A Phase I Clinical Study Evaluating the Safety, Tolerability, Pharmacokinetic (PK) Characteristics, and Preliminary Efficacy of Intratumoral Injection of Ferrous Ion Adsorbed Carbon Nanoparticle Suspension Injection (CNSI-Fe) in Patients with Advanced Solid Tumors

Study Protocol

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Screening Number:

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Sponsor: Sichuan Enray Pharmaceutical Technology Company

Study Protocol

Indication: Patients with advanced solid tumors, such as colorectal cancer, pancreatic cancer, breast cancer, gastric cancer, cervical cancer, lung cancer, head and neck cancer, cholangiocarcinoma, renal cancer, prostate cancer, vulvar cancer, etc., who have failed standard treatment (disease progression after treatment or unable to tolerate treatment) or have no standard treatment available.

Study Objectives

Primary Objectives: To evaluate the safety and tolerability of intratumoral injection of different doses of the ferrous iron adsorbed carbon nanoparticle suspension injection (CNSI-Fe) in patients with advanced solid tumors, and to observe the dose-limiting toxicity (DLT) of CNSI-Fe, and to determine the maximum tolerated dose (MTD) or the maximum injectable dose in humans, which will provide a basis for the selection of doses in subsequent clinical studies.

Secondary Objectives: To evaluate the pharmacokinetic (PK) characteristics of intratumoral injection of different doses of CNSI-Fe in patients with advanced solid tumors;

To preliminarily evaluate the efficacy of intratumoral injection of different doses of CNSI-Fe in patients with advanced solid tumors.

Exploratory Objectives: To evaluate the intratumoral pharmacodynamic (PD)

characteristics of intratumoral injection of different doses of CNSI-Fe in patients with advanced solid tumors (not mandatory);

To evaluate the intratumoral PK characteristics of intratumoral injection of different doses of CNSI-Fe in patients with advanced solid tumors (not mandatory);

To explore the relationship between tumor size and CNSI-Fe injection dose, as well as the dose-effect relationship of CNSI-Fe concentration, in patients with advanced solid tumors receiving intratumoral injection of CNSI-Fe (not mandatory).

Study Endpoints

Primary Endpoints:

Safety

Adverse events (AEs), vital signs, physical examinations, laboratory tests, Eastern Cooperative Oncology Group (ECOG) performance status (see Appendix 1), 12-lead electrocardiogram (ECG), echocardiography, etc. during the study period;

Incidence of dose-limiting toxicities (DLTs).

Secondary Endpoints:

Pharmacokinetics (PK)

Including but not limited to area under the curve (AUC_{0-t}), peak concentration (C_{max}), $AUC_{0-\infty}$, time to peak concentration (T_{max}), apparent volume of distribution (V_d), elimination rate constant (K_e), half-life ($t_{1/2}$), apparent clearance (CL), mean residence time (MRT), Vd/bioavailability (F), CL/F, accumulation ratio, etc.

Efficacy

Evaluation of anti-tumor efficacy of the injected lesions based on the revised Response Evaluation Criteria in Solid Tumors version 1.1 (revised RECIST v1.1) (see Appendix 2), including objective response rate (ORR) and disease control rate (DCR);

Evaluation of overall anti-tumor efficacy based on the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) (see Appendix 3).

Note: The primary efficacy endpoints are ORR and DCR of the injected lesions based on the revised RECIST v1.1.

Exploratory Endpoints

Exploration of the levels of hydrogen peroxide (H_2O_2), peroxidase (POD), malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase 4 (GPX4), hydroxyl radical (·OH), etc. in tumor tissue samples after administration (optional);

Exploration of the intratumoral PK study in the injected lesions, if available (not mandatory);

Exploration of the dose-response relationship between tumor size and administered dose/concentration using three-dimensional reconstruction technology (3D-CTA) to guide rational dosing in future clinical trials (not mandatory).

Total number of subjects: up to 30 cases, the final sample size will depend on the number of dose groups evaluated and the tolerability in each dose group.

Number of study centers: expected to be 3-6 centers.

Subject Selection Criteria

Inclusion Criteria：

Patients must meet all of the following criteria to be eligible for enrollment:

Understand and voluntarily sign the written informed consent form (ICF), have the willingness and ability to comply with all trial requirements;

Signed the ICF and are male or female aged 18-80 years (inclusive) at the time of signing the ICF;

Patients with histologically or cytologically confirmed advanced solid tumors, for whom standard treatment is ineffective (disease progression after treatment or unable to tolerate treatment) or there is no effective standard treatment available, such as colorectal cancer, pancreatic cancer, breast cancer, gastric cancer, cervical cancer, lung cancer, head and neck cancer, cholangiocarcinoma, renal cell carcinoma, prostate cancer, vulvar cancer, etc.

Note: Patients with advanced solid tumors who are unable to receive standard treatment due to any reason leading to disease progression, or patients with tumor types insensitive to current standard treatments (such as pancreatic cancer, undifferentiated thyroid cancer, and sarcoma) who have disease progression after receiving the first course of standard treatment, can be included.

According to RECIST v1.1, have at least one measurable lesion, and this lesion has not received prior radiation therapy (unless there is clear progression of the lesion

after radiation therapy), nor has it undergone a tissue biopsy within 7 days prior to the first dose;

Have an injectable lesion (e.g. can be directly injected or assisted by medical imaging instruments);

ECOG performance status of 0-1 within 7 days prior to the first dose;

Expected survival time ≥12 weeks;

Drug-related adverse reactions (ADRs) from prior treatments have recovered to ≤Grade 1 per NCI CTCAE v5.0 (except for alopecia) prior to screening;

Left ventricular ejection fraction (LVEF) ≥50%;

Within 7 days prior to the first dose, have adequate hematologic and end-organ function, with laboratory tests meeting the following criteria:

a) Hematology

No use of granulocyte colony-stimulating factor (G-CSF) within 14 days prior to hematology laboratory tests, and absolute neutrophil count (ANC) ≥1.5×109/L;

