diagnosis using next generation sequencing for carcinoma of unknown primary site

Clinical Study Protocol

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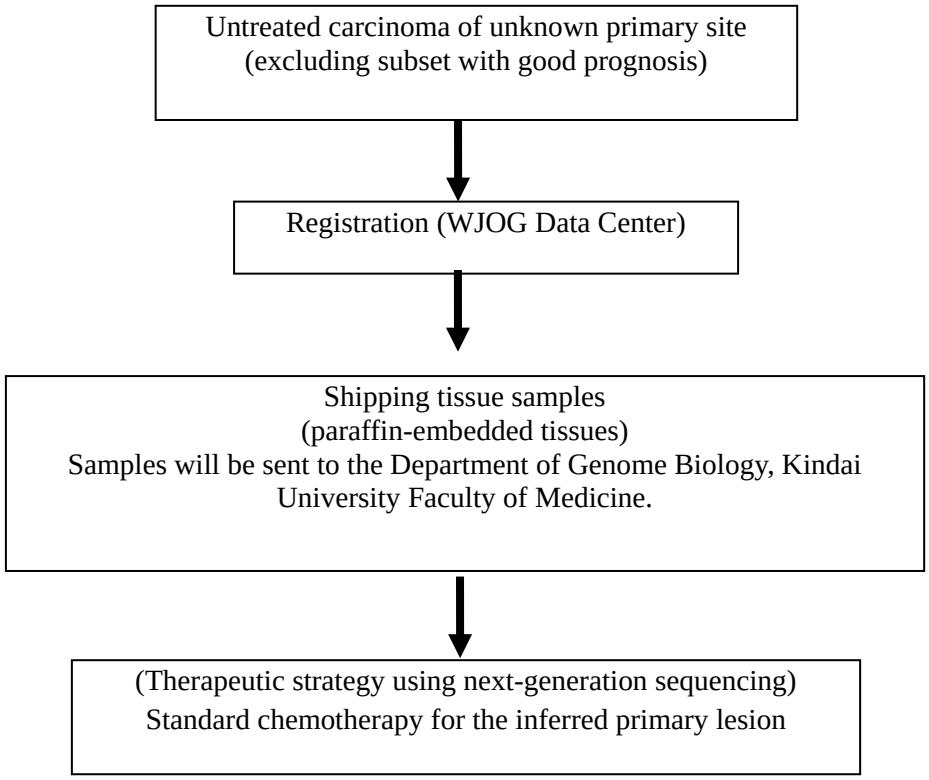
1. Outline of the protocol

1.1. Schema of this study



In principle, post-treatment will not be given unless the disease is exacerbated again.

1.2. Study type

Multicenter phase II clinical study using a central registration system

1.3. Purpose of the study

To examine whether the use of next-generation sequencing (NGS) to infer the primary site is useful in patients with previously untreated carcinoma of unknown primary site in a phase II study

Primary endpoint: 1-year survival rate

Secondary endpoints: Overall survival, progression-free survival, antitumor effect, safety, the relationship between the inferred primary site and therapeutic effect (exploratory investigation), and the frequency of gene mutations/amplifications in carcinoma of unknown primary site

1.4. Target number of cases and study period

Target number of cases: 110

Study period: March 2015 to February 2020 (3 years, 1 year, and 1 year for registration, follow-up, and analysis, respectively)

1.5. Subjects

Patients with untreated carcinoma of unknown primary site

1.6. Patient inclusion and exclusion criteria

1.6.1. Inclusion criteria

Eligible patients should satisfy all of the following conditions.

(1) Epithelial carcinoma of unknown primary site with histologically confirmed metastatic tumors (Any patients histologically diagnosed with malignant melanoma, malignant lymphoma, or sarcoma should be excluded, and the investigator should do the utmost to exclude patients with these conditions.)

(2) Chemotherapy-naïve patients (However, patients who received chemotherapy for malignant tumors cured more than 5 years previously may be enrolled.)

(3) Patients from whom a pre-treatment tumor tissue can be obtained as a paraffin-embedded tissue

(4) Patients aged 20 years or older (as of the date of informed consent)

(5) Patients with an ECOG performance status (PS) of 0 to 2

(6) Patients with no major organ damage (bone marrow, heart, lungs, liver, kidneys) and laboratory test values at the time of treatment initiation satisfying the following criteria (based on data within 14 days before enrollment; the study enrollment date is counted as Day 1, and the same day of the week two weeks prior is acceptable.)

Hemoglobin	$\geq$ 9.0 g/dL
White blood cell count	$\geq$ 4,000/mm^3
Platelet count	$\geq$ 100,000/mm^3
GOT and GPT (AST and ALT)	$\leq$ 100 IU/L
Total bilirubin	$\leq$ 1.5 mg/dL
Serum creatinine	$\leq$ 1.5 mg/dL

(7) Patients who were fully informed of the study content and voluntarily provided written consent prior to the start of the study

1.6.2. Exclusion criteria

(1) Patients for whom radical surgery or definitive radiation therapy is indicated

(2) Patients receiving palliative radiation therapy for bone and brain metastases except the primary lesion. However, patients with a 2-week interval between irradiation and the initiation of study treatment may be enrolled.

(3) A certain population of patients with carcinoma of unknown primary site have a good prognosis, and a standard treatment modality has been established for such patients. Therefore, the following patients will be excluded from this study.

[1] Adenocarcinoma of unknown primary site in women with axillary adenopathy alone (Treated similarly to breast cancer)

[2] Adenocarcinoma in women with peritoneal dissemination (ascites) alone (Treated similarly to ovarian cancer)

[3] Squamous cell carcinoma with cervical lymphadenopathy alone (Treated similarly to head and neck cancer)

[4] Squamous cell carcinoma with inguinal lymphadenopathy alone (Excision)

[5] Patients with features of germ cell neoplasms or neuroendocrine tumors

[6] A high PSA level as a serum/intratumoral tumor marker in men with sclerotic bone metastases alone (Treated similarly to prostate cancer)

(4) Patients with active, serious infections

(5) Patients with symptomatic brain metastases. Patients requiring antiedema drugs such as steroids for symptom control. (However, patients with clinically stable, asymptomatic, known brain metastases that do not require treatment may be enrolled.)

(6) Patients with interstitial pneumonia or pulmonary fibrosis evident on chest x-ray

(7) Patients with a history of bone marrow transplantation or peripheral blood stem cell transplantation
(8) Patients with poorly controlled diabetes
(9) Patients with a history of clinically significant serious drug allergy
(10) HBs antigen-positive patients
(11) Patients receiving continuous systemic administration of steroids or other immunosuppressant drugs (orally or intravenously)
(12) Patients with mental disorder or psychiatric symptoms who are assumed to have difficulty participating in the study
(13) Pregnant women, lactating women, patients who may be pregnant, and patients who wish to become pregnant
(14) Patients with unstable angina (angina that has developed or has worsened in the last 3 weeks) or a history of myocardial infarction within 6 months
(15) Patients with poorly controlled hypertension
(16) Patients with active gastrointestinal hemorrhage
(17) Patients with active double cancer
Note: Double cancer is defined as synchronous multiple cancers and metachronous multiple cancers with a disease-free interval of 5 years or less. Carcinoma in situ or lesions equivalent to intramucosal carcinoma that are considered cured by local treatment are not included in active double cancer.
(18) Patients with reproductive potential who are not willing to use contraception during the study
(19) Other patients deemed inappropriate by the investigator or subinvestigator

1.7. Dosing regimen

1.7.1 The primary site will be inferred by using NGS. Based on the inference, treatment will be provided with a regimen that is considered standard. Examples are shown below for reference. The standard treatment for the primary lesion should also be employed in second-line chemotherapy after disease progression whenever possible.

Examples for reference

(1) Non-small cell lung cancer as inferred by using NGS:
Histological type: Squamous cell carcinoma
<u>Carboplatin plus paclitaxel therapy</u>
Carboplatin AUC 6 and paclitaxel 200 mg/m^2, both on Day 1, every 3 weeks

Histological type: Non-squamous cell carcinoma (adenocarcinoma, large cell carcinoma)
<u>Cisplatin plus pemetrexed therapy</u>
Cisplatin 75 mg/m^2 and pemetrexed 500 mg/m^2, both on Day 1, every 3 weeks
Premedication with vitamin B12 and folic acid preparation should be administered.
Additionally, bevacizumab 15 mg/kg on Day 1 every 3 weeks should be considered if possible in non-squamous cell carcinoma.

EGFR mutation-positive:
<u>Gefitinib:</u> 250 mg once daily, or
<u>Erlotinib:</u> 150 mg once daily, or
<u>Afatinib:</u> 40 mg once daily

(2) Gastric cancer as inferred by using NGS:
<u>Cisplatin plus S-1 therapy</u>

Cisplatin 60 mg/m^2 on Day 8 and S-1 80 mg/m^2 on Days 1-21, every 5 weeks

HER2-positive patients (If NGS analysis suggests HER2 amplification, it is recommended to verify the results based on immunohistochemistry [IHC] or fluorescence in situ hybridization [FISH] in line with the routine practice of the study site.)

