Clinical Study Protocol

A Phase III, Multi-Center, Randomized, Open-Label, Active-Controlled Trial to Compare the Efficacy and Safety of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein (F-627) and Recombinant Human Granulocyte Colony Stimulating Factor (GRAN®) in the Prophylactic Treatment for Chemotherapy-Induced Neutropenia

Protocol Number:	SP11631
Investigational Drug:	Recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627)
Clinical Phase:	Phase III
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Sponsor:	Generon (Shanghai) Corporation Ltd.

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Protocol Revision History

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Synopsis

Protocol Number	SP11631
Title	A Phase III, Multi-Center, Randomized, Open-Label, Active-Controlled, Trial to Compare the Efficacy and Safety of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein (F-627) and Recombinant Human Granulocyte Colony Stimulating Factor (GRAN®) in the Prophylactic Treatment for Chemotherapy-Induced Neutropenia
Sponsor	Generon (Shanghai) Corporation, Ltd.
Clinical Phase	Phase III
Study Drug	Investigational Drug: Recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627) Reference Drug: Recombinant Human Granulocyte Colony Stimulating Factor for Injection (GRAN®)
Study Population	Female patients with breast cancer will be enrolled to receive at least 4 cycles of EC chemotherapy, that is: epirubicin 100 mg/m^2 and cyclophosphamide 600 mg/m^2.
Study Design	A multi-center, randomized, open-label, active-controlled phase III clinical trial
Number of Subjects	240
Primary Objective and Endpoints	The primary objective of this study is to compare the efficacy of recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627) versus recombinant human granulocyte colony stimulating factor (GRAN®) in the first cycle of prophylactic therapy in patients with breast cancer receiving EC chemotherapy. The primary endpoint is defined as the duration (days) of grade 3 or 4 (moderate and severe) neutropenia, that is, the number of days in which ANC < 1.0 × 10^9/L in cycle 1.
Secondary Objectives and Endpoints	• The incidence of grade 3 or 4 neutropenia (ANC < 1.0 × 10^9/L and ANC < 0.5 × 10^9/L, respectively) in each cycle • The durations (days) of grade 3 or 4 neutropenia (ANC < 1.0 × 10^9/L and ANC < 0.5 × 10^9/L, respectively) in cycles 2-4 • The incidence and duration (days) of grade 4 neutropenia (ANC < 0.5 × 10^9/L) in each cycle • The overall duration (days) of grade 3 or 4 neutropenia (ANC < 1.0 × 10^9/L and ANC < 0.5 × 10^9/L, respectively) in overall 4 cycles • The incidence and duration (days) of grade 2 or above neutropenia (ANC < 1.5 × 10^9/L) in each cycle • Incidence of febrile neutropenia (FN) (defined as ANC < 1.0×10^9/L; a single measurement of body temperature > 38.3°C or a temperature ≥ 38.0 °C sustained over 1 h) • Profile of neutrophil count over time • The neutrophil count nadir from day 3 to day 13 of cycle 1 • The time (days) of ANC nadir returns to 2.0 × 10^9/L in each cycle
Safety Objectives and Endpoints	To compare the safety of F-627 and GRAN® by monitoring adverse events/serious adverse events, laboratory measurements, 12-lead ECG, abdominal ultrasound, physical examination, vital signs and symptoms.
Exploratory Objectives and Endpoints	To evaluate the potential immunogenicity of F-627 by testing anti-F-627 antibodies in serum.

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Study Design	This is a multi-center, randomized, open-label, and active-controlled phase III clinical trial with 240 enrollment of subjects with breast cancer to receive 4 cycles of EC chemotherapy, that is: epirubicin 100 mg/m^2, i.v. + cyclophosphamide 600 mg/m^2, i.v. on day 1, repeat cycle every 21 days for 4 cycles. After completing the evaluation for overall 4 cycles, subjects will receive subsequent treatments according to routine practice. The investigator must ensure that a recommended dose is given in cycle 1, delayed or reduced doses due to toxicities other than myelotoxicity (such as cardiotoxicity) in cycles 2-4 are permitted. Eligible subjects are randomized to F-627 or GRAN® in a 1:1 ratio before day 1 of cycle 1. Treatment allocations remained unchanged during the entire treatment period. Subjects will receive F-627 (20 mg/dose, s.c.) or GRAN® [5 μg/kg/day, s.c., once daily (± 4 h) up to 2 weeks or until neutrophil count returns to 5.0×10^9/L] on day 3 of each cycle, i.e., 48 ± 4 h after the start of chemotherapy. During the trial, except for the blood routine test (neutrophil count) in the first cycle of treatment performed by the central laboratory, the other laboratory measurements are performed locally; adverse events/serious adverse events are collected, along with examinations such as laboratory measurements, 12-lead ECG and abdominal ultrasound. The last visit should be completed 3 weeks after the last chemotherapy dose. A follow-up visit by telephone should be completed 30 days after the last dose.
Inclusion Criteria	Patients satisfying all the following criteria can be included: 1. Willing to sign the informed consent form and able to comply with protocol requirements; 2. 18-75 years old; 3. Female postoperative patients with breast cancer who require adjuvant chemotherapy, and are planned to receive 4 cycles of EC chemotherapy, (epirubicin 100 mg/m^2 + cyclophosphamide 600 mg/m^2); 4. ECOG performance status ≤ 2; 5. Absolute neutrophil count (ANC) ≥ 2.0×10^9/L, hemoglobin (Hb) ≥ 11.0 g/dL, and platelet (PLT) ≥ 100×10^9/L prior to enrollment; 6. Hepatic and renal function: Total bilirubin ≤ 1.5 × ULN, ALT and AST ≤ 2.5 × ULN, serum creatinine ≤ 1.5 × ULN; 7. Left ventricular ejection fraction greater than 50%; 8. Women without child-bearing potential, i.e., women who have had menopause for at least 1 year or who have undergone sterilization (bilateral tubal ligation, double oophorectomy or hysterectomy); patients with child-bearing potential should agree to take appropriate contraceptive measures, including condoms, spermicidal condoms, foams, gels, contraceptive barrier, intrauterine devices (IUD), and contraceptives (oral or injection), starting from 1 month before the start of the study until 30 days after the end of the study.
Exclusion Criteria	Patients who meet any of the following criteria must be excluded from this study: 1. Radiation therapy within 4 weeks prior to enrollment; 2. Breast cancer patients who have received neoadjuvant chemotherapy before surgery; 3. Prior bone marrow or stem cell transplant; 4. With other malignant tumors other than breast cancer; 5. Patients who have received a treatment with recombinant human granulocyte stimulating factor within 6 weeks prior to randomization; 6. Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches; 7. With any disease that may cause splenomegaly; 8. With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment), or Hepatitis C; 9. Women in pregnancy or breastfeeding; 10. Known HIV positive or AIDS;

	11. With active tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray; 12. With sickle cell anemia; 13. With alcohol or drug abuse that may affect the compliance with the study; 14. With known hypersensitivity to granulocyte colony stimulating factor or excipients; 15. Patients treated with other investigational products within 1 month or 5 half-lives of the other investigational products prior to enrollment (whichever is longer); 16. Patients with diseases or symptoms unsuitable for participating in the trial. For example, the study drugs may compromise the health of the patient or the assessment of adverse events may be affected.
Criteria for Withdrawal	Subjects have the right to withdraw from the study at any time without any reason. Subjects who withdraw from the study at any time will be required to complete the last evaluation and relevant examinations. 1. Subject withdraws voluntarily; 2. Occurrence of uncontrolled grade 3 or 4 adverse events related to the study drug as judged by the investigator; 3. Subjects fail to comply with the study protocol; 4. Discontinuation by the investigator under safety considerations or in the best interest of the subject when the subject's condition changes; 5. Pregnancy; 6. Death.
Dosing Regimen	<u>Chemotherapy Regimen</u> All enrolled breast cancer patients are treated with at least 4 cycles of EC chemotherapy, namely: epirubicin 100 mg/m^2, i.v. + cyclophosphamide 600 mg/m^2, i.v. on day 1, repeat cycle every 21 days for 4 cycles. The investigator must ensure that a recommended dose is given in cycle 1, delayed or reduced doses due to toxicities other than myelotoxicity (such as cardiotoxicity) in cycles 2-4 are permitted. After 4 cycles of treatment and evaluation, subjects will receive subsequent treatment according to routine practice. <u>Study Drug</u> F-627 treatment arm: 20 mg/dose, one subcutaneous injection on day 3 of each cycle (48 ± 4 h after the initiation of chemotherapy) for 4 cycles. GRAN® treatment arm: 5μg/kg/day, one subcutaneous injection daily (± 4 h) starting from day 3 of each cycle (48 ± 4 h after the initiation of chemotherapy) for ≤ 2 weeks or until neutrophil count recovers from nadir to 5.0 × 10^9/L, for a total of 4 cycles. GRAN® can be discontinued by the investigators according to the ANC test results obtained from local laboratories. Treatment allocation will remain unchanged during the entire treatment period (4 cycles).
Concomitant Therapy	The following medications are prohibited during the study: recombinant human granulocyte colony stimulating factor (rhG-CSF) and recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF); traditional Chinese medicines known clearly with neutropoiesis-inducing potential; lithium, which may contribute to the release of neutrophils; and prophylactic antibiotics. The investigator may choose appropriate treatment at their discretion.
Randomization, Statistical Hypothesis, and Stratification	Central randomization is performed using the Interactive Web-Response System (IWRS). Breast cancer patients who meet the inclusion criteria are randomized to 2 treatment arms (i.e., F-627 and GRAN® treatment arms) in a 1:1 ratio. Efficacy analyses will be based on the Intent-to-Treat (ITT) and Per-Protocol (PP) analysis sets. Safety analyses will be based on the Safety set. Non-inferiority analysis will be used for the primary efficacy analysis, with an equivalence margin of 1 day. There is no plan for interim analysis.

Abbreviations

Abbreviations and Terms	Definitions
AE	Adverse Event
AKP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
AST	Aspartate Transaminase
AUC	Area Under Concentration-Time Curve
BSA	Body Surface Area
CFDA	China Food and Drug Administration
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
ECG	Electrocardiography
EC	Name of Chemotherapy Regimen (Epirubicin + Cyclophosphamide)
ECOG	Eastern Cooperative Oncology Group (to determine Performance Status)
FDA	U.S. Food and Drug Administration
IEC	Independent Ethics Committee
IRB	Independent Review Board
GCP	Good Clinical Practice
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICF	Informed Consent Form
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
NCI	National Cancer Institute
NOEAL	No-Observed-Adverse-Effect-Level
RECIST	Response Evaluation Criteria in Solid Tumors
PD	Progression of Disease
PD	Pharmacodynamics
PK	Pharmacokinetics
rhG-CSF	Recombinant Human Granulocyte Colony Stimulating Factor
SAE	Serious Adverse Event
SAS	Statistical Analysis Plan
TAC	Name of Chemotherapy Regimen (Taxotere + Adriamycin + Cyclophosphamide)
T_{max}	Time to Peak
ULN	Upper Limit of Normal
WBC	White Blood Cell Count
WHO	World Health Organization
WHODD	WHO Drug Dictionary

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1.0 BACKGROUND AND PRINCIPLES

1.1 Introduction to F-627

1.1.1 Name, structure, and physicochemical properties

F-627, a recombinant human granulocyte colony stimulating factor-Fc fusion protein (rhG-CSF-Fc), is a biological product (category 1) with intellectual property rights developed by Generon (Shanghai) Corporation Ltd. This rhG‐CSF‐Fc fusion protein is a rhG‐CSF dimer expressed in CHO cells and based on the technology of Fc fusion protein, and has long-acting properties. The Fc fragment is derived from human immunoglobulin. The half-life of IgG immunoglobulins can be up to 3 weeks in human blood.