No platelet transfusion within 14 days prior to hematology laboratory tests, and platelet count (PLT) ≥90×109/L;

No blood transfusion or use of erythropoietin within 14 days prior to hematology laboratory tests, and hemoglobin (Hb) ≥90 g/L;

b) Renal function

Serum creatinine (Cr) ≤1.5×upper limit of normal (ULN) or calculated creatinine clearance (Ccr) ≥50 mL/min using the Cockcroft-Gault formula (only calculated if baseline Cr >1.5×ULN);

c) Hepatic function

Total bilirubin (TBIL) ≤1.5×ULN (≤3.0×ULN for patients with Gilbert's syndrome or liver metastases);

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) ≤3×ULN, patients with confirmed liver or bone metastases must meet the following criteria:

Patients with confirmed liver metastases: AST and ALT ≤5×ULN;

Patients with confirmed bone metastases: ALP ≤5×ULN;

Serum albumin ≥2.8 g/dL;

d) Coagulation function

International normalized ratio (INR) or prothrombin time (PT) and activated partial thromboplastin time (aPTT) ≤1.5×ULN;

Note: For patients with injectable lesions in the skin and/or subcutaneous tissue who are receiving anticoagulant therapy, prolonged INR, PT and aPTT are allowed, as bleeding can be controlled by direct pressure application, as determined by the investigator.

For female patients of childbearing potential (WOCBP), a negative pregnancy test result must be obtained within 7 days prior to the first dose of the study drug, and they must agree to use highly effective contraception or abstinence during the study drug treatment and for 6 months after the end of study drug treatment. In addition, female patients must be non-lactating and agree not to donate eggs during this period;

Note: WOCBP is defined as a female who has experienced menarche but has not

undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as a female over the age of 45 years who has not had menses for 12 consecutive months in the absence of other biological or physiological causes. Additionally, females 55 years of age or younger must have a serum follicle-stimulating hormone (FSH) level >40 mIU/mL to be considered post-menopausal.

Hormone replacement therapy (HRT) may artificially suppress a woman's FSH levels and may require a washout period before the physiological FSH level can be restored. The duration of the washout period depends on the type of HRT. The following washout period durations are suggested as guidance, and the investigator should use their judgment based on the FSH level test results:

At least 1 week for vaginal hormonal products (rings, creams, gels);

At least 4 weeks for transdermal products;

At least 8 weeks for oral products;

Up to 6 months for other non-oral, non-transdermal products.

If during the washout period the serum FSH level is >40 mIU/mL, the patient may be considered post-menopausal.

Male patients must agree to use highly effective contraception or abstinence during the study drug treatment and for 6 months after the end of study drug treatment. In addition, male patients must agree not to donate sperm during this period.

Exclusion Criteria:

Patients who meet any of the following criteria will not be eligible to participate in this clinical study:

History or current presence of iron metabolism disorders (except for iron deficiency anemia), such as thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency (favism), etc.;

History or current evidence of hollow organ perforation at the injection site;

History or current skin breakdown, redness, swelling, necrosis, or bleeding at the injection site that may affect the administration of the investigational drug;

Received radiotherapy or any anti-cancer therapy within 4 weeks prior to the first administration of the investigational drug, or the time since the last anti-cancer treatment [including but not limited to chemotherapy, targeted therapy, immunotherapy, National Medical Products Administration (NMPA)-approved anti-cancer Chinese patent medicines, and Chinese medicines with anti-tumor effects] is less than 5 half-lives, whichever is shorter;

Underwent major surgery, significant trauma, or have persistent unhealed wounds and ulcers within 4 weeks prior to the first administration of the investigational drug;

Presence of life-threatening clinical manifestations of brain and central nervous system metastases at the time of enrollment;

Uncontrolled hypertension (systolic blood pressure ≥150 mmHg or diastolic blood pressure ≥100 mmHg);

Uncontrolled cancer-related pain;

Uncontrolled pleural effusion, pericardial effusion, or ascites requiring repeated

drainage (once/month or more frequently);

Concurrent malignancy within 5 years prior to the first administration of the study treatment, except for curatively treated non-melanoma skin cancer, localized prostate cancer, ductal carcinoma in situ or stage I uterine cancer, cervical carcinoma in situ, or breast carcinoma in situ;

Received live virus vaccines within 4 weeks prior to the first administration of the investigational drug;

Note: Seasonal influenza vaccines are usually inactivated influenza vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are attenuated live vaccines and are not allowed.

History of immunodeficiency, including positive for human immunodeficiency virus (HIV) or other acquired or congenital immunodeficiency diseases, or history of organ transplantation;

Active hepatitis B virus (HBV) infection [positive for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb) and HBV DNA >500 IU/mL], hepatitis C virus (HCV) infection [positive for HCV antibody and HCV RNA by polymerase chain reaction (PCR) above the upper limit of normal], or positive for anti-human immunodeficiency virus antibody (Anti-HIV);

Serious chronic or active infections (including tuberculosis infection) requiring systemic antibacterial, antifungal, or antiviral treatment within 4 weeks prior to the start of the study treatment;

Note: Patients with viral hepatitis are allowed to receive antiviral treatment.

Severe cardiovascular diseases, including but not limited to:

History of acute coronary syndrome or coronary artery angioplasty/stent placement/bypass surgery within the past 6 months;

New York Heart Association (NYHA) (see Appendix 4) class II-IV congestive heart failure (CHF), or history of NYHA class III or IV CHF.

Active psychiatric disorders (schizophrenia, severe depressive disorder, bipolar disorder, etc.);

Known allergy or intolerance to the active ingredient, excipients, or other iron supplementation products of the investigational drug;

Participated in other interventional clinical studies within 4 weeks prior to the start of the study treatment (calculated from the first day after the last dose of the previous study, except for those who did not use any investigational drugs or medical devices);

Any other condition that the investigator deems unsuitable for the patient to participate in this study.