Cisplatin plus capecitabine plus trastuzumab therapy

Cisplatin 80 mg/m^2 on Day 1, capecitabine 2,000 mg/m^2 on Days 1-14, and trastuzumab 6 mg/kg on Day 1 (8 mg/kg for the first dose)

(3) Colorectal cancer as inferred by using NGS:

FOLFOX plus bevacizumab therapy

Fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2 on Day 1, oxaliplatin 85 mg/m^2 on Day 1, folinic acid 200 mg/m^2 on Day 1, and bevacizumab 5 mg/kg on Day 1, every 2 weeks

In the absence of Ras mutations

FOLFIRI plus cetuximab/panitumumab therapy

Irinotecan 150 mg/m^2 on Day 1, fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2 on Day 1, and folinic acid 200 mg/m^2 on Day 1

Plus

Cetuximab 250 mg/m^2 (400 mg/m^2 for the first dose only) on Days 1 and 8

Or

Panitumumab 6 mg/kg on Day 1

Every 2 weeks

(4) Breast cancer as inferred by using NGS:

In HER2-positive patients (in cases where NGS analysis suggests HER2 amplification), one of the following will be administered. (It is recommended to verify the analysis results based on immunohistochemistry [IHC] or fluorescence in situ hybridization [FISH] in line with the usual practice of the study site.)

(Pertuzumab plus trastuzumab plus docetaxel therapy)

Docetaxel 75 mg/m^2 plus trastuzumab 6 mg/kg plus pertuzumab 420 mg/body

The first dose of trastuzumab should be 8 mg/kg.

Docetaxel, trastuzumab, and pertuzumab will be administered every 3 weeks (on Day 1, every 3 weeks).

For other HER2 statuses, one of the following will be administered.

Anthracyclines or taxanes will be used.

Anthracyclines:

AC therapy Doxorubicin 50 mg/m^2 on Day 1 and cyclophosphamide 500 mg/m^2 on Day 1, every 3 weeks, or

EC therapy Epirubicin 60 mg/m^2 on Day 1 and cyclophosphamide 500 mg/m^2 on Day 1, every 3 weeks

Taxanes:

Paclitaxel therapy Paclitaxel 80 mg/m^2 on Days 1, 8, and 15, every 4 weeks, or

Docetaxel therapy Docetaxel 75 mg/m^2 on Day 1, every 3 weeks

(5) Pancreatic cancer as inferred by using NGS:

Gemcitabine therapy

Gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 4 weeks
FOFIRINOX therapy
Fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2, oxaliplatin 85 mg/m^2 on Day 1, and irinotecan 180 mg/m^2 on Day 1, every 2 weeks

(6) Biliary tract cancer as inferred by using NGS:
Split-dose cisplatin plus gemcitabine therapy
Cisplatin 25 mg/m^2 on Days 1 and 8 and gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 3 weeks

(7) Hepatocellular carcinoma as inferred by using NGS:
Sorafenib 400 mg/dose, twice daily (oral daily administration)

(8) Bladder cancer as inferred by using NGS:
Cisplatin plus gemcitabine therapy
Cisplatin 70 mg/m^2 on Day 2 and gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 4 weeks

(9) Renal cancer as inferred by using NGS:
Sunitinib 50 mg/day for 4 weeks, followed by a 2-week washout period

(10) Prostate cancer as inferred by using NGS:
LH-RH analogue:
Leuplin 3.75 mg, subcutaneous injection, every 4 weeks

(11) Cervical cancer as inferred by using NGS:
Carboplatin plus paclitaxel therapy
Carboplatin AUC 5 and paclitaxel 175 mg/m^2, both on Day 1, every 3 weeks

(12) Ovarian cancer as inferred by using NGS:
Carboplatin plus paclitaxel therapy
Carboplatin AUC 5 and paclitaxel 175 mg/m^2, both on Day 1, every 3 weeks
Additionally, bevacizumab 15 mg/kg on Day 1 every 3 weeks should be considered if possible.

(13) Head and neck cancer as inferred by using NGS:
FP plus cetuximab therapy
Cisplatin 80 mg/m^2 on Day 1, fluorouracil 800 mg/m^2 on Days 1-4 (continuous administration), and cetuximab 250 mg/m^2 (400 mg/m^2 for the first dose only) on Day 1, 8, and 15, every 3 weeks

(14) Esophageal cancer as inferred by using NGS:
FP therapy
Cisplatin 80 mg/m^2 on Day 1 and fluorouracil 800 mg/m^2 (continuous administration) on Days 1-5, every 4 weeks

(15) Malignant lymphoma as inferred by using NGS:
(R)-CHOP therapy Rituximab may be added at the discretion of the study site.
The presence of CD20 expression will be determined by expression analysis in cases where malignant lymphoma is inferred.

Rituximab may be added.
Cyclophosphamide 750 mg/m^2 on Day 1, doxorubicin 50 mg/m^2 on Day 1, vincristine 1.4 mg/m^2 (maximum: 2 mg/body) on Day 1, and prednisolone 100 mg/body on Days 1-5, oral administration, every 3 weeks
(If rituximab is added) Rituximab 375 mg/m^2 on Day -1,

(16) Neuroendocrine carcinoma as inferred by using NGS:
Cisplatin plus etoposide therapy
Cisplatin 80 mg/m^2 on Day 1 and etoposide 100 mg/m^2 on Days 1-3, every 3 weeks

(17) Germinoma as inferred by using NGS:
BEP therapy
Cisplatin 20 mg/m^2 on Days 1-5, etoposide 100 mg/m^2 on Days 1-3, and bleomycin 30 U on Days 2, 9, and 16, every 3 weeks (for all three drugs)

(18) Soft tissue sarcoma as inferred by using NGS:
Doxorubicin monotherapy
Doxorubicin 75 mg/m^2 on Day 1, every 3 weeks
c-KIT mutation-positive (gastrointestinal stromal tumor [GIST] as inferred by using NGS):
Imatinib: 400 mg once daily

2. Background

2-1 Carcinoma of unknown primary site

Carcinoma of unknown primary site is, as the name suggests, an all-inclusive term for any cancer in which metastatic lesions have been identified but not the primary site despite extensive search. Carcinoma of unknown primary site is believed to account for about 3% to 5% of all cancers, and the patient population is highly heterogeneous because the site of origin varies from patient to patient. The prognosis is generally poor, and the median survival time (MST) is considered to be 6 to 12 months. In Western countries, the concept of carcinoma of unknown primary site has been established, and chemotherapy, which is systemic treatment, is generally indicated since only metastatic lesions are present. Surgery and radiotherapy, which are local treatment modalities, may be employed as part of multimodality therapy in combination with chemotherapy, but it is not unusual that they are not indicated.
Thus far, several clinical studies have been reported on the treatment of carcinoma of unknown primary site. In general, however, these studies excluded sarcomas, malignant lymphomas, and malignant melanomas, and dealt with narrowly defined carcinoma of unknown primary site, focusing on epithelial tumors such as adenocarcinoma, anaplastic carcinoma, and squamous cell carcinoma.

2-2. Standard treatment for carcinoma of unknown primary site

As mentioned in the previous section, the mainstay of treatment for carcinoma of unknown primary site is chemotherapy. To date, however, there have been no reports of large-scale phase III controlled studies that address the value of chemotherapy, including those in which palliative care (best supportive care: BSC) is subjected to comparison. Therefore, no standard treatment for this type of tumor is currently available, and the usefulness of chemotherapy in patients with this condition is even unclear.
On the other hand, certain groups of patients with carcinoma of unknown primary site are thought to have a good prognosis, and there are accepted appropriate treatment modalities for such groups of patients.

(1) Adenocarcinoma of unknown primary site in women with axillary adenopathy alone

(Treated similarly to breast cancer)

(2) Adenocarcinoma in women with peritoneal dissemination (ascites) alone (Treated similarly to ovarian cancer)

(3) Squamous cell carcinoma with cervical lymphadenopathy alone (Treated similarly to head and neck cancer)

(4) Squamous cell carcinoma with inguinal lymphadenopathy alone (Excision)

(5) Patients with features of germ cell neoplasms or neuroendocrine tumors

(6) A high PSA level as a serum/intratumoral tumor marker in men with sclerotic bone metastases alone (Treated similarly to prostate cancer)

These patients, as well as those with sarcoma, malignant lymphoma, and malignant melanoma, have been excluded from recent clinical studies of carcinoma of unknown primary site.

Regarding chemotherapy for carcinoma of unknown primary site, fluorouracil (5-FU), cyclophosphamide, doxorubicin, and other agents have been tried since the 1980s, but the response rate was disappointingly low at 20% to 30%, with an MST of 4 to 9 months. Later on, cisplatin was developed and studied in an extremely small controlled study (involving less than 100 patients, albeit conducted as a phase III study), but no superiority of cisplatin was demonstrated and no conclusive results were shown that would warrant the inclusion of cisplatin in treatment strategies[1-2]. As new agents, such as taxanes, gemcitabine, and irinotecan hydrochloride, were introduced for the treatment of various cancer types in the 1990s, a number of phase II studies were conducted for carcinoma of unknown primary site, mainly in combination with platinum-containing drugs[3-7]. Although such studies were nothing more than phase II studies, the results were far superior to those for previous drugs, with a 30–40% response rate and an MST of longer than 12 months in some studies. In particular, combination therapy using a platinum-containing drug (carboplatin) and a taxane (paclitaxel) showed the most promising results, and carboplatin plus paclitaxel is currently the most frequently used regimen in clinical practice. However, once again, the usefulness of chemotherapy for carcinoma of unknown primary site remains unclear, and there are no drugs that can be positioned as the standard treatment for this condition.

2-3. Current status and issues on the microarray-based inference of primary lesions in carcinoma of unknown primary site

Recent advances in molecular biology have suggested the possibility of identifying the primary site from the genetic information of tumor tissue, and methods have been developed to identify the primary site by combining gene chips with current diagnostic imaging techniques.