In the construction of the G-CSF-Fc fusion protein, a peptide linker comprising 16 amino acids was added between G-CSF and IgG2-Fc, so as to keep the Fc domain of the fusion protein away from the G‐CSFR binding site and improve its bioactivity. In addition, the peptide linker may keep one G-CSF away from another G-CSF domain, and produce an rhG-CSF-Fc in a dimeric form with 2 G-CSF molecules on a single Fc fusion protein. The rhG-CSF-Fc dimer may have a stronger receptor activation compared with an rhG-CSF monomer, and thereby the bioactivity of the fusion protein can be further improved.

1.1.2 Mechanism of action

The human granulocyte colony stimulating factor receptor (G-CSF receptor, G-CSFR) is a specific, single-chain receptor with high affinity to G-CSF. The density of G-CSF receptors on the cell surface becomes higher as the neutrophils grow more mature. G-CSF: G-CSFR compound exists in a 2:2 ratio, i.e., 2 ligands bind to 2 receptors. Each G-CSF molecule binds to one receptor. Only when two receptors binding to G-CSF ligands interact with each other to form a 2:2 dimer, the C-terminal of the G-CSF receptor may activate the downstream signaling molecule, JAK2 (Janus tyrosine kinase 2). JAK2 then initiates gene transcription by activating STAT3, resulting in cell proliferation. Theoretically, dimeric ligands may activate downstream signaling pathways faster and stronger than monomeric ligands.

G-CSF receptors on neutrophils can not only transmit G-CSF signal, but also regulate the G-CSF concentration in the blood. During an infection or a neutropenic period, G-CSF concentrations in the blood will increase to stimulate the production of neutrophils. When absolute neutrophil count increases, the binding of G-CSF in the blood also increases, thereby maintaining a relatively stable G-CSF concentration in vivo.

In addition, a clinical study of rhG-CSF showed that hematopoietic precursor cells and hematopoietic stem cells in peripheral blood increased significantly after the injection of rhG-CSF. This process is known as peripheral blood precursor cell (PBPC) and/or peripheral blood stem cell (PBSC) mobilization. Many hematopoietic growth factors such as SCF, FL, GM-CSF, and IL-3, as well as reagents affecting bone marrow matrix proteins and adhesion molecules have the similar function of PBPC and/or PBSC mobilization.

1.1.3 Preclinical pharmacokinetics study

Pharmacokinetic studies of F-627 in rats and cynomolgus monkeys showed that F-627 is a long-acting drug in vivo. F-627 demonstrated nonlinear pharmacokinetics. Increases in blood drug concentration and ANC (PD response) are dose-dependent. F-627 (100 μg/kg) adminstered to rats via subcutaneous injection showed $T_{1/2}$ of 7.6 ± 1.3 hrs, C_{max} of 162 ng/mL and AUC of 4217 ± 641 ng/mL·hr; pegfilgrastim (Neulasta, 100 μg/kg) administered to rats showed $T_{1/2}$ of 7.1 hrs and AUC of 1600 ng/mL·hr (FDA IND).

In the cynomolgus monkey model with cyclophosphamide-induced neutropenia, we compared the PK parameters of F-627 and Pegfilgrastim at the equivalent dose after the first dose (as shown in Table 1). Results showed that the PK parameters of F-627 and pegfilgrastim at the equivalent dose were comparable. However, F-627 resulted in a faster neutrophil recovery compared with pegfilgrastim.

Table 1. Comparison of PK parameters between F-627 and pegfilgrastim.

Dose (60 μg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC (ng/mL*hr)	$T_{½}$ (h)
F-627 (Female)	8.0	776±155	21289±5744	8.9
F-627 (Male)	8.0	566±162	14515±7324	11
Pegfilgrastim (Female)	12.0	482±88	25571±474	8.2
Pegfilgrastim (Male)	8.0	574±87	21894±2616	8.7

Note: The table shows the PK data after the first dose in the cynomolgus monkey model with cyclophosphamide-induced neutropenia.

1.1.4 Preclinical pharmacology study

In vitro bioactivity study of F-627

The in vitro bioactivity study of F-627 showed that the ED_{50} of F-627 was 0.68 ng/mL (7.58 pM) in the proliferative response of M-NFS-60 cell line. The effect of F-627 on M-NFS-60 proliferation can be neutralized by anti-human G-CSF monoclonal antibodies. The activation of intracellular signaling pathways following G-CSF ligand-receptor binding was studied by detecting G-CSF-activated phosphorylated STAT3. Similar to filgrastim (Neupogen) and pegfilgrastim (Neulasta), F-627 can effectively activate phosphorylated STAT3 signaling and stimulate cell proliferation in M-NFS-60 cell line.

In vivo pharmacodynamics study of F-627

In vivo bioactivity study of F-627 showed that: a single subcutaneous injection of F-627 in normal mice, rats, and cynomolgus monkeys resulted in increases of peripheral blood white blood cell count (WBC) and absolute neutrophil count (ANC) in a dose-dependent manner. The minimum effective dose of F-627 in rats was 3.0 μg/kg. A single subcutaneous injection of F-627 100μg/kg in mice showed that phosphorylated STAT3 levels in bone marrow cells increased 17-fold compared to baselines. Peak ANC in peripheral blood appeared at 48 hrs after the dose, and recovered to normal levels at 72 hrs. F-627 was significantly superior to Neulasta and rhG-CSF at the same molar dose of G-CSF in terms of ANC increase and duration.

In the cyclophosphamide-induced neutropenic model of cynomolgus monkeys, F-627 not only shortened the recovery time of ANC, but also reduced the ANC decrease compared with filgrastim (rhG-CSF) and pegfilgrastim (Neulasta), thereby preventing severe neutropenia. F-627 demonstrated superior pharmacological effects over the same dose (60 μg/kg) of pegfilgrastim.

1.1.5 Preclinical toxicology study

A systematic preclinical safety evaluation of F-627 has been conducted. Refer to Table 2 for the details of safety evaluation.

Table 2. Summary of F-627 preclinical safety evaluation tests.

Animal	Study Title	Dose (μg/kg)	Administration	Observation Time	NOAEL (μg/kg)
Rat	Acute toxicity study	100,500,2500,7500	Single	2 weeks	
Rat	Dose range finding toxicity study	30,100,300,1500	Repeat	4 weeks	
Rat	Repeat dosing toxicity study	100,300,1000	Repeat	Administration: 3 months Recovery period: 1 month	1000
Cynomolgus Monkey	Acute toxicity study	300,3000	Single	2 weeks	
Cynomolgus Monkey	Repeat dosing toxicity study	75,225,675	Repeat	Administration: 3 months Recovery period: 1 month	675
Mouse	Safety pharmacology study - spontaneous activity	30,120,480	Single	10 minutes	
Mouse	Safety pharmacology study - synergy of sodium pentobarbital hypnotic effect in mice	30,120,480	Single	30 minutes	
Mouse	Safety pharmacology study - hypnotic effect of sodium pentobarbital of subthreshold dose	30,120,480	Single	30 minutes	
Rabbit	Muscular irritation study	400,1600	Single	48 hours, 16 days	
Rabbit	Vascular irritation study	400,1600	Single	48 hours, 16 days	
Guinea Pig	Active anaphylaxis test	Sensitization phase: 100, 500; Challenge phase: sensitization dose × 2	Sensitization: 5 times, once every other day	30 minutes, 3 hours	
Rabbit	RBC in vitro hemolysis test	1000μg/mL	In vitro incubation	3 hours	

In the 3-month repeat dosing toxicity studies, the no-observed-adverse-event-level (NOAEL) of F-627 was 1000 μg/kg in rats and 675 μg/kg in cynomolgus monkeys, which were the high dose selected in the dose design.

1.1.6 Previous clinical studies

At present, a total of 7 clinical studies involving F-627 either have been completed or are ongoing, which include 2 phase I clinical trials in healthy subjects, 3 phase I clinical trials in patients with breast cancer, and 2 phase II clinical trials in patients with breast cancer. Refer to Table 3 for the summary of F-627 clinical studies. Six of these clinical trials have been completed. One clinical trial has completed subject recruitment and follow-up, and is now in the process of data analysis.

A total of 54 healthy subjects and 410 breast cancer patients have participated in the F-627 clinical trials sponsored by Generon (Shanghai) Corporation Ltd. The doses used in these trials ranged from 30 to 360 μg/kg/dose, or were fixed doses of 10 and 20 mg/dose. The longest duration of treatment was 6 cycles, with one subcutaneous injection per cycle. The chemotherapy regimens used for treating breast cancer patients include TAC (docetaxel + doxorubicin + cyclophosphamide), EC (epirubicin + cyclophosphamide), and TC (docetaxel + cyclophosphamide).

Table 3 Summary of F-627 clinical studies.

Study No. (Status)	Study Population/No. of Subjects	Study Design	Treatment Arm	Study Period
SP-CDR-1-1302 (Completed)	Randomized 138 female patients with breast cancer Location: China	A multi-center, randomized, open-label, active-controlled, dose-finding **phase II clinical trial**	Chemotherapy regimen: EC F-627 10 mg/dose and 20 mg/dose, administered subcutaneously on day 3 after chemotherapy for 4 continuous cycles. Comparator arm: GRAN® 5 μg/kg/day, administered subcutaneously from day 3 after chemotherapy for no longer than 2 weeks, for 4 continuous cycles	Jul. 3, 2014–Nov. 22, 2015
GC-627-02 (Completed)	Randomized 232 patients with stage I-IV invasive breast cancer Location: U.S., Ukraine, and Russia	A multi-center, randomized, open-label, active-controlled, dose-finding **phase II clinical trial**	Chemotherapy regimen: TC or TAC F-627 80, 240, and 320 μg/kg/dose, administered subcutaneously on day 2 after chemotherapy for 4 continuous cycles. Comparator arm: Neulasta (pegfilgrastim) 6 mg/day, administered subcutaneously on day 2 after chemotherapy for 4 continuous cycles	Aug. 10, 2012–Oct. 24, 2014
GC-F-627-01 (Completed)	30 healthy male subjects Location: Australia	A single-center, open-label, single-dose, dose-escalation **phase I clinical trial**	F-627 30, 60, 120, 240, and 320 μg/kg, administered subcutaneously on day 1 of the treatment period	May 25, 2010–Nov. 18, 2010
2012-F-627-CH1 (Completed)	Randomized 18 female patients with breast cancer Location: China	A single-center, open-label, single- and repeated-dose, dose-escalation **phase I clinical trial**	Chemotherapy regimen: EC F-627 80, 240, 320 μg/kg/dose, administered subcutaneously on day 3 after chemotherapy for 4 continuous cycles	Dec. 27, 2012–Dec. 3, 2013
SP-CDR-1-1301 (Completed)	Randomized 15 female patients with breast cancer Location: China	A multi-center, open-label **phase Ib clinical trial**	Chemotherapy regimen: TAC F-627 240 and 320 μg/kg/dose, administered subcutaneously on day 3 after chemotherapy for 6 continuous cycles	Feb. 25, 2014–Aug. 19, 2015
SP11502 (Study completed, currently undergoing data analysis)	Randomized 7 female patients with breast cancer Location: China	**A phase Ib clinical trial**	Chemotherapy regimen: TAC F-627 320 μg/kg/dose, administered subcutaneously on the same day following chemotherapy (4 hrs within the completion of chemotherapy) for 6 continuous cycles	Aug. 25, 2015–Mar. 30, 2016
GC-F-627-03 (Completed)	15 healthy male subjects Location: Australia	A multi-center, randomized, open-label **phase Ib clinical trial**	F-627 lyophilized powder or pre-filled syringe, 20 mg/dose, administered subcutaneously on day 1 of the treatment period	Feb. 3, 2016–Feb. 22, 2016

EC = epirubicin + cyclophosphamide; TAC = Taxotere® [docetaxel] + doxorubicin + cyclophosphamide; TC = Taxotere® [docetaxel] + cyclophosphamide

Safety of F-627

Generally, F-627 is safe and well tolerated. The incidence rate of serious adverse events (SAEs) is low with no report of death and significantly abnormal laboratory measurements. The most common SAE is febrile neutropenia. All SAEs are unrelated to the study drug except for 2 cases in the SP-CRD-1-1302 trial. In this study, one case in the F-627 10 mg/dose arm and one case in the GRAN® arm were determined to be related to the study drug (causality with study drug was "cannot be determined" in both cases, which was classified as "related").