Criteria for Suspension of Clinical Trials:

The trial will be suspended until the cause is confirmed and the safety review committee (SRC) composed of the sponsor and investigators, etc. discusses and determines that the trial can be restarted, under the following circumstances:

Any death of a subject not due to disease progression within 21 days (inclusive) after injection of CNSI-Fe;

Multiple subjects discontinue study drug treatment for reasons other than disease

progression;

The incidence and severity of adverse drug reactions indicate that the risks of the study drug regimen outweigh the benefits;

The quality of data recording is too poor, inaccurate and/or incomplete.

Withdrawal Criteria:

Withdrawal Decided by Investigators: Withdrawal decided by investigators refers to the situation where a selected participant is deemed unsuitable to continue the study during the research process, and the investigator decides to withdraw the participant from the study. This includes, but is not limited to, the following situations:

The participant has seriously deviated from or violated the protocol, and this has affected the evaluation of the drug's efficacy or safety, so the participant must be withdrawn from the study;

The investigator believes that the participant should be withdrawn from the study for other reasons.

Withdrawal Decided by Participants: According to the Good Clinical Practice (GCP) for Pharmaceutical Trials and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline E6, all participants have the right to withdraw from the study at any time during the trial, or if the participant no longer accepts medication and testing but does not explicitly request withdrawal, this is also considered "withdrawal" (or "dropout"). The reasons for withdrawal should be understood as much as possible and recorded, such as:

unable to tolerate certain adverse events; unable to continue the clinical study due to other reasons; or lost to follow-up without explanation, etc.

Handling of Withdrawn Cases

Investigators must record the reason for participant withdrawal in the electronic case report form (eCRF), and try to contact the participant as much as possible to complete the assessments that can be done. Regardless of the reason, the eCRF of the withdrawn case should be retained. For withdrawn cases where adverse events exist at the time of study termination, the follow-up requirements for the adverse events are described in Section 8.6.

The basis for dose selection: According to the "Guidance Principles for Estimating the Maximum Recommended Starting Dose for First-in-Human Clinical Trials of Healthy Adult Volunteers" issued by the NMPA, and with reference to the Maximum Recommended Starting Dose (MRSD) for anticancer drugs in ICH S9 "Nonclinical Evaluation for Anticancer Pharmaceuticals", the design of the dosage regimen depends on the data from the long-term toxicity studies of CNSI-Fe in animals. In the toxicity study with 2 intramuscular injections on Day 1 and Day 4, followed by a 4-week recovery period in rats, the Highest Non-Severely Toxic Dose (HNSTD) was 20 mg/kg (calculated as Fe^{2+}), corresponding to a Human Equivalent Dose (HED) of 3.6 mg/kg; in the toxicity study with Beagle dogs, the HNSTD was 15 mg/kg, corresponding to an HED of 8.7 mg/kg. Assuming an adult body weight of 60 kg and a safety factor of 6, the MRSD for humans is estimated to be approximately 0.6

mg/kg and 1.45 mg/kg, respectively.

Considering the effective doses in preclinical studies, in multiple solid tumor xenograft models (human colon cancer COLO 205, human breast cancer MDA-MB-231, human breast cancer HCC1954, and human colon cancer HT-29 cells), CNSI-Fe (calculated as Fe^{2+}, 0.1875 mg, 0.375 mg, 0.50 mg, 0.75 mg, administered twice on Day 1 and Day 4) significantly inhibited tumor growth. The effective dose and the maximum effective dose were 0.1875 mg and 0.75 mg, respectively. Assuming a mouse body weight of 20 g and an adult human body weight of 60 kg, the corresponding human equivalent doses are 0.84 mg/kg and 3.36 mg/kg, respectively.

Considering the above factors and the safety of the first-in-human trial, this study selected 0.5 mg/kg as the starting dose, with escalating doses of 0.5 mg/kg, 1.0 mg/kg, and 1.5 mg/kg. Calculated based on an average human body weight of 60 kg, the escalating doses are 30 mg, 60 mg, and 90 mg (calculated as Fe^{2+}). If ≥2 subjects experience DLT at the 90 mg dose, the dose will be reduced to 75 mg for safety and tolerability observation; if the dose escalation to 90 mg does not reach the MTD, the SRC will discuss whether to further escalate the dose to 120 mg, 150 mg, or even higher, based on the available safety, PK/PD, and preliminary efficacy data (if 120 mg still does not reach the MTD, the SRC will discuss whether to stop the dose escalation or directly proceed to 150 mg or even higher doses).

Study Design: This is an open-label, dose-escalation Phase I clinical study conducted in patients with advanced solid tumors, aiming to evaluate the safety, tolerability, PK/PD characteristics, and preliminary efficacy of CNSI-Fe intratumoral injection in patients with advanced solid tumors.

Study Period: The study includes a screening period, a treatment period (including DLT evaluation period), and a follow-up period.

Screening Period: -28 to -1 days, to confirm that the subjects meet the inclusion criteria and do not meet the exclusion criteria.

Treatment Period (including DLT Evaluation Period): The DLT evaluation period is the first treatment cycle (duration: 21 days). During the DLT evaluation period, all subjects will receive CNSI-Fe intratumoral injection according to the protocol plan (preferentially selecting 1 suitable lesion for injection, tentatively with 1 injection on Day 1; if the corresponding dose of the investigational drug cannot be completed in 1 lesion, other lesions can be injected simultaneously; the dosing regimen may be adjusted by the sponsor and investigator based on the PK characteristics of this study). Safety assessments will be performed on Day 7, Day 14, and Day 21 after the first dose to evaluate DLT.

After the DLT evaluation is completed, subjects who do not experience unacceptable toxicity, if the investigator judges that the benefit of continued treatment outweighs

the risk, will enter the maintenance treatment period. In the maintenance treatment period, subjects will continue to receive one more dose of the investigational drug according to the DLT evaluation period dosing regimen (injection into the same lesion as the DLT evaluation period, tentatively with 1 injection on Day 1, the dosing regimen may be adjusted by the sponsor and investigator based on the PK characteristics of this study), and safety assessments will be performed on Day 7, Day 14, and Day 21 after dosing. Samples will be collected at the predetermined timepoints according to the study flow chart.