One of the most proactively developed techniques to date is microarray technology, which is characterized by the ability to search for unknown useful genes in the whole genome.

It has been reported that it is possible to locate the primary site with 78–85% accuracy by examining the expression of 110 to 16,000 genes through a microarray approach in a range of solid tumors with an established primary lesion[8-11]. At present, there are only a few retrospective reports on whether these microarray techniques can be used to infer the primary site in carcinoma of unknown primary site. Nonetheless, microarray techniques may be useful for inferring the primary site in this condition. Since 2008, a group led by Nakagawa has been conducting a phase II prospective controlled study to examine whether a treatment strategy based on the inference of primary site using microarray can lead to the improvement of prognosis of carcinoma of unknown primary site. On the basis of this phase II study, treatment strategies involving the inference of primary site may be further investigated for suitability as standard treatment for carcinoma of unknown primary site in phase III studies.

Microarray is useful for inferring the primary lesion in carcinoma of unknown primary site, but there are some problems. Microarrays using cDNA, which is obtained from mRNA (obtained from tumor tissue) via reverse transcription, require plenty of high-quality RNA, and thus extraction of RNA from frozen tissue is often essential. Tissues obtained by tumor biopsy in

daily clinical practice are often paraffin-embedded (FFPE) blocks/sections, and microarray analysis of RNA obtained from these blocks and sections does not guarantee accuracy. It is therefore necessary to establish more optimum methods to search for gene expression in order to utilize the inference of primary site in patients with carcinoma of unknown primary site in day-to-day clinical practice in the future.

2-4. Inference of primary lesions by next-generation sequencing in carcinoma of unknown primary site

Over the past decade, it has been shown that a broad array of gene mutations and gene amplifications are involved in malignant traits of various cancers. Particularly in cases where malignant traits depend on a single or a small number of genetic abnormalities (oncogene addiction), molecular-targeted drugs that inhibit such genetic abnormalities have been shown to be useful. Examples include EGFR-TKIs for non-small cell lung cancer with EGFR mutations. Next-generation sequencing (NGS) may be used as a means for searching for such gene mutations and amplifications.

Sanger sequencing has been used to decode biological DNA sequences for more than 20 years. While Sanger sequencing is highly accurate in decoding DNA sequences, its processivity is low, with the number of bases that can be decoded in a single analysis being limited. In recent years, NGS with an extremely high processivity has been developed, enabling ultra-high-speed DNA sequencing and mass decoding. RNA-Seq, which uses NGS to analyze the sequence of RNA, allows quantification of gene expression with a small amount of RNA and can be used as an alternative to the quantification of RNA expression by microarray. More recently, the development of a technology called target sequencing, which analyzes several dozen to 300 useful genes for diagnosis and treatment, has made it possible to analyze gene mutations and gene expression by using a fairly small amount of samples. NGS also has a function to count the number of gene reads, and gene amplification can be estimated from the number of reads. Specifically, the analysis can be performed using 10 ng equivalent of gDNA extracted from an FFPE sample for gene mutation and 5 ng equivalent of RNA extracted from an FFPE sample for gene expression analysis. Thus, NGS may be highly useful for carcinoma of unknown primary site because it can analyze multiple gene expression, gene mutation, and gene amplification using FFPE samples in a short time and at a low cost.

2-5. Significance of this study

Recent advances in molecular biology have suggested the possibility of locating the primary site based on genetic information in tumor tissue. This study aims to identify the primary site and cancer-specific genetic abnormalities (mutations/amplifications) by using NGS in conjunction with the current diagnostic imaging techniques. The gene mutations and amplifications (EGFR mutation in non-small cell lung cancer, KIT mutation in GIST, HER2 amplification in gastric cancer and breast cancer, and RAS mutation in colorectal cancer) that are currently used in clinical practice will be identified. Then, it will be verified whether the application of treatment strategies for these mutations to the treatment of carcinoma of unknown primary can help improve the outcome of patients. In this study, a highly reproducible analysis of gene expression profile will be carried out with a fairly small amount of samples in order to infer the primary site by performing RNA-Seq with an original panel (CUPanel) containing approximately 150 primary-specific gene signatures and reference genes for correction using NGS. This study is designed to determine whether such a treatment strategy can be useful as a treatment strategy for carcinoma of unknown primary site.

3. Ethical conduct of the study

The ethical principles of the Declaration of Helsinki and the Ethical Guidelines for Clinical Research will be observed in conducting this study, and the human rights,

welfare, and safety of study subjects will be ensured to the full extent.

3.1. Protection of the subjects' privacy

The names of subjects will not be disclosed by the participating sites to the data center. Subjects will be identified and referred to using the case registration number (anonymized number) issued at the time of enrollment, and no information that can be used to identify the patient by a third party, such as the patient's name, will be entered into the database.

3.2. Approval by the institutional review board

This study protocol and the written informed consent form should be approved by the institutional review board of each participating institution.

3.3. Compliance with the study protocol

Researchers participating in this study should comply with the study protocol to the extent that such actions do not undermine the safety and human rights of the patients.

3.4. Management of conflicts of interest (COI)

The COI of persons involved in clinical practice at participating institutions, such as facility representatives and liaison officers, should be handled in accordance with the regulations of the respective participating institutions.

Conflicts of interest of researchers who play an important role in this study, such as the Study Chair, will be managed by the Academic Support Division of the Kindai University Faculty of Medicine.

4. Purpose of the study

To examine whether the use of NGS to infer the primary site and to detect specific gene mutations and gene amplifications is meaningful in patients with previously untreated carcinoma of unknown primary site in a phase II study

Primary endpoint: 1-year survival rate

Secondary endpoints: Overall survival, progression-free survival, antitumor effect, safety, the relationship between the inferred primary site and therapeutic effect (exploratory investigation), and the frequency of gene mutations/amplifications in carcinoma of unknown primary site.

5. Study design

Multicenter phase II study

6. Drugs used in the study

Commercially available drugs used at medical institutions will be used as study drugs in this study.

7. Patient inclusion and exclusion criteria

7.1. Inclusion criteria

Eligible patients should satisfy all of the following conditions.

(1) Epithelial carcinoma of unknown primary site with histologically confirmed metastatic tumors (Any patients histologically diagnosed with malignant melanoma, malignant lymphoma, or sarcoma should be excluded, and the investigator should do the utmost to exclude patients with these conditions.)

(2) Chemotherapy-naïve patients (However, patients who received chemotherapy for

malignant tumors cured more than 5 years previously may be enrolled.)
(3) Patients from whom a pre-treatment tumor tissue can be obtained as a paraffin-embedded tissue
(4) Patients aged 20 years or older (as of the date of informed consent)
(5) Patients with an ECOG performance status (PS) of 0 to 2
(6) Patients with no major organ damage (bone marrow, heart, lungs, liver, kidneys) and laboratory test values at the time of treatment initiation satisfying the following criteria (based on data within 14 days before enrollment; the study enrollment date is counted as Day 1, and the same day of the week two weeks prior is acceptable.)

Hemoglobin	≥ 9.0 g/dL
White blood cell count	≥ 4,000/mm^3
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Total bilirubin	≤ 1.5 mg/dL
Serum creatinine	≤ 1.5 mg/dL

(7) Patients who were fully informed of the study content and voluntarily provided written consent prior to the start of the study

7.2. Exclusion criteria

(1) Patients for whom radical surgery or definitive radiation therapy is indicated
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[6] A high PSA level as a serum/intratumoral tumor marker in men with sclerotic bone metastases alone (Treated similarly to prostate cancer)
(4) Patients with active, serious infections
(5) Patients with symptomatic brain metastases. Patients requiring antiedema drugs such as steroids for symptom control. (However, patients with clinically stable, asymptomatic, known brain metastases that do not require treatment may be enrolled.)
(6) Patients with interstitial pneumonia or pulmonary fibrosis evident on chest x-ray
(7) Patients with a history of bone marrow transplantation or peripheral blood stem cell transplantation
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(9) Patients with a history of clinically significant serious drug allergy
(10) HBs antigen-positive patients
(11) Patients receiving continuous systemic administration of steroids or other immunosuppressant drugs (orally or intravenously)
(12) Patients with mental disorder or psychiatric symptoms who are assumed to have

difficulty participating in the study

(13) Pregnant women, lactating women, patients who may be pregnant, and patients who wish to become pregnant

(14) Patients with unstable angina (angina that has developed or has worsened in the last 3 weeks) or a history of myocardial infarction within 6 months

(15) Patients with poorly controlled hypertension

(16) Patients with active gastrointestinal hemorrhage

(17) Patients with active double cancer

Note: Double cancer is defined as synchronous multiple cancers and metachronous multiple cancers with a disease-free interval of 5 years or less. Carcinoma in situ or lesions equivalent to intramucosal carcinoma that are considered cured by local treatment are not included in active double cancer.

(18) Patients with reproductive potential who are not willing to use contraception during the study

(19) Other patients deemed inappropriate by the investigator or subinvestigator

Contact for inquiries about the protocol:

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8. Informed consent

8.1. Creation of informed consent form

The physician in charge at each participating institution should create a site-specific informed consent form with reference to the master informed consent form. The informed consent form and the study protocol should be approved by the institutional review board.

8.2. Obtaining consent

Before a subject participates in the study, the physician in charge should hand the subject the informed consent form (after approval by the institutional review board) and provide a thorough explanation of the items listed in the following section. Prospective subjects should also be given the opportunity to ask questions and enough time to decide whether to participate in the study.