Two subjects withdrew from the study due to treatment-emergent adverse events (TEAEs). In the GC-627-02 study, one subject in the F-627 80 µg/kg/dose arm withdrew due to moderate uveitis, which was probably related to the study drug. In addition, in the SP-CDR-1-1302 study, one subject in the GRAN® arm withdrew due to fever. Causality with study drug could not be determined by the investigator.

The most common drug-related TEAEs were back distress, osteodynia, pain in extremity, pain joint, and general malaise. These TEAEs are also commonly seen with other recombinant human granulocyte colony stimulating factors (rhG-CSFs). F-627 has a low incidence rate of injection site reactions compared with GRAN® and Neulasta® (PEGylated rhG-CSF), and a dose-response relationship was not observed.

The incidence rate and severity of TEAEs of F-627 are similar with those of Neulasta® and GRAN®. In the SP-CDR-1-1302 study, F-627 had a lower incidence rate of TEAEs compared with GRAN®.

The incidence rate and severity of TEAEs are similar across different doses of F-627.

In the analysis of serum anti-F-627 antibodies, the incidence rate of positive result in binding assay was very low and the results of the following confirmatory assay were negative. Neutralizing antibodies were not detected.

Abnormalities with clinical significance in ECGs were not observed. During the study, the incidence rate of cardiovascular events was low.

In summary, the incidence rate of SAEs was low and unrelated to the study drug. There were only 2 subjects who withdrew due to TEAEs. Abnormalities with clinical significance were not found in vital signs, laboratory measurements, and ECGs. F-627 has good safety and is similar with other marketed rhG-CSFs such as GRAN® and Neulasta. Significant immunogenicity was not observed for F-627.

Efficacy of F-627

SP-CDR-1-1302 is a randomized, open-label, active-controlled, dose-finding phase II clinical study which randomized 138 breast cancer patients to receive EC chemotherapy and F-627 10 mg/dose, F-627 20 mg/dose, or GRAN® 5 µg/kg/dose (up to 2 weeks or when ANC recovers to 5.0×10^9/L from nadir) 24 hours (day 3) after chemotherapy for 4 continuous cycles.

The primary endpoint is the duration (days) of grade 3 or 4 neutropenia in cycle 1, that is, the number of days in which ANC < 1.0×10^9/L. Non-inferiority was established when comparing F-627 10 mg/dose and F-627 20 mg/dose with GRAN®. No significant differences were observed between F-627 20 mg/dose arm, F-627 10 mg/dose arm, and GRAN® arm in the incidence rate and duration of neutropenia, ANC nadir, as well as the time of ANC recovered to 2.0×10^9/L from nadir. F-627 20 mg/dose was

slightly superior to F-627 10 mg/dose, supporting the use of F-627 20 mg/dose as the recommended dose for phase III clinical trials. The ANC-time profile was similar between F-627 10 mg/dose and 20 mg/dose, and GRAN®. Febrile neutropenia was not observed in overall 4 cycles.

In addition, a randomized, open-label, active-controlled, dose-finding phase II clinical study (GC-627-02) recruited 232 patients with stage I-IV breast cancer in Europe and in the United States. These patients were treated with either TC or TAC chemotherapy. The primary endpoint was duration of grade 3 or 4 neutropenia. Non-inferiority was established when comparing 3 dose levels of F-627 (80, 240, and 320 μg/kg) with Neulasta. There were no significant differences between F-627 240 and 320 μg/kg and Neulasta in the incidence rate and duration of neutropenia, ANC nadir, as well as the time of ANC recovered to 2.0×10^9/L from nadir.

Pharmacokinetics/pharmacodynamics (PK/PD) of F-627

Five studies evaluated the PK/PD of F-627, of which 2 involved healthy subjects and 3 involved female subjects with breast cancers.

GC-F-627-01: This study evaluated the safety and PK/PD of 5 dose levels of F-627 in healthy males (30, 60, 120, 240, and 360 μg/kg). Results suggested that F-627 demonstrated nonlinear PK. C_{max} and $T_{1/2}$ increased with the increasing dose. T_{max} and $AUC_{(0\text{-inf})}$ increased with the increasing dose from 30 to 240 μg/kg, but leveled off at the dose of above 240 μg/kg. Mean $T_{1/2}$ was between 43.9–71.4 hrs. Clearance (CL) decreased with the increasing dose but leveled off at 240 and 360 μg/kg, suggesting that clearance peaked at high concentrations of F-627.

The study also found dose-dependent increases in leukocytes, neutrophils and CD34+, which peaked at 36–96 hrs after dose administration. T_{max} was delayed to 96–120 hrs for high-dose F-627.

2012-F-627-CH1: This study evaluated the PK of F-627 80, 240, and 320 μg/kg given on day 3 (48 hrs) after chemotherapy for 4 continuous cycles in breast cancer patients receiving epirubicin/ cyclophosphamide (EC) chemotherapy. In cycles 1 and 3, C_{max} and AUC increased with the increasing dose, with the increases higher in cycle 1 than in cycle 3. In cycle 1, C_{max} in cycle 1 leveled off between 240–320 μg/kg; clearance decreased with the increasing dose and leveled off between 240–320 μg/kg. In cycle 3, AUC showed a dose-dependent increase between 240–320 μg/kg. Mean $T_{1/2}$ was 34–56 hrs.

SP-CDR-1-1301: This study evaluated the PK of F-627 (7 subjects in the 240 μg/kg arm and 8 subjects in the 320 μg/kg arm) in breast cancer patients receiving docetaxel/doxorubicin/cyclophosphamide (TAC) chemotherapy in cycles 1 and 3. F-627 was administered subcutaneously on day 2 (24 hrs) after chemotherapy for 6 continuous cycles. In cycles 1 and 3, C_{max} and AUC increased in a dose-dependent manner, with the increases higher in cycle 1 than in cycle 3. Mean $T_{1/2}$ was 26–33 hrs.

In these two studies, pharmacodynamics endpoints such as the shortening of the duration of neutropenia, the increase of ANC nadir, and the shortening of the time ANC recovered to normal from nadir, were dose-dependent.

1.2 Principles

Cancer is a threat to human health with an increasing incidence rate. Cytotoxic chemotherapeutic agents are still the major therapy for cancer. Myelosuppression is one of the most common toxicities in chemotherapy. The inhibition of granulocytes, such as neutropenia, is a primary concern by clinical physician. Neutropenia not only increases the risk of infections and death in cancer patients, but also has detrimental effects on quality life of patients. Neutropenia is also the main factor leading to chemotherapy delay or dose reduction. The treatment of neutropenia is still an unmet clinical need.

Recombinant human granulocyte colony stimulating factor (rhG-CSF) has been in use for approximately 25 years. Filgrastim (Neupogen) and Lenograstim have a half-life of only 3 hours and thus require daily administration. The average cycle is 11 days before ANC returns to normal levels. PEGylated rhG-CSF (Pegfilgrastim, Neulasta) has a half-life of 30–80 hours and requires just one administration for each chemotherapy cycle. Clinically, G-CSF can be used as primary prophylaxis (starting from cycle 1 until the chemotherapy treatment is completed) or secondary prophylaxis (in the following cycles if severe neutropenia or FN occurs after chemotherapy). The European Organization for Research and Treatment of Cancer (EORTC), American Society of Clinical Oncology (ASCO), and National Comprehensive Cancer Network (NCCN) all recommend to use G-CSF as prophylaxis in chemotherapy regimens if the incidence rate of FN ≥ 20%, or if the incidence rate of FN is 10–20% and chemotherapy is considered to be a risk factor.

Among the adjuvant chemotherapy regimens recommended by the NCCN 2014 guideline for breast cancer, preferred regimens include docetaxel + doxorubicin + cyclophosphamide (TAC), dose-dense doxorubicin + cyclophosphamide (AC) → dose-dense paclitaxel (P), doxorubicin + cyclophosphamide (AC) → paclitaxel/docetaxel (P/T), epirubicin + cyclophosphamide (EC) → paclitaxel/docetaxel (P/T), docetaxel + cyclophosphamide (TC), epirubicin + cyclophosphamide (EC), and doxorubicin + cyclophosphamide (AC)[2]. Common regimens used in China are AC/EC→P or AC/EC→T for they have milder myelotoxicity compared with TAC/TEC or TA/TE regimens; TC is also one of the common chemotherapy regimens. A large phase III clinical trial showed that 61% of patients developed grade 3-4 neutropenia and 5% developed febrile neutropenia when receiving the TC regimen, while 55% of patients developed grade 3-4 neutropenia and 2.5% developed febrile neutropenia (FN) when receiving the AC regimen[3, 4]. In another phase III clinical trial, the incidence rates of grade 3-4 neutropenia were 54% with EC regimen (E:120 mg/m^2+C:600 mg/m^2) and 64% with sequential D (docetaxel) followed by EC; the incidence rates of FN were 2.8% and 6.6% for EC regimen and sequential D (docetaxel) followed by EC respectively.[8]Also, a phase III clinical trial comparing EC regimen (E:90 mg/m^2 + C:600 mg/m^2) and ED regimen (E:120 mg/m^2 + D:75 mg/m^2) found that the incidence rates of leukopenia were 74% and 81%, respectively and the incidence rates of infections (including FN) were 6.3% and 7.2%, respectively. Chemotherapy regimens with high-dose epirubicin require supportive care with G-CSF.[9].

The study population for this trial is breast cancer patients receiving EC chemotherapy. Data show that the incidence rate of FN in EC chemotherapy regimen is between 10–20%.

F-627 (recombinant human granulocyte colony stimulating factor-Fc fusion protein, rhG-CSF-Fc), a rhG-CSF dimer, is a biological product developed by Generon (Shanghai) Corporation Ltd. PK/PD studies in breast cancer patients showed that median $T_{1/2}$ of F-627 was 34.7–56.4 hours after a single dose for each cycle. A phase II clinical trial confirmed that F-627 is non-inferior to standard doses of pegfilgrastim (Neulasta) or filgrastim (GRAN®) when used as prophylactic treatment for chemotherapy-induced neutropenia. The main adverse reactions were osteodynia, pain joint, pain in extremity, and injection site rash, similar to the side effects of rhG-CSF. F-627 had lower incidence rates of osteodynia, pain joint, and pain in extremity compared with GRAN®. This confirmatory phase III clinical trial will be carried out with a larger sample size, with breast cancer patients receiving EC chemotherapy as the study population. This study will compare the efficacy and safety of F-627 pre-filled syringes (20 mg) with GRAN® to support the grant of marketing approval of F-627 in China.

2.0 OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to compare the efficacy of recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627) versus recombinant human granulocyte colony stimulating factor (GRAN®) in the first cycle of prophylactic treatment in subjects with breast cancer receiving chemotherapy. The primary endpoint is the duration (days) of grade 3 or 4 (moderate and severe) neutropenia in cycle 1, that is, the number of days in which ANC < 1.0×10^9/L in cycle 1.