Follow-up Period: Safety follow-up until 28 days after the last dose.

Dose Escalation

This study will enroll patients with advanced solid tumors and use the traditional "3+3 method" for dose escalation. The dose escalation principle is: in any dose cohort, 3 subjects will be enrolled first. If none of the first 3 subjects experience DLT during the DLT evaluation period, the next dose cohort can be entered. If ≥2 of the first 3 subjects experience DLT during the DLT evaluation period, dose escalation will be stopped (for the 30 mg cohort, if 2 DLTs occur, the sponsor and investigator will jointly decide whether to reduce the dose and continue exploration). If 1 of the first 3 subjects experiences DLT during the DLT evaluation period, an additional 3 subjects will be enrolled in that dose cohort, and if ≤1 of the 6 subjects in that dose cohort experience DLT during the DLT evaluation period, the next dose cohort can be entered; if ≥2 of the 6 subjects in that dose cohort experience DLT during the DLT

evaluation period, dose escalation will be stopped. The dose prior to the dose at which ≥2/3 or ≥2/6 subjects experience DLT during the DLT evaluation period will be determined as the MTD (if only 3 subjects are enrolled in a cohort, an additional 3 subjects will need to be enrolled).

The "3+3" dose escalation principle

The number of subjects who experienced a dose-limiting toxicity (DLT) within a particular dose cohort	**dose escalation principle**
0/3	no DLT is observed in the 3 newly enrolled subjects
0/3	no DLT is observed in the 3 newly enrolled subjects
≥2/3	Dose escalation should be stopped, and that dose will be considered the Maximum Administered Dose (MAD). If the previous lower dose was only administered to 3 subjects, then 3 more subjects need to be enrolled
1/3	If the 3 newly enrolled subjects in the dose group do not erience a DLT (Dose-Limiting Toxicity), then the study will ceed to the next dose group. ● ● If ≥1 of the 3 newly enrolled subjects in the dose group experience a DLT, then dose escalation will be stopped, and that dose level will be considered the MAD (Maximum Administered Dose). The previous dose level will be considered the MTD (Maximum Tolerated Dose) (if the dose group only had 3 subjects, then an additional 3 subjects will need to be enrolled).

The DLT (Dose-Limiting Toxicity) evaluation period is in the first cycle, i.e., within 21 days after the first dose. This stage is tentatively set with 3 dose groups (calculated as Fe^{2+}, 30 mg, 60 mg, 90 mg). If ≥2 subjects in the 90 mg group observe DLT, the dose will be reduced to 75 mg for safety and tolerability observation; if the dose is increased to 90 mg and MTD (Maximum Tolerated Dose) is still not reached, the SRC

(Safety Review Committee) will discuss continuing to escalate the dose to 120 mg, 150 mg, or even higher (if MTD is still not reached at 120 mg, the SRC will discuss whether to stop dose escalation or directly proceed to 150 mg or even higher doses).

After 3-6 subjects in each dose group have completed the DLT evaluation, the SRC will evaluate the overall safety of each dose group based on the data obtained and determine whether to continue escalating to the next dose group or to determine the recommended dose for subsequent clinical development.

Enrollment Interval: To fully protect the safety of the subjects, during the dose escalation process, the first subject in each dose group must be dosed at least 3 days after the first dose, and the remaining subjects in that dose group can then start the first dose. The interval for subsequent subjects in the same dose group can be controlled at the discretion of the investigator. The last subject in each dose group must complete the DLT evaluation, and the safety and tolerability must be reviewed by the SRC before the first subject in the next dose group can receive the first dose.

Subject Replacement: During the DLT evaluation period, if a subject is unable to be evaluated for DLT (e.g., the subject's dose of the study drug did not reach at least 80% of the protocol-specified dose, or the subject discontinued study treatment before completing the DLT evaluation period for reasons other than DLT), additional subjects will need to be enrolled to make up the corresponding number in that dose

group.

DLT Definition: This study will grade toxicities according to NCI CTCAE v5.0. DLT refers to the following toxicity reactions that are considered by the investigator and/or the sponsor to be definitely or possibly related to the study drug CNSI-Fe, occurring within the first cycle (i.e., within 21 days after the first dose):

Hematological toxicity:

(1) Any grade 4 hematological toxicity;

(2) Any grade 3 hematological toxicity that persists for >7 days after best supportive care, or grade 3 thrombocytopenia with bleeding tendency or requiring platelet transfusion;

(3) Grade 3 neutropenia with fever, defined as: ANC<1×10^9/L and a single temperature >38.3°C or sustained temperature ≥38°C for >1 hour.

Non-hematological toxicity:

(1) Grade 3 cardiac toxicity that persists for >3 days after best supportive care, or grade 4 cardiac toxicity;

(2) Grade 3 hepatic toxicity (for subjects with liver or bone metastases, and baseline grade 2 liver transaminases, ALT, AST or ALP >10×ULN) that persists for >3 days after best supportive care, or grade 4 hepatic toxicity;

(3) Any grade 3 non-hematological toxicity (excluding laboratory tests) that persists

for >7 days after best supportive care, except for grade 3 nausea, vomiting or diarrhea;

(4) Any grade 4 non-hematological toxicity (excluding laboratory tests);

(5) Any grade 3 or 4 non-hematological laboratory abnormality, if:

It requires medical intervention;

Or leads to hospitalization;

Or persists at grade 3 or above for ≥7 days after drug discontinuation.

Others:

(1) Any grade 5 toxicity;

(2) Other grade toxicities that the investigator and sponsor agree require early termination of CNSI-Fe treatment.

This study will not only observe the safety and tolerability of CNSI-Fe, but also preliminarily evaluate the efficacy of CNSI-Fe in patients with advanced solid tumors.