After confirming that the subject has fully understood the content of the study, the physician in charge should obtain the subject's written consent for voluntary participation in the study. The physician in charge should also hand the subject a copy of the signed (or signed and sealed) informed consent form immediately. The original consent form should be properly retained at each study site for three years after the end of the study period.

8.3. Items to be explained to subjects by using informed consent form

(1) Disease name and the condition of the disease

(2) The nature of this study, which is to be conducted as a clinical study involving translational research

(3) Rationale and purpose of this study

(4) Methods of the study and treatment details (Drug name, administration method, dose amount, treatment cycle, overall study duration)

(5) Expected effects and potential adverse reactions
(6) Burden of expense and compensation (Explanation that treatment expenses are covered by the health insurance system and that compensation for health damage is handled in the same way as in general clinical practice)
(7) Presence/absence of alternative therapies and their details
(8) Expected benefits and potential disadvantages to patients participating in the study
(9) Direct access to medical history (Explanation on the acceptance of facility inspection for the sake of accuracy control, including direct access by medical personnel at other facilities to medical records with the permission of the facility director)
(10) Refusal and withdrawal of consent (Patients will not be disadvantaged even if they do not agree to participate in the study, and they can freely withdraw their consent after participation.)
(11) Protection of human rights (Confidentiality of the names and other personal information of patients)
(12) Freedom to ask questions (Written contact information of not only the attending physician but also the Study Chair [or the Study Coordinator] will be provided, and patients will be allowed to freely ask questions about the study or treatment details.)
(13) Management of conflicts of interest
(14) Storage, disposal, and secondary use of samples (Anonymized clinical information will be stored at the WJOG Data Center and the Department of Medical Oncology, Kindai University Faculty of Medicine, and samples will be stored at the Department of Genome Biology, Kindai University Faculty of Medicine. They will be stored for five years after the end of the study period and then discarded. If a subject withdraws consent to the use of clinical information and samples, the samples will be immediately discarded.)

9. Registration of patients

9.1. Registration procedure

(1) After confirming that a prospective subject satisfies all of the inclusion criteria and does not fall under any of the exclusion criteria, the participating institution will fill out the Registration Form and fax it to the WJOG Data Center (information to be included: facility name, department, physician in charge, patient's initials, height, weight, date of consent, facility ID, date of birth, and age).
(2) The Data Center will check the Registration Form for eligibility and fax a case registration number (anonymized number) as well as the sex of the subject to the participating institution, the Study Coordinator, and the Department of Genome Biology, Kindai University Faculty of Medicine.
(3) Participating institutions should send tumor tissue samples with a case registration number to the Department of Genome Biology, Kindai University Faculty of Medicine, together with a report from the pathologist at the site where the subject has been registered (a photomicrograph of hematoxylin-eosin stained specimens should be appended whenever possible).
(4) The Department of Genome Biology, Kindai University Faculty of Medicine will report the analysis results to the Study Coordinator. Then, the Study Coordinator will send the Notification of Primary Site Inference Result with the case registration number (anonymized number) to the Study Coordinator by email.
(5) Upon receiving the Notification of Primary Site Inference Result, the Data Center will report the result of primary site inference to the participating institution.

(6) The participating institution will start treatment within two weeks after receipt of the Notification of Primary Site Inference Result.

Contact for case registration and reception hours:
WJOG Data Center
Tel.: +81-6-6633-7400; Fax: +81-6-6633-7405
Email: datacenter@wjog.jp
Reception hours: 9 a.m. to 5 p.m., Monday to Friday (except national holidays and year-end and New Year holidays [December 29 to January 3])

9.2. Notes on registration

(1) A Registration Form will not be accepted unless all entries in it have been completed.
(2) A Notification of Registration Result with a case registration number will be issued only after eligibility has been confirmed by the Data Center. The sending of a Notification of Registration Result should be deemed to constitute an official registration.
(3) The Notification of Registration Result faxed from the Data Center to the physician in charge of registration should be stored.
(4) Once registered, patients will not be de-registered (i.e., they will not be deleted from the database). In the case of duplicate registration, only the information obtained at the first registration (and the first registration number) should be used in all instances.
(5) If misregistration or duplicate registration is found, the Data Center should be contacted immediately.
(6) Body surface area and dose amount should be calculated and confirmed at the facility.

10. Protocol treatment

In principle, protocol therapy should be initiated within two weeks (the same day of the week two weeks later is acceptable) after receipt of the Notification of Primary Site Inference Result from the WJOG Data Center. It is desirable to reconfirm that the eligibility criteria are still satisfied within one week before the start of administration.

10.1 Treatment regimen by cancer type as inferred by using NGS

The primary site will be inferred by using NGS. Based on the inference, treatment will be provided with a regimen that is considered standard. Standard treatment for the primary lesion as inferred at the time of enrollment will be provided. Example are shown below. The standard treatment for the primary lesion should also be employed in second-line chemotherapy after disease progression whenever possible.

Examples for reference

(1) Non-small cell lung cancer as inferred by using NGS:
Histological type: Squamous cell carcinoma
Carboplatin plus paclitaxel therapy
Carboplatin AUC 6 and paclitaxel 200 mg/m^2, both on Day 1, every 3 weeks

Histological type: Non-squamous cell carcinoma (adenocarcinoma, large cell carcinoma)
Cisplatin plus pemetrexed therapy
Cisplatin 75 mg/m^2 and pemetrexed 500 mg/m^2, both on Day 1, every 3 weeks

Premedication with vitamin B12 and folic acid preparation should be administered.
Additionally, bevacizumab 15 mg/kg on Day 1 every 3 weeks should be considered if possible in non-squamous cell carcinoma.

EGFR mutation-positive:
Gefitinib: 250 mg once daily or
Erlotinib: 150 mg once daily or
Afatinib: 40 mg once daily

(2) Gastric cancer as inferred by using NGS:
Cisplatin plus S-1 therapy
Cisplatin 60 mg/m^2 on Day 8 and S-1 80 mg/m^2 on Days 1-21, every 5 weeks

HER2-positive patients (If NGS analysis suggests HER2 amplification, it is recommended to verify the result based on immunohistochemistry [IHC] or fluorescence in situ hybridization [FISH] in line with the routine practice of the study site.)
Cisplatin plus capecitabine plus trastuzumab therapy
Cisplatin 80 mg/m^2 on Day 1, capecitabine 2,000 mg/m^2 on Days 1-14, and trastuzumab 6 mg/kg on Day 1 (8 mg/kg for the first dose)

(3) Colorectal cancer as inferred by using NGS:
FOLFOX plus bevacizumab therapy
Fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2 on Day 1, oxaliplatin 85 mg/m^2 on Day 1, folinic acid 200 mg/m^2 on Day 1, and bevacizumab 5 mg/kg on Day 1, every 2 weeks

In the absence of Ras mutations
FOLFIRI plus cetuximab/panitumumab therapy
Irinotecan 150 mg/m^2 on Day 1, fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2 on Day 1, and folinic acid 200 mg/m^2 on Day 1
Plus
Cetuximab 250 mg/m^2 (400 mg/m^2 for the first dose only) on Days 1 and 8
Or
Panitumumab 6 mg/kg on Day 1
Every 2 weeks

(4) Breast cancer as inferred by using NGS:
In HER2-positive patients (in cases where NGS analysis suggests HER2 amplification), one of the following will be administered. (It is recommended to verify the analysis result based on immunohistochemistry [IHC] or fluorescence in situ hybridization [FISH] in line with the routine practice of the study site.)
(Pertuzumab plus trastuzumab plus docetaxel therapy)
Docetaxel 75 mg/m^2 plus trastuzumab 6 mg/kg plus pertuzumab 420 mg/body
The first dose of trastuzumab should be 8 mg/kg.
Docetaxel, trastuzumab, and pertuzumab will be administered every 3 weeks (on Day 1).

For other HER2 statuses, one of the following will be administered.
Anthracyclines or taxanes will be used.

Anthracyclines:

AC therapy Doxorubicin 50 mg/m^2 on Day 1 and cyclophosphamide 500 mg/m^2 on Day 1, every 3 weeks, or

EC therapy Epirubicin 60 mg/m^2 on Day 1 and cyclophosphamide 500 mg/m^2 on Day 1, every 3 weeks

Taxanes:

Paclitaxel therapy Paclitaxel 80 mg/m^2 on Days 1, 8, and 15, every 4 weeks, or

Docetaxel therapy Docetaxel 75 mg/m^2 on Day 1, every 3 weeks

(5) Pancreatic cancer as inferred by using NGS:

Gemcitabine therapy

Gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 4 weeks

FOFIRINOX therapy

Fluorouracil (bolus) 400 mg/m^2 on Day 1, fluorouracil (continuous infusion over 46 hours) 2,400 mg/m^2, oxaliplatin 85 mg/m^2 on Day 1, and irinotecan 180 mg/m^2 on Day 1, every 2 weeks

(6) Biliary tract cancer as inferred by using NGS:

Split-dose cisplatin plus gemcitabine therapy

Cisplatin 25 mg/m^2 on Days 1 and 8 and gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 3 weeks

(7) Hepatocellular carcinoma as inferred by using NGS:

Sorafenib 400 mg/dose, twice daily (oral daily administration)

(8) Bladder cancer as inferred by using NGS:

Cisplatin plus gemcitabine therapy

Cisplatin 70 mg/m^2 on Day 2 and gemcitabine 1,000 mg/m^2 on Days 1, 8, and 15, every 4 weeks

(9) Renal cancer as inferred by using NGS:

Sunitinib 50 mg/day for 4 weeks, followed by a 2-week washout period

(10) Prostate cancer as inferred by using NGS:

LH-RH analogue

Leuplin 3.75 mg, subcutaneous injection, every 4 weeks

(11) Cervical cancer as inferred by using NGS:

Carboplatin plus paclitaxel therapy

Carboplatin AUC 5 and paclitaxel 175 mg/m^2, both on Day 1, every 3 weeks

(12) Ovarian cancer as inferred by using NGS:

Carboplatin plus paclitaxel therapy

Carboplatin AUC 5 and paclitaxel 175 mg/m^2, both on Day 1, every 3 weeks

Additionally, bevacizumab 15 mg/kg on Day 1 every 3 weeks should be considered if possible.