2.2 Secondary Objectives

- The incidence rate of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L and < 0.5×10^9/L, respectively) in each cycle
- The duration (days) of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L and < 0.5×10^9/L, respectively) in cycles 2–4
- The incidence rate and duration (days) of grade 4 neutropenia (ANC < 0.5×10^9/L) in each cycle
- The overall duration (days) of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L and < 0.5×10^9/L, respectively) in overall 4 cycles
- The incidence rate and duration (days) of grade 2 or greater neutropenia (ANC < 1.5×10^9/L) in each cycle
- Incidence rate of febrile neutropenia (FN) (defined as ANC < 1.0×10^9/L; a single measurement of body temperature > 38.3 °C or a temperature ≥ 38.0 °C sustained over 1 hr)
- ANC-time profile
- The ANC nadir from day 3 to day 13 of cycle 1
- The time (days) of ANC nadir recovers to 2.0×10^9/L in each cycle

2.3 Safety Objectives

To evaluate the safety of F-627 and GRAN®. Safety endpoints include adverse events and serious adverse events, laboratory measurements, physical examinations, vital signs, and symptoms.

2.4 Exploratory Objectives

To evaluate the immunogenic potential of F-627 by testing serum anti-F-627 antibodies.

3.0 STUDY DESIGN

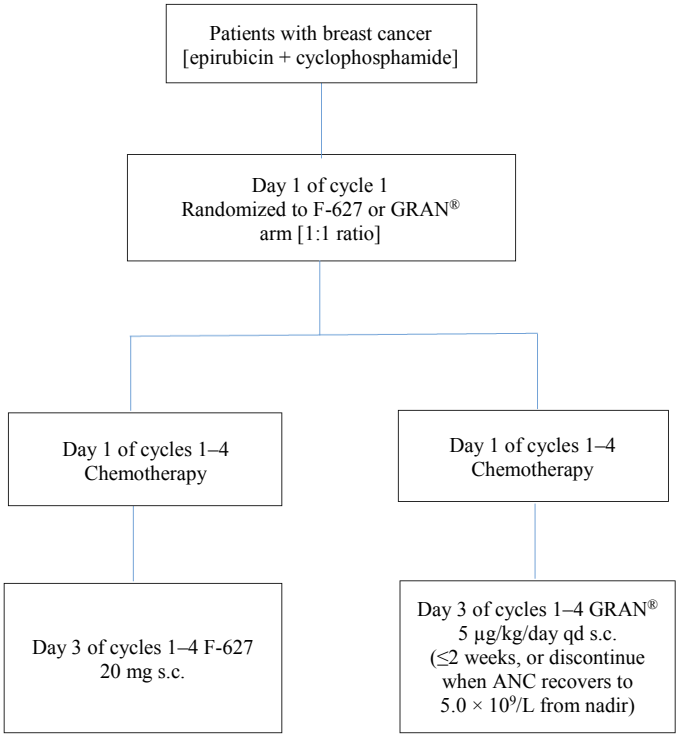
3.1 Overall Study Design and Treatment Plan

This is a multi-center, randomized, open-label, active-controlled phase III clinical trial. A total of 240 postoperative subjects with breast cancer will be enrolled to receive at least 4 cycles of EC chemotherapy, namely: epirubicin 100 mg/m^2 + cyclophosphamide 600 mg/m^2, i.v. on day 1, repeat cycle every 21 days for 4 cycles. After completing the evaluation for the first 4 cycles, subjects will receive subsequent treatments according to routine clinical practice. The investigator must ensure that the first cycle of treatment follows the recommended dosages for chemotherapy. In cycles 2–4, dose delays and reductions due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted. Dosages are permitted to be individualized based on subject conditions.

As shown in the flow diagram in Table 4, patients are randomized before day 1 of the cycle 1 to F-627 arm or GRAN® arm in a 1:1 ratio. Treatment allocation will remain unchanged during the entire treatment period (4 cycles). On day 3 of each cycle, that is, 48±4 hours after the start of chemotherapy, subjects will receive F-627 (20 mg/dose, s.c.) or GRAN® (5 μg/kg/day, s.c., once daily [±4 hrs] for ≤ 2 weeks or until ANC recovers to 5.0×10^9/L from nadir [investigators may refer to ANC results from respective study sites to decide when to discontinue GRAN®]). All laboratory measurements are performed at individual study sites except for the routine blood test (neutrophil count) in cycle 1, which is performed at the central laboratory. Adverse events/serious adverse events are recorded, along with laboratory measurements, 12-lead ECGs and abdominal ultrasounds.

The last visit will be completed 3 weeks after the last chemotherapy dose. A follow-up visit by telephone will be completed 30 days after the last dose.

Table 4. Study flow diagram.



3.1.1 Principles of study design

This is a multi-center, randomized, open-label, active-controlled phase III clinical trial. Randomization and the utilization of comparator conform to the standard methods.

3.1.2 Principles of dose selection

Selection of Chemotherapy Dose

Standard doses of EC chemotherapy will be used: epirubicin [Pharmorubicin®] 100 mg/m^2, i.v. + cyclophosphamide 600 mg/m^2, i.v. on day 1, repeat cycle every 21 days for 4 cycles.

Dose Selection of Investigational Drug

Results from 3-month repeat dosing toxicity studies showed that the no-observed-adverse-effect-level (NOAEL) of F-627 was 1000 µg/kg in rats and 675 µg/kg in cynomolgus monkeys.

Table 5. Rationale for F-627 doses in clinical trials.

	Pegfilgrastim	F-627
Repeat Dosing Toxicity Study in Rats	100, 300, and 1000 μg/kg, once a week for 3/6 months	100, 300, and 1000 μg/kg, once a week for 3 months, followed by a recovery period of 1 month
NOAEL in Rats	1000 μg/kg	1000 μg/kg
Repeat Dosing Toxicity Study in Monkeys	75, 250, and 750 μg/kg, once a week for 1 month, followed by a recovery period of 1 month	75, 225, and 675 μg/kg, once a week for 3 months, followed by a recovery period of 1 month
NOAEL in Monkeys	750 μg/kg	675 μg/kg
Phase I Clinical Trial in Healthy Volunteers	30, 60, 100, 300 μg/kg	30, 60, 120, 240, 360 μg/kg
Phase I Clinical Trial in Subjects with Breast Cancer	60,100,300 μg/kg	80,240,320 μg/kg
Recommended Dose for Clinical Trials	6 mg/dose	20 mg/dose

A total of 54 healthy subjects and 410 breast cancer patients have participated in F-627 clinical trials. The doses used in these trials ranged from 30-360 μg/kg/dose, or were fixed doses of 10 and 20 mg/dose (Refer to Table 5 for rationale for F-627 doses in clinical trials). Overall, the incidence rates of serious adverse events (SAEs) were low and unrelated to the study drug. Only 2 subjects withdrew due to TEAEs. F-627 has good safety. The most common drug-related TEAEs were back distress, osteodynia, pain in extremity, pain joint, and general malaise. These TEAEs are also commonly seen with other marketed recombinant human granulocyte colony stimulating factors (rhG-CSFs) such as GRAN® and Neulasta. In the SP-CDR-1-1302 study, F-627 had a lower incidence rate of TEAEs compared with GRAN®.

Data from 138 patients with breast cancer were analyzed in a phase II clinical trial (SP-CDR-1-1302). Results indicated that non-inferiority was established when comparing F-627 10 mg/dose and 20 mg/dose with GRAN® in terms of the primary endpoint, or, the duration of grade 3–4 neutropenia in cycle 1. No significant differences were observed between F-627 10 mg/dose, 20 mg/dose and GRAN® in the incidence rate and duration of neutropenia, ANC nadir, as well as the time of ANC recovered to 2.0×10^9/L from nadir. In addition, a phase II clinical study (GC-627-02) recruited 232 patients with stage I-IV breast cancer in Europe and in the United States. These patients were treated with either TC or TAC chemotherapy. Non-inferiority was established when comparing 3 dose levels of F-627 (80, 240, and 320 μg/kg) with Neulasta in terms of the primary endpoint. There were no significant differences between F-627 240 and 320 μg/kg and Neulasta in the incidence rate and duration of neutropenia, ANC nadir, as well as the time of ANC recovered to 2.0×10^9/L from nadir.

Moreover, PK/PD showed good correlation. C_{max} and $AUC_{0\text{-}inf}$ increased in a dose-dependent manner and pharmacodynamics indicators also improved. Thus, F-627 20 mg is used for this phase III clinical trial.

3.1.3 Selection of comparator

rhG-CSF has been used clinically for almost 20 years. There are multiple rhG-CSF products of different manufacturers approved in China, including originator products and biosimilars, of which 2 are PEGylated rhG-CSF biosimilars. GRAN® (manufactured by Kyowa Hakko Kirin China Pharmaceutical Co., Ltd.) has been selected as the comparator for this phase III clinical trial due to the reliability and stable quality of this originator product.

Dose Selection of Comparator

According to the product labeling, GRAN® is recommended to be given at a dose of 5 μg/kg, once a day from day 3 of each cycle, for ≤ 2 weeks or until ANC recovers to 5.0×10^9/L.

3.2 Randomization

After signing the informed consent form, subjects will be assigned a unique subject number in sequential order. The investigator should document the subject number in the electronic case report form (eCRF), original medical records, and study documents. Central randomization is performed in this study using the Interactive Web-Response System (IWRS). After eligible patients provide a signed informed consent form, authorized investigators will log in to the IWRS system and key in relevant information. The system will then randomize subjects to 2 different arms, F-627 or GRAN®.

3.3 Blinding

No blinding is applied in this study.

3.4 Study Sites and Number of Subjects

This is a multi-center clinical trial. Approximately 20 sites will participate in this study.

This study plans to recruit 240 patients with breast cancer and randomize them to F-627 or GRAN® arm at a 1:1 ratio.

4.0 STUDY POPULATION

4.1 Study Population

The study population is patients with breast cancer who plan on receiving at least 4 cycles of EC chemotherapy.

4.2 Inclusion Criteria

All of the following criteria must be met for a subject to be enrolled:

1. Willing to sign the informed consent form and able to comply with protocol requirements;
2. 18–75 years old;
3. Female postoperative patients with breast cancer who require adjuvant chemotherapy, and are planned to receive at least 4 cycles of EC chemotherapy, namely epirubicin 100 mg/m^2 + cyclophosphamide 600 mg/m^2;
4. ECOG performance status ≤ 2;
5. Absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, hemoglobin (Hb) ≥ 11.0 g/dL, and platelet (PLT) $\geq 100 \times 10^9$/L prior to enrollment;
6. Hepatic and renal functions: Total bilirubin $\leq 1.5 \times$ ULN, ALT and AST $\leq 2.5 \times$ ULN, serum creatinine $\leq 1.5 \times$ ULN;
7. Left ventricular ejection fraction > 50%;
8. Women without child-bearing potential, i.e., women who have had menopause for at least 1 year or who have undergone sterilization (bilateral tubal ligation, double oophorectomy or hysterectomy); patients with child-bearing potential should agree to take appropriate contraceptive measures, including condoms, spermicidal condoms, foams, gels, contraceptive barrier, intrauterine devices (IUD), and contraceptives (oral or injection), starting from 1 month before the start of the study until 30 days after the end of the study.

4.3 Exclusion Criteria

Patients who meet any of the following must be excluded from this study.