The investigators will evaluate the efficacy of CNSI-Fe based on the results of imaging examinations [computed tomography (CT), positron emission tomography (PET)/CT, or magnetic resonance imaging (MRI)], referring to the revised RECIST v1.1 and RECIST v1.1.

Contrast-enhanced CT or contrast-enhanced MRI is the preferred imaging method for evaluating tumor response. If the subject is known to be allergic to the contrast agent, if possible, obtain a contrast agent evaluation using local preventive standards, or use

alternative methods. In cases where contrast agents are strictly contraindicated, non-contrast scans should be performed. If the subject is contraindicated for contrast-enhanced CT scans, a non-contrast chest CT scan and contrast-enhanced abdominal and pelvic MRI scan can be performed.

The baseline imaging evaluation will be performed within 28 days prior to the first study treatment (if the subject has undergone imaging examinations at the study site during the routine clinical care process prior to signing the ICF, and the examination site and method meet the protocol requirements, and the examination time is within 28 days prior to the first study treatment, the corresponding examination results may be used for this trial's screening after the investigator's consent).

All subjects will undergo tumor assessment once every 3-4 weeks during the study treatment period, and once every 7-8 weeks for those who enter the maintenance treatment period (the investigator may decide whether to perform additional imaging examinations for special changes in the disease condition, and the first tumor assessment must be performed at 3-4 weeks).

Subjects who achieve a complete response (CR) or partial response (PR) in the first assessment will require confirmation at least 4 weeks later.

The baseline tumor evaluation must include the chest, abdomen, pelvis, and head (to

exclude brain metastases), as well as other suspected tumor sites. Subsequent evaluations should re-evaluate all measurable and evaluable lesions found at baseline, including the chest, abdomen, pelvis, and other suspected tumor sites.

The imaging examination methods used for efficacy evaluation of subjects during the study period must be consistent with the baseline imaging evaluation methods.

Investigational medicinal products

The CNSI-Fe involved in this study is composed of a ferrous iron adsorbed carbon nanoparticle, which are prepared and used on-site. The drug information is as follows:

Drug Name	Dosage Form	Specification	Storage Conditions	Shelf Life
CNSI-Fe(II)	Injection	2 mL：100 mg	Store at 2-8°C	24 months
Ferrous Sulfate for Injection Lyophilized	Powder for Injection	30 mg/vial (as ferrous)	Store at 2-8°C, protected from light	24 months

The carbon nanoparticle suspension injection and the ferrous sulfate injection are both provided by the applicant.

Packaging and labeling: The nano carbon suspension injection and the ferrous sulfate injection are labeled by the applicant according to the "Good Manufacturing Practice for Drugs (Revised in 2010)" (NMPA, 2011) and the "Good Clinical Practice for Drugs" (NMPA, 2020), and are marked as for clinical trial use only.

dosage regimen

Dose Escalation Phase (DLT Phase)

Dosing

Three dose groups are tentatively set (calculated as Fe^{2+}, 30 mg, 60 mg, 90 mg). If ≥2 subjects in the 90 mg group observe DLT, the dose will be reduced to 75 mg for safety and tolerability observation; if the dose is escalated to 90 mg and MTD is still not reached, the SRC will comprehensively discuss the available safety, tolerability, PK/PD, and preliminary efficacy data, and consider further dose escalation to 120 mg, 150 mg, or even higher (if 120 mg still does not reach MTD, the SRC will discuss whether to stop dose escalation or directly proceed to 150 mg or even higher doses).

Dosing Volume

Regardless of tumor size, number, and location, a single tumor lesion suitable for intratumoral injection will be selected, and the corresponding dose of the investigational drug will be administered by intratumoral injection. The dosing volume will be determined based on the size of the injected lesion. If the corresponding dose of the investigational drug cannot be fully administered in one lesion, other lesions can be injected simultaneously. When multiple lesions are injected, the amount allocated to each lesion should be proportional to the lesion size, or the investigator can evaluate an appropriate allocation.

During the study, the investigator may adjust the dosing regimen or add unplanned

doses based on the safety, efficacy, and PK/PD data of CNSI-Fe.

CNSI-Fe Preparation

Take a vial of ferrous sulfate injection, keep the aluminum cap and rubber stopper sealed, and only remove the plastic outer cap of the aluminum-plastic combination cap. Use a suitable syringe to slowly draw an appropriate amount of the nano-carbon suspension injection, and inject it into the vial containing ferrous sulfate. Mix the solution evenly. The CNSI-Fe preparation requires aseptic operation, shielding from air, and must be used within 6 hours after preparation at room temperature. See Appendix 5 in Chapter 15 for details.

Maintenance Treatment Period

After the DLT assessment is completed, subjects who do not experience unacceptable toxicity, if the investigator judges that the benefit of continued treatment outweighs the risk, will enter the maintenance treatment period. During the maintenance treatment period, subjects will continue to receive the investigational drug according to the DLT assessment regimen (tentatively, one intratumoral injection on Day 1, the dosing regimen may be adjusted based on the PK characteristics of this study, as discussed by the sponsor and investigator).

Dose Groups: Three dose groups are tentatively set, namely 30 mg, 60 mg, and 90 mg (calculated as Fe^{2+}). Subsequently, based on the safety, tolerability, PK/PD, and preliminary efficacy data of CNSI-Fe, additional doses may be added for further investigation.

Research Steps: Please refer to the experimental process flow chart

Sample collection: PK and PD studies will be conducted by a designated third-party laboratory. The sampling time points for each sample may be adjusted based on the results of the previous clinical studies (decided by the SRC discussion). The methods for sample collection, processing, preservation, transportation, and testing will be described in a separate laboratory operations manual.

PK samples

First cycle: Within 1 hour before CNSI-Fe dosing, 5 min ± 1 min, 10 min ± 2 min, 15 min ± 2 min, 30 min ± 5 min, 1 h ± 10 min, 2 h ± 10 min, 4 h ± 15 min, 8 h ± 15 min, 12 h ± 30 min, 24 h ± 30 min, 48 h ± 1 h, 72 h ± 2 h after CNSI-Fe dosing.