(13) Head and neck cancer as inferred by using NGS:

FP plus cetuximab therapy

Cisplatin 80 mg/m^2 on Day 1, fluorouracil 800 mg/m^2 on Days 1-4 (continuous administration), and cetuximab 250 mg/m^2 (400 mg/m^2 for the first dose only) on Day 1, 8, and 15, every 3 weeks

(14) Esophageal cancer as inferred by using NGS:

FP therapy

Cisplatin 80 mg/m^2 on Day 1 and fluorouracil 800 mg/m^2 (continuous administration) on Days 1-5, every 4 weeks

(15) Malignant lymphoma as inferred by using NGS:

(R)-CHOP therapy Rituximab may be added at the discretion of the study site.

The presence of CD20 expression will be determined by expression analysis in cases where malignant lymphoma is inferred.

Rituximab may be added.

Cyclophosphamide 750 mg/m^2 on Day 1, doxorubicin 50 mg/m^2 on Day 1, vincristine 1.4 mg/m^2 (maximum: 2 mg/body) on Day 1, and prednisolone 100 mg/body on Days 1-5, oral administration, every 3 weeks

(If rituximab is added) Rituximab 375 mg/m^2 on Day -1,

(16) Neuroendocrine carcinoma as inferred by using NGS:

Cisplatin plus etoposide therapy

Cisplatin 80 mg/m^2 on Day 1 and etoposide 100 mg/m^2 on Days 1-3, every 3 weeks

(17) Germinoma as inferred by using NGS:

BEP therapy

Cisplatin 20 mg/m^2 on Days 1-5, etoposide 100 mg/m^2 on Days 1-3, and bleomycin 30 U on Days 2, 9, and 16, every 3 weeks (for all three drugs)

(18) Soft tissue sarcoma as inferred by using NGS:

Doxorubicin monotherapy

Doxorubicin 75 mg/m^2 on Day 1, every 3 weeks

c-KIT mutation-positive (gastrointestinal stromal tumor [GIST] as inferred by using NGS):

Imatinib: 400 mg once daily

10.24 Concomitant medications and therapies

10.24.1. Prohibited concomitant medications and therapies

During the administration of the study medication, any concomitant use of anticancer drugs, biological response modifiers (BRM), hormone therapy, thermotherapy, radiation therapy, or other investigational drugs that may affect the study and are not planned at the time of enrollment should be prohibited. If drugs requiring particular attention to concomitant use are to be used in combination of the study drug, due caution should be exercised.

10.24.2. Supportive care

No specific restrictions will be posed on the use of antiemetics, blood transfusions, G-CSF preparations, analgesics, and tranquilizers used under health insurance coverage.

11 Completion or discontinuation of the study

The criteria for completion and discontinuation are defined as follows. The study should be discontinued if any of the following discontinuation criteria is met during the study period.

Study completion	None of the discontinuation criteria shown below is met during the specified study period, and subjects have received no fewer than the standard treatment courses of the standard regimen for respective types of cancer.

Discontinuation criteria	Presence of the necessity of discontinuing the study as judged by the attending physician
	Occurrence of grade 4 non-hematologic toxicity
	Occurrence of grade 3 or higher interstitial pneumonia
	Failure of protocol treatment (judged to be ineffective)
	Patient's refusal to continue treatment
	Death occurring prior to discontinuation of study treatment for other reasons

12 Post-treatment

In principle, patients who have discontinued or completed the study will not receive treatment specifically for carcinoma of unknown primary site unless re-exacerbation of the disease is noted. However, this does not apply when such treatment is provided as a result of prioritizing the patient's wishes and benefits.

13 Items to be measured and observed and the timing thereof

13.1 Definition of the study period and follow-up period

The study period and follow-up period for each subject are defined as follows.

Study period	From the date of registration until 4 weeks after the final dose or until the start of post-treatment initiated within 4 weeks after the final dose
Dosing period	From the first administration day until the last administration day
Follow-up period	From the end of the study period until 1 year after the registration of the last patient

13.2 Items to be measured and observed before the start of treatment

(1) Patient characteristics

Sex, height, weight (measured within 14 days before registration [the same day of the week 14 days prior as the registration date is acceptable]), presence/absence of a noteworthy past disease history and the name of the condition, presence/absence of a noteworthy complication and the name of the condition, presence/absence of double cancer (disease name and last treatment date if applicable), smoking history, presence/absence of a history of drug allergy and the name of the drug used for drug allergy, surgical findings, histopathological diagnosis, tumor marker, rationale for the diagnosis of carcinoma of unknown primary site, and primary lesion deduced from the clinical circumstances

(2) Determination of tumor localization site

Plain chest x-ray, thoracoabdominal CT (contrast media should be used in principle), brain MRI, or brain CT (contrast media should be used in principle) will be performed to determine the tumor localization site within 28 days prior to registration (the same day of the week four weeks prior is acceptable). Whole-body PET(/CT) will also be performed if possible.

(3) Subjective and objective findings

Subjective and objective findings will be confirmed within 14 days prior to registration. (The same day of the week 14 days prior as the registration date is acceptable.) It is desirable to reconfirm subjective and objective findings immediately before administration.

PS, nausea, vomiting, anorexia, fatigue, constipation, diarrhea, maculopapular rash, hyperpigmentation of the skin, allergic reactions, peripheral motor neuropathy, peripheral sensory neuropathy, oral mucositis, alopecia, pain, pneumonitis, phlebitis, pyrexia, febrile neutropenia, and other grade 3 adverse events

Note: Conditions immediately before dosing should be described in case report forms.

(4) Laboratory tests

HBs antigen, HBs antibody, HBc antibody, and HCV antibody will be determined before registration.

This may be performed at any time before registration.

The following tests will be performed within 14 days prior to registration. (The same day of the week 14 days prior as the registration date is acceptable.) It is desirable to reconfirm test values immediately before administration.

Peripheral blood counts: White blood cell, neutrophil (ANC: stab and segmented neutrophils), hemoglobin, and platelet

Blood biochemistry: Total protein, albumin, total bilirubin, AST (GOT), ALT (GPT), creatinine, LDH, BUN, Na, K, Cl, Ca, CRP, Ccr (Cockcroft-Gault method or 24-hour creatinine clearance)

Tumor markers (if elevation is noted), 12-lead ECG

13.3 Items to be measured and observed during the study period

The following tests and observations will be performed. The items described in 1) and 2) will be investigated at least once per course (every 3 to 5 weeks depending on the regimen) for the first 6 months after the start of treatment. Thereafter, they will be investigated at least once every 6 to 10 weeks. Regarding 3), assessment will be carried out every 2 to 3 courses. While 1) through 3) are mandatory, 4) is optional. For regimens in which the duration of 1 course cannot be clearly defined (e.g., daily oral administration), 1 course should be deemed to comprise 21 days.

1) Subjective and objective symptoms: Same as those described in Section 13.2(3)
2) Blood tests: Same as the blood tests described in Section 13.2(4) (except for Ccr, HBs antigen, HBs antibody, HBc antibody, and HCV antibody)
3) Tumor assessment (diagnostic imaging that allows the evaluation of antitumor effects)
4) Tumor markers (if elevation is noted) (once a month)
5) Type of treatment regimen, dose of each drug, and date of administration (in "/m^2" or "/body")

13.4 Items to be measured and observed during the follow-up period

The follow-up period is 1 year following the date of registration of the last patient. The tests and observations below will be performed during this period. In the event a subject dies, follow-up will be terminated at that time.

1) Until 1 year after registration, subjects will be examined and receive image assessment for antitumor effects at least once every 2 months until disease

progression is noted.

2) Tumor markers (elevation in CEA levels) should also be measured as often as possible, at least once every 2 months.

In principle, periodic follow-up investigations will be conducted twice a year. Case report forms (follow-up forms) should be completed and submitted to the Data Center during the periodic follow-up investigations. In this study, the first periodic follow-up investigation after the end of the follow-up period (which is 1 year after registration of the last patient) is the final investigation.

Items to be described in case report forms

i. Outcome (information about survival)
ii. Tumor markers (if measured) and imaging test findings (up to 2 years after registration)
iii. Recurrence status (The date of recurrence should be identified even in cases where post-treatment has been provided before recurrence is documented.)
iv. Presence/absence of post-treatment (The details should also be described if applicable.)
v. Primary site (if identified)

Note: Inferring the primary site by using NGS is a significant part of this study. Accordingly, in the event of death of a patient enrolled in this study, efforts will be made to obtain autopsy data whenever possible so that consistency with the inference results can be examined, although it is said that the primary site is rarely identified by autopsy in carcinoma of unknown primary site.