1. Radiation therapy within 4 weeks prior to enrollment;
2. Patients with breast cancer who have received neoadjuvant chemotherapy before surgery;
3. Prior bone marrow or stem cell transplant;
4. With other malignant tumors other than breast cancer;
5. Patients who have received a treatment with recombinant human granulocyte colony stimulating factor within 6 weeks prior to randomization;
6. Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches;
7. With any disease that may cause splenomegaly;
8. With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment), or Hepatitis C;
9. Women in pregnancy or breastfeeding;
10. Known HIV positive or AIDS;
11. With active tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray;

12. With sickle cell anemia;
13. With alcohol or drug abuse that may affect the compliance with the study;
14. With known hypersensitivity to granulocyte colony stimulating factor or excipients;
15. Have received any other investigational drug within 1 month or 5 half-lives of the investigational drugs prior to enrollment (whichever is longer);
16. Patients with diseases or symptoms unsuitable for participating in the trial. For example, the study drugs may compromise the health of the patient or the assessment of adverse events may be affected.

4.4 Withdrawal

Subjects have the right to withdraw from the study at any time without any reason. The medical rights of the subjects will not be affected by the withdrawal of the study.

4.4.1 Criteria for withdrawal

1. Subject withdraws voluntarily;
2. Occurrence of uncontrolled grade 3 or 4 adverse events related to the study drug as judged by the investigator;
3. Subjects fail to comply with the study protocol;
4. Termination by the investigator under safety considerations or in the best interest of the subject when the subject's condition changes;
5. Pregnancy;
6. Death.

4.4.2 Procedure for withdrawal

Subjects who withdraw from the study at any time will be required to complete the last evaluation and relevant examinations.

4.4.3 Visits for withdrawals

If a subject withdraws from the study, the investigator should ask if the subject is willing to continue follow-up visits, and document the discussion in the medical history and case report form. If a subject is lost to follow-up, the investigator should make every effort to contact the subject or her family members to understand and document the reason for the withdrawal, and complete the final evaluation.

If a subject voluntarily withdraws from the study, request for a telephone visit 30 days after the subject's last dose. If the subject refuses, no further data needs to be collected.

If the study for a subject is terminated due to an adverse event, the event should be followed until it recovers to normal or baseline levels and be documented in the electronic case report form (eCRF).

4.5 Replacement Principle

4.5.1 Subject replacement

This trial will not plan on replacing the subjects who terminate the trial early.

5.0 TRIAL PROCEDURE

The schedule of study procedures is detailed in Table 6.

Table 6. Schedule of study procedures.

Treatment Period / Items	Screening Day -21 to Day -1	Cycles 1–4			End of Study Day 21 of Cycle 4[j]
		Day 1	Day 3	Days 3–21	
Informed Consent Form	×				
Inclusion/Exclusion Criteria	×				
Tumor History	×				
Concomitant Diseases	×	×	×	×	×
Combined Medications	×	×	×	×	×
Physical Examination[a]	×	×			×
Weight/Height[b]	×	×			×
Blood Pressure, Heart Rate	×	×			×
Body Temperature[c]	×	×	×	×	×
Abdominal Ultrasound[d]	×	×			×
12-lead ECG[d]	×	×			×
Echocardiography	×				
Chest X-Ray or CT	×				
Laboratory Measurements					
Routine Blood Test[e]	×	×	×	×	×
Blood Biochemistry	×	×			×
Urinalysis	×	×			×
Pregnancy Test	×				×
Serum Anti-F-627 Antibodies[f]		×			×
Chemotherapy[g]		×			
Study Drug[h]			×	×	
Adverse Events[i]	×	×	×	×	×

Note:

a. A complete physical examination is required during screening. Corresponding physical examination is completed based on subject condition on day 1 of cycles 1–4 (vital organs);

b. Height is only measured during screening. Weight is measured on day 1 of each cycle (measurements within 3 days prior to treatment are acceptable) and at the end of the study;

c. Temperature is measured during screening, on day 1 of each cycle, and at the end of the study. On days 3–21 of each cycle, temperature is measured when ANC < 1.0×10^9/L;

d. Abdominal ultrasound should be performed during screening or before the start of cycle 1 and at the end of the study. The time window for abdominal ultrasound is +/– 3 days. The 12-Lead ECG should be performed during screening (ECGs performed within 7 days prior to randomization can be used as the result for cycle 1), before the start of each cycle, and at the end of the study. The time window for 12-Lead ECG is +/–3 days;

e. Routine blood test is performed on days 3, 5, 7, 8, 9, 10, 11, 13, 15, and 21 of cycle 1, and on days 3, 5, 7, 9, 11, 13, 15, and 21 of cycles 2–4. Laboratory measurements (routine blood test, blood biochemistry, urinalysis, and pregnancy test) within 7 days before cycle 1 (day 1) were acceptable and did not need to be repeated. The time window for laboratory measurements (routine blood test, blood biochemistry, urinalysis, and pregnancy test) before cycles 2–4 was –within –3 days;

f. Blood samples are collected on day 1 (±1 day) of each cycle and day 21 (±1 day) of cycle 4, as well as 90 days (±7 days) after the last visit (day 21 of cycle 4), to test for serum anti-F-627 antibodies. Results from the test on day 1 of cycle 1 are used as baseline.

g. The investigator will decide on the dose and time of the next chemotherapy based on the patient's actual conditions. Results of routine blood test must meet the following criteria prior to the start of the next cycle of chemotherapy: ANC $\geq 2.0 \times 10^9$/L, PLT $\geq 80 \times 10^9$/L, and liver and renal functions should meet the standard as described in "Inclusion Criteria". A 14 day recovery period is permitted if these criteria are not met prior to the scheduled cycle 2, cycle 3, and cycle 4. If the criteria described above are still not met after 14 days, the patient is not eligible to enter the cycle 2, cycle 3, or cycle 4 of this study.

h. On day 3 of each cycle (48±4 hours after the start of chemotherapy), subjects will receive F-627 20 mg subcutaneously or GRAN® (5 μg/kg/day, s.c., once daily [±4 hrs] for ≤ 2 weeks or until ANC recovers to 5.0×10^9/L from nadir [investigators may refer to ANC results from respective study sites to decide when to discontinue GRAN®]);

i. Adverse events are evaluated according to NCI CTCAE 4.03. Adverse events that occur within 30 days after the last dose are included in the safety evaluation. Serious adverse events should be reported;

j. The last visit should be completed on day 21 of cycle 4. A follow-up visit by telephone should be completed 30 days (±3 days) after the last dose;

Note: Routine blood test during cycle 1, as well as all serum anti-F-627 antibody assays are performed at the central laboratory. Routine blood test, blood biochemistry, pregnancy test, and urinalysis during screening and cycles 2–4, and at the end of the study are performed at study sites. In cycle 1, when necessary, investigators can conduct routine blood test at their respective study sites based on subject conditions, in order to provide necessary treatment to prevent the risks from neutropenia. Appropriate measures are taken for chemotherapy-related toxicities based on the investigator's clinical judgment and routine clinical practice.

5.1 Schedule of Study Procedures and Evaluations

5.1.1 Evaluations during screening

In principle, all subjects must sign the informed consent form approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) prior to any study-related examinations. Since the examinations and evaluations during screening are often part of the routine clinical practice, results of echocardiography and chest x-ray or CT prior to signing the informed consent form can be used to avoid repeated examinations.

The screening period is 21 days before the start of study. The following examinations and evaluations are required to be completed prior to dosing:

1) Signing the informed consent form
2) Medical history inquiry
3) Record treatment measurements for concurrent diseases within the past 2 weeks and anti-neoplastic treatments received within the past year
4) Physical examination
5) Measurement of height (cm)
6) ECOG performance status scoring
7) Measurement of body temperature
8) Measurement of blood pressure and heart rate
9) Laboratory measurements:

    - Routine blood test
    - Blood biochemistry
    - Urinalysis
    - Pregnancy test

10) Abdominal ultrasound (include at least the liver and spleen)
11) 12-lead ECG
12) Left ventricular ejection fraction (LVEF) measured by echocardiography to assess cardiac function
13) Chest x-ray (frontal and lateral) or chest CT

Patients must satisfy all the eligibility criteria, i.e. meet all the inclusion criteria and not meet any of the exclusion criteria, to participate in this trial.

5.1.2 Enrollment of eligible patients

An eligible patient will be assigned a subject code which is unique to identify the patient. This code will remain constant throughout the study.

The investigator should reconfirm the eligibility of the subject prior to the chemotherapy. The investigator will log in to the IWRS system for central randomization. Subjects will be randomized to 2 different arms, F-627 or GRAN®. Treatment allocation will remain unchanged during the entire treatment period.

5.1.3 Evaluations during treatment

The following examinations and evaluations are required on day 1 of cycle 1:

1) Randomization (before cycle 1)
2) Measurement of height and weight for body surface area
3) Blood pressure and heart rate
4) Body temperature
5) Physical examination
6) Laboratory measurements:

    - Routine blood test
    - Blood biochemistry
    - Urinalysis

7) Serum anti-F-627 antibody assay
8) 12-Lead ECG (not required if performed during screening)
9) Abdominal ultrasound (not required if performed during screening)
10) Record and evaluate AEs and SAEs (defined in Section 7.0);
11) Record combined medications

The following examinations and evaluations are required on days 3–21 of cycle 1:

1) Laboratory measurements:

    - Routine blood test: on days 3, 5, 7, 8, 9, 10, 11, 13, 15, and 21

2) Body temperature (when ANC $< 1.0 \times 10^9$/L)
3) Record and evaluate AEs and SAEs
4) Record combined medications

The following examinations and evaluations are required on day 1 of cycles 2–4:

1) Measurement of weight
2) Blood pressure and heart rate
3) Body temperature
4) Physical examination
5) Laboratory measurements:

    - Routine blood test
    - Blood biochemistry
    - Urinalysis

6) Blood sampling for serum anti-F-627 antibody assay
7) 12-lead ECG
8) Record and evaluate AEs and SAEs (defined in Section 7.0);
9) Record combined medications

The following examinations and evaluations are required on days 3–21 of cycles 2–4:

1) Temperature (when ANC < 1.0×10^9/L)
2) Laboratory measurements:
    - Routine blood test: on days 3, 5, 7, 9, 11, 13, 15, and 21
3) Record and evaluate AEs and SAEs
4) Record combined medications

5.1.4 Evaluations at end of study

The following examinations and evaluations are required to be performed at the completion of 4 chemotherapy cycles (on day 21±1 of cycle 4):

1) Physical examination
2) Measurement of weight
3) Body temperature
4) Blood pressure and heart rate
5) Laboratory measurements:
    - Routine blood test
    - Blood biochemistry
    - Urinalysis
    - Pregnancy test
6) Serum anti-F-627 antibody assay
7) Abdominal ultrasound
8) 12-lead ECG
9) Record and evaluate AEs and SAEs
10) Record combined medications

The following evaluations should be completed in the follow-up visit by telephone (30 days after the last dose): Subjects who withdraw from the study or complete the study are required to have a follow-up visit by telephone 30±3 days after the last dose to follow their general conditions, disease recurrence, combined medications, doctor's visits, hospitalizations and other information, and evaluate the AEs/SAEs.

5.1.5 Unscheduled evaluations

Additional visits may be arranged accordingly during treatment for potential safety concerns or necessary tumor assessments.

After the visit on day 21 of cycle 4, the investigator may arrange an unscheduled visit 2 weeks later based on the subject conditions in order to evaluate the AEs.

5.2 Definitions and Evaluation Criteria of Study Endpoints

5.2.1 Efficacy evaluation

5.2.1.1 Efficacy endpoints

Primary endpoint:

The primary endpoint is the duration (days) of grade 3 or 4 (moderate or severe) neutropenia in cycle 1, that is, the number of days in which ANC < 1.0×10^9/L.