Second cycle (if any): The sampling time points may be adjusted appropriately based on the PK results from the first cycle (adjusting sampling time, increasing/decreasing sampling frequency, etc.).

Collect 3 mL of blood sample at each sampling point.

PD samples (exploratory tumor tissue samples)

Before the first CNSI-Fe dose, 24 h to 17 days after the first CNSI-Fe dose (specific time points to be determined based on clinical practice), and at disease progression.

Subject to the subject's willingness, try to collect tumor tissue samples from the injected lesion before and after the first CNSI-Fe dose; the pre-dose tumor tissue sample can be the most recent archived sample or the sample collected during the screening period; post-dose tumor tissue samples will be collected according to clinical practice.

Tumor PK samples (exploratory tumor tissue samples)

Before the first CNSI-Fe dose, within 21 days after the first CNSI-Fe dose (specific time points to be determined based on clinical practice).

Subject to the subject's willingness, try to collect tumor tissue samples from the injected lesion before and after the first CNSI-Fe dose; the pre-dose tumor tissue sample can be the most recent archived sample or the sample collected during the screening period; post-dose tumor tissue samples will be collected according to clinical practice as much as possible to complete the tumor PK study (not a mandatory requirement).

Statistical Analysis: Analysis of the Data Set

Full Analysis Set (FAS)

The FAS includes all enrolled subjects who received at least one dose of the study drug and had a measurable lesion at baseline.

Dose-Limiting Toxicity (DLT) Analysis Set (DLTS)

The DLTS includes subjects who completed the DLT evaluation period as per the protocol (defined as receiving at least one dose of the study drug at ≥80% of the protocol-specified dose and did not prematurely discontinue or experience a DLT during the DLT evaluation period).

Safety Analysis Set (SS)

The SS includes all subjects who received at least one dose of the study drug and had at least one post-dose safety assessment.

Pharmacokinetic (PK) Analysis Set (PKS)

The PKS includes all subjects who received at least one dose of the study drug and had at least one valid PK measurement.

Pharmacodynamic (PD) Analysis Set (PDS)

The PDS includes all subjects who received at least one dose of the study drug and had at least one valid PD endpoint measurement.

Statistical Analysis Methods

The Statistical Analysis Plan (SAP) will provide detailed specifications and descriptions of all planned statistical analyses in accordance with the protocol, and will be finalized prior to database lock.

The statistical analyses will include demographic and baseline characteristics, safety and tolerability, PK, PD, and efficacy.

The analyses will be primarily descriptive, without formal statistical hypothesis testing.

The statistical analyses will be performed using SAS® version 9.4 or higher.

The following descriptive statistical analyses will be conducted based on the nature of the variables:

Continuous variables: number of non-missing observations, mean and standard deviation (SD), median, minimum, and maximum.

Categorical variables: frequency and percentage.

Safety Analysis: For the primary estimand, the number of subjects, incidence, and number of events of each type of adverse event will be summarized by dose group. Numerical laboratory data at each visit will be described using mean, SD, median, extremes, and changes from baseline, and categorized as normal, clinically insignificant abnormal, or clinically significant abnormal, with the incidence of clinically significant abnormalities tabulated. The same approach will be used for ECG. Vital signs will be described using mean ± SD.

For the secondary estimand, the incidence of DLTs will be summarized by dose

group.

PK Analysis: The primary PK endpoints for CNSI-Fe intratumoral injection will include the iron ion concentration in serum. PK analyses will provide descriptive statistics of the relevant measurements and concentration-time profiles. For the first dose of CNSI-Fe, AUC, Cmax, Tmax, and other parameters may be calculated. Descriptive statistics will be provided for these parameters, and informal testing of AUC may be performed to explore the relationship with dose.

Efficacy Analysis: Based on best lesion response, ORR, DCR, and their two-sided 95% confidence intervals (Clopper-Pearson method) will be statistically described.

PD Analysis: Descriptive statistics will be provided for the PD endpoint levels before and after dosing, as well as the changes (absolute or percent) from baseline. Corresponding time course profiles will be generated. Comparisons of the PD endpoint levels and changes from baseline at each timepoint will be performed, with normality testing and using paired t-tests or Wilcoxon signed-rank tests as appropriate.

Research on time：

After this study has been reviewed and approved by the Ethics Committee (EC), it

will continue until the expected sample size is reached, based on the actual situation.

The study is expected to end 28 days after the last participant receives a maximum of two doses. For participants who still achieve CR (complete response) or PR (partial response) at the end of the study, if the investigator determines that they can still benefit from continued treatment, they can receive the study drug free of charge until disease progression, development of intolerable toxicity, the investigator determines it is no longer appropriate for the participant to continue receiving the study drug, or the sponsor terminates the study.

Table 1-1. Clinical Trial Flow Diagram

Study Procedure	**Screening Period[1]**	**Therapeutic Period（DLT Evaluation Period）[2]**				**Maintenance Therapy Phase（Secondary Cycle）[3]**				**Early Termination/Early Completion of Treatment Visits[4]**	**Safety Follow-up[5]**
		DLT Evaluation Period				**Secondary Cycle（if tolerated, 21 days/cycle）**					
follow-up visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**		**V10**
date（D）	**D-28~D-1**	**D1**	**D7**	**D14**	**D21**	**D1**	**D7**	**D14**	**D21**	**confirm end/termination of treatment**	**28 days after the last administration**
period（d）			**±1d**	**±1d**	**±1d**	**+7d**	**±1d**	**±1d**	**±1d**	**+7 d**	**±3 d**
Research management process											
informed consent	×										
evaluation of inclusion and exclusion criteria	×										
demographic data	×										
medical history and treatment history[6]	×										
Clinical procedures/evaluations											