13.5 Storage of clinical information

The clinical information obtained in this study will be anonymized at each site and then forwarded to the WJOG Data Center. The WJOG Data Center and the Study Coordinator will retain the clinical information for five years after the end of the study.

Study schedule

<table>
<tr><th rowspan="2"></th><th rowspan="2">Before registration</th><th colspan="3">Study period</th><th rowspan="2">Follow-up period
(Up to 1 year after registration of the last patient)</th></tr>
<tr><th>From registration until the start of administration</th><th>From the start of administration until 6 months</th><th>From 6 months on</th></tr>
<tr><td>Patient characteristics</td><td>✓
Height and weight should be measured within 14 days prior to registration</td><td></td><td></td><td></td><td rowspan="3">Medical consultation should be performed at least once every 2 months</td></tr>
<tr><td>Subjective and objective symptoms</td><td>✓
Within 14 days prior to registration</td><td rowspan="2">To be reconfirmed immediately before administration whenever possible</td><td>✓
At least once per course</td><td>✓
At least once per 2 courses</td></tr>
<tr><td>Blood test</td><td>✓
Within 14 days prior to registration</td><td>✓
At least once per course
Day before the start of the course, or before administration on the day of administration</td><td>✓
At least once per 2 courses
Day before the start of the course, or before administration on the day of administration</td></tr>
<tr><td>Plain chest x-ray</td><td>✓
Within 28 days before registration</td><td></td><td colspan="2">Every 2 to 3 courses</td><td rowspan="2">Every 2 months</td></tr>
<tr><td>CT and MRI</td><td>✓
Within 28 days before registration</td><td></td><td colspan="2">Every 2 to 3 courses
(Imaging that allows the evaluation of antitumor effects)</td></tr>
<tr><td>PET*</td><td>✓
Within 28 days before registration</td><td></td><td colspan="2"></td><td></td></tr>
<tr><td>Tumor markers</td><td>✓
Within 14 days prior to registration</td><td></td><td colspan="2">Once a month</td><td>Once every 2 months whenever possible</td></tr>
</table>

*PET(/CT) is desirable but not mandatory.

14 Ensuring safety

14.1 Basic matters to ensure the safety of subjects

The treating physician should perform necessary and appropriate tests while the subject is participating in the study, and exercise caution to ensure subjects' safety. If an adverse event occurs, appropriate measures should be taken as necessary, and the cause of the event should be investigated with due consideration to ensuring the safety of the subject.

14.2 Expected adverse reactions

Refer to the package insert of each anticancer drug.

15 Efficacy endpoint:

15.1 Progression-free survival

Progression-free survival is defined as the period from the date of registration until (1) the date of death from any cause, (2) the date on which disease progression is confirmed by

periodic testing, or (3) the date on which exacerbation of clinical symptoms is documented, whichever comes first.
Even in cases where protocol treatment is discontinued for reasons other than disease progression, imaging-based evaluation should be continued to check for disease progression. However, in subjects who have received post-treatment before disease progression is confirmed, the cut-off date will be defined as the day of post-treatment initiation.
For subjects in whom death or disease progression has not been confirmed at the time of analysis or in whom the date of the onset of these events is unknown, the most recent outpatient visit date or inpatient treatment date before loss to follow-up will be defined as the cut-off date.
Definition of disease progression:
Disease progression is defined as follows: ≥ 20% increase in one direction and 5-mm absolute increase in the size of a measurable lesion on regular CT compared with the size at the most shrinked state; unequivocal progression of a non-target lesion; appearance of a de novo lesion; and exacerbation of clinical symptoms.

15.2 Overall survival

Overall survival is defined as the period from the date of registration until death from any cause. For subjects who are alive at the time of analysis and those lost to follow-up, the last day on which they were confirmed to be alive should be handled as the cut-off date.

15.3 Best overall response

In determining tumor response, the best overall response will be evaluated only in subjects with a measurable lesion by using the Response Evaluation Criteria In Solid Tumor (RECIST) version 1.1 (translated into Japanese by the JCOG). A response duration of 4 weeks or longer is not a requirement for complete response (CR) or partial response (PR) as the best overall response. However, stable disease (SD) as the best overall response requires that overall response be maintained at SD state from the time of registration until adjudication carried out at 6 weeks or later.

16. Safety endpoints

16.1 Incidence of adverse events/reactions

The incidence of adverse events/reactions by worst grade in each course will be investigated by using the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v 4.0; translated into Japanese by the JCOG/JSCO). Adverse events will be graded according to the closest match to the definition given in grades 1 to 4. In this study, treatment-related death will be recorded as a grade 4 event, although the original NCI-CTCAE specifies that this event should be classified into the grade 5 events.

In the event of treatment-related death, the causal relationship between the adverse event(s) observed and death will be investigated and subjected to adverse event reporting, and the details will be described in the “circumstances at the time of death” section of the treatment discontinuation form and the follow-up form. (A decision will be made separately in a post-hoc review as to whether the event should be handled as a grade 5 event.)

16.2 Definition of adverse events

An adverse event is any undesirable unintended sign (including abnormalities in clinical laboratory values), symptom, or disease observed during a treatment or procedure, regardless of the causal relationship with the treatment or procedure. This study will follow the grading system of the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v 4.0; translated into Japanese by the JCOG/JSCO).

16.3 Records of subjective and objective findings and laboratory tests during the study period

(1) Regarding the items described in the subjective and objective findings in Section 13.2(3), the grade and date of onset will be described in case report forms, regardless of whether they are adverse events or not.

(2) Regarding the items described in the blood tests in Section 13.2(4) (except Ccr, HBs antigen, HBs antibody, HBc antibody, and HCV antibody), test values and date of testing will be recorded in case report forms, regardless of whether the findings constitute adverse events.

(3) Regarding subjective and objective findings and blood tests not specified above, the event name, grade, and date of onset will be described in case report forms, only if there is a causal relationship with the study drug.

17 Reporting of adverse events

17.1 Adverse events requiring expedited reporting

Adverse events that fall under any of the following are subject to expedited reporting.

(1) Any death occurring during the study period or within 30 days after the final dose. It may or may not be causally related to the study treatment. The death of a patient in whom study treatment has already been discontinued is still subject to expedited reporting as long as the event occurred within 30 days after the final dose. (In counting "30 days," the final dosing day should be regarded as day 0.)

(2) Death occurring on or after day 31 following the final dose whose causal relationship with the study treatment cannot be ruled out. This applies to deaths suspected of being treatment-related. Unequivocal cases of death from cancer will be excluded.

(3) Unexpected grade 4 non-hematologic toxicity (adverse events not included in the Blood/Bone Marrow category in the CTCAE). Adverse events not listed in the package insert of each drug are applicable.

17.2 Adverse events requiring non-expedited reporting

Adverse events that fall under any of the following are subject to non-expedited reporting.

(1) Unexpected grade 2-3 adverse events

Grade 2-3 adverse events not listed in the package insert of the drug

(2) Irreversible and significant disorders

Aplastic anemia, myelodysplastic syndrome, secondary cancer

(3) Other significant medical events

Information that does not fall under any of the above but is judged to be important and should be shared by the principal investigator and study group members

17.3 Responsibilities of the facility representative

If an adverse event for mandatory reporting occurs, the facility representative should report it to the Study Coordinator in accordance with the following rules.

1) Expedited report

In the event of an adverse event subject to expedited reporting, the attending physician should promptly inform the facility representative. If the facility representative cannot be contacted, the attending physician should act on behalf of the facility representative.

i. Primary report

The facility representative should complete the Adverse Event Form (expedited primary reporting; timeframe: within 72 hours), send a fax or email to the Study Coordinator, and inform the Study Coordinator by phone within 72 hours after becoming aware of the occurrence of an adverse event of concern.

ii. Secondary report

The facility representative should complete the Adverse Event Form and separately prepare a case report (A4 size; no particular format specified) containing more detailed information. Both of these should be mailed, faxed, or emailed to the Study Coordinator within 7 days after the facility representative becomes aware of the adverse event of concern. Submission of a report with some incomplete sections is acceptable at this stage since prompt communication of information should be given priority.

iii. Tertiary report

The facility representative should complete all blanks in the Adverse Event Form and mail, fax, or email the completed Adverse Event Form to the Study Coordinator within 15 days after becoming aware of the adverse event of concern. An autopsy report should also be submitted if autopsy has been performed. (An autopsy report may be submitted at a later date.)

2) Non-expedited report

The facility representative should complete the Adverse Event Form and mail, fax, or email it to the Study Coordinator within 15 days after becoming aware of the occurrence of the adverse event of concern.

17.4 Responsibilities of the Study Coordinator

After confirming the reliability of the expedited report, the Study Coordinator should immediately report to the Chairperson of the Data and Safety Monitoring Board. At the same time, depending on the urgency and seriousness of the report, expedited reporting should be made known to the participating sites. If it is judged that the report is urgent and serious and that it is dangerous to continue the study, the Study Coordinator should direct the WJOG Data Center to suspend registration. If instructed by the Data and Safety Monitoring Board, the Study Coordinator will collect additional information and disseminate such information to study sites, and order the suspension or continued suspension of registration. Participating sites may be contacted by phone depending on the degree of urgency; however, such phone communication should be followed by a notice in writing (fax, mail, or email) as soon as possible.

17.5 Responsibilities of the Data and Safety Monitoring Board

The Data and Safety Monitoring Board will assess the progress of the study, safety data, and the efficacy of study treatment. It will also make recommendations to the Study Coordinator to continue, modify, or discontinue the study. The Board will review the Study Coordinator's views on reportable adverse events and the appropriateness of response to adverse events. Where necessary, the Board will recommend study discontinuation or protocol revision, and will also take charge of reviewing and approving proposed revisions. The Study Coordinator will be informed by the Board in writing of future actions, including handling of cases and whether to continue the study.