Secondary endpoints include:

- The incidence rate of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L) in each cycle
- The duration (days) of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L) in cycles 2–4
- The incidence rate and duration (days) of grade 4 neutropenia (ANC < 0.5×10^9/L) in each cycle
- The duration (days) of grade 3 or 4 neutropenia (ANC < 1.0×10^9/L and < 0.5×10^9/L, respectively) in the 4 cycles
- The incidence rate of febrile neutropenia (FN) [FN is defined as ANC < 1.0×10^9/L with the body temperature in a single measurement > 38.3 °C or a fever ≥ 38.0 °C lasting for greater than 1 hour]
- ANC-time profile
- ANC nadir between days 3–13 in cycle 1
- The incidence rate and duration (days) of grade 2 or greater neutropenia (ANC < 1.5×10^9/L) in each cycle
- The time (days) of ANC nadir recovers to 2.0×10^9/L in each cycle

5.2.1.2 Methods for evaluating efficacy endpoints

Efficacy endpoint should be determined by routine blood test (including ANC). The sampling time points for routine blood test specified in the protocol meet the evaluation of efficacy endpoint.

Routine blood test is completed at the central laboratory during cycle 1 and at the study sites for cycles 2–4. Results of ANC in cycle 1 are the primary efficacy endpoint. The central laboratory will provide each study site with materials and Central Laboratory Service Manual, as well as appropriate guidance and training.

5.2.2 Safety evaluation

5.2.2.1 Safety endpoints

Safety evaluations include all observed and recorded AEs, physical examinations, vital signs, ECOG performance status score, laboratory measurements, ECG, and abdominal ultrasound change.

5.2.2.2 Methods for evaluating safety endpoints

Adverse events (AEs)

Refer to Section 7.0 for the definitions of adverse events, which are graded based on NCI CTCAE v4.03. Corresponding interventions should be given timely by the investigators.

Physical status based on ECOG PS scoring

Performance status score (ECOG score): 5 grades, 0 = Fully active, able to carry on all performance without restriction; 1 = Restricted in physically strenuous activity but ambulatory and able to carry out work of a light nature or office work; 2 = Ambulatory and capable of all self care but unable to carry out any work activities; up and about more than 50% of waking hours; 3 = Capable of only limited self care; confined to bed or chair more than 50% of waking hours; 4 = Completely disabled; cannot carry on any self care; totally confined to bed or chair; 5 = Death.

Laboratory Measurements

Routine blood test measurements and F-627 antibodies are tested at the central laboratory in cycle 1. Routine blood test, blood biochemistry, serum pregnancy test, and urinalysis are completed at each study site during cycles 2–4 and at the end of study. In cycle 1, when necessary, investigators can conduct routine blood test at the clinical laboratory of their respective study sites based on subject conditions, in order to provide necessary treatment to prevent the risks from neutropenia.

The central laboratory will provide the materials and brochures, and also appropriate training and guidance. Refer to Table 7 for all laboratory measurements.

Table 7. Laboratory measurements.

Routine blood test	Red blood cell, hemoglobin, white blood cell, platelet count, differential blood count (neutrophils, lymphocytes, monocytes, basophils and eosinophils), hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), etc.
Blood biochemistry	Total protein (TP), albumin (ALB), blood glucose, blood urea nitrogen, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, aspartate transaminase (AST), γ-glutamyl transpeptidase, alanine transaminase (ALT), Ca, P, K, and Na, etc.
Urinalysis	pH, specific gravity, protein, urinary casts, RBC, and urine glucose and ketones; Microscopic urinalysis is required if the urinary dipstick is positive for WBC and/or hemoglobin

The investigator must document the sampling time points and test results in the electronic case report form (eCRF).

Refer to Section 7.0 to determine whether a laboratory abnormality with clinical significance meets the criteria for an adverse event and needs to be documented in the eCRFs.

12-lead ECG:

Including heart rate, PR interval, QRS interval, QT interval, and QTc;

Abdominal ultrasound:

Including at least the liver and spleen; special attention to splenomegaly if any.

Physical examinations:

Including general conditions, skin, lymph nodes, eyes, ears, nose, mouth, throat, neck, thyroid, chest, lungs, cardiovascular system, abdomen, limbs, nervous system, musculoskeletal system, etc.

Body temperature:

Oral temperature is the standard, but tympanic, axillary, or rectal temperatures are also acceptable.

Clinically significant abnormalities in physical examinations, vital signs, ECOG score, 12-lead ECG, and abdominal ultrasound during the study have to be reported as AEs.

5.3 Immunogenicity Evaluation

5.3.1.1 Immunogenicity endpoints

To evaluate the immunogenic potential of F-627 by testing serum anti-F-627 antibodies.

5.3.1.2 Methods for evaluating immunogenicity

Serum anti-F-627 antibody assay primarily consists of three sequential assays, that is, binding assay - electrochemiluminescence by MSD (ECL), confirmatory assay - binding inhibition assay, and neutralization assay - cell-based neutralization assay. Firstly, all collected serum samples are subjected to a binding assay for antibody detection by ECL. Secondly, serum samples with positive binding assays are further subjected to a confirmatory assay, in which the antibody should be blocked and inhibited before ECL detection. After titering assay, serum samples with positive confirmatory assays are further subjected to the neutralizing assay to determine whether the biological function of G-CSF is neutralized by the antibodies in the serum sample using a cell-based platform. Criteria for immunogenicity analysis results are shown in Table 8.

Serum anti-F-627 antibody assays are performed at the central laboratory according to the laboratory SOP.

The central laboratory will provide each study site with materials and Central Laboratory Service Manual, as well as appropriate guidance and training.

Table 8. Criteria for immunogenicity of F-627.

	Binding Assay	Confirmatory Assay	Neutralizing Assay	Judgment of Results
Step One	-			Negative
Step Two	+	-		Negative
Step Three	+	+	-	Negative
	+	+	+	Positive

6.0 TREATMENT PLAN

6.1 Study Drugs

6.1.1 Dose and mode of administration

Refer to Table 9 below for a summary of dose and mode of administration of study drug.

Table 9. Dose and mode of administration.

Study Drug	Dose	Route of administration	Dosing Interval
F-627	20 mg/dose	Subcutaneous	Day 3 of each cycle, i.e., 48±4 hrs after the start of chemotherapy
GRAN®	5 μg/kg/day*	Subcutaneous	Since day 3 of each cycle, i.e., 48±4 hours after starting chemotherapy, continuous treatment for ≤ 2weeks or until ANC recovers to 5.0×10^9/L from nadir (investigators may refer to ANC test results from the clinical laboratory of respective study sites to decide when to discontinue GRAN®)

Note: *: The actual dose is rounded to the nearest whole number, but the difference between the actual and theoretical doses should be within ± 5%.

6.1.2 Dosage form, packaging, labeling, and storage of study drugs

Investigational Drug: Recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627)

The investigational drug used in this study is recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627), 20 mg/mL prefilled syringes. The product information of F-627 is presented in Table 10.

Prior to use, the safety cap should be removed before subcutaneous injection. The medication should not be placed at room temperature for more than 1 hour.

Table 10. F-627 product information.

Product	Dosage Form	Strength	Ingredients and Excipients
F-627	Prefilled syringe	20 mg/mL	20 mg/mL of F-627, sodium acetate, sorbitol, polysorbate 20, and Ethylenediaminetetraacetic acid (EDTA), pH 5.2

The label of the investigational drug should contain the following information: name of the investigational drug, medication number, protocol number, storage conditions, mode of administration, batch number, expiration date, and standard text "for clinical trial use only". The investigational drug is provided by the sponsor.

The drug should be stored as specified in a sealed container at 2–8 °C away from light, and under the management of designated personnel. If the temperature exceeds the storage condition during transport and storage, record and report the incident to the sponsor.

Reference Drug: Recombinant human granulocyte colony stimulating factor (GRAN®)

The specification of the reference drug GRAN®: 300 μg/0.7 mL/vial, liquid preparation (ampoules), stored at 2–8 °C and managed by designated staff. The drug must not be frozen.

The reference drug is provided by the sponsor. If the temperature exceeds the storage condition during transport and storage, record and report the incident to the sponsor.

6.1.3 Drug accountability

The receipt, dispense (use), return, and disposal of the study drug should be accurately documented in the drug accountability record and drug inventory sheet. The investigator must ensure that the study drug is restricted to study subjects.

The study drug may be disposed at the study sites or by a third-party designated by Generon (Shanghai) Corporation Ltd. If the study drug is to be disposed at study site, the study site's SOP for study drug disposal must be strictly followed. The method and location of disposal, as well as the quantity of disposed drugs must be documented.

6.2 Chemotherapy

6.2.1 Dose and mode of administration of chemotherapy drugs

Each subject is treated with 4 cycles of EC chemotherapy. The dose and mode of administration of the chemotherapy drugs are shown in Table 11:

Table 11. Dose and administration of chemotherapy drugs.

Study Drug	Dose	Route of administration	Dosing Interval	Cycle
Epirubicin [Pharmorubicin®]	100 mg/m^2	i.v.	Day 1 of each 3-week cycle	4
Cyclophosphamide	600 mg/m^2	i.v.	Day 1 of each 3-week cycle	4

After 4 cycles of EC treatment, each subject will receive subsequent treatment according to routine clinical practice.

Note: 1. The actual total dose of chemotherapy drugs should be maintained at 95–105% of the theoretical total dose;

2. The recommended time for the start of cycle 1 is within 1 month of the surgery. Subsequent cycles should not be delayed for more than 2 weeks. The following criteria must be met prior to starting the next cycle of chemotherapy: ANC $\geq 2.0 \times 10^9$/L, PLT $\geq 80 \times 10^9$/L, and liver and renal functions meeting "Inclusion Criteria". A 14 day recovery period is permitted if these criteria are not met prior to the scheduled cycle 2, cycle 3, and cycle 4. If the criteria described above are still not met after 14 days, the patient is not eligible to enter the cycle 2, cycle 3, or cycle 4 of this study.

6.2.2 Principles of dose adjustment and management

The treatment-emergent toxicities are evaluated using NCI CTCAE v4.03.

The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in cycles 2–4, dose delay and reduction due to toxicities (such as cardiotoxicity) other than myelotoxicity are permitted, or the investigator may individualize the dosage based on subject conditions.

Investigators should assess the subject conditions before starting each treatment cycle, including the severity, duration, and recovery of toxicities. Investigators will then take appropriate actions, such as adjusting, delaying, or suspending the dose.

6.3 Combined and Prohibited Medications

The investigators may decide on the appropriate treatment for infusion reactions caused by the study drug, such as paracetamol, meperidine (dolantin), or chlorpheniramine or other antihistamines.

The following medications are prohibited in the study: recombinant human granulocyte colony stimulating factor (rhG-CSF) and recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF); traditional Chinese medicines with indications for promoting neutrophil production; lithium, which may contribute to the release of neutrophils; and prophylactic antibiotics.

7.0 SAFETY

7.1 Adverse Events (AEs) and Abnormal Values of Laboratory Measurements

7.1.1 Clinical AEs

An adverse event is defined as any untoward medical condition in a patient or clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptoms, or disease temporally associated with the use of a medicinal product (study drug), whether or not considered related to the medicinal product.

If an elective surgery/treatment has been scheduled prior to the study, then it is not considered as an AE. However, if the disease worsens during the trial (e.g. surgery/treatment is required earlier than scheduled), then the elective surgery/treatment due to disease worsening is considered as an AE.

AEs and corresponding severity during the trial should be recorded in the eCRFs. Refer to Section 7.1.1.1 for detailed severity. A causality evaluation between AEs and treatment should also be performed.