vital signs [7]	×	×	×	×	×	×	×	×	×	×	×
physical examination [8]	×	×	Based on clinical indications, determined by the researcher			×	Determined by the researcher based on clinical indications			×	×
weight[9]	×	×				×				×	×
height	×										
ECOG	×[10]	×			×	×			×	×	×
12-lead ECG [11]	×	×	×	×	×	×	×	×	×	×	×
echocardiogram [12]	×	Determine and conduct based on clinical indications as judged by the investigator throughout the study period									
combination therapy [13]	×	×	×	×	×	×	×	×	×	×	×
Adverse envents[14]	×	×	×	×	×	×	×	×	×	×	×
Laboratory procedures/evaluations											
complete blood count [15]	×[10]		×	×	×		×	×	×	×	×
urinalysis [16]	×[10]		×	×	×		×	×	×	×	×
blood biochemistry [17]	×[10]		×	×	×		×	×	×	×	×
coagulation function [18]	×[10]		×		×		×		×	×	×
pregnancy test [19]	×[10]									×	×
FSH[20]	×										
glucose-6-phosphate dehydrogenase (G6PD) test	×										
viral serology [21]	×										
blood iron test [22]	×	×	×	×	×	×	×	×	×	×	×

tumor markers [23]	×[10]				×				×	×	×
cardiac enzyme profile [24]	×	During the entire study period, decisions were made by the researchers based on clinical indications									
Investigational drug											
CNSI-Fe		×				×					
PK/PD											
PK sampling [25]		×				×					
collection of tumor tissue samples (PD/intra-tumoral PK sampling)[26]	×	×									
Efficacy evaluation											
tumor assessment [27]	×	×								×	

Note: Unless otherwise specified, all assessments, examinations, and sample collections should be performed on the same day before drug administration.

Screening Period: Routine examinations or examination results obtained at a tertiary hospital (or this hospital) within 28 days prior to the first dose (unless otherwise specified) and before signing the informed consent form (ICF) can be used for screening, and do not need to be repeated.

Treatment Period (DLT Evaluation Period): For the first intratumoral injection of CNSI-Fe, safety assessments will be performed on Day 7 (±1 day), Day 14 (±1 day), and Day 21 (±1 day) after the first dose to evaluate DLT.

Maintenance Treatment Period: After the DLT evaluation is completed, subjects without unacceptable toxicity will continue to the maintenance treatment period if the investigator determines that the benefit of continued treatment outweighs the risk. During the maintenance treatment period, subjects will receive one more dose of the investigational drug (intratumoral injection to the lesion, tentatively scheduled for Day 1, the dosing regimen may be adjusted based on the PK characteristics of this study by the sponsor and investigator).

Early Termination/End of Treatment (EOT) Visit: This will be scheduled within 7 days after the decision to discontinue treatment. If the assessments required for the

EOT visit were performed within 7 days prior to the EOT visit, they do not need to be repeated.

Safety Follow-up: Subjects who have received at least one dose of the study drug will undergo a safety visit 28 days (±3 days) after the last dose. If the assessments required for the safety visit were performed within 3 days prior to the safety visit, they do not need to be repeated.

Medical History and Treatment History: Collected during the screening period, including past medical history, medication history (concomitant treatments within 4 weeks prior to screening and currently ongoing), history of all previous tumors (including tumor stage, diagnosis date, genetic test results), history of all previous anti-tumor treatments, family history, surgical history, immunization history, allergy history, smoking history, alcohol history, history of drug abuse/dependence, etc.

Vital Signs: Including respiratory rate, pulse/heart rate, blood pressure, and body temperature. Measured during screening, within 2 hours before CNSI-Fe administration, 1.5 hours (±30 minutes) after administration, on Days 7, 14, and 21 of each cycle, at the End of Treatment (EOT) visit, and during the safety follow-up visit (excluding telephone visits).

Physical Examination: Including general condition, skin and mucosa, lymph nodes,

head, neck, chest, abdomen, spine, limbs, and nervous system. A complete physical examination will be performed during screening, before CNSI-Fe administration, at the EOT visit, and during the safety follow-up visit (excluding telephone visits). Symptom-directed physical examinations will be performed during the treatment period as clinically indicated.

Body Weight: Measured during screening and before CNSI-Fe administration.

ECOG performance status, complete blood count, urinalysis, blood chemistry, coagulation function, pregnancy test (for women of childbearing potential), and tumor marker tests should be completed within 7 days prior to the first dose of the investigational drug.

12-lead ECG: Performed during screening, within 2 hours before CNSI-Fe administration, 0.5 hours (±5 minutes), 4 hours (±30 minutes), 8 hours (±30 minutes), 24 hours (±30 minutes), 48 hours (±30 minutes), and 72 hours (±30 minutes) after administration, on Days 7, 14, and 21 of each cycle, at the EOT visit, and during the safety follow-up visit (excluding telephone visits). Continuous cardiac monitoring will also be performed for 8 hours after CNSI-Fe administration. Additional 12-lead ECG assessments may be added during the study to ensure subject safety, at the discretion of the investigator.

Echocardiography: Left ventricular ejection fraction (LVEF). Performed during screening, and as needed during the study period if there are clinically significant ECG abnormalities.

Concomitant Medications: All concomitant medications and treatments from the time of ICF signing until 28 days after the last dose or the start of a new anti-cancer therapy (whichever occurs first) should be recorded in the patient's medical records and reported in the electronic case report form (eCRF), including the name, reason for use, dose, route of administration, frequency, start date, and stop date.

Adverse Events (AEs): Only serious adverse events (SAEs) related to the protocol-specified procedures should be reported before the start of study drug treatment. After the start of study treatment, all AEs should be reported, regardless of their relationship to the study drug, until 28 days after the last dose. The investigator should follow up on all AEs until the event resolves to baseline or better, is considered stable by the investigator, the subject is lost to follow-up, or the subject withdraws consent. For all SAEs related to the investigational product or study procedures, every effort should be made to follow up until a final outcome can be reported.