17.6 Reporting obligation based on the Ethical Guidelines for Medical Research

Involving Human Subjects

1) Reporting to the head of the study site

Adverse events subject to expedited reporting under the provisions of "17.1 Adverse events requiring expedited reporting" will be handled as "serious adverse events" in the Ethical Guidelines for Medical Research Involving Human Subjects. If these adverse events occur, the principal investigator of the study site should immediately report to the head of the study site in accordance with the regulations of the site.

2) Reporting to the Minister of Health, Labour and Welfare

Among the adverse events that are subject to expedited reporting under the provisions of "17.1 Adverse events requiring expedited reporting," unexpected adverse events for which a causal relationship with protocol treatment cannot be ruled out should be reported to the Minister of Health, Labour and Welfare through the head of the study site where the adverse event of concern occurred in accordance with the Ethical Guidelines for Medical Research Involving Human Subjects.

18 Case report forms

(1) The treating physician or clinical research coordinator (CRC) should complete the case report form without delay and submit it to the Data Center

(2) Submission due date

Pre-treatment report form: Within 28 days after registration

Course-specific report: Within 28 days after completion of each course

Completion/discontinuation report form: Within 28 days after completion/discontinuation

6- (12-, 18-, or 24-) month investigation form: Within 28 days after the investigation has been conducted

Follow-up form: Within 21 days after the request

(3) If any deviation from or non-compliance with the study protocol is found, the details and future actions should be described in the Remarks column of the relevant case report forms.

19 Monitoring and auditing

19.1 Monitoring

The Study Coordinator will monitor the progress of the study and review collected case reports to confirm the protocol compliance status of the participating sites. The occurrence of adverse events will be confirmed by means of expedited reports and non-expedited reports. If the number of mortality cases for which a causal relationship with the study treatment cannot be ruled out reaches 3, advice from the Data and Safety Monitoring Board will be sought on whether to continue the study.

19.2 Necessary measures

If, as a result of monitoring, the study implementation status of a participating site is judged to be inappropriate, the Study Coordinator will request the site to take appropriate measures to improve the situation.

20 Statistical considerations

20.1 Number of cases and the rationale therefor

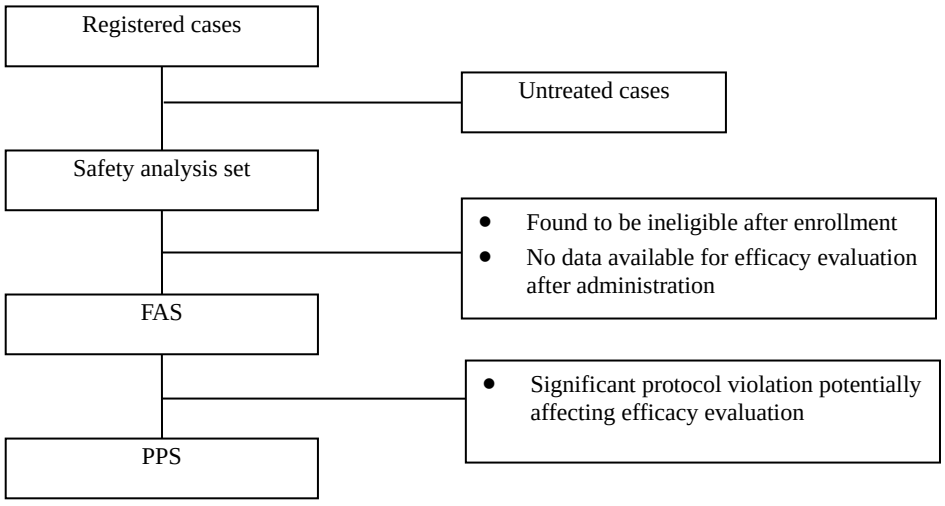
The threshold 1-year survival rate in this study has been set at 40% based on the fact that the median survival of patients treated with chemotherapy was 6 to 10 months in previous clinical studies of carcinoma of unknown primary site. Assuming that the new treatment strategy in this study will yield a similar outcome to that expected in

previous studies in which DNA chips were used to infer the primary site, the expected 1-year survival rate in this study has been set at 50%. With a β error of 0.2 and an α error of 0.05, a total of 102 cases would be necessary, assuming that the registration period is 2 years and the follow-up period is 1 year. Allowing for deviation cases, the number of cases has been set at 110.

20.2 Analysis population

The patient populations subject to statistical analysis are shown in the figure below. In principle, the classification of subjects is determined according to the following criteria. Efficacy analysis will be performed primarily in the full analysis set. Safety analysis will be performed primarily in the safety analysis set.

Registered cases	All subjects who have been registered by the Data Center after confirmation that they meet all inclusion criteria and do not fall under any of the exclusion criteria.
Safety analysis set	Among all registered subjects, those who received at least one dose of the study drug.
Full analysis set (FAS)	Population obtained by excluding the following subjects from the safety analysis set: ● Subjects who have been found to be ineligible after enrollment ● Subjects in whom efficacy has not been evaluated at all after administration
Per protocol set (PPS)	Population obtained by excluding the following subjects from the FAS: ● Subjects in whom a significant protocol violation has been noted that may affect efficacy evaluation ● Subjects in whom adequate efficacy evaluation is impossible due to insufficient observation



20.3 Final analysis

All endpoints will be subject to analysis 1 year after registration of the last subject. The details of the analysis will be provided separately in the final analysis plan. The analysis results will be compiled by the Data Center in the form of a final analysis

report and submitted to the Study Coordinator. The results will be presented in accordance with the rules of our study group.

21 Costs associated with the clinical study and compensation

This study will be conducted within the scope of usual health insurance coverage, and medications, tests during the study period will be covered by the subject's health insurance.

22 Responsibilities for addressing health damage to subjects

In the event that any health damage occurs in subjects as a result of the conduct of this study, the (sub)investigator and study site should take necessary measures such as medical treatment.

23 Approval and modification of the protocol

This protocol and written information for patients should be approved by the institutional review board of participating sites. If modification of an important part of the protocol becomes necessary after the start of the study, the Study Coordinator will report to the team members, discuss the matter, and carry out modifications. This does not apply to minor changes, however. Modifications of matters described in appendices such as changes in participating sites will not be handled as a revision of the protocol.

24 Discontinuation or suspension of the entire study

Discontinuation or suspension of the entire study may be considered in the following instances.

1. The Data and Safety Monitoring Board has made a recommendation to discontinue the study, and it has been determined that the continuation of the study is impossible as a result of deliberations at the member meeting.
2. There is nothing for it but to discontinue the study for certain reasons.

25 Publication of study results

After the end of the study, the results of the study will be promptly compiled and published in appropriate academic conferences and English journals in Japan and overseas.

26. Method for inferring the primary site by using NGS

26.1 Type and amount of samples

Biopsy samples:

Tumor tissue of metastatic lesions (including lymph nodes) will be collected by endoscopic biopsy, and percutaneous needle biopsy. Coelomic fluid samples such as pleural fluid and ascitic fluid are not acceptable because of quality problems.

26.2 Methods and procedures

26.2.1 Timing and method of collection

In principle, samples obtained at the time of diagnosis will be used. However, if it is impossible to collect a sufficient amount of samples for analysis at the time of diagnosis or if the samples obtained are not suitable for analysis, samples may be collected once again for analysis specifically, provided that the patient's consent is obtained and the patient safety is ensured. Samples will be collected by endoscopic biopsy and percutaneous needle biopsy.

26.2.2 Shipment of samples

- For biopsy samples subject to analysis, at least 10 (preferably 20) paraffin-embedded sections (FFPE sections, approximately 4 to 5 μm thick) with

tumors confirmed by a pathologist should be sent by courier (at room temperature). Even in cases where 10 or more sections cannot be prepared, efforts should be made to submit as many sections as possible. It is desirable to submit sections mostly made up tumors.

- Samples should be labeled with the case registration number.
- A report from the pathologist should be attached, and a photomicrograph of hematoxylin-eosin stained specimens should also be appended whenever possible. Personal information (name, date of birth) included in the report should be masked so that it is not disclosed to the outside.
- The Nakagawa Group will bear the costs of shipment (including newly purchased slide cases). (Original receipts should be obtained for these expenses.)
- Shipment should be arranged in a way that samples are delivered between 9 a.m. and 5 p.m. on weekdays (not on Saturday, Sunday, or national holidays).

Delivery address

Tissue biopsy samples should be sent to:

Kazuto Nishio, Department of Genome Biology, Kindai University Faculty of Medicine
knishio@med.kindai.ac.jp
377-2, Ohno-higashi, Osakasayama, Osaka 589-8511
Tel.: 072-366-0221, ext.: 3150
Fax: 072-367-6369

26.2.3 Method for inferring the primary site

Delivered samples will be subjected to DNA and RNA extraction and analyzed for gene mutation, amplification, and expression at the Department of Genome Biology, Kindai University Faculty of Medicine. The flow chart below will be followed in inferring the primary site.