7.1.1.1 Severity

AEs are graded according to NCI CTCAE v4.03.

Investigators should use the following guideline to assess the severity of adverse events that are not graded by NCI CTCAE (Table 12) :

Table 12. Grading of adverse events.

Grade	Definition
Mild	The AE causes discomfort but does not affect activities of daily living
Moderate	The AE causes discomfort and affects activities of daily living
Severe	The subject cannot work or perform activities of daily living
Life-Threatening/Disabling	The AE is life-threatening, or results in disability or incapacity, severely affecting activities of daily life
Fatal	Death

7.1.1.2 Causality with study drug

The investigator will use "definitely related", "probably related", "possibly related", "unlikely related", and "unrelated" to determine the degree of causality between treatment and an AE. Refer to the Table 13 for the criteria.

1) Definitely/probably related:

This category applies to AEs that are considered highly likely to be caused by the study drug. The event is considered probably related if three of the followings are met:

a) Plausible time relationship to administration.
b) Cannot be reasonably explained by known signs and symptoms, environmental or toxic factors, or other treatments received.
c) The AE resolved or improved after treatment suspension or dose reduction (with one exception where the AE does not resolve after suspending treatment, such as (1) myelosuppression, (2) delayed dyskinesia).

d) Consistent with the recognized toxicity of the suspected drug.
e) Positive rechallenge.

2) Possibly related

This category applies to AEs that are not likely to be caused by the study drug, but cannot be ruled out with certainty. The AE is considered possibly related if two of the followings are met:

a) Plausible time relationship to administration.
b) Can be explained by disease, environmental or toxic factors, or other treatments received.
c) Consistent with the recognized toxicity of the suspected drug.

3) Unlikely related (meet 2 criteria)

This category of adverse events generally meets the following criteria:

a) Implausible time relationship to administration.
b) Plausibly explained by disease, environmental or toxic factors, or other treatments received.
c) Not consistent with the recognized toxicity of the suspected drug.
d) Negative rechallenge.

4) Unrelated

This category applies to AEs that are clearly caused by other factors (disease, environment, etc.) and does not meet the criteria for "unlikely related", "possibly related", "probably related", and "certainly related".

Table 13. Criteria for causality evaluation.

Criteria	Definitely/Probably Related	Possibly Related	Unlikely Related	Unrelated
Clearly Caused by Other Factors	-	-	-	+
Plausible Time Relationship to Administration	+	+	-	-
May be Explained by Disease	-	+	+	+
Recognized Toxicity of the Suspected Drug	+	+	-	-
Positive Dechallenge	+	-	-	-
Positive Rechallenge	+	-	-	-

7.1.1.3 Serious adverse events (SAEs)

An AE meets any one of the followings is considered as an SAE. The investigator should report the SAE to the sponsor and regulatory authorities according to relevant regulations within 24 hours of learning of the event.

Table 14. Criteria for serious adverse events.

Serious Adverse Event	Definition
Death	An adverse event that causes the death.
Life-threatening	An AE in which the subject is at immediate risk of death at the time of event if no medical interventions are taken as judged by the investigator, not an event that hypothetically might cause death or worsening.
Hospitalization	An AE that leads to hospitalization, excluding emergency or outpatient visits.

Prolonged hospitalization	An AE occurs during the hospitalization and results in prolonged hospitalization.
Congenital anomaly	An abnormality present at birth or after birth, or any malformations leading to abortion.
Permanent or significant disability/incapacity	An AE that has important effect on the subject's daily life. Incapacity does not include medical events of relative minor importance, such as headache, nausea, vomited, diarrhoea, influenza, or accidental injury (e.g. ankle sprains).
Important Medical Events Requiring Pharmaceutical or Surgical Interventions to Prevent Serious Outcomes	Important medical events may not be immediately life-threatening, or result in death or hospitalization, but may jeopardize the subject and require pharmaceutical or surgical interventions to prevent any of the above outcomes (i.e. death, life threat, hospitalization or prolonged hospitalization, and congenital anomaly). These events may include allergic bronchospasm requiring treatment in an emergency room or at home, convulsions that do not result in hospitalization, or drug dependence or abuse.

A severe AE refers to the severity of the event, and is not necessarily an SAE, e.g. persistent vomiting for several hours is considered as a severe AE, but not an SAE clinically.

After the initial report of an SAE, medical history, autopsy report, and other necessary documents should be provided as required.

The severity, causality with the study drug, interventions taken and outcome of an SAE should be included in the report.

7.1.2 Treatment and follow-up of AEs

An AE should be followed until it returns to the baseline level or until it is stabilized. If an AE does not return to the baseline level or cannot be stabilized, a reasonable explanation should be recorded in the eCRFs.

7.1.3 Abnormal values of laboratory measurements

Results of laboratory measurements should be recorded in the eCRFs. Abnormal values of laboratory measurements that meet the criteria for SAEs should be recorded in the SAE reports and the CRFs as AEs simultaneously.

Clinically significant abnormal values of laboratory measurements should be recorded in eCRFs as independent AEs if at least one of the followings is met:

- Accompanied by clinical symptoms
- Resulting in changes in administration (such as dose adjustment, treatment suspension, or permanent discontinuation)
- Requiring changes in combined treatment (such as the addition, suspension, discontinuation of, or changes to combined medication or treatment)

7.1.3.1 Follow-up of abnormal values of laboratory measurements

Any clinically significant abnormal values of laboratory measurements that cannot be explained should be retested and followed until returning to the baseline levels, or provided with a reasonable explanation and recorded in the CRFs.

7.2 AE Management

7.2.1 AE reporting

The investigator should closely monitor the clinical and laboratory evidence of each AE in the study. All AEs should be evaluated and recorded in detail, including the date of onset, symptoms, severity, outcome, duration, result of the event, relationship to the study drug, the diagnosis of the AE, and measures taken. The investigator should provide other potential information on causes of SAEs that are considered possibly unrelated to the study drug.

For an AE considered intermittent, the nature and severity should be similar between each occurrence. All AEs should be recorded, regardless of whether they are collected from the subject's medical history, investigator's observation, or self-reporting by the subject.

All AEs should be followed until satisfactory resolution.

All SAEs that occur from signing the informed consent form and randomization until 30 days after the last dose of the study drug should be recorded, including AEs that occurred prior to the randomization and worsened during the treatment period, regardless of whether they are collected from the investigator's observation or self-reporting by the subject.

7.2.2 SAE reporting (immediate)

If any AEs or abnormal values of laboratory measurements that occur from signing the informed consent form until 30 days after the last dose is considered as an SAE, the investigator must report the SAE to the sponsor and regulatory authorities according to relevant regulations within 24 hours of learning of the event, regardless of whether interventions are given.

For SAEs occurring within the above-mentioned period, those considered related to the study drug should also be reported.

7.2.3 Special non-SAE reporting

Progression of disease:

Tumor progression should not be reported as an AE or SAE. A second cancer can be instead.

Infusion reaction:

Other than reporting an "infusion reaction", symptoms and AEs associated with the study drug or chemotherapy should also be reported. An AE may be related to the infusion if the event occurs during or within 24 hours from the chemotherapy infusion.

The following data should be collected:

- Special symptoms to be recorded in the eCRFs;
- Severity of each AE;
- Symptoms that occur within 24 hours from the infusion, e.g. fever, rigours, and hypotension.

7.2.4 Pregnancy

Subjects who become pregnant during the trial must notify the investigator immediately and discontinue the study drug and chemotherapy. Subjects who become pregnant within 90 days after the completion of treatment should also notify the investigator. The investigator must report the pregnancy to the sponsor within 24 hours. The investigator should discuss with the subject about the risks of continuing pregnancy and potential effects on the fetus. The pregnancy should be followed until satisfactory resolution.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan

8.1.1 Sample size estimation

The primary endpoint is the duration of grade 3 or 4 neutropenia in cycle 1. The non-inferiority/ equivalence margin for F-627 and GRAN® is defined to be 1 day[5-7]. Although the maximum standard deviations for the two F-627 phase II clinical trials SP-CDR-1-1302 and GC-627-02 were 1.33 and 1.58 days, respectively, it is conservatively assumed that the standard deviation for this study is 1.75 days. In the case of a one-sided $\alpha = 0.025$ and a power of 95%, 94 subjects are required for each arm, and in consideration of a 15-20% drop-out rate, 120 subjects are needed for each arm (a total of 240 subjects).

8.1.2 Analysis sets

Efficacy analyses will be based on the ITT and PP analysis sets. The safety analysis will be performed using the safety set. Definitions of each analysis set are as follows:

Intent-to-treat (ITT) set: all randomized subjects who have received the study drug and underwent at least one post-baseline efficacy evaluation. The ITT set is primarily used for efficacy evaluation.

Per-protocol (PP) set A: subjects in the ITT analysis exclusive of subjects with "major protocol violations, serious medication noncompliance, lost to follow-up, or withdrawal" during cycle 1. The PP set A is only used for analyses of endpoints related to cycle 1.

Per-protocol (PP) set B: subjects in the ITT set exclusive of subjects with "major protocol violations, serious medication noncompliance, lost to follow-up, or withdrawal" in any cycle. The PP set B is used for PP set analyses of endpoints only related to overall cycles. The PP sets are primarily used to evaluate treatment efficacy.

Safety set: all randomized subjects who received at least one dose of the study drug. The safety set is primarily used for safety evaluation.

Major protocol violations are confirmed before the database locking. The definitions of major protocol violations are as follows:

- Serious violations of inclusion/exclusion criteria;
- Administration of a wrong study drug (not the originally randomized treatment);
- Administration of a wrong dose
- Other major protocol violations considered by the sponsor.

8.1.3 Statistical analysis methods

8.1.3.1 Significance level and statistical software

Unless otherwise specified, a one-sided test with a significance level of 0.025 for the non-inferiority test will be used. Other tests will use a two-sided test with a significance level of 0.05. Statistical analyses are performed using SAS 9.1.3 or above.

8.1.3.2 Statistical analysis

Efficacy analyses will be performed using both the intent-to-treat (ITT) and per-protocol (PP) sets. Safety analyses will be performed using the safety set. Appropriate statistical methods will be selected according to the nature of data distribution. Refer to the statistical analysis plan for detailed explanations regarding the statistical analysis methods.

8.1.4 Demographics and baseline characteristics

Demographics and baseline characteristics of each dose arm will be summarized using descriptive statistics; continuous variables will be summarized using numbers of cases, means, standard deviations, medians, minimums, and maximums; categorical variables will be summarized using frequencies and percentages.

8.1.5 Efficacy analysis

8.1.5.1 Primary efficacy analysis

The primary efficacy endpoint will test the non-inferiority of F-627 to GRAN® in terms of the duration of grade 3 neutropenia (including grade 4 or severe neutropenia) in cycle 1. The hypotheses for noninferiority between F-627 and GRAN® are as follows:

H_0: $\mu_{\text{F-627 (20 mg/dose)}} - \mu_{\text{GRAN®}} > 1$ day; H_1: $\mu_{\text{F-627 (20mg/dose)}} - \mu_{\text{GRAN®}} \leq 1$ day

This is a non-inferiority test. A two-sided 95% confidence interval will be established using the Hodges-Lehmann estimator (F-627 - GRAN®). Non-inferiority is established if the upper limit of the two-sided 95% confidence interval for the difference in the duration of grade 3 or 4 neutropenia is ≤ 1 day.

Refer to the statistical analysis plan for detailed explanations regarding the statistical analysis methods.

8.1.5.2 Secondary efficacy analysis

The duration of grade 3 neutropenia in cycles 2–4

The duration of neutropenia in cycles 2-4 will be compared between F-627 and GRAN® using the same method as that for the primary endpoint analysis. The 95% confidence interval will be calculated.