Blood routine: Red blood cell count (RBC), hemoglobin (Hb), hematocrit, white blood cell count (WBC) and differential count (neutrophils, eosinophils, lymphocytes, monocytes, basophils and others) and platelet count (PLT). To be performed within 7

days prior to first dose of study drug in the screening period, on Day 7, Day 14, Day 21 of each cycle, at the End of Treatment (EOT) visit, and at the safety follow-up visit (excluding telephone visits). Additional blood routine tests can be performed as needed based on the investigator's clinical judgment.

Urinalysis: Urine specific gravity, urine pH, urine glucose, urine protein, urine ketone, urine red blood cells and urine white blood cells. To be performed within 7 days prior to first dose of study drug in the screening period, on Day 7, Day 14, Day 21 of each cycle, at the EOT visit, and at the safety follow-up visit (excluding telephone visits).

Biochemistry: Glucose, blood urea nitrogen (BUN) or urea, creatinine (Cr), sodium, potassium, magnesium, chloride, calcium, phosphorus, total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin, triglycerides, total cholesterol, amylase, lipase. To be performed within 7 days prior to first dose of study drug in the screening period, on Day 7, Day 14, Day 21 of each cycle, at the EOT visit, and at the safety follow-up visit (excluding telephone visits). Additional biochemistry tests can be performed as needed based on the investigator's clinical judgment.

Coagulation function: International normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen (FIB). To be performed within 7 days prior to first dose of study drug in the screening period,

on Day 7, Day 21 of each cycle, at the EOT visit, and at the safety follow-up visit (excluding telephone visits).

Pregnancy test: For female subjects of childbearing potential only; to be performed within 7 days prior to first dose of study drug in the screening period, at the EOT visit, and at the safety follow-up visit (excluding telephone visits). Pregnancy tests other than screening can be either serum or urine pregnancy tests, as per the investigator's judgment. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test.

FSH test: For post-menopausal female subjects over 45 years of age but less than 55 years of age (see Section 4.1), a serum FSH test will be performed during the screening period to confirm post-menopausal status. If the subject is confirmed to be post-menopausal, no further pregnancy testing will be required; otherwise, she will undergo pregnancy testing like female subjects of childbearing potential.

Viral serology: Including hepatitis B virus (HBV) - [hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb)], hepatitis C virus (HCV) - [HCV-Ab] and human immunodeficiency virus (HIV) - [Anti-HIV] testing. HBV quantitative requirement: During screening, test the 5 HBV markers, if HBsAg is positive, HBV DNA testing is required; if HBsAg is negative but HBcAb is positive,

HBV DNA testing is also required. HCV quantitative requirement: During screening, test for HCV-Ab to determine if the subject has HCV infection, if HCV-Ab is positive, HCV RNA quantitative testing is required.

Iron tests: Serum iron (Fe), total iron binding capacity (TIBC), serum ferritin (SF), transferrin saturation (TSAT). To be performed during screening, within 24 h prior to CNSI-Fe dosing, at 5 min ± 1 min, 15 min ± 2 min, 30 min ± 5 min, 2 h ± 30 min, 6 h ± 30 min and 10 h ± 30 min post-dosing, on Day 7, Day 14, Day 21 of each cycle, at the EOT visit, and at the safety follow-up visit (excluding telephone visits). Additional iron tests may be performed as needed for subject safety during the study.

Tumor marker detection: carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), cancer antigen 125 (CA125), cancer antigen 15-3 (CA15-3), cancer antigen 19-9 (CA19-9), etc. Researchers will select appropriate tumor markers based on the situation. These will be tested within 7 days before the first dose of the study drug, on Day 21 of each cycle, at the End of Treatment (EOT) visit, and during the safety follow-up (excluding telephone visits).

Cardiac enzymes: lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), hydroxybutyrate dehydrogenase (HBDH). These will be tested during the screening period, and supplementary testing will be performed during the study period if the subject

experiences chest pain, palpitations, or ECG abnormalities.

PK blood sample collection: In Cycle 1: within 1 hour before CNSI-Fe dosing, and at 5 min ± 1 min, 10 min ± 2 min, 15 min ± 2 min, 30 min ± 5 min, 1 h ± 10 min, 2 h ± 10 min, 4 h ± 15 min, 8 h ± 15 min, 12 h ± 30 min, 24 h ± 30 min, 48 h ± 1 h, and 72 h ± 2 h after dosing. In Cycle 2 (if applicable), the sampling schedule may be adjusted based on the PK results from Cycle 1.

Collection of tumor tissue samples (PD/intratumoral PK sampling): For subjects who consent to provide archived or fresh tumor tissue samples from the target lesion, the samples will be obtained during the screening period. For PD samples, they should be collected within 24 hours to 17 days after the first dose (specific timepoint to be determined based on clinical practice), and at disease progression as clinically indicated. For intratumoral PK samples, they should be collected within 21 days after the first dose (specific timepoint to be determined based on clinical practice). Tumor tissue samples will only be transported for subjects who are eligible for enrollment.

Tumor assessment: Baseline imaging assessments will be performed within 28 days prior to the first study treatment (if the patient underwent imaging as part of routine clinical care prior to signing the ICF, and the imaging modality and location meet the protocol requirements, the results may be used for screening upon investigator approval). Baseline tumor evaluation should include the chest, abdomen, pelvis, head (to exclude brain metastases), and any other suspected tumor sites. Subsequent evaluations should re-assess all measurable and evaluable lesions identified at

baseline, including the chest, abdomen, pelvis, and any other suspected tumor sites. The imaging modality used for tumor assessment during the study must be consistent with the baseline assessment. Subjects will undergo tumor assessment every 3-4 weeks during the treatment period, and every 7-8 weeks during the maintenance period (the investigator may decide to perform additional imaging assessments based on changes in the subject's condition; the first tumor assessment must be performed at weeks 3-4). Subjects with a confirmed complete response (CR) or partial response (PR) at the first assessment will require confirmation at least 4 weeks later. Tumor imaging may be omitted at the early termination/withdrawal visit if it was performed within th