A. The primary site will be inferred based on gene expression data obtained from extracted RNA. This process involves an algorithm that applies the actual analysis results from the Randomized Phase II Study Comparing Chemotherapy Led by Using the Classification of Primary Tumor Origin with Empirical Chemotherapy for Patients with Unknown Primary Cancer, which we previously conducted using the cancer classification algorithm constructed by using Gene Expression Omnibus, a public database, and learning data at the Department of Genome Biology, Kindai University. This previously used algorithm is constructed from an analysis of 22,215 probe sets commonly measured in 2,280 organ-specific carcinomas with a diagnosis of primary site, and cross-validation has confirmed that appropriate cancer classification is enabled by reduction of variables and selection of cancer type. The reduction of variables is implemented in two stages. In the first stage, the average of the highly correlated probe sets is obtained and variables are reduced to about 1,000. In the second stage, they are reduced to some 10 variables by the partial least square method. Cancer type selection is based on reduced variables, and classification is done according to the closest expression profile. However, classification is impossible if the information entropy of the sample is higher than a certain level. In the analysis of more than 120 patients in an actual clinical study, there were several genes that were poorly expressed and could not be used for inference of primary sites. It was therefore considered essential to narrow down the gene candidates in view of future applications to clinical practice, as well as for the sake of convenience. Accordingly, an algorithm in which the candidates were narrowed down to about 150 genes was constructed anew wherein the weighted voting method is used for score calculation. Different algorithms are applied depending on the sex. Verification using the database has shown that almost equivalent results to the old algorithm can be obtained.

B. Cancer-specific gene mutations and gene amplifications that have a significant impact on treatment strategies will be explored based on DNA analysis. Specifically, active EGFR mutation in lung adenocarcinoma, active c-KIT mutation in sarcoma (GIST), HER2 gene amplification in breast cancer and gastric cancer, and RAS mutation in colorectal cancer will be investigated. If a cancer-specific gene mutation or amplification is present that is relevant to treatment, the probability of each cancer as calculated by Bayes' inference is multiplied by the cancer inference score in the algorithm mentioned in A above, and the one with the highest score is adopted as the inferred type of cancer. Gene information will be communicated to the treating physician if it is associated with the cancer type identified as a result of inference (EGFR mutation for lung adenocarcinoma, c-KIT mutation for sarcoma, and HER2 gene amplification for breast cancer or gastric cancer). If these gene mutations and amplifications are present, it can be presumed that patients with lung cancer are EGFR mutation-positive, patients with sarcoma have c-KIT mutation-positive gastrointestinal stromal tumors, and patients with breast cancer or gastric cancer are HER2-positive, and medical care on health insurance may be provided on such assumptions (treatment with gefitinib, erlotinib, or afatinib for lung cancer; treatment with imatinib or sunitinib for sarcoma; and HER2 therapy with trastuzumab for breast cancer or gastric cancer). RAS gene mutations in colorectal cancer will be used for the decision as to whether to use an anti-EGFR antibody.

The WJOG Data Center will be informed of the primary site inference results thus obtained within about 7 business days (not including Saturdays and Sundays), and the site in charge of the subject of concern will be also notified immediately.

26.3 Sample storage location and period

Samples will be stored at a laboratory within the Department of Genome Biology, Kindai University Faculty of Medicine. Kazuto Nishio of the Department of Genome Biology, Kindai University Faculty of Medicine will assume responsibility for the storage of samples. DNA and RNA will be stored in a -80°C deep freezer. Paraffin slides of surgical samples will be stored in the same laboratory within the Department of Genomic Biology, Kindai University Faculty of Medicine. Samples will be stored until the end of the period of five years from the date on which the end of the study is announced or until the end of three years from the date on which the final report of the study results is published, whichever comes later. Samples for which the storage period has expired are discarded unless there is a particular reason. The security of the storage area will be ensured with two or three protective measures, namely access control at the entrance to the lab building, a locked lab door, and a key-controlled deep freezer.

26.4 Disposal of samples

Samples will be disposed of if the consent of the contributing patient is withdrawn, if the anonymized number becomes invalid due to a flaw in labeling or computer glitch, if there is a documented/suspected mix-up or contamination of the sample, or if the investigator recognizes the necessity of disposal. In such cases, the anonymized number should be removed before disposal. If a contributing patient withdraws his/her consent and applicable samples are stored at a study site, the person in charge at the study site will be informed of the consent withdrawal and should dispose of the samples. If the applicable samples are stored at Kindai University, the Study Coordinator will be contacted for disposal. Samples for which the storage period has expired (five years from the date on which the end of the study is announced or three years from the date on which the final report of the study results is published, whichever is later) will be

disposed of, unless there is a particular reason for further storage.

26.5 Use of surplus samples

If consent is obtained, surplus samples will be stored as valuable research resources and made available for research purposes. However, the use of surplus samples in a way not specified in this protocol should be limited to cancer research only, and it is also essential that the study protocol be approved by our study group as well as the institutional review board of the facility providing the samples. No germline genetic analysis will be performed. Surplus samples will be stored at a laboratory within the Department of Genome Biology, Kindai University Faculty of Medicine until the end of the period of five years from the date on which the end of the study is announced or until the end of three years from the date on which the final report of the study results is published, whichever comes later. Surplus samples for which the storage period has expired are discarded unless there is a particular reason for further storage.

26.6 Analytical techniques that may be employed in the future

NGS, microarrays, RT-PCR, immunostaining, and ELISA. Any of these can be used for the analysis of cancer cells and the detection of somatic mutations or acquired genomic aberrations. It should be noted that the “structure or function of the human genome and genes that can be inherited by offspring” will not be subject to analysis. However, analysis of normal tissue to identify mutations will be allowed since it is not within the scope of the Ethical Guidelines for Human Genome/Gene Analysis Research. On the other hand, analysis of normal tissue for the “structure or function of the human genome and genes that can be inherited by offspring,” such as SNP analysis, will not be allowed. Review and approval by the participating site and Nakagawa Group are required whenever samples collected in this study are to be used.

26.7 Anticipated results and attendant risks

This study will increase the likelihood that patients with carcinoma of hitherto unknown primary site can receive more effective treatment by adding another tool to infer the primary lesion, and this not only greatly contributes to the improved treatment of carcinoma of unknown primary site but also potentially helps clarify the characteristics of this condition. However, it is highly likely that new tissue samples will be collected for this study specifically, and there is a potential risk that physical hazards and disadvantages may arise out of new sample collection. It is conceivable that the risks and disadvantages related to the human rights and privacy of patients are extremely small for the following reasons: (1) the study will be conducted in accordance with this study protocol and subjects will be fully informed of the study content and give their consent; (2) personal information will be anonymized and strictly controlled; (3) the “structure or function of the human genome and genes that can be inherited by offspring” will be outside the scope of this study; and (4) the genomes of normal tissues will not be analyzed except as controls.

26.8 Method of protecting personal information

Personal information is anonymized and strictly controlled by the WJOG Data Center. Genetic information linked to personal information will not be provided to the analysis facility or any third party.

26.9 Disclosure of genetic information

The disclosure of genetic information will be handled as follows. The result of genetic analysis in each patient will not be disclosed without a request for disclosure from the

patient. The reason for this is that whether the newly identified evidence from the samples stored in this study has clinical significance is unclear unless correlation analysis with clinical information is carried out. Moreover, such significance depends in a large part on future research, such as evaluation in follow-up studies. Nonetheless, if a patient requests disclosure of genetic analysis results despite being informed of the above, only his/her data will be disclosed. In such cases, the attending physician should contact the Study Coordinator by phone or email.

26.1 Genetic counseling

This study does not fall under the scope of research on single-gene disorders and does not involve the "structure or function of the human genome and genes that can be inherited by offspring." Therefore, gene counselling is not applicable.

26.11 Definition of terms

The definition of terms in this study protocol is congruent with that in the Ethical Guidelines for Human Genome/Gene Analysis Research, which was established through joint efforts by the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labour and Welfare, and the Ministry of Economy, Trade and Industry.

Samples:

Parts of human body such as blood, tissues, cells, body fluids, and excrement, as well as human DNA extracted therefrom, that are intended to be used in human genome/gene analysis research, information of medical treatment in contributing patients, and other information used in the research.

Human genome/gene analysis research:

A type of research that attempts to clarify the structure or function of the human genome and genes that are commonly present in the cells making up individual donors and can be inherited by their offspring by using samples This includes cases where only samples used in this study are provided. (Detailed rules) In principle, these guidelines do not apply to research targeting genomic or genetic mutations that arise after birth only in lesions in diseases such as cancer and are not inherited by offspring (i.e., research analyzing somatic mutations, including analysis of normal tissue to identify mutations), research on gene expression, and research on the structure or function of protein.

Anonymization:

Removal of identifying information so that the original source cannot be known. Donors can be identified when necessary through a correspondence table between the donors and newly assigned codes or numbers.

27. Study organization

This study will be conducted as a group study of the Nakagawa Group. The study results will be shared and published regardless of the results.

Study Chair

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Statistical analysis manager: Yasutaka Chiba, Clinical Research Center, Kindai University Hospital

Data and Safety Monitoring Board Chairperson
Tetsuya Mitsudomi, Department of Thoracic Surgery, Kindai University Faculty of Medicine

Data and Safety Monitoring Board Member
Taro Sato, Department of Frontier Science for Cancer and Chemotherapy, Osaka University Graduate School of Medicine

Data and Safety Monitoring Board Member
Naruo Yoshimura, Department of Respiratory Medicine, Osaka City University Faculty of Medicine

28. Administrative expenses for the study

The administrative expenses for this study will be covered by consigned research expenses of the Japan Agency for Medical Research and Development (Practical Research Project for Innovative Cancer Medicine) for fiscal 2016.

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