The incidence rate of grade 3 neutropenia in cycles 2–4

The frequency and incidence rate of grade 3 neutropenia in cycles 2–4 will be compared between F-627 and GRAN®. The differences between F-627 and GRAN® are compared using Fisher's exact test.

The overall duration of grade 3 neutropenia in overall 4 cycles

The method is the same as the secondary endpoint analysis of overall duration of grade 3 neutropenia in **over**all 4 cycles.

The duration of grade 4 neutropenia in cycles 1–4

The method is the same as the secondary endpoint analysis of the duration of grade 3 neutropenia in cycles 2–4.

The incidence rate of grade 4 neutropenia in cycles 1–4

The frequency and incidence rate of grade 3 neutropenia in each cycle will be compared between F-627 and GRAN®. The differences between F-627 and GRAN® are compared using Fisher's exact test.

ANC - time profile

The efficacies of F-627 and GRAN® are compared by plotting ANC - time logarithmic curves.

The time of ANC recovered to 2.0×10^9/L from nadir

The time of ANC recovered to 2.0×10^9/L from nadir is compared by the same analysis method, calculating 95% confidence intervals.

Febrile neutropenia

The frequency and incidence rate of febrile neutropenia are summarized by different dose arms and cycles. The differences between F-627 and GRAN® are compared using Fisher's exact test.

ANC nadir

The ANC nadir in cycle 1 was compared between different dose arms using the same method as that for the primary endpoint with 95% confidence intervals calculated.

8.1.6 Safety analysis

Safety analysis is performed using the safety set. AEs and laboratory measurements are evaluated based on NCI CTC AE 4.03. All AEs that occur during the treatment period 30 days after the last dose will be tabulated in nature of the event, frequency, severity, and causality with the study drug. All clinically significant laboratory measurements, physical examinations, vital signs, and treatment suspensions, and permanent discontinuations due to AEs will be reported and tabulated. All safety data and comparisons with the baseline values will be tabulated and summarized in detail. AEs and laboratory data will be summarized using descriptive statistics.

In addition, any abnormal values in physical examinations, vital signs, abdominal ultrasound, and ECG should be presented.

8.1.7 Exploratory analysis of F-627 immunogenicity

Analysis of F-627 immunogenicity should be performed in a population with at least one result of serum F-627 antibody assay after F-627 treatment. All results of serum anti-F-627 antibody assays will be tabulated and summarized for descriptive statistics only.

8.1.8 Interim analysis

This study has no scheduled interim analysis.

8.1.9 Data quality assurance

The standard operating procedure (SOP) of contract research organization (CRO) describes the quality assurance procedure of the clinical trial data.

The clinical research associate will check the eCRFs and drug dispensing log to ensure the data accuracy and reliability.

Data from the eCRFs is entered into the database and checked and verified by the computer system. Any inconsistent data should be resolved by the investigator.

8.2 Terminology

AEs, medical history, and surgical history are coded by system organ class (SOC) and preferred term (PT) in ICH Medical Dictionary for Regulatory Activities (MedDRA).

Combined medications are coded using the Anatomical Therapeutic Chemical (ATC) Classification System of the WHO Drug Dictionary (WHODD).

9.0 ETHICS

9.1 Local Regulations and Declaration of Helsinki

The study should be conducted in accordance with the Good Clinical Practice (ICH GCP) based on the Declaration of Helsinki and national laws and regulations applicable to clinical trials in order to maximize the protection of subjects.

9.2 Informed Consent

The investigator or its authorized representative must obtain the informed consent forms signed by the subjects participating in this study. The investigator should explain to the subjects the nature, objectives, potential risks and benefits of this trial, and should inform the subjects that they may withdraw from the study at any time for any reason.

The subject's legal representative must sign the informed consent form if the subject is unable to do so. If both the subject and legal representative are unable to read the informed consent form, a neutral witness must be present during the entire informed consent discussion. After the subject and legal representative verbally agree to participate in the study, the witness must sign the informed consent form and ensure that the subject and legal representative have fully understood the content.

The informed consent will be documented in the eCRFs. If the safety results lead to major changes in the risk/benefit assessment, the informed consent form must be reviewed and revised whenever necessary. All subjects (including those who are receiving treatment) must be informed of the latest information and sign the revised informed consent form prior to trial continuation.

Subjects should be aware that they may withdraw from the study at any time for any reason.

9.3 Privacy Protection

The investigator must ensure that subjects' privacy is not disclosed to any unauthorized third parties. The eCRFs and other documents submitted to the sponsor should not contain the names of the subjects and the subjects are distinguished only by their identification codes. The investigator can retain the enrollment list containing the identification code, name, and address of each subject. Documents such as the informed consent forms should be kept strictly confidential and should not be submitted to the sponsor.

9.4 Independent Ethics Committee/Review Board

The clinical study protocol and amendments, Investigator’s Brochure, informed consent form, clinical trial information for subjects (such as subject recruitment advertisements), and other necessary documents must be reviewed by the Independent Ethics Committee/Review Board (IEC/IRB).

IEC/IRB approval must be obtained prior to the trial commencement. The date of the committee meeting and approval should be indicated in the approval letter to the investigator.

Any protocol amendments must be officially approved or filed by the IEC/IRB.

All SAEs must be reported to the ethics committee and regulatory authorities according to relevant regulations.

The investigator must also report to the IEC/IRB immediately if a protocol violation that may increase subject risks occurs during the trial.

9.5 Protocol Amendment

The protocol amendment must be submitted and approved by the IEC/IRB. Before the amendment is approved by the IEC/IRB, the investigators should still follow the original study protocol, unless the content of the protocol amendment can immediately eliminate potential harms to the subjects, or the content of the protocol amendment only involves changes in study management (such as changes in phone numbers, etc.) or typos.

10.0 STUDY TERMINATION

The sponsor has the right to terminate the study. Both the sponsor and investigator must arrange the related procedures after review and negotiation. After deciding to terminate the study, the sponsor and investigators must give full consideration to protecting the interests of the subjects.

After the study is terminated, the sponsor must notify the regulatory authorities in writing, and the investigator or sponsor must notify the IEC/IRB in writing.

Additionally, the investigator must return, destroy, or retain the trial information as requested by the sponsor.

11.0 RETENTION OF TRIAL DOCUMENTS, ELECTRONIC CASE REPORT FORMS (eCRFs), AND RECORDS

In accordance with ICH GCP requirements, the sponsor or its authorized representative should conduct monitoring and audits to ensure that the trial materials and records are available on site.

11.1 Trial Documents and Document Retention

The investigators must document the trial process adequately and accurately for data verification. These documents can be divided into two categories, investigators' documents and subjects' source data.

Investigators' documents include the study protocols and amendments, case report forms and data clarification forms, approval certificates and correspondences with the IEC/IRB and regulatory authorities, template of the informed consent form, drug records, resumes and authorization forms of the study personnel, and other necessary documents and correspondences.

Subjects' source documents (pre-defined key efficacy/safety data to be recorded) include inpatient/outpatient medical records, doctor's and nurse's orders, appointment dates of visits, original laboratory measurement results, ECG, EEG, X-ray, pathology reports and special assessment reports, signed informed consent forms, consultation records, and subject screening and enrollment lists.

The investigator must keep these documents for 5 years after the completion or termination of the trial. After 5 years, please consult with the sponsor regarding the retention of these documents.

If the investigators are willing to transfer the trial documents to other parties or places, the sponsor should be informed in advance.

If the investigator cannot ensure that these documents are kept safely at the study site, the investigators and sponsor can cooperatively transfer the documents to other places for storage. These documents must be sealed in order for retrieve at the time of regulatory audits. If these documents are still in use, photocopies may be kept at other places.

11.2 Source Documents and Data

When CRFs are illegible or an error occurs during data transfer, the investigator is required to provide the source data of trial documents or medical records upon the request from the sponsor. If there are special concerns or queries and/or requests from regulatory authorities, complete study records should be available in the case that subject privacy is protected.

Each study site is responsible for collecting and keeping the source documents for each subject.

11.3 Electronic Case Report Forms (eCRFs)

In this study, data will be documented on electronic case report forms (eCRFs). All study sites will receive training and guidance on how to fill out eCRFs correctly.

The investigator/study coordinator should be authorized and trained to fill in the eCRFs. The eCRFs should be completed timely and owned by the sponsor. Except for the sponsor's authorized representatives or drug administration personnel, the contents of eCRFs cannot be disclosed to any third parties in any way without the written consent of the sponsor.

The principal investigator is responsible to ensure the completion, review, and approval of eCRFs. The eCRFs must be signed by the principal investigator or authorized investigator to signify that the information in the eCRFs is completed and accurate. The principal investigator is ultimately responsible for the accuracy and authenticity of the clinical and laboratory data in the eCRFs at any time. Subjects' source data are kept at the study sites. The information documented in the eCRFs must be consistent with subjects' source data.

12.0 MONITORING AND AUDITING

12.1 Trial Monitoring and Verification of Source Data

The clinical research associate (CRA) appointed by the sponsor will conduct regular on-site monitoring to ensure that the study protocol is strictly implemented and the study data is accurate and complete. Besides, regular communication (through email, telephone, or fax) is required to ensure that all activities of the study meet the requirements of the study protocol and regulations.

During the daily visits, the clinical research associate should verify that the CRFs are filled in according to the protocol requirements and that data are complete, consistent, and accurate. The clinical research associate should also obtain laboratory measurements and other subject records to verify the accuracy of data filled in the CRFs. All clinical trial conclusions should be ensured to be based on the source data. The investigators (or their assistants) should assist the clinical research associate in resolving any queries raised during monitoring.

12.2 On-Site Audits/Inspections

The investigator should ensure that when the sponsor, IEC/IRB, or the drug administration department is performing on-site audits/inspections, they can directly obtain the source documents related to the trial, eCRFs, and other study documents, and be fully supported and cooperated with. The data in the eCRFs should be directly derived from the source data. The purpose of audits/inspections is to systematically and independently examine all the activities and documents related to the study, ensuring that the activities have been carried out and the data have been documented and analyzed in accordance with the study protocol, ICH GCP guidelines and regulatory requirements.

The investigator must immediately notify the sponsor when regulatory authorities request to inspect the study site.

13.0 CONFIDENTIALITY AND USE OF TRIAL RESULTS

All unpublished information of the investigational drug provided by the sponsor, such as indications, dosage forms, production processes, and other study data, are the proprietary intellectual property and confidential materials belonging to the sponsor. The investigators should agree that all the disclosed information are used for this study only and should not be used for other purposes without the written consent of the sponsor. The study results are owned by the sponsor.

The investigator should agree that study results will be used for drug registration and publication in China and other countries. The names, addresses, qualifications, and responsibilities of investigators must be reported to the drug administration department.

The sponsor is responsible for writing the final clinical study report. The principal investigator is required to state that the study report accurately describes the trial process and results. Trial results should be submitted to the drug administration department or IEC/IRB.

All documents and information provided by the sponsor and from this trial are intellectual properties belonging to the sponsor. The investigator has the right to publish the results. However, a complete copy of the paper or abstract should be sent to the sponsor at least 45 days before the submission. The sponsor reserves the right to review and approve the paper to be published, including the presentation or abstract that uses data from this trial. The investigator must comply with the confidentiality requirements of the sponsor for such information, and agree to retain it for an additional 90 days if necessary, in order that the sponsor has enough time to apply for patent or other intellectual property protection.

The sponsor and the investigator should reach an agreement regarding the way and time to publish the paper prior to the trial commencement.

14.0 REFERENCES

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